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Description of *Aplectana sriniketanensis* sp. nov. (Nematoda: Cosmocercidae) from *Hoplobatrachus crassus* (Anura: Dicroglossidae) from Birbhum District, West Bengal, India

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ABSTRACT

Aplectana sriniketanensis sp. nov. (Nematoda, Cosmocercidae) was collected from the rectum of *Hoplobatrachus crassus* from Illambazar town, Birbhum district of West Bengal, India. The males are characterized by presence of twenty pairs of caudal papillae of which five pairs are pre-anal, two pairs adanal and thirteen pairs postanal in position. Spicules are equal, 0.18×Ù0.20 long, well-sclerotized and non-alate with sharply pointed distal end and bluntly rounded proximal end. Gubernaculum is absent. *Aplectana sriniketanensis* sp. nov. represents 57th species assigned to the genus, 5th from India and 6th from the Indian subcontinent.

Figures : 02 References : 26 Table : 01

KEY WORDS : *Aplectana sriniketanensis* sp. nov; Frogs; *Hoplobatrachus crassus*; West Bengal.

Introduction

Hoplobatrachus crassus, is popularly known as Jerdon's bullfrog and South Indian bullfrog[8,12]. In India, these frogs are found in seasonally flooded grasslands and also in cultivated areas and near human settlements[8]. Works on amphibian nematode parasites in Eastern India, particularly in West Bengal are very rare[19,22,23]. In the Indian subcontinent a number of species described and assigned to the genus *Aplectana*[15], *Oxysomatium*[16] and *Neoxysomatium* have been synonymised with×Ù *Aplectana macintoshii*[3,4,24,25]. It should be noted that five species of *Aplectana* have so far been reported from the Indian subcontinent. These are: *A. macintoshii*, *A. duttaphryni*[20], *A. dubrajpuri*[19], *A. hoplobatrachusia*[21] in India and *Aplectana akhrami*[9].

In the present study, nematodes recovered from the rectum of *H. crassus*, are described and assigned to a new species of the genus *Aplectana*; the name *Aplectana sriniketanensis* sp. nov. is proposed for them. The new species represents the 57th species assigned to the genus and 5th from India. The purpose of this article is to describe a new species of *Aplectana* from West Bengal, India.

Materials and Methods

During an amphibian host survey for nematode parasites in West Bengal, India, one, out of two frogs, *H. crassus*, collected from Sriniketan, Birbhum district, was found to be positive for nematodes. After proper anaesthesia, the frogs were killed and examined for nematode parasites. A longitudinal slit of oesophagus, stomach and intestine helped in examination of contents for nematode parasites. One frog was infected with 8 male and 13 female nematodes. The specimens were cleared from debris by shaking them in 0.67% NaCl solution and

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TABLE-1: Selected diagnostic features of *Aplectana* spp. without gubernaculum[21]

