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Morphological and molecular analysis of *Spinitectus notopteri* (Nematoda: Cystidicolidae) from freshwater fish in Thailand

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Abstract: The nematode *Spinitectus notopteri* Karve et Naik, 1951 was collected from two species of freshwater fish belonging to the family Notopteridae, namely *Chitala ornata* (Gray) and *Notopterus notopterus* (Pallas), at Khun Thale Swamp in Surat Thani province of southern Thailand. The overall prevalence of the parasite was found to be 88% (94 fish infected/106 fish examined). A higher prevalence was found in *C. ornata* (96%), while a higher intensity was noted for *N. notopterus* (117 parasites/fish). The most important morphological characters were the presence of cephalic and cuticular structures, precloacal ridges, and genital papillae. Specimens were genetically characterised using cox1 mtDNA. Morphological characteristics were most similar to *Spinitectus petterae* Boomker, 1993 from *Clarias gariepinus* (Burchell) in Africa, while the genetic data were dissimilar to all available data for the genus. Therefore, the identification of nematode specimens using a combination of morphological and molecular techniques is stressed. This study also presents the first molecular analysis of *S. notopteri* infecting Thai freshwater fish and a new geographical record for *S. notopteri*.

Keywords: Nematode, *Chitala ornata*, *Notopterus notopterus*, morphology, molecular techniques, cytochrome oxidase subunit 1 (*cox*1)

Fish diseases caused by parasites are important, as it is known that parasites may cause considerable damage to their hosts. Parasite infections may be caused by protozoans, helminths or arthropods, of which the helminths are dominant among fish parasites. Three groups of helminths are parasitic in fishes: Platyhelminthes, Acanthocephala and Nematoda. In particular, nematode infections can be an economic threat to the market value and health of fishes in natural habitats.

The nematode genus *Spinitectus* Fourment, 1884 (Nematoda: Cystidicolidae) contains numerous species described mainly from the gastrointestinal tract of freshwater and marine fish species worldwide (Moravec et al. 2002, 2004, 2023, Barrios-Gutiérrez et al. 2019, Austin et al. 2023). In Thailand, two species of *Spinitectus* have been reported in freshwater fish, namely *S. thaiensis* Moravec et Yooyen, 2011 and *Spinitectus notopteri* Karve et Naik, 1951 (Moravec and Yooyen 2011, Moravec et al. 2016).

However, specifically unidentified *Spinitectus* spp. have been reported in many species of freshwater fish in Thailand such as *Channa striata* (Bloch), *Discherodontus ashmeadi* (Fowler), *Hemibagrus nemurus* (Valenciennes), *Notopterus notopterus* (Pallas), *Schistura buccalentus* (Smith), *S. breviceps* (Smith), *S. poculi* (Smith) and *Systomus orphoides* (Valenciennes) (Wongsawad et al. 2004, Purivirojkul and Areechon 2008, Sriwongpuk 2013, Phalee 2018, Juntaban et al. 2021). Moreover, *Spinitectus* sp. has been reported from *N. notopterus* in Pakistan (Sethar et al. 2020).

The identification to the species level of these worms is difficult because the descriptions of most species are based solely on light microscopy (LM). The body surface, cephalic and cuticular structures, precloacal ridges and genital papillae or phasmids have so far been observed by scanning electron microscopy (SEM) only in several species. In addition, many species of these worms are closely similar in appearance.

Even though molecular analysis of this species has been reported in India by using the ribosomal 18S rRNA gene, the current study uses cox1 mtDNA for characterisation (Kashyap et al. 2024). Interestingly, no *Spinitectus* specimens have been previously reported from *Chitala ornata* (Gray). Therefore, the present study aimed to identify and describe the collected specimens of *S. notopteri* using both morphological and molecular analysis to confirm species identity of nematode species in freshwater fish belonging to the family Notopteridae.

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MATERIALS AND METHODS

Parasite examination

Two species of fish belonging to the family Notopteridae, namely *Notopterus notopterus* and *Chitala ornata*, were collected by a local fisherman at Khun Thale Swamp, Surat Thani province, southern Thailand. These fish were caught using a gill net from January to December 2020. They were then transported to the laboratory in an icebox. The fish were measured for both their weight and total length. The digestive tract, including the stomach and intestine were dissected and placed in separate Petri dishes containing 0.85% normal saline and then examined under a stereo microscope.

