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Morphological Redescription of the *Spinitectus notopteri* Karve and Naik, 1951 from the Bronze Featherback *Notopterus notopterus* (Pallas, 1769) from Muzaffarnagar (U.P.), India

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ABSTRACT

The parasitic nematode *Spinitectus* Fourment, 1883 is redescribed from specimens collected from stomach of freshwater fish *Notopterus notopterus* (Pallas) (Notopteridae, Osteoglossiformes) in district Muzaffarnagar (Uttar Pradesh) India. Based on the morphological and morphometrical comparisons, the results indicate that it is *Spinitectus notopteri* Karve and Naik, 1951.

Keywords: Nematodes, *Spinitectus notopteri,* fish, *Notopterus*,

INTRODUCTION

Parasitic nematodes are one of the important group of helminths in fishes. Nematodes infect almost all species of fishes in almost all locations. Genus *Spinitectus* Fourment, 1883 belongs to the family Cystidicolidae, a group of nematodes that have cosmopolitan distribution. *Spinitectus* comprises a rich diversity of cystidicolid nematodes that are parasites of fishes (Yamaguti, 1935; Soota, 1983; Sood, 1989; Moravec, 1998; Moravec et al., 2010; Moravec and Nagasawa, 2021). In India, species of *Spinitectus* are poorly described based only on the literature data. About six species of *Spinitectus* are reported from India from the host *N. notopterus* i.e., *S. notopteri* Ali, 1957; *S. thapari* Ali, 1957; *S. bengalensis* Chakravarty et al., 1961; *S. alii* Kalyankar, 1970; *S. agrawali* Verma, 1971 and *S. muelleri* Gupta and Verma, 1979. Although by Sood (1989), *S. agrawali* and *S. muelleri* were synonymized with *S. mastacembeli* Karve and Naik, 1951. Specimens of *Spinitectus* were recovered during a survey of freshwater fish nematode parasites in district Muzaffarnagar (Uttar Pradesh), India and are described on the basis of morphological features herein.

MATERIAL AND METHOD

Fish *Notopterus notopterus* (Osteoglossiformes: Notopteridae) were collected from the fish market of the district Muzaffarnagar (29.4727° N, 77.7085° E) Uttar Pradesh, India in November and December, 2020. Name of fish follows FishBase (Froese and Pauly 2009). Nematodes recovered from the stomach of the host were fixed in hot 4% formaldehyde solution. For light microscopy, they were cleared in glycerin and drawings were made with the aid of camera Lucida. Photomicrography of the specimen was done and is presented herewith. All measurements given in the description are in micrometers except which are stated otherwise.

OBSERVATION AND DESCRIPTION

Spinitectus notopteri Karve and Naik, 1951 (Fig. 1 and 2)

Host - *Notopterus notopterus* (Osteoglossiformes: Notopteridae).

Site of infection - Stomach.

Locality - District Muzaffarnagar (29.4727° N, 77.7085° E) Uttar Pradesh, India.

Total prevalence - 61% (13 fish infected/21 fish examined.

Depository of voucher specimens - Department of Zoology, D.A.V. College, Muzaffarnagar (Uttar Pradesh), India.

Synonyms: S. thapari Ali, 1957; *S. bengalensis* Chakravarty et al., 1961; *S. alii* Kalyankar, 1970; *S. gomalensis* Siddiqui and Kattak, 1984.