	Male			Female		
***Aplectana* spp.**	**Body length (mm)**	**Spicule length (mm)**	**Caudal papillae pattern in pairs unless otherwise mentioned (Pre- : ad- : post-anal)**	**Body length (mm)**	**Vulva from anterior end (mm)**	**References**
A. akhrami[9]	1.80- 3.40	0.15- 0.18	8: 0: 6+ 2 unpaired papillae on the tail tip	3.10- 3.60	0.90- 1.10	Baker, 1987; Islam *et al.* 1979
A. artigasi[14]	3.00- 4.00	0.322- 0.364	14: 1: 10-14+1 unpaired on upper lip of anus + 2 unpaired papillae on tail	3.80- 6.00	2.30- 3.30	Puga & Torres, 1997
A. chilensis[11]	2.30- 2.70	0.260- 0.410	12: 0: 6	3.30- 3.40	1.60	Lent & Freiras, 1948
A. crossodactyli[3]	2.80- 3.70	0.130- 0.140	20: 0: 5+ 1 unpaired preanal papilla	3.40- 5.30	2.10- 2.60	Baker, 1980
A. crucifer[25]		0.330	6: 1: 4	4.20	2.10	Travassos, 1925
A. delirae[7]	4.00- 4.10			3.80- 4.60	2.00- 2.50	Fabio, 1971; Baker, 1980
A. dubrajpuri[19]	1.06– 1.15	0.147- 0.167	3: 1: 14+ 1 unpaired on upper lip of anus	1.37– 1.51	0.63– 0.66	Sou & Nandi, 2015
A. hoplobatrachusia[21]	1.10–1.62	0.16–0.21	4:1-2:10+ 1 unpaired preanal papilla	2.27–4.50	1.09– 2.29	Sou *et al.* 2018
A. meridionalis[11]	2.30- 2.60	0.130- 0.136	7: 2: 5	3.4- 4.0	1.8-2.0	Lent & Freiras, 1948
A. papillifera[1,3]	4.40- 5.20	0.40	10: 1: 8+ 1 unpaired preanal papilla	5.90- 8.50	3.50- 5.00	Araujo, 1977; Baker, 1980
A. praeputialis[18]	1.50- 2.20	0.220- 0.270	5: 0: 6	3.00- 4.50	1.50- 2.20	Skrjabin, 1916; Travassos, 1931; Baker, 1980
A. tarija Ramallo[6]	3.00- 3.10	0.110- 0.120	1: 0: 4+ 1 unpaired median papilla	4.70- 5.80	2.70- 3.30	Ramallo *et al.* 2007
A. vercammeni[10]	2.30	0.240- 0.250	8: 0: 12	8.00	5.30	Le Van Hoa, 1962
Aplectana sriniketanensis sp. nov	1.45- 1.52	0.18- 0.20	5: 2: 13	5.01- 5.10	1.69- 1.73	Present study

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were killed and fixed in steaming 4% formaldehyde solution; 5% glycerine was used as a mounting medium for examination using a light microscope. After examination, the glycerine treated specimens, were transferred directly to 4% formalin and stored. For SEM studies, nematodes after washing thoroughly in physiological saline (0.67% NaCl) were fixed in 2.5% gluteraldehyde in 0.2 M sodium cacodylate buffer (pH-7.2), post fixed in 2% osmium tetroxide in the same buffer, dehydrated in ascending series of ethanol, transferred to 100% isoamyl acetate through the mixtures of ethanol and isoamyl acetate, and finally critical point dried. Then the nematodes were coated with gold and examined with a Hitachi S-530 (Japan) SEM at a resolution of 50 ú and operating at an accelerating voltage of 15 kV. All measurements of nematode parasites are given in millimetres unless otherwise stated. All type specimens have been submitted in Helminthological collection, Parasitology Laboratory, The University of Burdwan, Purba Bardhaman, West Bengal, India.

Results

Aplectana sriniketanensis sp. nov. (Fig.1a-i; Fig.2a-h)

General morphology: Small worms. Cuticle moderately thick with prominent transverse striations. Lateral alae well developed and extend from a level slightly anterior to the cervical papillae posteriorly to the anal region in both sexes. Mouth surrounded by three large lips, one dorsal and two subventral. Four submedian cephalic papillae present at the base of the lips. Dorsal lip bears two cephalic papillae and each of the subventral lips bears one cephalic papilla and an amphid. Four submedian rows of small sized somatic papillae present, starting from the pharyngeal region to the anal region in both sexes. Oesophagus divided into a short pharynx, a long cylindrical corpus, a small isthmus and a large valved oesophageal bulb. Nerve ring located at the posterior half of the corpus. Excretory pore situated just posterior to the oesophageal bulb. Cervical papillae spine-like and situated anterior to the nerve ring.

