Ultra-Morphological Structure and Molecular Characterization of *Contracaecum* larvae (Nematode) Parasitic of Some Fishes in Sulaimani Province, Kurdistan Region-Iraq

A Dissertation
Submitted to the Council of
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in Biology
(**Parasitology)**

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

وَاللَّهُ أَخْرَجَكُمْ مِنْ بُطُونِ أُمَّهَاتِكُمْ لا تَعْلَمُونَ شَيْئاً وَجَعَلَ لَكُمْ السَّمْعَ وَالأَبْصَارَ وَالأَفْئِدَةَ لَعَلَّكُمْ تَشْكُرُونَ۞

سورة النحل (اية ٧٨)

In the name of Allah, the Entirely Merciful, the Especially Merciful

And Allah has brought you out from the wombs of your mothers while you know nothing. And he gave you hearing, sight, and hearts that you might give thanks (to Allah)۞

An-Nahl (Verse 78)

Supervisors Certification

We certify that the preparation of dissertation titled "Ultra-Morpho and Molecular Structure of *Contracaecum* larva (Nematode) Parasitic of Some Fishes in Sulaimani Province, Kurdistan Region-Iraq" accomplished by **Younis S. Abdullah**, was prepared under our supervision in the College of Science at the University of Sulaimani as partial fulfillment of the requirements for the degree of Doctor of Philosophy of Science in Biology **(Parasitology).**

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Abstract

During the current study, random samples of fishes were taken from 26 localities mostly in the Lesser Zab and Sirwan tributaries within Sulaimani Province, Kurdistan Region-Iraq. A total of 2122 freshwater fishes, belonging to 36 species in 26 genera and 10 families were collected randomly from January to the end of December 2018. The fishes were examined for *Contracaecum* larvae parasitizing them.

The study demonstrated that the most diverse family was Cyprinidae with 14 species (38.88%) followed by Leuciscidae with eight species (22.22%) then Nemacheilidae with six species (16.66%), then Xenocyprididae with two species (5.55%), Bagridae, Heteropneustidae, Mastacembelidae, Mugilidae, Siluridae and Sisoridae each with only one species (2.77% for each).

In addition, the study revealed that the most abundant and wide spread species recorded in this investigation was known *Cyprinion macrostomum* with prevalence 15.17%, followed by *Capoeta trutta* with the prevalence of 10.46%, then *Cyprinus carpio* as a third rank with the prevalence 9.18%. It was clarified that *Leuciscus vorax* was scarce with the prevalence 0.047%.

Furthermore, in the present investigation, *Alburnoides velioglui* was recorded for the first time in Iraq. Morphometric, meristic and molecular characterization of this fish were done. A mitochondrial cytochrome c oxidase subunit I (COX-1) was used as DNA barcode marker to clarify the taxonomic status of this fish. The genetic characterization of *A. velioglui* in the present study is available in the GenBank database under the accession number (MN893770).

Larval nematodes of the genus *Contracaecum* (n=140) were collected from 30 infected fishes belonging to 10 different fish species

(*Acanthobrama marmid*, *Arabibarbus grypus*, *Capoeta trutta*, *Carasobarbus luteus*, *Chondrostoma regium*, *Cyprinus carpio*, *Luciobarbus barbuls*, *L. esocinus*, *L. xanthopterus*, and *Mastacembelus mastacembelus*). This investigation revealed that 35%, 0.81%, 0.90%, 4.49%, 5.76%, 2.05%, 0.92%, 1.92%, 19.35%, and 1.06% of the fish species were infected with *Contracaecum* larvae respectively.

The third larval stages (L3) were studied morphologically by optical microscope and ultra-structurally with scanning electron microscope. In addition, the molecular analysis study were done by amplification, sequencing and comparing different gene loci including internal transcribed spacers (ITS-1 and ITS-2) and cytochrome oxidase c subunit II (COX-2) of different isolated *Contracaecum* larvae. These sequences were also compared with closely related nematode sequences from the GenBank. Thirty sequences were obtained for this study from collected *Contracaecum* larvae. ITS-1, ITS-2 and COX-2 were amplified by polymerase chain reaction (PCR) and sequenced. The sequences of ITS-1, ITS-2 and COX-2 reveal that the collected *Contracaecum* larval specimens from all 10 infected fish species represented exactly one species (*Contracaecum rudolphii* type-B) based on identity percentage in Gene Bank database. Phylogenetic analysis of the genotypes were described. The genetic characterization of the parasite in the present study is available in the GenBank database. ITS-1, ITS-2 and COX-2 sequences obtained were deposited in GenBank and their accession numbers were demonstrated.

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CHAPTER ONE
INTRODUCTION

INTRODUCTION

Fish is an important source of food for human in the world. It is a healthy food being rich in essential nutrients like quality animal proteins, polyunsaturated fatty acids (PUFA) especially the omega 3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and micronutrients. There is a high tendency among people to consume fish due to its great health benefits (Mohanty *et al.*, 2019).

Most fish, regardless of geography, harbor a diversity of pathogenic organisms including viruses, bacteria, fungi and parasites to human and causes a great concern to human health when it is consumed raw or lightly preserved (Hoole *et al.*, 2001).

Nematodes are one of the most important parasitic animal taxa (Ruppert *et al*., 2004). Nematodes from family Anisakidae, commonly named anisakids, are parasites of many water organisms, the low specificity in the choice of hosts (both intermediate and definitive) cause that their geographical worldwide distribution (Szostakowska *et al.*, 2005). The most widespread genera from this family are *Anisakis*, *Pseudoterranova*, and *Contracaecum* which they have similar life cycles. The third larval stage (L3) of *Contracaecum* is usually found in the body cavity, mesenteries, and branchial chambers of a wide range of fish species. In addition, this larva have been reported in great variety of invertebrates such as gastropods, cephalopods, copepods, echinodermis and chaetognaths (Norris & Overstreet, 1976), while the adult stage found in the intestine of piscivorous birds and mammals associated with fresh, brackish and sea water (Whitfield and Heeg, 1977; Anderson, 2000).

Nematodes are rarely life threatening to their fish hosts. They may however damage the parasitised organs and cause general poor health such as emaciation, reduced growth and reduced fecundity. The range and degree of these detrimental effects varies depending upon the nematode species involved, the intensity of infection, and whether the host is final or intermediate (Hoole *et al.*, 2001).

Anisakid larvae (L3) may accidentally infect human through eating raw, smoked, or undercooked fish such as sushi, sashimi, ceviche, and gravlax and leading to a sever disease known as anisakidosis (anisakiasis), a zoonotic disease characterized by stomach pains, fever, diarrhea and vomiting, particularly the species belonging to *Anisakis*, *Contracaecum* and *Pseudoterranova* (Oshima, 1987; Arslan *et al*., 1995; Yagi *et al*., 1996; Shamsi & Butcher, 2011). This disease has been reported worldwide, and it is endemic in Southeast Asia (Audicana & Kennedy, 2008; Mattiucci & Nascetti, 2008).

Valles-Vega *et al*. (2017) mentioned the first human case of contracaecosis in the medical literature in Germany in 1967, and the first human case of anisakidosis acquired from eating locally caught fish in Australia recorded from a 41-year-old woman (Shamsi & Butcher, 2011). Two cases of human infection with *Contracaecum* have been reported in Hokkaido island from Japan between 1980 and 1996 (Ishikura *et al*., 1996), if compare human cases caused by *Anisakis* and *Pseudoterranova* with the number of cases caused by *Contracaecum* is quite small (Nagasawa, 2012). Although, this group of nematodes is harmful to humans, scientists use some species of Anisakidae nematodes (*Anisakis*) as a biological tag for host-stock characterization (Mattiucci *et al*., 2008a).

The *Contracaecum* larvae were recorded for the first time in Iraq from ten fish species from different inland waters of Iraq by Herzog (1969). After this record these larvae were recorded continuously in many freshwater fish in different Iraqi water bodies by many researchers. So far, a total of 42 freshwater fish host species are known for *Contracaecum* spp. larvae as well as from five marine fish in Iraq (Mhaisen, 2019). Furthermore, a total of 21 fish host species are known for *Contracaecum* larvae in Kurdistan Region of Iraq (Mhaisen & Abdullah, 2017). However, research to date has not yet investigated the specific identification of *Contracaecum* larvae in fishes based on molecular approach in Iraqi waters, and this requiring further investigation. So, this study becomes the first molecular investigation on this group in Iraq.

Adult worms of *Contracaecum* spp. were detected from six species of aquatic birds: *Egretta alba*, *E. garzetta*, *Ardeola ralloides*, *Botaurus stellaris*, *Ardea purpurea* and *Ceryle rudis* from Bahr Al-Najaf depression (Al-Awadi *et al.*, 2010) without diagnosed at species level.

The main problems regarding diagnosis of this parasitic larval nematode in freshwater fish of Iraq can be summarized as follows:

1- Identification of nematodes based on morphometric and meristic of sex organs and their appendages in both male and female of nematode, hence the larva not reach the sexual maturity and the sex organs not developed in both sex.

2- There is no molecular genetic characterization according to current standards for accurate identification to support taxonomy of this nematode in Iraq.

The present study is a preliminary investigation toward molecular genetic characterization and description of *Contracaecum* larvae in Iraq, by

using a combined molecular and ultra-morphological approach (scanning electron microscopy) based on sequence data of well-identified adults and *Contracaecum* larval types in GenBank. Previous studies showed that this approach is useful for reliable identification of *Contracaecum* larvae to species level (Shamsi *et al.*, 2011).

The main purpose of this study is:

1- To know the prevalence of *Contracaecum* larvae among fishes in Sulaimani Province and to discover the genetic diversity of *Contracaecum* larvae that widely distributed nematodes of fishes.

2- To know the relationship between these recorded parasites with those recorded internationally.

3- Identification of the freshwater fishes in Sulaimani Province, Kurdistan Region-Iraq and compared with those reported in the Iraqi literature and to report possible new fish species, and new locality records.

4- To search new fish species in this region and their phylogenetic relationship by using morphological and molecular data.

CHAPTER TWO
LITERATURE REVIEW

LITERATURE REVIEW

Importance of fish

Fishes are cold-blooded vertebrates; they inhabit different kinds of environment ranging from deep water of ocean to boundless surface of the open sea and fast running stream, the muddy waters, brackish, estuaries, stagnant pools and under the ground in caves (Shammi & Bhatnagar, 2006). Fish have a global significance as a source of food, for sport, for their ornamental appeal, and as experimental models for research. The most important of fish are serving as food for human, their lipids, which usually contains high amount of omega-3 fatty acids (e.g. EPA and DHA), they are a good source of easily digestible protein, and its amino acid profile usually contains most of the essential amino acids which is required to humans for balanced diet. They are also rich source of fat-soluble and B-group vitamins. In addition, they possess a great economic, medicinal, and industrial, values as well as providing employment for millions of people in the world (Hoole *et al.*, 2001; Erkan & Bilen, 2010; Pal *et al.*, 2018).

The first taxonomic studies of ichthyofauna in Iraq started with Johann J. Heckel in 19th century. He described 17 species from Tigris River at Mosul City in northern Iraq (Jawad, 2012; Kaya *et al.*, 2016). There are a few works on ichthyofauna in Kurdistan Region of Iraq including the study of ichthyofauna in Lesser Zab by Al-Rawi *et al.* (1978) and Dokan and Derbandikhan Lakes by Ciepielewski *et al.* (2001); Abdullah (2006); Abdullah *et al*. (2007); Abdullah & Abdullah (2018) and Greater Zab by Agha (2017).

The knowledge concerning the fish fauna of Kurdistan Region of Iraq is limited to fish parasitic studies carried out by Abdullah & Rasheed (2004a;

2004b); Abdullah (2002; 2005); Bashê (2008); Shwani (2009); Nasraddin (2013); Abdullah & Abdullah (2013a; 2013b; 2015a; 2015b; 2016) and Bilal *et al.* (2017).

Recently new fish species are being described from this Region. For instance, Freyhof *et al.* (2014) described two new species *Paracobitis molavii* in Zalm Stream in Sulaimani Province and *Paracobitis zabgawraensis* in Rean Stream near Ziraran in Erbil Province. Freyhof *et al*. (2016) recorded *Eidinemacheilus proudlovei* a subterranean loach from an aquifer into an ephemeral spring flowed into a small stream, which belongs to the Tabeen drainage in Sulamani Province. Freyhof & Abdullah (2017) recorded two new loaches *Oxynoemacheilus gyndes* and *O. hanae* in headwater streams of the upper Sirwan in Sulamani Province. Also, Freyhof & Geiger (2017) recorded *Oxynoemacheilus zarzianus* in a spring fed stream in the Qalachulan River drainage in Sulamani Province. In this investigation, we have summarized the available information on the biodiversity of fishes in Sulaimany Province that were collected in one year alone.

Importance of Nematode

Nematodes are among the most abundant animals on earth, they commonly known as roundworms and belong to the phylum Nematoda. They are bilaterally symmetry, elongated and cylindrical in shape, tapering at each end and lacks body segmentation and they possess pseudocoel (Hoole *et al*. 2001; Gerald & Roberts, 2009). The nematodes show a very wide range of ecological adaptation. Most of them are free-living found in soil, fresh, brackish and sea waters, other are facultative or obligate parasites attacking both animals and plants (Anderson, 1988).

Nematodes represent the most frequent and the most important parasites of fishes in fresh, brackish, and marine environments throughout the world. They attack most body organs and parasitizing them as adults and larvae. Some nematode species are known as the agents of serious fish diseases causing considerable losses in fish cultures and in some regions and some of them cause important public health problems for human such as anisakidosis (anisakiasis), gnathostomosis or paracapillariosis (Moravec, 2007). The nematode parasites infect various tissues and organs of fish such as stomach, intestine, liver, gonads, visceral mesenteries, peritoneum body cavity, blood vessels, swim bladder, connective tissues, fins, orbits of the eye and brain. Most species of the nematodes in adult stage live in the alimentary canal except the family Philometridae which are found in body cavity, liver and gonads (Akhater, 2008).

The family Anisakidae has been universal distributed, it is found in marine fish in temperate and cold regions and few has been recording freshwater fish (Berland, 1991; Moravec, 1994).

Family: Anisakidae Skryabin and Karokhin, 1945

The Family Anisakidae is identified by their cuticle without spines or supplementary ridge-like or finger-like structures, esophagus with ventricle, either esophageal or intestinal caeca present or both. Blind processes are occasionally absent or several in number. They are parasites of freshwater and migratory fish.

The genus belong this family are determined by presence or absence and the number of intestinal caeca (Fig. 1). Anterior intestinal caecum present in *Procaecum*, posterior esophageal caecum present in *Raphidascaris*, two caeca present, intestinal pointing anteriorly and esophageal pointing

posteriorly found in *Contracaecum*, intestinal canal without caeca found in *Anisakis* (Bykhovskaya-Pavlovskaya *et al*., 1962).

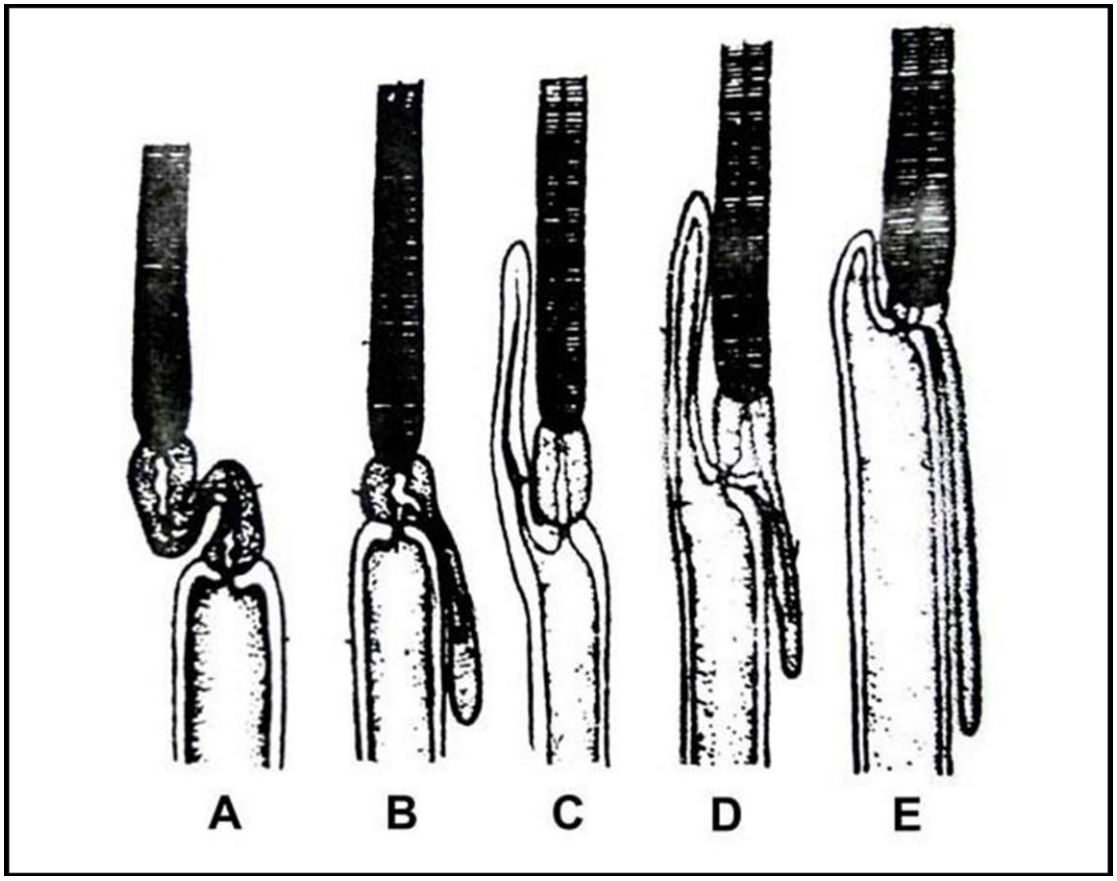

Fig. (1): Anterior parts of the digestive canal of some Anisakidae according to Bykhovskaya-Pavlovskaya *et al.* (1962).
A= *Anisakis*; **B=** *Raphidascaris*; **C=** *Procaecum*; **D=** *Contracaecum*; **E=** *Goezia*

Genus: *Contracaecum* Railliet and Henry, 1912

The genus *Contracaecum* is charachterized by intestinal canal sends off two blind caeca at boundary between esophagus and midgut, esophageal caecum longer than intestinal caecum. Six labia present. Intermediate labia are relatively short and most half as long as main labia. Numerous forms with scoop-like depressions present on internal surface of labia. Male has

pre-anal and post-anal papillae. Spicules are equal size. Vulva most often found in anterior half body. They are oviparous; their larvae parasitize freshwater fishes (Bykhovskaya-Pavlovskaya *et al*., 1962).

Classification of *Contracaecum*

The classification of *Contracaecum* is shown below according to Schmidt & Roberts (2009).

Kingdom: Animalia

Phylum: Nematoda

Class: Chromadorea

Subclass: Chromadoria

Order: Rhabditida

Suborder: Spirurina

Infraorder: Ascaridomorpha

Superfamily: Ascaridoidea

Family: Anisakidae

Subfamily: Contracaecinae

Genus: *Contracaecum*

Life Cycle of *Contracaecum*

The life cycle of *Contracaecum* follows the general Anisakid nematode life cycle pattern, including eggs, four larval stages (L1–L4) and the adult stages in the final host (Fig. 2). The life cycle involves a variety of hosts that are transferred through the aquatic food chain (Anderson, 2000).

The nematode eggs are excreted through feces of final hosts and embryonate in the water, the developing larva undergoes two moults and attaining the third larval stage (L3). Both hatched L3 (Fig. 3) or those still

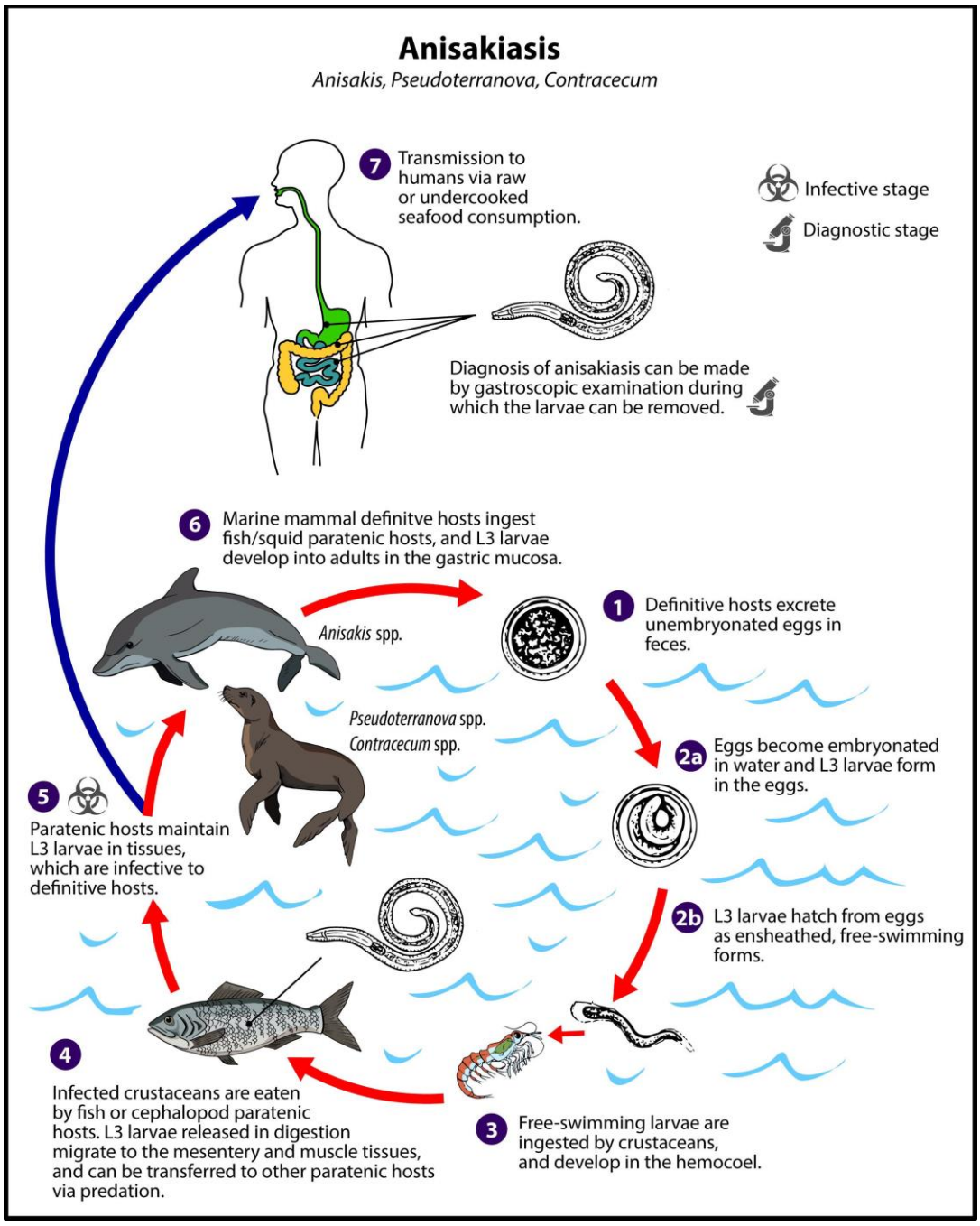

Fig. (2): Life cycle of *Contracaecum* according to Center for Diseases Control and Prevention (CDC) (https://www.cdc.gov/)

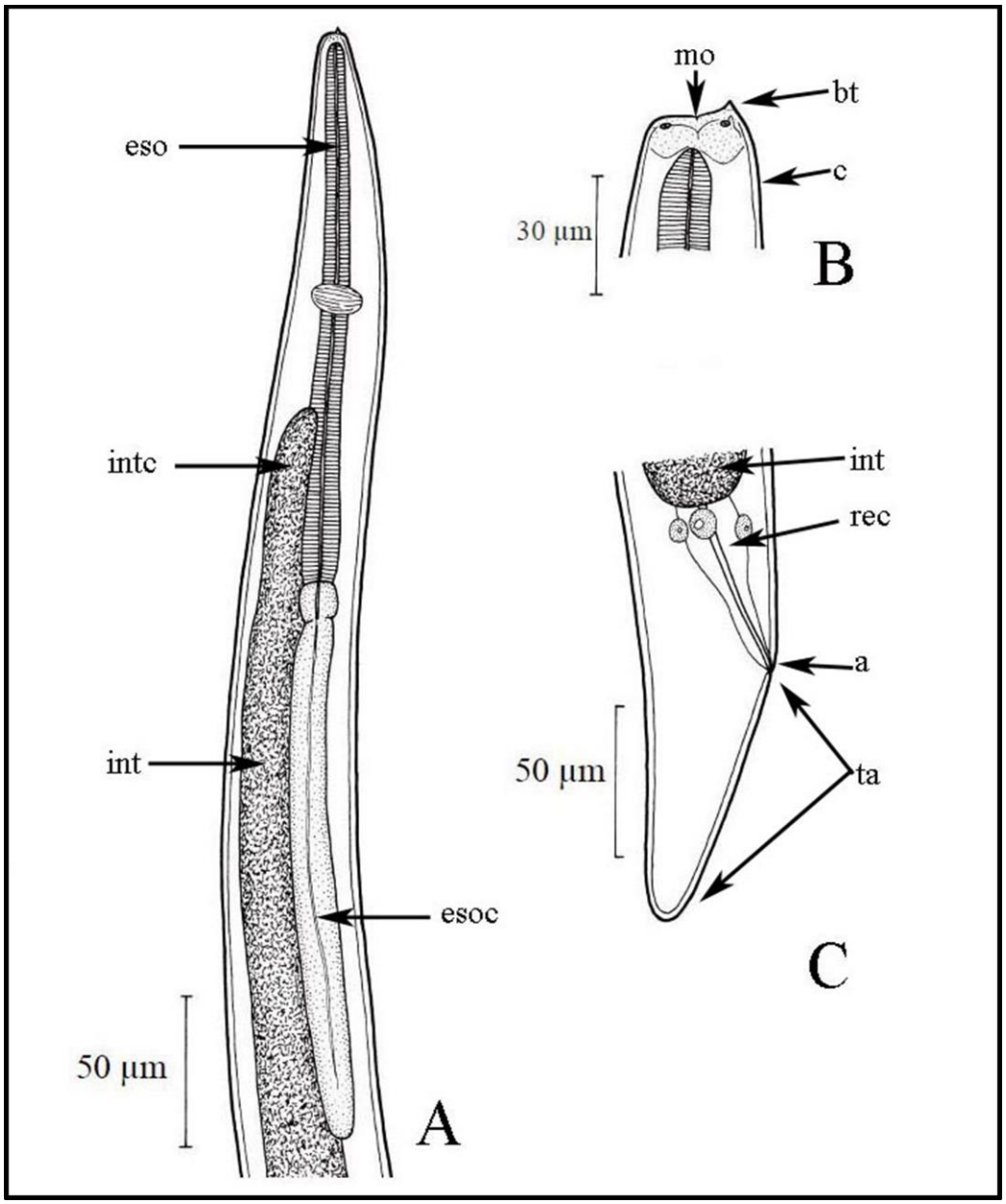

Fig. (3): Third larval stage of *Contracaecum* larva according to Moravec (2009).

A- Anterior part of the larva

B- Head region of the larva

C- Tail region of the larva.

a= anus; **bt=** boring tooth; **c=** cuticle; **eso=** esophagus; **esoc=** esophageal caecum; **int=** intestine; **intc=** intestinal caecum; **mo=** mouth opening; **rec=** rectum; **ta=** tail.

inside the egg shells can infect crustacean (copepods) and fish which both can serve as a paratenic hosts (Køie, 2001; Moravec, 2009). The various predatory fish species serve as paratenic hosts. Inside the paratenic hosts the larvae are capable of re-infecting the paratenic hosts without further molting. Consequently, piscivorous birds and mammals when eating the paratenic hosts may accumulate enormous of the larvae (Lile, 1998).

Some study on *Contracaecum* larvae around the world

The information about *Contracaecum* parasitize in fish around the world are very tremendous. For this reason, the present review will be limited to cover about the last two decades, and will cover only those which parasitize in fishes only. Although there are a few publications on the specific identification of the larval stages of *Contracaecum* in fishes from the world. The review in the present study is divided in to two categories as follows:

1- Optical microscopy study of *Contracaecum* larvae

Barson (2004) recorded *Contracaecum* larvae in the body cavity of catfish *Clarias gariepinus* from Lake Chivero in Zimbabwe. Bergmann & Motta (2004) isolated *Contracaecum* larvae from abdominal cavity of Mayan cichlid fish *Cichlasoma urophthalmus* in southern Florida in USA. Barson & Avenant-Oldewage (2006) noted *Contracaecum* larvae in the abdominal cavity of catfish *Clarias gariepinus* from the Rietvlei Dam near Pretoria, South Africa.

Nagasawa (2012) reviewed two main types of a single species of *Contracaecum* larvae in 13 species of marine teleost and six species of pinnipeds based on morphology under optical microscope, *C. osculatum* sensu lato and *C. osculatum* A, in Japan. Gholami *et al.* (2014) discovered

encysted *Contracaecum* larvae attached to the outer part of intestine of *Capoeta damascina* caught in the Kor River Basin, southwestern Iran.

During compared parasite communities study in two fish species (*Hoplias malabaricus* and *Hoplerythrinus unitaeniatus*), Alcântara & Tavares-Dias (2015) isolated *Contracaecum* larvae in both fishes which they collected them from Amazon River system in Brazil.

In USA, Valles-Vega *et al.* (2017) recorded *Contracaecum* larvae in mullet fish (*Mugil curema*) collected from Bahía de La Paz, Baja California Sur, Mexico. Ribeiro *et al.* (2017) observed third larval stage of *Contracaecum* in the viscera (mucosa of the stomach, mesentery, pancreas and peritoneal fat) of introduced fish in Brazil (*Clarias gariepinus*) collected from a lake in the disctrict of Tocos, in the city of Campos dos Goytacazes, State of Rio de Janeiro, Brazil.

During observation for parasitic nematodes Sorour & Hamouda (2019) recorded third larval stage of *Contracaecum* that attached to alimentary canal and mesentery of infected African sharp-tooth catfish (*Clarias gariepinus*) that collected in Lake Nasser in Egypt. Lima *et al*. (2019) reported *Contracaecum* larvae in Pyloric cecum, intestines, liver, mesentery and gonads of two exotic fish species *Acestrorhynchus pantaneiro* and *Trachelyopterus lucenai* collected from the Tramandaí River Basin in southern Brazil.

2- Scanning electron microscopy and Molecular study of *Contracaecum* larvae

In Poland, Szostakowska & Fagerholm (2007) recorded the third larval stage of *C. rudolphii* B from crucian carp *Carassius carassius* collected from Selment Wielki Lake in Mazury and two strains (*C. rudolphii* A and B) from Caspian round goby *Neogobius melanostomus* collected from a brackish-water region in Baltic Sea, Gdańsk Bay at the Polish coast by using molecular approach by amplifying and sequencing internal transcribed spacers (ITS-1 and ITS-2) regions. Shamsi & Aghazadeh-Meshgi (2011) used molecular approach for identification of *Contracaecum* laravae obtained from intestine and body cavity of barboid fishes caught in Parishan Lake, the largest freshwater lake in Iran. ITS-1 region of the larvae were amplified and sequenced (452 bp) and the molecular study indicated that the larvae were *C. multipapillatum*.

Abdul Jabbar *et al*. (2013) sequenced the ITS-1 and ITS-2 of the ribosomal DNA (rDNA) for *Contracaecum* larvae collected from three fish species (*Aldrichetta forsteri*, *Mugil cephalus* and *Pseudocaranx dentex*) in southern Western Australia. The ITS sequences determined the larva collected from *Aldrichetta forsteri* and *Mugil cephalus* were belong to *C. multipapillatum* while those collected from *Pseudocaranx dentex* was belongs to *C. ogmorhini*. Garbin *et al*. (2013) collected 16 *Contracaecum* L3 larvae isolated from anchovy fish (*Engraulis anchoita*) collected from Bahía Engaño, Chubut, Argentina. The entire larva sequenced for three genes: mitochondrial cytochrome oxidase-2 (COX-2), mitochondrial ribosomal RNA (rrnS), ITS-1 and ITS-2 of the nuclear ribosomal DNA region. The sequence alignments obtained from ITS-1 and ITS-2 regions of the rDNA

indicated *C. pelagicum.* In addition, the ultra-structural studies under scanning electron microscope (SEM) were carried out on L3 larvae.