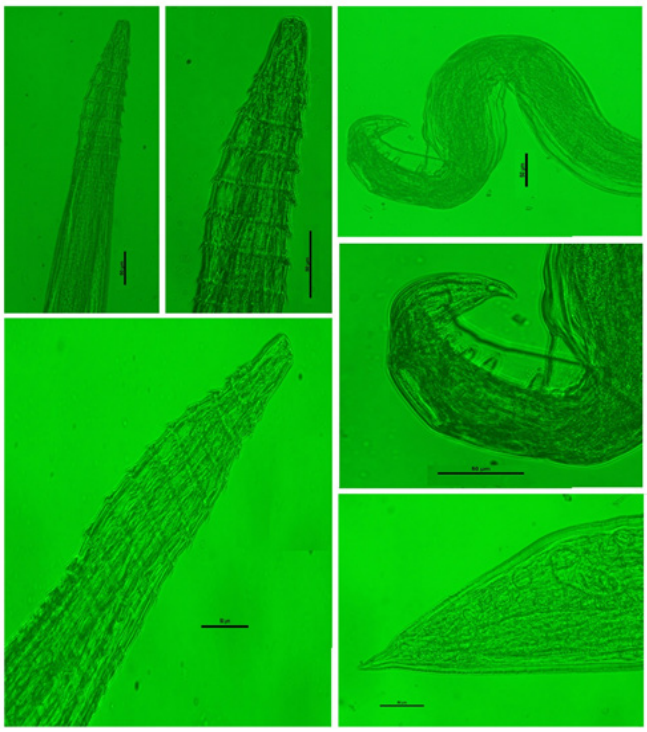

Fig. 1 Spinitectus notopteri Karve and Naik, 1951. A – anterior view of male, B – anterior end of male enlarged view, C – tail of male, D – tail of male showing papillae with enlarged view, E- anterior end of female, F – posterior end of female showing eggs.

DESCRIPTION

Specimens of *S. notopteri* small, white with elongate body, blunt anterior cephalic end and conical posterior end. Transverse rings of spines on body surface. At one side view, first ring with 21-22 spines. The longest spines present in anterior 5th to 7th ring, after that they are gradually reducing posteriorly.

Male (3 specimens) (Fig. 1, Table 1). Length of body measures 1.33–2.21 mm with maximum width of 104–115 respectively. Spines maximum length measures 8-9.83. From anterior extremity, first ring of spines situated 40-49.28. Oesophagus divided into muscular and glandular. Muscular oesophagus 80-120 in length, 10-16 in width while glandular oesophagus 325–350 in length, 40-50 in width. From anterior extremity, nerve ring and excretory pore measures 49.35-70 and 117.90–123.50, respectively. Body posterior end curved of ventrally with preanal and postanal papillae present. Spicule length measures 279–315 with tail 75-109 long respectively.

Female (3 specimens) (Fig. 1, Table 2). Body length measures 3.43–3.85 mm with a maximum width of 164–206. Spines maximum length 10.7–12.5. From anterior extremity, first ring of spines measures 51–54.25. Oesophagus divided into: muscular oesophagus 149.10–194 long, 16.4–23 wide while glandular oesophagus measures 485–550.8 long, 55–65.3 wide. From anterior extremity, nerve ring and excretory pore measures 80.37–102 and 110–155.7 respectively. Vulva not bulging, placed in posterior part of body, measures 2.84–3.55 mm from anterior extremity. Muscular vagina present with anteriorly directed from vulva. Amphidelphic uterus is present. Developed eggs present in uterus, oval in shape, thick-walled, measures 27.94–32 in length and 17–27.43 in width respectively. Tail 49–71.84 in length, covered by minute spines.

Table 1. Comparative measurement of male and female *Spinitectusnotopteri* KarveandNaik, 1951 from *Notopterunotopterus*.

Body features	***Spinitectusnotopteri* Moravec et al., 2016 (female)**	***Spinitectusnotopteri* Moravec et al., 2016 (male)**	Spinitectusnotopteri **Present specimen (female)**	***Spinitectusnotopteri* Present specimen (male)**
Body length	3.30-4.08mm	2.43-3.26	3.43-3.85mm	1.33-2.21mm
Body width	177-231	99-135	164-206	104-115
Spines length	9-15	9-12	10.7-12.5	8-9.83
First ring of spines from anterior region	51-63	39-57	51-54.25	40-49.28
Muscular oesophagus length	171-202	108-171	149.10-194	80-120
Muscular oesophagus width	21-30	12-24	16.4-23	10-16
Glandular oesophagus length	501-570	396-492	485-550.8	325-350
Glandular oesophagus width	57-78	36-75	55-65.3	40-50
Nerve ring from anterior region	90-111	69-99	80.37-102	49.35-70
Excretory pore from anterior region Tail length	141-156	117-135	110-155.7	117.90-123.50
Spicule length		1:3.57-4.52	-	279-315
Vulva from anterior region	3.06-3.82	-	2.84-3.55	-
Eggs length	33-36	-	27.94-32	-
Eggs width	27-30		17-27.43	-

