Journal of Helminthology (2012) **86,** 353–362
doi:10.1017/S0022149X11000472

The morphology and genetic characterization of *Iheringascaris goai* n. sp. (Nematoda: Raphidascarididae) from the intestine of the silver whiting and spotted catfish off the central west coast of India

A. Malhotra[1*†], N. Jaiswal[2], A.K. Malakar[3], M.S. Verma[3], H.R. Singh[4], W.S. Lakra[3], S.K. Malhotra[1] and S. Shamsi[5]

[1]Department of Zoology, University of Allahabad, Allahabad 211002, UP, India: [2]Department of Zoology, Nehru Gram Bharati University, Allahabad, UP, India: [3]National Bureau of Fish Genetic Resources, Canal Ring Road, PO Dilkusha, Lucknow, UP, India: [4]College of Fisheries, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India: [5]School of Animal and Veterinary Sciences, Charles Sturt University, Borooma St, Estella, New South Wales 2678, Australia

(Received 18 September 2010; Accepted 4 July 2011; First Published Online 17 August 2011)

Abstract

In this study a new species of nematode, *Iheringascaris goai* n. sp., is reported from two fish hosts, including silver whiting, *Sillago sihama*, and spotted catfish, *Arius maculatus*, caught off the Central West Coast of India at Goa. The new species can be differentiated morphologically from *I. inquies*, the most closely related species collected from cohabiting marine fish. The distinguishing characteristics are distinct cuticular striations, a unilateral excretory system, the presence of dentigerous ridges on the inner margin of the lips and the ratio of oesophagus to body length. In males, the ratio of spicules to body length is higher and the number of pre-anal papillae is less in comparison to those in *I. inquies*. In addition, the tail curves ventrad in males, while in females, the vulva is post-equatorial. The sequence alignment of 18S rDNA and cytochrome *c* oxidase subunit I with sequences of known species selected from the same superfamily shows a significant difference. The morphological and molecular differences reported here can, therefore, be used to assign the specimen to a new species.

Introduction

The genus *Iheringascaris* was established by Pereira (1935) with *I. iheringascaris* as type species based on the characters of a distinctly plicated cuticle devoid of spines, cuticular alae, equal spicules, two lateral doubled papillae on dorsal lip, subventral lip with amphid, adjacent mediolateral doubled papilla and single lateral papilla, absence of dentigerous ridges, presence of interlabia, excretory pore opening near level of nerve ring, absence of gubernaculum, caudal papillae in lateral row as well as regular pre- and post-anal papillae, and a pre-equatorial vulva. Mozgovoi (1950) studied the taxonomy of Ascaridata where the genus *Iheringascaris* was invalidated as a synonym.

Ascaris inquies was described by Linton (1901) from *Rachycentron canadum* (L.) and cobia (Rachycentridae). Later on, Rasheed (1965) redescribed the species and

*E-mail: anshu.malhotra@hotmail.com
†Present address: Department of Biochemistry and Cancer Biology, Meharry Medical College, Nashville, Tennessee, USA.
Nucleotide sequence data reported in this paper are available in the GenBank database under the accession numbers GQ265674, GQ265671, GQ265673 and FJ172978.

placed it under the genus *Thynnascaris* (Dollfus, 1933). The genus *Iheringascaris* was considered a junior synonym to *Thynnascaris* by Anderson *et al.* (1974), and later *I. inquies* (Linton) was accommodated as a new combination by Deardorff & Overstreet (1981) with *I. iheringascaris* as its synonym. These authors also proposed transfer of *Neogoezia elacateiae* (Khan & Begum, 1971) as a junior synonym of *I. inquies.* They also proposed a key to species of *Iheringascaris* to differentiate it from the species of *Hysterothylacium* Ward & Magath (1917) on the basis of deep transverse annulations in cuticle that were supposedly absent in the latter genus.

Although Akther *et al.* (2004) have reported *Goezia bangladeshi* from fish of the River Ganges in neighbouring Bangladesh, the first report of *Thynnascaris* was published by Kalyankar (1971) from the Arabian Sea at Goa, India. This, however, was later transferred to *Iheringascaris*, as *I. inquies.* The first report of these parasites from Indian fish was published as larvae of *I. inquies* by Kalyankar (1972) from the gills of sea-crabs in Ratnagiri. This identification was later questioned by Deardorff & Overstreet (1981). Bruce & Cannon (1989) were not convinced about the fragility of evidence for differentiation between *Iheringascaris* and *Hysterothylacium*. However, they upheld the revival of the former genus by Deardorff & Overstreet (1981) on the basis of the presence of distinct cuticular annulations. Their work also validated the presence of a bilateral excretory system in the former as against a unilateral excretory system in the latter, as the essential characters to separate the two genera from each other. Deardorff & Overstreet (1980) transferred several species of genus *Thynnascaris* to *Hysterothylacium* and retained the separate identity of the genus *Iheringascaris*.

