Morphological and genetic characterisation of selected *Contracaecum* (Nematoda: Anisakidae) larvae in Iran

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In their life cycle, *Contracaecum* spp infect marine mammals and piscivorous birds as definitive hosts and crustaceans and a wide range of fish species as their intermediate hosts (Anderson, 2000). Humans can accidentally be infected with larval stages of these nematodes, leading to a sever disease generally known as anisakidosis (Shamsi and Butcher, 2011). The disease now is considered as an emergence zoonotic disease and therefore, these parasites attracted attention of scientists in different parts of the world and various aspects of their biology and lifecycle is being investigated (Audicana et al., 2002; Nadler et al., 2005; D'Amelio et al., 2007; Shamsi et al., 2008; Shamsi et al., 2009a, b).

In Iran, there is a paucity of information on these socioeconomically important parasites. Iranian anisakids are known principally from early works by Eslami and Mokhayer (Mokhayer, 1973; Eslami and Mokhayer, 1977; Eslami and Kohneshahri, 1978; Mokhayer, 1981; Eslami and Kohneshahri, 1994). Recently, other investigators also reported occurrence of larvae in various fish in the country (Sattari et al., 2000; Peyghan et al., 2004; Sattari et al., 2006; Jalali et al., 2008). However, there is no detailed morphological description or a specific identification based on genetic characterisation, according to current standards for identification of these parasites (Paggi and Bullini, 1994; Shamsi et al., 2009b). The present study is a preliminary study toward genetic characterisation and description of anisakid nematodes in Iran, using a combined molecular and morphological approach. Previous studies showed that this approach is useful for reliable identification of *Contracaecum* nematodes to species level (Shamsi et al., 2011). Nematode larvae were collected from intestine and body cavity of barboid fishes (n=4) caught in Parishan Lake, the largest freshwater lake in Iran. Nematodes were washed in physiological saline. A small piece of the mid-body were removed with a scalpel for molecular study, and the rest of the nematode were cleared in

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lactophenol for morphological examination.

All samples were studied morphologically by light microscopy and characters of systematic importance were measured. All drawings were made to scale with the aid of a camera lucida and measurements were made directly with an eyepiece micrometer. All measurements are given in millimetres, unless stated otherwise. Mean measurements are given, followed by the range in parentheses.

Genomic DNA was isolated from individual specimen by sodium dodecyl-sulphate/proteinase K treatment, column-purified (Wizard™ DNA Clean-Up, Promega) and eluted into 30 µl of water. Control DNA was also isolated from the liver of cormorants using the same method. The PCR was used to amplify the ITS-1 region with primer sets SS1 and NC13R (Shamsi et al., 2008). The PCR (in a volume of 50 µl) was performed in 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 3 mM $MgCl_2$, 250 µM each of dNTP, 50 pmol of each primer and 1.5 U *Taq* polymerase (Promega) in a thermocycler (Biometra) under the following conditions: 94°C for 5 min (initial denaturation), followed by 30 cycles of 94°C, 30 sec (denaturation), 55°C, 30 sec (annealing), 72°C, 30 sec (extension) and a final extension of 72°C for 5 min. One microlitre (10-20 ng) of genomic DNA was added to each PCR reaction. Samples with cormorants DNA or without genomic DNA were included in the PCR as negative controls; no amplicons were produced in the PCR from these samples. An aliquot (5 µl) of each amplicon was examined on a 1.5 % w/v agarose gel, stained with ethidium bromide and photographed using a gel documentation system.

Amplicons were purified over mini-columns (Wizard™ PCR Prep, Promega, WI, USA), eluted in 30 µl H_2O and then subjected to automated sequencing (BigDye® chemistry, Applied Biosystems), in both directions, using the same primers as for PCR. Sequences were aligned using the computer program ClustalX (Thompson et al., 1997) and then adjusted manually. Polymorphic sites were designated using IUPAC codes. Nucleotide sequence data reported in this paper are available in the GenBank database under the accession numbers FM210433 to FM210436.

Light microscopic examination revealed larval type consistent with *Contracaecum* type in all fish (Fig. 1). Larvae were in third stage of development. Body was relatively thick. Cuticle annulated, forming collar at the anterior end. Lateral interruption at this area in some specimens. Excretory pore opening at anterior end. Oesophagus ends in short, subglobular ventriculus. Intestinal caecum much shorter than ventricular appendix. Intestine broad, filling remainder of body. Gonads not developed. Three anal glands present. Tail sharply pointed, with spine. Table 1 summarizes measurements of systematically important features of larvae.

