International Journal for Parasitology: Parasites and Wildlife 17 (2022) 127–132

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Genetic characterisation of *Tanqua* (von Linstow, 1879) (Nematoda: Gnathostomatidae) larval forms including new host and locality records

Michelle Williams [a,*], Marta Hernandez-Jover [a], Md Shafaet Hossen [a,b], Shokoofeh Shamsi [a]

[a] School of Agriculture, Environmental and Veterinary Sciences & Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, NSW, 2678, Australia

[b] Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh

ARTICLE INFO

Keywords:
Channa punctata
Tanqua species
Bangladesh

ABSTRACT

In an unrelated study of spotted snakehead fish *Channa punctata* (Bloch) of family Channidae (N = 103) from Bangladesh, ten fish had taupe and clear coloured cysts attached to the intestinal mesentery. Investigation of the cysts revealed larval nematodes. The larvae were damaged and not suitable for detailed morphological study, however, key features such as tooth like projections of the pseudolabia and lateral pseudolabium were observed in specimens with undamaged cephalic regions. Molecular characterisation was undertaken and although the parasite genetic material was poor, five of the twelve nematode larvae through sequencing of the 18S ribosomal RNA gene, showed 98.17% match with sequences assigned for *Tanqua tiara* (accession number JF934728) deposited in GenBank. The prevalence of infection was 9.7% and the mean intensity 2.70. *Tanqua* has not previously been identified in fish, or from the definitive host, the Asian water monitor *Varanus salvator* (Laurenti, 1768) of family Varanidae (class Reptilia), in Bangladesh. Therefore, this study represents a new host and locality record for this nematode species. In many previous reports from this region, nematode larvae have been identified morphologically and assigned to a diverse range of nematode genera. Some confusion therefore exists regarding their accuracy and further investigations are required using molecular methodology to clarify the species of larval nematodes which infect edible fish in Bangladesh.

1. Introduction

Spotted snakehead fish *Channa punctata*, is a hardy, air breathing, benthopelagic and potamodromous freshwater species (Prasad et al., 2011; Karnatak et al., 2020) which is distributed throughout the Indian sub-continent (Pakistan, India, Sri Lanka, Nepal, Bangladesh) (Qadir and Malik, 2011; Karnatak et al., 2020), Yunnan in China and Myanmar (Chaudhry et al., 2019; Islam et al., 2020). Due to the plethora of freshwater environments in Bangladesh, species of small indigenous fish are widely available and provide a dietary source of high-quality protein, which is also cheap to purchase (Islam et al., 2020). In a study of *C. punctata* from Bangladesh, commercially obtained from a fish market in Dhaka, fish were found to have a proximate protein composition of 15.91 ± 0.34% (Hossain et al., 1980). *Channa* are also claimed to be medically important and consumption of *C. striata*, for example, has been anecdotally linked to rapid wound healing and reduced pain after surgery (Gam et al., 2006).

Channa punctata is distributed in swamps, ponds and ditches and as adults in muddy streams and stagnant water bodies (Alam et al., 2019). *Channa* spp. are known as carnivorous and voracious predators of small fish/fries, frogs, young turtles and may even prey on ducklings (Mustafa et al., 2012; Deshmukh et al., 2020). Due to these feeding habits, *C. punctata* may become highly infected with intestinal parasites (Chowdhury and Hossain, 2015). Many nematode species have been described in *C. punctata* however, there is a great deal of taxonomic confusion regarding larval nematodes identified from *C. punctata* in Bangladesh (Table 1). It is recognised that identification of larval nematodes to a species level morphologically is not reliable (Borges et al., 2012; Shamsi and Suthar, 2016a; Moravec and Nagasawa, 2018; Mazzone et al., 2019; Abro et al., 2020) because most features are absent in larval specimens. Therefore, the aim of this study was to identify larval nematodes recovered from the intestinal mesentery of *C. punctata* (Bangladesh) using molecular methodology.

* Corresponding author.
E-mail address: miwilliams@csu.edu.au (M. Williams).

https://doi.org/10.1016/j.ijppaw.2022.01.001
Received 17 October 2021; Received in revised form 17 December 2021; Accepted 3 January 2022
Available online 6 January 2022

2. Materials and methods

2.1. Parasite collection

Fish were thawed before dissection. It is unknown how long the fish had been frozen preceding examination. The methods used for fish examination, parasite collection and isolation followed the description in Shamsi and Suthar (2016b). Fish were then lightly blended and artificially digested in a pepsin physiological saline solution (concentration of 15 mg/L) following the method described in Bier et al. (2001). The pepsin/fish solution was processed at 37 °C, 100–120 rpm for 16–20 h. The digesta was passed through a 1000-μm mesh strainer and the clarity adjusted with physiological saline before examining under a dissecting microscope (Leica EZ4 Stereo Microscope). Collected parasites were stored in 1.5 mL sterile Eppendorf® tubes containing 70% ethanol pending molecular identification.