Bruce & Cannon (1989) enumerated issues related to the nomenclature problems associated with ascaridoid nematodes due to inadequate descriptions or poorer state of specimens of ascaridoid nematodes studied by Yamaguti (1961) and Fujita (1940). Moravec *et al.* (1985) reported 11 species of *Contracaecum* to be synonymous with *H. aduncum*. Soota (1983) placed 11 species as *species inquirenda* and expressed reservations about the validity of an additional 29 species of ascaridoid roundworms. Bruce & Cannon (1990) later proposed a key to uphold the validity of genus *Iheringascaris* using the characters of clearly defined posterior margin of interlabia and a bilateral excretory system. The characteristics of the worms reported here, especially with respect to the details of the unilateral excretory system and marked cuticular striations on the body would, therefore, be critical to validate genera segregation in the taxonomy of ascaridoid nematodes.

A combination of morphological and molecular strategies has been advocated recently for accurate identification of nematodes (e.g. Paggi *et al.*, 2001; Shamsi *et al.*, 2009a, b). Several reports envision that the rRNA gene can be useful for taxonomic differentiation among helminth parasites. However, the conservation status of genes in the ribosomal region does not provide an impeccable conclusion as regards the exact identification of some helminth parasites. Hence, the need for more conserved genes has surfaced, and the mitochondrial genes are now being considered for deriving a consolidated phylogenetic structure of helminth parasites (Hebert *et al.*, 2003; Santamaria *et al.*, 2007).

The present study, aims at describing a new species of *Iheringascaris* morphometrically and its molecular characterization based on gene sequences of 18S ribosomal DNA and cytochrome *c* oxidase subunit I (coi) mitochondrial DNA.

Materials and methods

Collection and examination of fish for parasites

A total of 219 specimens of worms were collected from the silver whiting, *Sillago sihama* ($n = 255$), spotted catfish, *Arius maculatus* ($n = 246$), and whale shark, *Rhinchodon typus* ($n = 657$), at Dona Paula Beach, Goa off the Central West coast of India. Adult nematodes were washed thoroughly in physiological saline. A small piece of the mid-body of each individual nematode was removed with a scalpel and preserved in 75% ethanol for molecular analysis. The remainder of the roundworms were processed for morphological examination after Malhotra (1986). Some specimens were fixed in 70% ethanol for scanning electron microscopy (SEM).

Drawings were made with the aid of a drawing tube and measurements were made directly with an eyepiece micrometer. Measurements of roundworms were recorded in millimetres and expressed as range, followed by mean + SE in parentheses, unless otherwise stated. Photomicrographs were taken using Biovis Image Analysis software (http://www.motic.com). The SEM examination was conducted on specimens that were rehydrated and the head, tail and body parts post fixed in osmium tetroxide in 1 M sodium phosphate buffer (3 h), then passed through graded alcohols, followed by two steps of amyl acetate. SEM analysis was conducted at SIF-AIIMS, Department of Anatomy, All India Institute of Medical Sciences, New Delhi. Adult nematodes were identified to species based on the available keys and descriptions (Anderson *et al.*, 1974; Deardorff & Overstreet, 1981; Gibson, 1983; Sprent, 1983; Bruce & Cannon, 1989, 1990). Specimens have been deposited in collections of the Zoological Survey of India, Dehradun, India.

Molecular analyses

The total genomic DNA was isolated by using the standard phenol–chloroform procedure (Sambrook *et al.*, 1989), followed by an overnight digestion with proteinase-K at 37°C, alcohol precipitation and washing with 70% ethanol or the DNeasy Tissue kit (Qiagen Inc.; www.qiagen.com). The pellet of DNA was suspended with 50 µl of Tris-EDTA (TE) buffer. The concentration of isolated DNA was estimated using a UV spectrophotometer. The DNA was diluted to get a final concentration of 50 ng/µl.

The mtDNA cytochrome *c* oxidase subunit I (coi) gene was amplified in 50 µl volume with 2 µl (50 mM) $MgCl_2$, 0.25 µl (2.5 mM) of each deoxynucleotide triphosphate (dNTP), 0.5 µl (10 pM) of each primer, 0.6 U of *Taq* polymerase and 50 ng of genomic DNA. The primers used for the amplification of the *coi* gene were: forward primer LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′; reverse primer HCO2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer *et al.*, 1994). The thermal

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regime consisted of an initial denaturation at 94°C for 1 min, followed by 30 cycles at 94°C for 1 min, 48°C for 1 min, 72°C for 2 min, and a post-amplification extension for 7 min at 72°C.

The small subunit ribosomal RNA (18S rRNA) gene was also amplified in 50 µl volume with 5 µl of 10 × *Taq* polymerase buffer, 2 µl of $MgCl_2$ (25 mM), 0.25 µl of each dNTP (0.05 mM), 0.5 µl of each primer (0.01 mM), 0.6 U of *Taq* polymerase and 2 µl of genomic DNA. The primers used for the amplification of the 18S rRNA gene were: Nem18SF, 5′-CGCGAATRGCTCATTACAACAGC-3′; and Nem18SR, 5′-GGGCGGTATCTGATCGCC-3′ (Floyd *et al.*, 2005). The thermal regime consisted of an initial denaturation step of 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 54°C and 1 min at 72°C followed, in turn, by a final extension of 10 min at 72°C.

The polymerase chain reaction (PCR) products were run on 1.2% agarose gel and visualized by ethidium bromide staining. The purification of products in the range of 50–75 ng/µl was done using MiniElute PCR purification Kit (Qiagen Inc.). Then they were dissolved in distilled water and amplified using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, California, USA). The purification of products was done again using Centri-Sep spin columns (Princeton Separations, Adelphia, New Jersey, USA) and sequenced by ABI 3730 autosequencer (Applied Biosystems).