The collected nematodes were fixed in 4% formaldehyde solution, with some additionally preserved in 70% alcohol. In addition, some nematodes (in 70% alcohol) were obtained from *N. notopterus* at the Mekong River, Nakhon Phanom province, northeastern Thailand. For identification, the worms were cleared in glycerine and compared to the descriptions provided by Yamaguti (1961) and Moravec et al. (2016). Drawings were made with the aid of a UDA-60 drawing attachment (Japan).

Parasite infections were recorded and the infection rate was calculated according to Margolis et al. (1982). All measurements are given in micrometres unless otherwise stated. Scientific names of fish hosts follow FishBase (Froese and Pauly 2024). The voucher specimens were deposited at the Helminthological Collection of the Institute of Parasitology (IPCAS), Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (Cat. No. N_1095).

Scanning electron microscopy observations

The specimens were washed three times in 0.1 M phosphate buffer at pH 7.2, then fixed with 2.5% glutaraldehyde at 4 °C for 24 h and post-fixed in 1% osmium tetroxide (OsO_4) for 2 h. The worms were dehydrated with a graded alcohol series and dried in a critical point dryer. Specimens were sputter coated with gold at 10 milliamperes for 3 minutes. Micrographs were taken using a SEM-Quanta (FEI Quanta 250) scanning electron microscope at an acceleration voltage of 15 kV.

Molecular analysis

Specimens for DNA sequencing were kept in 100% ethanol. DNA extraction was done on individual nematodes using the Genomic DNA Mini Kit (Tissue) (GF-1 kit, Vivantis Technologies, Selangor Darul Ehsan, Malaysia). Usually, 25 mg of each sample material were lysed with 20 µl of Proteinase K and 200 µl of lysis buffer solution in a heating block at 60 °C for half an hour. Then, after lysis, 200 µl of lysis buffer was added and incubation continued at 70 °C for 10 minutes. The mixture was eluted with 50 µl of elution buffer. The concentration was determined by nanodrop and adjusted by dilution to 20 ng/µl.

A partial fragment of the cytochrome c oxidase subunit 1 (cox 1) gene was amplified with the forward primer (507) 5′-AGTTCTAATCATAA(R) GATAT(Y)GG and the reverse primer (HCO2198) 5′- TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al. 1994, Nadler et al. 2006, Barrios-Gutiérrez et al. 2019). The reaction mixture contained 10–20 ng of extracted DNA, 10 pmol of each primer, 1× PCR reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 25 mM MgCl2, 10 mM dNTPs, and 5 units/µl of Taq DNA polymerase (Finnzymes, Espoo, Finland).

Amplification of DNA was done with a Perkin-Elmer (Gene Amp PCR system 9700). Thermocycling conditions included denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 0.5 min, annealing at 52 °C for 0.5 min, and extension at 72 °C for 0.5 min, followed by a post-amplification incubation at 72 °C for 7 min. PCR products were separated by gel electrophoresis. DNA bands were stained with SYBR Safe and photographed using a Gel Documentation machine (GEL DOC 1000, Hongkong), and the PCR products were subjected to DNA sequencing. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer.

Sequences generated in this study were compared to sequences in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank) using the BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi.). The sequences were submitted to GenBank, and phylogenetic and molecular evolutionary analyses were conducted using MEGA version 11 (Tamura et al. 2021). The evolutionary history was inferred by using the Maximum Likelihood method and the Jukes-Cantor model (Juke and Cantor 1969). The bootstrap consensus tree inferred from 1,000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the taxa analysed.

RESULTS

Parasite infection

Spinitectus notopteri from *Chitala ornata* and *Notopterus notopterus* (Notopteridae) was studied. A total of 106 fish were examined for parasites in the stomach. The infection rate showed prevalences of 96% and 85% with mean intensities of 68 and 117 parasites/fish, respectively (Table 1).

Morphological description of *S. notopteri* from Thailand (Figs. 1, 2) (based on 20 specimens from *C. ornata* and 20 specimens from *N. notopterus*)

General description. Body elongated; cephalic end blunt, posterior end conical. Body surface covered with transverse non-interrupted rings spines (Fig. 1A,B,E,F). First ring with 20–28 spines (Fig. 1C). Specimens from *N. notopterus* with 20–26 spines (n = 10; Surat Thani = 20–26; Nakhon Phanom = 20–25), specimens from *C. ornata* with 21–28 spines (n = 10).

