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Research Note

Procamallanus spiculogubernaculus Agarwal, 1958 (Nematoda: Camallanidae) from Stinging catfish, *Heteropneustes fossilis* in India: morphological characterization and molecular data

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Summary

The nematode, *Procamallanus spiculogubernaculus* Agarwal, 1958 was found from the Stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) from Ghazipur, Delhi, India. Morphological characterization, including scanning electron microscope observation supplemented with DNA sequences is provided. Specimens recently found are characterized by the presence of an unlined buccal capsule having a small basal ring, esophagus muscular and glandular, vulva position is slightly post-equatorial, tail conical, long, and ending in three digit-like processes, phasmids present at about mid-length and cloaca located at the posterior end. In this study the species *P. spiculogubernaculus* is validated on the basis of molecular data after 47 years from its original description. In the scanning electron microscope examination, the topology of mouth and sensory pits in anterior portion, while the phasmids and digit like processes in posterior portion is clearly observed. Molecular data of the 18S ribosomal RNA and mitochondrial cytochrome c oxidase subunit 1 (*cox*1) gene were analyzed. Molecular phylogenetic analyses supported the validity of *Procamallanus spiculogubernaculus* and confirmed the paraphyletic status of the members of *Procamallanus, Spirocamallanus, Camallanus* and *Paracamallanus*. Taxonomic status of members of the family Camallanidae are briefly discussed along with the results of the systematic evaluation of *P. spiculogubernaculus* based on molecular data.

Keywords: Nematode; *Procamallanus*; Scanning electron microscopy; Molecular analyses; 18S; *cox*1; India

Introduction

The genus *Procamallanus* Baylis, 1923 (Nematoda: Camallanidae) includes species that parasitize freshwater, brackish-water and marine fishes, less often amphibians. More than 20 species of this genus have been described from Bangladesh, India and Sri Lanka (Sood, 1989) from *Heteropneustes fossilis* (Bloch, 1794). *H. fossilis* have high economic importance as food fish and for medicinal value (Talwar & Jhingran, 1991; Sherwani & Parwez, 2000; Acharya & Mohanty, 2014). Morphological identification of *Procamallanus* species is based on characters such as esophagus structure, presence of spicules, and gubernaculum in males, while in females it is based on the conical tail with digit-like tail processes, vulva position and shape of the eggs. The rich diversity of the species of *Procamallanus* from *H. fossilis* in India made the species identification difficult, as many descriptions are inadequate for diagnosis. Due to the lack of proper morphological identification and descriptions, species of this genus remain unclear (De & Moravec 1980). Therefore, morphological identification needs the use of molecular analyses to supplement the proper identifica-

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tion and validation of species. Molecular tools are frequently used in nematode identification and systematics (Gasser, 2001; Nadler *et al*., 2005; Mattiucci & Nascetti, 2008; Borges *et al*., 2012; Jones *et al*., 2012; Liu *et al*., 2013; Pekmezci *et al*., 2014; Di Azevedo *et al*., 2015; Goswami *et al.*, 2015, 2016).

In this study, supporting morphological and molecular information is provided for *P. spiculogubernaculus* Agarwal, 1958 from the freshwater fish *H. fossilis* collected from Ghazipur, Delhi, India.

Materials and Methods

A total of 25 specimens of *H. fossilis* (body length 14 – 19 cm) were collected during January 2013 to March 2014 from Ghazipur (28.7342° N, 77.2728° E), Delhi, India for examination of parasites, from which 14 fish were found infected. Host specimens were kept alive in aerated jars at 24 °C until dissection. Nematodes (7 female specimens) from the small intestine of fish were collected in 0.6 % saline solution and then stored in 70 % ethanol until processing for morphology. For the light microscopy study, nematodes were cleared and mounted with glycerin. Drawings were made with the aid of a camera lucida. All measurements were taken in mm from the camera lucida diagram. Photos were taken with a light microscope equipped with differential interference contrast and digital image analysis system (Nikon, Eclipse 50*i*, Kawasaki, Japan). Specimens were deposited in the Museum d' Histoire Naturelle, Geneva, Switzerland (MHNG-INVE-91853) and the Museum, Department of Zoology, Chaudhary Charan Singh University, U.P., India under the voucher number Nem/2015/02. For scanning electron microscopy (SEM) studies, nematodes were fixed in 4 % formaldehyde, post-fixed in 1 % osmium tetraoxide, dehydrated through ascending grades of ethanol series, dried using the critical point dryer and sputter-coated with gold. The specimens were examined with a JEOL Neoscope JCM5000 scanning electron microscope (Tokyo, Japan) at an accelerating voltage of 10 kV.

Samples for molecular analysis were fixed in 95 % ethanol. Genomic DNA extraction from female worm was conducted using the Qiagen DNeasy™ tissue kit (Qiagen, Germany) according to the manufacturer's instruction. PCR amplification for partial 18S (900bp) and *cox*1 (574bp) gene regions were performed using two different sets of the primers, Nem 18SF (5'-CGCGAATRGCTCATTACAACAGC-3') and Nem 18SR (5'-GGGCGGTATCTGATCGCC-3') (Floyd *et al*. 2005); LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*. 1994) respectively. Each 25 µl PCR amplification reaction contained 3 µl of DNA, 2.5 µl of 10× PCR buffer, 0.9 µl of each primer, 3.4 µl of deoxyribonucleotide triphosphates and 1.5 U of Taq DNA polymerase (Biotools, Spain). The amplification was carried out in an Eppendorf Mastercycler (Eppendorf, Germany) with the following cycling profile: 94 °C for 3 min (hot start), followed by 35 cycles of denaturation at 94 °C for 50 s, annealing for 1 min on 54 °C for 18S and 48 °C for *cox*1, followed by 1 min extension at 72 °C and a final extension at 72 °C for 5 min. The PCR products were electrophoresed on 1.5 % agarose in TAE buffer stained with ethidium bromide and visualized under UV transilluminator. For sequencing, the PCR product was purified using Purelink™ Quick Gel Extraction and

