BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 9(16)

Morphological redescription and molecular characterization of three species of Travassosinematidae (Nematoda: Oxyurida: Thelastomatoidea) from *Gryllotalpa africana* Beauv (Orthoptera: Gryllotalpidae)

Neetu Singh, Anshu Chaudhary* & Hridaya Shanker Singh

Molecular Taxonomy Laboratory, Department of Zoology, University Road, Chaudhary Charan Singh University, Meerut (UP), 250004, India; Anshu Chaudhary - Email: anshu8282@rediffmail.com; *Corresponding author

Received August 29, 2013; Accepted August 31, 2013; Published September 23, 2013

Abstract:
Binema mirzaia (Basir, 1942a) Basir, 1956, *Cameronia nisari* (Parveen and Jairajpuri, 1985) Adamson and Van Waerebeke, 1992a and *Mirzaiella meerutensis* Singh and Malti, 2003 are redescribed morphologically along with molecular identification from the intestine of mole cricket *Gryllotalpa africana*. Molecular characterization was carried out using the D2–D3 expansion domains of the 18S ribosomal DNA region. This study first time presents molecular data for the above three nematode species.

Keywords: Nematode, Travassosinematidae, Molecular characterization, Meerut, India.

Background:
Several species of nematodes have been discovered from the host *G. africana* (mole crickets) in Russia, Brazil, India, France, Germany and Spain **[1-4]**. In India, from *G. africana* a number of nematode species were described that belong to the various genera viz., *Binema* Travassos, 1925 **[1]**; *Basirella* Biswas and Chakravarty, 1963 **[5]**; *Cameronia* Basir, 1948a **[6]**; *Chitwoodiella* Basir, 1948b **[7]**; *Gryllophila* Basir, 1942a **[8]**; *Indiana* Chakravarty, 1943 **[9]**; *Isobinema* Rao, 1958 **[10]**; *Mirzaiella* Basir, 1942a **[8]**; *Mohibiella* Farooqui, 1970 **[11]**; *Pteronemella* Rao, 1958 **[10]**; *Singhiella* Rao, 1958 **[10]**. The species *B. mirzaia* (Basir, 1942a) Basir, 1956 **[8, 12]**; *C. nisari* (Parveen and Jairajpuri, 1985a) Adamson and Van Waerebeke, 1992a **[13, 14]** and *M. meerutensis* Singh and Malti, 2003 **[15]** were also reported under the above genus. Previously, all of the taxonomic studies of these parasites were based on adult morphology only **[13, 16-19]**.

During the last decade, for making nematode differentiation and identification easier, molecular methods have provided additional tools. Advances in molecular-biology techniques allowed an objective analysis of the taxonomy of Nematoda for identification and phylogenetic analyses in nematode species. Recently, phylogenetic analyses based on the 18S ribosomal DNA sequences provided new discernment into relationships within the Nematoda and showed that studies based on morphological characters need revision **[20, 21]**. Phylogenetic relationship within nematodes has been studied frequently **[22-24]** but phylogenies based on molecular data are still contradictory in the genus of nematodes harboring *G. africana*. 18S ribosomal RNA gene represents a well-conserved gene that has recently been used in the studies resolving the phylogenetic relationship between nematode species and was also shown to be a suitable marker for barcoding of nematodes **[25-27]**.

The present study re-examines female parasites of *B. mirzaia*, *C. nisari* and *M. meerutensis* morphologically and presents molecular data. Limited data on nematodes of Travassosinematidae is available till date on databases. For that reason, D2-D3 domains of SSU rDNA and phylogenetic analyses were done in this study. The 18S rDNA sequences for three isolates *B. mirzaia*, *C. nisari* and *M. meerutensis* were obtained to validate their phylogenetic position.

Methodology:

Sample collection and morphological study

Host insects *G. africana* collected in Meerut (29° 01' N, 77° 45' E), U.P., India, were placed in individual vials from the fields. Insects were anaesthetized with chloroform and dissected immediately in normal saline under a stereoscopic microscope. Gut was teased out with fine needle and the contents were mixed with saline. Nematodes were picked up and being fixed in hot 70% alcohol for slide preparation. For light microscopy examination, the nematodes were cleared gradually in glycerine. Collected samples were preserved in 95% ethanol, until DNA extraction. Voucher specimens have been deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut, India.