DISCUSSION

Genus Spinitectus Fourment, 1883 include nematode species infecting, freshwater and marine fishes, some amphibians and one species also reported from mammal (Boomker, 1993, Moravec et al., 2002, 2009, 2010). Species were also described from freshwater fishes of India (Soota 1983, Moravec and Sey 1988, Sood 1989, Singh and Tandon 2002). Validity of nematode species that belong to the genus *Spinitectus,* from India, have taxonomic confusions due of lack of proper morphological and morphometrical data. This is the reason why many species are in category of *species inquirendae* (Moravec and Yooyen 2011).

The original description of *Spinitectus notopteri* Karve and Naik, 1951 was based on morphology of single male specimen. Due to lack of morphometrical data it was not possible to compare it with nematodes of the present specimen collected from *N. notopterus*. Although, Moravec et al., 2016 described *S. notopteri* infecting *N. notopterus,* from Thailand, much more extensively, supported with light and scanning electron microscopy. *S. notopteri* is easily distinguished from other *Spinitectus* species from India, in number and arrangement of caudal papillae. According to Moravec et al., 2016, the number and arrangement of papillae described by Karve and Naik (1951) is somewhat unfamiliar for *Spinitectus* species. Moravec et al., 2016 consider *S. thapari* Ali, 1957; *S. bengalensis* Chakravarty et al., 1961; *S. alii* Kalyankar, 1970; *S. gomalensis* Siddiqui and Kattak, 1984 to be the synonyms of *S. notopteri*.

The biodiversity of *Spinitectus* species from India should be explored with proper description, for future studies. For a better understanding of their phylogenetic relationship, the morphological and morphometrical data should be supported by molecular data.

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Varietal Screening of Tomato Crop with Reference to Symptoms & Physiological properties due to TLCV in Ayodhya (U.P.)

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ABSTRACT

Tomato (*Solanum lycopersicum* L) is an important vegetable crop and cultivated at large scale in India. However, this crop suffers by a number of disease from nursery phase to an adult plant. Identification of viral of viral infection in tomato is characterized by the symptoms viz; leaf curl and stunting. The infected plant shows brittle older leaves, smaller internodes, pale colour and bushy appearance. Symptoms vary little in different varities studied under investigation. Physical properties viz; Dilution End Point (DEP), Thermal inactivation point (TIP), Longevity in vitro (LIV) have been observed and found varied with two varieties of tomato viz; Pusa ruby and Money maker.

Keywords : Varietal Screening, TLCV, Symptomatology, Physiological properties.

Vegetable crops are playing an important role in solving food problem throughout the world. Tomato (*Solanum lycopersicum* L) is one widely grown vegetable crops in India. Among the vegetables, tomato stands next to potato in world acreage. Tomato occupies an area of 4-85 m.ha. in the world with the production 182.3 m. tones and the productivity of 37.6 t/ha. The crop is grown for its edible fruits. Tomato fruits are used raw either as salad, or cooked as vegetables or processed as sauce and prickles. Tomato is a valuable source of fibre, vitamin A,C and minerals like iron and phosphorus.

Tomato leaf curl has been considered as the most common disease of tomato causing heavy losses in quality of the fruits and large quantity of tomato fruits unfit for consumption (Hasan, 2005). This disease in severe cases can lead to complete defoliation. Assessment of loss about 21 to 68% also has been reported due to Tomato leaf curl disease Simons (1957) reported two distinct types of leaf curl symptoms, the first which react with plant leaves dark green and second type the shape of the leaves that become oral and rounded.

Symptomatology is one of the important criteria used in preliminary identification of virus. The symptoms of leaf curl disease is characterized by severe stunting of the plants with downwards rolling and crinkling of the leaves. The newly emerging leaves exhibit slight yellow coloration and later on they also show curling symptoms. Older leaves become healthy and brittle. The nodes and internodes are significantly reduced in size. The infected plants look pale and produce more lateral branches giving bushy appearance. The fruits from infected plants are small and deformed.