2.2. Parasite processing

The prevalence (P), mean intensity (MI), and mean abundance (MA) of larval nematodes were calculated following Bush et al. (1997). For molecular study, a small piece was excised from the mid-body of twelve larval nematodes using a sterile scalpel blade and the excised piece transferred to 1.5 mL sterile Eppendorf® tubes as described in Shamsi et al. (2008). The anterior and posterior portion of each nematode were then slide mounted and cleared with lactophenol for image capture using an Upright Motorized Microscope ECLIPSE Ni-E, Nikon, Japan.

2.3. Genetic characterisation

Genomic DNA from 12 larval nematodes were extracted according to the method described in Shamsi et al. (2019) using DNeasy Blood & Tissue Kits (QIAGEN, Germany) and eluted by 40 μl of elution buffer. Since the 18S gene has been successfully used as a genetic marker for studying *Tanqua* and other Spirurina parasites in freshwater aquatic species (Laetsch et al., 2012; Choudhury and Nadler, 2018; Schoeman et al., 2020), samples from 12 larval nematodes were amplified using the primer sets Forward: 35 fm (5′-TATAATGGTGAAACCGCGAACGGC-3′) and Reverse: 18gMm (5′- GGAAACCTTGTTACGACTTTTGCC-3′). The cycling conditions for PCR were as follows, 95 °C for 2 min followed by 95 °C for 30 s, 48 °C for 45 s, 72 °C for 1 min for 40 cycles and a final extension of 72 °C for 10 min.

For each amplicon, a 3 μl aliquot was examined on a 1.5% w/v agarose gel, was stained with GelRed™ after which a photograph was taken using a gel documentation system. Five of the 12 amplified samples were sent for sequencing to the Australian Genome Research Facility (AGRF). The chromatogram and sequence data were observed using the Sequence Scanner software (Applied Biosystems® Genetic Analysers). BLAST search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to conduct homology searches. The sequences generated from AGRF were cleaned first and then aligned using MUSCLE (in MEGA v. 10) (Kumar et al., 2016, 2018) to verify any variation between sequences.

The 18S sequences of Gnathostomatidae available in GenBank were included in phylogenetic analyses. Five new sequences generated in the present study for *Tanqua* sp. from the type-host *Channa punctata* in Bangladesh were also added (Table 2). *Cucullanus robustus* (accession number JF934726) was used as the outgroup based on earlier Gnathostomatidae-related phylogenetic analysis (Laetsch et al., 2012). The phylogenetic tree was inferred using the Maximum Likelihood method (MEGA v. 10) and is presented as Fig. 1.

3. Results

Laval nematodes were encysted in or on the intestinal mesentery, some in round taupe-coloured resilient sacks, others in fragile transparent cysts and three were encysted in musculature. A total of ten *C. punctata* were infected with 27 larval nematodes, prevalence was 9.7%; mean intensity 2.70 and mean abundance 0.30 (Table 3).

3.1. Larval morphology

No morphological description was provided for adult *Tanqua tiara* from *Varanus indicus*, Australia in Laetsch et al. (2012 Supp. data 18_S. haplotypes) however Schoeman et al. (2020) provided morphological description for larval stage (L3) *Tanqua* sp. from *Xenopus laevis*, South Africa. Twelve of the specimens in the present study used for DNA extraction were entire, and although internal structures were damaged (Fig. 2A) the cephalic region of the 12 entire specimens followed the description of the cephalic region of adult *Tanqua tiara* in Sou (2020) in which large lateral pseudolabia and tooth like projections were described. Fig. 2A and B shows the anterior of two of the 12 specimens used for DNA extraction and identified at *Tanqua* sp. Fig. 2B at 40X magnification shows the tooth like projections of the pseudolabia (*tl*) and the large lateral pseudolabia (*lp*). Schoeman et al. (2020) describes the pseudolabia as minute and although tooth like projections are not described in text these can be observed in Fig. 6B of the publication. The entire body, of all of the 12 specimens used for DNA extraction in the

Table 1
Previous reports of nematodes including nematodes with uncertain taxonomic status (in bold) infecting *Channa punctata* in Bangladesh.

Hosts	Parasite taxa	Family	Site	Localities	Reference
C. punctata	***Ascaridia* sp.**	Compositae	Digestive tract, viscera, body cavity	Dhaka, Bangladesh	Huq et al. (1985)
	Ascaridia sp.	Compositae	Intestine	Mymensingh, Bangladesh	Farzana et al. (2019)
	Ascaris sp.	Ascarididae	Stomach	Dhaka, Bangladesh	Huq et al. (1985)
	Camallanus sp.	Camallanidae	Unknown	Bangladesh	Chandra (2006)
	Camallanus intestinalus	Camallanidae	Intestine	Dhaka and Sylhet, Bangladesh	Bashirullah (1974) and Khalil et al. (2014)
	Camallanus pearsi	Camallanidae	Unknown	Bangladesh	Chandra (2006)
	Contracaecum sp. larva	Anisakidae	Intestine	Mymensingh, Bangladesh	Ali (1968) and Farzana et al. (2019)
	Echinocephalus sp.	Gnathostomatidae	Unknown	Bangladesh	Chandra (2006)
	***Neocamallanus* sp.**	–	Pyloric caeca	Chittagong, Bangladesh	Arthur and Ahmed (2002)
	Neocamallanus ophiocephali	–	Pyloric caeca, intestine	Dhaka or Sylhet, Bangladesh	Ahmed (1981); Bashirullah (1973)
	Paracamallanus spiculogubernaculus	Camallanidae	Unknown	Bangladesh	Chandra (2006)
	Paracamallanus sweeti	Camallanidae	Unknown	Bangladesh	Chandra (2006)
	Porrocaecum sp.	Ascarididae	Intestine	Mymensingh, Bangladesh	Farzana et al. (2019)
	Procamallanus sp.	Camallanidae	Intestine	Dhaka, Bangladesh	Huq et al. (1985)
	Procamallanus (*Spirocamallanus*) sp.	Camallanidae	Stomach, intestine	Chittagong, Dhaka, Sylhet, Bangladesh	Bashirullah (1973) and Ahmed (1981)

