The Bioscan 18(3): 159-162, 2023
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

REDESCRIPTION OF *OSCHIOUS* SP. AROUND SUGARCANE SOIL IN OSMANABAD DISTRICT(M.S) INDIA.

KOMAL DALVE AND SUNITA BORDE
Department of Zoology, Dr, Babasaheb Ambedkar Marathwada University, Aurangabad - 431004
e-mail: komaldalve110491@gmail.com

KEYWORDS
Nematoda
Sugarcane
Soil
Osmanabad

Received on :
24.03.2023

Accepted on :
29.06.2023

***Corresponding author**

ABSTRACT
Redescription of the genus, *Oschious* was found in the soil around root of sugarcane crops in Osmanabad district (M. S) India. Molecular and morphological analysis were performed. The species is characterized by body size medium and length measures 2587mm, a = 18.9, b = 3.43, c = 19.9, c'= 1.66, V=55.62%Length of stoma 65μm.Amphids circular, female reproductive system amphidelphic and pointed tail.

INTRODUCTION

Plant parasitic nematodes are important pathogens of all cultivated crops including sugarcane, which is a major agricultural crop produced in many countries with tropical and sub-tropical climates.The problem caused by Phytonematodes are common, which was highlighted by (Severino *et al*., 2010) Nematode diversity on sugarcane is greater than most other cultivated crops with more than 310 species and 48 genera of endo-and ectoparasitic nematodes reported from the root and rhizosphere of the plant (Spaull and Cadet 1991). Meloidogyne and Pratylenchus are the two species of the plant-parasitic nematodes most frequently reported as highly pathogenic to sugarcane world-wide. Sugarcane is most important cash- crop of agricultural field in India. It is also important food crop of the tropics and subtropics (Sivaesan and waller,1986). Sugarcane is a renewable, natural agricultural resource because it provides sugar, biofuel, fiber fertilizer with ecological sustainability. Its juice is used for making white sugar, brown sugar and jaggery. It is one of the main crops of earning foreign exchange. The main product is sugar which is used universally as sweeteners and as preservatives. It has also become an essential part in many diets and almost indispensable in the food manufacturing and pharmaceutical industries. Girei and Giroh (2012)described a number of factors that could be responsible for the low production of sugarcane from the sugar industries. One of the outstanding problems of sugarcane cultivation caused by nematodes has been the rapid yield decline of successive ratoon crops (Bock *et al*.,1968). The plant-parasitic nematodes can damage roots and reduce the length of cane stalks leading to sugarcane yield loss. Several nematode species particularly those belonging to the orders Rhabditida, Tylenchida and Dorylaimida have been associated with sugarcane roots (Afolami *et al*.,2014).

The present communication deals with the genus *Oschious tipulae*. In 1976, Andrassy described *Oschious* as a sister taxon of Rhabditids. Sudhaus (1976) placed *Oschious* in Rhabditidae and divided it into two groups Insectivora and Dolichura. He synonymized the genus Dolicorhabditis. several *Oschious* species have been described bringing it a total no of 42 of which 22 species belongs to Insectivora group and 14 to Dolichura group along with six new species were recovered as follows. *O.citrin,*sp, *O.cynodonti,* n. sp*O.cobb,* n. sp. O.*esculents n*. sp. *O. punctata,* n. sp and *O. sacchari n* sp. Sudhaus (2011) gave idea of Rhabditidae on morphological and molecular basis, which consist three group namely pleiorhabditis, synarhabditis and anarhabditis and placed the genus *Oschious* in synarhabditis. Tabassum and Shahina (2002) described *O. maqbooli* from sugarcane plantation of chatta goth, Baluchisthan, while O, Andrassy was described as a new species from sugarcane field of Jhang, Punjab by Tabassum and Shahina (2008).
The present communication deals with molecular and morphological identification of nematodes that is *Oschious tipulae* sp. From the soil around sugarcane roots, Tuljapur, Dist. Osmanabad (M.S). India.

MATERIALS AND METHODS

For extraction of nematodes, soil samples were processed through Cobb's sieving and decanting method followed by Baermann's funnel technique (Thorne, 1961). After 24h, the nematode suspension was collected in a tube and was allowed to stand for at least 1-2h to allow nematodes to settle

at the bottom of the tube. The nematodes were then transferred in a small amount of water in cavity blocks, the excess water was removed and the nematodes were killed and fixed in hot F.A. (4:1). The nematode specimens were dehydrated by Seinhorst's (1959) rapid glycerine method and mounted permanently on slides using dehydrated glycerine as mounting. Then they were observed under a compound microscope, measured using ocular micrometre and drawings were drawn with a camera lucida. Then, ratio was calculated according to De Man's (1884) formula and De Grisse's (1964) symbols.

