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A New Species of *Neyraplectana* Ballesteros Marquez, 1945 (Oxyuridae: Cosmocercinae) From Intestine of Bull Frog, *Rana tigrina* (Anura: Dicroglossidae) in North India

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ABSTRACT- A new species of oxyuridean nematodes, *Neyraplectana fatehpurensis* n.sp. (Oxyuridae: Cosmocercinae) has been collected from small intestine of bull frog, *Rana tigrina* (Anura: Dicroglossidae) in North India during one year (2008-2009) uninterrupted investigation and substantiated by the application of polythetic divisive classificatory system. Sums of 257 bull frogs (male 159; female 98) were examined and 239 specimens, *Neyraplectana* sp. (male 97; female 142) recovered during parasitological investigation. The numerical validation of newer nematodes *N. fatehpurensis* n.sp. were based on the morphotaxometry of 33 specimens (male 12; female 21). The worms were typically characterized by paired equal spicules, absence of a gubernaculum and plectane, 5 pairs of preanal, 2 pairs adanal and 10-11 pairs post anal papillae, sub-equatorial vulva and *in utero* embryonated eggs.

Key-words- Cosmocercinae, Neyraplectana fatehpurensis n.sp., Dicroglossidae, Rana tigrina, Polythetic divisive classificatory system, Morphotaxometry

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INTRODUCTION

The earlier workers on amphibian's taxonomy have worked out more than 5,000 species of frogs [1] and these are the organisms that have great risk on their existence and survivals. The decline in the populations of frogs and amphibians worldwide is due to the loss and fragmentation of habitat, water, soil, radiation and chemical pollutions, global warming and climate changes, introduction of non-native species and in the last but not the least helminthes populations as parasites also been responsible for the decline of aquatic, amphibious and terrestrial hosts [2-6]. A comprehensive or in-depth investigation on helminthes parasite fauna of anurans was carried out in rural areas of western region of Nagaland state and species of worms recovered during study was summarized as checklist [7].

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But the helminthes worm of present investigation was not explored by the earlier worker, therefore, taken in consideration for the establishment and validation of this newer fauna. A very interesting and economically important genera of nematoda, Aplectana [8] was investigated in the old world and it has been revised and redescribed by so many taxonomists time to time. Thus the supplementary species of the old world are either debarred from the genus or synonymized with various Aplectana spp. But latest world species are reviewed and revised and finally about 21 species are considered as valid species. Out of that one species, A. travassosi [9] was proposed for Neyraplectana travassosi [10]. In the early taxonomic investigation of helminthes fauna, Neyraplectana [11] was proposed for Aplectana spp. and characterized by the devoid of a gubernaculum. Finally the Neyraplectana as a synonym of Aplectana was recommended by Chabaud [12]. Various species of Neyraplectana described by earlier workers in vesteryears are: N. punctata [13], N. schneideri [14], N. chilensis [15], N. travassosi [10], N. crucifer [16], N. delirae [17], N. meridionalis [15], N. papillifera [18], N. vellardi [19], and N. pintoi [16].

MATERIALS AND METHODS

The host bull frogs, Rana tigrina were collected from fresh water ponds at Fatehpur district of Uttar Pradesh, north India for parasitological investigations during 2008-2009 in Parasitology Laboratory, Department of Zoology, University of Allahabad, Allahabad, Uttar Pradesh, India. The recovered nematodes were preset and processed for preservation of worms [20]. Nematodes were for the interim mounted in glycerol for morphometric examination under microscope. Microphotographs were captured by image analyzer unit "MOTIC" using Biovis image plus software Nikon trinocular computerized and photomicrography unit. Drawings of roundworms were prepared with SIPCON SP-14 camera lucida. Polythetic Divisive Classificatory System (PDCS) was applied to conduct taxometric analysis with earlier described taxa of same genera to evaluate and establish the present worms as newer species [21-24]). PDCS worked out with the application of various formulae such as: Coefficient of Dissimilarity [25], Coefficient of Divergence [26], Mean Character Difference [27-28], heretofore mentioned as C. Dis., C. D. and M.C.D. in text respectively.

RESULTS AND DISCUSSION

Description: The genus *Neyraplectana* [11] was characterized by the flask-shaped oesophagus, typical equal spicules, absence of gubernaculums and plectane [29-30]. The description of newer round worms *N. fatehpurensis* n.sp. were based on the morphometry of 12 mature male and 21 female round worms collected from the small intestine of bull frogs, *Rana tigrina*.