Table 1. Comparative morphometric data of *P. spiculogubernaculus* female specimens

	P. spiculogubernaculus **Agarwal, 1958**	***P. spiculogubernaculus*** **(De & Moravec, 1980)**	***P. spiculogubernaculus*** **present study**
Infecting:			
Host	*Heteropneustes fossilis*	*H. fossilis*	*H. fossilis*
Organ	Intestine	Stomach	Intestine
Body:			
Length	4.84	3.56 – 7.49 (3.44)	4.71 (3.25 – 5.31)
Width	0.088	0.090 – 0.189 (0.096)	0.090 – 0.189 (0.096)
Buccal capsule:			
Length	0.08	0.069 – 0.084 (0.063)	0.069 – 0.084 (0.063)
Width	0.048	0.048 – 0.069 (0.057)	0.048 – 0.069 (0.057)
Length of basal ring:	–	0.009	0.009
Muscular oesophagus:			
Length	0.032	0.264 – 0.333 (0.282)	0.264 – 0.333 (0.282)
Width	–	0.057 – 0.066 (0.057)	0.057 – 0.066 (0.057)
Glandular oesophagus:			
Length	0.046	0.318 – 0.450 (0.336)	0.318 – 0.450 (0.336)
Width	–	0.042 – 0.072 (0.048)	0.042 – 0.072 (0.048)
Nerve ring distance from anterior end:	–	0.129 – 0.156 (0.126)	0.129 – 0.156 (0.126)
Vulva distance from posterior end:	2.2	1.58 – 3.48 (1.46)	3.26 (2.98 – 3.57)
Tail length:	0.1	0.093 – 0.138 (0.090)	0.06 (0.05 – 0.07)
Tail process length:	–	0.003	0.002

PCR purification Combo Kit (Invitrogen, Germany) following manufacturer's instructions. Purified PCR products were sequenced (in both directions) using the Big Dye Terminator vr. 3.1 cycle sequencing kit in ABI 3130 Genetic Analyzer, Applied Biosystems (Foster City, USA) with the same primers cited above.

Obtained sequence fragments of 18S rRNA and mt *cox*1 gene were assembled using MEGA V6.06 (Tamura *et al*., 2013). Ambiguous bases were clarified using their corresponding ABI chromatograms. The 18S rDNA and mt *cox*1 gene sequences were compared for similarities with other available sequences from GenBank using BLAST (www.ncbi.nlm.nih.gov) and the related sequences based on nucleotide similarities were aligned with the software CLUSTAL W (Thompson *et al*., 1994). DNA pairwise distances for *cox*1 and 18S rDNA were calculated in MEGA V6.06 using the *p*-distance model. The dataset was tested prior to phylogenetic analysis for the nucleotide substitution model of best fit using ModelTest (Posada & Crandall, 1998) and the best-fitting model was chosen using the Akaike Information Criterion (AIC). The model GTR + G + I (general time reversible) for 18S sequences and Hasegawa-Kishino-Yano model (HKY) for mtDNA *cox*1 were selected. The phylogenetic trees were inferred by Maximum Likelihood (ML) using MEGA vr. 6 (Tamura *et al*., 2013) with bootstrap analysis based on 1000 replicates. Bayesian Inference (BI) analyses were also carried out in Topali 2.5 (Milne *et al*., 2009) using MCMC chains searches on two simultaneous runs of four chains over 1,000,000 generations with every 100th tree saved. The first 25 % of the sampled trees were discarded as 'burn in'. The members belong to the family Physalopteridae, Dracunculidae, Philometridae and Micropleuridae and *Dracunculus insignis* (EU646538) were used as out-groups for 18S rDNA and *cox*1 mt DNA phylogenetic reconstructions respectively.

Results and Discussion

A total of 7 *P. spiculogubernaculus* specimens were collected from 14 infected host fish, *H. fossilis*. All specimens were found in the small intestine, none was recovered from the stomach, liver or body cavity. The prevalence was 56 % and mean intensity of in-