Table 2
Details of the accession numbers **used to construct the phylogenetic tree in the present study.** Information at GenBank only from unpublished PhD in Spanish indicated with (*). Laetsch et al. (2012) used all sequences (*) for phylogenetic analysis of nSSU sequences of Spirurina B. Information from GenBank only for sequence KT894809 indicated with (#). Sequence KT894809 used as an outgroup in Maldonado et al. (2020).

Nematode specimen	GenBank accession number	Host species	Geographical origin of the sample	Reference
Cucullanus robustus (Outgroup)	JF934726	Isolate N624	-	Laetsch et al. (2012) in Supp. data 18_S. haplotypes
Echinocephalus carpiae	KC493258	*Cyprinus carpio*	Burullus Lake, Egypt	Abdel-Ghaffar et al. (2013)
Echinocephalus overstreeti	JF934729	*Heterodontus portusjacksoni*	Adelaide, South Australia	Laetsch et al. (2012)
Gnathostoma binucleatum	Z96946	Pool of eight larvae	–	*Unpublished
Gnathostoma turgidum	Z96948	–	–	*Unpublished
Gnathostoma neoprocyonis	Z96947	–	–	*Unpublished
Gnathostoma turgidum	KT894809	*Philander opossum*	Santa Catarina, Brazil	#Unpublished
Spiroxys contortus	MN629917	*Emys orbicularis*	Polesie National Park (South-Eastern Poland)	Demkowska-Kutrzepa et al. (2021)
Spiroxys hanzaki	AB818383	*Andrias japonicus*	Hyogo, Japan	Hasegawa et al. (2013) in Japanese
Spiroxys japonica	AB818382	*Rana nigromaculata*	Niigata, Tokamachi, Japan	Hasegawa et al. (2013) in Japanese
Spiroxys japonica	KF844295	*Pelophylax nigromaculatus*	Yingtan, Jiangxi Province, China	Li et al. (2014)
Tanqua tiara	JF934728	*Varanus indicus* (Isolate N691)	Maningrida, Northern Territory, Australia	Laetsch et al. (2012) in Supp. data 18_S. haplotypes
Tanqua sp.	MN526252	*Xenopus laevis*	Limpopo Province, South Africa	Schoeman et al. (2020)
Tanqua sp.	OL830839	*Channa punctata*	Bangladesh	This study, isolate 674-1
Tanqua sp.	OL830840	*Channa punctata*	Bangladesh	This study, isolate 678-1
Tanqua sp.	OL830841	*Channa punctata*	Bangladesh	This study, isolate 678-9
Tanqua sp.	OL830842	*Channa punctata*	Bangladesh	This study, isolate 678-10
Tanqua sp.	OL830843	*Channa punctata*	Bangladesh	This study, isolate 678- 12

present study, were heavily annulated and although characteristic of a number of other genera of nematodes (Moravec et al., 2007; Pereira et al., 2014; Velarde-Aguilar et al., 2015; Ocampo-Salinas et al., 2021), are also described in adult *Tanqua tiara* (Sou, 2020). Specimens identified as *Tanqua* sp. (Fig. 2C and D) in the present study show the trunk is heavily annulated and these annulations cover the entire parasite. Schoeman et al. (2020) did not describe annulations in the morphological description of L3 *Tanqua* sp.

3.2. Molecular identification

The 18S sequences of three specimens (voucher numbers 674-1; 678-1 and 678-9), were explored at 1581 bp long. Voucher numbers 678-12 and 678-10 showed bp of 1405 and 1001 long, respectively. However, no nucleotide variability was observed among sequences obtained from 674-1 (OL830839); 678-1 (OL830840); 678-9 (OL830841); 678-10 (OL830842) and 678-12 (OL830843). A search in GenBank for one of the representative sequences generated in the present study showed a 98.17% match with GenBank sequences assigned for *Tanqua tiara* (accession number JF934728) from the mangrove monitor (*Varanus indicus*), Maningrida, Northern Territory, Australia in (Laetsch et al., 2012 Supp. data 18_S. haplotypes). Phylogenetic analysis of the *Tanqua* sp. in the present study showed that they are a distinct taxon within Gnathostomatidae and clustered with previously identified adult *Tanqua tiara* (JF934728) and larval *Tanqua* sp. (MN526252).