Mattiucci *et al*. (2015) collected 272 *Contracaecum* larvae from some marine fish (*Chionodraco hamatus*, *Trematomus bernacchii*, *Trematomus hansoni*, *Trematomus newnesi*) collected from the natural Antarctic marine ecosystem. The larvae sequenced for COX-2 gene with 519 bp. The genetic analysis was revealed that the larvae belong to *C. osculatum*. In addition they recovered a significant genetic variation among the larvae of *C. osculatum* recovered in fish hosts, the sibling species were *C. osculatum* D and *C. osculatum* E.

Shamsi *et al.* (2017) recorded third larval stage of *Contracaecum* in the intestinal tissue of carp caught from Coonancoocabil Lagoon, New South Wales, Australia, and described genetically as *C. bancrofti*. Younis *et al.* (2017) isolated encysted third larval stage of *Contracaecum* from the body cavity of *Lates niloticus* and *Hydrocynus forskahlii* and free third larval stage in brancheal chamber of *Oreochromis niloticus* and *Tilapia galilaea* collected from Lake Nasser, Egypt. The ITS-1 and ITS-2 of nuclear ribosomal DNA from isolated larvae sequenced and phylogenetic analyses showed that the larval nematode belonged to *C. multipapillatum*. Also, light and scanning electron microscope (SEM) studies were done.

Zuo *et al*. (2018) sequenced the COX-1 and COX-2 genes in the third larval stage of *Contracaeum* collected from liver of Baltic fish (*Gadus morhua*) in Baltic Sea. The sequencing indicated that the larva belongs to *Contracaecum osculatum*. In addition, SEM was conducted for the larva. Shamsi *et al*. (2018) recorded the third stage larva of *Contracaecum* in eight fish species (*Carassius auratus*, *Melanotaenia fluviatilis*, *Misgurnus*

anguillicaudatus, *Cyprinus carpio*, *Gambusia holbrooki*, *Hypseleotris* sp., *Nematalosa erebi*, *Retropinna semoni*) collected from Murrumbidgee River, South-eastern Australia. The nematode larvae were identified as *Contracaecum bancrofti* using a combined morphological and molecular approach. The ITS-1 and ITS-2 regions of ribosomal DNA used for the molecular study.

Molnár *et al.* (2019) recorded *Contracaecum rudolphii* type B larvae in bream *Abramis brama* collected from Balaton Lake in Hungary, and in common carp *Cyprinus carpio* collected from Hévíz Lake directly connected to Balaton Lake by using ITS as a genetic marker. Pekmezci & Yardimci (2019) recorded *Contracaecum* L3 larvae isolated from *Mugil cephalus* collected in the Aegean Sea in Turkey. The *Contracaecum* L3 larvae were specifically identified as *C. overstreeti* based on molecularly characterization of ITS, rrnS and COX-2 genes. Pinheiro *et al.* (2019) studied morphology, morphometry, prevalence and ultramorphology (using SEM) of *Contracaecum* larvae in cichilid fish (*Astronotus ocellatus*) collected in Tapajós River in the municipality of Santarém in Pará State, Brazil.

Study on *Contracaecum* larvae in Iraq

In Iraq, the first record of *Contracaecum* larvae was done by Herzog (1969) who studied 16 species of fishes collected from different inland water of Iraq, and isolated *Contracaecum* larvae in 10 different freshwater fishes, these were: *Arabibarbus grypus* (reported as *Barbus grypus*), *Carasobarbus luteus* (reported as *B. luteus*), *Heteropneustes fosilis*, *Leuciscus vorax* (reported as *Aspius vorax*), *Luciobarbus esocinus* (reported as *B. esocinus*), *L. xanthopterus* (reported as *B. xanthopterus*), *Mesopotamichthys sharpeyi*

(reported as *B. sharpeyi*), *Mystus pelusius*, *Planiliza abu*. (reported as *Mugil abu*) and *Silurus triostegus*.

Shamsuddin *et al*. (1971) published the second paper on fish parasite in Iraq and isolated *Contracaecum* larvae in *S. triostegus* and *P. abu* (reported as *M. abu*) collected from several local fish market in Baghdad in which the fishermen caught them from Amara, Habbaniyah, Kut Thar thar Lakes, and Tigris and Euphrates Rivers.

Mhaisen (1986) recorded *Contracaecum* larvae from 14 fish species in Sahtt Al-Arab River and the north west of the Arab Gulf. Mhaisen *et al.* (1986) isolated the same larvae from intestine, liver, gonads and body cavity of *C. luteus*, *A. vorax* and *L. abu* collected from Mehajeran Greek Western tributaries of Shatt Al-Arab River, South of Basrah city.

Ali *et al*. (1987) observed the third larval stage of *Contracaecum* from coelom of *Acanthobrama centisquama*, *C. macrostomus*, *H. fossilis*, and *Leuciscus cephalus* collected from Tigris River in Baghdad Province. Khalifa *et al.* (1987) found *Contracaecum* larva from the stomach wall of Jirri (*S. triostegus*) in several parts of Tigris River from Northwest of Baghdad City.

Ali *et al*. (1989) recorded *Contracaecum* larvae from *Planiliza abu* in Babylon fish farm from Hilla City. Khalifa (1989) isolated *Contracaecum* larvae in *Barbus grypus* and *B. xanthopterus* collected in some ponds in several parts of Baghdad and Samarra regions and Al-Tharthar Canal.

Balasem *et al.* (1993) found encysted *Contracaecum* larvae from kidney of *P. abu* and the body cavity of *H. fossilis* collected from Tigris River at Al-Zaafaraniya South of Baghdad, Iraq. Mhaisen *et al*. (1993) recorded *Contracaecum* larvae in some freshwater and marine fishes collected from Basrah Province including *Aspius vorax*, *Barbus grypus*, *B. luteus*, *B.*

sharpeyi, B. xanthopterus, Heteropneustes fossilis, Liza abu, L. clussumieri, Mystus pellusius and Silurus triostegus.

Tahir *et al.* (1994) recorded the epidemiology of *Contracaecum* larvae in *Planiliza abu* collected from five different region of Al-Najaf Province (Al-Najaf City center, Al-Kufa, Al-Mikhshab, Al-Hurriah, and Al-Qadisyah). Rahemo & Al-Abbadi (1994) isolated it from body cavity and intestine of *P. abu* from Al-Gharaf River passing through Shatra Town from Thi-Qar district, South of Iraq.

Mhaisen *et al*. (1997) recorded *Contracaecum* larvae from *Aspius vorax* collected from Euphrates River in Anbar Province, Iraq. Mhaisen *et al*. (1999) found *Contracaecum* larvae from intestine of *Liza abu* collected from Al-Habbaniya Lake.

Rahemo & Al-Niaeem (2001) recorded one encysted *Contracaecum* larvae from external wall of European catfish (*Silurus glanis*) collected from Tigris River in Al-Rasheedya from Mosul City. Al-Nasiri *et al.* (2002) collected *Contracaecum* larvae from intestine wall, liver, spleen and gall bladder of common carp (*Cyprinus carpio*) from artificial lake at Baghdad Province. Al-Niaeem & Al-Azizz (2002) isolated *Contracaecum* larvae in *Barbus sharpeyi* from Qarmat Ali River north of Basrah City.

Abdu-Fraj & Ftohe (2008) collected *Contracaecum* larvae in the stomach of *Silurus glanis* caught in the Tigris River in Musul City. Al-Jadoa (2008) discovered the *Contracaecum* larvae in *P. abu* (reported as *Liza abu*) from a local drainage net from north of Al-Diwanyia Province. Al-Zubaidy (2009) found these larvae in mugilid fish (*P. abu*) from three different localities (Hilla River, Al-Furat fish farm and Al-Mahaweel Drain) in Babylon Province in the middle of Iraq, the larvae were found as a free in the body cavities, most often in the abdominal or pericardial cavity, some

encapsulated on the external walls of stomach, intestine, liver, heart, and gonads of fishes.

Al-Awadi & Mhaisen (2010) found encysted *Contracaecum* larvae from kidneys, liver and mesenteries of both *Aphanius dispar*, *Barbus grypus* in the body cavity of *Gambusia affinis* and in the intestine, kidneys, liver, mesenteries and spleen of *Liza abu* collected from Bahr Al-Najaf depression, Southwest of Al-Najaf Al-Ashraf City, middle of Iraq. Al-Saadi *et al*. (2010) isolated *Contracaecum* larvae from mesenteries and liver of *A. vorax*, intestinal wall of *B. grypus*, body cavity of both *B. sharpeyi* and *B. xanthopterus* and from the intestinal wall and gonads of *L. abu*. The fish samples were collected from Al-Husainia Creek, north east of Karbala Province, middle of Iraq.

Al-Alusi (2011) founded *Contracaecum* larvae in the body cavity of *C. carpio* and the external surface of intestine of *B. xanthopterus* and *A. vorax* which they collected from Euphrates River at Al-Haklania District, Al-Anbar Province.

Mhaisen *et al*. (2012) reported *Contracaecum* larvae in the intestine, liver and body cavity of *C. carpio* and *P. abu* collected from Al-Furat fish farm in Babylon Province. Mhaisen & Al-Nasiri (2012) listed the fish parasites of Salah Al-Deen Province included *Contracaecum* larvae isolated from body cavity, intestine, liver and gonads of seven host species including: *A. marmid*, *A. vorax*, *B. grypus*, *B. xanthopterus*, *C. regium*, *S. triostegus*, *Varicorhinus trutta*.

Awad & Al-Tameemi (2013) isolated *Contracaecum* larvae in the coelom of *Poecilia latipinna* which collected in the crop of pied kingfisher (*Ceryle rudis*) were collected from Al-Mashab Marsh north of Basrah Province. Eassa *et al*. (2014) recorded *Contracaecum* larvae in the digestive

tract of common carp (*C. carpio*) collected from three different regions (Qurna, Dayer and Abu Al-Khaseeb) in Basrah Province. Ali *et al*. (2014) recorded two types of third larval stages (L3) of *Contracaecum*. *Contracaecum* sp. 1 recorded from body cavity, internal organs and mesenteries of 20 fish species in different water bodies in Basrah Province including *Aphanius dispar*, *Arabibarbus grypus*, *C. luteus*, *M. sharpeyi*, *Luciobarbus xanthopterus*, *A. sellal*, *Carassius auratus*, *C. idella*, *C. carpio*, *H. fossilis*, *Johnius* (*Johnius*) *belangerii*, *L. vorax*, *L. abu*, *L. subviridis*, *M. mastacembelus*, *Mystus pelusius*, *Otolithes ruber*, *Silurus triostegus*, *Synaptura orientalis*, *Tenualosa ilisha*. While, *Contracaecum* sp. 2 larvae recorded from body cavity and mesentries of *H. fossilis* from Shatt Al-Arab River near Nahr Khooz Village.

Mhaisen *et al.* (2015) listed the *Contracaecum* larvae of fishes from the Euphrates River at Al-Musaib City, middle of Iraq and recorded the *Contracaecum* larvae from the intestine of *Alburnus orontis*, *Arabibarbus grypus*, *Carasobarbus luteus*, *Coptodon zillii*, *Leuciscus vorax*, *Luciobarbus xanthopterus*, *Mastacembelus mastacembelus*, *Mystus pelusius* and *Silurus triostegus*. Mohammad (2016) discovered *Contracaecum* larvae in the pericardial cavity of exotic fish (*Tilapia zillii*) that collected from Al-Dalmaj Marsh from Al-Diwaniya Province in the middle of Iraq and from central marshes of Thi Qar Province in south of Iraq. Al-Mayali & Al-Mahi (2016) isolated third larval stage of *Contracaecum* from intestine of *Liza abu* collected from Diwaniya River in Diwaniya.

Al-Kinanny & Al-Obaidy (2017) found *Contracaecum* larvae in the intestine of *Liza abu* collected from Euphrates River in The-Qar Province. Mhaisen *et al*. (2017) listed the parasite fauna of fishes from different marshlands and markets of Basrah Province and reported the *Contracaecum*

larvae from body cavity, mesenteries and different internal organs of *C. luteus*, *C. carpio*, *H. fossilis*, *L. vorax*, *M. sharpeyi*, *P. abu*, *Poecillia latipinna* and *S. triostegus*.

Al-Moussawi *et al*. (2018) isolated encapsulated *Contracaecum* larvae in the intestine wall of Asian catfish (*S. triostegus*) were caught from the Tigris River in Baghdad Province. Mhaisen & Rubaie (2018) listed the parasites of native fishes of Babylon Province and reported encysted third larval stage of *Contracaecum* in the intestine, body cavity, stomach, intestine, liver, heart and gonad of *A. orontis*, *A. grypus*, *C. luteus*, *C. zillii*, *L. vorax*, *L. xanthopterus*, and in the intestine of *M. mastacembelus*, *M. pelusius*, *P. abu* and *S. triostegus* from the Euphrates river at Al-Musaib City.

Mhaisen *et al*. (2018) listed the parasites of freshwater fishes of Salah Al-Din Province and recorded *Contracaecum* larvae from body cavity, viscera, liver body cavity, and muscular layer of stomach and external surface of intestine of *A. marmid*, *A. grypus*, *C. trutta*, *C. regium*, *L. vorax*, *L. xanthopterus* and *S. triostegus*.

Mhaisen (2019) listed the fish parasites of Thi-Qar Province in south of Iraq and recorded the *Contracaecum* larvae from body cavity and intestine of *C. luteus*, visceral cavity of *C. zillii*, body cavity and intestine of both *L. vorax* and *L. xanthopterus* body cavity and intestine of *P. abu* and body cavity and intestine of *S. triostegus.* Mhaisen *et al*. (2019) listed the fish parasites of Al-Diwaniyah Province and reported *Contracaecum* larvae in the intestine, body cavity, liver, spleen of *A. grypus*, *C. luteus*, *C. carpio*, *L. vorax*, *L. xanthopterus*, and *P. abu.*

Study on *Contracaecum* larvae in Kurdistan Region-Iraq

The first study of *Contracaecum* larvae in Kurdistan Region-Iraq was done by Ali (1989) who collected *Contracaecum* larvae in the liver, body cavity, intestine and intestinal wall of *Carasobarbus luteus* (reported as *Barbus luteus*) from Greater Zab River near Iski-Kalak, Erbil city. Abdullah (1990); Abdullah & Rasheed, (2004b) recorded large numbers of different stages of *Contracaecum* larvae were obtained from the stomach and intestine of *Cyprinus carpio*, *Luciobarbus esocinus* and *Arabibarbus grypus*, from coelom of *Luciobarbus subquincunciatus*, *Squalius lepidus* and *Luciobarbus kersin*, from liver and intestine of *Luciobarbus barbulus* and *Chondrostoma regium*, from liver of *Cyprinion macrostomum* and *Carasobarbus luteus*, and from coelom and gonad of *Luciobarbus xanthopterus* collected from Dokan Lake in Sulaimani Province.

Nawab Al-Deen (1994) isolated *Contracaecum* larvae from mesenteries of *Leuciscus vorax* (reported as *Aspius vorax*) from Lesser Zab River near Altun Kupri, Erbil Province.

Abdullah (2000) recorded *Contracaecum* larvae from intestinal wall of *L. esocinus* (reported as *Barbus esocinus*), *C. luteus* (reported as *B. luteus*) and *L. xanthopterus* (reported as *B. xanthopterus*), and in the liver of *A. grypus* (reported as *B. grypus*) and *C. carpio* collected from local market in Erbil City.

Abdullah (2002); Abdullah & Mhaisen (2011) made a survey on fish parasites from both Lesser Zab and Greater Zab River, *Contracaecum* larvae were recorded from body cavity of *A. marmid* from Lesser Zab and Greater Zab Rivers, from intestine and intestinal wall of *Capoeta damascina* (reported as *Barbus belayewi*). From *C. luteus* (reported as *B. luteus*), intestinal wall of *C. carpio, Garra rufa, H. fossilis,* liver, gonads and

intestine of *L. barbulus* (reported as *B. barbulus*), muscles of *Mastacembelus mastacembelus*, body cavity, gonads and liver of *Squalius lepidus* (reported as *Leuciscus lepidus*).

Abdullah (2004) recorded *Contracaecum* larvae from intestinal wall of *Planiliza abu* (reported as *Liza abu*) collected from Mortuka Stream from southeast of Erbil Province and from a fish farm pond located in south of Erbil Province.

Abdullah (2005) surveyed the parasites of 17 species of fishes from Darbandikhan Lake in Sulaimani Province and detected the third larval stage (L3) of *Contracaecum* in body cavity of *A. marmid*, body cavity, liver and intestine of *C. regium* (misspelled as *C. regius*), *H. fossilis*, stomach, intestine and external wall of intestine of *L. esocinus* (reported as *B. esocinus*), muscles of *M. mastacembelus*, intestine and liver of *S. cephalus* (reported as *L. cephalus*) from Darbandikhan Lake.

Rahemo *et al.* (2005) recorded encysted *Contracaecum* larvae from the liver of *S. cephalus* (recorded as *Leuciscus cephalus*) caught from Serchinar Stream in Sulaimani Province. Abdullah & Mhaisen (2006) recorded *Contracaecum* larvae from liver, intestine and external wall of intestine of *C. carpio* collected from Lesser Zab River.

Shwani (2009); Shwani & Abdullah (2010) recorded many encysted third larval stage of *Contracaecum* found in ovary and liver of *S. triostegus* collected from Greater Zab River near Guwer District southwest of Erbil City. Abubaker (2015) reported *Contracaecum* larvae in the intestine of *C. trutta* and *C. macrostomum* collected from Greater Zab River near Aski-Kalak, Erbil Province.

Scanning Electron Microscopy study on *Contracaecum* in Iraq

There is no any ultra-structural study of *Contracaecum* larvae except a single study were done by Rahemo & Nawab Al-Din (2009) used scanning electron microscopy (SEM) for observation of three nematode including *Contracaecum* larvae, that collected from *Acanthobrama marmid* and other fishes that collected from Tigris River. According to Mhaisen (2019) a total of 42 fish host species are known for *Contracaecum* larvae in Iraq including Kurdistan Region of Iraq. However, there is no detailed morphological study and a specific identification based on molecular characterization according to current standards for identification of this parasites.

CHAPTER THREE
MATERIALS AND METHODS

MATERIALS AND METHODS

Description of study area

Sulaimani Province is a mountainous province in Kurdistan Region, located in the northeast of Iraq. It is situated between the latitudes of 35^0 05' and 36^0 30' and between longitudes of '44^0 25' and 46^0 20' (Fig. 4)**.** It is located close to the Iraqi-Iranian border. There are many water bodies in this province in addition to the two large rivers, namely, the Lesser Zab (Little Zab) and Sirwan Rivers which they pass through this province and both originate in Iran and they finally feed the Tigris River (Al-Saudi, 1976). The sampling areas were divided into eight area and 26 localities (Table 1).

I- Collection, Examination and Identification of Fishes

Fishes were caught weekly by pulsed DC electro-shock device (SAMUS 1000). The device was set up as follow: frequency of output pulses 50 Hz, duration of output pulses 5 milliseconds, amplitude of the output pulses 320V, output power 220W . This device just anesthetizes the fishes for a few seconds without harming the fishes or other water creatures. Also, gill netting, and hook were used (SAMUS 1000).

Fishes were kept in a cool box with river water and transferred to the laboratory of Parasitology, Department of Biology, College of Science, University of Sulaimani. The fishes were identified based on their morphometric and meristic characters, so the measurements made point to point never by projections, according to Beckman (1962), Kottelat & Freyhof (2007) and Coad (2010) and the scientific names for fishes were identified according to Froese & Pauly (2020).

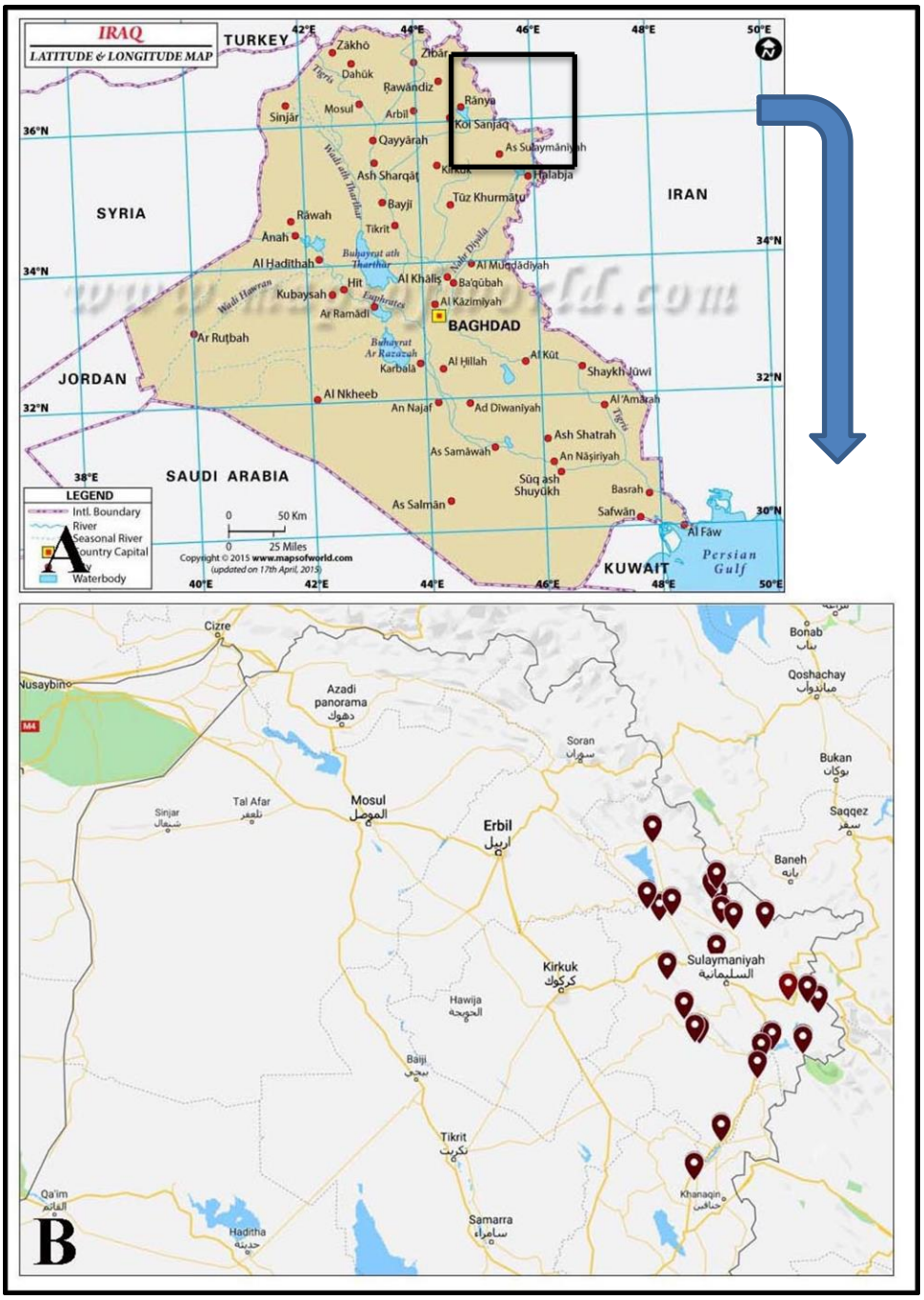

Fig. (4): A- Map of Iraq.

B- Map of north Iraq, the spots showing the study area (Google map).

Table (1): List of sampling locations and their geographical specification in the present study

Areas	Localities	Coordinates
Sulaimani city	Sarchnar	N 35.586029^0, E 45.381129^0
Sharbazher	Awkurte	N 35.964140^0, E 45.396998^0
	Bardbard	N 35.915020^0, E 45.367732^0
	Kareza	N 35.78366^0, E 45.418174^0
	Khewata	N 35.75105^0, E 45.7062^0
	Kunamasi	N 35.79695^0, E 45.41370^0
	Qashan	N 35.867406^0, E 45.403254^0
	Wazha	N 35.750105^0, E 45.496155^0
Sharazwr	Darbandikhan lake	N 35.11315^0, E 45.70650^0
	Kawta	N 35.095823^0, E 45.92079^0
	Reeshen	N 35.354654^0, E 45.961988^0
	Saraw	N 35.3722^0, E 45.8351^0
	Shameran	N 35.117285^0, E 45.719307^0
	Taparezina	N 35.30135^0, E 46.0284^0
	Zmkan	N 35.089628^0, E 45.918118^0
Qaradagh	Astely Ashty	N 35.1634^0, E 45.2600^0
	Hazar Kani	N 35.1713^0, E 45.2252^0
Garmyan	Banikhelan	N 35.063475^0, E 45.648604^0
	Kalar	N 34.649567^0, E 45.379714^0
	Kulajo	N 34.452221^0, E 45.197145^0
	Sangaw	N 35.292413^0, E 45.160485^0
Bazyan	Basara	N 35.5001^0, E 45.0621^0
Dukan	Chami Rezan	N 35.8084^0, E 45.021689^0
	Swrqawshan	N 35.872773^0, E 44.944338^0
	Tabin	N 35.8336^0, E 45.104544^0
Ranya	Darbany Ranya	N 36.216218^0, E 44.99143^0

Some unrecognized fishes, after anesthesia (by electro-shock device), were fixed in 5% formalin and stored in 70% ethanol, a pieces of its' muscle were directly fixed and stored in 99% ethanol, at room temperature for molecular studies (Freyhof *et al*., 2016).

Fish Identification

Fishes can be identified according to morphological features and/or molecular based identification.

1- Morphological features

Fishes are primarily classified according to morphological characters such as body shape, mouth structures, mouth position, types of caudal fin, types of fish scale, color, size, presence or absence of scales, barbel ….. etc. The morphological characteristics for the fish species identification based on two main criteria:

A- Morphometric measurements

Morphometric measurement refers to the quantitative analysis of fish form (shape). Morphometric characters are measurable characters of a fish and they have an important value in fish identification. All measurements were to the nearest 0.1 mm using dial calipers. Measurements were taken on the left side. All measurements were taken in a straight line and not over the curve of the head or body (Fig. 5) (Freyhof & Abdullah, 2017).

The basic measurements include:

1- Total length.
2- Fork length.
3- Standard length.
4- Pre-dorsal length

5- Post dorsal length
6- Head length
7- Body depth
8- Snout length
9- Eye diameter
10- Postorbital length
11- Length of dorsal fin
12- Length of pectoral fin
13- Length of pelvic fin
14- Pre-pelvic length
15- Pre-anal length
16- Length of anal fin
17- Depth of caudal peduncle
18- Length of caudal peduncle
19- Length of base of anal fin
20- Length of base of dorsal fin
21- Inter-orbital width
22- Dorsal head length

B- Meristic measurements

According to Kottelate & Freyhof (2007), most fish species can be adequately described by meristic (countable) characters (Fig. 6) such as:

1- Scale counts (e.g. lateral line scales, transverse scales between lateral line and origin of dorsal fin …etc.)
2- Numbers of gill rakers
3- Number of vertebrae
4- Fin ray counts (dorsal, pelvic, anal …etc.)

5- Pharyngeal tooth formula

6- Presence and number of barbels

7- Fin numbers and positions

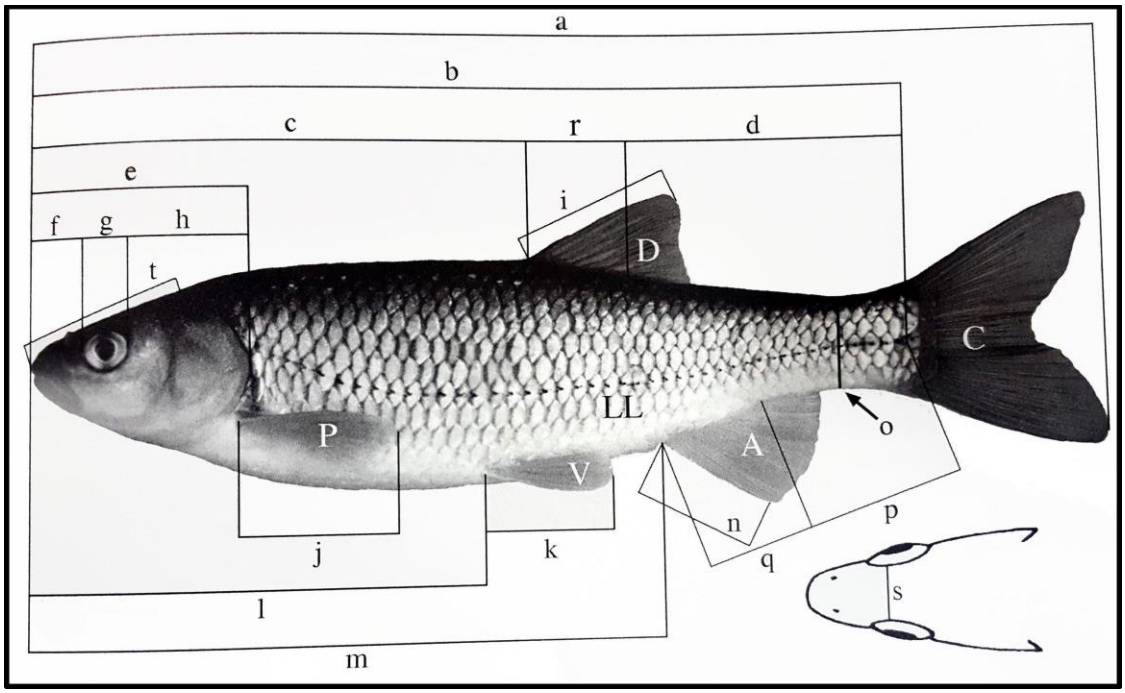

Fig. (5): Morphometric measurements of fish according to Kottelate and Freyhof (2007).
A= anal fin; **C=** caudal fin; **D=** dorsal fin; **P=** pectoral fin; **V=** pelvic fin; **LL=** lateral line; **a=** total length; **b=** standard length; **c=** predorsal length; **d=** postdorsal length; **e=** head length; **f=** snout length; **g=** eye diameter; **h=** postorbital length; **i=** dorsal fin length; **j=** pectoral fin length; **k=** pelvic fin length; **l=** pelvic length, **m=** preanal length; **n=** anal fin length; **o=** caudal peduncle depth; **p=** caudal peduncle length; **q=** base of anal fin length; **r=** base of dorsal fin length; **s=** interorbital width; **t=** dorsal head length.

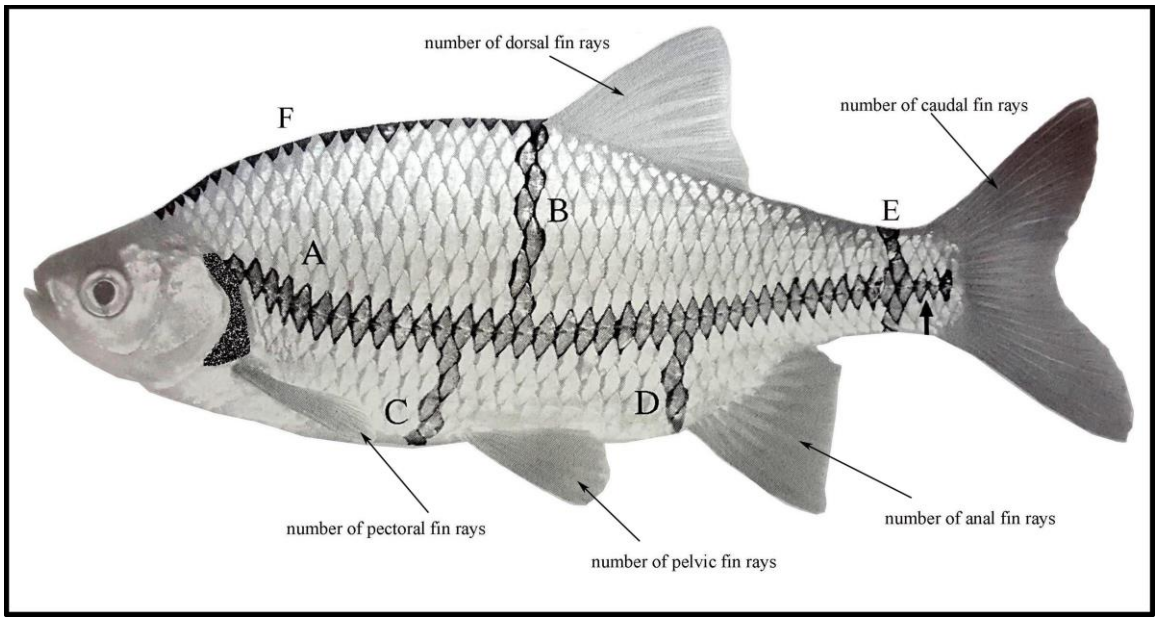

Fig. (6): Meristic measurement of fish according to Kottelate and Freyhof (2007).
A= lateral line scales; **B=** transverse scales between lateral line and origin of dorsal fin; **C=** transverse scales between lateral line and midline of belly; **D=** transverse scales between lateral line and origin of anal fin; **E=** circumpedunclar scales; **F=** predorsal scales; **Short arrow=** last scale on body and first scale on caudal fin.