Following the morphological identification, the ITS-1 region was amplified from genomic DNA samples from individual larvae. ITS-1 was 452 bp in length. The G+C content was 46.02%. Alignment of sequences of *Contracaecum* larva in this study showed nucleotide variations in alignment positions 20, 161,

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372 and 373 (Fig. 2). Based on previous studies on *Contracaecum* spp in Australia (Shamsi et al., 2009b), it seems that nucleotide variation in alignment position 161 is due to a polymorphism. It is very likely that the *Contracaecum* larvae in the present study in fact belong to two distinct species. Alignment of these larvae with those in GenBank™ showed that they are the most similar to *C. multipapillatum* (Accession number AM940058) (Shamsi et al., 2008). However no identical sequence was present in the GenBank database.

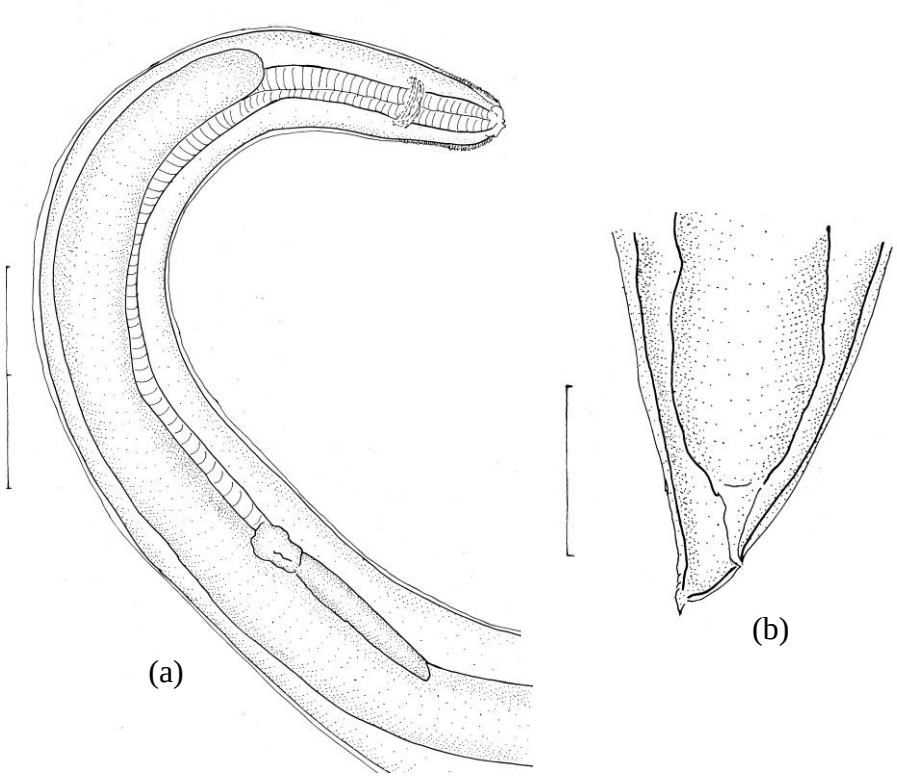


Figure 1: *Contracaecum* larval type from *Barbus* sp. *(*a) anterior end of larva (scale-bar= 0.16 mm); (b) posterior end of larva (scale-bar= 0.16 mm).

C. multipapillatum is a species complex and it was shown that it comprised at least 3 distinct species occurring in USA, Europe and Australia (D'Amelio et al., 2007; Shamsi et al., 2008). It seems that *Contracaecum* larvae in Iran belong to *C. multipapillatum sensu lato*. However, it is necessary to examine adult species and analyse more specimens for a final and precise conclusion. Although, in the present study ITS-1 of larval anisakid from barboid fish did not genetically match with known reference sequences (ITS-1 rDNA) derived from adult nematodes available in current gene databases, the sequences obtained can be used as a basis for future work on anisakid nematodes in other Iranian hosts. Given that sequence data for all *Contracaecum* spp. are not available in GenBank yet, it is not surprising that not

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all sequences obtained from larvae can be matched with sequences obtained from adult nematodes.