Seeds of different varieties of naturally infected tomato plant with tomato leaf curl virus (TLCV) were sown in 45 cm. pots and in an insect proof chamber. Symptoms were regularly observed till maturity in the first ratoon crop. The behaviour of reaction on different varieties of tomato shows the symptoms consisted as severe leaf pattern, chlorotic area giving a coarse pattern of greenish mottle forming short streak towards the tip. These streaks however more discrete.

Pusa Early dwarf. The symptoms were more prominent from July to November with lower in temperature. The symptoms on older leaves become diffused. The symptoms almost get disappear when the temperature remains below 10°C.

Arca Vikas : The symptoms of this disease show severe mosaic pattern. The symptoms were mixed broadly elongated, yellowish green discrete streaks running on the full length of the leaf lamina.

Money maker : The leaf of the plant shows a greenish yellow mottle and may be slightly curled and irregular in shape. TMV often caused a brown streaking of the some of the branch. This cause yellowing and curled drooping of the leaves.

However, fruits on streak branch that have dropped leaves often are greenish and wrinkled. One strain of this virus cause a brilliant yellow mottle of the leaves. It may also cause green curling of some small fruits.

Selection 22.

The infected plants with the common form of cucumber mosaic virus develop a mottling and mosaic like that of TMV, but with more contrast between the light and dark portion of the leaves. The leaves curled upward at the edge and often are abnormally narrow and pointed. At the time when yellowing ring are produced in the leaves and fruit, the rings are a quarter to half an inch in diameter. The infected plants at 10-20 days after transplanting did not yield any fruit and loss in yield was 100%.

Navodaya : The plants were infected and show a green and yellow mottling like TMV. Although at time the symptoms may be less pronounced. There are no symptoms on the fruits but yield is reduced. Some of the older leaves become yellow and then drop from such plants. Yellow green circular spot about quarter inch in diameter often appear below the smooth surface of the green fruits. As the fruits ripen the spotted part become more yellow and they shrivel slight. (Joshi and Dubey, 1975)

Morglobe : The symptoms induce clearing of vein of younger leaves of tomato plants later occur yellowing of whole leaf followed by irregular pattern of light yellow and dark green patches. The stunting of diseased plants is accompanied by defective development of reproductive parts. Distortion of vein has also been noticed. Infected plants produce very few fruits.

PHYSICAL PROPERTIES

The study of physical properties is to establish and identify the sap transmissible viruses. Therefore, properties such as Dilution End Point (DEP), Thermal Inactivation Point (TIP), longevity in vitro (LIV) and desiccation have been investigated in the present study, using *Nicotiana tobacum*-78 and 78 A as the test plant.

Young leaves with distinct leaf curl symptoms from two weeks old systemically infected plants were used as the source of virus inoculation. Such leaves were grinded in a morter and pestle, the sap obtained was passed through double folds of muslin cloth and the filterate was centrifuged at 3000 rpm, for 5 min. to separate particulate clumps in the mascerate. A clear supernatant thus obtained was used as the source of virus inoculums in virus studies. (Badwen & Kassaris, 1950)

1. Dilution End Point (DEP) : Seven dilutions (10^{-10}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) of the crul clarified supernatant of infective sap were prepared by using the following procedure.

Eight test tube were kept in a test tube rack and seven of these were filled with nine ml distilled water. One ml infective sap was poured in the first test tube. One ml sap from Ist test tube was transferred to the second tube with nine ml water (Dilution 10^{-10}). After thorough mixing of this dilution, 1 ml 10^{-1} dilution sap was transferred to the next test tube with 9 ml distilled water to obtain 10^{-2} dilution. This process was repeated to get further dilutions.

During preparation of each dilution, a fresh pipette was used. Inoculation were done with the highest dilution down to the undiluted control on the leaves of 10days old test plants.

Appearance of the symptoms of these plant was recorded and the data are presented in Table.