2- Molecular Study of fish

A- DNA extraction

In order to assess DNA analysis of the collected fish (*Alburnoides velioglui*), four specimens of this fish species fixed in 99% ethanol (the specimens were collected from Zalm Stream near Tapazerina Village) and were analyzed molecularly. Prior to molecular studies, each specimen was identified on the basis of morphometric and meristic characters.

Genomic DNA was extracted from individual fish muscle tissues using QIAamp® DNA Mini Kit. According to the manufacturer's protocols as follow:

Procedure

1) The fish muscle (≤ 25 mg) were cut into small pieces and placed in a 1.5 ml microcentrifuge tube. Added 180 µl of tissue lysis buffer (ATL buffer) and 20 µl protenase K, mixed by vortex and incubated at 56°C for 1-3 hours until completely lysed.

Note: the mixture mixed by vortex during incubation to aid the digestion (lysis) process.

2) Two hundred microliters (200 µl) of lysis buffer (AL buffer) added. Mixed thoroughly by vortex for 15 seconds.
3) The mixture incubated at 70°C for 10 minutes. Briefly centrifuged the tube to remove drops from lid.
4) Two hundred micro liters (200 µl) ethanol (96-100%) added. Mixed by vortex for 15 seconds. Briefly centrifuged the tube to remove drops from the lid.
5) The mixture pipetted on to the QIAamp Mini spin column (in a 2 ml collection tube). Centrifuged at 8,000 rpm for 1 minute. The flow-through and collection tube discarded.
6) The QIAamp Mini spin column placed in a new 2 ml collection tube and 500 µl washing buffer 1 (AW1 buffer) added. Centrifuged at 8,000 rpm for 1 minute. The flow-through and collection tube discarded.
7) The QIAamp Mini spin column placed in a new 2 ml collection tube and 500 µl washing buffer 2 (AW2 buffer) added. Centrifuged at full speed (14,000 rpm) for 3 minutes. The flow-through and collection tube discarded.

Note: The QIAamp Mini spin column placed in a new 1.5 ml microcentrifuge tube and centrifuged at full speed (14,000 rpm) for 1 minute. This eliminates the chance of possible buffer AW2 carryover.

8) The QIAamp Mini spin column placed in a new 1.5 microcentrifuge tube. 100 µl Elution buffer (AE buffer) or distilled water added and incubated at room temperature for 1 minute. Centrifuged at 8,000 rpm for 1 minute to elute the DNA.

Note: step 8 was repeated for increased DNA yield with a further 100 µl AE buffer or distilled water (QIAamp® DNA Mini Kit).

B- DNA amplification

The polymer chain reaction technique (conventional PCR) was used to amplify the cytochrom c oxidase I (COX-1) gene using primers, forward FishF1-(5′-TCAACCAACCACAAAGACATTGGCAC-3′) and reverse FishR1-(5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) designed by Ward *et al*. (2005).

The PCR reactions (in a volume of 30 µl) was performed in 25 mM Tris-HCl, pH 9.0, at 25°C, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mg/ml gelatin, 200 µM de dATP, dGTP, dTTP, 100 µM [α32-P]dCTP (0.05 µCi/nmol) and 12.5 µg activated salmon sperm DNA and 10 pmol of each primer and 1.5 U *Taq* polymerase (Canvax Biotech, S.L.). The PCR reactions were done in a thermocycler (Applied Biosystems 2720, USA) using the following cycling instructions: 94°C for 5 min (initial denaturation), 35 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 7 min, 4°C ∞. Two microliters of genomic DNA and water free deionized distilled water (ddH_2O) were added to each PCR reaction.

C- **Gel Electrophoresis**

All PCR products obtained from thermocycler machine were verified on a 2% agarose gel with power supply 80V for 30 minutes.

The Materials and Equipment Used

1) Agarose powder.
2) 1X TAE buffer (Tris-Acetate EDTA buffer).
3) DNA stain (Good View^{TM}) (SBS Genetech Beijing, China).
4) Electrophoresis equipment.
5) 6X Gel loading dye (50 mM EDTA, 0.2% SDS, 50% glycerol, 0.05% w/v bromophenol blue).
6) 1Kb DNA ladder (Vivantis, Malaysia).

Preparation of 50X TAE buffer (0.04 M, pH 8.5)

1) 800 ml of dH_2O prepared in a suitable container.
2) 242 g of Tris base (SBS Genetech Beijing, China) added to the distilled water.
3) 18.61 g of Disodium EDTA added to the mixture.
4) 57.1 g of Acetic acid added to the mixture.
5) Distilled water added until the volume is reached 1L.

Procedure

1) 300 ml 1X TAE prepared (Prepared from the 50X stock of TAE by dissolving 1ml of the stock in 49 ml of autoclaved distilled water).
2) For a 2% w/v gel, 2 g agarose added to 100 ml 1X TAE buffer.
3) The solution heated to boiling in the microwave to dissolve the agarose, until the agarose gel becomes clear in texture.

4) 5 µl of DNA stain (Good View^{TM}) (SBS Genetech Beijing, China) added to the dissolved agarose and mixed gently.

5) The combs fixed into electrophoresis tray, and then poured the melted agarose onto the electrophoresis tray box.

6) Let the gel to cool in room temperature. It became solid and looked like cloudy.

7) The combs removed carefully, and put the gel into the electrophoresis box, 1X TAE (electrophoresis buffer) poured over the gel until covered the wells properly.

8) 6 µl of the prepared samples (5 µl of product and 1 µl of 6X loading dye) added for each well. The samples should carefully place into adjacent wells by using a micropipette and a steady hand.

9) The samples were electrophoresed at 80 V for 30 min.

10) The gel removed from the box carefully, and then put into a gel documentation system, SMARTDOC^{TM} blue light box (ACCURIS^{TM} instrument).

11) Protective glasses wore or covered the light box with protective shield when the blue light is on, then exposed the gel to camera to took a picture of the bands.

12) The genome size detected by comparing the obtained band with the 1 kb DNA ladder (Vivantis, Malaysia) that applied on the gel plate. The expected size of the PCR amplicon was 655 bp for *Alburnoides velioglui* (COX-1).

D- Gel purification

After detecting the DNA bands (amplicons) on the gel, the amplicon were purified using EasyPure® Quick Gel Extraction Kit (TRANSGEN BIOTECH), according to the manufacturer's protocols as follow:

Procedure

1) The DNA fragments (amplicon) were cut from the gel using a razor blade. The gel slice weighted, and put the gel slice into a 1.5 ml eppendorf tube.

Note: the gels were cut into as small pieces as possible, to ensure the gel to be completely dissolved.

2) 3 volume of gel solubilization buffer (GSB) added to 1 volume of gel (100 mg ≃ 100 µl). Incubated at 55 ^{0}C for 6-10 minutes until the gel slice has completely dissolved. The tube mixed every 2-3 minutes to help dissolve the gel during the incubation.

Note: once the gel is completely dissolved, watch the color of solution. The color of solution should be the same as GSB.

3) When the solution temperature fell back to room temp., transferred the solution to spin column. Incubated for 1 minute at room temp., then centrifuged 10,000 rpm for 1 minute. The flow-through discarded.
4) 650 µl of Wash buffer (WB) added, centrifuged at 10,000 rpm for 1 minute. The flow-through discarded.
5) The empty column centrifuged at 10,000 rpm for 1-2 minutes to remove the residual WB.
6) The spin column placed in a clean 1.5 ml microcentrifuge tube. 30-50 µl of Elution buffer (EB) or sterile distilled water added directly to the center of the spin column. Incubated the column at room temperature for 1 minute. Centrifuged at 10,000 rpm for 1 minute to elute the DNA. The purified DNA is ready to use or can be stored at -20°C (EasyPure® Quick Gel Extraction Kit).

E- DNA sequencing

The resulted products (purified amplicons) sent to the Macrogen Company in South Korea for nucleotide sequence analyses by a dideoxy termination method using Genetic analyzer 3500, an Applied Biosystems (USA) DNA Sequencer in the two directions (forward and reverse) by the same PCR-used primers.

F- Computer based sequence analysis

The resulted COX-1 sequences (forwards) were compared with their complements (reverses) and then adjusted using online software tool (bioinformatics.org\sms\rev_comp.html) to obtain reverse complement. Then the resulted sequences were aligned to each other using multiple sequence alignment program by using the online software tool CLUSTALW (genome.jp/tools-bin/clustalw) to get the most homologous sequences (one sequence). Subsequently, the obtained sequence put into the NCBI Blast program for homology search (http://www.ncbi.nlm.nih.gov/).

The sequence results of COX-1 fragments obtained from four *Alburnoides velioglui* were installed into the MEGA X version 10.7.1 software program (Kumar *et al*., 2018). To unify the length of the sequences, the common 605 bp length of COX-1 segment was selected and used for phylogenetic analysis to determine the most appropriate sequence evolution model for the given data, treating gaps and missing data with the partial deletion option. The sequences were aligned using CLUSTALW alignment for constructing the trees of evolutionary development. The trees of all isolated species were constructed based on the Maximum Likelihood (ML) method and Tamura-Nei model (Tamura & Nei, 1993).

II- Fixation, Preservation and Examination of *Contracaecum* larvae

The fishes were examined for encysted and free *Contracaecum* larval nematode parasites. The fishes were opened from the ventral side. Body cavity, stomach, spleen, liver, kidneys, heart, muscles, swim bladder and gonads were all examined for presence of larval *Contracaecum* cysts. The gastrointestinal tract was dissected out from the rectum to the esophagus and opened longitudinally and examined internally and externally carefully under dissecting microscope (Amlacher, 1970).

1- Light microscopy (LM)

The encysted *Contracaecum* larvae were collected from different organs and washed with normal saline solution (0.9%) in a glass petri dish, with the aid of very fine needles tear them and they were fixed in hot 4% formalin solution (60°C) for relaxing their bodies and stored in 70% ethanol. A small piece of the mid-body of some larvae were excised for molecular study (Shamsi & Aghazadeh-Meshgi, 2011) and the rest of the larval nematodes were cleared with glycerine 5%, 10%, 50% (each for 1 hr.) and 100% (for 1-2 hr.) then mounted in Jelly glycerine (Moravec *et al.*, 2009; Moravec & Yooyen, 2011).

All measurements of parasite were made with an eyepiece ocular micrometer (Olympus, Japan) and given in millimeters. Photos were taken with Sony Optical Steady Shot Digital camera model DSC-W570, 16.1 mega pixels. The detected parasites were identified according to their morphology and key features and descriptions of Bykhovskaya-Pavlovskaya *et al*. (1962); Hoffman (1998); Anderson (2000); Shamsi *et al*. (2011).

2- Scanning Electron Microscopy (SEM)

The larvae were removed from the cyst for scanning electron microscopy (SEM) study, specimens were fixed in 4% (v/v) hot formalin solution (60°C), then preserved in 70% (v/v) ethanol and later they were post fixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series with ethanol (1:1), (1.5-0.5) and absolute acetone, 15 minutes for each concentration. For drying a critical-point method was used by shaking the samples for 24 hours in centrifuge to pulled out the acetone, then embedded on the targets and sputter-coated with gold (Moravec *et al.*, 2009; Moravec & Yooyen, 2011). The specimens were examined in Geology Department, College of Science, University of Sulaimani by using a FEI Quanta 400 SEM at an accelerating voltage of 25 kV.

3- Molecular Study of *Contracaecum* larvae

A- DNA extraction

Prior to the molecular studies, each specimen was identified on the basis of morphological characteristics under an optical microscope. Genomic DNA was extracted from mid piece of individual larvae after being preserved directly in absolute ethanol (99%). A total of 30 larvae (3 for each infected fish species) were prepared for molecular study. The genomic DNA was extracted by using a QIAamp® DNA Mini Kit with slight modifications. In brief, the mid piece of individual larval parasites were cut into small pieces, digested for 1-3 h at 56°C with proteinase K in ATL buffer and eluted in 50 µl of AE buffer (QIAamp® DNA Mini Kit).

B- DNA amplification, Gel purification and Sequencing

The PCR technique (conventional) was used to amplify the ITS-1, ITS-2, and COX-2 regions. The specific primer sets, forward SS1F (5′-GTTTCCGTAGGTGAACCTGCG-3′) and reverse, NC13R (5′-GCTGCGTTCTTCATCGAT-3′), forward SS2F (5′-TTGCAGACACATTGAGCACT-3′) and reverse NC2R (5′-TTAGTTTCTTTTCCTCCGCT-3′) (Shamsi *et al.*, 2008), forward 210F (5′-CACCAACTCTTAAAATTATC-3′) and reverse 211R (5′-TTTTCTAGTTATATAGATTGGTTCAT-3′) (Nadler & Hudspeth, 2000) were used to amplify the two nuclear ribosomal markers (ITS-1 and ITS-2) and cytochrome oxidase II (COX-2), respectively.

The PCR reaction (in a volume of 30 µl) was performed in 25 mM Tris-HCl, pH 9.0 at 25°C and contained 50 mM KCl, 2 mM $MgCl_2$, 0.1 mg/ml gelatin, 200 µM dATP, dGTP and dTTP, 100 µM [α32-P] dCTP (0.05 µCi/nmol), 12.5 µg of activated salmon sperm DNA, 10 pmol of each primer and 1.5 U *Taq* polymerase (Canvax Biotech, S.L.). The PCR reactions were carried out in a thermocycler (Applied Biosystems 2720, USA) using the following cycling instructions: 94°C for 5 min (initial denaturation), 35 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 7 min, followed by holding at 4°C. Two microliters of genomic DNA (20-40 ng) in nuclease-free deionized distilled water were added to each PCR reaction. Samples with fish genomic DNA (extracted from muscle) were included in the PCR as negative controls; no amplicons were produced from these samples. Five microliters of each PCR product was examined on a 2% w/v agarose gel, stained with DNA stain (Good View^{TM} SBS Genetech Beijing, China) with power supply 80V for 30 minutes and photographed using a gel

documentation system. A 1000 bp DNA ladder (Vivantis, Malaysia) was used. The expected size of the PCR amplicon was 530 bp for *Contracaecum* larvae (ITS-1), 430 bp for ITS-2 and 629 bp for COX-2.

The amplicon were then purified using the EasyPure® Quick Gel Extraction Kit (TRANSGEN BIOTECH), according to the manufacturer's protocols. The resulting products were sent to Macrogen in South Korea for nucleotide sequence analysis by a dideoxy termination method using a Genetic Analyzer 3500 DNA sequencer (Applied Biosystems, USA) in both directions (forward and reverse) using the same PCR primers.

C- Computer based sequence analysis

The resulted ITS-1, ITS-2 and COX-2 sequences (forwards) were compared with their complements (reverses) and then adjusted using online software tool (bioinformatics.org\sms\rev_comp.html) to obtain reverse complement. Then the resulted sequences were aligned to each other using multiple sequence alignment program by using the online software program CLUSTALW (genome.jp/tools-bin/clustalw) to get the most homologous sequences (one sequence). Subsequently, the obtained sequence put into the NCBI Blast program for homology search (http://www.ncbi.nlm.nih.gov/). In addition, the multiple sequence alignment were done for each obtained sequences from each gene (ITS-1, ITS-2 and COX-2) in all *Contracaecum* larvae collected in the 10 different fish hosts by using the online software program CLUSTALW (genome.jp/tools-bin/clustalw), in order to obtain nucleotide variation among *Contracaecum* larvae in different fish host.

D- Phylogenetic analysis

For the phylogenetic study, the sequence data of ITS-1, ITS-2 and COX-2 fragments obtained from *Contracaecum* larvae collected from all different fish host species were installed into the MEGA X version 10.7.1 software program (Kumar *et al*., 2018). To unify the length of the sequences, the common 447, 268 and 475 bp length of ITS-1, ITS-2 and COX-2 segments respectively were selected and used for phylogenetic analysis to determine the most appropriate sequence evolution model for the given data, treating gaps and missing data with the partial deletion option. The sequences were aligned using CLUSTALW alignment for constructing the trees of evolutionary development. The trees of all isolated species were constructed based on the Maximum Likelihood (ML) method and Tamura-Nei model (Tamura and Nei, 1993).

Ethical Approval and/or Informed Consent

The care of experimental animals was consistent with Republic of Iraq animal welfare laws, guidelines and policies approved by University of Sulaimani Local Ethics Committee (Permit reference number 122/2020). All fishes were collected from both Lesser Zab and Sirwan River drainages, with required permissions of the Directorate Police of Forest and Regional Sulaimani Province (Permit reference number 1060/2018).

Criteria of Infection

The ecological terms (prevalence and mean intensity of infection) were used in the present study based on the terminology of Margolis *et al.* (1982):

A- Prevalence of Infection:

The percentage of investigated fish infected with a particular parasite species per the total number of host examined.

B- Mean intensity of infection:

Mean number of particular parasite species per infected host in a sample.

CHAPTER FOUR
RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

I- Identification of fishes

A total of 2122 freshwater fishes were collected randomly from different water bodies in Sulaimani Province during the period from January to the end of December 2018. Table (2) shows number of fish species in different families, their abundance in this area and status. In the present study the fish fauna of this area comprised 36 species in 26 genera and 10 families. The most diverse family was Cyprinidae with 14 species (38.88%) followed by Leuciscidae with 8 species (22.22%) then Nemacheilidae with 6 species (16.66%), then Xenocyprididae with 2 species (5.55%), Bagridae, Heteropneustidae, Mastacembelidae, Mugilidae, Siluridae and Sisoridae each with only one species (2.77%) (Fig. 7).

The native species comprised 31 species (86.11%) in eight families namely *Arabibarbus grypus*, *Barbus lacerta*, *Capoeta trutta*, *C. umbla*, *Carasobarbus kosswigi*, *C. luteus*, *Cyprinion kais*, *C. macrostomum*, *Garra rufa*, *Leuciscus vorax*, *Luciobarbus barbulus*, *L. esocinus*, *L. xanthopterus* (Cyprinidae), *Acanthobrama marmid*, *Alburnoides velioglui*, *Alburnus caeruleus*, *Alburnus mossulensis*, *Chondrostoma regium*, *Leuciscus vorax*, *Squalius cephalus* and *S. lepidus* (Leuciscidae), *Mystus pelusius* (Bagridae), *Mastacembelus mastacembelus* (Mastacembelidae), *Planiliza abu* (Mugilidae), *Eidinemacheilus proudlovei*, *Oxynoemacheilus gyndes*, *O. hanae*, *O. kurdistanicus*, *O. zarzianus* and *Turcinoemacheilus kosswigi* (Nemacheilidae), *Silurus triostegus* (Siluridae), and *Glyptothorax kurdistanicus* (Sisoridae). While, five exotic species (13.88%) were listed in three families including: *Carassius auratus*, *Cyprinus carpio* (Cyprinidae),

Hemiculter leucisculus and *Hypophthalmichthys molitrix* (Xenocyprididae), and *Heteropneustes fossilis* (Heteropneustidae).

Table (2): List of fishes collected from different water bodies in Sulaimani Province with their numbers and status.

Family and Scientific Names	Number	Status according to IUCN
Family: Cyprinidae Rafinesque, 1815		
Arabibarbus grypus **(Heckel, 1843)**	123	VU
Barbus lacerta **Heckel, 1843**	7	LC
Capoeta trutta **(Heckel, 1843)**	222	LC
Capoeta umbla **(Heckel, 1843)**	161	LC
Carasobarbus kosswigi **(Ladiges, 1960)**	5	VU
Carasobarbus luteus **(Heckel, 1843)**	89	LC
Carassius auratus **(Linnaeus, 1758)***	54	LC
Cyprinion kais **Heckel, 1843**	10	LC
Cyprinion macrostomum **Heckel, 1843**	322	LC
Cyprinus carpio **Linnaeus, 1758***	195	VU
Garra rufa **(Heckel, 1843)**	57	LC
Luciobarbus barbulus **(Heckel, 1849)**	108	NE
Luciobarbus esocinus **Heckel, 1843**	52	VU
Luciobarbus xanthopterus **Heckel, 1843**	31	VU
Family: Leuciscidae Bonaparte 1835		
Acanthobrama marmid **Heckel, 1843**	20	LC
Alburnoides velioglui **Turan, Kaya, Ekmekçi & Doğan, 2014****	22	NE
Alburnus caeruleus **Heckel, 1843**	7	LC
Alburnus mossulensis **Heckel, 1843**	62	NE
Chondrostoma regium **(Heckel, 1843)**	52	LC
Leuciscus vorax **(Heckel, 1843)**	1	LC
Squalius cephalus **(Linnaeus, 1758)**	37	LC
Squalius lepidus **Heckel, 1843**	62	LC
Family: Xenocyprididae Günther 1868		
Hemiculter leucisculus **(Basilewsky, 1855)***	121	LC
Hypophthalmichthys molitrix **(Valenciennes, 1844)***	2	NT
Family: Bagridae Bleeker, 1858		
Mystus pelusius **(Solander, 1794)**	8	LC
Family: Heteropneustidae Hora, 1936a		
Heteropneustes fossilis **(Bloch, 1794)***	8	LC
Family: Mastacembelidae Swainson, 1839		
Mastacembelus mastacembelus **(Banks & Solander, 1794)**	94	LC

Family: Mugilidae Cuvier, 1829 *Planiliza abu* **(Heckel, 1843)**	76	LC
Family: Nemacheilidae Regan, 1911 *Eidinemacheilus proudlovei* **Freyhof, Abdullah, Ararat, Hamad & Geiger, 2016**	40	NE
Oxynoemacheilus gyndes **Freyhof & Abdullah, 2017**	14	NE
Oxynoemacheilus hanae **Freyhof & Abdullah, 2017**	5	NE
Oxynoemacheilus kurdistanicus **Kamangar, Prokofiev, Ghaderi & Nalbant, 2014**	12	NE
Oxynoemacheilus zarzianus **Freyhof & Geiger, 2017**	2	NE
Turcinoemacheilus kosswigi **Bănărescu & Nalbant, 1964**	2	LC
Family: Siluridae Cuvier, 1816 *Silurus triostegus* **Heckel, 1843**	20	LC
Family: Sisoridae Bleeker, 1858 *Glyptothorax kurdistanicus* **(Berg, 1931)**	19	DD
Total	**2122**	**-**

=** Exotic fish; *=**New record in Iraq; **DD**= Data Deficient; **LC=** Least Concern; **NE**= Not Evaluated; **NT**=Near Threatened; **VU**= Vulnerable

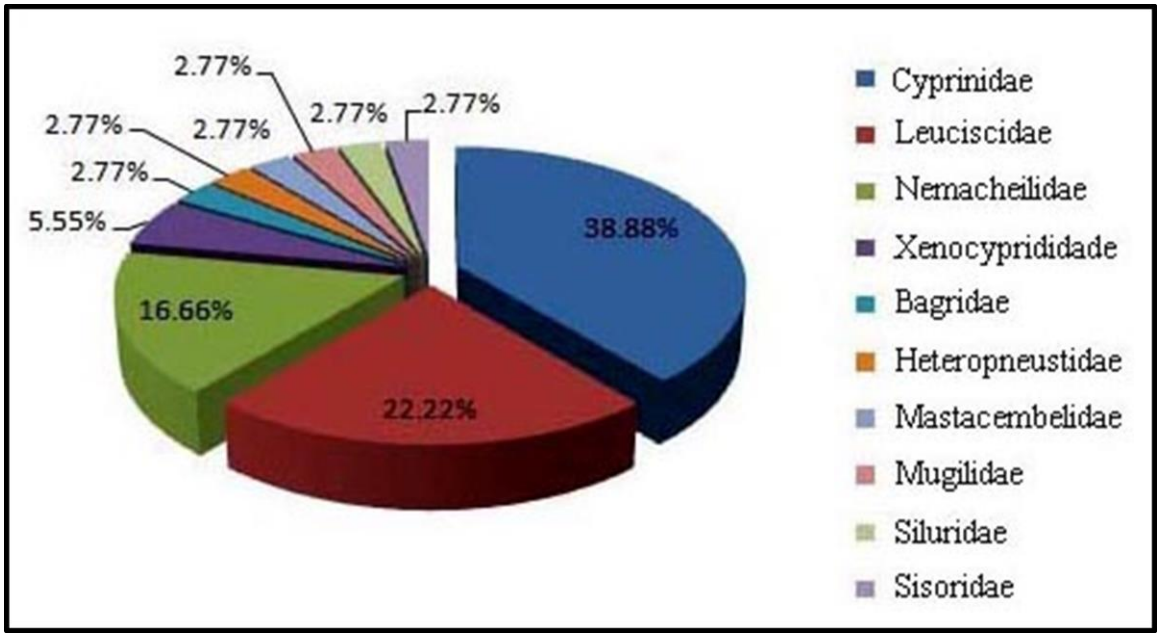

Fig. (7): Percentage composition of fish families collected in the present study

The native distribution of *C. auratus* is in northern Asia and China. *C. carpio* naturally found in Europe and Asia, In Iraq they were first introduced from Holland and Indonesia. *H. leucisculus* was originally described from Peking, China. The native range of this species is from Maritime Russia south through China to Korea and Vietnam. *H. molitrix* was originally described from China and the natural distribution is from the Amur River in the former U.S.S.R. southward to southern China. Also, *H. fossilis* was described from Tranquebar, Tamil Nadu, India. These fishes were introduced into Iraqi water bodies for different purposes such as food fish, phytoplankton control, and as a biological control of mosquito and snail in order to control the parasitic diseases especially malaria and bilharzia (Coad, 2010).

The most abundant and wide spread species recorded in this investigation was known *C. macrostomum* with prevalence 15.17%, followed by *C. trutta* with the prevalence of 10.46%, then *C. carpio* as a third rank with the prevalence 9.18%. It was clarified that *L. vorax* was scarce with the prevalence 0.047%.

According to International Union for Conservation of Nature (IUCN) red list of threatened species, four of the native species are vulnerable including *A. grypus, C. kosswigi*, *L. esocinus* and *L. xanthopterus* (Table 2).

Apparently, many factors may affect decreasing these fish species in Sulaimani Province water bodies such as illegal way of fishing, overfishing, fishing in a spawning season, climate change, flood, water pollutions, instruction of gravel mining on streams and rivers, and introducing the exotic species annually especially common carp which they compete the native species for the place and food. Moreover, demanding of local people on these types of fishes is another reason for more fishing by fisherman.

It is expected that the ichthyofauna of Sulaimani Province could be more than this investigation and need more ichthyologists to find them.

In the past years there were a few works on ichthyofauna in Sulaimani Province; Ciepielewski *et al.* (2001) mentioned the name of 20 species (*Barbus grypus*, *B. barbulus,*, *B. esocinus*, *B. kersin*, *B. longiceps*, *B. luteus*, *B. pectoralis*, *B. rajanonim*, *B. xanthopterus*, *Chondrostoma nasus*, *C. regium*, *Cyprinus carpio*, *Leuciscus cephalus*, *Mastacembelus mastacembelus*, *Silurus glanis*, *S. triostegus*, *Varicorhinus barroisi*, *V. damascinus*, *V. trutta* and *V. umbla*) during their investigation in both Dokan and Derbandikhan Lakes.

Abdullah (2006) recorded 23 species (*Acanthobrama marmid*, *Alburmus mossulensis*, *A. sellal*, *Barbus barbulus*, *B. belayewi*, *B. esocinus*, *B. grypus*, *B. kersin*, *B. luteus*, *B. subquincunciatus*, *B. xanthopterus*, *Capoeta trutta*, *Chondrostoma regium*, *Cyprinion macrostomum*, *Cyprinus carpio*, *Garra rufa*, *Leuciscus cephalus*, *L. lepidus*, *Varicorhinus trutta*, *Glyptothorax kurdistanicus*, *Heteropneustes fossilis*, *Liza abu* and *Mastacembelus mastacembelus*.) from Dokan Lake. Abdullah *et al*. (2007) recorded 26 species (*Acanthobrama marmid*, *Aspius vorax*, *Barbus barbulus*, *B. esocinus*, *B. grypus*, *B. kersin*, *B. lacerta*, *B. luteus*, *B. xanthopterus*, *Capoeta damascinus*, *C. trutta*, *Chondrostoma regium*, *Cyprinion macrostomum*, *Cyprinus carpio*, *Garra rufa*, *Hypophthalmicthyes molitrix*, *Leuciscus cephalus*, *L. lepidus*, *L. spurious*, *Varicorhinus barroisi*, *V. umbla*, *Silurus glanis*, *Glyptothorax kurdistanicus*, *Heteropneustes fossilis*, *Liza abu* and *Mastacembelus mastacembelus*) in Derbandikhan Lake. Rasheed (2011) recorded five species (*Barbus grypus*, *B. esocinus*, *Capoeta damascinus*, *Carassius auratus* and *Cyprinus carpio*) from Derbandikhan Lake, Abdullah & Abdullah (2018) recorded 17 species (*Arabibarbus grypus*, *Barbus*

barbulus, *Capoeta trutta*, *C. umbla*, *Carasobarbus luteus*, *Carassius auratus*, *Chondrostoma regium*, *Cyprinion macrostomum*, *Cyprinus carpio*, *Garra rufa*, *Hemiculter leucisculus*, *Hypophthalmichthys molitrix*, *Luciobarbus esocinus*, *Squalius lepidus*, *Mystus pelusius*, *Silurus triostegus*, and *Mastacembelus mastacembelus*) in the same lake.

It seems from the previous study that mentioned above the biodiversity of fish species in Sulaimani Province which recorded by researchers was very limited and nearly all of them were recorded the same species and they were not recording a new species, this is due to the way of specimen collection, nearly all researchers depended on the fisherman whom they use gillnetting or hock for fishing, and they couldn't collect and record those fishes which they never reach to enough size in order to capture by gillnet. Moreover, the place of fishing is another reason, most of researcher only collected the fish from the lakes and the large rivers, but they didn't collect fishes from small streams and springs. The evidence supporting this idea is the size of those fishes which they recorded by the researchers, most of them were fishes which they use as a food by local people and they present in the local markets.

In the present study, *Alburnoides velioglui* was recorded for the first time in Iraq which collected in Zalm Stream, Sirwan River drainage in Sulaimani Province, Kurdistan Region-Iraq. The following is an account on morphometric and meristic of the genus and the species of this fish:

Alburnoides Jeitteles, 1861

Sprilins of the genus *Alburnoides* Jeitteles, 1861 are small sized fish, which inhabit fast flowing waters especially small streams and is less frequent in the main flow of large rivers. It prefers well-oxygenated water, low in pollution, with hard streambeds. Also, found in the surf zone of lakes. The Genus *Alburnoides* is distinguished by having elongate anal fin, decurved lateral line often with a distinct spotting pattern (small black spot) on each sides of the lateral line pore and presence the orange base of the pectoral, pelvic and anal fins (Kottelate & Freyhof, 2007; Bogutskaya & Coad 2009; Coad 2010).

Alburnoides velioglui Turan, Kaya, Ekmekçi & Doğan, 2014

1- Morphological study of *A. velioglui*

A total of 22 *Alburnoides velioglui* (Fig. 8) were collected from Zalm Stream at Taparezina Village.

Morphological investigation of *A. velioglui* populations from Zalm Stream revealed as small fish, body moderately deep and slightly compressed in both sides. Head short less than its width, eyes large, Mouth is terminal, tip of both lips are equal, rounded tip snout. Lateral line pigmentation is distinct. Pectoral fins are short not reaching pelvic-fin origin. Pelvic fins are short, not reaching the origin of anal fin, but reaching the anus. Anal fin is slender. Ventral keel poorly developed between pelvic and anal fins and completely scaled. Caudal fin moderately forked.

Total length 50-55 mm, standard length 40-46 mm, head length 7-12 mm, body depth at dorsal fin origin 14-19 mm, eye diameter 4 mm, snout length 2.5-3 mm, post orbital length 5 mm, interorbital length 4 mm. The number of scales on lateral line is 45-50. The number of branched dorsal fin

ray 8½, branched pectoral fin ray 10-11, branched pelvic fin ray 6½, branched anal fin ray 13½, branched caudal fin ray 17.