The DNA sequences were aligned for phylogenetic analysis using the Clustal W computer program (Thompson *et al.*, 1997) and DNA sequences were edited in BioEdit (Hall, 1999). The evolutionary distances were computed by Kimura's two parameter method (Kimura, 1980). The neighbour-joining tree (Saitou & Nei, 1987) was constructed using MEGA version 4.0 (Tamura *et al.*, 2007). The tree was evaluated using the bootstrap test (Felsenstein, 1985) based on 1000 replications.

The trees were outgroup-rooted using the nucleotide sequences from *Ascaridia* (GenBank accession no.: EF180058). Partial *coi* sequences of specimens in the present study were compared for homology with sequences present in the GenBank of the National Center for Biotechnology Information (NCBI) by using BLAST (Basic Local Alignment Search Tool) (Altschul *et al.*, 1990).

Results

A detailed examination of the worms collected predominantly from silver whiting, *S. sihama*, as well as from Indian sharks, *R. typus* and spotted catfish, *A. maculatus*, showed that they can be divided into two

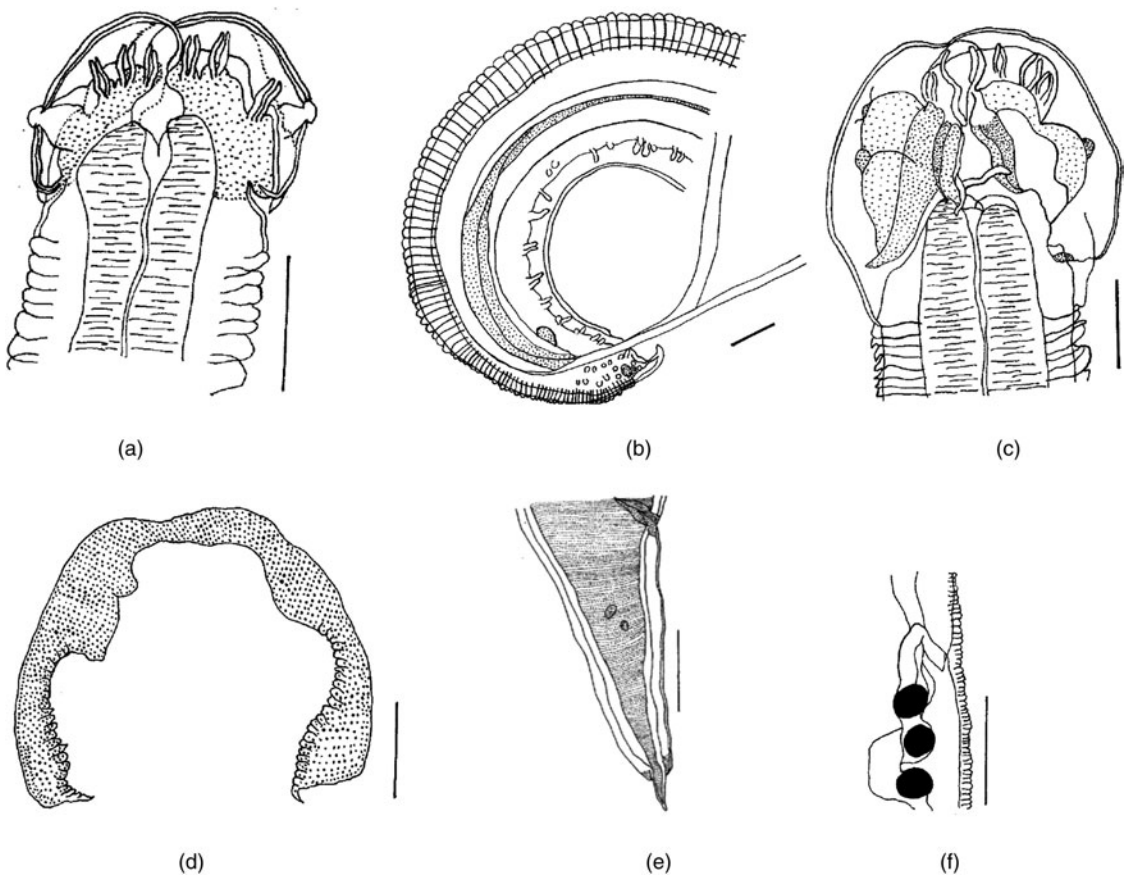

Fig. 1. *Iheringascaris goai* n. sp.: (a) male head; (b) male tail extremity with spicules; (c) female head; (d) interlabia; (e) tail extremity in a female; (f) female post-equatorial vulva. Scale bars: 0.05 mm (a, c, d), 0.1 mm (b, e, f).

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groups. Those from *R. typus* were identified as *I. inquies* ($n = 372$) and those from *S. sihama* and *A. maculatus* were placed into a new species named herein as *Iheringascaris goai* n. sp.

The genetic characterization of newly proposed species, *I. goai* n. sp. using 18S rRNA and *coi* sequence analyses validated morphological differentiation of characters of these nematodes from *I. inquies* that was collected from sharks and *A. maculatus* in the same area of study in India.