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Whole body of adults were lysed for DNA extraction and genomic DNA was extracted using a DNeasy Tissue Kit (Qiagen, Germany) as per by manufacturer's instruction. A partial region of the 18S nuclear DNA was amplified using the primers Nem18SF and Nem18SR **[28]**. PCR amplification was performed in 25 µL of reaction mixture containing 1 U Taq polymerase (Biotools, Spain), 10 X PCR reaction buffer (Biotools), 0.4 mM dNTPs mix, 3 µL template, and 10 pM of each forward and reverse primers, using Thermal Cycler (Eppendorf Mastercycler personal). The amplification conditions followed were as an initial denaturation at 94°C for 3 min, followed by 35 cycles each of denaturation at 94°C for 30 s, annealing at 54°C for 45s, and extension at 72 °C for 1 min with final extension at 72°C for 10 min. Amplicons were visualized in agarose gel under UV-illumination by ethidium bromide staining. Amplicons of the 18S region were subsequently purified using Purelink™ Quick Gel Extraction Kit (Invitrogen) followed the manufacturer's instruction. Purified PCR products were sent to Chromous Biotech Ltd. (Bangalore, India) for DNA sequencing.

Phylogenetic analysis

A BLAST search was performed at the NCBI (http://www.ncbi.nlm.nih.gov/) using the sequences obtained as queries for compared to reported nematode 18S sequences which were then used in the phylogenetic analysis. Alignment of sequences was carried out using the ClustalW application of MEGA vr. 5. Phylogenetic trees obtained for 18S D2–D3 datasets were inferred with Maximum Likelihood (ML), Maximum Parsimony (MP) and Neighbour-Joining (NJ) methods using software MEGA Vr. 5 **[29]**. For the ML method analysis, a DNA substitution model was selected using neighbor-joining tree as a result, the GTR+G model was selected and applied with a default gamma parameter of 5. The ML method was conducted using the NNI search strategy and the gap-containing positions were excluded from the analysis. The MP method was conducted using the CNI search strategy. The trees were bootstrapped 1,000 times to assess the degree of support for the branching indicated by the phylogenetic tree for each method.

Nucleotide sequence accession numbers

Sequences of 18S rDNA generated from this study were submitted in GenBank and are available by the following accession numbers KC763367–KC7663369.