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           >ITS-1
FM210435   TCGAGCTAAACCATAAAAGCCTCCATACTTGCATACCTTCCATTTGCAAGTACTAGTGA 60
FM210436   ............................................................
FM210434   ...................T........................................
FM210433   ...................T........................................

FM210435   CCATGCAGTAAGGCACGCACATGTGGTGGCAGCCGTTCGCTGTTGCTTATTTAAGCGTA 120
FM210436   ............................................................
FM210434   ............................................................
FM210433   ............................................................

FM210435   TTGGCGAACAATGGCTCGTGCTTTACTGGGTGTTGAGGGGAAATAGGTGATTCACCGTG 180
FM210436   ............................................................
FM210434   ............................................................
FM210433   ........................................R...................

FM210435   TAGTAGGTGATTCGAGAGCGCCTATTTCCTCGTTACGTTAACAACGGTGTCCACTTTGG 240
FM210436   ............................................................
FM210434   ............................................................
FM210433   ............................................................

FM210435   GTCTACGCTCCACCTAGCTACTGCCTGGACCGTCGGTAGCAATGAAAGGTGGGGAGATA 300
FM210436   ............................................................
FM210434   ............................................................
FM210433   ............................................................

FM210435   TTCCTCCTCGTGAGTTGAGCAGACTTAATGAACCCGGCTAGAGGGTCGCCAAAAACCAA 360
FM210436   ............................................................
FM210434   ............................................................
FM210433   ............................................................

FM210435   CACACACACACACATTCATTTTAAACTTATGTATATCGATGAATCATGATGTGCTCCAA 420
FM210436   ............................................................
FM210434   ...........--.....................................................
FM210433   ...........--.....................................................

FM210435   TTATGGAGACATGTATGTGATGAGTCGTTAAGTA 454
FM210436   ................................
FM210434   ................................
FM210433   ................................
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Figure 2 : Alignment of sequences of ITS-1 regions of *Contracaecum* larval type in the present study. Polymorphic sites were designated using International Union of Pure and Applied Chemistry (IUPAC) codes.

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Table 1: Measurements of systematically important features in larvae in the present study (in millimetre)

Parameters	Number examined	Minimum	Maximum	Mean
Total length	11	13.28	24.54	19.26
Maximum width	12	0.52	0.89	0.74
Length of oesophagus	10	1.56	2.28	1.98
Intestinal caecum	10	1.07	1.95	1.45
Ventricular appendix	10	0.36	2.08	0.74
Distance of nerve ring to anterior end	12	0.23	0.36	0.28
Distance of anus to posterior end	12	0.09	0.16	0.12
Ventriculus	7	0.08	0.13	0.11

Characterisation of adult parasites from definitive hosts in the area of symatry and alignment of the sequences between larval stages and adult nematodes can be promising for identification of these parasites which in turn will be beneficial for the management of the fisheries resources and wildlife populations.The ability to accurately identify and distinguish among species of anisakid nematodes, including *Contracaecum* spp in different hosts and at any developmental stage has important implications for studying their systematics, population biology and ecology as well as for controlling the diseases they cause.

In conclusion, this is the first step toward molecular study of anisakids in Iran. While the prevalence of this parasite group is usually high, there is no information on their ecology or human health significance in the country. While the interpretations from the present data are limited due to the relatively small sample size, the molecular approach provides a unique basis for exploring the composition of their populations in a broader range of fish species as well as in their definitive hosts, including their genetic structure and their ecology.

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References

Anderson, R. C., 2000. *Nematode parasites of vertebrates: Their development and transmission,* Wallingford, UK: CABI Publishing.

Audicana, M. T., Ansotegui, I. J., Fernandez de Corres, L. and Kennedy, M. W., 2002. *Anisakis simplex*: Dangerous - dead and alive? *Trends in Parasitology,* 18(**1**)**,** 20-25.

D'Amelio, S., Barros, N. B., Ingrosso, S., Fauquier, D. A., Russo, R. and Paggi, L., 2007. Genetic characterization of members of the genus contracaecum (nematoda: Anisakidae) from fish-eating birds from west-central florida, USA, with evidence of new species. *Parasitology,* 134(**7**)**,** 1041-1051.