Morphology: Iheringascaris goai *n.sp*

Description. Worms medium-sized with stout body (male, fig. 1a and b). Body enclosed in a cuticular envelope with prominent cephalic flanges (fig. 1a and c). The head distinctly demarcated from the rest of the body. Head with three lips, each with marked indentations. The dorsal lip as well as the two subventral lips with a pair of cephalic papillae each. Interlabia present (fig. 1d). The alae run from immediately posterior to subventral lips to the tail (figs 1e, f, 2a and b). Excretory pore opens latero-ventrally (fig. 2c), located at almost one-third of the distance along the total length of the oesophagus, from anterior extremity. The dentigerous ridges on each of the lips on the head, distinctly marked on the inner edge (fig. 2d), and each lip has filamentous outgrowths distally (fig. 2e). Buccal cavity with a triangular opening, bearing a chitinized rim. Interlabia bifurcated at the base, not distally (fig. 3a), without deep interlabial grooves. The hind extremity of the oesophagus terminates into a smaller bulbar ventriculus. Nerve ring located at a distance of 0.18–0.20 (0.188 ± 0.004) from the anterior extremity. The intricate cuticularized valvular configuration is distinct at the junction of the oesophagus and intestine. The body reaches greatest width at about the anterior third of the body length. Cuticular striations are prominent (figs 3a and b), particularly in the anterior to mid region of the body, but annulations become inconspicuous in the hind region of the body (fig. 3c). Caudal alae narrower (fig. 3d). Rectal glands, two pairs pre-anal and one pair post-anal and smaller, present in both sexes of worms. Two long, sub-equal spicules, 15–17% of the length of body of worms. The wavy cuticular margins of folds on spicules were characteristically typical. The caudal papillae comprise a lateral row along with a medial post-anal row (fig. 3c) as they approach the extremity. The caudal end of the male is strongly curved ventrally, terminating in a bluntly pointed tip at the extremity, devoid of minute spines. Excretory vesicle is elongated pyriform, enveloped in a double-layered sac. Rectal glands oval.

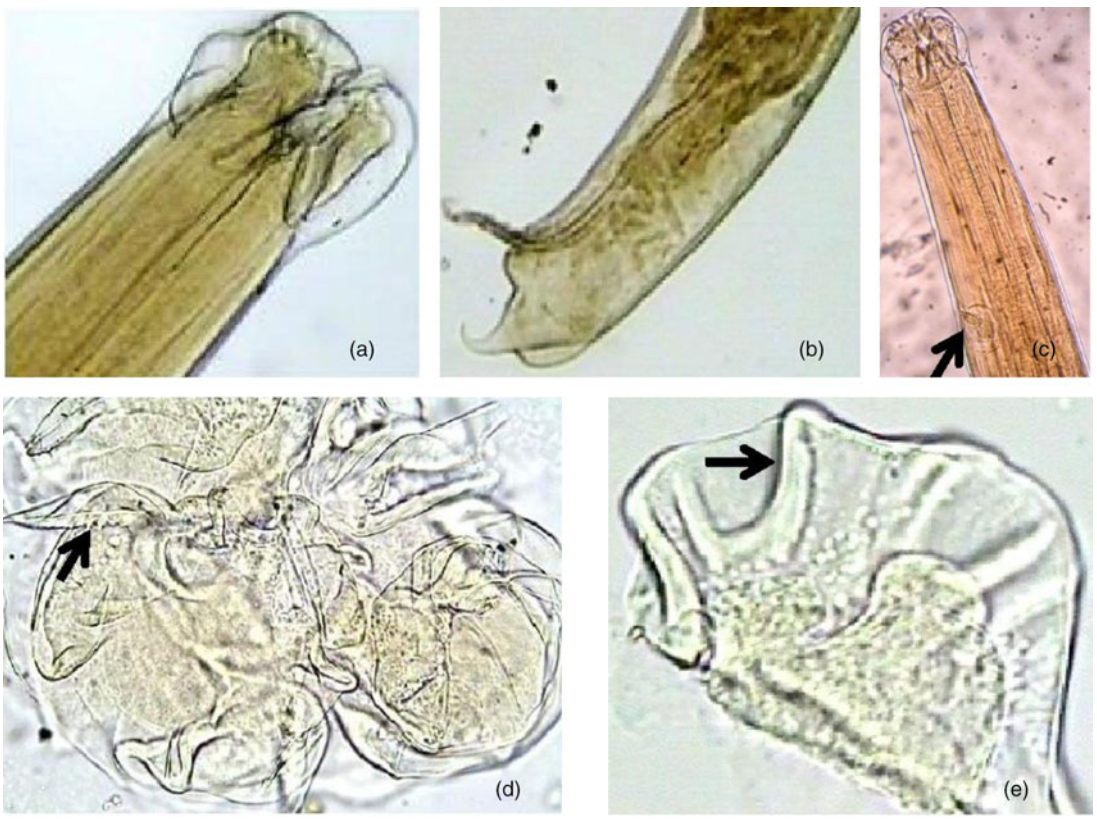

Fig. 2. *Iheringascaris goai* n. sp.: (a) male head (× 400); (b) posterior end of male showing curved tail extremity and spicule (× 100); (c) anterior end of female showing location of excretory vesicle (× 100); (d) transverse section of oral end of female showing dentigerous ridges on inner margin of lips; (e) transverse section of oral end of female showing filamentous indentations on lips. (See online at http://journals.cambridge.org/jhl for a colour version of this figure.)