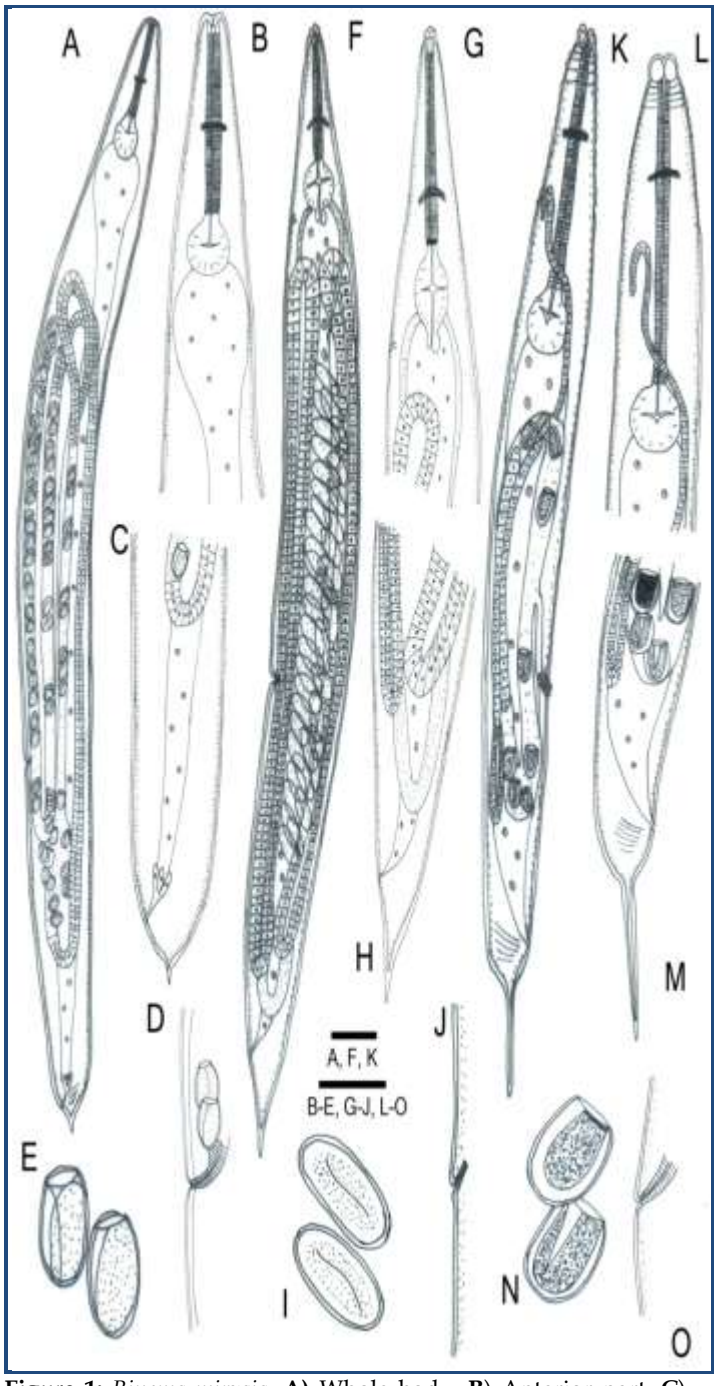

Figure 1: *Binema mirzaia.* **A)** Whole body; **B**) Anterior part; **C**) Posterior part; **D)** Enlarged vulva; **E**) Eggs. *Cameronia nisari***. F)** Whole body; **G)** Anterior part; **H)** Posterior part; **I)** Egg**; J)** Enlarged vulva. *Mirzaiella meerutensis;* **K)** Whole body; **L)** Anterior part; **M)** Posterior part; **N**) Egg; **O**) Enlarged vulva. *Scale-bars*: A, F, K (10X), 0.10 mm; B-E, G-J, L-O (40X) 0.10 mm

Results & Discussion:

***Binema mirzaia* (Basir, 1942a) Basir, 1956**

Host and site of infection: *Gryllotalpa africana* Beauv (Orthoptera: Gryllotalpidae); intestine.

Locality: Meerut (29^0 01'N, 77^0 45'E), U.P., India.

Voucher material: The slides have been deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India, under the voucher no. Nem/2013/02.

Female redescription (**Figure 1A–E; Table 1 see supplementary material)**: Gravid female long, wide, body striations limited to anterior part and with smooth cuticle. Nerve-ring surrounds the corpus, located towards its anterior end. Esophagus long, consisting of a corpus, isthmus and esophageal bulb. Tail small, vulva slight posteriorly, vagina muscular and directed anteriorly. Two ovaries present, one anterior and other posterior, both are reflexed with loops. Each capsule usually contained two eggs. The population of this species were characterized by a comparatively broad and longer female body, two reflexed ovaries, one anterior and other posterior. Tail short. Vulva situated at about middle third of the body about 70% of body length. The morphology and morphometrics of these *B. mirzaia* population agree closely with the original description of this species by Basir, 1942a **[8]** and redescribed by Basir, 1956 **[12] (Figure 1, Table 1)**.

***Cameronia nisari* (Parveen and Jairajpuri, 1985a) Adamson and Van Waerebeke, 1992a**

Host and site of infection: *Gryllotalpa africana* Beauv (Orthoptera: Gryllotalpidae); intestine.

Locality: Meerut (29^0 01'N, 77^0 45'E), U.P., India.

Voucher material: The slides have been deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India, under the voucher no. Nem/2013/03.