Molecular analysis

Nematodes intended for molecular analysis were fixed with 95% ethyl alcohol. Sample were sent to Codon Bio-science lab Panji, Goa. for further analysis.

DNA extraction was carried out using Gene lute Mammalian Genome DNA extraction kit (Sigma G1N70-1KT).10 mg of ground tissue is added to 400ul of lysis buffer. RNAse was added to make final concentration to 0.6/ul and incubated at 370°c for 30mins. Proteinase K was added to make concentration of 1ug/ul and incubated until the tissue was digested and formed a viscous liquid. Equal volume of phenol was added and shaken for 15 mins. This solution was then centrifuged 6500rpm for 4min. 360ul of supernatant was transferred to a clean tube and equal volume of phenol was added and shaken for 15 mins. This solution was then centrifuged 6500rpm for 4min. 340ul of supernatant was transferred to a clean tube and equal volume of phenol was added and shaken for 15 mins. This solution was then centrifuged 6500rpm for 4 min. 340ul of supernatant was transferred to a clean tube and 30uL of 6M NaCl was added while gently being stirred and kept in cold to precipitate. DNA was precipitated 2.5 volume of very cold absolute ethanol, and this was then centrifuged for 4 mins at 13,000 rpm. The supernatant was drained off and pellet was washed with 70% ethanol. The pellet was air dried and then resuspended in 50uL of TE buffer and refrigerated at 0ºc.PCR was performed using 18S primers. In PCR, 100ng of total DNA amount was used. Total reaction mixture of 25uL consisted of 1ul of template, 12,5uL of Promega gotaq green mastermix,1uLof primers, and 9.5uL of nuclease free water. The PCR was performed in Takara PCR thermocycler Dice Gradient PCR with the following parameters. The primer for amplification of ITS gene were (forward) TCA TTT AGA GGA AGT AAA GTC. (reverse) GTT AGT TTC TTT TCC TCC GCT. The PCR profile were used as denaturation at 1min at 92ºC followed by 42ºC for 1min, re-annealing, extension at 72ºC for 1min and final extension 72ºC for 5min respectively.

Sequences have been deposited in NCBI Gen Bank under the following inclusive accession numbers (KP756939.1) The DNA sequence were analysed using online BLAST (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. Phylogenetic trees were generated using the maximum-Parsimony, minimum-Evolution, and neighbour-joining method in MEGA 6 (Tamura *et al.*,2013). Neighbour -joining trees (Saitou N and Nei M., 1987).

43 Specimens of the plant nematode collected from the soil around the sugarcane crops, Tuljapur, Dist. Osmanabad, M.S. India in the month of November 2021. Collected specimen were observed and identified.

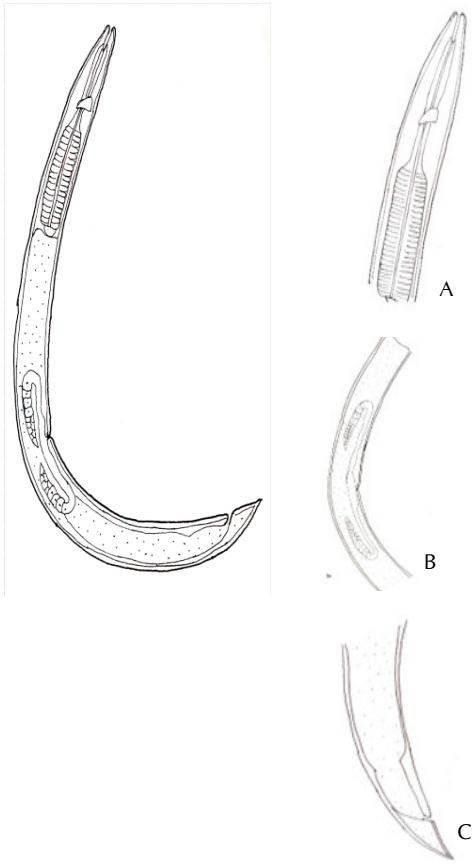

Fig 1 A. Whole body, B. Anterior region, C. Middle region, D. posterior region.

Collected plant nematodes are preserved in hot (90ºC - 100ºC), diluted FAA (Formalin acetic acid) solution. Mounted in glycerine, and drawings are made with the aid of camera Lucida. All measurements are in 'μm' except 'L' in mm.

Nematode body is elongate, spindle shaped, tapering toward both body ends. The buccal cavity lies between the mouth and pharynx, pharynx is muscular. Intestine is complete mouth to anus. sexes are separate.