Male: (based on holotype and seven paratypes; measurement of holotype in parentheses): Body 1.45×1.52 (1.50) long and 0.19×0.21 (0.22) wide. Total length of oesophagus 0.34×0.37 (0.35), of pharynx 0.043×0.045 (0.045) long and 0.052×0.057 (0.056) wide, of cylindrical corpus 0.22×0.24 (0.23) long and 0.04×0.06 (0.05) wide, of oesophageal bulb 0.08×0.09 (0.08) long and 0.07×0.08 (0.07) wide. Distance to nerve ring 0.13×0.135 (0.131), to cervical papillae 0.10×0.102 (0.10), to excretory pore 0.23×0.29 (0.28) from anterior end. Tail 0.21×0.23 (0.22) long, ventrally curved, tapering gradually to a fine spine-like terminal end. Twenty pairs of caudal papillae of which five pairs are pre-anal (three pairs ventral and two pairs subventral), two pair adanal (lateral to cloacal opening) and thirteen pairs postanal (two pairs ventral and eleven pairs lateral) in position. In morphological view, caudal papillae larger in size than the somatic papillae. Spicules equal, 0.18×0.20 (0.19) long and 0.01×0.012 (0.01) wide, well-sclerotized and non-alate with sharply pointed distal end and bluntly rounded proximal end. Cloacal opening transverse and semilunar in structure. Gubernaculum absent.

Female: (based on allotype and twelve paratypes; measurement of allotype in parentheses): Body 5.01×Ù5.10 (5.05) long and 0.45×Ù0.47 (0.45) wide. Total length of oesophagus 0.54×Ù0.61 (0.57), of pharynx 0.06×Ù0.08 (0.07) long and 0.07×Ù0.09 (0.08) wide, of cylindrical corpus 0.38×Ù0.41 (0.39) long and 0.04×Ù0.06 (0.05) wide, of oesophageal bulb 0.10×Ù0.12 (0.11) long and 0.11×Ù0.12 (0.11) wide. Distance to nerve ring 0.34×Ù0.40 (0.37), to cervical papillae 0.22×Ù0.27 (0.24), to excretory pore 0.49×Ù0.53 (0.51) from anterior end. Tail 0.42×Ù0.50 (0.46) long, relatively thick in anterior quarter, tapers gradually through last three quarters to a spine-like terminal end. Anus broad and transverse opening. Vulva located at the junction of anterior and middle third of the body, 1.69×Ù1.73 (1.70) from anterior end. Reproductive system prodelphic with two ovaries. Eggs thin-shelled and embryonated when released.

Taxonomic Summary

Family: Cosmocercidae Railliet, 1916

Subfamily: Cosmocercinae Railliet, 1916

Genus: *Aplectana* Railliet and Henry, 1916

Species: *Aplectana sriniketanensis* sp. nov.

Host: *Hoplobatrachus* crassus (Jerdon, 1853)

Location: Rectum

Locality: Sriniketan, Birbhum, West Bengal, India

Holotype: One male; Regn. No: BUPL- 159A

Allotype: One female; Regn. No: BUPL- 159B

Paratypes: Seven males and twelve females; Regn. No.: BUPL-159C

Etymology: The new species is named after the locality of collection of the host.

Discussion

In SEM micrograph of the head in enface view triangular mouth opening is seen to be bordered by a thick cuticularised margin formed by the free end of the lips. The dorsal lip bears two large submedian cephalic papillae and each of the ventro-lateral lips bears one submedian cephalic papilla and a lateral amphid. In SEM micrograph of the tail, the caudal papillae are elevated on the cuticle and in lateral view of the tail phasmids are