The morphometric and meristic data of the present specimen are closely similar to the specimens of Turan *et al.* (2014) who recorded it for the first time from the northern Euphrates drainage (Sırlı, Karasu, Divriği and Sultansuyu Streams) in eastern Anatolia, Turkey. Based on the consulting with the specialized scientific side Dr. Jörg Freyhof, Museum für Naturkunde Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany and Dr. Cüneyt Kaya, Recep Tayyip Erdogan University, Faculty of Fisheries and Aquatic Sciences, Turkey, it was confirmed that this fish is *A. velioglui*.

In Iraq, there was only one species of *Alburnoides* recorded (*A. bipunctatus*) as a complex species (Coad, 2010). One specimen from Iraq in the Natural History Museum, London has no locality data and a second is listed as from Sarchinar in Sulaymani City. Although, Coad (2010) mentioned that it has probably been misidentified or confused with other species in Iraq. So, *Alburnoides velioglui* has been now recorded for the first time in Iraq from Zalm Stream near Taparezina Village a tributary of the Sirwan River in Sulaimani City, Kurdistan Region, Iraq during an expedition April 2018.

Alburnoides is belongs to Leuciscidae, according to Tan & Armbruster (2018) there are six genera with eight species of this family in Iraq. *Alburnoides* are wide spread from France to Afganistan (Kottelat & Freyhof 2007) and five species (*A. diclensis, A. emineae, A. idignensis, A. nicolausi, A.velioglui*) are known to occur in the Euphrates and Tigris River drainages (Turan *et al.* 2016). In addition, from adjacent Iran, 12 species (Jouladeh-

Roudbar *et al*., 2016; Esmaeili *et al*., 2018) and from Turkey 12 species of *Alburnoides* were recorded (Turan & Kaya, 2019; Kaya, 2020).

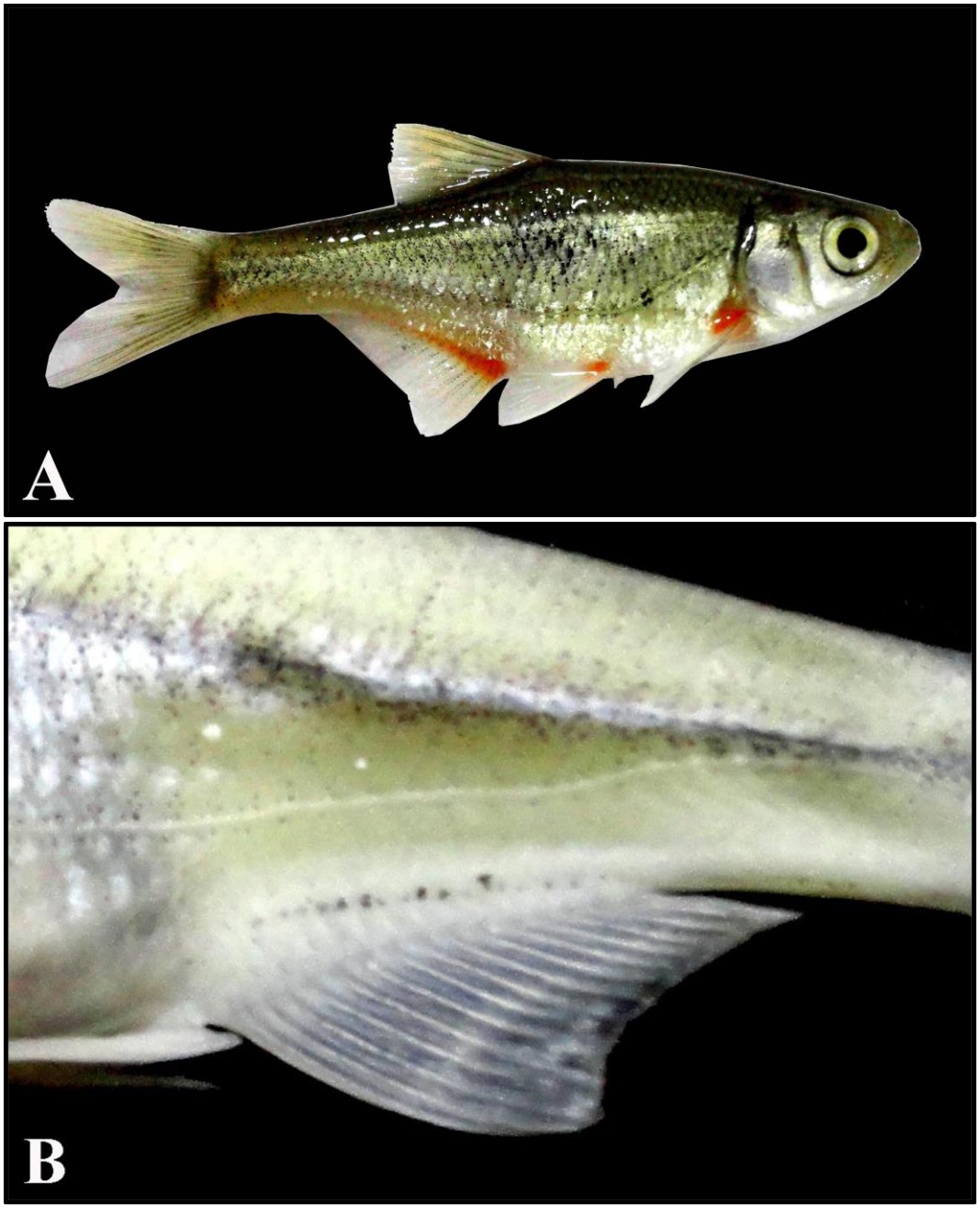

Fig. (8): *Alburnoides velioglui*
A- *Alburnoides velioglui*, 46 mm SL; Iraq: Zalm stream at Tapazerina village.
B- Anal fin of *Alburnoides velioglui*.

2- Molecular study of *A. velioglui*

The molecular examination of *A. velioglui* in the present study was done by amplifying the COX-1 gene region and sequencing the amplicon by using Genetic analyzer 3500, an applied Biosystems (USA). After the morphological identification, the COX-1 regions from individual fish were amplified by PCR from genomic DNA samples. Agarose gel electrophoresis demonstrated the size of COX-1 region was 655 bp (Fig. 9).

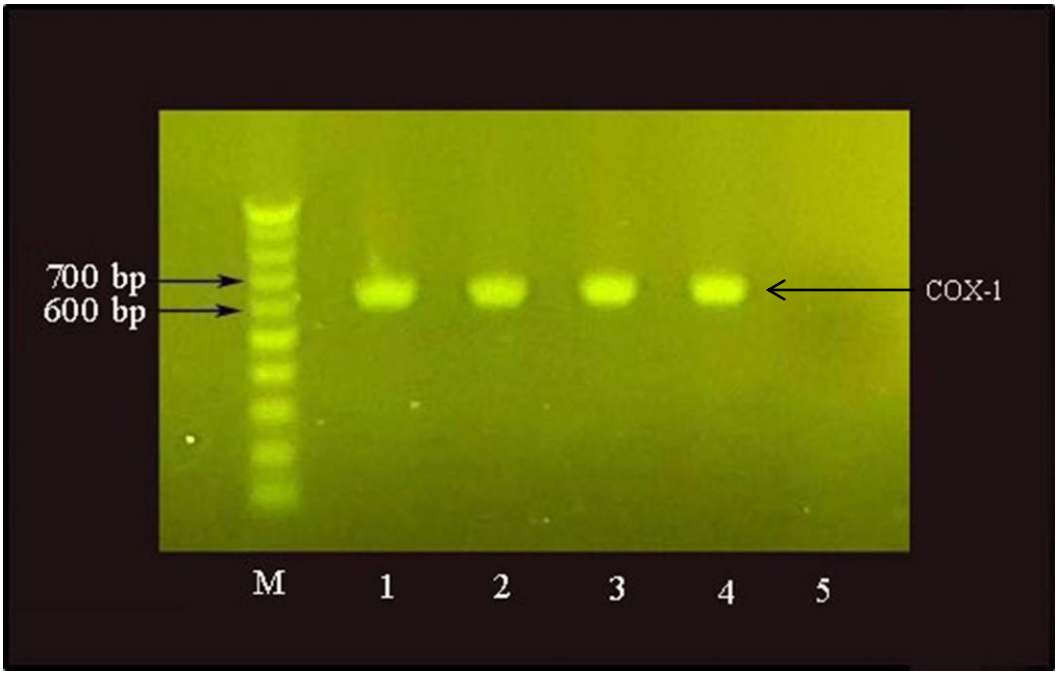

Fig. (9): PCR product of COX-1 sequences of *Alburnoides velioglui* on a 2% TAE agarose gel.
M= DNA ladder (1Kb); lanes **1,2,3,4=** PCR product of COX-1 sequences of *A. velioglui* ; **5=** negative control.

Sequence and phylogenetic analysis of *A. velioglui*

Alignment of resulted sequences revealed that there is no significant variation of COX-1 regions, which indicate the presence of only one fish species. The resulted COX-1 sequences obtained from the present fish show the highest similarity (97.19%) to COX-1 sequence of *Alburnoides qanati* (Accession number: KU705266) from the Gene Bank were blasted (online) which sequenced by Roudbar *et al.* (2016).

The results are in agreement with Roudbar *et al.* (2016) who used mtDNA COX-1 625 locus to study nine species of the genus *Alburnoides* from different river drainage in Iran, and Levin *et al*. (2018) who used COX-1 as a DNA barcode marker to create a reference dataset of Caucasian *Alburnoides.*

For many years, only subspecies of *A. bipunctatus* had been recognized (Barbieri *et al*., 2017), while recent molecular and phylogenetic studies described several populations in Europe and Asia (Bogutskaya and Coad 2009; Geiger *et al*. 2014; Stierandova *et al*. 2016; Roudbar *et al*. 2016; Levin *et al*. 2018; Esmaeili *et al*., 2018).

Alburnoides velioglui was discovered for the first time in 2014 by Turan *et al.* (2014) in Turkey. There is no molecular study on *Alburnoides* in Iraq before. This is the first molecular approach toward this fish in Iraq. The genetic characterization of *A. velioglui* in the present study is available in the GenBank database. COX-1 sequence obtained was deposited in GenBank under the accession number (MN893770).

In order to verify the morphological identities, the obtained sequence data of COX-1 from *A. velioglui* collected in stream Zalm were subjected to phylogenetic analysis. The sequence data aligned with the 31 data sequence of COX-1 form other *Alburnoides* including 13 genotypes that detected in

GenBank (Accession numbers: HQ600666 *A. prespensis*, KJ552616 *A. fangfangae*, KJ552639 *A. devolli*, KU705247 *A. idignensis*, KU705255 *A. namaki*, KU705256 *A. coadi*, KU705259 *A. nicolausi*, KU705266 *A. qanati*, KU705271 *A. samiii*, KX189528 *A. eichwaldii*, KX189559 *A. gmelini*, KX189569 *A. fasciatus*, and KX189574 *A. kubanicus*), and 18 sequences of different genotypes (*Alburnoides diclensis*, *A. emineae* and *A. velioglui*) obtained from Dr. Jörg Freyhof, Museum für Naturkunde Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany (unpublished data), also *Alburnus caeruleus* (MG775321) was used as outgroup detected in GenBank.

Phylogenetic analysis was conducted in MEGA X (Kumar *et al*., 2018). The evolutionary histories were inferred by using the Maximum Likelihood (ML) method and Tamura-Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (-1986.68 for COX-1) are shown (Fig. 10). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The *A. velioglui* in the present study were clustered in the same clade of *A. velioglui* which previously collected by Dr. Jörg Freyhof in both Iraq and Turkey. Moreover, the phylogenetic tree of the COX-1 sequences using ML analysis indicated that *Alburnoides velioglui* clades were distinct species by high bootstrap values (Fig. 10).

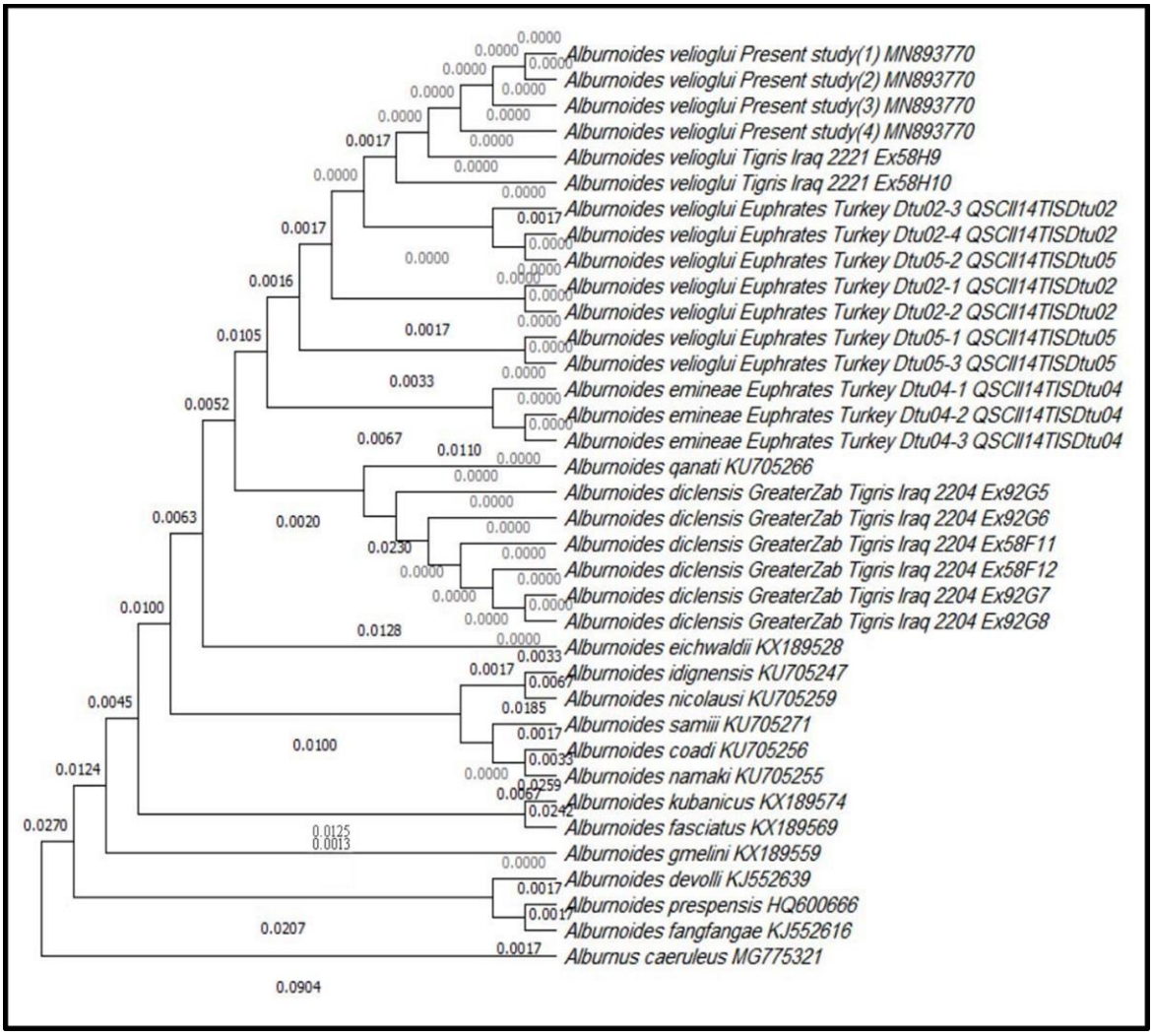

Fig. (10): Phylogenetic relationships between *Alburnoides velioglui* from the present study and other *Alburnoides* species as inferred by maximum likelihood obtained from COX-1. The species number (1-4) indicates the isolated species sequenced from the present study. *Alburnus caeruleus* was used as outgroup.

II- *Contracaecum* larvae

1- Prevalence of *Contracaecum* larvae

A total of 2122 fish were examined for parasitic larval *Contracaecum* nematodes, 30 fishes belonging to 10 different fish species were infected. The parasitic larvae were found in the intestinal wall, liver, ovaries, swim bladder, gallbladder, and mesenteries of the infected fishes. The prevalence of *Contracaecum* larva and the mean of intensity per infected fish varied among different fish species (Table 3).

A total of 7 fish (prevalence 35%, mean intensity 5.57) out of the 20 *Acanthobrama marmid* specimens examined were infected with *Contracaecum* larvae, which represented the highest prevalence among the collected fishes. While, only one fish (prevalence 0.81%, mean intensity 7) out of 123 *Arabibarbus grypus* specimens examined was infected with this larvae, which represented the lowest prevalence among the collected fishes in the present study. These results agreed with Abdullah & Mhasein (2011), who recorded *Contracaecum* larvae in *Acanthobrama marmid* and *Chondrostoma regium* with prevalences of 16.6% and 36.4%, respectively, among ten fish species in the Lesser Zab River. This variation in the prevalence may be related to the temperature, water level, intensity of both the intermediate host and migratory bird (final host), and the types of food and feeding habits of the fishes (Younis *et al*., 2017).

The present investigation shows that prevalence and intensity of *Contracaecum* larvae in the examined fishes are relatively low. It is considered to mention that only visual examination was used to isolate *Contracaecum* larvae in this study. The prevalence and number of larvae may have been higher than *Contracaecum* larvae found in the collected fishes in the present study if an incubation method had been used (Shamsi &

Suthar 2016). Third larval stage of the anisakid nematode *Contracaecum rudolphii* Hartwich, 1964, are commonly infect a range of fish species and mainly cyprinids particularly in area where the final host (piscivorous birds such as cormorants) are found (Moravec, 1994).

Table (3): Prevalence of *Contracaecum* larvae and mean of intensity among fish species.

Hosts	Fish		Prevalence %	Mean intensity	Site of infection
	No. Examined	No. Infected			
A. marmid	20	7	35	5.57	Gallbladder and liver
A. grypus	123	1	0.81	7	Intestinal wall and ovaries
C. trutta	222	2	0.90	3	Liver and mesentery
C. luteus	89	4	4.49	4.25	Liver and mesentery
C. regium	52	3	5.76	9	Intestinal wall and liver
C. carpio	195	4	2.05	2.75	Intestinal wall and swim bladder
L. barbulus	108	1	0.92	6	Intestine wall and liver
L. esocinus	52	1	1.92	6	Intestine wall and liver
L. xanthopterus	31	6	19.35	2.66	Intestine wall and liver
M. mastacembelus	94	1	1.06	5	Liver and mesentery

2- Morphological identification of *Contracaecum* larvae

Morphological examination and measurements were done by optical microscope and showed that the Anisakid larva of the present study were *Contracaecum* larvae (L3) as described by Moravec (2009). Additionally, there were no any significant morphological differences among the larvae which were recorded in different fishes.

The *Contracaecum* larvae were light brownish-yellow in color. They have elongated cylindrical body, forming collar at the anterior end, short tail with rounded tip. The bodies have fine, dense transverse striation of the cuticle. The larvae were encapsulated within slender body, they have distinct boring tooth. Excretory pore was situated anteriorly, cuticular striations observed through the whole length of the body. Esophagus consists of a long muscular part and a short glandular ventriculus. Esophageal caecum was extended posteriorly and the intestinal caecum was extending anteriorly. The intestine was filled with numerous small brownish granules. Gonads and other parts of reproductive system not developed (Fig. 11, 12).

Total length of the larvae is 3.5-11 mm, width 0.10-0.35 mm. Esophagus length 0.45-1.3 mm. Intestinal caecum length 0.20-0.48 mm. esophageal caecum length 0.30-0.70 mm (Table 4). Since this parasite in the larval stage the reproduction system not developed yet, it is difficult to determine the exact classification status in the level of species morphologically.

The present specimens show close resemble to those specimens which have been recorded by Abdullah (1990) from *Arabibarbus grypus* (reported as *Barbus grypus*) and *Carasobarbus luteus* (reported as *Barbus luteus*) from Dukan Lake, and by Moravec (2009) studied in *Cyprinus carpio* from Czech Republic in both measurements and characters. During microscopical

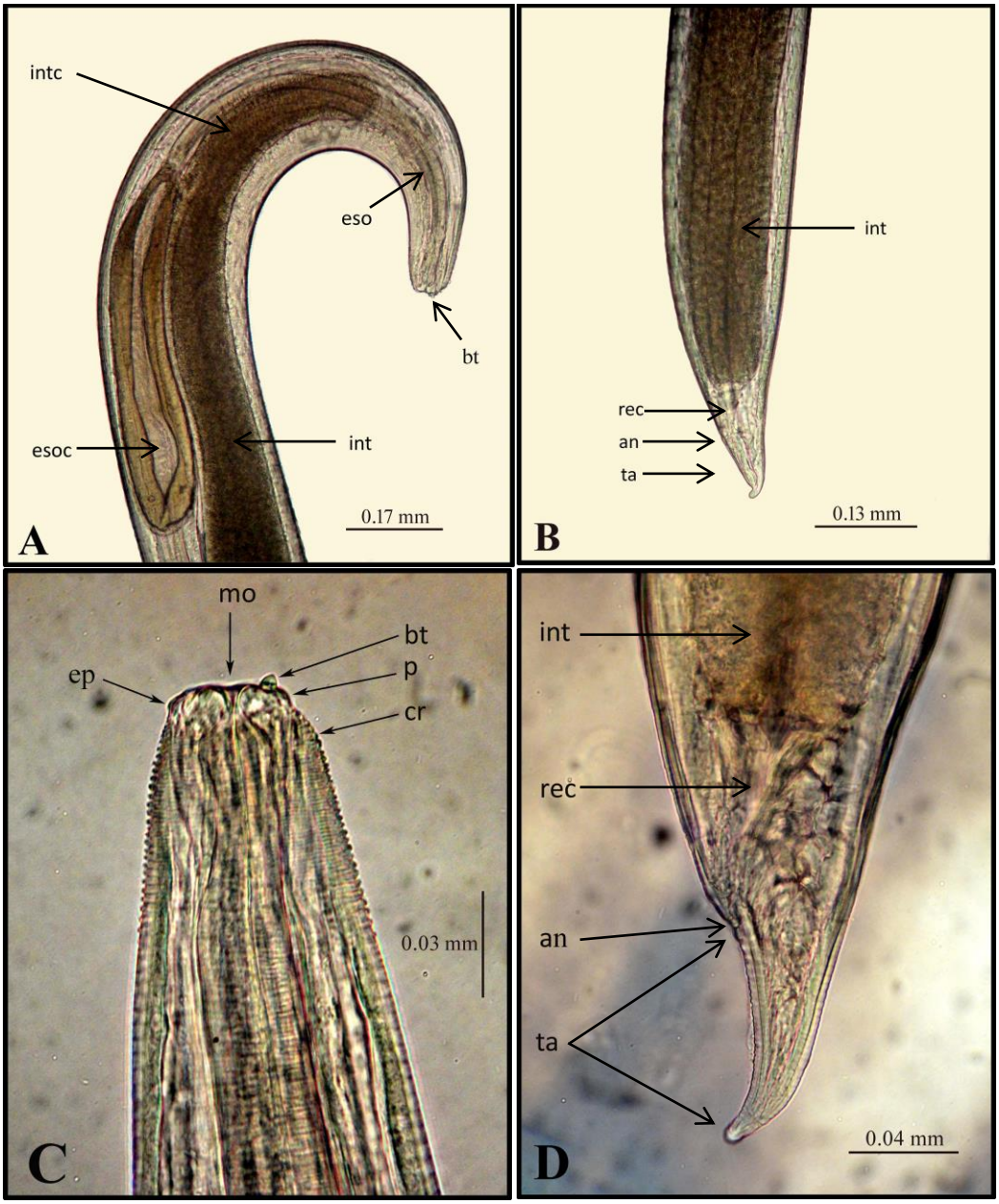

Fig. (11): Photomicrograph of *Contracaecum* larva in *Carasobarbus luteus*

A- Anterior region of the larva
B- Posterior region of the larva
C- Mouth region of the larva
D- Anal region of the larva

an= anus; **bt**= boring tooth; **cr**= cuticle ridges; **ep=** excretory pore; **eso**= esophagus; **esoc**= esophageal caecum; **int**= intestine; **intc**= intestinal caecum; **mo**= mouth opening; **p**= papillae; **rec**= rectum; **ta**= tail.

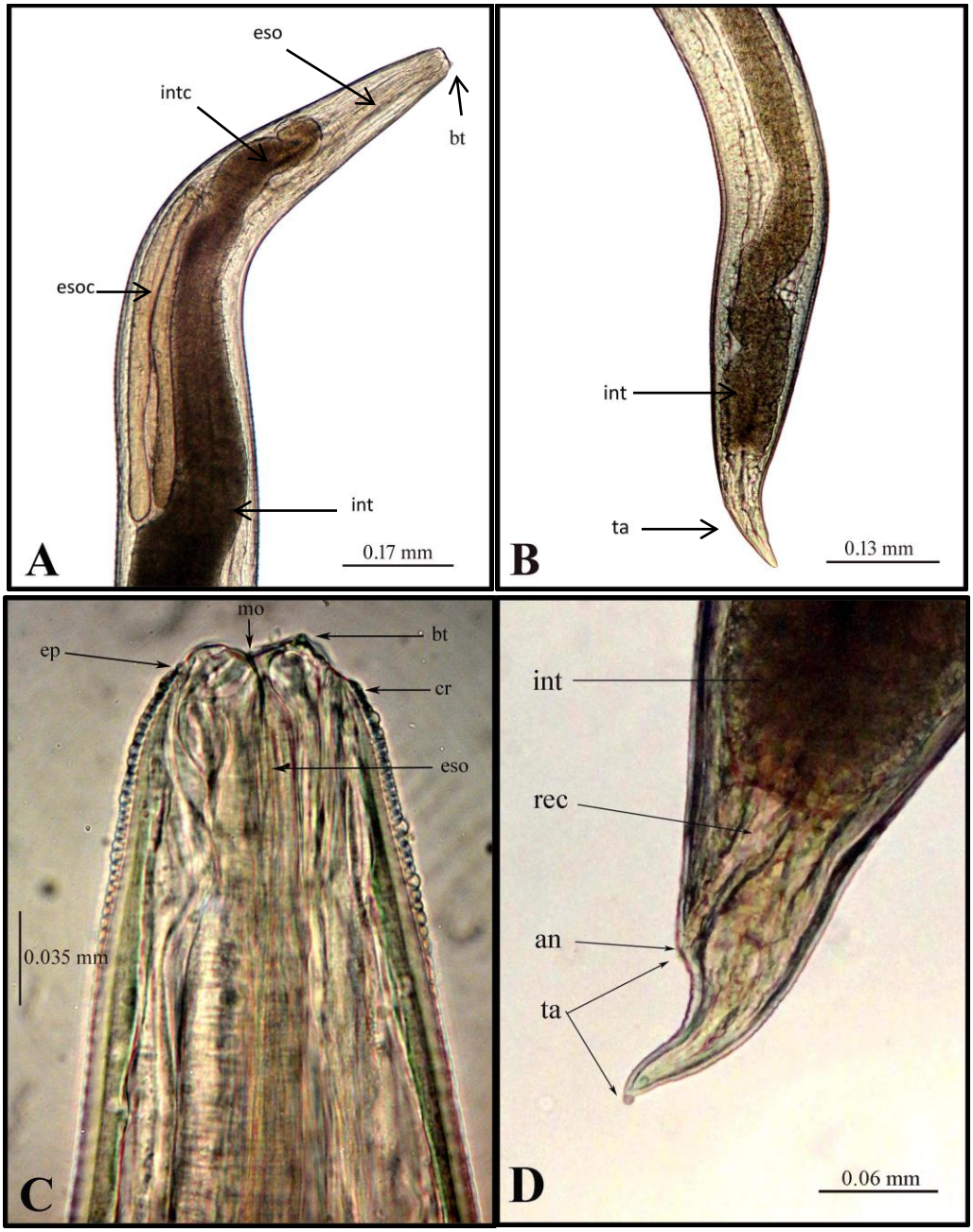

Fig. (12): Photomicrograph of *Contracaecum* larva in *Luciobarbus xanthopterus*

A- Anterior part of the larva
B- Posterior part of the larva
C- Mouth region of the larva
D- Tail region of the larva

an= anus; **bt**= boring tooth; **cr**= cuticle ridges; **ep**= excretory pore; **eso**= esophagus; **esoc**= esophageal caecum; **int**= intestine; **intc**= intestinal caecum; **mo**= mouth opening; **rec**= rectum; **ta**= tail.

Table (4): Comparison of measurements of systematically important features in *Contracaecum* larvae in different fish species in the present study (in millimeter)

***Contracaecum* larvae / Hosts**	**Total Length**	**Maximum width**	**Tail length**	**Rectum length**	**Boring tooth length**	**Esophagus length**	**Esophageal caeca length**	**Intestinal caeca length**
A. marmid	4.10	0.20	0.07	0.07	0.005	0.48	0.50	0.38
A. grypus	5.07	0.25	0.07	0.07	0.01	0.65	0.30	0.20
C. trutta	4.20	0.23	0.08	0.07	0.005	0.45	0.48	0.33
C. luteus	4.62	0.25	0.08	0.07	0.005	0.600	0.55	0.40
C. regium	7.75	0.35	0.08	0.09	0.01	0.600	0.60	0.45
C. carpio	3.50	0.25	0.085	0.07	0.005	0.70	0.70	0.48
L. barbulus	3.50	0.20	0.08	0.07	0.005	0.68	0.50	0.30
L. esocinus	5.00	0.25	0.08	0.07	0.005	0.75	0.52	0.40
L. xanthopterus	3.35	0.17	0.09	0.07	0.005	0.68	0.50	0.30
M. mastacembelus	5.20	0.75	0.04	0.03	0.0075	0.70	0.50	0.37

studies, there were no significant morphological differences among the *Contracaecum* larvae which they recorded in present investigation in these 10 different fish species, and the photomicrograph of the third larval stage (L3) of *Contracaecum* in both *Carasobarbus luteus* and *Luciobarbus xanthopterus* were put just as examples (Fig. 11; 12 respectively). The larvae morphologically identified as belonging to the genus *Contracaecum* and they were subjected to further molecular characterization to identify their species. The specific identification of *Contracaecum* larvae is not possible based solely on morphological description (Shamsi *et al*., 2017).

3- Ultra-morphological study of *Contracaecum* larvae

The scanning electron microscopy (SEM) study revealed that the patterns of cuticular striations of larvae from all fishes are striated regularly, which they are narrow in both of the anterior larvae and in pre tail region and become wider gradually when extended posteriorly. There are no significant different ornamentations among the larvae etween the striated cuticular rings there are transverse striation (Fig. 13).