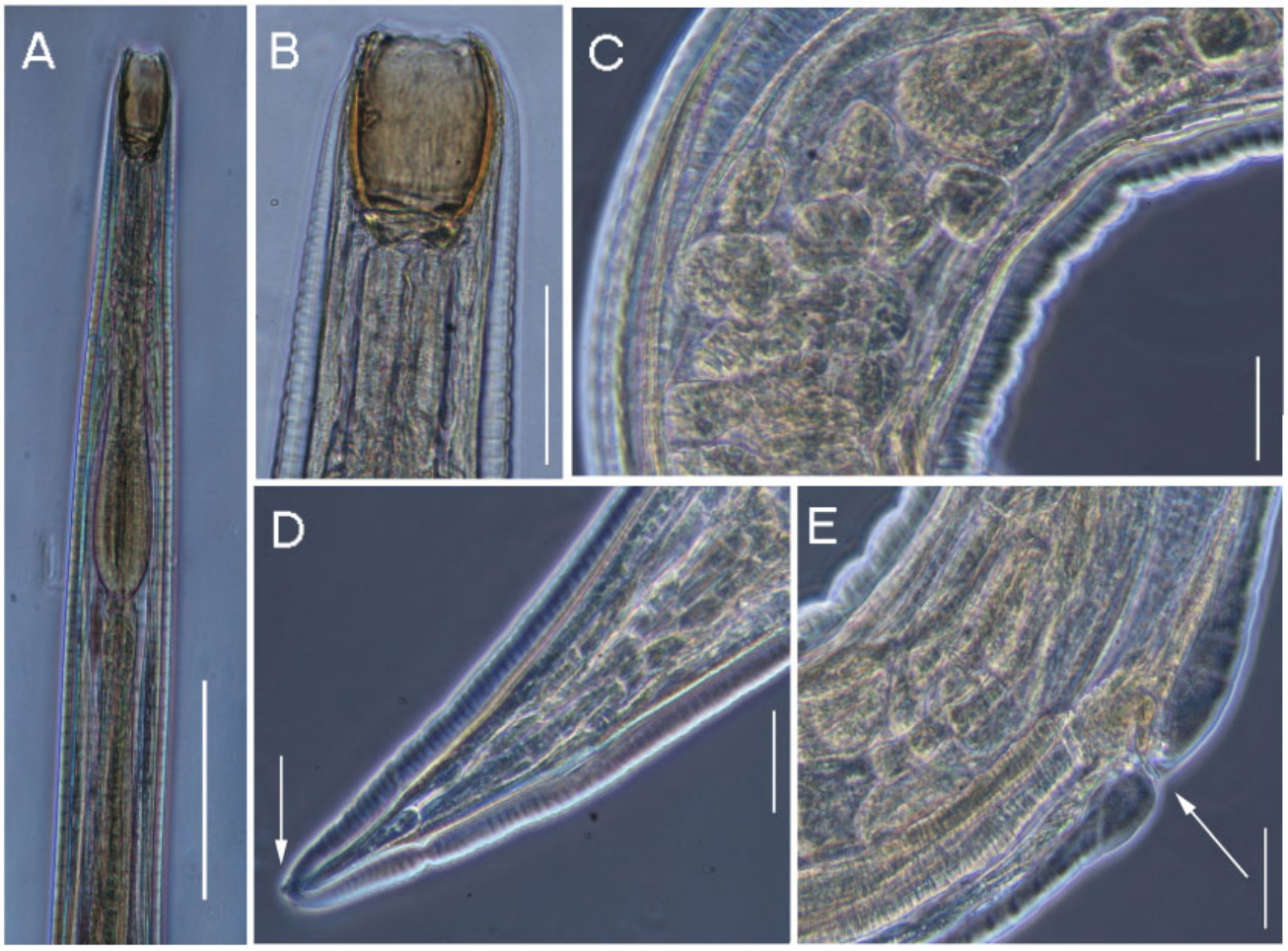

Fig. 1. Differential interference contrast images of female *Procamallanus spiculogubernaculus* (dorsal view). A – anterior end; B – buccal capsule of female; C – eggs; D – female tail with processes; E – Vulva. Scale bars: Fig. A = 0.33 mm; Fig. B = 0.11 mm; Figs. C – E = 0.11 mm.

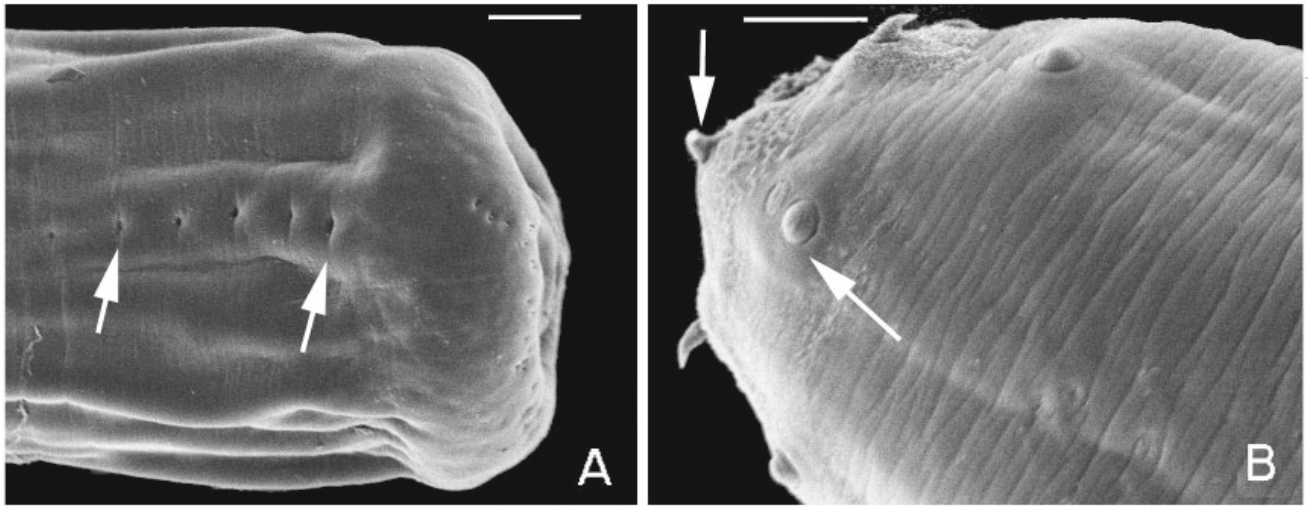

Fig. 2. Scanning electron micrographs of *Procamallanus spiculogubernaculus* (female). A – anterior part of female showing mouth and sensory pits; B – tail of female showing posterior papillae (phasmids) and digit-like processes. Scale bars: Fig. A = 50 µm; Fig. B = 20 µm.

fection was 2.7. Morphological identification was based on female worms and the specimens were tentatively identified as *P. spiculogubernaculus* due to the presence of an unlined buccal capsule with a small basal ring, muscular and glandular esophagus, the position of vulva slightly post-equatorial, tail conical, long, ending in three digit-like processes (one dorsal and two ventrolateral), phasmids occur at about mid-length, cloaca located at the posterior end in female (Fig. 1 and Fig. 2). These specimens are similar to the description of *P. spiculogubernaculus* and the redescription by De & Moravec (1980). Measurements were taken and compared to other described species in the Table 1.