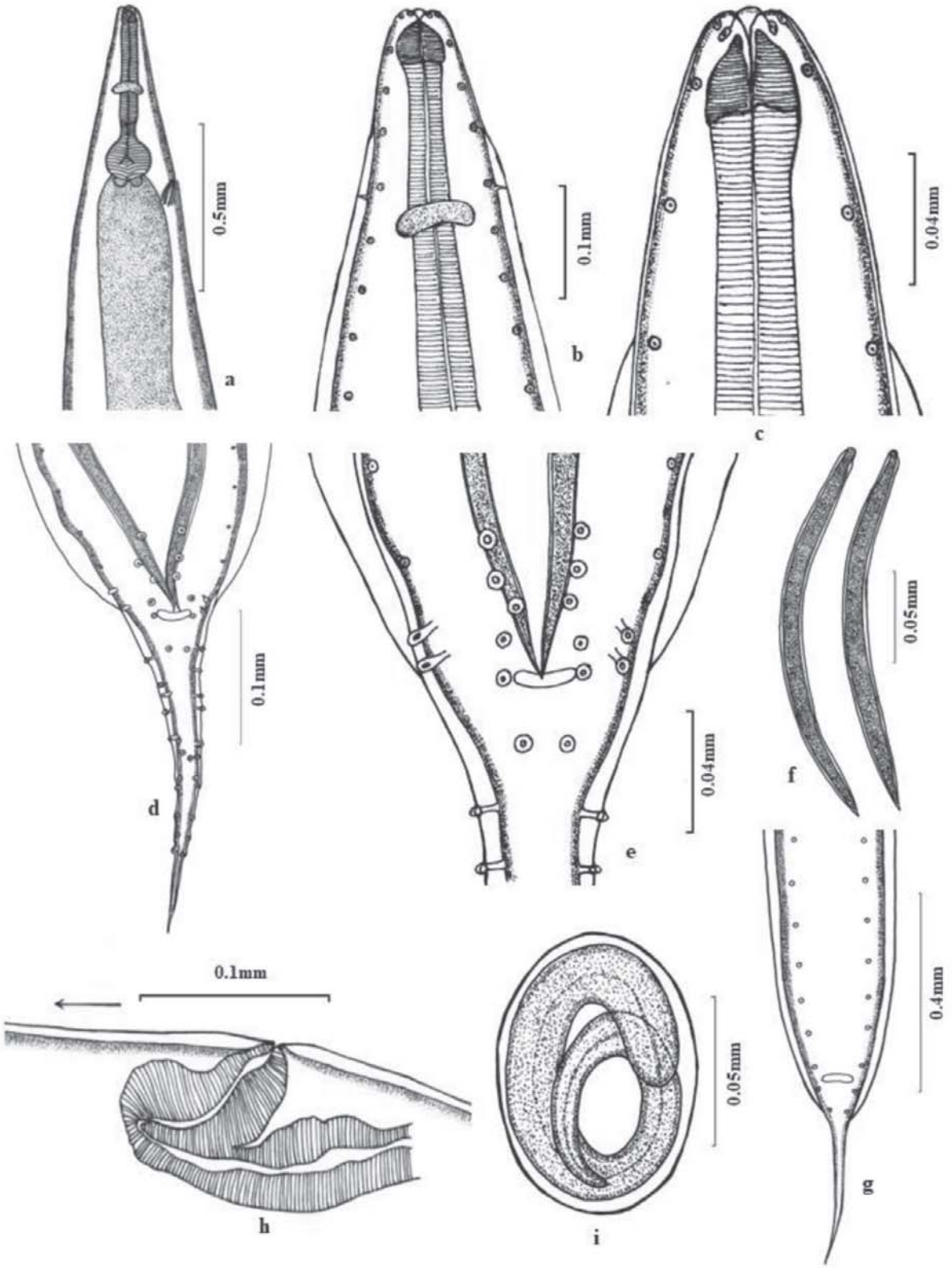

Fig. 1: ***Aplectana sriniketanensis*** **sp. nov. a. Anterior end of a female, lateral view. b. Anterior end of a male, dorsalview. c. Enlarged view of the anterior end of a male, dorsal view. d. Posterior end of a male, ventral view. e. Enlarged view of the cloacal region of a male, ventral view. f. Spicules (Isolated). g. Posterior end of a female, lateral view. h. Vulvar region of a female, lateral view; arrow points towards the cephalic end. i. Embryonated Egg.**