4. Discussion

In the present study, 27 larval nematode specimens were recovered from cysts attached to the mesentery or the musculature of ten *C. punctata* from Bangladesh. Five of the twelve nematodes used for molecular characterisation, were identified as *Tanqua* sp. representing a new host and locality record for this genus. Molecular identification of seven of the 12 larval nematodes used for DNA extraction was not possible in the present study due to the poor quality of genetic material. However, all of the 12 specimens used for DNA extraction, despite having damage to the trunk and internal structures, had the head and tail intact. All of the 12 specimens were annulated over the entire body and had similar toothlike projections and large lateral pseudolabia described in adult *T. tiara* by Sou (2020). Of the remaining 15 larval nematodes, not subjected to DNA extraction, and with undamaged cephalic regions the same features were identified. This may suggest that many of the remaining 15 specimens were also be *Tanqua* sp. Specimens in the present study were heavily annulated which differed from the description of larval *Tanqua* sp. in Schoeman et al. (2020). Intraspecific morphological variations of adult *T. tiara* has been reported (Sou, 2020). The specimens in the present study showed low genetic variability with adult *T. tiara* (1.8%: JF934728) in Laetsch et al. (2012) and by L3 *Tanqua* sp. (1.2%: MN526252) in Schoeman et al. (2020). The differences in body annulations and size of the lateral pseudolabia between L3 larvae in Schoeman et al. (2020) and the present study may be a consequence of a the difference in geographical locality, host or larval developmental stage. Further morphological and genetic study is required to explore these relationships.

The phylogenetic tree clustered the sequences obtained in the present study with the sequences registered for *Tanqua* species in GenBank with a strong bootstrap value (Fig. 1). Therefore, the present study confirms the *C. punctata* as a host of *Tanqua* species in Bangladesh. As the specimens in the present study were larval stage and not suitable for detailed morphological study authors have assigned our specimens to *Tanqua* sp.

A search of the literature for published records of *Tanqua* sp. in the definitive host, the Asian water monitor, *Varanus salvator*, or any species of fish from Bangladesh was unsuccessful. Previous records of nematodes identified in *Channa punctata* from Bangladesh are included in Table 1. The Asian water monitor *V. salvator*, commonly found in Asia and the Indian sub-continent, is considered the definitive host of *Tanqua* sp., although other members of *Varanus* genus have also been identified as hosts (Schoeman et al., 2020). In Bangladesh, *V. salvator* is distributed mainly in mangroves where they scavenge a huge array of prey, particularly during the wet season when most foraging activity occurs (Rahman et al., 2017b). Crabs, toads, small fishes, frogs, shrimp/-prawns, birds' eggs, water birds and kitchen scraps are all foraged by *V. salvator* in Bangladesh (Rahman et al., 2017a). Although distribution of *V. salvator* in Bangladesh is in mangroves this species also frequent, ponds, swamps, sewers and drains which reflects the habitat where *C. punctata* is commonly distributed (Froese and Pauly, 2018). Eggs shed in the faeces of *V. salvator* infect multiple aquatic species (Agustin et al., 2017) many of which are also foods predated by *C. punctata* (Deshmukh et al., 2020).