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[DOR: 20.1001.1.15622916.2011.10.2.18.9]

Eslami, A. and Mokhayer, B., 1977. Nematode larvae of medical importance found in market fish in iran. *Pahlavi Medical Journal,* 8(**3**)**,** 345-348.

Eslami, A. and Kohneshahri, M., 1978. Study on the helminthiasis of rutilus frisii katum from the south caspian sea. *Acta Zoologica et Pathologica Antverpiensia,* (**70**)**,** 153-155.

Eslami, A. and Kohneshahri, M., 1994. Study on the helminthiasis of rutilus frisiikatum from the south caspian sea. *Archive of the Faculty of Veterinary Medicine, Tehran University,* 6**,** 108-110.

Jalali, B., Barzegar, M. and Nezamabadi, H., 2008. Parasitic fauna of the spiny eel, mastacembelus mastacembelus banks et solander (teleostei: Mastacembelidae) in iran. *Iranian Journal of Veterinary Research,* 9(**2**)**,** 158-161.

Mokhayer, B., 1973. List of parasites of iranian sturgeons. *Journal of the Veterinary Faculty, University of Tehran,* 29(**1**)**,** 1-12.

Mokhayer, B., 1981. Parasites of fish in the sefid rud basin. *Journal of Veterinary Faculty University of Tehran,* 36(**4**)**,** 61-75.

Nadler, S. A., D'Amelio, S., Dailey, M. D., Paggi, L., Siu, S. and Sakanari, J. A., 2005. Molecular phylogenetics and diagnosis of *anisakis, pseudoterranova,* and *contracaecum* from northern pacific marine mammals. *Journal of Parasitology,* 91(**6**)**,** 1413-1429.

Paggi, L. and Bullini, L., 1994. Molecular taxonomy in anisakids. *Bulletin of the Scandinavian Society of Parasitology,* 4**,** 25-39.

Peyghan, R., Rod, N. H. and Desfuli, A. Y., 2004. Determination of parasitic helminths in persian gulf grouper, (epinephelus coioides), and silver pomfret, (stromateus cinereus). *Pajouhesh-va-Sazandegi. In Animal and Fisheries Sciences,* (**62**)**,** Pe49-Pe55.

Sattari, M., Mokhayer, B., Eslami, A. and Bokaei, S., 2000. Parasites of acipenser persicus (chondrostei: Acipenseridae) from south-west of caspian sea. *Journal of the Faculty of Veterinary Medicine, University of Tehran,* 55(**3**)**,** 19-24.

Sattari, M., Mokhayer, B. and Shafii, S., 2006. Parasitic worms of persian sturgeon (acipenser persicus borodin, 1897) from the southwest of the caspian sea. *Bulletin of the European Association of Fish Pathologists,* 26(**3**)**,** 131-136.

Shamsi, S., Gasser, R., Beveridge, I. and Shabani, A. A., 2008. *Contracaecum pyripapillatum* n. Sp. And a description of *c. Multipapillatum* (von drasche, 1882) from the australian pelican, *pelecanus conspicillatus*. *Parasitology Research,* 103(**5**)**,** 1031-1039.

Shamsi, S., Norman, R., Gasser, R. and Beveridge, I., 2009a. Genetic and morphological evidences for the existence of sibling species within *contracaecum rudolphii* (hartwich, 1964) (nematoda: Anisakidae) in australia. *Parasitology Research,* 105(**2**)**,** 529-538.

Shamsi, S., Norman, R., Gasser, R. and Beveridge, I., 2009b. Redescription and genetic characterization of selected *contracaecum* spp. (nematoda: Anisakidae) from various hosts in australia. *Parasitology Research,* 104(**6**)**,** 1507-1525.

Shamsi, S. and Butcher, A. R., 2011. First report of human anisakidosis in australia. *Medical Journal of Australia,* 194(**4**)**,** 199-200.

Shamsi, S., Gasser, R. B. and Beveridge, I., 2011. Mutation scanning-coupled sequencing of nuclear ribosomal DNA spacers as a tool

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[DOR: 20.1001.1.15622916.2011.10.2.18.9]

for the specific identification of different contracaecum (nematoda: Anisakidae) larval types. *Molecular and Cellular Probes,* 25(**1**)**,** 13-18.

Thompson, J. D., Gibson, T. J., Plewniac, F., Jeanmougin, F. and Higgins, D. G., 1997. The clustal x windows interface:Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research,* 24**,** 4876-4882.

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