The mouth was transverse in shape; the lips are not well developed and provided by four papillae, a well-defined boring tooth present. Excretory pore anteriorly located just below the mouth. Anal opening is located near the posterior end. Tail is short; the body is free of any projections like spine or papillae. The cloacal region has no any papillae because they are in larval stage and not mature yet. The ultra-structural characters of the present specimen were show a great similarity with the specimen (*Contracaecum* larvae) collected by Younis *et al.* (2017) who studied from different freshwater fishes from Lake Nasser in Egypt. Also, the ultra-structural feature of the present *Contracaecum* larvae are closed to those specimens

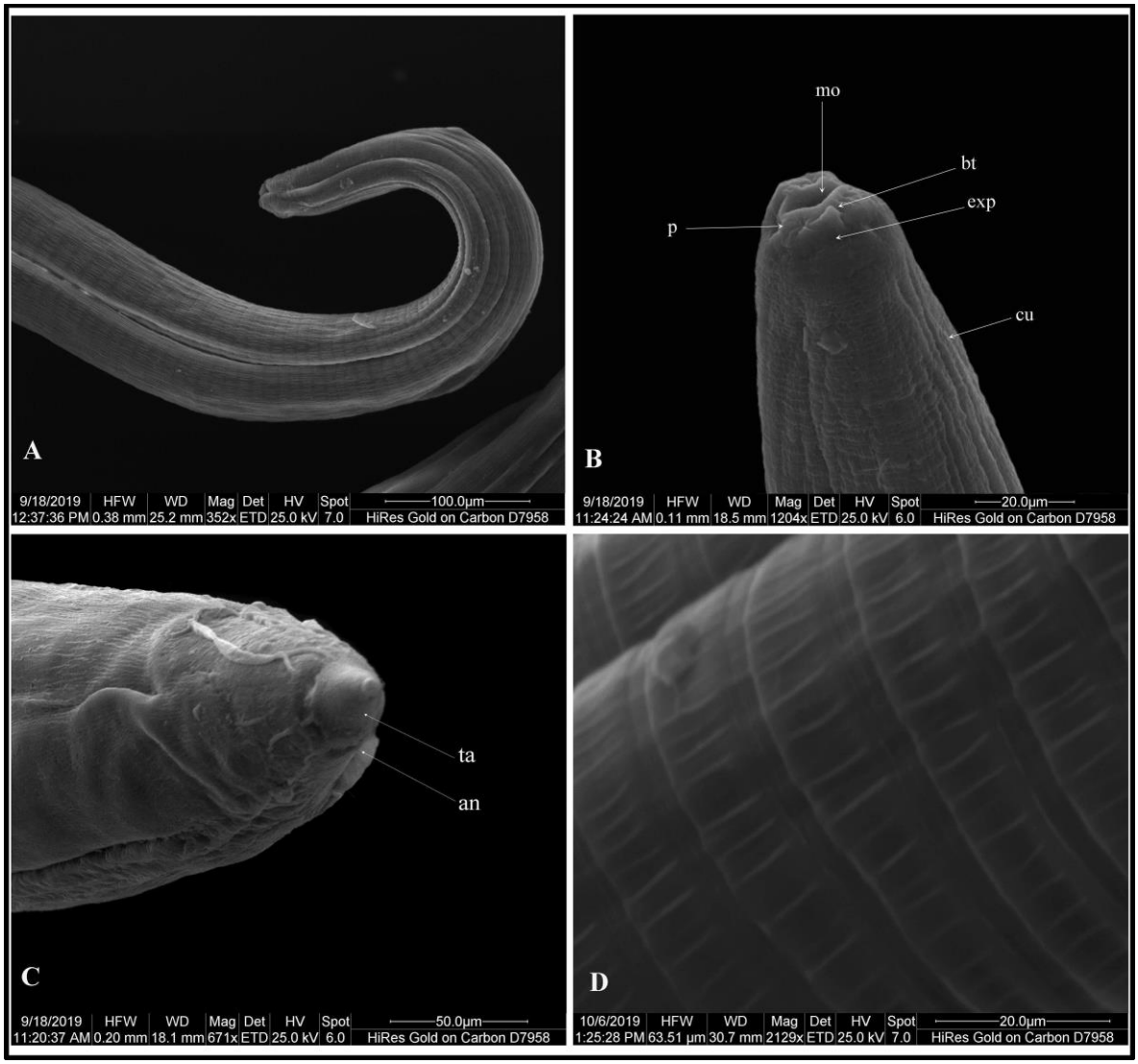

Fig. (13): Scanning Electron micrograph of *Contracaecum* larva in *Carasobarbus luteus*

A- Anterior region of the larva
B- Head region of the larva
C- Posterior region of the larva
D- Cuticlar striation of the larva

an= anus; **bt**= boring tooth; **cu**= cuticle; **exp**= excretory pore; **mo**= mouth opening; **p**= papillae; **ta**= tail.

were studied with SEM by Rahemo & Nawab Al-Din (2009) in *Acanthobrama marmid* from Tigris River. During Scanning electron microscopy studies, there were no significant ultra-morphological differences among the *Contracaecum* larvae which they recorded in present investigation from these 10 different previously mentioned fish species, and the scanning electron photomicrograph of the third larval stage of *Contracaecum* in *Carasobarbus luteus* put just as an example (Fig. 13).

4- Molecular study of *Contracaecum* larvae

The molecular examination of *Contracaecum* larvae in the present study included the mid pieces of three specimens from each host species (*A. marmid*, *A. grypus*, *C. trutta*, *C. luteus*, *C. regium*, *C. carpio*, *L. barbulus*, *L. esocinus*, *L. xanthopterus* and *M. mastacembelus*) were done by amplifying the ITS-1, ITS-2 and COX-2 gene regions and sequencing the amplicon by using Genetic analyzer 3500, an applied Biosystems (USA).

PCR of ITS-1, ITS-2 and COX-2 analysis

After the morphological identification, the ITS-1, ITS-2 and COX-2 regions from individual larva were amplified by PCR from genomic DNA samples (n = 30). Agarose gels electrophoresis demonstrated the same size for each ITS-1, ITS-2 and COX-2 region. Amplicons were 530 bp, 430 bp and 629 bp for the ITS-1, ITS-2, and COX-2 respectively confirming that all sequences are of the same genus (Figs: 14, 15, 16).

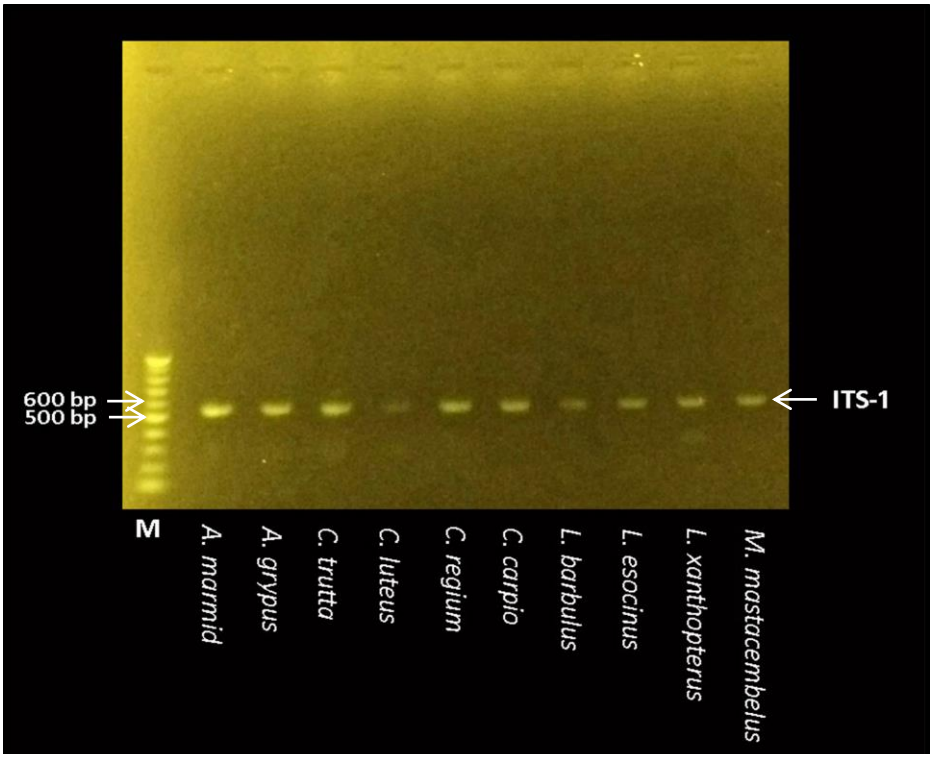

Fig. (14): PCR product of ITS-1 sequences of *Contracaecum* larvae in different fish species on 2% TAE agarose gel. **M**= DNA ladder (1Kb).

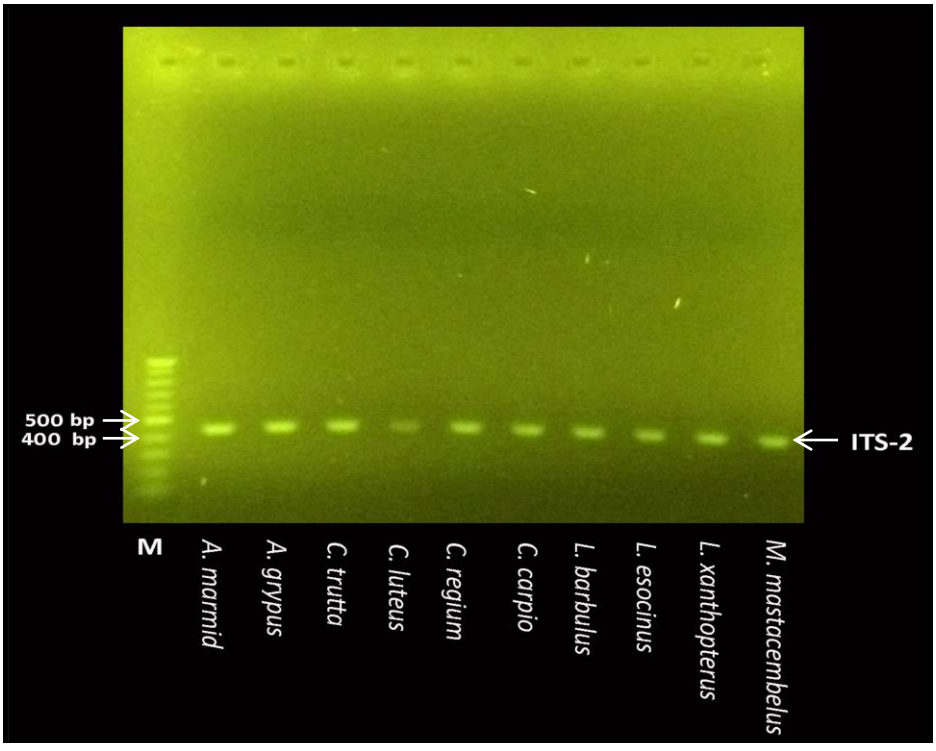

Fig. (15): PCR product of ITS-2 sequences of *Contracaecum* larvae in different fish species on 2% TAE agarose gel. **M**= DNA ladder (1Kb).

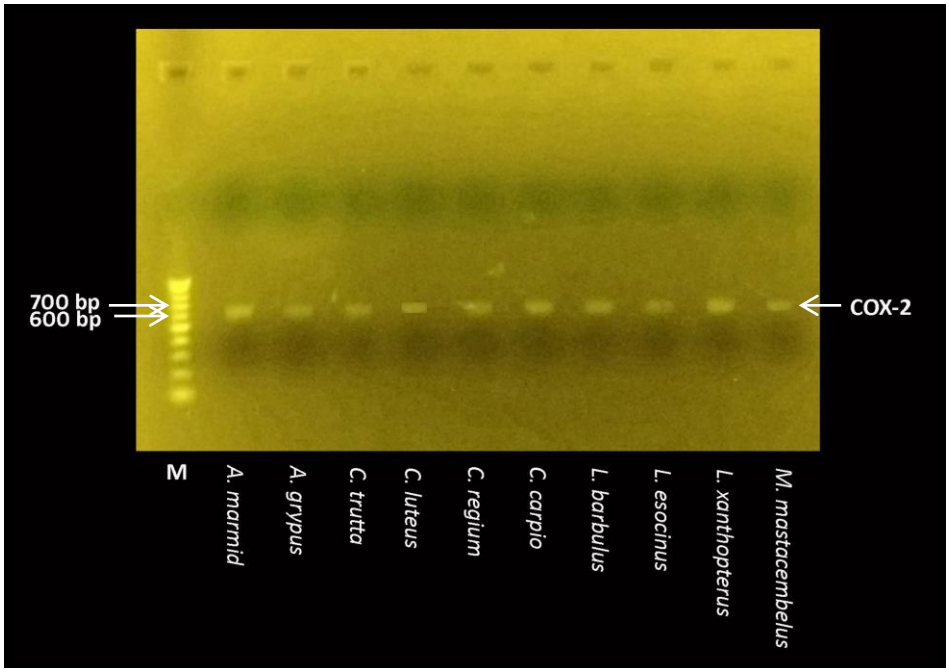

Fig. (16): PCR product of COX-2 sequences of *Contracaecum* larvae in different fish species on 2% TAE agarose gel. **M**= DNA ladder (1Kb).

Sequence analysis of *Contracaecum* larvae

Alignment of resulted sequences revealed that there is no significant variation of each ITS-1, ITS-2 and COX-2 regions, which indicate the presence of only one type of larva. Based on percentage identities of nucleotides from GenBank, the online BLAST tool showed the ITS-1 sequences obtained from larvae-infected *A. marmid*, *A. grypus*, *C. trutta*, *C. luteus*, *C. regium*, *C. carpio*, *L. barbulus*, *L. esocinus*, *L. xanthopterus* and *M. mastacembelus* matched 99.78%, 100%, 99.76%, 100%, 100%, 100%, 100%, 99.55%, 100% and 100% respectively to the previously reported reference gene sequences for the ITS-1 in *Contracaecum rudolphii* B (Zhang *et al*., 2009) from the stomachs of the final host great cormorant *Phalacrocorax carbo sinensis* from the Guangzhou Zoo in Guangdong in China, which was examined previously and deposed in GenBank

(FJ467618) (Zhang *et al*., 2009) (Appendix: Fig. 24 to 33), while the ITS-2 sequences obtained from larvae-infected the mentioned host species matched 100% to the previously reported reference gene sequences for the ITS-2 in *Contracaecum rudolphii* B (Zhang *et al*., 2009) from the same mentioned host and locality, which was deposed in GenBank (FJ467620) (Zhang *et al*., 2009) (Appendix: Fig. 34 to 43). Also, matched 100% to the previously reported reference gene sequences for the ITS-2 in *Contracaecum rudolphii* B (Li *et al*., 2005) from the same host from the Venice lagoon in northeastern Italy and from Monaci Lake in central Italy, which was examined previously and deposed in GenBank (AJ634786) (Li *et al*., 2005).

The COX-2 sequences obtained from larvae-infected *A. marmid* matched 98.52% to the previously reported reference gene sequences for the COX-2 in *Contracaecum rudolphii* B (Mattiucci *et al*., 2008b) from the stomachs of great cormorant *P. carbo sinensis* from Italy, which was examined previously and deposed in GenBank (EF122203) (Mattiucci *et al*., 2008b) (Appendix: Fig. 44). The COX-2 sequences obtained from larvae-infected *A. grypus*, *C. trutta*, *C. regium*, *L. barbulus*, *L. esocinus*, *L. xanthopterus* and *M. mastacembelus* matched 100%, 99.19%, 100%, 100%, 99.37%, 100% and 99.58% respectively to the previously reported reference gene sequences for the COX-2 in *Contracaecum rudolphii* B (Mattiucci *et al*., 2008b) from the great cormorant *P. carbo sinensis* from Italy, which was examined previously and deposed in GenBank (EF513509) (Mattiucci *et al*., 2008b) (Appendix: Fig. 45, 46, 48, 50, 51, 52 and 53). While, the COX-2 sequences obtained from larvae-infected *C. luteus* and *C. carpio* matched 99.79% to the previously reported reference gene sequences for the COX-2 in *Contracaecum rudolphii* B from the same host which was examined

previously and deposed in GenBank (EF558894) (Mattiucci *et al*., 2008b) (Appendix: Fig. 47 and 49).

The genetic characterization of the parasites in the present study are available in the GenBank database; the ITS-1, ITS-2 and COX-2 sequences obtained were deposited in GenBank and their accession numbers were demonstrated in Table (5). The ITS-1, ITS-2 and COX-2 sequence analyses confirmed that the third larval stage of *Contracaecum* (L3) parasitizing the fishes from the present study belong to species *Contracaecum rudolphii* type-B, a parasite at the adult stage of the great cormorant *Phalacrocorax carbo sinensis* mainly from Italy water (Li *et al*., 2005; Mattiucci *et al*., 2008b). ITS-1, ITS-2 and COX-2 markers may provide reliable evidence for specific species identification of *Contracaecum* larvae occurring in fish (Mattiucci *et al*., 2010). Therefore, the occurrence of *C. rudolphii* B larvae from Iraqi waters was also proved by molecular evidence inferred from the ITS-1, ITS-2 and COX-2 markers used in the present study.

Table (5): Accession numbers provided by NCBI for the collected *Contracaecum* larvae in different fish hosts in the present study

Host of *Contracaecum rudolphii* B	Accession numbers for ITS-1 sequences	Accession numbers for ITS-2 sequences	Accession numbers for COX-2 sequences
A. marmid	MN557376	MN526259	MN589997
A. grypus	MN557377	MN563727	MN589998
C. trutta	MN557378	MN563728	MN589999
C. luteus	MN557379	MN563729	MN590000
C. regium	MN557380	MN563730	MN590001
C. carpio	MN557381	MN563731	MN590002
L. barbulus	MN557382	MN563732	MN590003
L. esocinus	MN557383	MN563733	MN590004
L. xanthopterus	MN557384	MN563734	MN590005
M. mastacembelus	MN557385	MN563735	MN590006

The sequences of ITS-1, ITS-2 and COX-2 obtained from the collected larvae in different fish species were aligned with each other (the same gene). The results of multiple sequence alignment as follow:

Pairwise comparisons of all nucleotides sequence among the *Contracaecum* larvae collected in the 10 different fish hosts revealed that only three nucleotide variation (0.65%) in alignment position 31, 32 and 161 for ITS-1 (Fig. 17A; 17B), and there was no nucleotide variations in alignment for ITS-2 (Fig. 18A; 18B). While, COX-2 showed 18 nucleotide variations (3.27%) in alignment positions 27, 33, 36, 54, 57, 78, 117, 138, 144, 150,168, 177, 282, 318, 336, 384, 474 and 480 (Fig. 19A; 19B).

Contracaecum rudolphii Hartwich, 1964 is a species complex it consist of several sibling species. *C. rudolphii* sensu lato named *C. rudolphii* A and *C. rudolphii* B (D'Amelio *et al*. 1990), they could also be differentiated from each other based on the ITS-1 and ITS-2 sequence data (Li *et al*. 2005). D'Amelio *et al*. (2007) indicated the existence of a third cryptic species of *C. rudolphii* complex (*C. rudolphii* C) in double-crested cormorants from west-central Florida based on PCR-RFLP and sequencing of the rrnS mitochondrial gene and nuclear ribosomal spacers. Shamsi *et al*. (2009) described two new sibling species of the *C. rudolphii* complex, *C. rudolphii* D from *Phalacrocorax carbo* and *C. rudolphii* E from *Phalacrocorax varius* in Australia, based on the ITS-1 and ITS-2 sequence data. Recently, D'Amelio *et al*. (2012) recorded new isolate of *C. rudolphii* complex (*C. rudolphii* F) from brown pelican *Pelecanus occidentalis* in the northern Gulf of Mexico.

Various studies demonstrated that ITS-1 and ITS-2 of the nuclear ribosomal DNA (rDNA) provide genetic markers for the accurate identification of a range of species of Ascaridoids. In addition, more studies

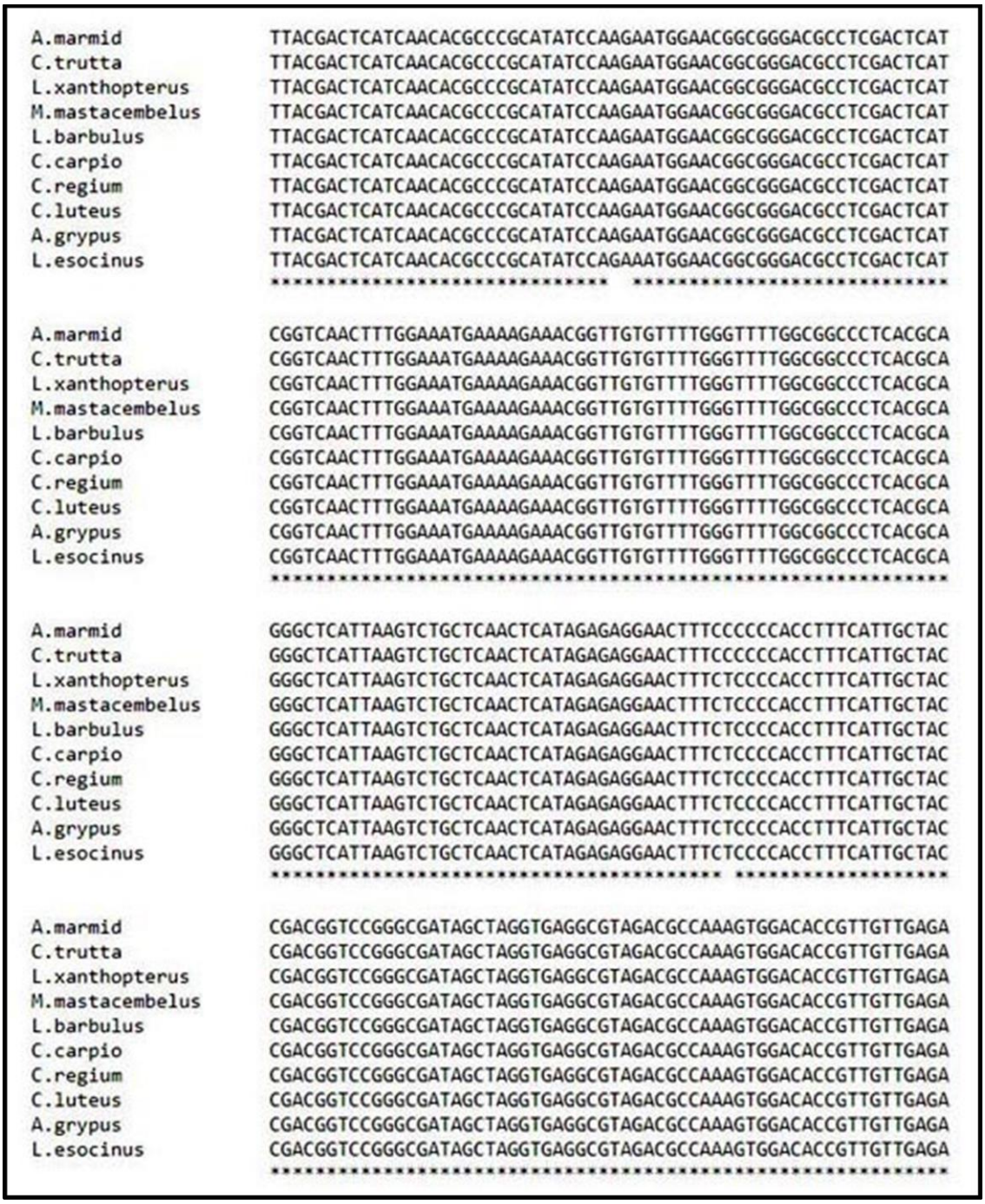

Fig. (17A): Nucleotide sequences alignment (ITS-1) of *Contracaecum* larvae obtained in different fish species in the present study.

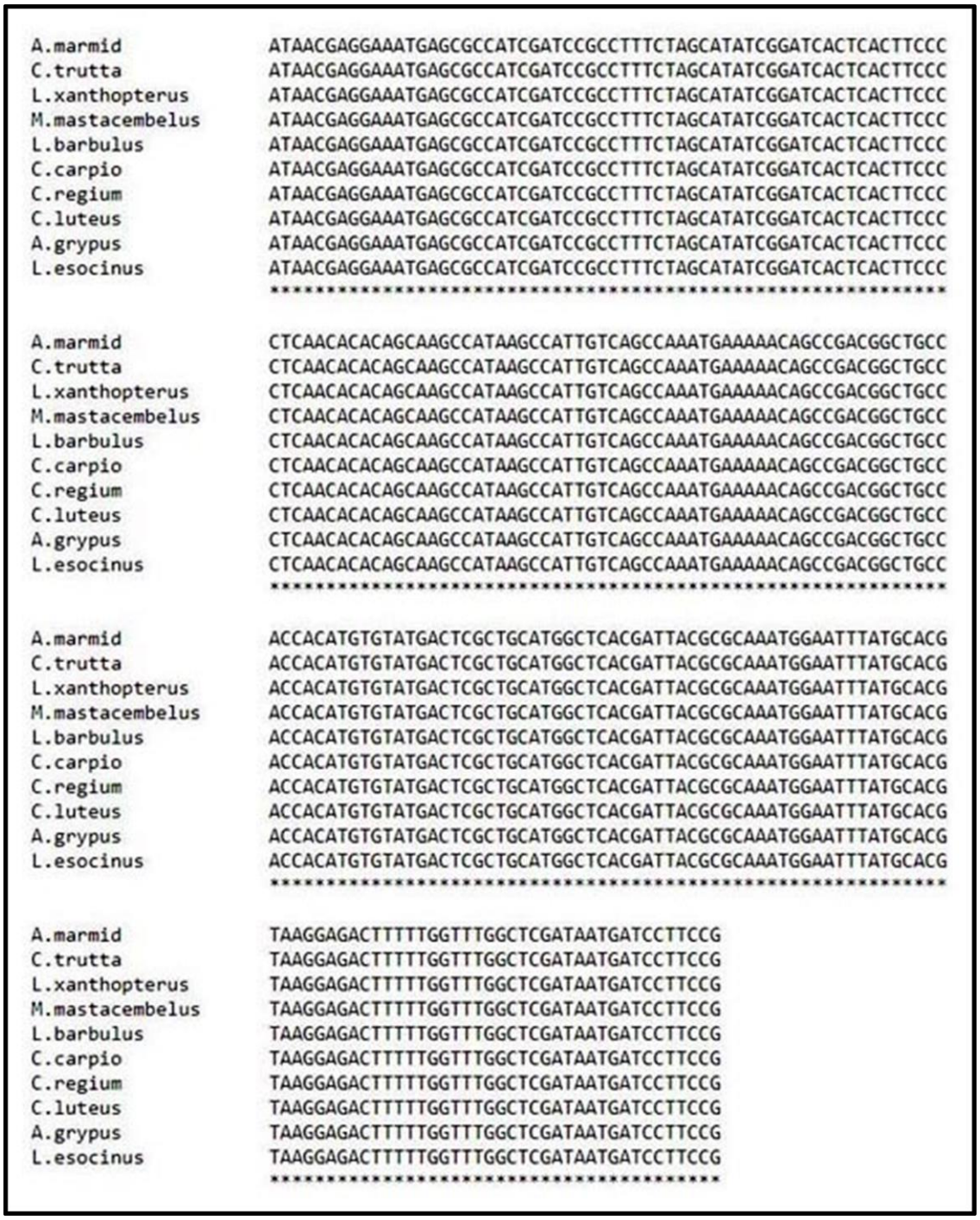

Fig. (17B): Nucleotide sequences alignment (ITS-1) of *Contracaecum* larvae obtained in different fish species in the present study (continued).

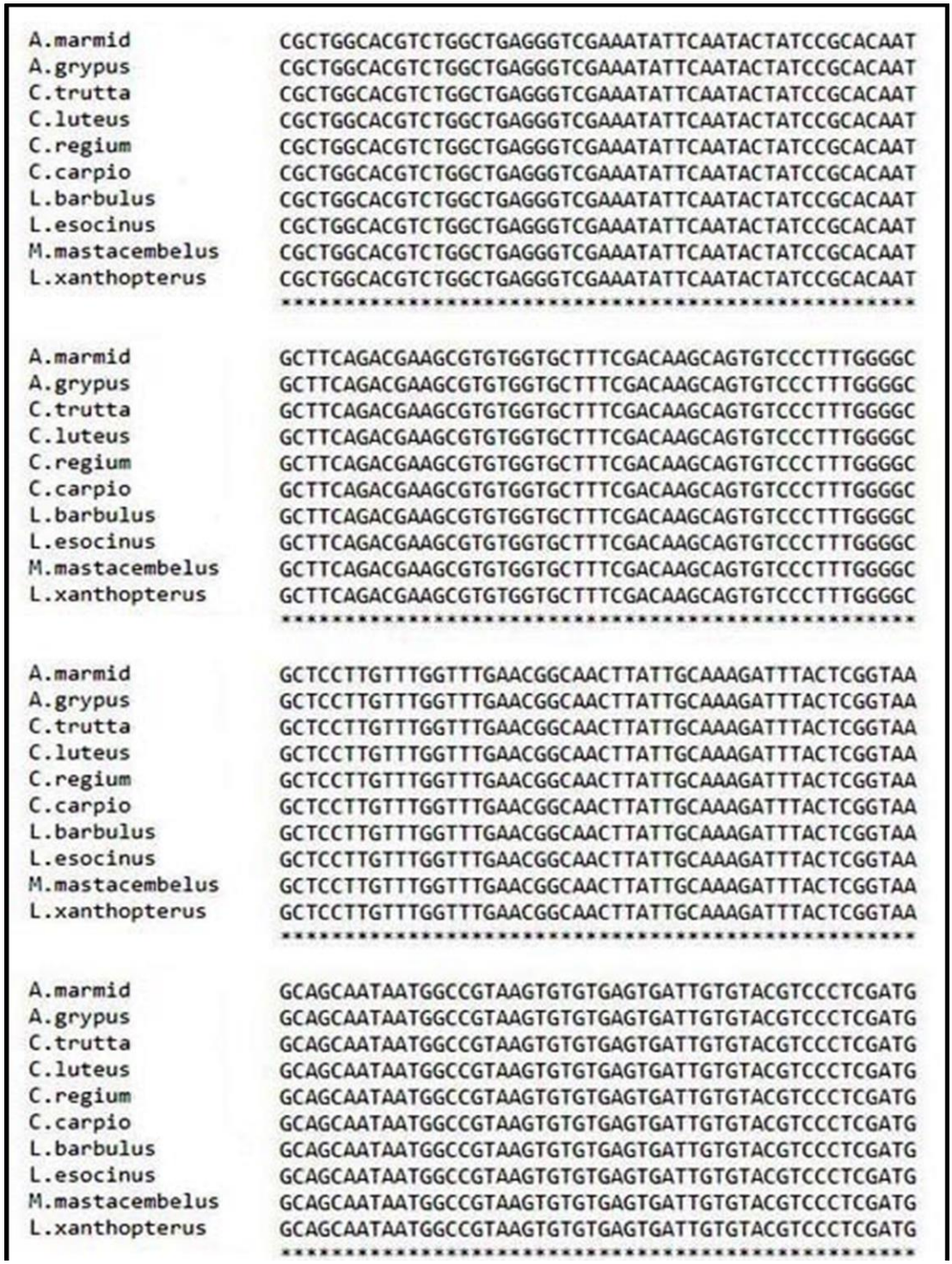

Fig. (18A): Nucleotide sequences alignment (ITS-2) of *Contracaecum* larvae obtained in different fish species in the present study.

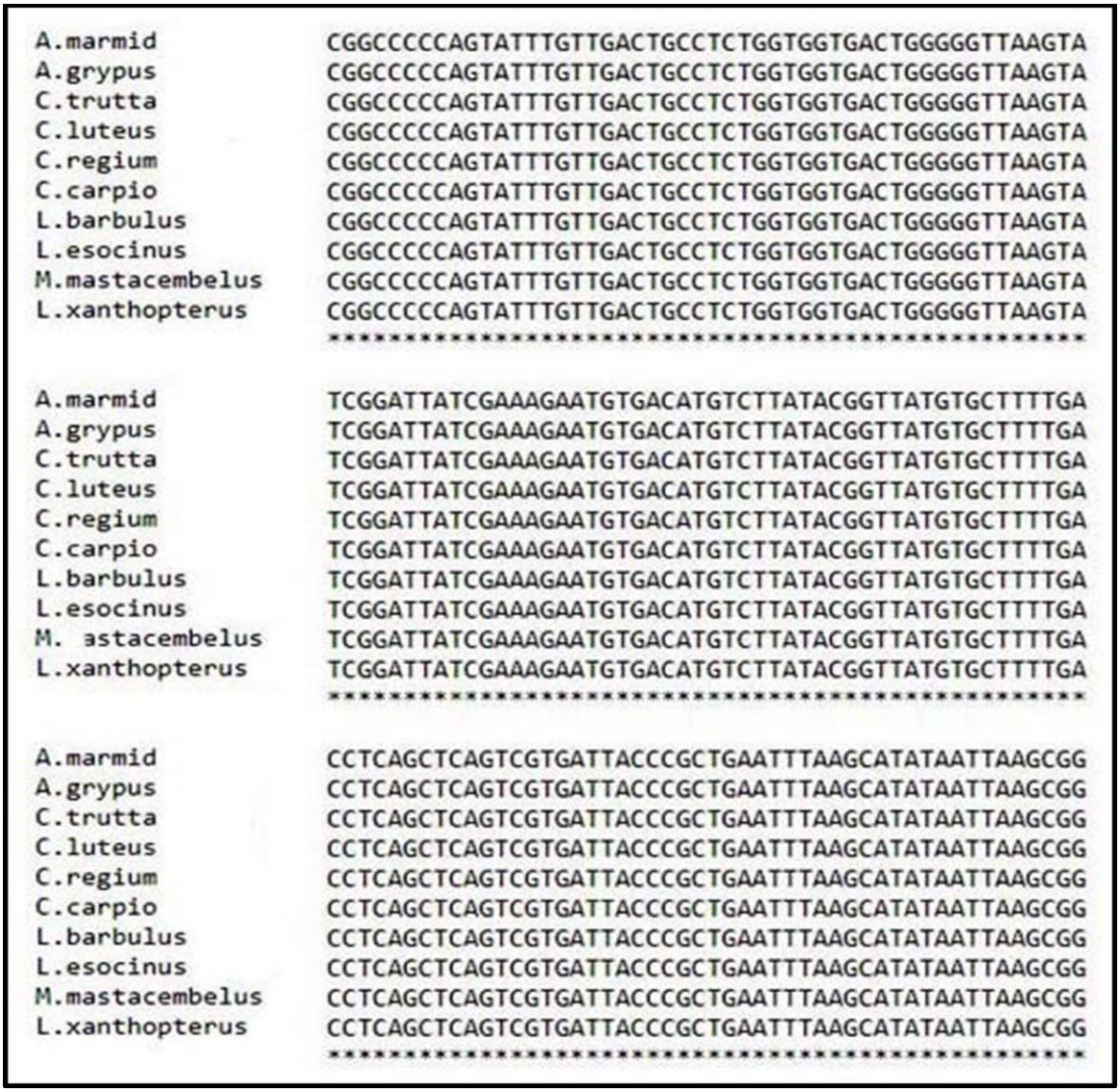

Fig. (18B): Nucleotide sequences alignment (ITS-2) of *Contracaecum* larvae obtained in different fish species in the present study (continued).