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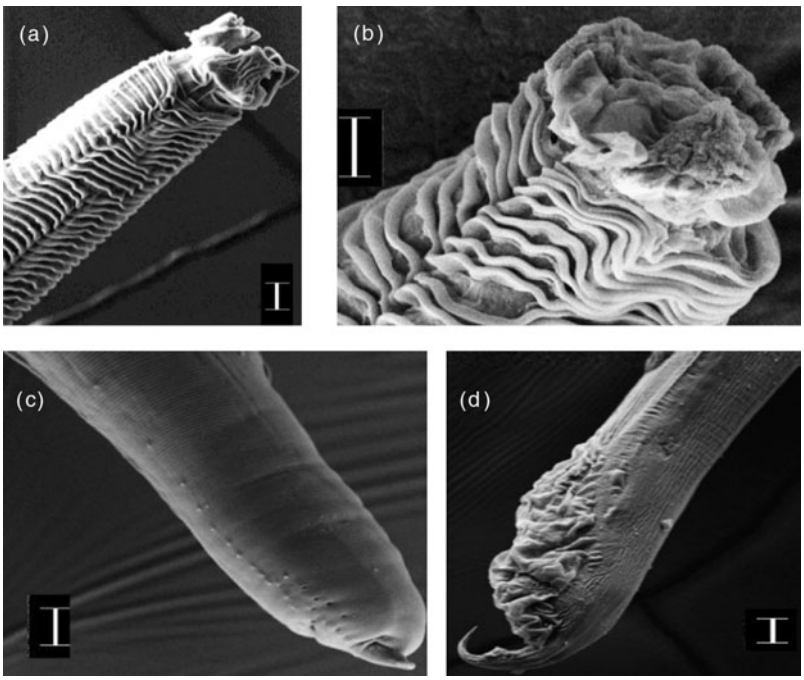

Fig. 3. Scanning electron micrographs of *Iheringascaris goai* n. sp.: (a) head with dorsal lip and ventral interlabium; (b) cephalic extremity with deep cuticular striations on anterior end; (c) tail extremity in a male with caudal papillae; (d) tail, lateral view with alae (scale bars: 10 μm).

Type. Holotype ZSI 1 ♂ ZSI/NRS/IV/N/392 and 1 ♀ ZSI/NRS/IV/N/393; paratypes AU 5 ♂ PNLS 116 and 5 ♀ worms- PNLS 117.

Type host. *Arius maculatus* (Siluroidea: Tachysuridae).

Other host. *Sillago sihama* (Percoidea: Sillaginidae).

Localization in host. Small intestine.

Type locality. Arabian Sea (coastal areas of Dona Paula beach), Goa, Central West coast of India.

Etymology. The new species has been named after its marine habitat in the coastal areas of Goa in Central West Coast of India.

Morphology: Iheringascaris inquies *(Linton, 1901)*

Specimens in this study were similar in morphology of taxonomically important features with those described by Bruce & Cannon (1989). The summary of measurements of specimens of *I. inquies* is presented in table 1.

Material examined. 6♂, 5♀ (ZSI/NRS/IV/N/394; ZSI/NRS/IV/N/395; PNLS118; PNLS119).

Host. *Rhincodon typus* (Orectolobidae, Laminiformes).

Localization in host. Small intestine.

Locality. Arabian Sea (coastal areas of Dona Paula beach), Goa, central west coast of India.

Gene sequence analyses

18S rRNA gene analysis

The read length of all 18S rDNA sequences was 864 bp, but a few insertions and deletions were also detected. The average K2P distance of individuals within *I. goai* n. sp. based on 18S gene sequence, was 0.0145, as compared with an average distance of 0.0117 and 0.0058 of the genera *Hysterothylacium* and *Ascaris*, respectively (fig. 4). On an average, therefore, there was 1.2 times more variation among the individuals of *I. goai* as compared to different species of *Hysterothylacium* and 2.5 times more variation as compared to different species of *Ascaris*. It may be noteworthy that there were 808 (93.5%) conserved domains and 17 parsimony informative sites within the 864 bp long 18S region of the same organism.

Analysis of the GC content

The overall GC content of *I. goai* n. sp., based on *coi* gene composition, was found to be 48% (fig. 5). This is consistent with the GC values of all other specimens taken into consideration for this study. Since 18S rDNA is a non-protein-coding gene, the GC content was of little significance in inferring the phylogenetics of this organism.

A neighbour-joining tree was generated using the Kimura 2 Parameter to calculate pairwise distances (fig. 4). The three specimens, GQ265674, GQ265673 and GQ265671 form a separate clade which does not show any significant similarity to any other organism listed in the GenBank. These may, therefore, be ascribed a separate entity, *I. goai* n. sp.

coi Gene analyses

The *coi* representative sequence of the three specimens studied using 18S rDNA gene (table 2) could be readily

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Table 1. Morphometric measurements of *I. goai* n. sp. and *I. inquies* in the present study.