Male (Table 2). Male body covered with spines, visible at anterior part of intestinal region. Length of body 2.93–3.87 mm in specimens from *C. ornata* and 2.45–4.45 mm in specimens from *N. notopterus*; maximum width 100–

Table 1. Prevalence and mean intensity of infection with *Spinitectus notopteri* Karve et Naik, 1951 in freshwater fish species from Khun Thale swamp, Surat Thani, Thailand.

Fish species	Infected fish examined fish	Prevalence (%)	Mean and range of intensity (parasites/fish)
Chitala ornata (Gray)	25/26	96	68 (6–428)
Notopterus notopterus (Pallas)	68/80	85	117 (9–459)
Total	94/106	88	-

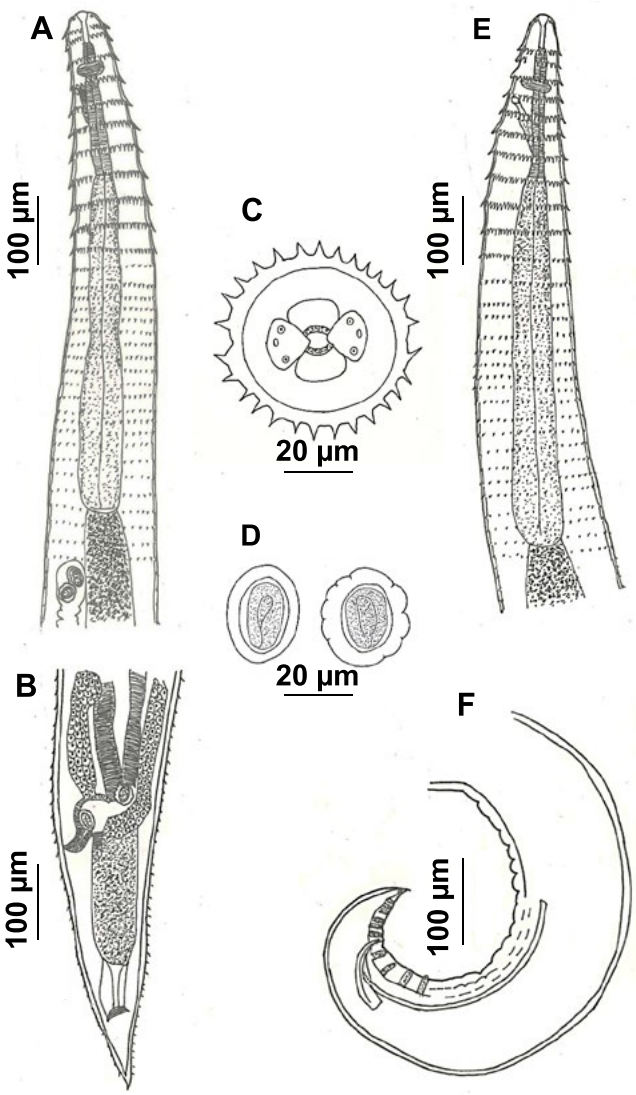

Fig. 1. *Spinitectus notopteri* Karve et Naik, 1951 from *Chitala ornata* (Gray). Light microscopy. **A** – anterior part of female; **B** – posterior part of female; **C** – cephalic end, apical view; **D** – mature eggs; **E** – anterior part of male; **F** – posterior part of male.

170 in nematodes from *C. ornata* and 100–160 in *N. notopterus*. Preanal papillae: four pairs of subventral pedunculate papillae. Postanal papillae: five pairs of subventral papillae and one pair of ventral papillae at level of last pair of subventrals. A pair of small phasmids situated posterior to genital papillae (Figs. 1E,F, 2A,D).

Female (Table 2). Female body covered with spines up to posterior extremity (Figs. 1A,C, 2E,F). Length of body 3.30–5.84 mm in specimens from *C. ornata* and 3.48–6.25 mm in specimens from *N. notopterus*; maximum width 150–290 in nematodes from *C. ornata* and 150–290 in nematodes from *N. notopterus*. Eggs oval to almost spherical, thick-walled, smooth or mostly with somewhat wrinkled sides, embryonated; eggs 22.5–30 long × 25–32.5 wide (n = 30) (Fig. 1D). Tail covered with minute cuticular spines, with small terminal spike (Figs. 1B, 2F).