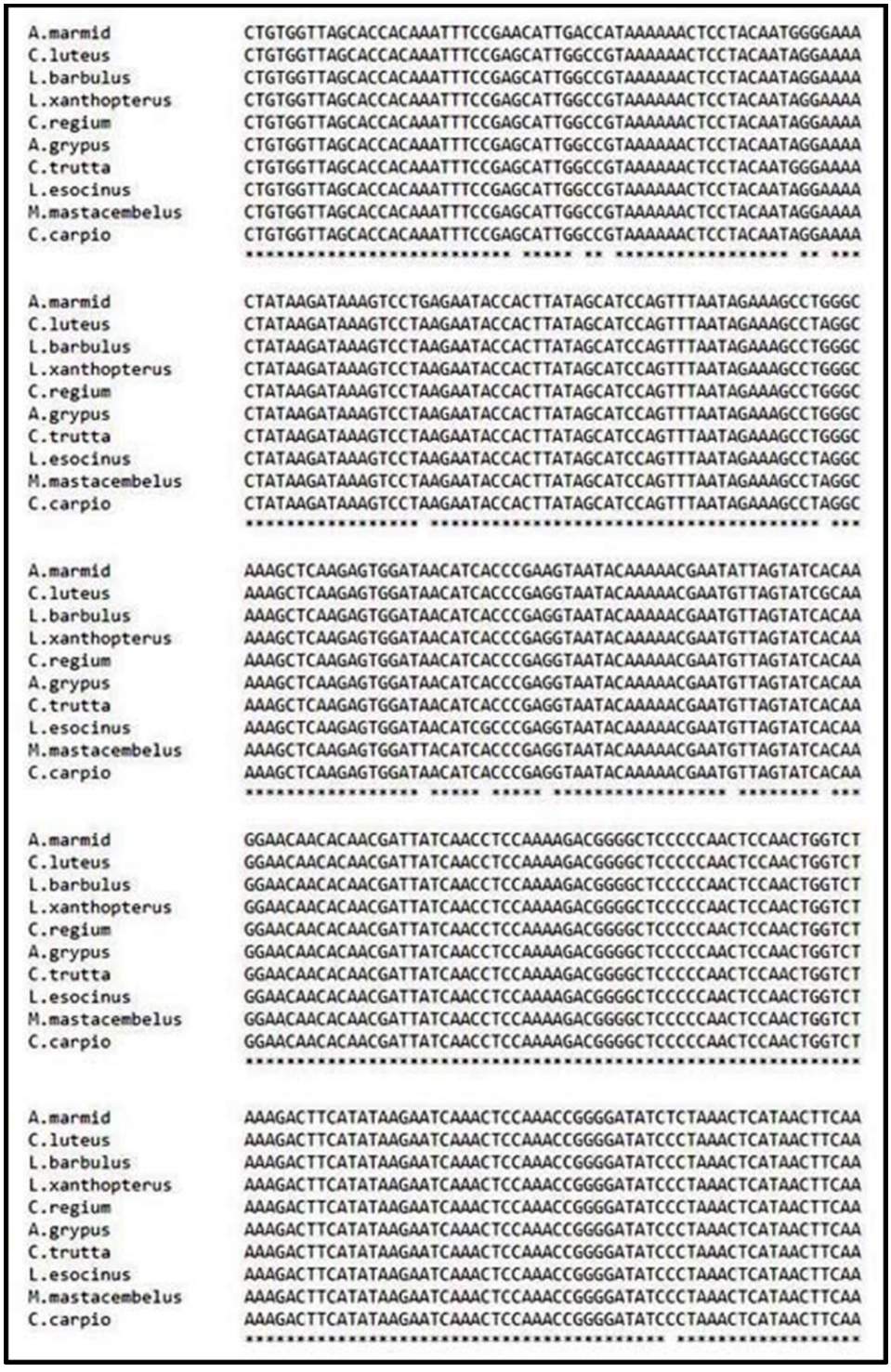

Fig. (19A): Nucleotide sequences alignment (COX-2) of *Contracaecum* larvae obtained in different fish species in the present study.

```
A.marmid          TACCATTGATGACCAGTAACCTTCACAGTTAAACTGCTATCAAGGTTTATTAAACCATAA
C.luteus          TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
L.barbulus        TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
L.xanthopterus    TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
C.regium          TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
A.grypus          TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
C.trutta          TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
L.esocinus        TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
M.mastacembelus   TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
C.carpio          TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA
                  **************** ****************** ************************

A.marmid          TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
C.luteus          TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
L.barbulus        TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
L.xanthopterus    TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
C.regium          TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
A.grypus          TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
C.trutta          TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
L.esocinus        TAATAAAGCAGACTCAAAGAAGGAATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
M.mastacembelus   TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
C.carpio          TAATAAAGCAGACTCAAAGAAGGAATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA
                  ********************** *************************************

A.marmid          CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTA
C.luteus          CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAGCGCCTG
L.barbulus        CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
L.xanthopterus    CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
C.regium          CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
A.grypus          CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
C.trutta          CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
L.esocinus        CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
M.mastacembelus   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
C.carpio          CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAACGCCTG
                  ***************************************************** *****

A.marmid          AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
C.luteus          AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
L.barbulus        AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
L.xanthopterus    AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
C.regium          AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
A.grypus          AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
C.trutta          AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
L.esocinus        AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
M.mastacembelus   AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
C.carpio          AATAACAAATAAGAAAATATAACAGAAACAAAAGATAAAACACCGAACAACAAACTACAA
                  ************************************************************

A.marmid          TTAAAATTATGAACCAATCT
C.luteus          TTAAAATTATGAACCAATCT
L.barbulus        TTAAAATTATGAACCAATCT
L.xanthopterus    TTAAAATTATGAACCAATCT
C.regium          TTAAAATTATGAACCAATCT
A.grypus          TTAAAATTATGAACCAATCT
C.trutta          TTAAAATTATGAACCAATCT
L.esocinus        TTAAAATTATGAACCAATCT
M.mastacembelus   TTAAAATTATGAACCAATCT
C.carpio          TTAAAATTATGAACCAATCT
                  ********************
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Fig. (19B): Nucleotide sequences alignment (COX-2) of *Contracaecum* larvae obtained in different fish species in the present study (continued).

indicated that sibling species can be differentiated based on the ITS sequences (Jacobs *et al*., 1997; Zhu *et al*., 2000; 2001; 2002). The ITS-1 sequences of the obtained larvae (*C. rudolphii* B) in the present study show 11 (2.46%) nucleotide differences with the previously reported reference gene sequence for the ITS-1 in *C. rudolphii* A which was examined and deposed in GenBank (Accession number: AJ634782). While, ITS-2 shows 14 (5.22%) nucleotide differences with the *C. rudolphii* A which previously reported reference gene sequence for the ITS-2 in *C. rudolphii* A which was examined and deposed in GenBank (Accession number: AJ634785) (Li *et al*., 2005) (Fig. 20). This clear genetic differentiation support previous sequence analyses (Li *et al*., 2005) that there are sequence differences (1.8%) in the ITS-1 and (5.1%) in ITS-2 between the sibling species of *C. rudolphii* A and B (Li *et al*., 2005). Extending these studies, we investigated that there is no significant sequence variation in the ITS1 and ITS2 within and among the larvae collected from different fish host in the present investigation. The molecular finding of the present investigation support that the present finding larvae belong to *C. rudolphii* type-B.

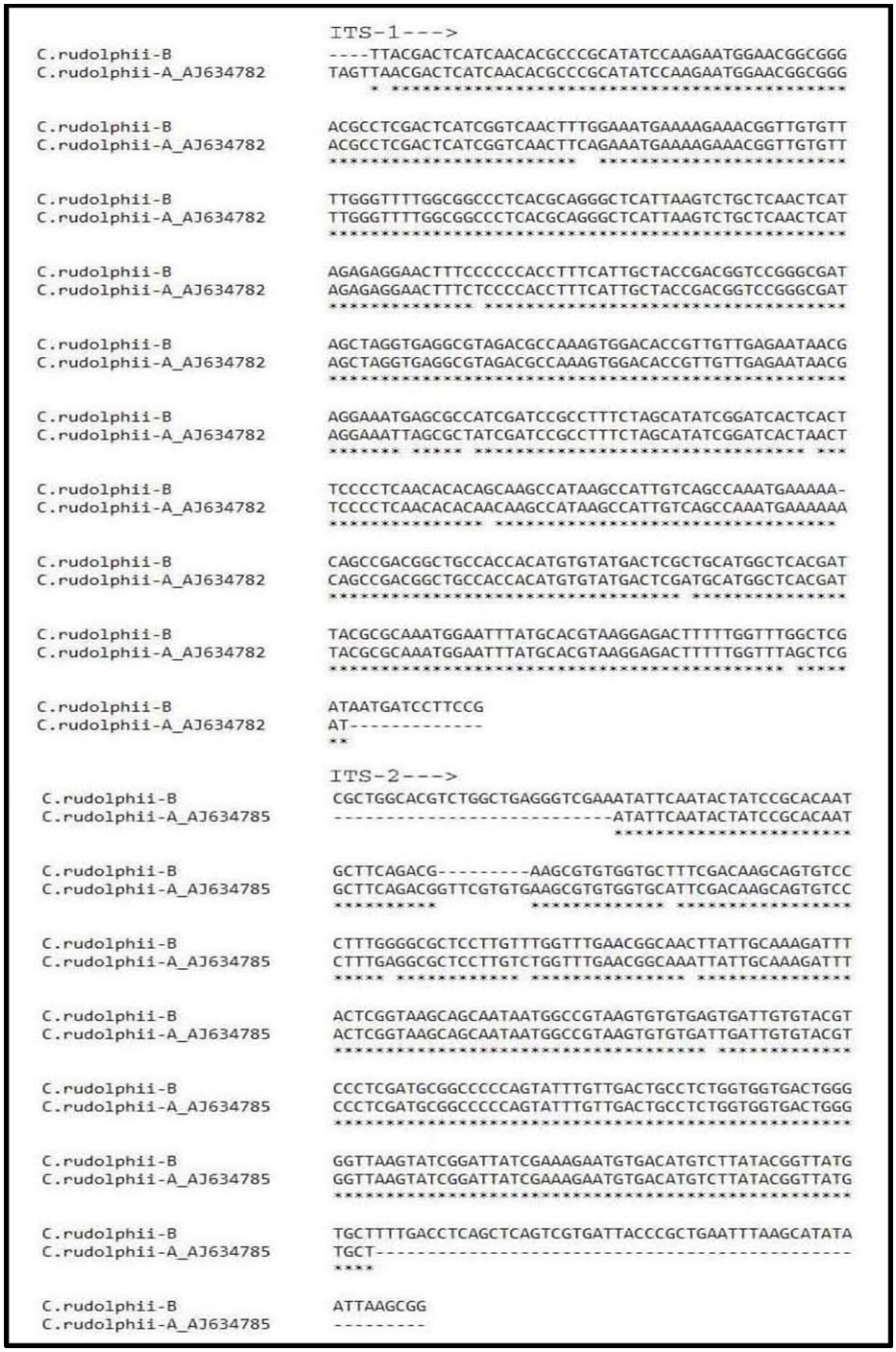

Fig. (20): Alignment of the ITS-1 and ITS-2 sequences representing genotype 1 (*Contracaecum rudolphii* B) from the present study and genotype 2 (*Contracaecum rudolphii* A) sequences have been deposited in GeneBank under the accession number AJ634782 and AJ634785 respectively. Nucleotide differences between the aligned sequences are indicated by having no asterisks.

Phylogenetic analysis of *Contracaecum* larvae

In the phylogenetic analysis, the sequence data aligned with the data sequences of ITS-1, ITS-2 and COX-2 form other different *Contracaecum* species (different genotypes) and *Ascaris sum* used as outgroup detected in GenBank (Table 6, 7). Phylogenetic analysis were conducted in MEGA X (Kumar *et al*., 2018). The evolutionary histories were inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (-6728.57, -3849.07 and -3890.27 for ITS-1, ITS-2 and COX-2 respectively) are shown (Fig. 21, 22, 23). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The *Contracaecum* larvae from all different fish hosts were clustered in the same clade of *Contracaecum rudolphii* B. Moreover, the phylogenetic tree of the ITS-1, ITS-2 and COX-2 sequences using ML analyses indicated that *Contracaecum* larvae clades were distinct species by high bootstrap values (Fig. 21, 22, 23).

Table (6): *Contracaecum* nematodes and specimens/accession number (ITS) of taxa used to build phylogenetic trees and the nucleotide differences with the *Contracaecum* larvae collected from the present study

Parasite	GeneBank accession no. (ITS)	No. of nucleotide differences	Host	Source
C. rudolphii A	ITS1 (AJ634782)	9	*Phalacrocorax carbo sinensis*	Li *et al.* (2005)
	ITS2 (AJ634785)	14	*Phalacrocorax carbo sinensis*	Li *et al.* (2005)
C. rudolphii B	ITS1 (AJ634783)	1	*Phalacrocorax carbo sinensis*	Li *et al.* (2005)
	ITS2 (AJ634786)	0	*Phalacrocorax carbosinensis*	Li *et al.* (2005)
C. rudolphii D	ITS1 (FM210251)	6	*Phalacrocorax varius*	Shamsi *et al.* (2009b)
	ITS2 (FM210268)	17	*Phalacrocorax carbo sinensis*	Shamsi *et al.* (2009b)
C. rudolphii E	ITS1 (FM210257)	6	*Phalacrocorax varius*	Shamsi *et al.* (2009b)
	ITS2 (FM210271)	15	*Phalacrocorax varius*	Shamsi *et al.* (2009b)
C. rudolphii F	ITS (JF424597)	21	*Pelecanus occidentalis*	D'Amelio *et al.* (2012)
		16	*Pelecanus occidentalis*	D'Amelio *et al.* (2012)
C. ogmorhini	ITS1 (AJ291468)	10	*Arctocephalus pusillus doriferus*	Zhu *et al.* (2001)
	ITS2 (AJ291471)	15	*Zalophus californianus*	Zhu *et al.* (2001)
C. eudyptulae	ITS1 (AJ007461)	8	-	Zhu *et al.*(unpublished)
	ITS2 (FM177565)	17	*Eudyptula minor*	Shamsi *et al.* (2009a)
C. chubutensis	ITS1 (HQ389546)	13	*Phalacrocorax brasilianus*	Garbin *et al.* (2011)
	ITS2 (HQ389548)	23	*Phalacrocorax atriceps*	Garbin *et al.* (2011)
C. variegatum	ITS1 (MK424804)	22	Bird	Hbaiel & Mohammad (unpublished)
	ITS2 (FM177537)	14	*Anhinga melanogaster*	Shamsi *et al.* (2009a)
C. microcephalum	ITS1 (FM177523)	50	*Phalacrocorax melanoleucos*	Shamsi *et al.* (2009a)
	ITS2 (FM177527)	72	*Phalacrocorax melanoleucos*	Shamsi *et al.* (2009a)
C. septentrionale	ITS1 (AJ634784)	24	*Phalacrocorax carbo sinensis*	Li *et al.* (2005)
	ITS2 (AJ634787)	35	*Alca torda*	Li *et al.* (2005)
C. bioccai	ITS (JF424598)	30	*Pelecanus occidentalis*	D'Amelio *et al.* (2012)
		40	*Pelecanus occidentalis*	D'Amelio *et al.* (2012)
C. radiatum	ITS (AY603529)	35	*Leptonechotes weddlli*	Kijewska *et al.* (2008)
		65	*Leptonechotes weddlli*	Kijewska *et al.* (2008)
C. osculatum	ITS (AB277825)	77	Arabesque greenling	Umehara *et al.* (2008)
		105	Arabesque greenling	Umehara *et al.* (2008)
C. multipapillatum	ITS1 (AM940056)	146	*Pelecanus conspicillatus*	Shamsi *et al.* (2008)
	ITS2 (AM940060)	132	*Pelecanus conspicillatus*	Shamsi *et al.* (2008)
C. pyripapillatum	ITS1 (AM940062)	141	*Pelecanus conspicillatus*	Shamsi *et al.* (2008)
	ITS2 (AM940066)	136	*Pelecanus conspicillatus*	Shamsi *et al.* (2008)
Contracaecum larva	ITS1 (MN557376)	-	*Acanthobrama marmid*	Present study
	ITS2 (MN526259)	-	*Acanthobrama marmid*	Present study
Contracaecum larva	ITS1 (MN557377)	-	*Arabibarbus grypus*	Present study
	ITS2 (MN563727)	-	*Arabibarbus grypus*	Present study
Contracaecum larva	ITS1 (MN557378)	-	*Capoeta trutta*	Present study
	ITS2 (MN563728)	-	*Capoeta trutta*	Present study
Contracaecum larva	ITS1 (MN557379)	-	*Carasobarbus luteus*	Present study
	ITS2 (MN563729)	-	*Carasobarbus luteus*	Present study
Contracaecum larva	ITS1 (MN557380)	-	*Chondrostoma regium*	Present study

	ITS2 (MN563730)	-	*Chondrostoma regium*	Present study
Contracaecum larva	ITS1 (MN557381)	-	*Cyprinus carpio*	Present study
	ITS2 (MN563731)	-	*Cyprinus carpio*	Present study
Contracaecum larva	ITS1 (MN557382)	-	*Luciobarbus barbulus*	Present study
	ITS2 (MN563732)	-	*Luciobarbus barbulus*	Present study
Contracaecum larva	ITS1 (MN557383)	-	*Luciobarbus esocinus*	Present study
	ITS2 (MN563733)	-	*Luciobarbus esocinus*	Present study
Contracaecum larva	ITS1 (MN557384)	-	*Luciobarbus xanthopterus*	Present study
	ITS2 (MN563734)	-	*Luciobarbus xanthopterus*	Present study
Contracaecum larva	ITS1 (MN557385)	-	*Mastacembelus mastacembelus*	Present study
	ITS2 (MN563735)	-	*Mastacembelus mastacembelus*	Present study
Ascaris suum	ITS1 (AB110023)	-	Pig	Ishiwata *et al*. (2004)
	ITS2 (FJ418786)	-	Pig	Wickramasinghe *et al*. (2009)

Table (7): *Contracaecum* nematodes and specimens/accession number (COX-2) of taxa used to build phylogenetic trees and the nucleotide differences with the *Contracaecum* larvae collected from the present study

Parasite	GeneBank accession no. (COX-2)	No. of nucleotide differences	Host	Source
C. rudolphii A	EF122201	34	*Phalacrocorax carbo sinensis*	Mattiucci *et al*. (2008)
C. rudolphii B	EF558894	1	*Phalacrocorax carbo sinensis*	Mattiucci *et al*. (2008)
C. rudolphii C	EF014283	272	*Phalacrocorax auritus*	D'Amelio *et al.* (2007)
C. rudolphii F	JF727879	40	*Pelecanus occidentalis*	D'Amelio *et al.* (2012)
C. ogmorhini	MN624184	32	*Zalophus californianus*	Mladineo *et al.* (under press)
C. chubutensis	HQ328504	46	*Phalacrocorax atriceps*	Garbin *et al.* (2011)
C. microcephalum	EF122208	71	*Phalacrocorax pygmaeus*	Mattiucci *et al*. (2008)
C. septentrionale	EF558898	60	*Phalacrocorax carbo carbo*	Mattiucci *et al*. (2008)
C. bioccai	EF558899	50	*Pelecanus occidentalis*	Mattiucci *et al*. (2008)
C. osculatum	KC412224	58	*Chionodraco hamatus*	Santoro *et al.* (2013)
C. multipapillatum	AF179910	72	-	Nadler & Hudspeth (2000)
C. micropapillatum	EU852350	70	*Pelecanus onocrotalus*	Mattiucci *et al*. (2010)
C. austral	GQ847539	55	*Phalacrocorax brasilianus*	Garbin *et al.* (2011)
C. pelagicum	EF122210	60	*Spheniscus magellanicus*	Mattiucci *et al*. (2008)
Contracaecum larva	MN589997	-	*Acanthobrama marmid*	Present study
Contracaecum larva	MN589998	-	*Arabibarbus grypus*	Present study
Contracaecum larva	MN589999	-	*Capoeta trutta*	Present study
Contracaecum larva	MN590000	-	*Carasobarbus luteus*	Present study
Contracaecum larva	MN590001	-	*Chondrostoma regium*	Present study
Contracaecum larva	MN590002	-	*Cyprinus carpio*	Present study
Contracaecum larva	MN590003	-	*Luciobarbus barbulus*	Present study
Contracaecum larva	MN590004	-	*Luciobarbus esocinus*	Present study
Contracaecum larva	MN590005	-	*Luciobarbus xanthopterus*	Present study
Contracaecum larva	MN590006	-	*Mastacembelus mastacembelus*	Present study
Ascaris suum	HQ704901	-	Swine	Liu *et al*. (2012)

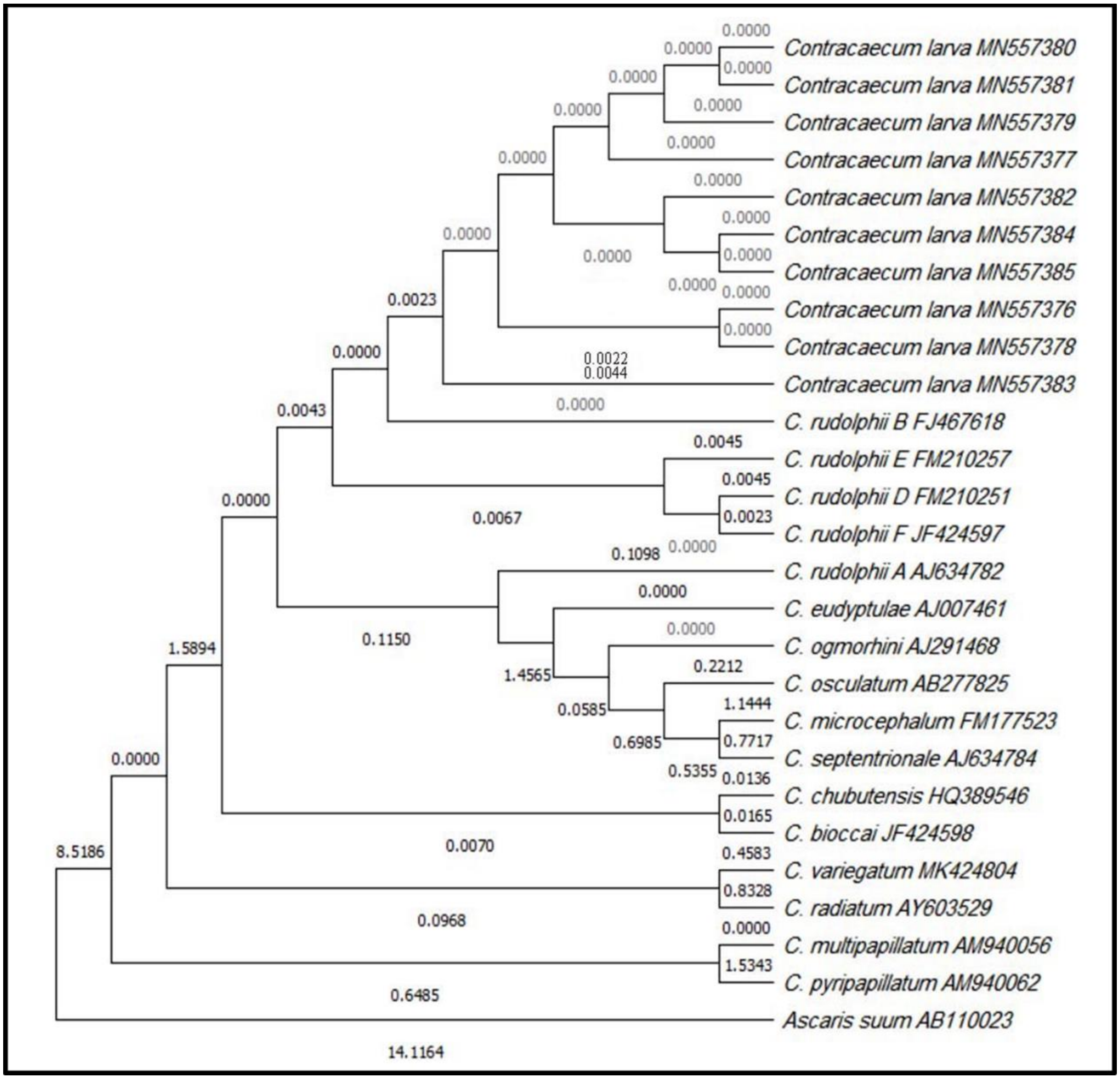

Fig. (21): Phylogenetic relationships between *Contracaecum* larvae from the present study and other *Contracaecum* species as inferred by maximum likelihood obtained from ITS-1. *Ascaris suum* was used as outgroup.