Table : Dilution End Point of the Virus

Dilution	No. of plant infected out of 20 inculated	
	Tomato var Pusa ruby	Tomato var Money maker
Undiluted infective	20	20
sap	20	20
1:10	16	17
1:100	11	12
1:1000	9	6
1:10,000	0	0
1:100,000	0	0
1:1,000,000	0	0
1:10,000,000		

VI.2 : Thermal Inactivation Point (TIP) :

The TIP of the virus was determined by taking 2 ml of fresh, clarified, infective sap from infected leaves of *Nicotiana tobacum* plant in seven thin walled tube (1cm. diam). These tubes were placed in thermostatically controlled water bath for 10 min. at various temperature (5^0C) between 45^0C-75^0C separately. Immediately after the heat treatment, the sap was cooled down at room temperature under running tap water. This sap was inoculated for two weeks old test plants. The temperature at which total inactivation of virus occurred was noted and has been presented in table. The unheated infective sap served as control. The experiment was repeated thrice.

Table : Thermal Inactivation Point of Virus

Temperature (°C)	No. of plant infected out of 20 inoculated	
	Tomato variety.. Pusa ruby	Tomato variety.. Money maker
Untreated infective sap	19	20
45	18	17
50	16	15
55	14	10
60	3	2
65	1	1
70	0	0
75	0	0

VI.3 : Longevity in vitro (LIV) : In order to determine the maximum duration for which the virus would service in vitro, 200 ml, clarified infective sap of the virus was taken separately in one small conical flask (250ml). Inoculations were made of each aliquot and appearance of symptoms was recorded. The data are presented in Table.

Table : Longevity in vitro of virus

Storage period No of plant infected out of 20 inoculated		
Day	Tomato Variety Pusa Ruby	Tomato variety Money Maker
0	20	20
1	19	18
2	18	16
3	15	15
4	13	13
5	11	09
6	10	08
7	08	08
9	06	07
10	06	06
11	05	05
12	05	04
13	05	05
14	04	02
15	04	02
16	03	01
17	03	01
18	0	0
19	0	0
20	0	0

RESULTS AND DISCUSSION :

The dilution end point of the virus was found between 10^{-3} to 10^{-4}, thermal inactivation point between 55°C -65° C and longevity in vitro 5-6 hrs at room temperature (30°C±2°C) and 5-6 Hrs. at 4^0C in Pusa Ruby and Money Maker at frequent intervals to have an adequate stock of virus inoculums.(Kauzmann K.W, 1959)

In order to determine the host range of the virus, it was observed that out of 53 spp. belonging to 11 different families, initial symptoms of the virus appeared only on 10 plants with in 8-10 days of inoculation. Plants like *Solanum tuberosum* L. and *Datura stramonium* L. carried the virus without any symptoms. This indicates a specified host of the virus mostly to plants of families *Solanaceae, Cucurbitaceae*, and *Compositae*. Common symptoms *on Capsicum annum L.* (Solanaceae) were vein clearing during younger period followed by severe mottling and light and dark green patches over the leaf surface, curling, marginal rolling in leaf and fruit size with damaged shape were also noticed. (Chant, SR, 1967)

During the course of the present study regarding the physical properties of the virus dilution end point (DEP) was 1:100,00 and the thermal inactivation point (TIP) was between 60^0C-65^0C. Regarding longevity in vitro the virus remained in effective in crude sap for 17 days in Pusa Ruby and 15 days in Money Maker. Virus isolated was easily transmitted by sap inoculation (Mechanically) and white fly were able to transmit the virus with 40% - 70% infection respectively. The virus could not be transmitted by soil, seed or root.

The cross protection tests for tomato leaf curl virus (TLCV) was done with natural tomato plant. On both tomato cultivars under investigations justified that the tomato leaf curl virus (TLCV) is clearly distinct virus of tomato and it was further identified and designated as tomato leaf curl virus.

During present investigation, it was found that the virus produced significant and severe symptoms in summer and rainy seasons (March-Oct.) when temperature was 20-42.5°C, but during winter (November to February) at temperature between 20°C-22°C, it produced only mild and diffused symptoms. (Andréae & Andréae, 1949)

It has also been observed that symptoms severity of the virus in the cultivars increased gradually infection period till 120 days of inoculation, after which it becomes moderate to mild. A close relationship between decrease or increase in virus concentration and symptom severity existed. The higher concentration of virus was noticed in

leaves harvested on 120 days of inoculation. This indicated that the virus concentration increase in leaves up to 120 days and then gradually declined.

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