Moreover, we obtained 18S rDNA sequence of *P. spiculogubernaculus* (900 bp), deposited in GenBank under the accession number KU292357 and compared it with the sequences available in the GenBank. The phylogenetic relationship of 18S rDNA was analyzed on the basis of ML and Bayesian inference (Fig. 3). The trees obtained by these two methods showed similar topologies, therefore, only the ML tree is presented (Fig. 3) together with posterior probability values for BI. The newly generated 18S rDNA sequence of *P. spiculogubernaculus* was used along with 67 published taxa for phylogenetic reconstruction. The 18S gene analysis comprises of total 68 sequences from which the members belong to the family Physalopteridae Leiper, 1908, Dracunculidae Leiper, 1912, Philometridae Baylis and Daubney, 1926 and Micropleuridae Baylis and Daubney, 1926 were taken as out-group from which the family Camallanidae arises. *P. spiculogubernaculus* is found to form a clade with representatives of genera *Procamallanus* Baylis, 1923, *Spirocamallanus* Olsen, 1952, *Camallanus* Railliet and Henry, 1915 and *Paracamallanus* Yorke and Maplestone, 1926. *P. spiculogubernaculus* represented sister taxa to the *Procamallanus laeviconchus* Wedl, 1861. This is the first sequence for any *Procamallanus* species from India available on GenBank.

Partial sequences of the *cox*1 (574 bp) gene obtained from *P. spiculogubernaculus* was deposited in GenBank under the accession number KU292358 in this study. Subsequently, we compared our *cox*1 sequence by BLAST similarity search with available sequences in the database. The sequences which show similarity with *cox*1 sequence of *P. spiculogubernaculus* were from *Spirocamallanus* and *Camallanus* genera (Fig. 4). There is no *cox*1 sequence available in the GenBank database for any species of *Procamallanu*s. Pairwise comparison between the *cox*1 sequence of *P. spiculogubernaculus* and *Spirocamallanus* and *Camallanus* species showed 0.17 – 0.21 % nucleotide differences. The phylogenetic analyses of the cox1 gene sequence of *P. spiculogubernaculus* isolated from *H. fossilis* were conducted with two methods: ML and Bayesian inference. We present only the ML tree as the BI tree shows a similar topology in the clade formation (Fig. 4). This phylogenetic tree was based on the sequences of *cox*1 mtDNA. This tree is focused on the representatives of the family Camallanidae with *Dracunculus insignis* (Leidy, 1858) (Dracunculidae) as an out-group species. The phylogenetic tree constructed from dataset containing representatives of genera *Camallanus* and *Spirocamallanus* displays in figure 4. The phylogenetic analyses gave a tree with two major clades (Fig. 4). *P. spiculogubernaculus* incorporates between clades of *Camallanus* and *Spirocamallanus*. This is unfortunate to encorporated any sequence of *cox*1 gene of *Procamallanus*, due to unavailability of *cox*1 sequence in GenBank. To bring out more detailed view on the relationships between species of *Procamallanus*, more sequences of *cox*1 gene have to be generated.

Both phylogenetic trees fairly illustrated the relationship of *P. spiculogubernaculus* with the genera *Camallanus* and *Spirocamallanus*. One represents the species of *Camallanus* and the other belonged to the species of *Spirocamallanus* and *Procamallanus* (Fig. 4). The specimens of *P. spiculogubernaculus* sequenced in the present study show close relationships with species of *Pro-*