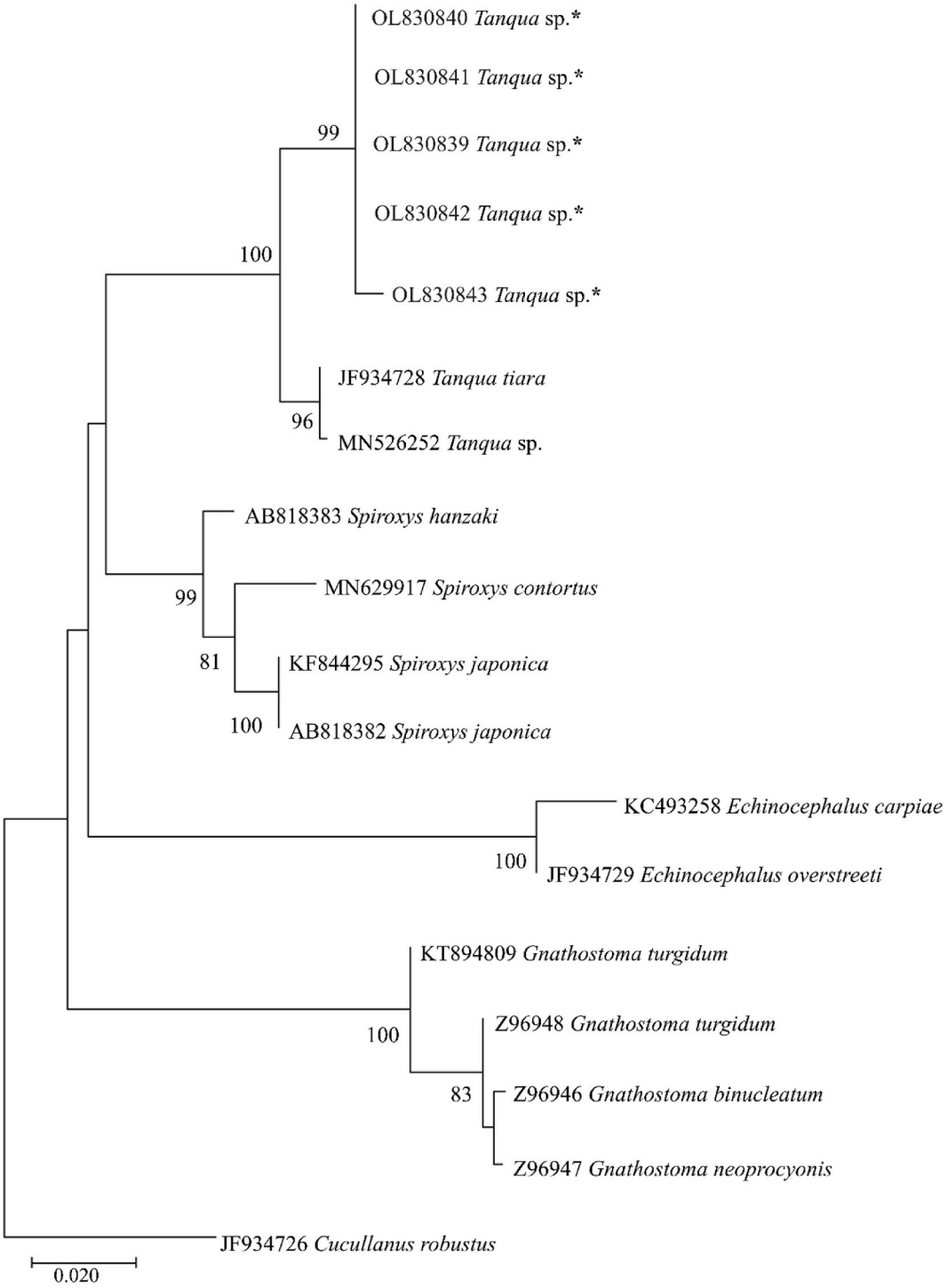

Fig. 1. Phylogenetic tree (of 18S sequences of nematodes) inferred using the Maximum Likelihood Method. The bootstrap values higher than 80 are indicated next to the branches. The new sequences generated from this study are indicated with asterisks.

Agustin et al. (2017) speculated that *T. tiara* has zoonotic potential and mammals may become infected from drinking worm eggs in contaminated water (Agustin et al., 2017). The authors of the present publication found no evidence to support this, however *T. tiara* is a species of one of four genera within the family Gnathostomatidae. One of the genera, *Gnathostoma*, has members species which have been identified in multiple cases of human infection globally (Chandenier et al., 2001; Chappuis et al., 2001; Chai et al., 2003; Górgolas et al., 2003; Basak et al., 2004; Barua et al., 2007). In addition, cases of gnathostomiasis in countries where *Gnathostoma* species have not been identified in local fish may have been misidentified according to Shamsi et al. (2021) and could be *Echinocephalus* sp., also of family Gnathostomatidae. *Channa punctata* in this study was infected with third stage larvae, which is the developmental stage infective to humans, and three specimens were embedded in the fish musculature. Perhaps the zoonotic potential of *Tanqua* sp. requires further investigation.

The habitat of *V. salvator* has declined in Bangladesh due to many anthropogenically driven factors (Khandakar et al., 2020). The consequence of this continued habitat degradation will be a blurring of boundaries between man and *V. salvator*. Fish, as the most favoured

Table 3
Infection data for larval nematodes and *Tanqua* sp. Genomic DNA was extracted from 12 of the 27 unidentified nematodes (row 1). Of these 12 nematodes used for extraction of DNA five were sent for sequencing and identified as *Tanqua* sp. (row 2).

	Fish and number (N =)	Parasite species	No. of fish infected	Range in infected fish	Prevalence (%)	Total no. of parasites found or identified	Mean intensity	Mean abundance	GenBank accession number this study	GenBank accession number match
1.	***Channa punctata* (N = 103)**	Larval nematodes	10	0–13	09.7	27.0	2.70	0.30		
2.		*Tanqua* sp.				5			OL830839 OL830840 OL830841 OL830842 OL830843	JF934728

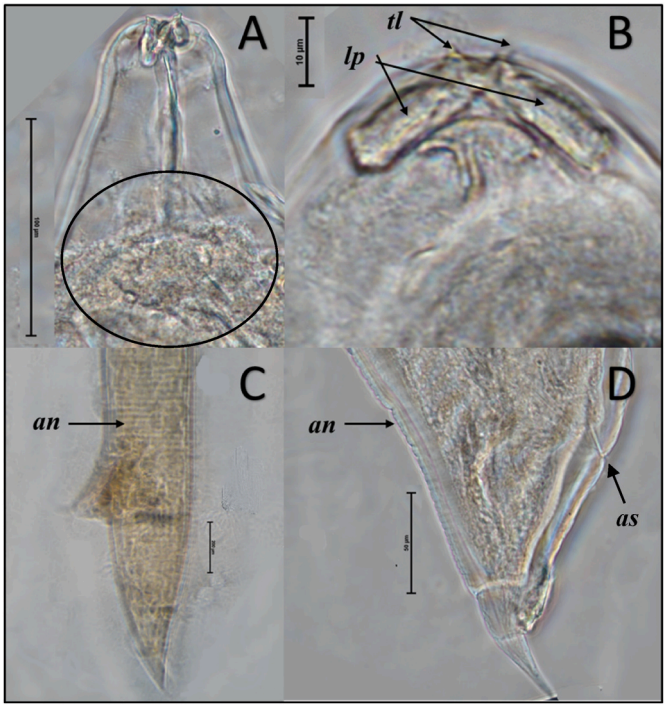

Fig. 2. Larval nematodes identified as Tanqua sp. 2A specimen 674-1 anterior tip (20x); 2B specimen 678-1 showing tooth like projections of pseudolabia (tl) and lateral pseudolabium (lp) (40x); 2C specimen 674-1 posterior trunk (4x) showing annulations (an). and 2D specimen 678-9 tail (20x) respectively showing annulations (an) and anus (as). The circled area in Fig. 2A is indicative of the damage to internal structures which precluded detailed morphological examination.