Female redescription **(Figure 1F–J; Table 1)**: Body cylindroid like, gradually tapering toward extremity with annulated cuticle. Oral opening surrounded by eight labio-papillae, two amphids and mouth leads into small, rectangular buccal cavity. Nerve-ring surrounds the esophagus which consists of a corpus, isthmus and esophageal bulb. Intestine dilated anteriorly to form cardia. Excretory pore post-oesophageal and intestine slightly enlarged anteriorly but the dilatation constantly remains less in diameter than the esophageal bulb. Anus situated at posterior end of the body with long, annulated tail, about 1/14th of the body length. Ovaries reflexed and two in number. Vulva post-equatorial with lips bulges out body contour and eggs ellipsoidal. The population of this species were characterized by a cylindroid shaped female body, taper towards the extremities, anteriorly dilated intestine forms cardia, ellipsoidal shaped eggs and annulated tail, about 1/14th of body length. In the original description of *C. nisari* by Parveen and Jairajpuri 1985a **[13]**, some measurements were missing, but the morphology and morphometrics of these *C. nisari* population are closely resemble with the present specimens **(Figure 1, Table 1)**.

***Mirzaiella meerutensis* Singh and Malti, 2003**

Host and site of infection: *Gryllotalpa africana* Beauv (Orthoptera: Gryllotalpidae); intestine.

Locality: Meerut (29^0 01'N, 77^0 45'E), U.P., India.

Voucher material: The slides have been deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India, under the voucher no. Nem/2013/01.

Female redescription **(Figure 1K–O; Table 1)**: Body cylindroid, cuticle striated, more prominent in cervical region. Oral pore surrounded by three lips and leads into buccal cavity. Nerve-ring located at the anterior end. Esophagus long, corpus constitutes the major part of esophagus, muscularized throughout its length. Isthmus short, non-muscular, connects corpus to esophageal bulb. Esophageal bulb pyriform in outline and anus located at posterior end. Tail short about 1/8th of the total body. Two ovaries present, anterior ovary arises in region of esophagus and posterior ovary reached posterior fourth of the body. Eggs oval, vagina long, muscular, anteriorly directed and vulva situated at 2/3rd of the body length. These species population were characterized by cylindrical female body, short tail about 1/8th of the total body, oval eggs and position of vulva 2/3rd of the body. The morphology and measurements of these population agree closely with the original description of the species by Singh and Malti, 2003 **[15] (Figure 1, Table 1).**

The amplification of the D2–D3 expansion segments of 18S rDNA region of *B. mirzaia*, *C. nisari* and *M. meerutensis* produced a fragment of 574, 765 and 740 bp respectively, based on sequencing. Phylogenetic relationship of *B. mirzaia*, *C. nisari* and *M. meerutensis* with closely related nematode species were carried out by means of ML, MP and NJ methods of tree reconstruction **(Figure 2A, B)**. BLAST at NCBI revealed a close relationship of *B. mirzaia* (KC763368) with another species of same genus, *B. korsakowi* (JX852712). Similarly, the 18S region matched *M. meerutensis* (KC763367) and *B. korsakowi* (JX852712) (96 % similarity). ML, MP and NJ analyses showed that *M. meerutensis* (KC763367) and *B. korsakowi* (JX852712) are sister taxa with bootstrap analysis also indicates that the sister relationship is reliably supported **(Figure 2A, B)**. The closest D2-D3 18S sequence similarity (94%) of C. nisari (KC763369) was found with parasites of *Periplaneta americana* viz., *Hammerschmidtiella indicus* (KC335147), *Leidynema portentosae* (EF180073) and *Thelastoma krausi* (EF180068) respectively. Not a wide range of data was available for comparison of parasites of nematodes from Travassosinematidae. This analyses evident three clades, with the *P. americana* nematodes forming one clade and potentially including C. *nisari*, the second clade included the *Binema* and *Mirzaiella* species with high bootstrap values. The *Cosmocercoides* and *Nemhelix* specimens represent third clade. The topologies of the ML, MP and NJ trees were congruent for 18S ribosomal marker. Thus, only the phylogenetic trees obtained by using the ML and NJ method are presented herein **(Figure 2)**.