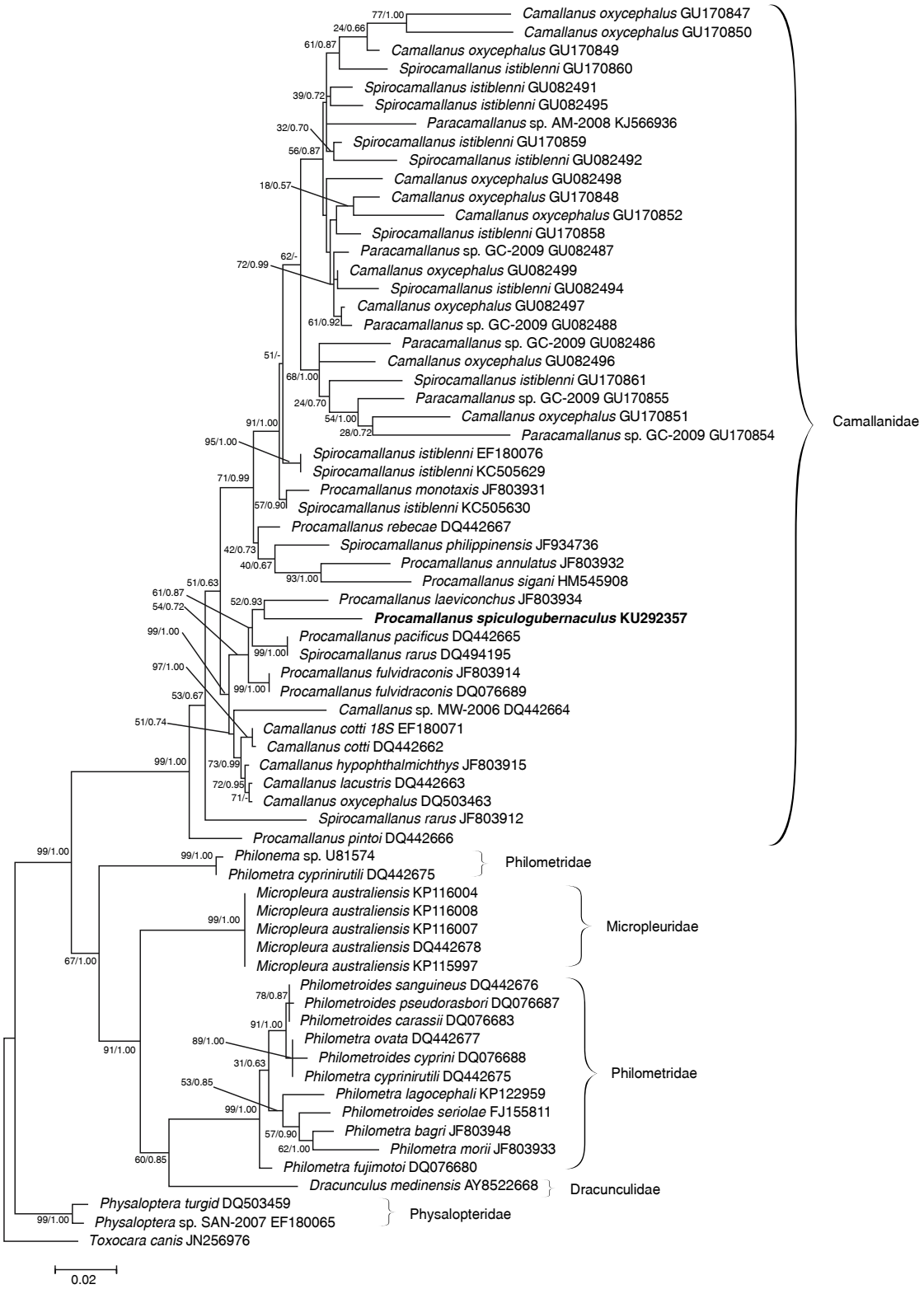

Fig. 3. A phylogenetic tree is based on the 18S rDNA sequences and demonstrating the position of *Procamallanus spiculogubernaculus* with other nematode species. The tree was generated by maximum likelihood (ML) method. Numbers of nodes indicate the bootstrap values ML and posterior probabilities (BI) respectively. GenBank accession numbers are listed along the species names. Species examined in this study are indicated in bold. Unsupported nodes by BI are marked with a hyphen.

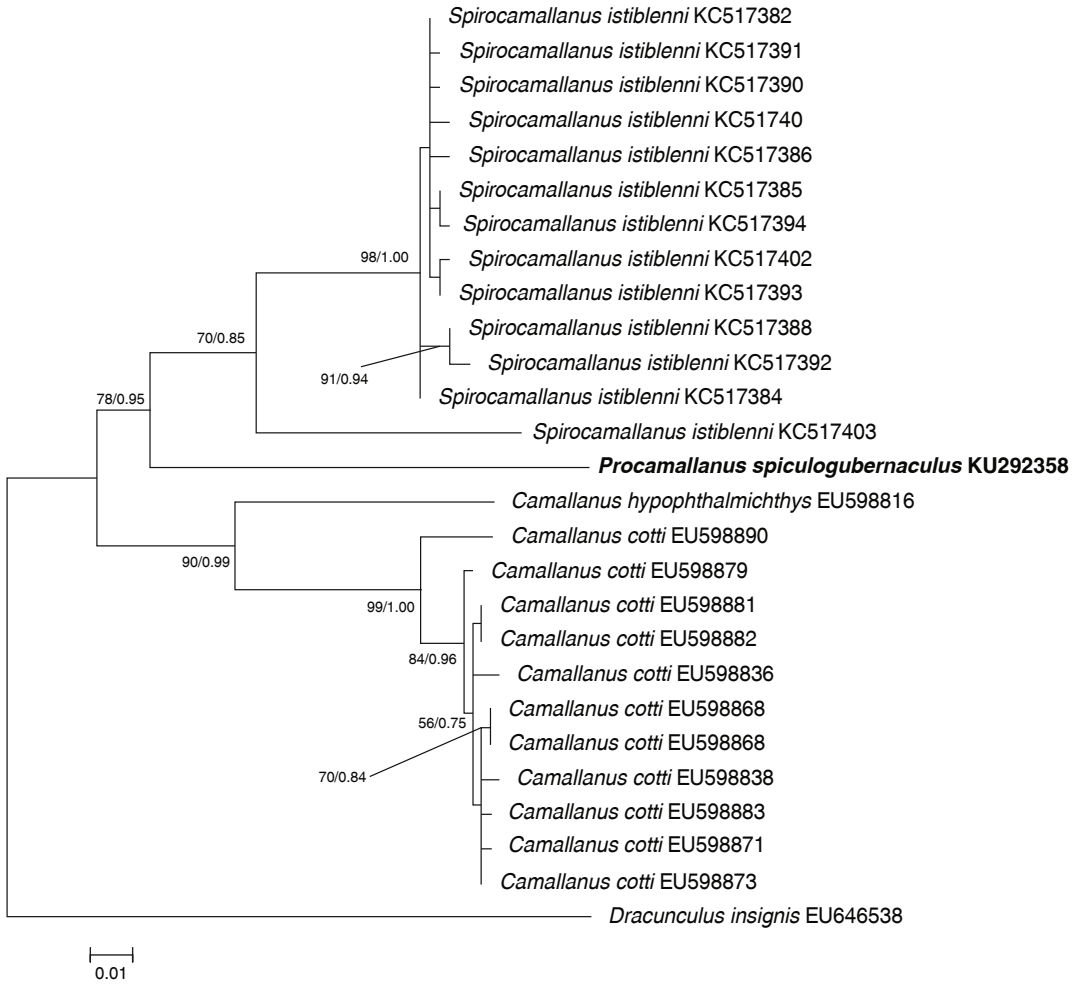

Fig. 4. Maximum Likelihood (ML) tree inferred from mtDNA *cox*1 gene sequence data from *Procamallanus spiculogubernaculus* obtained in this study. Numbers of nodes are bootstrap values higher than 50% and indicate the ML and posterior probabilities (BI) respectively. GenBank accession numbers are also given in the tree. *P. spiculogubernaculus* examined in this study are indicated in bold.

camallanus. It is also figured out the paraphyletic nature of *Procamallanus* from phylogenetic reconstruction as it grouped in four different clades included members of *Camallanus*, *Procamallanus* and *Spirocamallanus*. *P. spiculogubernaculus* in the present study shows the close relationships with species of *Spirocamallanus* that depicts them as putative sister species because the genus *Spirocamallanus* was retain as subgenus of *Procamallanu*s by Moravec & Sey (1988) to accommodate the species possessing a buccal capsule with spiral thickenings.