Hosts in Thailand: Clown featherback *Chitala ornata* (Gray) (Notopteridae, Osteoglossiformes); body length 25–49 cm (mean 35 cm), weight 119–900 g (mean 438 g) in Khun Thale Swamp. Bronze featherback *Notopterus notopterus* (Pallas) (Notopteridae, Osteoglossiformes); body length 21–35 cm (mean 25 cm), weight 76–250 g (mean 139 g) in Khun Thale Swamp.

Prevalence and mean intensity: 96% in *N. notopteri* (25 fish infected/ 26 fish examined); 68 (6–428) parasites/fish; 20 specimens measured. 85% in *C. ornata* (68 fish infected/ 80 fish examined); 117 (9–459) parasites/fish; 20 specimens measured.

Site of infection: Stomach.

Locality: Khun Thale Swamp, Surat Thani province, southern Thailand.

Genetic characterisation

Partial sequences of the cytochrome *c* oxidase subunit 1 (*cox*1) gene, 623 bp long, were obtained from one specimen of *S. notopteri* from *N. notopterus* (GenBank accession number OR984421) and one specimen from *C. ornata* in Surat Thani (southern Thailand) (OR984420), and one specimen from *N. notopterus* in Nakhon Phanom (northeastern Thailand) (PP838724).

Table 2. Morphological characteristics of *Spinitectus notopteri* Karve et Naik, 1951 from freshwater fish in Thailand.

Characteristic	Host: *Notopterus notopterus* (SR*)		Host: *Notopterus notopterus* (NP*)		Host: *Chitala ornata*	
	Male (n = 10)	Female (n = 10)	Male (n = 10)	Female (n = 10)	Male (n = 10)	Female (n = 10)
Body length (mm)	2.45–3.75	3.48–5.55	2.85–4.45	5.13–6.25	2.93–3.87	3.30–5.84
Body width	100–160	150–250	100–160	200–290	100–170	150–290
Vestibule + prostom	40–45	40–50	37.5–50	40–55	37.5–50	37.5–50
Prostom length	9–12.5	10–12.5	10–12.5	10–17.5	10–12.5	10–12.5
Prostom width	12.5–15	12.5–15	10–12.5	10–15	10–12.5	12.5–17.5
Muscular oesophagus length	130–170	150–260	135–195	140–230	110–187.5	160–223
Muscular oesophagus width	15–20	20–30	17.5–22.5	22.5–27.5	15–20	20–22.5
Glandular oesophagus length	330–580	490–620	385–530	545–705	480–530	420–640
Glandular oesophagus width	40–75	50–80	58–80	63–85	50–75	50–80
Nerve ring from anterior end	63–95	62.5–100	60–90	68–95	60–97.5	88–113
Excretory pore from anterior end	88–125	112.5–160	80–130	95–125	90–137.5	130–150
Large spicule length	288–370	-	313–350	-	300–395	-
Small spicule length	80–95	-	80–90	-	75–92.5	-
Vulva from anterior end (mm)	-	3.06–3.82	-	4.33–560	-	3.00–5.40
Eggs (n = 30)	-	22.5–27.5 × 25–32.5	-	22.3–30 × 27.5 –32.5	-	22.5–27.5 × 25–32.5

in µm except body length and distance of vulva; * SR – Surat Thani; *NP – Nakhon Phanom