Nematodes are amongst the most diverse and abundant organisms and are present everywhere. The Order Oxyurida contains parasites of both invertebrate and vertebrate hosts. Nematodes parasitizing the invertebrate hosts belong to the superfamily Thelastomatoidea. This family listed under the various superfamilies including Travassosinematidae **[14]**. Species of the genus of family Travassosinematidae viz., *Binema* Travassos, 1925 **[1]**; *Mirzaiella* Basir, 1942a **[8]** and *Cameronia* Basir, 1948a **[6]** are widely distributed in West and Northeast region of India. In India, the following nominal species of three

genera *Binema, Mirzaiella* and *Cameronia* are: *B. korsakowi* (Sergiev, 1923) Basir, 1956 **[30, 12]**; *B. ornata* Travassos, 1925 **[1]**; *B. mirzaia* (Basir, 1942a) Basir, 1956 **[8, 12]**; *B. anulinervus* Shah and Rizvi, 2004a **[31]**; *M. asiatica* Basir, 1942a **[8]**; *M. indicus* (Singh and Singh, 1955) Adamson and Waerebeke, 1992b **[32, 33]**; *M. gryllotalpae* (Singh and Singh, 1955) Adamson and Waerebeke, 1992b **[32, 33]**; *M. alii* Farooqui, 1967 **[34]**; *M. haroldi* Farooqui, 1968b **[35]**; *M. meerutensis* Singh and Malti, 2003 **[15]** and *C. biovata* Basir, 1948a **[6]**; *C. psilocephala* (Rao, 1958) Adamson and Van Waerebeke, 1992a **[10, 14]**; *C. nisari* (Parveen and Jairajpuri, 1985a) Adamson and Van Waerebeke, 1992a **[13, 14]**; *C. basiri* Rizvi and Jairajpuri, 2002 **[36]**; *C. travassosi* Farooqui, 1968a **[37]**; *C. aspiculata* (Farooqui, 1970) Adamson and Van Waerebeke, 1992a **[11, 14]**; *C. klossi* Parveen and Jairajpuri, 1984 **[38]**; *C. triovata* Shah, 2007b **[18]**; *C. manipurensis* Shah, 2007b **[18]**. *B. mirzaia* described by Basir in 1942a and again re-described in 1956 **[8, 12]**. This species was distinguished from other *Binema* species in India by the shape of the tail and having annulation that is prominent only in cervical region. *M. meerutensis* described by Singh and Malti 2003 **[15]** from Meerut, India having polar flagellated tuft at one end of the ova and distinguished it from other nominal species of this genus. In 1985, Parveen and Jairajpuri **[13]** described *P. nisari* from *G. africana* caught in Aligarh, India.

Currently, more than 30 nematode genome sequencing projects are going on **[39]**, however, no sequencing studies are for nematodes of Travassosinematidae, as molecular data of this group of parasites is scarce. Molecularly, *B. mirzaia* is closely related to the *B. korsakowi*, however, it is easily distinguishable by morphology. Surprisingly, difference in DNA sequence less than 1% showed between the species of *B. korsakowi* and *M. meerutensis*. In 1942a, Basir **[8]** described this genus as type species *M. asiatica* and also described another new genus *Gryllocola* with type species *G. gryllocola* from the same host and in the same year. Later, after a detailed study, he considered that the *Gryllocola* species female was synonym of *Binema* and male was of *M. asiatica*. The closeness of *M. meerutensis* and *B. korsakowi* species required further study to clarify that they are genuinely an independent biological species or not. This paper is the morphological redescription and first molecular description of *B. mirzaia*, *C. nisari* and *M. meerutensis* inhabiting *G. africana* in India. The results supported the validity of these three nematode species based on the morphological and molecular observation. In conclusion, nematode molecular phylogenetic studies of Travassosinematidae are still at an early stage as very limited amount of DNA sequence data is available in comparison to morphological data. Further addition of DNA sequences may change tree topology because currently only relatively limited data are available, therefore, more study is needed to revealed true homology and phylogenetic analyses.