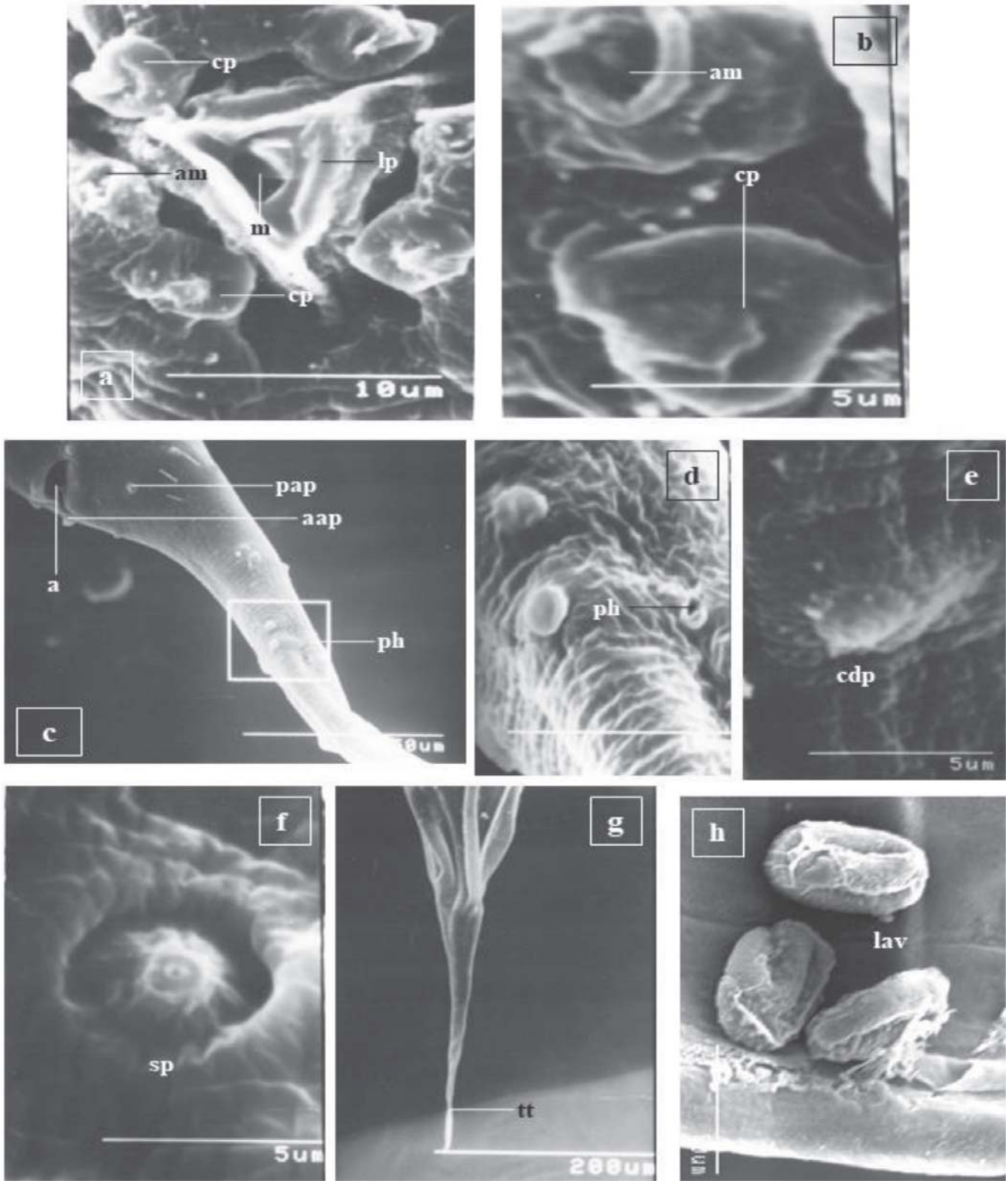

Fig. 2a-h (SEM micrographs): *Aplectana sriniketanensis* sp. nov. a. Enface view of head (female). b. Enlarged view of head showing amphid and cephalic papillae. c. Posterior region of male, ventro-lateral view. d. Enlarged view of tail showing phasmid. e. Structure of a caudal papillae in male. f. Structure of a somatic papillae in male. g. Posterior region of a female showing tail filament. h. Structure of embryonated eggs. (Abbreviation used: a- anus, aap- adanal papillae, am- amphid, cdp- caudal papillae, cp- cephalic papillae, lav- embryonated larvae, lp- lips, m- mouth, pap- postanal papillae, ph- phasmids, sp- somatic papillae, tt- tail