Character	*Iheringascaris goai* n.sp.		*Iheringascaris inquies*	
	Males	Females	Males	Females
Body length	10.78–12.04 (11.86)	7.60–28.9 (20.4)	19.90–40.10 (30.00)	18.51–43.68 (31.00)
Body width	0.10–0.17 (0.15)	0.071–0.35 (0.23)	0.36–0.77 (0.51)	0.07–1.07 (0.35)
Head length	0.06–0.12 (0.11)	0.03–0.12 (0.11)	0.14–0.26 (0.195)	0.09–0.39 (0.24)
Head width	0.10–0.17 (0.15)	0.08–0.12 (0.115)	0.068–0.25 (0.15)	0.03–0.211 (0.118)
Buccal cavity	0.01–0.03 (0.02) × 0.017–0.41 (0.23)	0.03–0.06 (0.04) × 0.01–0.05 (0.03)	0.01–0.03 (0.02) × 0.01–0.03 (0.019)	0.022–0.028 (0.025) × 0.02–0.053 (0.03)
Indentation on lip	0.01–0.09 (0.05)	0.02–0.04 (0.03)	0.01–0.02 (0.013)	0.01–0.02 (0.013)
Oesophagus	1.8–2.6 (2.18)	0.35–4.93 (2.65)	0.50–4.50 (2.50)	0.63–5.439 (3.0)
Oesophagus/body length	14.0–28.0 (21.05)%	10.11–16.60 (13.45)%	2.51–11.22 (6.86)%	3.40–12.43 (7.91)%
Ventriculus	0.059–0.11 (0.073) × 0.07–0.125 (0.083)	0.038–0.045 (0.039) × 0.045–0.053 (0.05)	–	–
Ventricular appendage	0.48–0.89 (0.67)	0.40–0.49 (0.45)	–	–
Intestinal caecum	–	0.06–0.16 (0.14)	–	–
Ratio of ventricular appendage to caecal lengths	1:04	1:03	–	–
Ratio of oesophagus to ventricular appendage lengths	1:03	1:02	–	–
Ratio of oesophagus to caecal lengths	–	1:07	–	–
Ovary	–	2.41–2.46 (2.44)	–	–
Vagina	–	0.136–0.217 (0.169) × 0.059–0.102 (0.068)	–	0.11–0.19 (0.15) × 0.02–0.04 (0.03)
Distance of vulva from anterior end	–	5.06–17.75 (15.89)	–	10.24–15.60 (13.16)
Distance of vulva from posterior end	–	2.50–12.8 (8.26)	–	18.28–28.79 (25.45)
Eggs	–	0.03–0.05 (0.04)	–	0.06–0.09 (0.075)
Left spicule (length)	1.61–2.11 (1.84)	–	2.1–2.96 (2.5)	–
Left spicule (width)	0.01–0.03 (0.02)	–	1.70–2.30 (2.0)	–
Right spicule (length)	1.59–1.9 (1.72)	–	–	–
Right spicule (width)	0.01–0.02 (0.017)	–	–	–
Ratio of left spicules to body length	15.0–16.1 (15.90)%	–	7.38–10.55 (8.96)%	–
Ratio of right spicules to body length	14.0–15.2 (14.80)%	–	5.73–8.54 (7.13)%	–
Distance of anus from tail tip	0.08–0.12 (0.11)	0.15–0.24 (0.22)	0.019–0.127 (0.073)	0.25–0.45 (0.35)
Rows of spines on tail	Absent	Absent	6–8	8–9
Tail process	0.05–0.08 (0.068)	0.03–0.11 (0.065)	0.02–0.05 (0.03)	0.02–0.06 (0.04)
Pre-anal papillae	15–20 pairs	–	19–24 pairs	–
Post-anal papillae	8–11 pairs	–	5–8 pairs	–
Distance of excretory pore from anterior end	–	0.358–0.363 (0.361)	0.59–0.71 (0.67)	–

distinguished based on DNA barcoding since the results obtained by *coi* gene complemented those obtained by 18S rDNA gene sequence analyses (fig. 6). Exact matches between different sequences of *I. goai* n. sp. could not be made due to non-availability of *coi* sequences of this genus in GenBank. However, analysis based on comparison with other, related genera is presented here.

Discussion

Worms of the new species had distinct cuticular striations on the body, a unilateral excretory system with the pore opening near the surface of the body, and a post-equatorial vulva, suggesting thereby that these did not belong to *Hysterothylacium*. Alignment of the 18S and *coi* gene sequences showed the existence of two distinct genotypes (figs 4 and 6), with differences in the morphology of the two species, the newer one named here as *I. goai* n. sp. A morphological comparison of worms of the new species with those of *I. inquies* is also included (table 1).

The morphological comparison of the new species with specimens of *I. inquies* collected from similar hosts, viz. sharks, *R. typus* as well as *A. maculatus* from the same habitat, i.e. Arabian Sea at Goa, revealed significant variations. In the new species, males possessed a smaller body compared to *I. inquies,* shorter head, smaller oesophagus (14–28% of body length versus 11%), narrower intestine and a greater number of post-anal papillae. In general terms, worms of the new species were significantly smaller in size than *I. inquies*. In consonance, measurements of various organs were generally smaller in the new species than in *I. inquies.* But measurements