scavenged food of Bengali *Varanus* spp. (Rahman et al., 2015), places this genus of monitors at the interface of fish polyculture in Bangladesh, essential to establish food security for the growing population (Bogard et al., 2015). It is important to identify parasites accurately using molecular method to better understand this developing dynamic between Bengali people, fish production, and wildlife.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Michelle Williams is the grateful recipient of the PhD scholarship from Australian Research Training Program Scholarship through Charles Sturt University.

References

Abro, M.M., Birmani, N.A., Brohi, G.H., Rajper, M., 2020. Detection of a pathogenic nematode from tire track eel, *Mastacembelus armatus* captured in the Indus River, Sindh, Pakistan. PAB (Pesqui. Agropecu. Bras.) 9, 91–95.
Agustin, A.L.D., Koesdarto, S., Lukiswanto, B.S., Suwanti, L.T., Arifin, Z., Putranto, E.D., 2017. Morphological identification nematodes *Tanqua tiara* found on Gastric *Varanus salvator* at East Java. KnE Life Sci. 668–676.
Ahmed, A.T.A., 1981. Helminth infection in freshwater fishes of Bangladesh. Fish Pathol. 15, 229–236.
Alam, M.J., Andriyono, S., Sektiana, S.P., Rahman, M.M., Kim, H.-W., 2019. The molecular characterization of complete mitochondrial genome of spotted snakehead fish, *Channa punctata* (Bloch 1793). Mitochondrial DNA Part B 4, 547–548.
Ali, M., 1968. Investigation on fish diseases and parasites in East Pakistan. Bull. Off. Int. Epizoot. 69, 1517.
Arthur, J.R., Ahmed, A.T.A., 2002. Checklist of the parasites of fishes of Bangladesh Food and Agriculture Organization of the United Nations Fisheries Technical Paper. Rome, Italy, pp. 1–88.
Barua, P., Hazarika, N., Barua, N., Barua, C., Choudhury, B., 2007. Gnathostomiasis of the anterior chamber. Indian J. Med. Microbiol. 25, 276–278.
Basak, S., Sinha, T., Bhattacharya, D., Hazra, T., Parikh, S., 2004. Intravitreal live gnathostoma spinigerum. Indian J. Ophthalmol. 52, 57–58.
Bashirullah, A., 1973. A brief survey of the helminth fauna of certain marine and freshwater fishes of Bangladesh. Bangladesh J. Zool. 1, 63–81.
Bashirullah, A., 1974. Two new nematode species of *Camallanus* Railliet and Henry 1915 from freshwater fishes of Dacca, Bangladesh. Norw. J. Zool. 22, 57–60.
Bier, J.W., Jackson, G.J., Adams, A.M., Rude, R.A., 2001. Bacteriological Analytical Manual (Chapter 19), Parasitic animals in foods.
Bogard, J.R., Thilsted, S.H., Marks, G.C., Wahab, M.A., Hossain, M.A.R., Jakobsen, J., Stangoulis, J., 2015. Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. J. Food Compos. Anal. 42, 120–133.
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, 40441-40414.
Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83, 575–583.
Chai, J.-Y., Han, E.-T., Shin, E.-H., Park, J.-H., Chu, J.-P., Hirota, M., Nakamura-Uchiyama, F., Nawa, Y., 2003. An outbreak of gnathostomiasis among Korean emigrants in Myanmar. Am. J. Trop. Med. Hyg. 69, 67.
Chandenier, J., Husson, J., Canaple, S., Gondry-Jouet, C., Dekumyoy, P., Danis, M., Riveau, G., Hennequin, C., Rosa, A., Raccurt, C., 2001. Medullary gnathostomiasis in a white patient: use of immunodiagnosis and magnetic resonance imaging. Clin. Infect. Dis. 32, e154–e157.
Chandra, K., 2006. Fish parasitological studies in Bangladesh: a review. J. Agric. Rural Dev. 4, 9–18.
Chappuis, F., Farinelli, T., Loutan, L., 2001. Ivermectin treatment of a traveler who returned from Peru with Cutaneous gnathostomiasis. Clin. Infect. Dis. 33, 587-587.
Chaudhry, S., de Alwis Goonatilake, S., Fernado, M., Otagama, O., 2019. Channa punctata, in: international union for conservation of nature. In: The IUCN Red List of Threatened Species 2019. IUCN, Gland, Switzerland.
Choudhury, A., Nadler, S.A., 2018. Phylogenetic relationships of spiruromorph nematodes (Spirurina: Spiruromorpha) in North American freshwater fishes. J. Parasitol. 104, 496–504.
Chowdhury, S.Z., Hossain, M.M.M., 2015. Isolation and characterization of internal parasites in snakehead. Int. J. Fish. Aquat. Stud. 2, 17–22.
Demkowska-Kutrzepa, M., Szczepaniak, K., Roczeń-Karczmarz, M., Palumbo, E.O., Studzińska, M., Różański, P., Tomczuk, K., 2021. The first case of *Spiroxys contortus* in European pond turtle (*Emys orbicularis*) in the wild in Poland. Int. J. Parasitol.: Parasites Wildl. 16, 26–29.
Deshmukh, S., Paliwal, G., Bhandarkar, S., Patankar, S., Rajankar, M., 2020. A report of epizootic ulcerative syndrom (EUS) in *Channa punctata* (Bloch, 1793) from