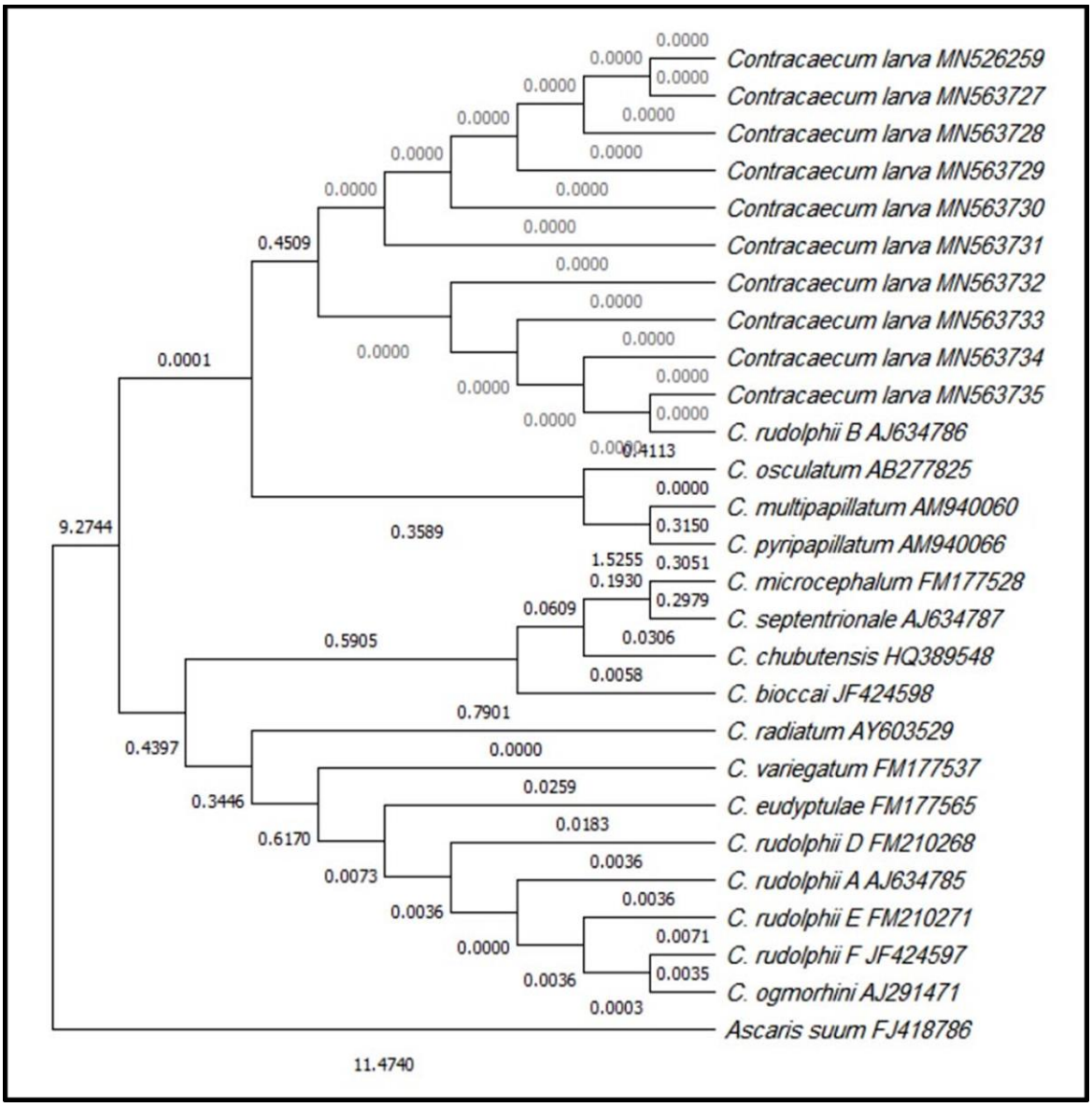

Fig. (22): Phylogenetic relationships between *Contracaecum* larvae from the present study and other *Contracaecum* species as inferred by maximum likelihood obtained from ITS-2. *Ascaris suum* was used as outgroup.

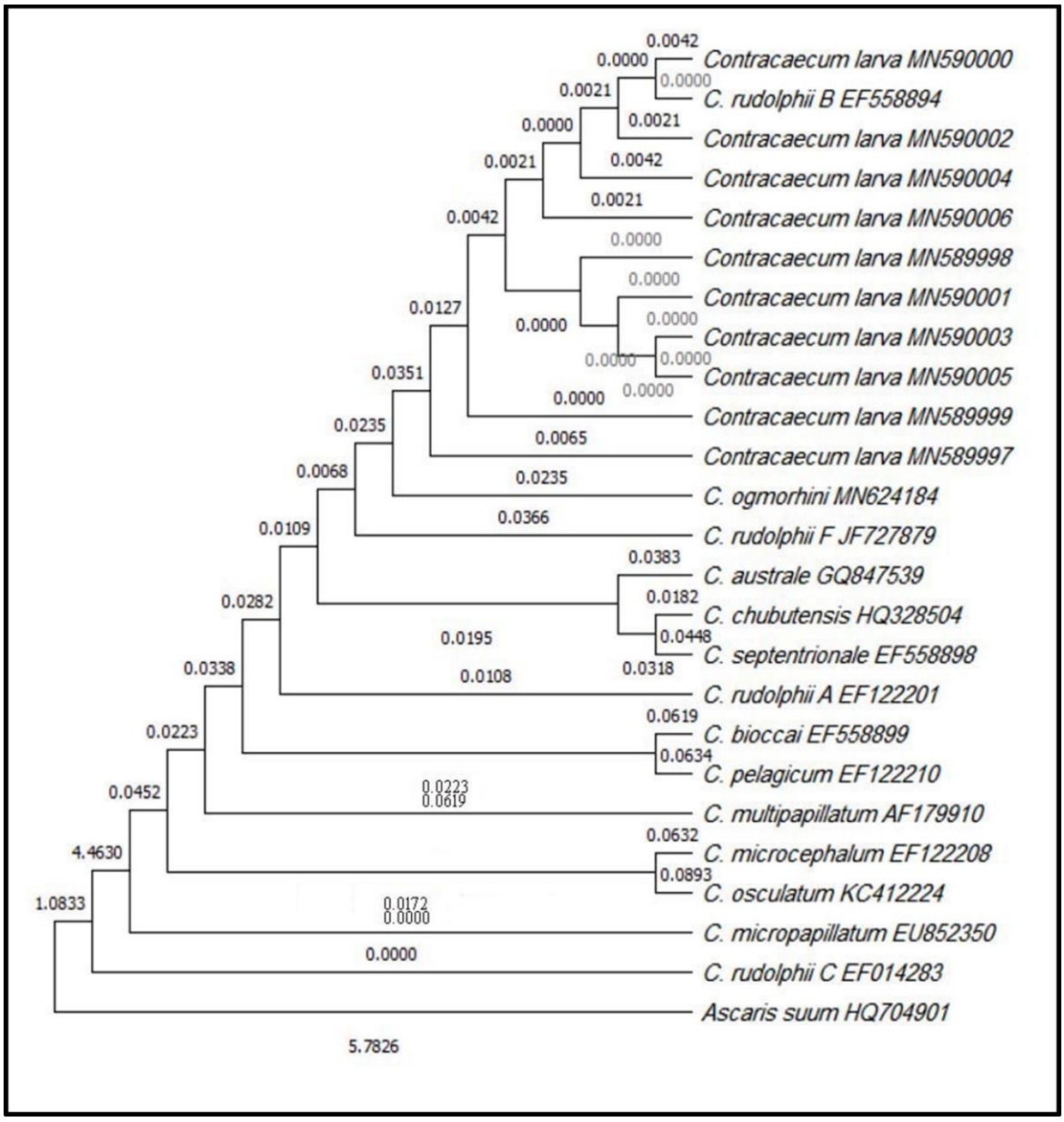

Fig. (23): Phylogenetic relationships between *Contracaecum* larvae from the present study and other *Contracaecum* species as inferred by maximum likelihood obtained from COX-2. *Ascaris suum* was used as outgroup.

The first information on *Contracaecum* larvae from the Iraqi freshwater fishes was given by Herzog (1969) which recorded *Contracaecum* larvae in 10 fish species from different inland waters of Iraq. While, In Kurdistan Region, *Contracaecum* larvae were recorded from Dukan Lake in Sulaimani Province by Abdullah & Rasheed (2004b) in *Arabibarbus grypus* (reported as *Barbus grypus*), *Carasobarbus luteus* (reported as *Barbus luteus*), *Chondrostoma regium*, *Cyprinion macrostomum*, *Cyprinus carpio*, *Luciobarbus barbulus*, *L. esocinus, L. kersin*, *L. subquincunciatus*, *L. xanthopterus*, and *Squalius lepidus*. So far, a total of 21 fish host species are known for *Contracaecum* larvae in Kurdistan Region of Iraq (Mhaisen & Abdullah, 2017). In addition, molecular identification of *Contracaecum* larvae in fish species have also not been studied, and there is still no specific identification of the *Contracaecum* species in fresh and marine water fish species in Iraq, as well as morphological identification based just on optical microscopy has been used to identify larval stage of *Contracaecum* only for genus level in this country. Recently, 42 different fish species were known as hosts for *Contracaecum* larvae in Iraq from north to south including marine water fish (Mhaisen, 2019).

It is considered to mention that the adult *Contracaecum rudolphii* sensu lato was recorded previously for the first time in Iraq in the digestive tract of the great black cormorant *Phalacrocorax carbo* from Baghdad Province in Iraq (Al-Moussawi & Mohammad, 2011). Furthermore, four other species of adult *Contracaecum* were reported in piscivorous bird (final host) from Barsrah Province, Iraq namely: *Contracaecum microcephalum* was recorded from the purple heron *Ardea purpurea* (Al-Hadithi & Habish, 1977; Habish, 1977; Awad *et al*., 1994), from the pygmy cormorant (*Phalocrocorax pygmeus*) and the little egret (*Egretta grazetta*) from Basrah

Marshes (Awad *et al*., 1994) and from *E. grazetta*, the bittern *Ardeola ralloides* and the little bittern *Ixobrychus minutus* from Al-Hammar Marsh (Ali, 2008). *C. micropapillatum* was isolated from the grey heron *Ardea cinerea* and *A. ralloides* from Al-Hammar Marsh (Ali, 2008). *C. multipapillatum* and *C. rudolphi* (reported as *C. spiculigerum*) were reported from the pygmy cormorant (*P. pygmeus*) from Basrah Marsh (Habish, 1977; Awad *et al*., 1994). *C. ovale* was reported from *A. purpurea* from Abu Zijri Marsh (Abdullah, 1988; Al-Hadithi & Abdullah, 1991) and from the bittern (*A. ralloides*) (Ali, 2008). In addition, unidentified adult *Contracaecum* species were also recorded from *Phalocrocorax carbo* in Shatt Al-Arab River (Abed, 2005) and from ten bird species in Meshab Marsh (Al-Tameemi, 2013).

The present investigation provides the first molecular and ultra-structural approaches toward characterization of larval ansakid nematodes (*Contracaecum*) in Iraq. Based on morphology, ultra-structure and molecular characters, all larvae in the present study which collected from *A. marmid*, *A. grypus*, *C. trutta*, *C. luteus*, *C. regium*, *C. carpio*, *L. barbulus*, *L. esocinus*, *L. xanthopterus*, and *M. mastacembelus* are belonging *Contracaecum rudolphii* B. It was noted that the *Contracaecum* larvae can infect more fish type in Iraq (Mhaisen, 2019). This unspecificity characters to infect a variety of different organs and different fish species may lead to infect a variety of piscivorous birds and mammals in the region. Anisakidosis is a disease caused by the accidental ingestion of larval anisakid nematodes in raw fish. All fishes which infected with *Contracaecum* larvae in the present study are edible, particularly *A. marmid* (prevalence 35%) and *L. xanthopterus* (prevalence 19.35%) in Sulaimani Province. This may affect human health in this region, because this fish is

used by local people and other consumers as a food source, as well as it is one of the delicious fish by local consumers.

Conclusions

In the view of the results of the present study, the following conclusions are drawn:

1. During this investigation 36 different fish species in 26 genera belonging to 10 families were recorded from different localities of Sulaimani Province. The most abundant species and wide spread species was *Cyprinion macrostomum* with prevalence 15.17% followed by *Capoeta trutta* 10.46%. While, *Leuciscus vorax* was scarce with the ratio 0.047%.
2. In the present study *Alburnoides velioglui* was recorded for the first time in Iraq. The molecular characterization of this fish deposed in the GenBank database under the accession number (MN893770).
3. During examination of fishes in Sulaimani Province, it appeared that there were 10 different fish species infected with the third larval stage (L3) of *Contracaecum.* Some fishes in Sulaimani Province showed a high degree of sensitivity towards the infection with *Contracaecum* larvae, for example, the prevalence of infection in *Acanthobrama marmid* and *Luciobarbus xanthopterus* reached 35% and 19.35% respectively. While, other species showed low degree for example the prevalence of infection in *Arabibarbus grypus* and *Capoeta trutta* reached 0.81% and 0.9% respectively.
4. The *Contracaecum* larvae cannot be exactly diagnose depending on morphology by using optical microscope even by ultra-structural study by using scanning electron microscopy. These larvae can be diagnose only by molecular study, and the *Contracaecum* larvae in Sulaimani Province are belonging to *Contracaecum rudolphii* type-B.
5. The differences in the measurements like intestinal caecum, esophageal caecum, rectum, and boring tooth due to a difference in the hosts, the molecular study showed that all belonging one species.
6. *Contracaecum* larvae can infect human by eating raw or undercooked fish.

Recommendations

1. Sulaimani Province and Kurdistan Region of Iraq are rich with water bodies and has a good fish biodiversity and not undergone much studies yet, so it is recommended to survey of its fish fauna by using a new and scientific fishing ways (electro shocking and snorkeling) in order to record new fish fauna and parasitic fauna of fishes in this country.
2. Confirmative diagnosis (molecular study) is necessary for some fish species which closely similar to each other.
3. Gravel mining, garbage dumping, oil dumping, wastewater pipeline to river, sewage pollution, introduction of exotic aquatic species, illegal and over fishing are the main threats to fish diversity in Kurdistan Region of Iraq. So, it is suggested avoiding gravel mining on the rivers, treat all waste before discarding, and prevent over and illegal fishing.
4. Identify and manage some geographical area, recognized and managed, through legal or other effective means, as a protected area to achieve long-term conservation of all fish species, particularly threatened species.
5. The molecular study has ability to accurate diagnose of parasites, so it is recommended to use this tool for confirmative diagnose of the previously diagnosed fish parasites particularly the larval stage of the parasites.
6. Determination of phylogenetic relationships among fish parasites in Iraq to understanding the relationships among them.
7. The *Contracaecum* larvae in Sulaimani Province are belonging *Contracaecum rudolphii* B. The study of complete life cycle of these larvae in the laboratory is necessary for examination of the morphology and ultra-structure of adult stage (male and female), also using additional genetic markers.

8. Due to the clinical importance of this parasite, it is necessary to study the impact of the ecological, biological and pathological aspects of these parasites and subsequently the mode of their control and treatment.

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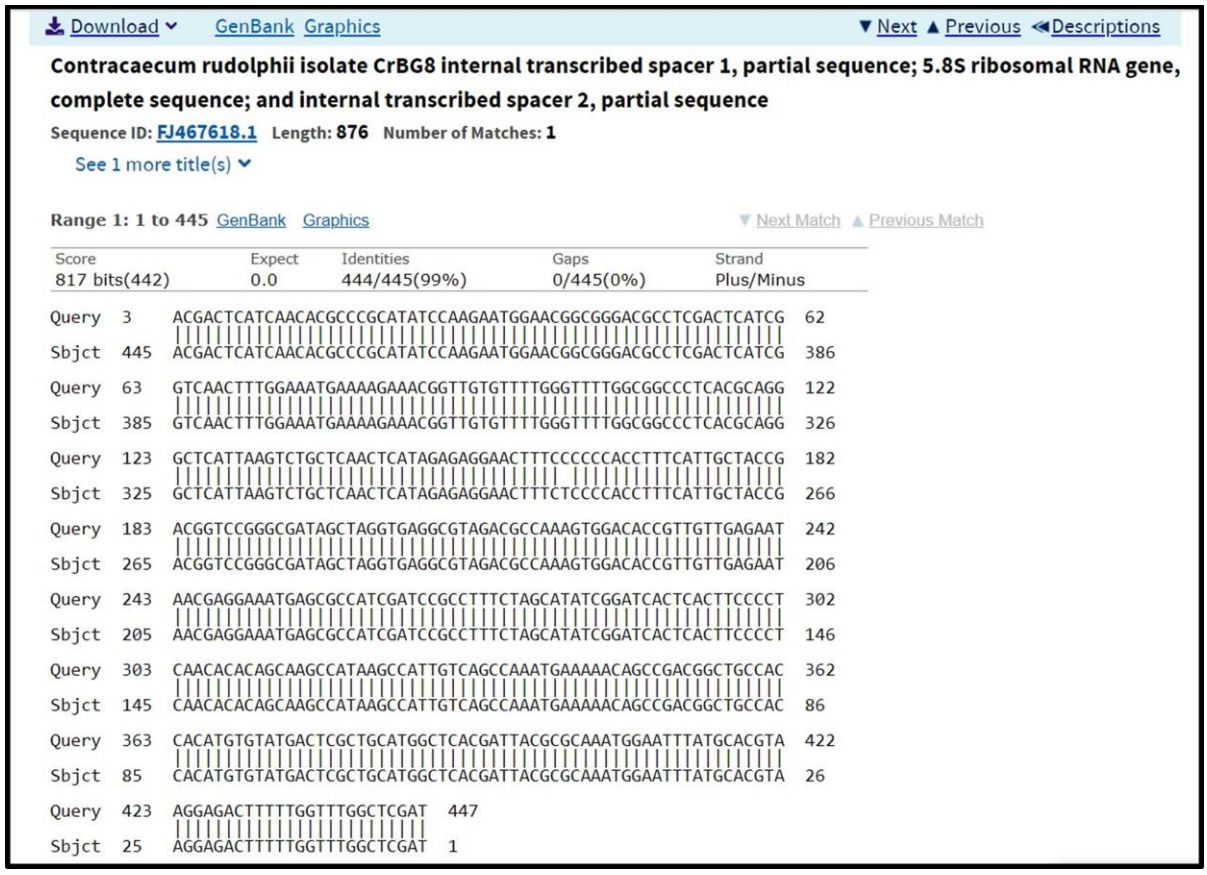
Appendices



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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

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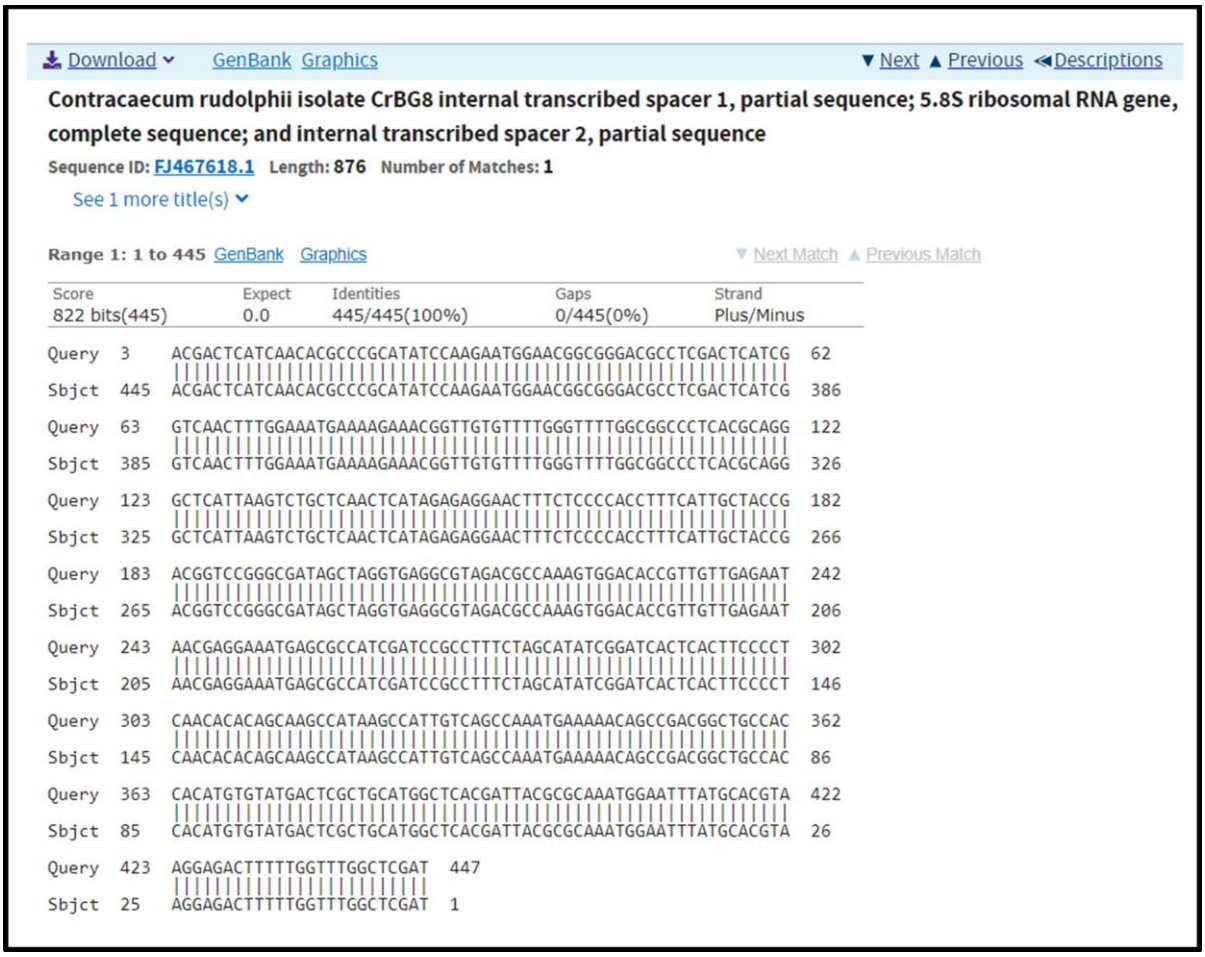

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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 Length: 876 Number of Matches: 1

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Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

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Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```
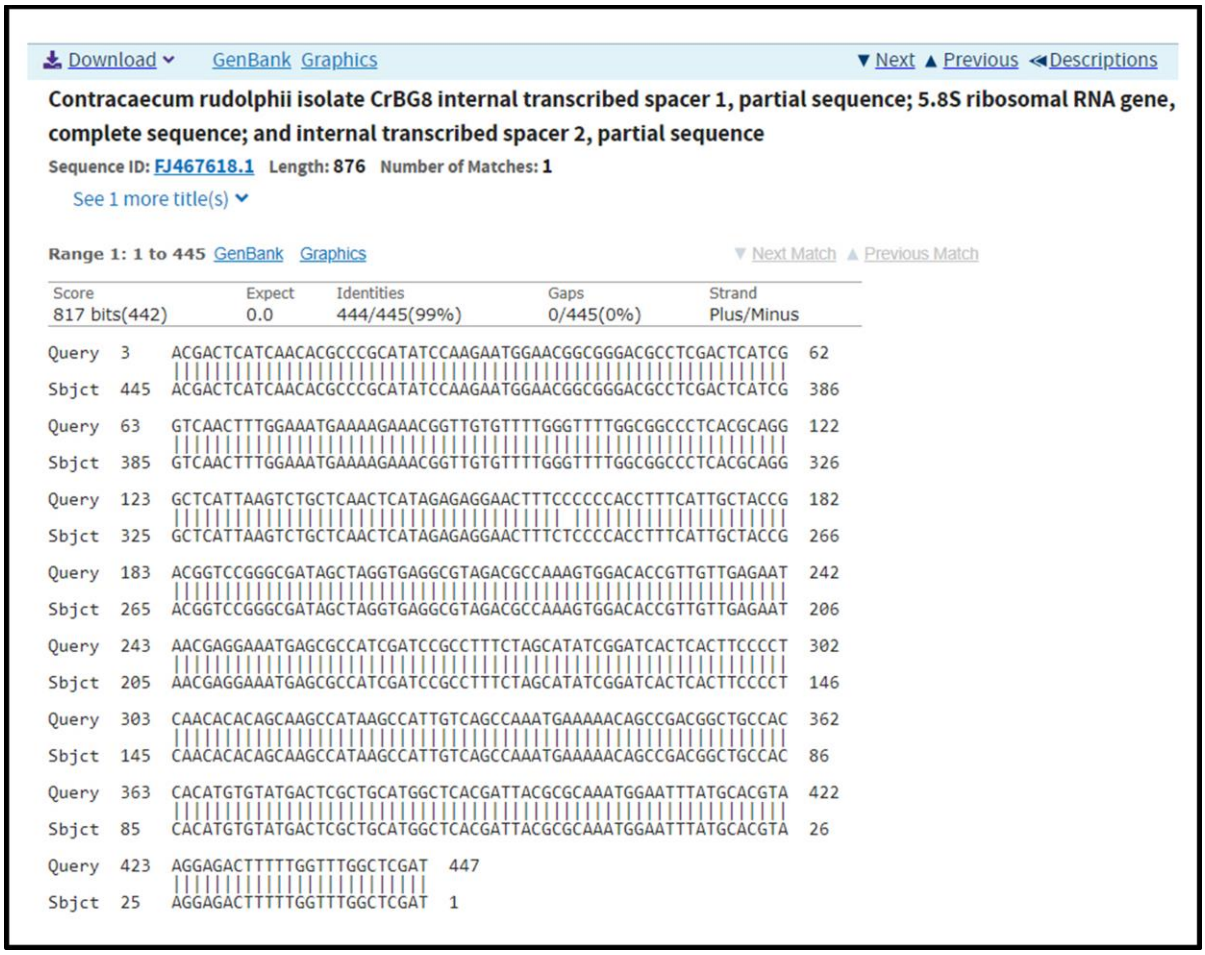

Fig. (25): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Arabibarbus grypus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

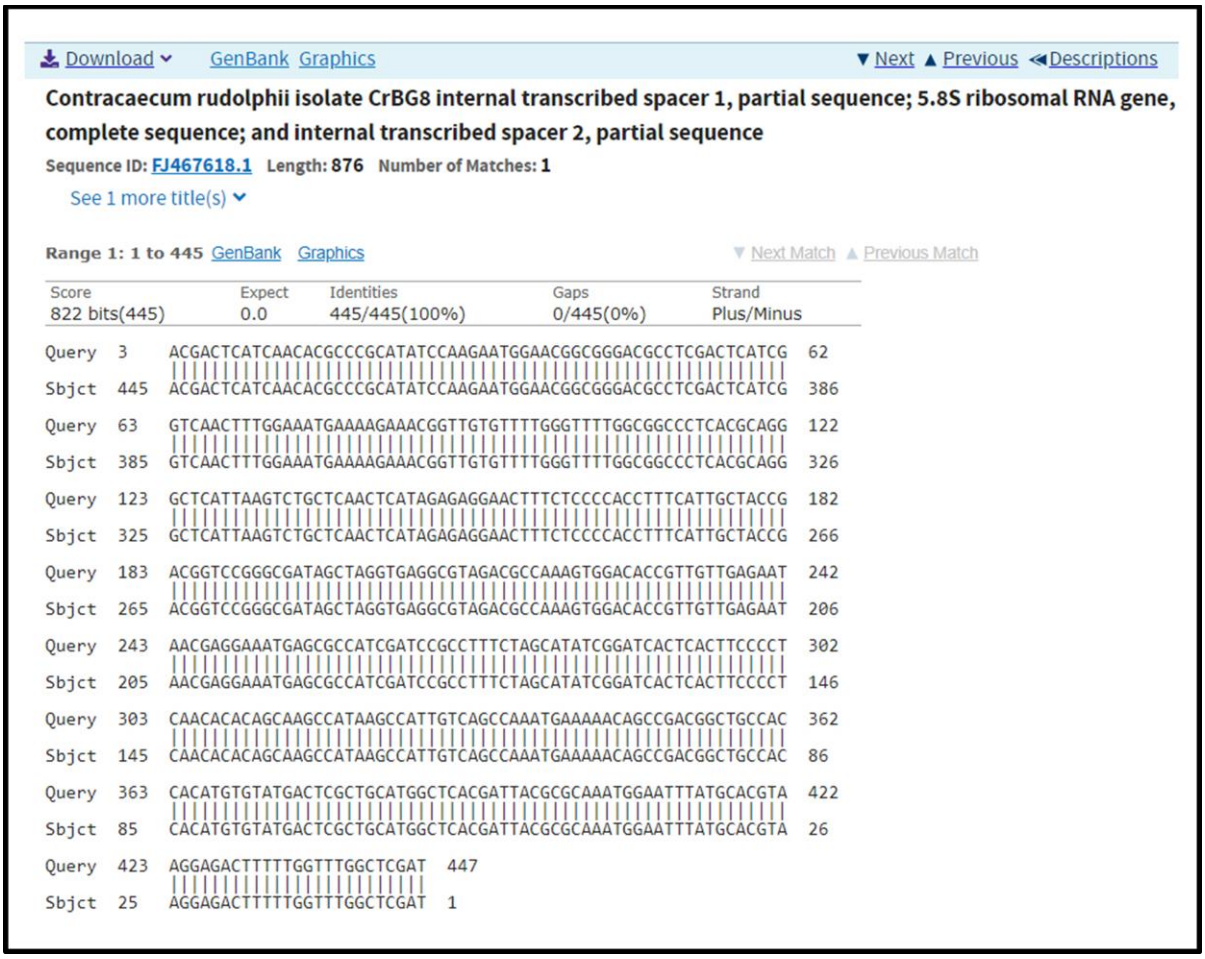
See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
817 bits(442)	0.0	444/445(99%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCCCCCCACCTTTCATTGCTACCG  182
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (26): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Capoeta trutta*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

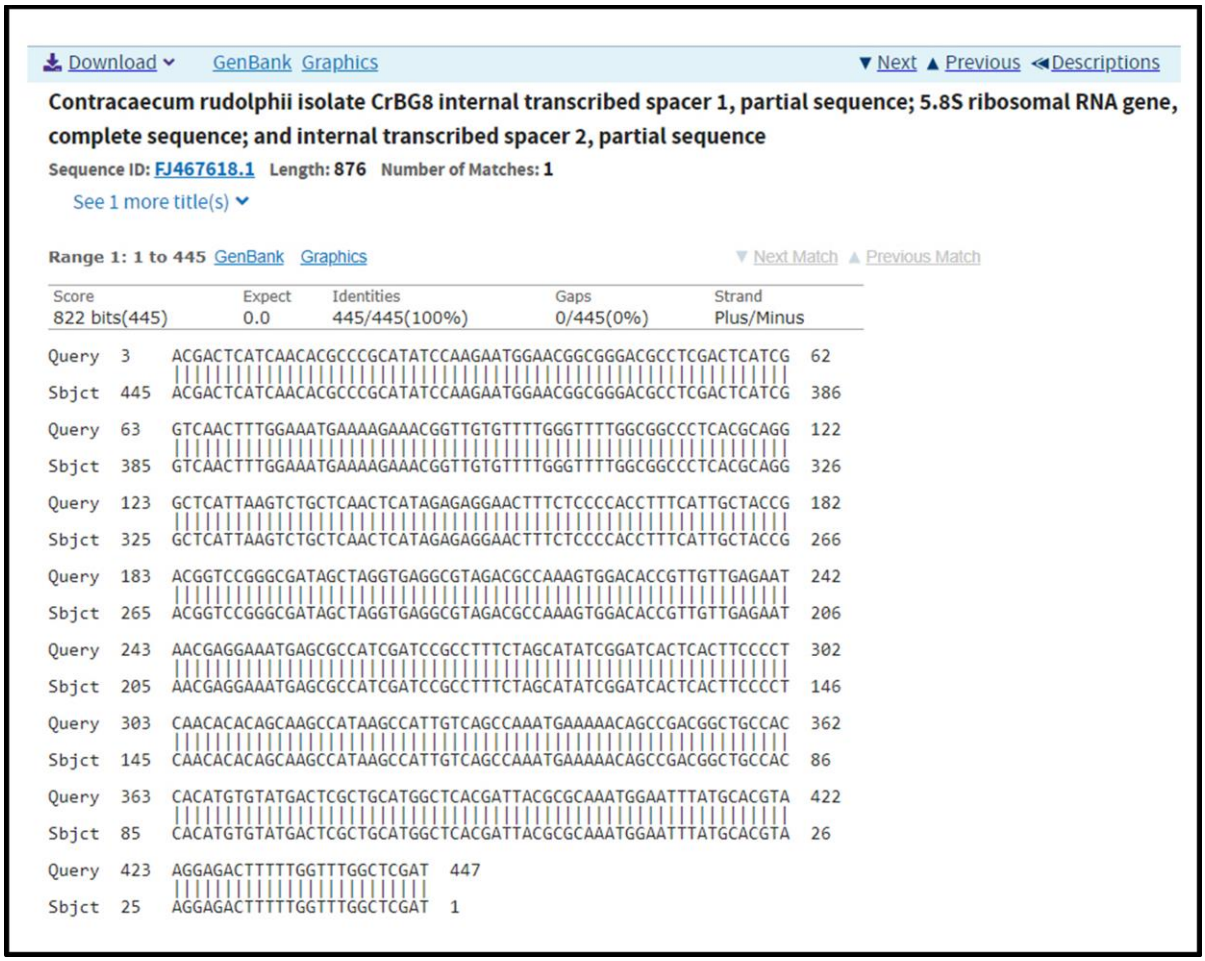
See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
822 bits(445)	0.0	445/445(100%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (27): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Carasobarbus luteus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
822 bits(445)	0.0	445/445(100%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (28): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Chondrostoma regium*, Query is the study or sample sequence and Subject is the GenBank sequence.

Download GenBank Graphics ▼ Next ▲ Previous ◀◀ Descriptions

Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 Length: 876 Number of Matches: 1

See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
822 bits(445)	0.0	445/445(100%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (29): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Cyprinus carpio*, Query is the study or sample sequence and Subject is the GenBank sequence.

Download GenBank Graphics ▼ Next ▲ Previous ◀◀ Descriptions

Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
822 bits(445)	0.0	445/445(100%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (30): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Luciobarbus barbulus*, Query is the study or sample sequence and Subject is the GenBank sequence.

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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
811 bits(439)	0.0	443/445(99%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAGAAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||  ||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (31): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Luciobarbus esocinus*, Query is the study or sample sequence and Subject is the GenBank sequence.

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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
822 bits(445)	0.0	445/445(100%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (32): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Luciobarbus xanthopterus*, Query is the study or sample sequence and Subject is the GenBank sequence.

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Contracaecum rudolphii isolate CrBG8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: FJ467618.1 **Length:** 876 **Number of Matches:** 1

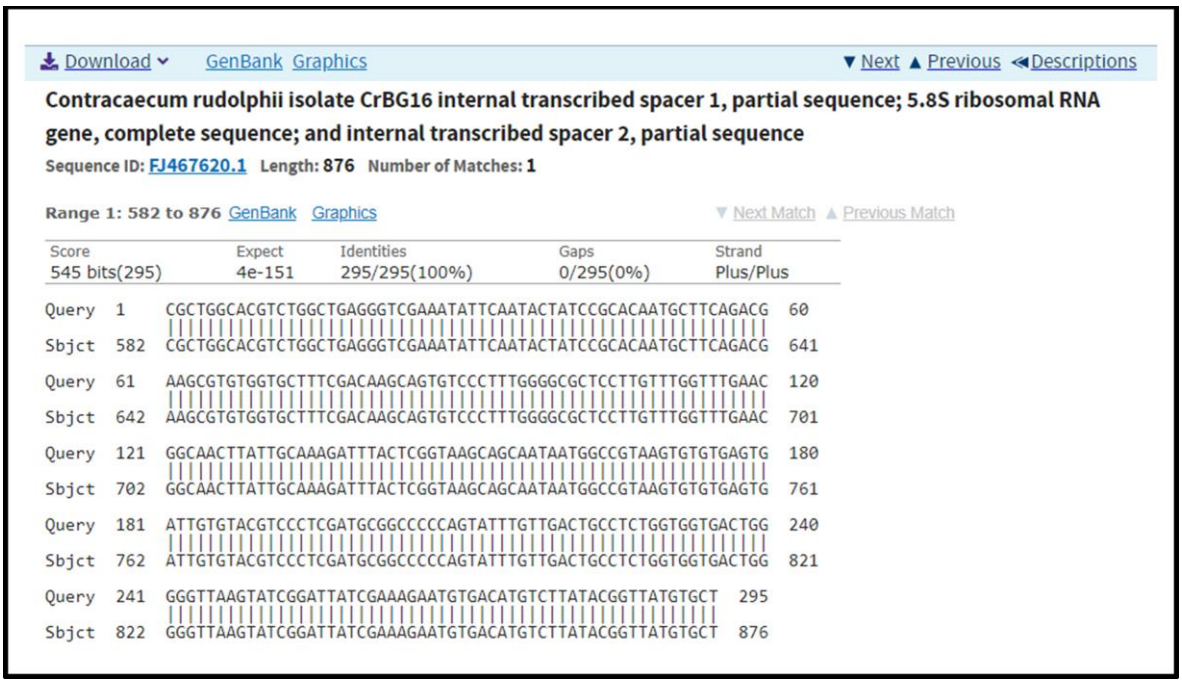
See 1 more title(s)

Range 1: 1 to 445 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
822 bits(445)	0.0	445/445(100%)	0/445(0%)	Plus/Minus

```
Query  3    ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  62
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  ACGACTCATCAACACGCCCGCATATCCAAGAATGGAACGGCGGGACGCCTCGACTCATCG  386

Query  63   GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  GTCAACTTTGGAAATGAAAAGAAACGGTTGTGTTTTGGGTTTTGGCGGCCCTCACGCAGG  326

Query  123  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  GCTCATTAAGTCTGCTCAACTCATAGAGAGGAACTTTCTCCCCACCTTTCATTGCTACCG  266

Query  183  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ACGGTCCGGGCGATAGCTAGGTGAGGCGTAGACGCCAAAGTGGACACCGTTGTTGAGAAT  206

Query  243  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  AACGAGGAAATGAGCGCCATCGATCCGCCTTTCTAGCATATCGGATCACTCACTTCCCCT  146

Query  303  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  CAACACACAGCAAGCCATAAGCCATTGTCAGCCAAATGAAAAACAGCCGACGGCTGCCAC  86

Query  363  CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   CACATGTGTATGACTCGCTGCATGGCTCACGATTACGCGCAAATGGAATTTATGCACGTA  26