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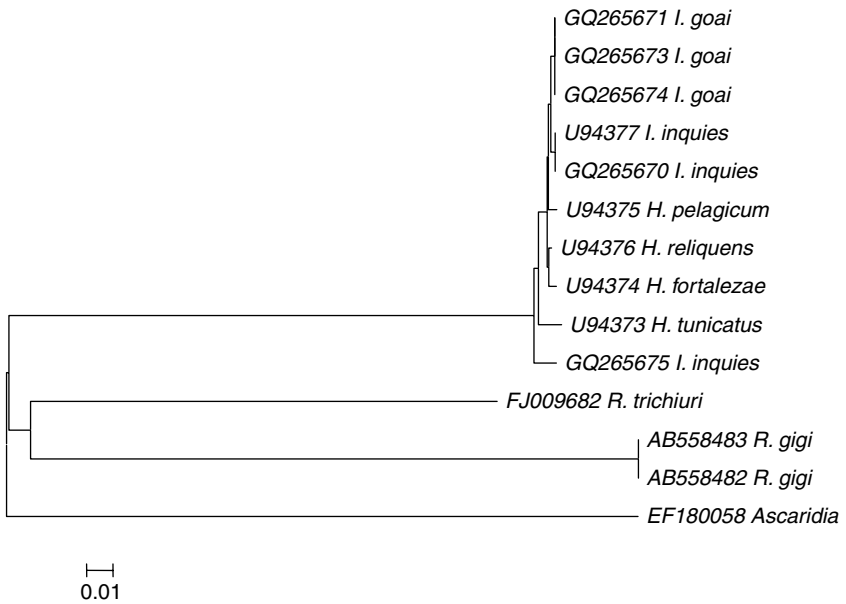

Fig. 4. Neighbour-joining tree based on nucleotide 18S rDNA sequences and reference species. Nucleotide 18S rDNA sequence data are as described in the text with GenBank accession numbers. Bootstrap values based on 1000 replicates were used (values not shown). Scale bar represents an interval of the Kimura two-parameter (K2P) model.

of a single, unusually larger male specimen showed a marked effect on the mean body size as well as length of spicules. One specimen measured 15.80 in body length and 0.22 in body width, with length of left spicule 4.67, and that of right spicule 4.16, their width being 0.04 and 0.03, respectively. The measurement of length of left and right spicule of all other worms studied were 1.59–1.9 (1.72) and 1.61–2.11 (1.84), respectively, if measurements of spicule length of the one unusually larger specimen were excluded. Variations in measurements of other organs of worms of the new species have been summarized in table 1.

In females, the body of the new species was also comparatively smaller than that in *I. inquires*, with a smaller head, wider buccal cavity, smaller oesophagus, post-equatorial vulva (versus pre-equatorial in *I. inquies*) and larger tail. The oesophagus was up to 17% of body length, compared to 12% in females of *I. goai* n. sp.

The new species could invariably be differentiated from the original description of type species (Pereira, 1935), *I. inquies* having indentations on lips as well as dentigerous ridges present on the inner margin of lips, excretory vesicle pyriform with a saccular component that opens in the anterior third part of the oesophagus distinctly behind the level of nerve ring, subequal spicules, post-equatorial vulva and the tail typically curved ventrad in male.

Worms of the new species further differed from the description given by Deardorff & Overstreet (1981) of specimens from Mississippi and Alabama, in diminishing striations on the body in the posterior part, excretory pore opening behind the level of nerve ring, larger oesophagus, greater ratio of spicules to body length, shorter but wider left and right spicules and lesser number of pre-anal papillae in male worms; and smaller body in female worms, possessing a larger oesophagus, a post-equatorial vulva and a longer tail. The ratio of caecal to ventricular appendage length was smaller in comparison to the ratio of caecal to oesophageal length, which was larger.

The variance in GC content among *I. goai* n. sp., based on their *coi* gene sequence, was much higher in the case of the second base (64.33%) as compared with the first and third base (0.27% and 6.54%, respectively). This could be attributed to the very low GC content at the second codon position of the specimen (fig. 5), largely due to the low GC_2 values recorded in the case of FJ172978 (*I. goai* n. sp.). Since more synonymous mutations are known to occur at position 3 and 1, the aberration obtained in the case of FJ172978 could play an important role in its delineation as an organism belonging to a separate distinct species.

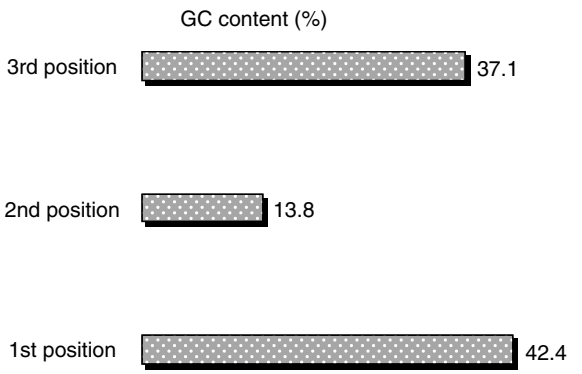

Fig. 5. Distribution of the GC content based on the codon position in the sequence.

https://doi.org/10.1017/S0022149X11000472 Published online by Cambridge University Press

Table 2. Representative groups of 18S and cytochrome *c* oxidase (coi) gene sequence selected for the study.