Male- Not found.

Female-

As shown in (Fig. A) Female body medium sized and body length measures 2587mm, slightly curved ventrally. In (Fig. B) shows Lip region continuous, labial papillae minute. Amphid opening circular, stoma rhabditid, with distinct cheilostome, gymnostome and stegmostome. Length of the stoma is 65 μm. Cuticle finely annulated. Nerve ring surrounding middle of isthmus, Length of the nerve ring 260 μm. Cardia conoid, long surrounded by intestinal tissue. Female reproductive system didelphic shows in (Fig.C) Both the sexual branches equally developed ovaries relaxed at both sides. Vulva transverse slit. In (Fig. D) shows Tail short and pointed at the end.

Table 2: Phylogenetic neighbours of *Oschious* Sp. based on partial 18s rRNA gene sequence

Sr. No.	Nearest phylogenetic neighbors	Percentage identity	Accession No.
1	*Oscheius tipulae* strain CEW1 18S ribosomal RNA gene, partial sequence	100	KP756939.1
2	*Oscheius* SP. 68_P29 18S ribosomal RNA gene, Partial sequence	100	KP756937.1
3	*Oscheius tipulae* isolate KS599 18S ribosomal RNA gene, partial sequence	100	HQ130502.1
4	*Oscheius* sp. PS113118S small subunit ribosomal RNA, partial sequence	100	U81587.1
5	*Oscheius* sp. 69_P20 18S ribosomal RNA gene,partial sequence	99.61	KP756938.1
6	*Oscheius* sp. 67_P20 18S ribosomal RNA gene, Partial sequence	99.61	KP756936.1
7	*Oscheius* sp. FVV-2 18S ribosomal RNA gene, partial sequence	99.61	HQ130503.1
8	Oscheius tipulae small Subunit ribosomal RNA gene, partial sequence	100	AF036591.1
9	*Oscheius tipulae* strain CEW1 18S ribosomal RNA gene, partial sequence	100	EU196009.1
10	*Oscheius* sp. FVV-2 voucher PS2068 small Subunit ribosomal RNA gene and internal transcribed spacer 1, partial sequence	100	MF196096.1

RESULTS AND DISCUSSION

The genus Oscheius has a large number of species which are morphologically very close to each other. (Sudhaus 2011) The genus *Oschious* was described by Andrassy 1976. Sudhaus 1976 placed *Oschious* in Rhabditidae and divided it into two groups Insectivoura and Dolichura. The genus *Oscheius* under the group Synrhabditis which consisted of 27 valid species, of which 13 species belonged to Dolichura group and 14 to insectivore group. Tabassum *et al*. (2016). Six species of Insectivoura group viz., O. *carolinensis*(Ye *et al*.,2010),O.*niazii* Tabassum and Shahina, 2010)O. *amsactae* (Ali *et al*.,2011), O. *siddiqii*(Tabassum and Shahina, 2010) *O. microvilli* (Zhou *et al*., 2007) O. safricana (Serepa- Dlamini and Gray, 2018). andone species of Dolichura group O. *onirici* Torrini *et al*.,2015 have been recognized. *Oschious tipulae* was described for the first time by Lam and Webster (1971). The nematode under discussion comes closer to *Oschious tipulae* in possessing body medium sized ventrally curved. Lip region continuous, stoma rhabditoid, cardia conoid reproductive system didelphic, vulva located posterior to middle part of body, tail short and pointed.

However, it differs from *Oschious tipulae* in value of a is 18.9 μm against (17-26) μm and also in value of b is 3.43 μm against (5.2-11.6) μm and the value of V% is 55.62 μm against 48-52 μm in present worm.

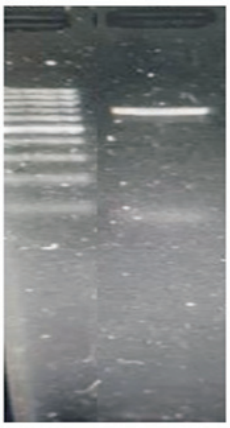

Fig 2 :PCR product using 18s b primer visualized on agarose gel under UV (band size 512bp)

Table1: Morphometric data of female *Oscheius tipulae*.(All measurement is in μm except L in mm)

Body Part	Measurements
L	2587
A	18.9
B	3.43
C	19.9
C′	1.66
V%	55.62
Length of lip region	78
Length of Stoma	65
Length of pharynx	442
Nerve ring length from anterior	260
Distance of vulva from anterior	15.34
Maximum body dimeter	130
Ovary length	234
Ovary width	65
Tail length	117

Molecular Data

A comparison of the partial sequences of the 18s rRNA gene of the present nematode with those of other nematode, in a phylogenetic context, provided further support for placing this species as a redescribed one within *Oschious tipulae*, thus confirming taxonomic conclusion based on morphological data (fig. 1).