seen to open as a pore like structure. The cloacal opening is semilunar in shape and it is guarded by thin cuticular margin. In SEM micrograph, mature eggs are thin shelled and some are embryonated. In SEM micrograph, the individual somatic papilla appears to be as a small knob projecting from a circular depression on cuticle.

The genus *Aplecta* was with *Ascaris acuminata*, as type species[14]. Since the name *Aplecta* was preoccupied, after that replaced the name *Aplecta* to *Aplectana*[15]. In the Oriental region only five species of the genus *Aplectana* have been reported, namely- *A. akhrami*, *A. dubrajpuri*, *A. duttaphryni*, *A. macintoshii, A. hoplobatrachusia* and *A. ranae*[3].

It should be noted that *Aplectana hainanensis*[5] was described as the 52nd species of the genus[5] but were not included two other species of *Aplectana* (in Table-1) that had been described previously: *A. nebulosa* from *Pleurodema nebulosum* from Argentina and *A. nordestina* Amorim from *Leposteron polystegum* from Brazil[1]. Recently described is *A. samarensis* from *Limnonectes magnus* in the Philippines[6]. Recently described is *A. hoplobatrachusia* from *H. crassus* in India. So the genus, *Aplectana* now comprises of 56 species of which only, *A. dubrajpuri*, *A. duttaphryni*, *A. hoplobatrachusia* and *A. macintoshii* have been reported from India[19-22].

The present nematodes are similar to *A. akhrami*, *A. artigasi*, *A. chilensis*, *A. crossodactyli*[3], *A. crucifer*[25], *A. delirae*[7], *A. dubrajpuri, A. hoplobatrachusia*, *A. meridionalis*[11], *A. papillifera*[2], *A. praeputialis*[17], *A. tarija* Ramallo[5] and *A. vercammeni*[10] by absence of a gubernaculum. Morpho-metric variations in features of the present species from those of other species[21] of *Aplectana* lacking a gubernaculum are presented in Table-1.

The new species described here is very close to *A. akhrami, A. dubrajpuri, A. hoplobatrachusia* and *A. praeputialis* in body length of males and in spicule lengths. The new species also is similar to *A. akhrami*, *A. chilensis*, *A. crucifer*, *A. vercammeni* and *A. praeputialis* in lacking papilla on the upper lip of the cloaca and additionally to *A. praeputialis* in having a pre-equatorial vulva. Finally, they differ from all above mentioned species in having a different number and arrangement of caudal papillae in males and a greater body length in females (except females of *A. vercammeni*). The females of the new species are smaller than that those of *A. vercammeni*. The vulva is more equatorial in the new species than that of *A. praeputialis*. Although the length of spicules in males of the new species is similar to that of *A. crossodactyli, A. meridionalis* and *A. tarija*, these species differ from *A. srinikatanensis* sp. nov. in other diagnostic metrical data and on the number and arrangement of caudal papillae.

The present nematodes, thus, appear to be new to science and the name *Aplectana sriniketanensis* sp. nov. is proposed for it. The new species is named in reference to its locality of collection. *A. sriniketanensis* sp. nov. represents the 57th species assigned to the genus and 6th species reported from the Indian subcontinent.

Compliance with ethical standards: Though there is no ethics committee in our Institution, frogs have been sacrificed for the present study following guidelines of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Act of the Indian Parliament.

Conflict of interest: Authors declared that there is no conflict of interest.

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