Male (Fig. 1 & 2): The male specimen of worms were 1.926-2.862(2.413±0.096) X 0.054-0.306(0.171±0.013) in dimension. The frontal beginning of body was tapering $0.013 - 0.034(0.023 \pm 0.002)$ with small head Х $0.027-0.042(0.034\pm0.001)$. The orifice was safeguarded by three distinct lips with one pairs of cephalic papillae. The buccal capsule was indistinguishable from pharynx $0.004-0.036(0.022\pm0.004)$ X $0.004-0.034(0.022\pm0.003)$ and leads into long round bottom flask-shaped oesophagus 0.296-0.379(0.338±0.009) X 0.027-0.108(0.048±0.006). The oesophagus was divided into two parts, anterior elongated glandular part 0.214-0.272(0.243±0.029) X $0.027-0.072(0.050\pm0.022)$ and a posterior distinct muscular bulb of $0.054 - 0.099(0.081 \pm 0.006)$ Х 0.045-0.108(0.079±0.004) size. Excretory pore was present at 0.256-0.423(0.316±0.015) and nerve ring at $0.126-0.416(0.271\pm0.145)$ distance from farthest anterior point of body. Intestine was simple and free of diverticula with 0.045-0.144(0.107±0.007) diameter. A pair of typical 0.189-0.369(0.285±0.019) equal spicules of X $0.004-0.027(0.013\pm0.002)$ in size was present. The male worms were devoid of a gubernaculum. There were, 5 pairs of preanal, 2 pairs adanal and 10-11 pairs post anal distinct papillae. Tail was longer tubular structure with 0.162-0.180(0.171±0.009) size and slit like anal aperture at a distance 0.168-0.270 (0.210±0.009) from tail tip.

Female (Fig. 3 & 4): Body was comparatively larger with 2.295-6.984(4.579±0.208) Х 0.090-0.464 (0.273±0.009) size. The anterior end was stout with broad head. $0.018 - 0.054(0.031 \pm 0.001)$ Х 0.036-0.126 (0.073 ± 0.004) with triangular slit like orifice fortified by three distinct lips and each bears a pair of papillae. Pharynx was very short 0.009-0.054(0.030±0.002) X 0.004-0.072 (0.040±0.003) and indistinct from buccal cavity. The pharynx was followed by long flask-shaped oesophagus 0.360-0.585(0.454±0.015) X 0.027-0.162(0.078±0.003) divided into two parts, anterior elongated glandular part 0.272-0.441(0.373±0.085) X 0.027-0.054(0.041±0.013) and a posterior distinct highly muscular bulb of 0.088-0.144(0.110±0.004) X 0.054-0.162(0.095±0.003) size. The gastrointestinal tract was trouble-free and its anterior part dilated at the region of oesophageo-intestinal junction, $0.032-0.204(0.089\pm0.004)$. The excretory pore and nerve ring was located at a distance of $0.392-0.684(0.494\pm0.015)$ and $0.126-0.198(0.166\pm0.008)$ respectively from anterior extremity. Vulva was sub-equatorial and placed comparatively more towards the centre of body and at 1.141-3.260(2.249±0.131) from anterior and 1.154-3.524(2.299±0.130) from posterior terminal. Vagina ran a short distance anteriorly before it moved back into uterus and became didelphic. The full-grown uterus was packed with large sized embryonated eggs 0.036-0.090 (0.061 ± 0.011) Х 0.045-0.090 (0.067±0.010). The anus was to be found at $0.333-0.846(0.513\pm0.025)$ detachment from tail tip. A long tubular, pointed tail was present measures 0.270-0.504 (0.367±0.019) in length. There were in utero coiled, slender, transparent larvae and $0.504-0.648(0.565\pm0.024)$ X $0.018-0.036(0.027\pm0.005)$ in size, proved the ovoviviparity in the present worms.