A large number of species are placed in *Procamallanus* Baylis, 1923 that are mostly parasites of freshwater and marine fishes (De & Moravec, 1980; Andrade-Salas *et al*., 1994; Rigby, 1999; De & Maity, 2000; Moravec *et al*., 2006; Moravec & Van As, 2015). In the present study, the collected specimens were all of the females; no male was found. According to De & Moravec (1980) many described species of the genus *Procamallanus* reported from the same host from India and nearby countries are very close to each other in morphology. The following species, *i.e*., *Procamallanus daccai* Gupta, 1959; *Neocamallanus heteropneusti* Chakravarty *et al*., 1961; *P. chakravartyi* Fernando & Furtado, 1963; *P. confuses* Fernando & Furtado, 1963; *P. mathurai* Pande *et al*., 1963; *P. hindenensis* Lal, 1965; *P. magurii* Lal, 1965; *P. devendri* Sinha & Sahay, 1966; *P. ottuei* Varma & Varma, 1971 and *P. sprenti* Bashirullah & Hafizuddin, 1974 were synonymized with *P. spiculogubernaculus* by De & Moravec (1980) as they all were morphologically similar. *P. spiculogubernaculus* was inadequately described before the study of De & Moravec (1980). This species is distinguished from others on the basis of taxonomic features such as the length of the body, spherical mouth opening, mouth papillae four in number, buccal capsule barrel shaped, esophagus divided into muscular anterior and glandular posterior parts; in females, tail ends conical with three small processes. As these digit-like processes were also found in female of *P. laeviconchus* and *P. pseudolaeviconchus* (Moravec & Van As, 2015) confirmed by the SEM

study. In the present study SEM observation was performed for *P. spiculogubernaculus* for the first time. It is evident that most *Procamallanus* species from India were inadequately described, making identification solely on the basis of light microscopy difficult, especially when many species of *Procamallanus* harbor the same host fish. Of many described *Procamallanus* species in India, only few studies are available using the SEM (Moravec *et al*. 2013). Structures like topology of mouth, sensory pits at the anterior region, the phasmids and digit like processes in posterior region visible by SEM can act as an important taxonomic feature through which *P. spiculogubernaculus* can easily be distinct from other species of *Procamallanus*. Although, morphological information contributes to species identification, but DNA sequence data are required for substantiating the findings. To date, no sequence is available in the GenBank database for any *Procamallanus* species from India. This study provides the phylogenetic status of *P. spiculogubernaculus* with combined use of 18S rDNA and *cox*1 mt DNA molecular data. Further, this is the first molecular data for this taxon and it does establish the phylogenetic relationship with some other members of *Procamallanus*. By comparing the sequence differences, it is easy to identify closely related nematode species. The molecular markers based on nuclear and mitochondrial genes are an excellent tool for differentiating nematode species (Floyd *et al*., 2002; Blaxter *et al*., 2005; McLean *et al*., 2012; Singh *et al*., 2013; Nakacwa *et al*., 2013; Chaudhary *et al*., 2015; Mikaeili *et al*., 2015; Goswami *et al*., 2015). The present molecular analyses using 18S rDNA and *cox*1 mtDNA genes support the placement of *P. spiculogubernaculus* in family Camallanidae. Comparison of a large number of sequences of representatives of Camallanidae also supports the paraphyly of genera *Procamallanus*. The current phylogenetic reconstruction also indicated the paraphyletic nature of Camallanid genera *Camallanus*, *Procamallanus* and *Spirocamallanus*. The genus *Spirocamallanus* was established as a distinct genus from *Procamallanus* with species having presence of spiral thickenings on the buccal capsule. Many authors agreed with the distinct status of *Spirocamallanus* (Ivashkin *et al.*, 1971; Chabaud, 1975; Soota, 1983; Andrade-Salas *et al.*, 1994). Whereas *Procamallanus* have smooth buccal capsule lack of spiral thickenings. Later Moravec & Sey (1988) recognized the genus *Procamallanus* and retain *Spirocamallanus* as a subgenus for accommodate the species in which both males and females have the buccal capsule with spiral thickenings. The close relationship of the 18S rDNA and mt *cox*1 sequence of *P. spiculogubernaculus* with species of *Procamallanus, Spirocamallanus*, *Paracamallanus,* and *Camallanus* but the differences found in the 18S and *cox*1 sequences suggest that they are closely related but distinct species. The two phylogenetic methods (ML and BI) yielded very similar topologies; differences were usually found at the nodes.

In summary, this is the first molecular study of species of *Procamallanus* from India and first *cox*1 gene sequence amplified in this study available in GenBank. Our findings suggest that along with morphology, molecular tools can be used as genetic markers for the accurate identification, distinction of species and for providing reliable phylogenies. Further studies in future with more sequence data are needed for congruent analysis and comparison which will clear phylogenetic position of parasitic nematodes of the family Camallanidae in India.

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References

Acharya, G., Mohanty, P.K. (2014): Comparative hematological and serum biochemical analysis of catfishes *Clarias batrachus* (Linnaeus, 1758) and *Heteropneustes fossilis* (Bloch, 1794) with respect to sex. *J. Entomol. Zool. Stud.*, 2: 191 – 197

Agarwal, S.C. (1958): On a new species of *Procamallanus* Baylis, 1923 (Nematoda). *Curr. Sci.*, 27: 348 – 349

Andrade-Salas, O., Pineda-López, R.F., García-Magaña, L. (1994): *Spirocamallanus rebecae* sp. n. Nematoda: Camallanidae) from freshwater fishes in south-eastern Mexico. *Folia Parasitol.*, 41: 259 – 270

Bashirullah, A.K.M., Hafizuddin, A.K.M. (1974): Two new nematode species of *Procamallanus* Baylis, 1923 from freshwater fishes of Dacca, Bangladesh. *Norw. J. Zool.*, 22: 53 – 55

Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., Abebe, E. (2005): Defining operational taxonomic units using DNA barcode data. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.*, 360: 1935 – 1943. DOI: 10.1098/rstb.2005.1725

Borges, J.N., Cunha, L.F.G., Santos, H.L.C., Monteiro-Neto, C., Santos, C.P. (2012): Morphological and Molecular Diagnosis of Anisakid Nematode Larvae from Cutlassfish (*Trichiurus lepturus*) off the Coast of Rio de Janeiro, Brazil. *PLoS ONE*, 7: e40447. DOI: 10.1371/journal.pone.0040447

Chabaud, A.G. (1975): *Keys to genera of the order Spirurida. Part 1. Camallanoidea, Dracunculoidea, Gnathostomatoidea, Physalopteroidea, Rictularioidea and Thelazioidea. CIH Keys to the nematode parasites of vertebrates 3.* Commonwealth Agricult. Bureaux, Farnham Royal, Bucks, UK, 27 pp.

Chakravarty, G.K., Majumdar, G., Sain, S.K. (1961): On a Camallanid nematode *Neocamallanus heteropneusti* n. gen. et sp. with amendation of the family. *Zool. Anz.*, 166: 221 – 224

Chaudhary, A., Kansal, G., Singh, N., Singh, H.S. (2015): New

molecular data for parasites *Hammerschmidtiella indicus* and *Thelandros scleratus* (Nematoda: Oxyurida) to infer phylogenetic position. *Turk. J. Zool.*, 39: 251 – 255. DOI:10.3906/zoo-1311-18

De, N.C., Moravec, F. (1980): Redescription of the nematode *Procamallanus spiculogubernaculus* with notes on related forms. *Věst. Československ. Zool. Společ.*, 44: 81 – 91

De, N.C., Maity, R.N. (2000): Development of *Procamallanus saccobranchi* (Nematoda: Camallanidae), a parasite of a freshwater fish in India. *Folia Parasitol.*, 47: 216 – 226. DOI: 10.14411/fp.2000.040

Di Azevedo, M.I., Knoff, M., Carvalho, V.L., Mello, W.N., Lopes Torres, E.J., Gomes, D.C., Iñiguez, A.M. (2015): Morphological and genetic identification of *Anisakis paggiae* (Nematoda: Anisakidae) in dwarf sperm whale *Kogia sima* from Brazilian waters. *Dis. Aquat. Org.*, 113: 103 – 11. DOI: 10.3354/dao02831

Fernando, C.H., Furtado, J.I. (1963): A study of some helminth parasites of freshwater fishes in Ceylon. *Z. Parasitenkd.*, 23: 141 – 163.

Floyd, R., Abebe, E., Papert, A., Blaxter, M. (2002): Molecular barcodes for soil nematode identification. *Mol. Ecol.*, 11: 839 – 850. DOI: 10.1046/j.1365-294X.2002.01485.x

Floyd, R.M., Rogers, A.D., Lambshead, J.D., Smith, C.R. (2005): Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Mol. Ecol. Notes*, 5: 611 – 612. DOI: 10.1111/j.1471-8286.2005.01009.x

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994): DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Marine Biol. Biotechnol.*, 3: 294 – 299

Gasser, R.B. (2001): Identification of parasitic nematodes and study of genetic variability using PCR approaches. In: Kennedy, M.W., Harnett, W. (Eds) *Parasitic nematodes: Molecular Biology, biochemistry and immunology*. CABI Publishing, London, pp. 53 – 82

Goswami, U., Chaudhary, A., Verma, C., Singh, H.S. (2015): First Molecular Characterization of *Aspiculuris tetraptera* (Nematoda: Heteroxynematidae) from *Mus musculus* (Rodentia: Muridae) in India. *Acta Parasitol.*, 60: 553 – 556. DOI: 10.1515/ap-2015-0079

Goswami, U., Chaudhary, A., Verma, C., Singh, H.S. (2016): Molecular and ultrastructure characterization of two nematodes (*Thelandros scleratus* and *Physalopteroides dactyluris*) based on ribosomal and mitochondrial DNA sequences. *Helminthologia*, 53: 165 – 171. DOI: 10.1515/helmin-2016-0013

Gupta, S.P. (1959): Nematode parasites of vertebrates of East Pakistan III. Camallanidae from fish, amphibian, and reptiles. *Can. J. Zool.*, 37: 771 – 779. DOI: 10.1139/z59-078

Ivashkin, V.M., Sobolev, A.A., Khromova, L.A. (1971): *Camallanata of animals and man and the diseases caused by them*. Nauka, Moscow, 388 pp. (In Russian)

Jones, R., Brown, D.S., Harris, E., Jones, J., Symondson, W.O.C., Bruford, M.W., Cable, J. (2012): First record of *Neoxysomatium brevicaudatum* through the non-native invasive sampling of *Anguis fragilis*: complementary morphological and molecular detection. *J. Helminthol.*, 86: 125 – 129. DOI: 10.1017/S0022149X11000174

Lal, C. (1965): Two new species of the genus *Procamallanus* Baylis, 1923 from North India. *Labdev J. Sci. Technol.*, 3: 199 – 200

Liu, G.H., Shao, R., Li, J.Y., Zhou, D.H., Li, H., Zhu, X.Q. (2013): The complete mitochondrial genomes of three parasitic nematodes of birds: a unique gene order and insights into nematode phylogeny. *BMC Genomics*, 14: 414. doi: 10.1186/1471-2164-14-414

Mattiucci, S., Nascetti, G. (2008): Advances and trends in the molecular systematics of Anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Adv. Parasitol.*, 66: 47 – 148. DOI: 10.1016/S0065-308X(08)00202-9