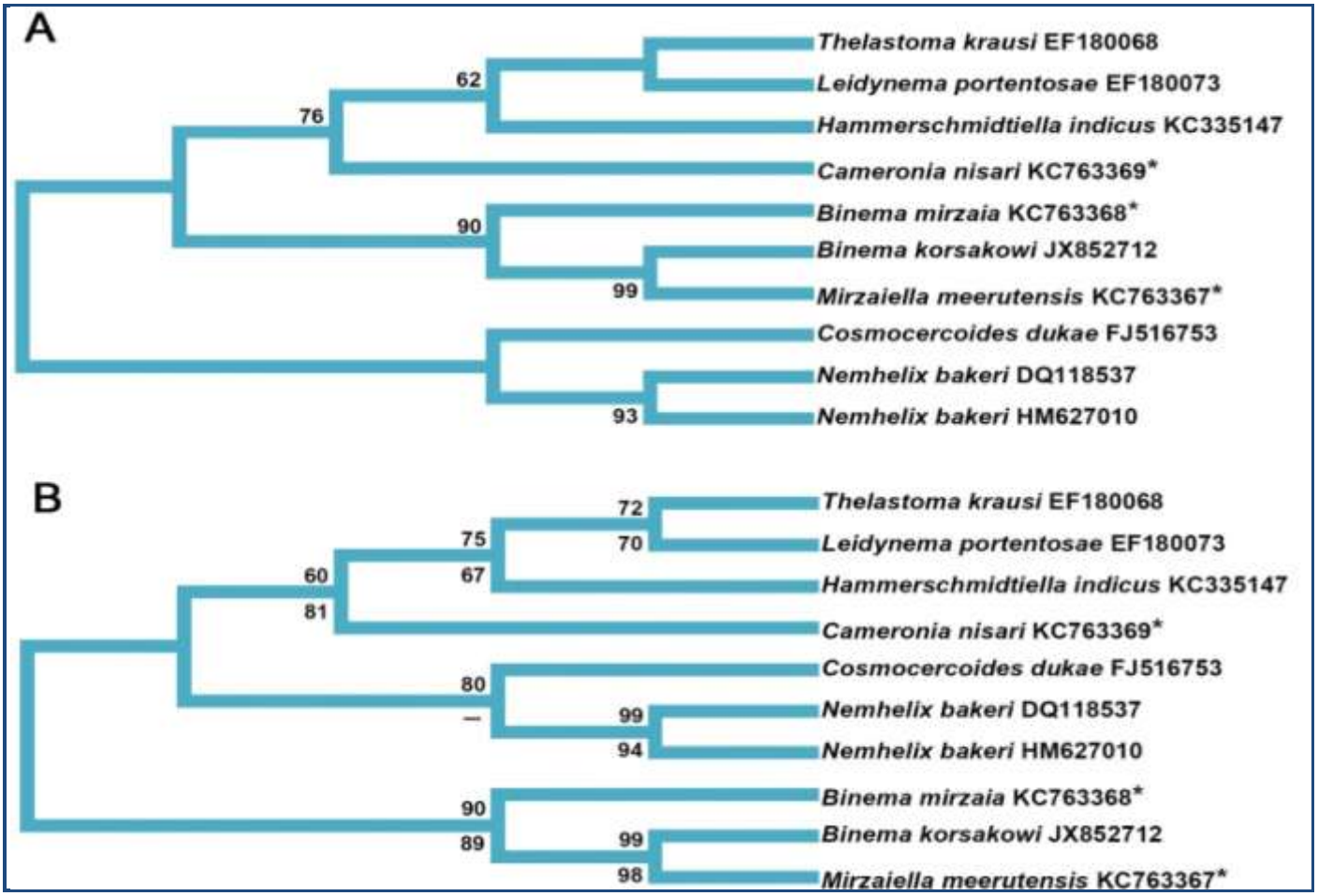

Figure 2: Phylogenetic trees of the partial 18S. **A)** by using the Maximum Likelihood (ML) method; **B)** Neighbour Joining (NJ) analysis, number at nodes are bootstrap values inferred from NJ (above) and MP (below), shown in each node respectively. Newly obtained sequences in this study are marked with asterisk.

Acknowledgement:
The present study was supported with funds provided by the University Grants Commission, New Delhi, India to finance Major Research Project no. 40-406/2011(SR). The authors are grateful to the Head, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India for resources.