Systematic summary:

SJ Sterre St.	
Order:	Oxyuridea [31]
Family:	Oxyuridae [32]
Subfamily:	Cosmocercinae [33]
Genus:	Neyraplectana [11]
Species:	Neyraplectana fatehpurensis n.sp.
-	(Fig. 1-4)



Fig. 1: Male *N. fatehpurensis* n.sp. Scale bars 0.1mm (A, C) and 0.05mm (B, D)



Fig. 2: Microphotographs of male *N. fatehpurensis* n.sp. (not to scale)



Fig. 3: Female *N. fatehpurensis* n.sp. Scale bars 0.1mm (A, C) and 0.05mm (B)



Fig. 4: Microphotographs of female *N. fatehpurensis* n.sp. (not to scale)

The newer worms were can be differentiated from earlier described species *N. multipapillata* [34] in possessing shorter body and pharynx, larger volvular machinery at oesophageo-intestinal junction and exceedingly powerfully built oesophageal bulb, farther site of excretory pore and closer setting of nerve ring from frontal end, position of vulva towards more equatorial and comparatively smaller *in utero* embryonated eggs and larvae, absence of sessile post anal papillae in female, however, larger body and comparatively longer spicules in male. The worms of current investigation were possessing volvular apparatus at oesophageo-intestinal junction in both the sexes of worms, however, absent in *N. crucifera* [16] and also differed in

greater number of preanal and post anal papillae in male as well as long tubular tail terminated into a pointed spine in female. The present worm also differed from the *N. linstowi* [30], *N. pintoi* [16], *N. schneideri* [14] and *N. vellardi* [19] in bearing cuticular configuration in oral capsule, spot of excretory stoma and nerve ring as well as superior numeral of caudal papillae. Aforementioned noteworthy points of morphotaxometric differences were substantiated and validated by the numerical application of Polythetic Divisive Classificatory System [21] after scheming C.D., Coefficient of Divergence [25], C. Dis., Coefficient of Dissimilarity [26] and M.C.D., Mean Character Difference [27] to elucidate taxonomic differentiation (Table 1).

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Character			Male			Female		
		C.D.	C.Dis	M.C.D.	C.D.	C.Dis	M.C.D.	
Body	L	2.314	0.479	0.385	-	0.606	0.427	
	W	_	0.964	0.729	_	0.683	0.638	
Pharynx	L	2.537	0.318	0.739	-	0.810	0.504	
·	\mathbf{W}							
		2.463	0.367	0.764	2.301	0.905	0.646	
Oesophageal bulb	L	2.373	0.398	0.321	-	0.851	0.671	
	W	2 2 2 1	0.621	0.464	2 202	0.661	0 472	
	• •	2.551	0.021	0.404	2.302	0.001	0.472	
Excretory pore from and	terior end	-	0.379	0.314	-	0.431	0.350	
Nerve ring from anterio	r end	-	0.763	0.534	2.412	0.455	0.370	
Intestine	W	2.449	0.698	0.492	2.323	0.865	0.541	
Anus to tail distance		2.335	0.364	0.319	2.318	0.619	0.458	
Tail process	L	-	-	-	-	0.763	0.548	
Spicules	L W	-	0.484	0.383	-	-	-	
	••	-	0.924	0.578	-	-	-	
Vulva from anterior end	l	-	-	-	2.448	0.794	0.559	
Vulva from posterior en	d	-	-	-	2.325	0.879	0.604	
Egg	L W	-	-	-	2.303	0.716	0.523	
	**	-	-	-	2.330	0.543	0.423	

Table 1: Taxometric analysis of observations of N. multipapillata [34] vis-a-vis N. fatehpurensis n.sp

Taxonomic summary:

Type host: *Rana tigrina* (Anura: Dicroglossidae)

Type locality: Fatehpur, U.P.

Habitat:Small intestine

- **Type specimen:** Holotype, PNLS 65/2008 (male) and Paratype PNLS 66/2008 (female) deposited with Parasitology Laboratory, Department of Zoology, University of Allahabad, Allahabad, U.P., India.
- **Etymology:** The worms of the newer species *N*. *fatehpurensis* n.sp. was named after habitat of its host in district Fatehpur U.P., north India.

CONCLUSIONS

The numerical validation of newer nematodes as *N. fatehpurensis* n.sp. (Oxyuridae: Cosmocercinae) were based on the morphotaxometry of collected specimens from gut of bull frogs, *R. tigrina* in north India. The worms were systematically characterized on the basis of taxonomically significant features as: paired equal spicules, absence of a gubernaculum and plectane, 5 pairs of preanal, 2 pairs adanal and 10-11 pairs post anal papillae, sub-equatorial vulva and *in utero* embryonated eggs. The differential numerical substantiation and validation of newly recovered worms with earlier described species of same genera based on polythetic divisive classificatory system were found to be significant. Hence the newer worm of the genus

Neyraplectana was proposed to accommodate as new species; *Neyraplectana fatehpurensis* n.sp.

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