freshwater fisheries lake in Gondia district Maharashtra. Int. J. Fish. Aquat. Stud. 8, 189–191.
Farzana, N., Naher, J., Zafar, M.A., Chandra, K.J., Ahmed, G.U., 2019. Study on infestations and biodiversity of metazoan parasites in *Channa punctatus* (Bloch), Mymensingh region. J. Bangladesh Agric. Univ. 17, 409–416.
Froese, R., Pauly, D., 2018. FishBase version February 2018.
Gam, L.-H., Leow, C.-Y., Baie, S., 2006. Proteomic analysis of snakehead fish (*Channa striata*) muscle tissue. Malays. J. Biochem. Mol. Biol. 14, 25–32.
Górgolas, M., Santos-O' Connor, F., Gárate, T., Guarch Troyas, R.M., Unzú, A.L., Grobusch, M.P., Fernández-Guerrero, M.L., 2003. Cutaneous and Medullar gnathostomiasis in travelers to Mexico and Thailand. J. Trav. Med. 10, 358–361.
Hasegawa, H., Sato, A., Kai, M., Uchida, A., 2013. Helminth parasites of bullfrogs, *Lithobates catesbeianus* (Shaw, 1802), Kanto District, Japan, with special reference to those introduced from North America. Jpn. J. Vet. Parasitol. 12, 1–10.
Hossain, M., Islam, M., Huq, M., 1980. Investigation into the parasitism of Bangladesh Agricultural University pond fishes. AGRIS 1, 15–20.
Huq, M., Hossain, M., Amin, M., Islam, M., 1985. A survey on the helminth parasites of lata (Channidae) in and around the Bangladesh Agricultural University campus, Mymensingh. AGRIS 10, 67–72.
Islam, M., Mawa, Z., Hossain, M., Rahman, M., Hasan, M., Khatun, D., Chowdhury, A., Rahman, O., Tanjin, S., Ohtomi, J., 2020. Morphometric and meristic characteristics of Spotted snakehead *Channa punctata* (Bloch, 1793) in a wetland ecosystem (NW Bangladesh) using multi-linear dimensions. Indian J. Geo Mar. Sci. 49, 1442–1446.
Karnatak, G., Sarkar, U.K., Naskar, M., Roy, K., Nandi, S., Mishal, P., Lianthuamluaia, L., Kumari, S., Das, B.K., 2020. Modeling pre-spawning fitness and optimal climate of spotted snakehead *Channa punctata* (Bloch, 1793) from a Gangetic floodplain wetland of West Bengal, India. Int. J. Biometeorol. 64, 1889–1898.
Khalil, S., Mamun, M., Bari, S., Haque, M., 2014. Endoparasitic infestation in *Channa punctatus* (Bloch, 1793) collected from different water bodies in Sylhet. J. Sylhet Agric. Univ. 1, 59–64.
Khandakar, N., Das, D.K., Galib, A.J., 2020. Occurrence of the Asian water monitor (*Varanus salvator* Laurenti, 1768) in Faridpur, Bangladesh. Biawak 14, 58–59.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
Laetsch, D.R., Heitlinger, E.G., Taraschewski, H., Nadler, S.A., Blaxter, M.L., 2012. The phylogenetics of Anguillicolidae (Nematoda: Anguillicoloidea), swimbladder parasites of eels. BMC Evol. Biol. 12, 60–76.
Li, L., Hasegawa, H., Roca, V., Xu, Z., Guo, Y.-N., Sato, A., Zhang, L.-P., 2014. Morphology, ultrastructure and molecular characterisation of *Spiroxys japonica* Morishita, 1926 (Spirurida: Gnathostomatidae) from *Pelophylax nigromaculatus* (Hallowell)(Amphibia: Ranidae). Parasitol. Res. 113, 893–901.
Maldonado, A., Simões, R., São Luiz, J., Costa-Neto, S., Vilela, R., 2020. A new species of *Physaloptera* (Nematoda: Spirurida) from *Proechimys gardneri* (Rodentia: Echimyidae) from the Amazon rainforest and molecular phylogenetic analyses of the genus. J. Helminthol. 94, 1–11.
Mazzone, A., Caffara, M., Gustinelli, A., Agnetti, F., Sgariglia, E., Lo Vaglio, G., Quaglio, F., Fioravanti, M.L., 2019. Morphological and molecular characterization of larval and adult stages of *Eustrongylides excisus* (Nematoda: Dioctophymatoidea) with histopathological observations. J. Parasitol. 105, 882–889.
Moravec, F., Hanzelová, V., Gerdeaux, D., 2007. New data on the morphology of *Comephoronema oschmarini* (Nematoda, Cystidicolidae), a little-known gastrointestinal parasite of *Lota lota* (Teleostei) in Palaearctic Eurasia. Acta Parasitol. 52, 135–141.