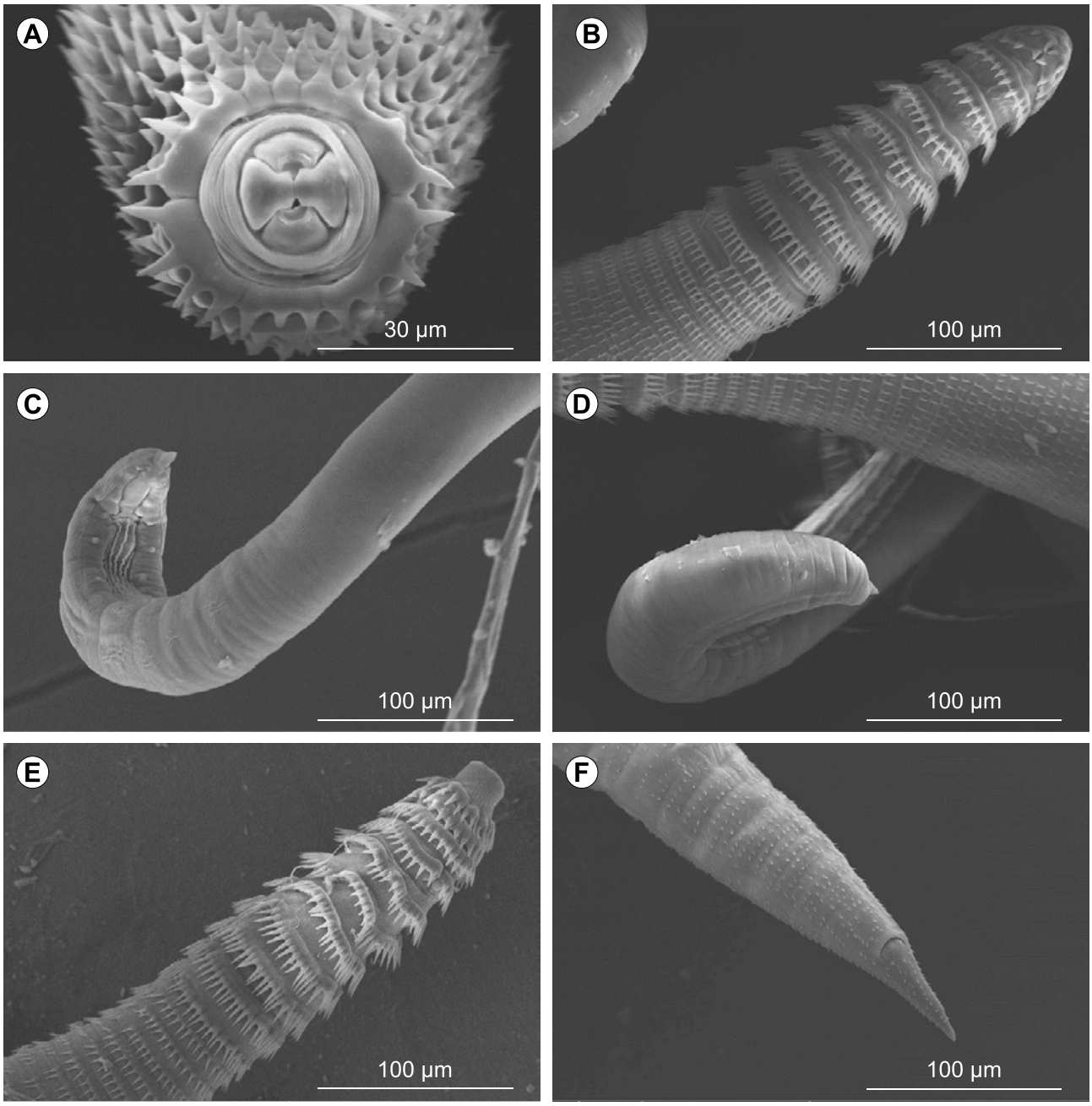

Fig. 2. *Spinitectus notopteri* Karve et Naik, 1951 from *Chitala ornata* (Gray). Scanning electron microscopical observations. **A** – mouth part of male; **B** – anterior part of male; **C** – posterior part of male with genital papillae; **D** – posterior part of male with ventral precloacal ridges; **E** – anterior part of female; **F** – posterior part of female.

A phylogenetic analysis of the *cox*1 dataset (381 bp long) yielded trees with similar topologies among the analysed species, all with well-supported nodes. The maximum likelihood (ML) analysis showed *S. notopteri* from Surat Thani to be reciprocally monophyletic in all analyses, with strong nodal support from bootstrap (100). *Spinitectus notopteri* from Nakhon Phanom also showed strong nodal support (97). The sequence divergences between *S. notopteri* from *N. notopterus* and *C. ornata* from Surat Thani was 0.79%, while divergence between Surat Thani and Nakhon Phanom samples was 7.15–8.00%. Haplotypes of *Spinitectus petterae* formed a clade with high bootstrap support (84). Haplotypes of *Spinitectus humbertoi* Caspeta-Mandujano et Moravec, 2000 formed a clade with high bootstrap support (98). Genetic divergences among *S. notopteri*, *S. humbertoi*, *S. petterae*, *Spinitectus osorioi* Choudhury et Pérez-Ponce de León, 2001 and Nematoda gen. sp. were 12.9–14.1%, 13.8–17.7 %, 14.7–17.3% and 12.2–14.4%, respectively (Fig. 3).