GenBank accession no.	Gene	Taxon	Reference
GQ265674	18S rDNA	*Iheringascaris goai* n. sp.	Present study
GQ265671	18S rDNA	*Iheringascaris goai* n. sp.	Present study
GQ265673	18S rDNA	*Iheringascaris goai* n. sp.	Present study
U94377	18S rDNA	*Iheringascaris inquies*	Nadler & Hudspeth (1998)
GQ265670	18S rDNA	*Iheringascaris inquies*	Present study
U94375	18S rDNA	*Hysterothylacium pelagicum*	Nadler & Hudspeth (1998)
U94376	18S rDNA	*Hysterothylacium reliquens*	Nadler & Hudspeth (1998)
U94374	18S rDNA	*Hysterothylacium fortalezae*	Nadler & Hudspeth (1998)
GQ265675	18S rDNA	*Iheringascaris inquies*	Present study
FJ009682	18S rDNA	*Raphidascaris trichiuri*	Damin & Heqing (2001)
AB558483	18S rDNA	*Raphidascaris gigi*	Abe (unpublished)
AB558482	18S rDNA	*Raphidascaris gig*	Abe (unpublished)
EF180058	18S rDNA	*Ascaridia gallii*	Nadler *et al.* (unpublished)
U94373	18S rDNA	*Hysterothylacium tunicatus*	Nadler & Hudspeth (1998)
AM411108	*coi*	*Toxocara canis*	Nadler *et al.* (2007)
EU730761	*coi*	*Toxocara canis*	Jex *et al.* (2008)
X54253	*coi*	*Ascaris suum*	Okimoto *et al.* (1990)
GU112207	*coi*	*Anisakis physeteris*	Liu (unpublished)
FJ172979	*coi*	*Iheringascaris goai*	Current authors (unpublished)
EU741046	*coi*	*Iheringascaris inquies*	Current authors (unpublished)
FJ907318	*coi*	*Raphidascaris trichiuri*	Liu (unpublished)
FJ172978	*coi*	*Strongylida cox1*	Current authors (unpublished)
U57030	*coi*	*Ancylostoma caninum*	Sukhdeo *et al.* (1997)
FJ483518	*coi*	*Ancylostoma caninum*	Jex *et al.* (2009)
FJ172979	*coi*	*Iheringascaris goai* n. sp.	Present study

As is evident, the representative specimen taken for *coi* gene analyses falls on a separate branch and shows no similarity with any other organism in GenBank (fig. 6). Similarity with other organisms cannot be substantiated at this point due to paucity of sequences of organisms of the family Raphidascarididae.

The read length of all *coi* sequences was 650 bp. No insertions, deletions or stop codons were detected in any of the sequences generated during the course of this study. The lack of stop codons in the sequence confirmed the observation that no pseudogenes or NUMTs (nuclear DNA sequences originating from mitochondrial sequences) were sequenced along with the desired gene sequence (Zhang & Hewitt, 1996). It may be noteworthy that there were 422 conserved domains and 171 parsimony informative sites within the 650 bp long *coi* region of *I. goai* n. sp.

The differences in morphology of the worms of both species were amply supported by 18S rDNA and *coi* gene sequence differentiation. Therefore, on the basis of the aforementioned significant points of morphological differences, besides simultaneously presented distinctive 18S rDNA and *coi* gene sequences, the new species of worms is proposed as *I. goai* n. sp.

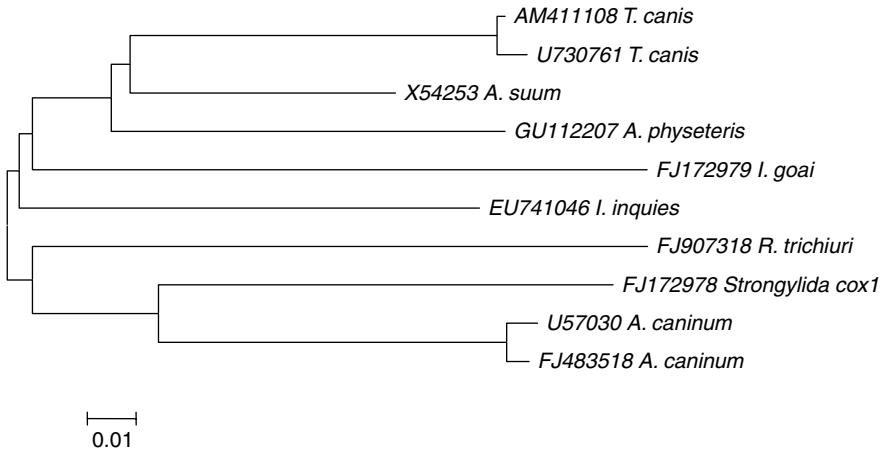

Fig. 6. Neighbour-joining tree based on nucleotide *coi* gene sequences and reference species. Nucleotide *coi* sequence data are as described in the text with GenBank accession numbers. Bootstrap values based on 1000 replicates were used (values not shown).

https://doi.org/10.1017/S0022149X11000472 Published online by Cambridge University Press

Acknowledgements

A.M. thanks Professor Ms I. Karunasagar for Research mentorship and Department of Biotechnology (DBT) for a Research Associateship. S.K.M. is thankful for the University Grants Commission, Govt of India research grant. N.J. is grateful for a fellowship under DBT research project no. BT/PR9651/SPD/09/818/2007, and to the Department of Science and Technology, Govt of India for a travel grant to visit Charles Sturt University, Australia. The authors would also like to thank Craig Farish at Charles Sturt University for preparing histological sections.

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