Moravec, F., Nagasawa, K., 2018. *Rhabdochona angusticaudata* sp. n.(Nematoda: Rhabdochonidae) from the Japanese eel *Anguilla japonica*, and new records of some other nematodes from inland fishes in Japan. Folia Parasitol. 65, 1–22.
Mustafa, A., Widodo, M.A., Kristianto, Y., 2012. Albumin and zinc content of snakehead fish (*Channa striata*) extract and its role in health. IEESE Int. J. Sci. Technol. 1, 1–8.
Ocampo-Salinas, J.M., Rosas-Valdez, R., Martínez-Salazar, E.A., 2021. Gastrointestinal Helminths of four Lizard species (Squamata: Phrynosomatidae and Teiidae) from Mixteca region, Oaxaca, Mexico. Comp. Parasitol. 88, 51–66.
Pereira, F.B., de Nazaré Pereira, A., Luque, J.L., 2014. A new species of *Comephoronema* (Nematoda: Cystidicolidae) from the squirrelfish *Holocentrus adscensionis* (Beryciformes: Holocentridae) off Brazil. Folia Parasitol. 61, 55–62.
Prasad, L., Dwivedi, A.K., Dubey, V.K., Serajuddin, M., 2011. Reproductive biology of freshwater murrel, *Channa punctatus* (Bloch, 1793) from river Varuna (A tributary of Ganga River) in India. J. Ecophysiol. Occup. Health 11, 69–80.
Qadir, A., Malik, R.N., 2011. Heavy metals in eight edible fish species from two polluted tributaries (Aik and Palkhu) of the River Chenab, Pakistan. Biol. Trace Elem. Res. 143, 1524–1540.
Rahman, K.M., Khan, M., Rakhimov, I., 2015. Scavenging Behavior of the Bengal monitor (*Varanus bengalensis*) in Jahangirnagar university campus, Bangladesh. J. Scientific Res. Rep. 7, 539–550.
Rahman, K.M., Rakhimov, I.I., Khan, M.M.H., 2017a. Activity budgets and dietary investigations of *Varanus salvator* (Reptilia: Varanidae) in Karamjal ecotourism spot of Bangladesh Sundarbans mangrove forest. Basic Appl. Herpetol. 31, 45–56.
Rahman, K.M., Rakhimov, I.I., Khan, M.M.H., 2017b. Observation of a *Varanus salvator* consuming potentially dangerous waste refuse in Karamjal, Bangladesh Sundarbans mangrove forest. Herpetol. Bull. 139, 33-33.
Schoeman, A.L., Joubert, T.-L., Du Preez, L.H., Svitin, R., 2020. *Xenopus laevis* as UberXL for nematodes. Afr. Zool. 55, 7–24.
Shamsi, S., Dang, M., Zhu, X., Nowak, B., 2019. Genetic and morphological characterization of *Mawsonascaris vulvolacinata* n. sp.(Nematoda: Anisakidae) and associated histopathology in a wild caught cowtail stingray, *Pastinachus ater*. J. Fish. Dis. 42, 1047–1056.
Shamsi, S., Gasser, R., Beveridge, I., Alizadeh Shabani, A., 2008. *Contracaecum pyripapillatum* n. sp. (Nematoda: Anisakidae) and a description of *C. multipapillatum* (von Drasche, 1882) from the Australian pelican, *Pelecanus conspicillatus*. Parasitol. Res. 103, 1031–1039.
Shamsi, S., Steller, E., Zhu, X., 2021. The occurrence and clinical importance of infectious stage of *Echinocephalus* (Nematoda: Gnathostomidae) larvae in selected Australian edible fish. Parasitol. Int. 83, 102333.
Shamsi, S., Suthar, J., 2016a. Occurrence of *Terranova* larval types (Nematoda: Anisakidae) in Australian marine fish with comments on their specific identities. PeerJ 4, e1722.
Shamsi, S., Suthar, J., 2016b. A revised method of examining fish for infection with zoonotic nematode larvae. Int. J. Food Microbiol. 227, 13–16.
Sou, S.K., 2020. Redescription of *Tanqua tiara* (von Linstow, 1879) Blanchard, 1904 (Nematoda: Gnathostomatidae) from *Varanus flavescens* (Hardwicke and Gray, 1827) (Reptilia: Varanidae) from Birbhum district, West Bengal, India. J. Parasit. Dis. 44, 381–387.
Velarde-Aguilar, M.G., Mata-López, R., Guillén-Hernández, S., León-Règagnon, V., 2015. *Parapharyngodon* n. spp.(Nematoda: Pharyngodonidae) parasites of hylid frogs from Mexico and review of species included in the genus. J. Parasitol. 101, 212–230.