DISCUSSION

This study revealed the occurrence of the nematode *Spinitectus notopteri* in two species of freshwater fish of the family Notopteridae, namely *Notopterus notopterus* and *Chitala ornata*. The present study reports the first discovery of *S. notopteri* in *C. ornata*. *Spinitectus notopteri* was

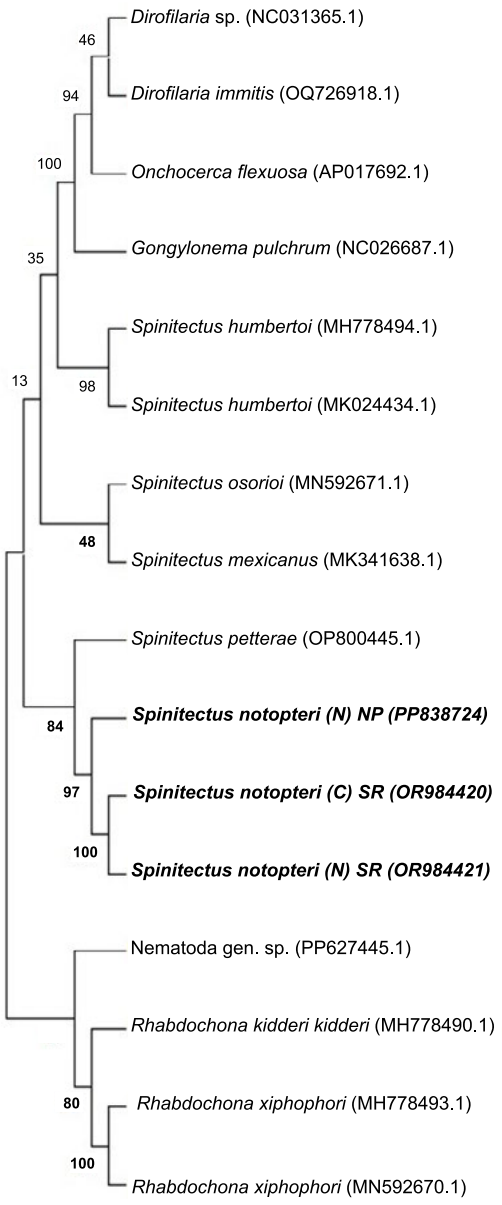

Fig. 3. Evolutionary analysis of *Spinitectus notopteri* by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and Jukes-Cantor model. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Jukes-Cantor model, and then selecting the topology with superior log likelihood value. This analysis involved 16 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + noncoding. There were a total of 381 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

previously found in freshwater fish from India and Thailand (Moravec et al. 2016, Kashyap et al. 2024). In Thailand, *S. notopteri* was already reported in *N. notopterus* by

Table 3. Reference for constructing phylogenetic analysis.

GenBank Accession	Parasite species
MH778494.1	*Spinitectus humbertoi* Mandujano-Caspeta et Moravec, 2000
MK024434.1	*Spinitectus humbertoi* Mandujano-Caspeta et Moravec, 2000
MH778493.1	*Rhabdochona xiphophori* Caspeta-Mandujano, Moravec et Salgado-Maldonado, 2001
OP800445.1	*Spinitectus petterae* Boomker, 1993
MN592671.1	*Spinitectus osorioi* Choudhury et Pérez-Ponce de León, 2001
NC_031365.1	*Dirofilaria* sp.
MH778490.1	*Rhabdochona kidderi* Pearse, 1936
MN592670.1	*Rhabdochona xiphophori* Caspeta-Mandujano, Moravec et Salgado- Maldonado, 2001
MK341638.1	*Spinitectus mexicanus* Caspeta-Mandujano, Moravec et Salgado-Maldonado, 2000
AP017692.1	*Onchocerca flexuosa* Abd-Ellatieff, Bazh, Hussin, Yamamoto,Yanai et AbouRawash, 2022
OQ726918.1	*Dirofilaria immitis* Leidy, 1856
NC_026687.1	*Gongylonema pulchrum* Molin, 1857
OR984420	*Spinitectus notopteri* (C*) SR*
OR984421	*Spinitectus notopteri* (N*) SR*
PP838724	*Spinitectus notopteri* (N*) NP*
PP627445.1	Nematoda gen. sp. BOLD:AFB1067