References:

[1] Travassos L, *Sciencia Medica (Rio de Janeiro)*. 1925 **3**: 416
[2] Leibersperger E, *Parasitologische Schriftenreihe*. 1960 **11**: 1
[3] Camino NB & Reboredo GR, *Mem Inst Oswaldo Cruz*. 1999 **94**: 311 [PMID: 10348979]
[4] Camino NB & Maiztegui B, *Mem Inst Oswaldo Cruz*. 2002 **97**: 655 [PMID: 12219130]
[5] Biswas PK & Chakravarty GK, *Z ParasitenKe*. 1963 **23**: 411 [PMID: 14111299]
[6] Basir MA, *Can J Res*. 1948 **26**: 201 [PMID: 18939166]
[7] Basir MA, *Can J Res*. 1948 **26**: 4 [PMID: 18913927]
[8] Basir MA, *Records of the Indian Museum*. 1942 **44**: 95
[9] Chakravarty GK, *Current Science*. 1943 **12**: 257
[10] Rao PN, *Arquivos do Museu Nacional, Rio de Janeiro*. 1958 **46**: 33
[11] Farooqui MN, *Riv Parassitol*. 1970 **31**: 195 [PMID: 5498224]
[12] Basir MA, *Zoologica (Stuttgart)*. 1956 pp. 1
[13] Parveen R & Jairajpuri DS, *Helminthologia*. 1985 **22**: 263
[14] Adamson ML & Waerebeke DV, *Systematic Parasitology*. 1992 **21**: 21
[15] Singh HS & Malti, *J Exp Zool India*. 2003 **6**: 329
[16] Manjur Shah M & Rizvi AN, *Parassitologia*. 2004 **46**: 317 [PMID: 15828437]
[17] Shah MM, *Acta Parasitologica*. 2008 **53**: 145
[18] Shah MM, *Acta Parasitologica*. 2007 **52**: 346
[19] Shah MM *et al. InTech*, China. 2012 pp. 163
[20] Aleshin VV *et al.* J *Mol Evol*. 1998 **47**: 597 [PMID: 9797410]
[21] Blaxter ML *et al. Nature*. 1998 **392**: 71 [PMID: 9510248]
[22] Subbotin SA *et al. Mol Phylogenet Evol*. 2001 **21**: 1 [PMID: 11603932]
[23] Bhadury P & Austen MC, *Hydrobiologia*. 2010 **641**: 245
[24] Perera A *et al. Journal of Helminthology*. 2012 doi:10.1017/S0022149X12000181
[25] De Ley P & Blaxter M, Leiden, E J Brill. 2004, pp. 633
[26] Ross JL *et al. Mol Phylogenet Evol*. 2010 **55**: 738 [PMID: 20132899]
[27] Chaudhary A *et al. J Helminthol*. 2013 **4**: 11 [PMID: 23452615]
[28] Floyd RM *et al. Molecular Ecology Notes*. 2005 **5**: 611
[29] Tamura K *et al. Mol Biol Evol*. 2011 **28**: 2731 [PMID: 21546353]
[30] Sergiev PG, *Trudy Gosundarstvennogo Instituta K Experimental noi Veterinarii*. 1923 **1**: 183
[31] Manjur Shah M & Rizvi AN, *Parassitologia*. 2004 **46**: 317 [PMID: 15828437]
[32] Singh KS & Singh KP, *Records of the Indian Museum*. 1955 **53**: 37
[33] Adamson ML & Waerebeke DV, *Systematic Parasitology*. 1992 **21**: 169
[34] Farooqui MN, *Zoologischer Anzeiger*. 1967 **176**: 276
[35] Farooqui MN, *Rivista di Parassitologia*. 1968 **29**: 21
[36] Rizvi AN & Jairajpuri DS, *Revista Ibérica de Parasitología*. 2002 **62**: 1
[37] Farooqui MN, *Parassitologia*. 1968 **29**: 269
[38] Parveen R & Jairajpuri DS, *Parasitologia*. 1984 **44**: 153
[39] Mitreva M *et al. Trends Genet*. 2005 **21**: 573 [PMID: 16099532]

Edited by P Kangueane
Citation: **Singh *et al.*** Bioinformation 9(16): 818-823 (2013)

BIOINFORMATION open access

Supplementary material:

Table 1: Morphometrics of various body parts of *Binema mirzaia* (Basir, 1942a) Basir, 1956, *Cameronia nisari* Parveen & Jairajpuri, 1985a and *Mirzaiella meerutensis* Singh & Malti, 2003 infesting *Gryllotalpa africana* from India. All measurements are given in millimeters (mm) and in the format: mean ± standard deviation (range).