McLean, E.R., Kinsella, J.M., Chiyo, P., Obanda, V., Moss, C., Archie, E.A. (2012): Genetic identification of five strongyle nematode parasites in wild African elephants (*Loxodonta africana*). *J. Wildl. Dis.*, 48: 707 – 716. DOI: 10.7589/0090-3558-48.3.707

Mikaeili, F., Mirhendi, H., Mohebali, M., Hosseini, M., Sharbatkhori, M., Zarei, Z., Kia, E.B. (2015): Sequence variation in mitochondrial cox1 and nad1 genes of ascaridoid nematodes in cats and dogs from Iran. *J. Helminthol.*, 89: 496 – 501. DOI: 10.1017/S0022149X14000133

Milne, I., Lindner, D., Bayer, M., Husmeier, D., McGuire, G., Marshall, D.F., Wright, F. (2008): TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics*, 25: 126 – 127. DOI: 10.1093/bioinformatics/btn575

Moravec, F., Justine, J.L., Würtz, J., Taraschewski, H., Sasal, P. (2006): A new species of *Procamallanus* (Nematoda: Camallanidae) from Pacific eels (*Anguilla* spp.). *J. Parasitol.*, 92: 130 – 137. DOI: 10.1645/GE-3509.1

Moravec, F., Sey, O. (1988): Nematodes of freshwater fishes from North Vietnam. Part 1. Camallanoidea and Habronematoidea. *Acta Soc. Zool. Bohemoslov.*, 52: 128 – 148

Moravec, F., Sheeba, S., Kumar, A.B. (2013): Observations on nematodes from the Indonesian shortfin eel *Anguilla bicolor bicolor* McClelland in India, including a revalidation of Heliconema ahiri Karve, 1941 (Physalopteridae). *Acta Parasitol.*, 58: 496 – 503. DOI: 10.2478/s11686-013-0168-0

Moravec, F., Van As, L.L. (2015): *Procamallanus* (*Procamallanus*) spp. (Nematoda: Camallanidae) in fishes of the Okavango River, Botswana, including the description of *P.* (*P.*) *pseudolaeviconchus* n. sp. parasitic in *Clarias* spp. (Clariidae) from Botswana and Egypt. *Syst. Parasitol.*, 90: 137 – 149. DOI: 10.1007/s11230-014-9541-0

Nadler, S.A., D'Amelio, S., Dailey, M.D., Paggi, L., Siu, S., Sakanari, J.A. (2005): Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from Northern Pacific marine mammals. *J. Parasitol.*, 91: 1413 – 1429. DOI: 10.1645/GE-522R.1

Nakacwa, R., Kiggundu, A., Talwana, H., Namaganda, J., Lilley, C., Tushemereirwe, W., Atkinson, H. (2013): Nematode 18S rRNA gene is a reliable tool for environmental biosafety assessment of

transgenic banana in confined field trials. *Transgenic Res.*, 22: 1003 – 1010. DOI: 10.1007/s11248-013-9712-9

Pande, B.P., Bhatia, B.B., Rai, P. (1963): On the Camallanid genus *Procamallanus* Baylis, 1923 in two of the freshwater fishes. *Indian J. Helminthol.*, 16: 105 – 108

Pekmezci, G.Z., Onuk, E.E., Bolukbas, C.S., Yardimci, B., Gurler, A.T., Acici, M., Umur, S. (2014): Molecular identification of *Anisakis* species (Nematoda: Anisakidae) from marine fishes collected in Turkish waters. *Vet. Parasitol.*, 201: 82 – 94. doi: 10.1016/j.vetpar.2014.01.005

Posada, D., Crandall, K.A. (1998): MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14: 817 – 818

Rigby, M.C. (1999): The Camallanidae (Nematoda) of Indo-Pacific fishes: Taxonomy, ecology and host-parasite coevolution. In: Séret, B., Sire, J.Y. (Eds). *Proceedings of the 55th Indo-Pacific Fish Conference, Nouméa, 1997*. Societé Francaise de Ichthyologie, Paris, France, pp. 633 – 644

Sherwani, F.A., Parwez, I. (2000): Effects of stress and food deprivation on catfish, *Heteropneustes fossilis* (Bloch). *Indian J. Exp. Biol.*, 38: 379 – 384

Singh, N., Chaudhary, A., Singh, H.S. (2013): Identification of two species of *Binema* Travassos, 1925 (Oxyurida: Travassosinematidae) based on morphological and sequence analysis of genomic (18S) and mitochondrial (Cox1) gene markers. *J. Nematode Morphol. System.*, 16: 173 – 180

Sinha, D.P., Sahay, U. (1966): On a new species of *Procamallanus* (Camallanidae, Nematoda) with a discussion on the validity of the genus *Indocamallanus* Chakravarty et al., 1961. *Zool. Anz.*, 176: 384 – 388

Sood, M.L. (1989): *Fish nematodes of South Asia*. New Delhi-Ludhiana, India, Kalyani Publishers, 227 pp.

Soota, T.D. (1983): Studies on nematode parasites of Indian vertebrates I. Fishes. *Records of the Zoological Survey of India*, Occasional Paper No. 54, Calcutta, 352 pp.

Talwar, P.K., Jhingran, A.G. (1991): *Inland Fishes of India and Adjacent Countries*, *Vol. 1 & 2*. New Delhi, India, Oxford & IBH Publishing Co. Pvt. Ltd., 1158 pp.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.*, 30: 2725 – 2729. DOI: 10.1093/molbev/mst197

Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22: 4673 – 4680

Troccoli, A., De Luca, F., Handoo, Z.A., Di Vito, M. (2008): Morphological and molecular characterization of *Pratylenchus lentis* n. sp. (Nematoda: Pratylenchidae) from Sicily. *J. Nematol.*, 40: 190 – 196

Varma, S., Varma, S. (1971): On *Procamallanus ottuei* sp. nov. (Nematoda Spiruroidea). *Indian J. Anim. Res.*, 5: 29 – 32