*C – Host: *Chitala ornata*; *N – Host: *Notopterus notopterus*; *NP – Nakhon Phanom; *SR – Surat Thani

Moravec et al. (2016). Nevertheless, this study conducted a comparative analysis of samples from various hosts to verify the species.

The number and arrangement of cuticular spines on the body are generally considered to be important features for the identification of *Spinitectus* spp., but the number of spines per ring may show some intraspecies variability (Moravec 1996). The number of spines in the first ring in *S. notopteri* was reported to be 20–23 by Moravec et al. (2016) and Kashyap et al. (2024). In our study, specimens had 21–28 (in *C. ornata*) and 20–26 spines (in *N. notopterus*) in the first ring, which extends the range of intraspecific variation in this species.

Spinitectus notopteri occurred in *C. ornata* and *N. notopterus* from Khun Thale Swamp, southern Thailand, with high prevalence (88–96%), which suggests that both fish serve as suitable definitive hosts of this nematode. It is probable that *Spinitectus* sp. collected from *N. notopterus* in northeastern Thailand by Sriwongpuk (2013) belong to *S. notopteri* but voucher specimens were not studied by the present authors to confirm identification.

Two unnamed species of *Spinitectus* were reported from *N. notopterus* in Pakistan. *Spinitectus* sp. I has many more spines in the first ring (58–60) and almost certainly represents another species. In contrast, *Spinitectus* sp. II has the number of spines (20–23) identical with *S. notopteri* as characterised by Moravec et al. (2016) and Kashyap et al. (2024) and is probably conspecific. Moravec et al. (2016) synonymised four different species of parasites, *Spinitectus alii* Kalyankar, 1970, *S. bengalensis* Chakravarty, Sain et Majumdar, 1961, *S. gomalensis* Siddiqui et Khattak, 1984, and *S. thapari* Ali, 1957, all found in *N. notopterus,* as one and the same species, with *S. notopteri*. However, this synonymy was based only on morphological characteristics in the original descriptions, without a study of type specimens or genetic comparison of individual taxa. Molecular data

are necessary to more reliably confirm validity of individual species of *Spinitectus* described from freshwater fish in India and South-East Asia.

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Author contributions. KK: sampling, data collection, analysis, drafting and revision of the manuscript. WH: molecular analysis, drafting, revision of the manuscript and submitting to GenBank. PP: DNA extraction and analysis. AP: sampling and analysis.

REFERENCES

Austin L., Santos Q.M.D., Avenant-Oldewage A. 2023: Additional data on *Spinitectus petterae* (Nematoda: Rhabditida) from *Clarias gariepinus* (Siluriformes: Clariidae) in the Vaal River system: conserved morphology or high intraspecific genetic variability? Folia Parasitol. 70: 002.

Barrios-Gutiérrez J.J., Santacruz A., Martínez-Ramírez E., Rubio-Godoy M., Pinacho-Pinacho C.D. 2019: *Spinitectus mixtecoensis* sp. nov. (Nematoda: Cystidicolidae), from the Oaxaca killifish *Profundulus punctatus* (Osteichthyes: Profundulidae) from Mexico, with comments on the distribution of *Spinitectus humbertoi* in the genera *Profundulus* and *Tlaloc*. Rev. Mex. Biodivers. 90: e902684.

Felsenstein J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

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. Mar. Biol. Biotechnol. 3: 294–299.

Froese R., Pauly D. (Eds.) 2024: FishBase. World Wide Web electronic publication, www.fishbase.org.

Jukes T.H., Cantor C.R. 1969: Evolution of protein molecules. In: H.N. Munro (Ed.), Mammalian Protein Metabolism, Academic Press, New York, pp. 21–132.

Juntaban J., Prisingkorn W., Wongmaneeprateep S., Wiriyapattanasub P. 2021: A survey of parasites in freshwater fishes from Nong Han wetland, Udon Thani Province, Thailand. Parasitol. Res. 120: 3693–3708.