In molecular analysis the phylogenetic neighbours of *Oschious* Sp. based on partial 18s rRNA gene are shown in table no. 2 and Fig.3 on the basis of position of sequence of present form in phylogenetic tree, the sample showed 100% similarity with the Oschious sp. that is 68 P29, having accession no. KP756937.1. Oscheius sp.69 P20, Oscheius sp.67 P20.

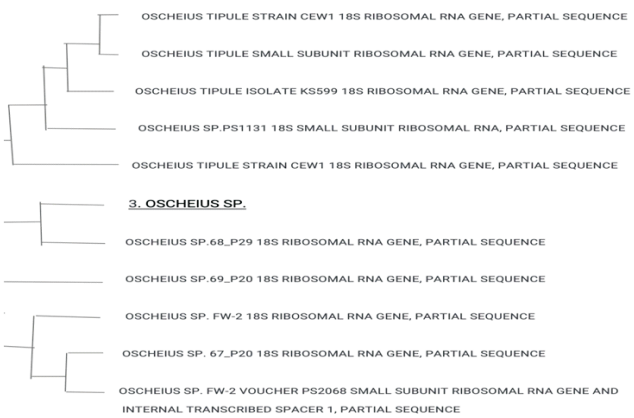

Fig. 3: Phylogenetic tree for *Oschious* Sp. using partial 18s rRNA gene sequence

And Oscheius sp. FVV-2 is clear with a maximum identity 99.61%. After partial 18s rRNA gene sequence of present form DNA sequence length is 512bp in Fig.2.

CONCLUSION

As both morphological and molecular observations show the characters of present form closer to *Oschious tipulae*. Therefore, it is redescribed here as *Oschious tipulae*.

AKNOLEDGEMENT

Author is thankful to the Head, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing the laboratory facilities during this research work and also thanks Codon Bio-science lab Panji, Goa for providing me necessary facilities.

REFERENCES

Afolami, Solomon and F. Daramola. 2014. Biodiversity of plant-parasitic nematodes of sugarcane in Bacita, *Nigeria, J. Entomology and Nematology*. 6(6): 71-79.

Andrássy I. 2005. Free Living Nematodes of Hungary. Vol. 1: Nematoda errantia. Hungarian Natural History Museum, Budapest, 495 pp.

Bedding, R. A. and R. J. Akhurst. 1975. A simple technique for the detection of insect paraistic rhabditid nematodes in soil. *Nematologica* .**21:**109- 110.

Bock, K.R, Ngundo ,B.W and Othieno, S.M.1968. Nematology IN Record of Research Annual Report, East African Agriculture Forestry Research Organization, East African Community. pp.97-10.

Lalfakawma, Bharat Chandra Nath, L. C. Bora and Seweta Srivastava 2014. Integrated disease management of zingiber officinale rosc. *rhizome rot*.**9(1):** 307-311

Cobb, N.A. 1918. Estimating the nematode population of soil. U.S Department of Agriculture. *Agriculture Circular*. **1:**1-48.

Darsouei, R. J.Karimi ,and E.Shokoohi.2014. Oscheius rugaoensis and Pristionchusmaupasi, two new records of entomophilic nematodes from Iran. *Russian J. Nematology*. **22:**141–155.

Dorris, M., P. De Ley and M. L. Blaxter. 1999. Molecular analysis of nematode diversity and the evolution of parasitism. *Parasitology Today* **15:**188-93.

De Grisse andA. T. 1964. Morphological observations on Criconemoides with a description of four new species found in Belgium (Nematoda). Meded Landb. Hoogesch. *Opzoek. Stns Gent*. **29:** 734-761.Brill, Leiden, The Netherlands. p. 206.

Gaugler, R. 2002. Entomopathogenic Nematology. Wallingford, UK: CABI Publishing. 388 p

Grewal, P.S., R.-U. Ehlers and D. I. Shapiro-Ilan. (eds.) 2005. Nematodes as Biocontrol Agents. CABI Publishing, Oxon, UK: CAB International. pp. 363-381.

Girei, A.A and Giroh, D.J. 2012. Analysis of the factors affecting sugarcane (Saccharum officinarum) production under the out-grower's scheme in numen local government area Adamawa State, Nigeria. Jr. Education Practice. 3: 8.

Karimi, J., N. Rezaei and E. Shokoohi 2018. Addition of a new insect parasitic nematode, *Oscheius tipulae*, to Iranian fauna. *Nematropica* .pp.48-45. 54.