Nematode species	***Binema mirzaia* (Basir, 1942a) Basir, 1956**	***Binema mirzaia* (Basir, 1942a) Basir, 1956 (present observations)**	***Cameronia nisari* Parveen & Jairajpuri, 1985a**	***Cameronia nisari* Parveen & Jairajpuri, 1985a (present observations)**	***Mirzaiella meerutensis* Singh & Malti, 2003**	***Mirzaiella meerutensis* Singh & Malti, 2003 (present observations)**
Locality	Hyderabad, AP, India	Meerut, UP, India	Aligarh, UP, India	Meerut, UP, India	Meerut, UP, India	Meerut, UP, India
Character	Females	Females (n = 4)	Females	Females (n = 7)	Females	Females (n = 5)
BDL	2.44–4.6	3.51–3.53 (3.519±0.0089)	1.6–1.79	2.22–2.241 (2.231±0.00702)	2.05–2.07	1.97–2.00 (1.984±0.012)
BDW	0.30–0.42	0.37–0.39 (0.379±0.009)	–	0.32–0.335 (0.327±0.0051)	0.26–0.27	0.22–0.223 (0.221±0.0012)
BUC	–	0.09–0.098x0.07–0.077 (0.094±0.0035x0.0735±0.0031)	–	0.06–0.0 66x0.08 – 0.085 (0.0637±0.0019x0.0835 ±0.0017)	0.039–0.040x0.010 – 0.012	0.09–0.093x0.07–0.073 (0.0913 ± 0.0012x0.0713±0.0012)
ESPL	0.4–0.54	0.45–0.47 (0.459±0.0088)	–	0.39–0.42 (0.404±0.010)	0.47–0.48	0.58–0.585 (0.582 ±0.00 19)
ESPB	0.09x0.12	0.13–0.135x0.12–0.126 (0.132±0.0020x0.1227± 0.0025)	0.074–0.079x0.068–0.076	0.1–0.115x0.1 – 0.115 (0.110 ± 0.0046x0.109 ±0.00599)	0.06–0.07x0.09–0.10	0.13–0.131x0.12–0.122 (0.130 ± 0.00045x0.121 ±0.00079)
ISL	0.012–0.02	0.01–0.012 (0.0107±0.00096)	0.018–0.125x0.022–0.029	0.02–0.025 (0.022±0.0016)	0.003–0.004	0.01–0.011 (0.0105±0.00035)
COL	0.3–0.408	0.31–0.322 (0.315± 0.0053)	0.20–0.23	0.27–0.28 (0.275±0.0033)	0.35–0.37	0.44–0.443 (0.44±0.0011)
NVR	0.18–0.22	0.2–0.21 (0.205±0.004)	–	0.2–0.22 (0.210 ±0.00645)	0.145–0.146	0.15–0.154 (0.152 ±0.00158)
EXPD	–	–	–	0.39-0.399 (0.395±0.0030)	0.45–0.47	–
VLD	3.06	2.24–2.282 (2.256±0.0188)	1.00–1.11	1.3–1.32 (1.31±0.0062)	1.2–1.5	1.22–1.25 (1.234±0.012)
AND	–	0.1–0.13 (0.1177±0.0134)	–	0.23–0.237 (0.233±0.00244)	0.235	0.3–0.328 (0.318±0.010)
TL	0.09–0.11	0.06–0.086 (0.0715±0.0108)	0.114– 0.370	0.18–0.192 (0.186±0.00388)	–	0.23–0.26 (0.245±0.011)
EG	–	0.05–0.056x0.045–0.051 (0.053 ± 0.0025x0.048± 0.00258)	–	0.09–0.11x0.04–0.05 (0.0971± 0.0058x0.0457 ±0.00 319)	0.1–0.12x0.066–0.067	0.07–0.082x0.06–0.0 71 (0.076 ± 0.0047x0.065 ± 0.0044)

All other abbreviations used for measurements are as: BDL, body length; BDW, body width; BUC, buccal cavity; ESPL, oesophageal length; ESPB, oesophageal bulb; ISL, isthmus length; COL, corpus length; NVR, Nerve ring from the anterior end of the body; EXPD, Excretory pore from the anterior end of the body; VLD, Vulva distance from anterior region; AND, Anus distance from posterior region; TL, tail length; EG, egg.