Kashyap D., Baliyan R., Panwar A., Kumar Y., Sharma B., Singh H.S., Chaudhary A. 2024. Occurrence and first molecular characterization of *Spinitectus notopteri* Karve et Naik, 1951, infected bronze featherback (*Notopterus notopterus*) in India. Acta Parasitol. 69: 1067–1072.

Margolis L., Esch G.W., Holmes J.C., Kuris A.M., Schad G.A. 1982: The use of ecological terms in parasitology (Report of an *ad hoc* Committee of the American Society of Parasitologists). J. Parasitol. 68: 131–133.

Moravec F. 1996: Some new data on the morphology of *Spinitectus inermis* (Nematoda: Cystidicolidae), a rare parasite of eels, as revealed by SEM. Parasite 3: 169–174.

Moravec F., García-Magaña L., Salgado-Maldonado G. 2002: *Spinitectus tabascoensis* sp. nov. (Nematoda: Cystidicolidae) from *Ictalurus furcatus* (Pisces) in southeastern Mexico. Acta Parasitol. 47: 224–227.

Moravec F., Huffman D.G., de Buron I., González-Solís D. 2023: Redescriptions of *Spinitectus acipenseri* and *S. micracanthus* (Nematoda, Cystidicolidae), with notes on the taxonomy of *Spinitectus*-like nematodes parasitising North American fishes. Parasite 30: 33.

Moravec F., Nie P., Wang G. 2004: New data on the morphology and systematic status of *Spinitectus petrowi* and *Spinitectus gigi* (Nematoda: Cystidicolidae) parasitic in catfishes in central China. Folia Parasitol. 51: 346–358.

Moravec F., Pachanawan A., Kamchoo K. 2016: Redescription of two species of cystidicolid nematodes (Spirurina: Cystidicolidae) from *Notopterus notopterus* (Osteichthyes) in Thailand. Acta Parasitol. 61: 278–280.

Moravec F., Yooyen T. 2011: Observations on two nematode species parasitizing freshwater fishes in Thailand, including *Spinitectus thaiensis* sp. nov. (Cystidicolidae) from *Pseudomystus siamensis* (Bagridae). Acta Parasitol. 56: 58–66.

Nadler S.A., Bolotin E., Stock S.P. 2006: Phylogenetic relationships of *Steinernema* Travassos, 1927 (Nematoda: Cephalobina: Steinernematidae) based on nuclear, mitochondrial and morphological data. Syst. Parasitol. 63: 161–181.

Phalee A. 2018: [Prevalence of helminth infection in fishes from Nong Yat Reservoir, Mueang District.] Nakhon Phanom Province. Burapha Science Journal. 23: 1757–1769. (In Thai.)

Purivirojkul W., Areechon N. 2008: Parasitic diversity of siluriform fishes in Mekong River, Chiang Rai Province. Kasetsart J. (Nat. Sci.). 42: 34–39.

Sethar R., Birmani N.A., Abro M.M. 2020: New record of *Spinitectus* (Nematoda: Cystidicolidae) from *Notopterus notopterus* (Osteoglossiformes: Notopteridae) in River Indus, Sindh, Pakistan. Pakistan J. Parasitol. 70: 11–16.

Sriwongpuk S. 2013: [Parasites of knife fish (*Notopterus notopterus*, Pallas) from the Huajarake Mark Reservior in Buriram province.] Khon Kaen Agr. J. 41 (Suppl. 1): 446–452. (In Thai.)

Tamura K., Stecher G., Kumar S. 2021: MEGA 11: Molecular Evolutionary Genetics Analysis version 11. Mol. Biol. Evol. 38: 3022–3027.

Wongsawad C., Rojtinnakorn J., Wongsawad P., Rojanapaibul A., Marayong T., Suwattanacoupt S., Sirikanchana P., Sey O., Jadhav B.V. 2004: Helminths of vertebrates in Maesa Stream, Chiang Mai, Thailand. Southeast Asian J. Trop. Med. Publ. Health 35 (Suppl. 1): 140–146.

Yamaguti S. 1961: The Nematodes of Vertebrates. Part I, II. Systema Helminthum III. Interscience Publishers, New York, London, 679 pp.

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