Kaya, H. K. and R. Gaugler. 1993. Entomopathogenic nematodes. *Annual Review of Entomology*. **38:**181-206.

Kaya, H. K and S. P. Stock. 1997. Techniques in insect nematology. Pp. 281–324 in Lacey, L. A. (ed.) Manual of Techniques in Insect Pathology. London: Academic Press.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Molecular Evolution*. **16:**111-120.

Kumari, S. and M. Lišková. 2009. Molecular confirmation of Xiphinema sitaliaeMeyl, 1953 (Nematoda: Longidoridae) from the Slovak Republic. *Helminthologia*. **46:**131–134.

Lam, A. B. Q. and J. M. Webster. 1971. Morphology and biology of Panagrolaimus tipulae n. sp. (Panagrolaimidae) and Rhabditis (Rhabditella) tipulae n. sp. (Rhabditidae), from leatherjacket larvae, Tipulapaludosa (Diptera: Tipulidae). *Nematologica*. **17:**201- 212.

Bina, L., Mohilal,N.,Pramodini, M. and Manjur Shah,M. 2012. Two new species of BOLEODORUS (NEMATODA: TYLENCHIDA) from Manipur, India. *The Bioscan*.**7(1):**115-118.

Gosh,P.P and Dutta,S . 2014. Effect of sole and combined inoculation of Ralstonia solanaacearum and Meloidogyne javanica on tomato. **9(1):** 93-100

Rana, A., Bhat, A. H., Chaubey, A.K., Machado, RAR. 2020. Morphological and molecular characterization of Heterorhabditis bacteriophora isolated from Indian soil and their biocontrol potential.PP.77-102.

Seinhorst, J. W.1959.A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*. **41:**67-69.

Severino, J. J, Dias- Arieira, C. R and Tessmann, D.J. 2010. Nematodes associated with sugarcane (Saccharum. spp) in sandy soils in Parana, Brazil, *Nematropica*. **40:** 111-119.

Sivanesan, A and Waller, J.M. 1986. Sugarcane Diseases. Commonwealth Agricultural Bureaux Commonwealth Mycological Institute (Phytopathological Paper 29). *Experimental Agriculture*. **24:** 131-131.

Spaull, V.W and Cadet, P. 1991. Nematode Parasites of Sugarcane. In: Plant-Parasitic Nematodes in subtropical and tropical Agriculture. M.LVC, Sikora RA, J. Bridge tds), pp. 461-491. Wallingford, U.K CAB International.

Stock, S. P and A. P. Reid. 2003. Biosystematics of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae): Current status and future directions. Cook, R. and D. J. Hunt (eds). Proceedings of the Fourth International Congress of Nematology 8-13 June 2002, Tenerife, Spain. Nematology Monographs and Perspectives. **2:**435-446.

Sudhaus, W. 2011. Phylogenetic systemization and catalogue of paraphylentic Rhabditidae *J. Nematode Morphology and Systematics* 14. **2:**113-178.

Sudhaus, W. and D. Fitch. 2001. Comparative studies on the phylogeny and systematics of the Rhabditidae (Nematoda). *J. Nematology*. **33:**1-70.

Sudhaus, W and D. J. Hooper. 1994. Rhabditis (Oscheius) guentherisp.n., an unusual species with reduced posterior ovary, with observations on the Dolichura and Insectivora groups (Nematoda: Rhabditidae). *Nematologica*. 40:508–533.

Tabassum, K. A.and F. Shahina. 2010. Oscheius siddiqii and O. niazii, two new entomopathogenic nematode species from Pakistan, with observations on O. shamimi. *International J. Nematology*. **20:**75- 84.

Tamura, K., G. Stecher, D. Peterson, A., Filipski and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*. **30:**2725- 2729.

Tabassum, K.A., Shahina, F., Nasira, k. and Eeum, Y. I.2016 Discription of six new species of *Oscheius*Andrassy 1976 (Nematoda: Rhabditidae) from Pakistan with a key and diagnostic compendium to species of the genus. *Pakisthan J. Nematology*. **342:** 109-161.

Torrini, G., Mazza, G., Carletti, B., Benvenuti, C., Roversi, P. F., Fanelli, E., Luca, F. D., Troccoli, A. & Tarasco, E. (2015). Oscheiusonirici sp. n. (Nematoda: Rhabditidae): a new entomopathogenic nematode from an Italian cave. *Zootaxa*.**26:** 533-548.

Valizadeh, A and Goldasteh, S. 2017. The occurrence of three species of the genus *Oscheius*Andrassy, 1976, in *Iron. J. plant protection research*. **57:**248-55.