Query  423  AGGAGACTTTTTGGTTTGGCTCGAT  447
            |||||||||||||||||||||||||
Sbjct  25   AGGAGACTTTTTGGTTTGGCTCGAT  1
```

Fig. (33): Pair wise alignment ITS-1 sequence of *Contracaecum* larva collected from *Mactacembelus mastacembelus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

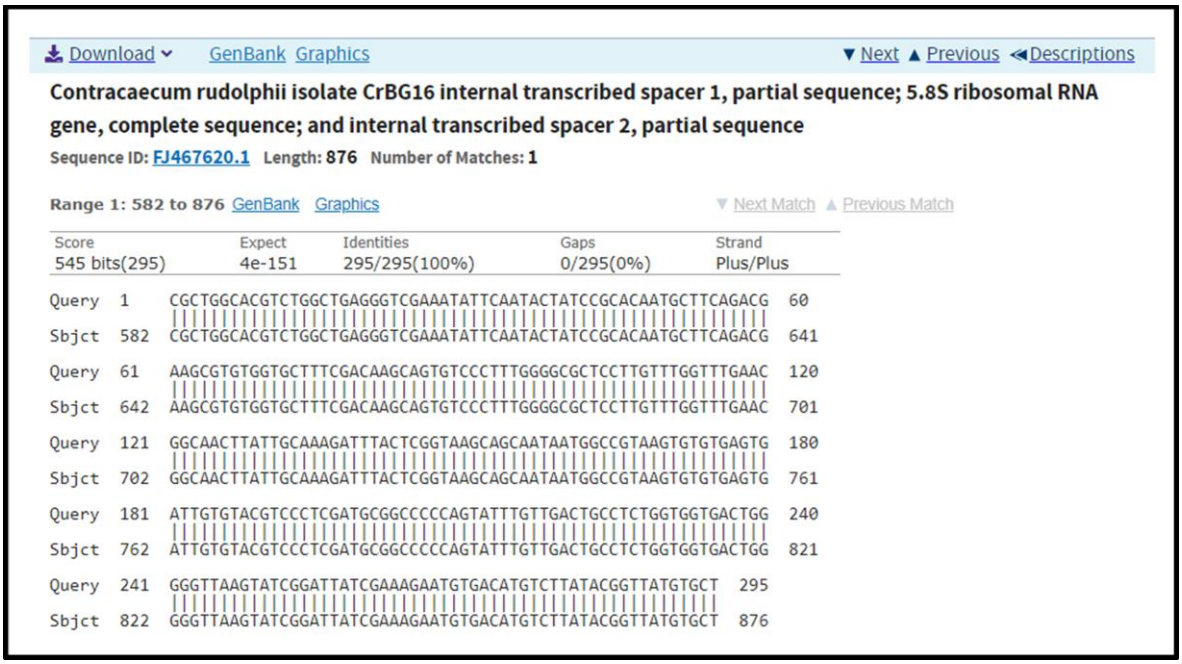
Sequence ID: FJ467620.1 Length: 876 Number of Matches: 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (34): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Acanthobrama marmid*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

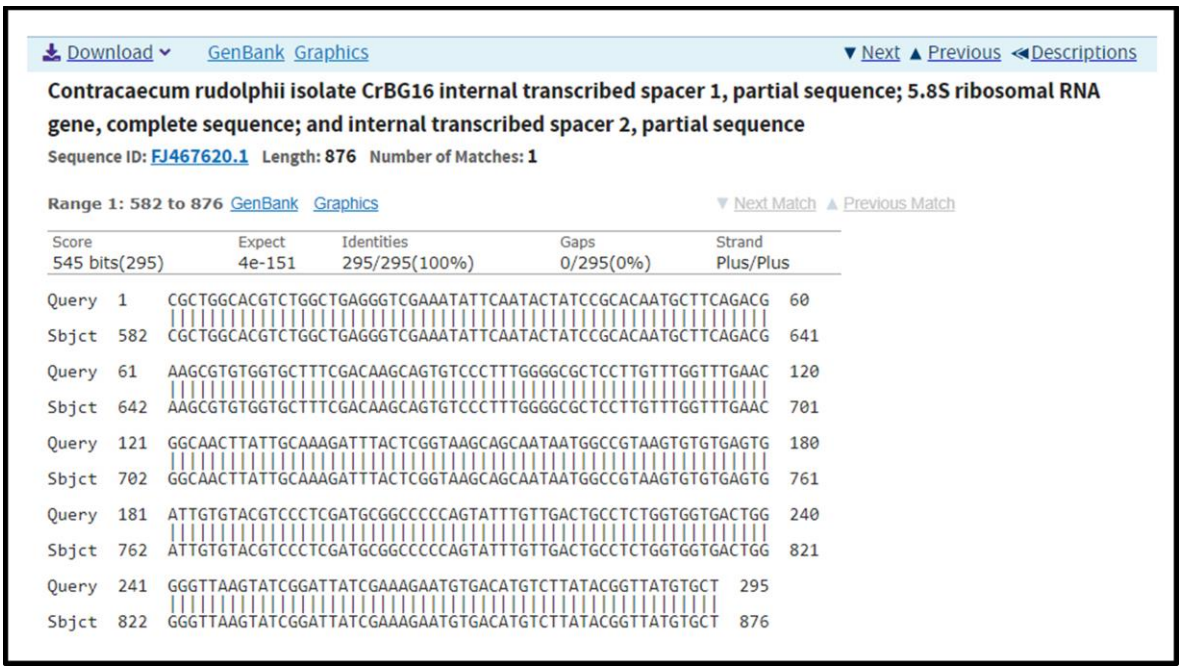
Sequence ID: FJ467620.1 Length: 876 Number of Matches: 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (35): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Arabibarbus grypus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

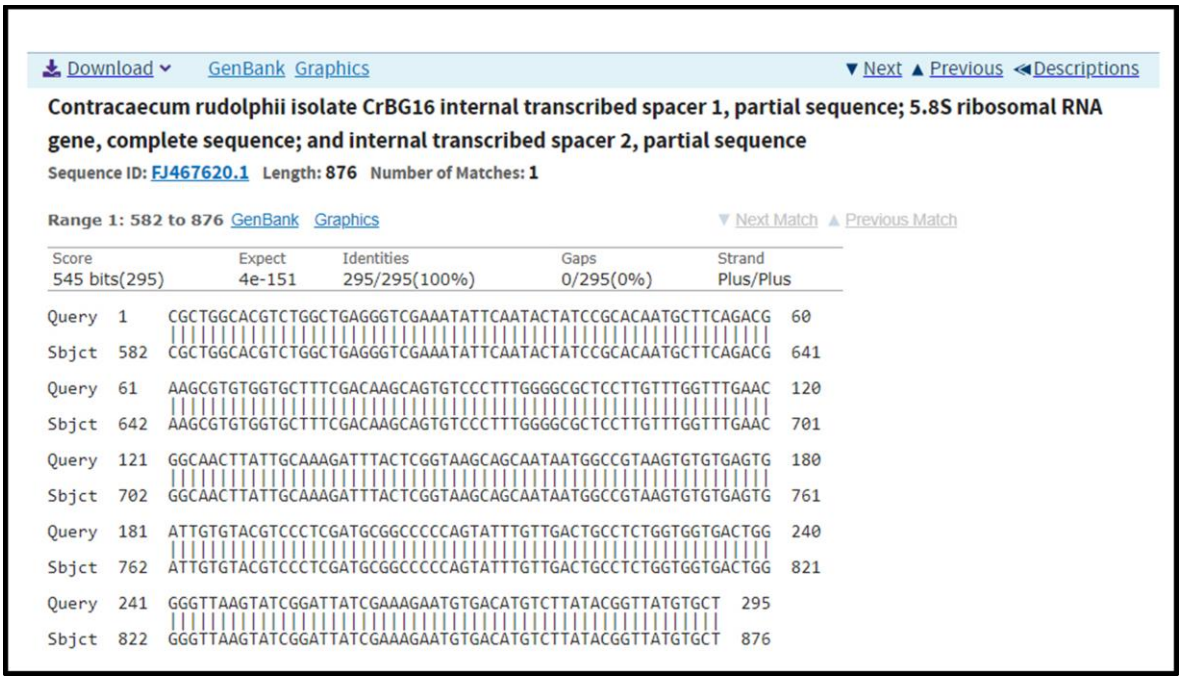
Sequence ID: FJ467620.1 **Length:** 876 **Number of Matches:** 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (36): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Capoeta trutta*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

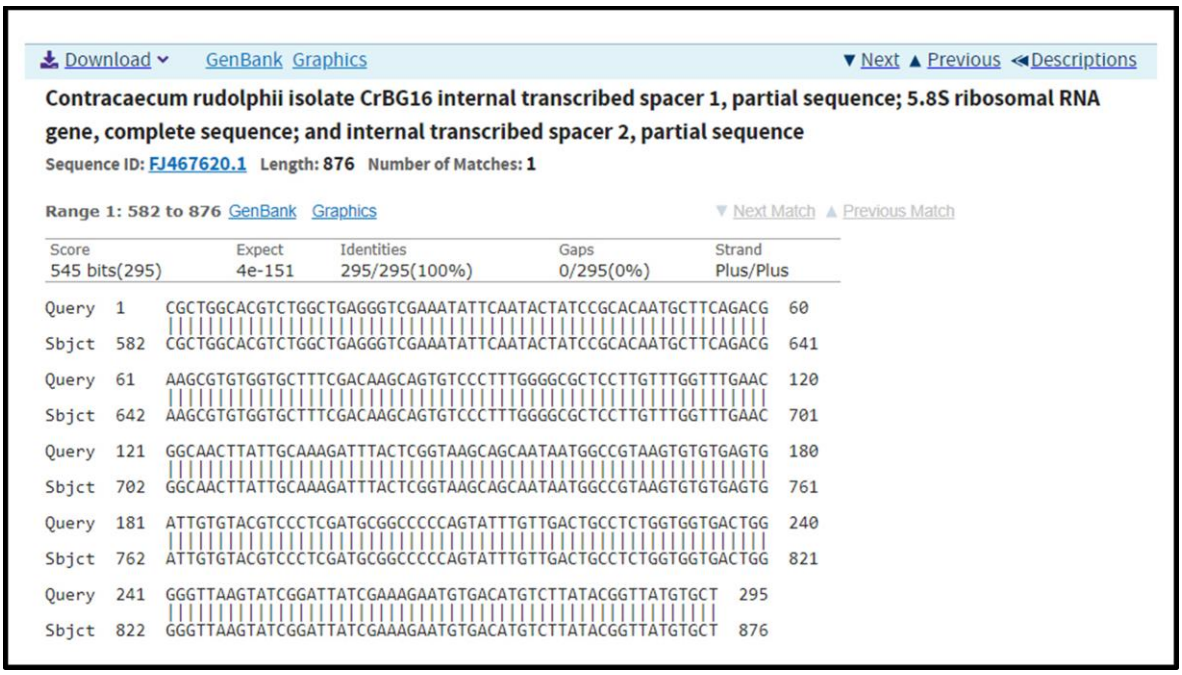
Sequence ID: FJ467620.1 **Length:** 876 **Number of Matches:** 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (37): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Carasobarbus luteus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

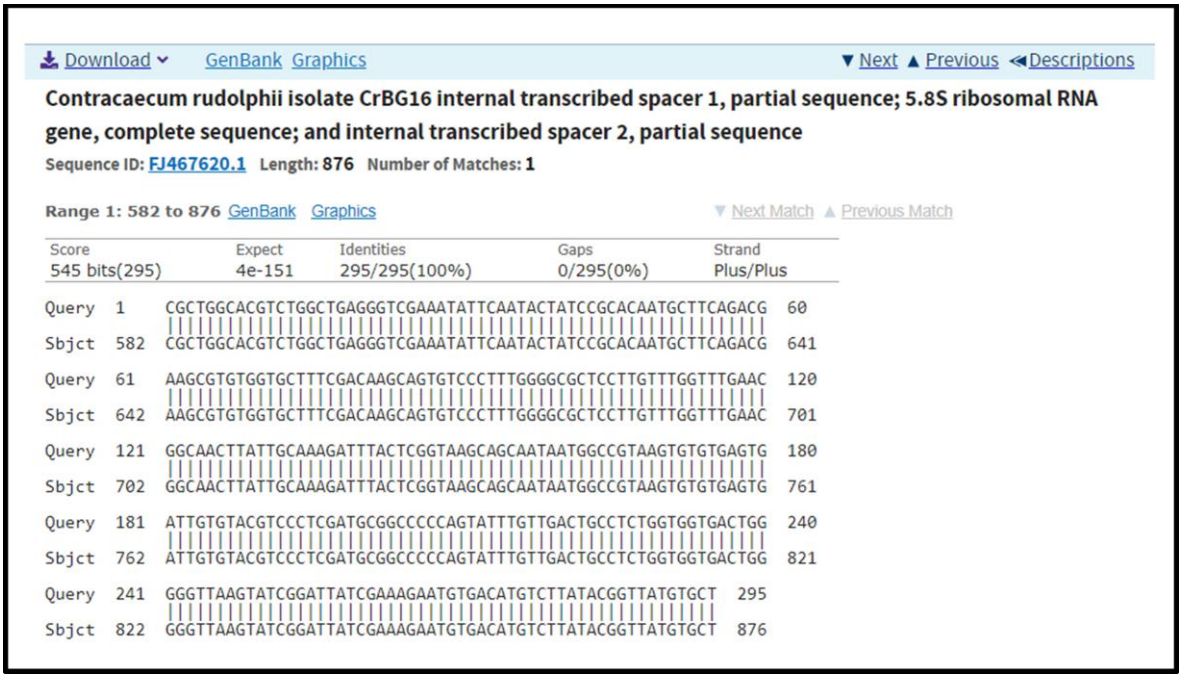
Sequence ID: FJ467620.1 **Length:** 876 **Number of Matches:** 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (38): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Chondrostoma regium*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

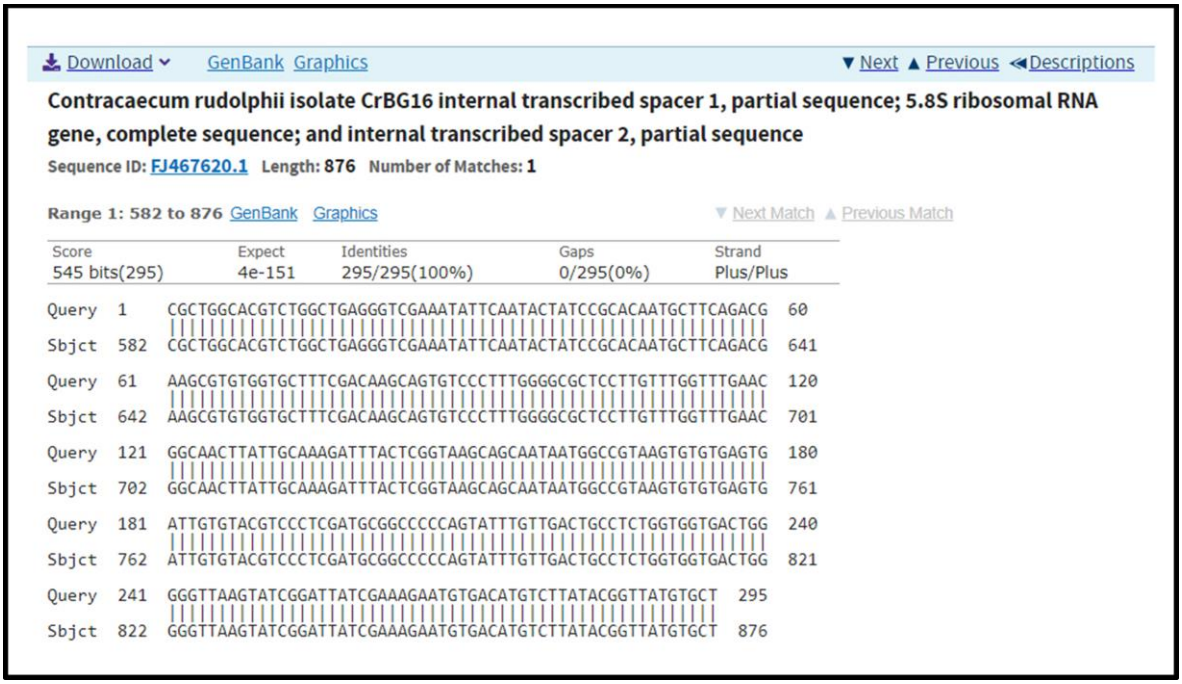
Sequence ID: FJ467620.1 **Length:** 876 **Number of Matches:** 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (39): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Cyprinus carpio*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

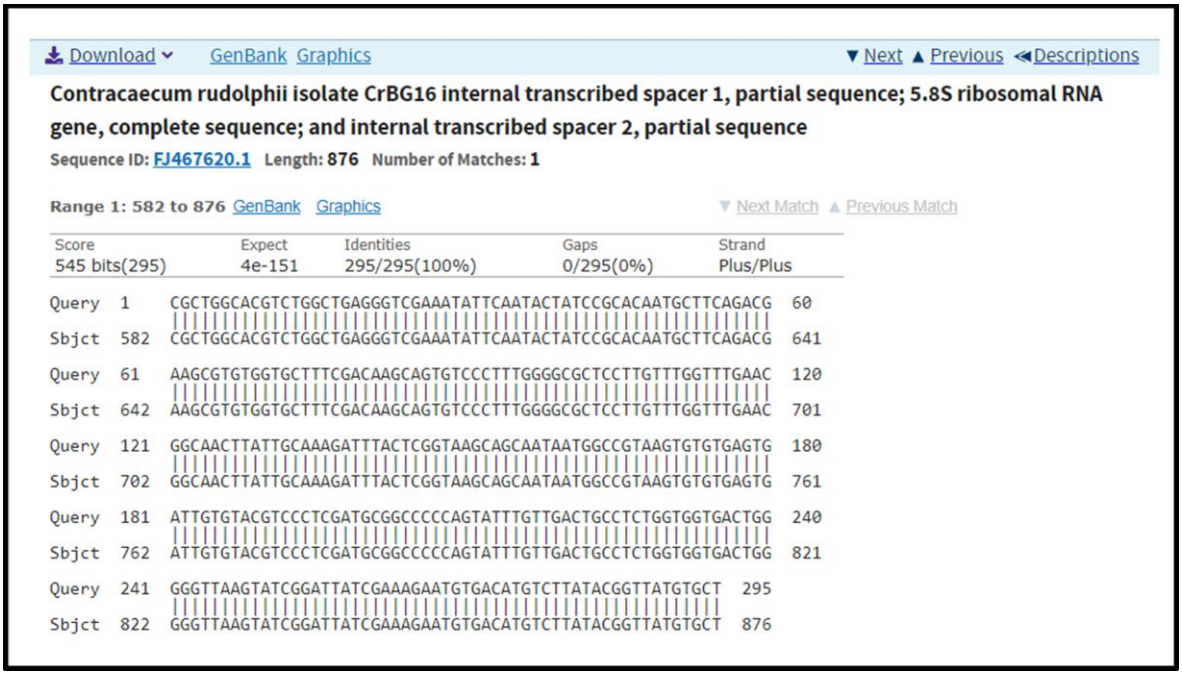
Sequence ID: FJ467620.1 Length: 876 Number of Matches: 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (40): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Luciobarbus barbulus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

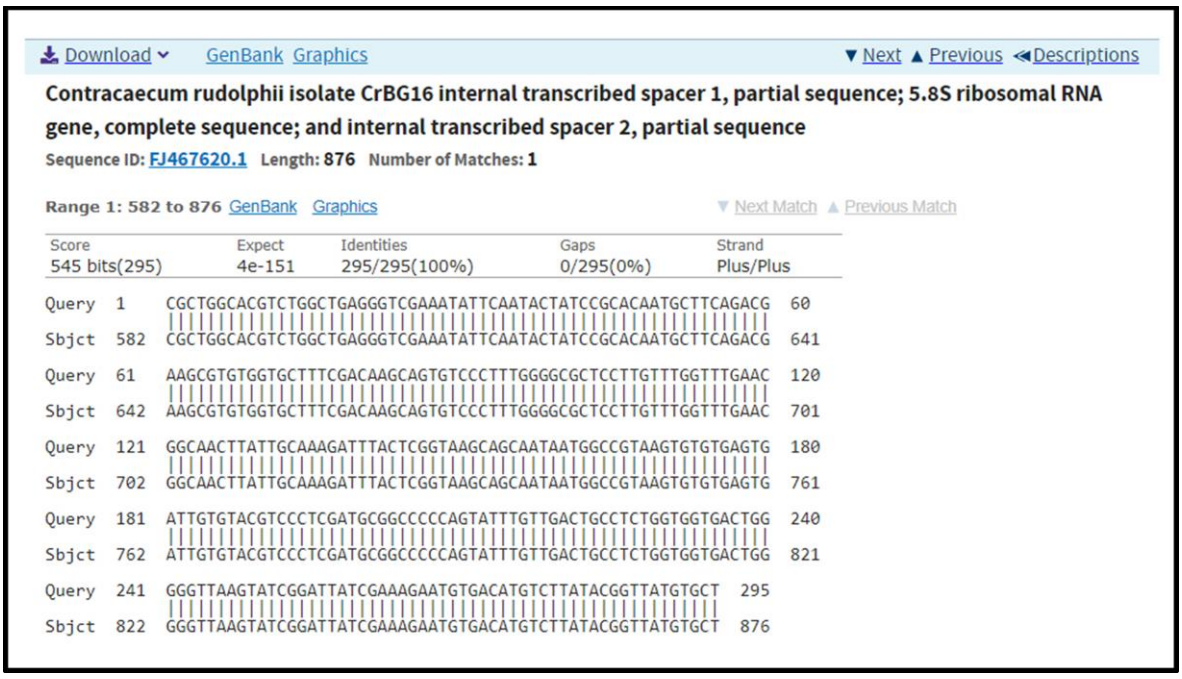
Sequence ID: FJ467620.1 Length: 876 Number of Matches: 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (41): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Luciobarbus esocinus*, Query is the study or sample sequence and Subject is the GenBank sequence.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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

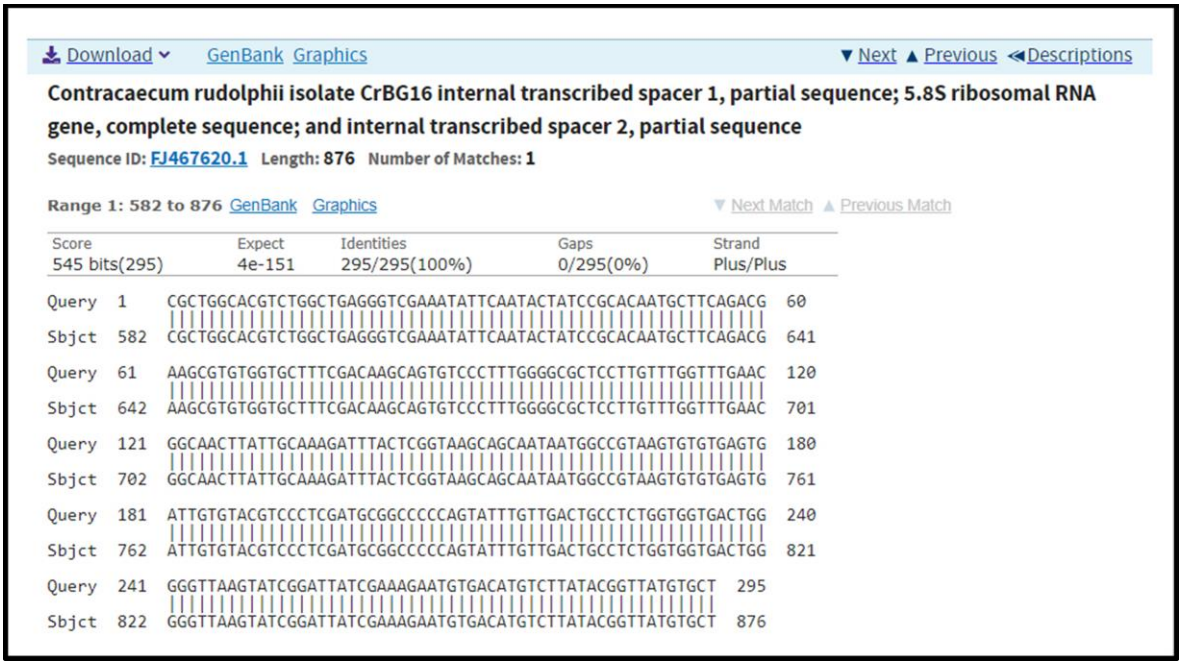
Sequence ID: FJ467620.1 Length: 876 Number of Matches: 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (42): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Luciobarbus xanthopterus*, Query is the study or sample sequence and Subject is the GenBank sequence.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Contracaecum rudolphii isolate CrBG16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

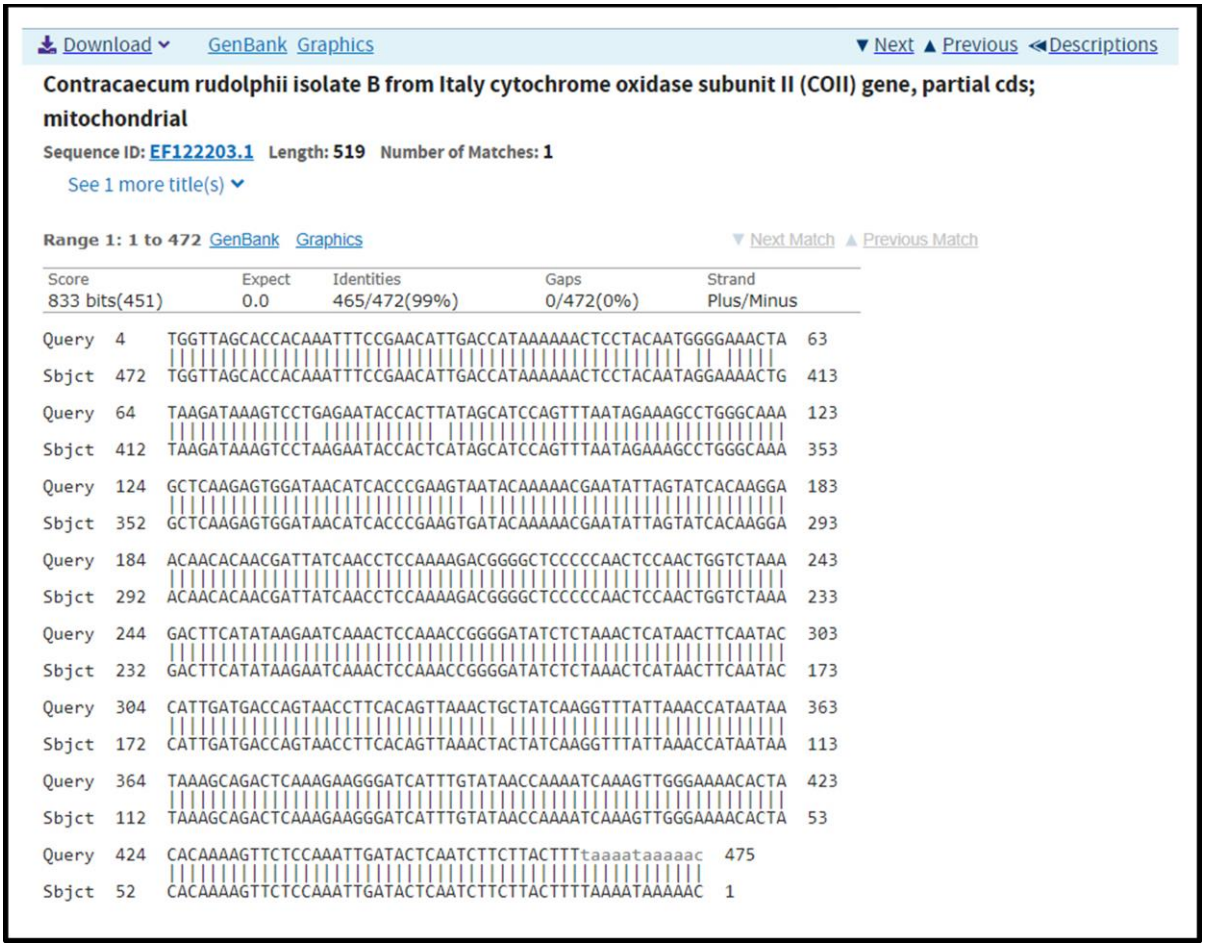
Sequence ID: FJ467620.1 Length: 876 Number of Matches: 1

Range 1: 582 to 876 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
545 bits(295)	4e-151	295/295(100%)	0/295(0%)	Plus/Plus

```
Query  1    CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582  CGCTGGCACGTCTGGCTGAGGGTCGAAATATTCAATACTATCCGCACAATGCTTCAGACG  641

Query  61   AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  642  AAGCGTGTGGTGCTTTCGACAAGCAGTGTCCCTTTGGGGCGCTCCTTGTTTGGTTTGAAC  701

Query  121  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  702  GGCAACTTATTGCAAAGATTTACTCGGTAAGCAGCAATAATGGCCGTAAGTGTGTGAGTG  761

Query  181  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  762  ATTGTGTACGTCCCTCGATGCGGCCCCCAGTATTTGTTGACTGCCTCTGGTGGTGACTGG  821

Query  241  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  295
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822  GGGTTAAGTATCGGATTATCGAAAGAATGTGACATGTCTTATACGGTTATGTGCT  876
```

Fig. (43): Pair wise alignment ITS-2 sequence of *Contracaecum* larva collected from *Mastacembelus mastacembelus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii isolate B from Italy cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial

Sequence ID: EF122203.1 **Length:** 519 **Number of Matches:** 1

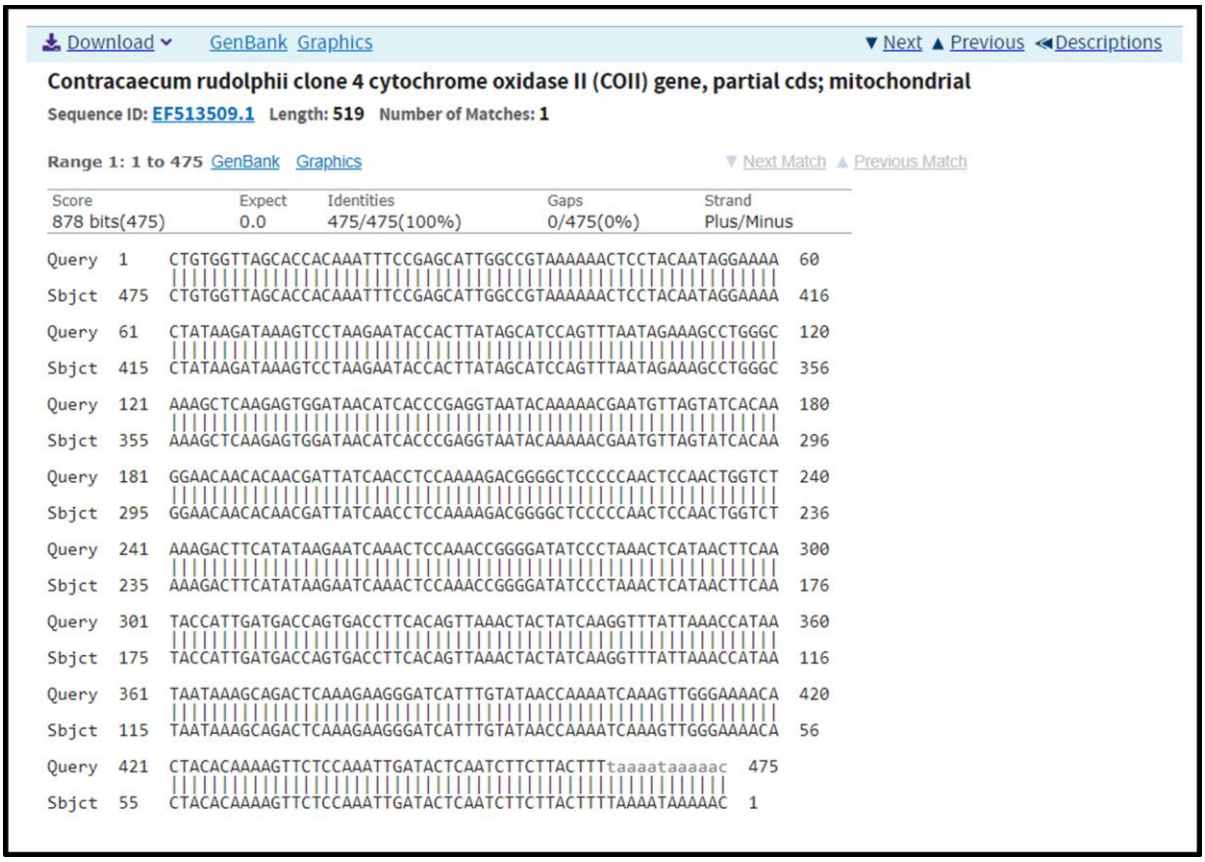
See 1 more title(s)

Range 1: 1 to 472 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
833 bits(451)	0.0	465/472(99%)	0/472(0%)	Plus/Minus

```
Query  4    TGGTTAGCACCACAAATTTCCGAACATTGACCATAAAAAACTCCTACAATGGGGAAACTA  63
            ||||||||||||||||||||||||||||||||||||||||||||||||||| || |||||
Sbjct  472  TGGTTAGCACCACAAATTTCCGAACATTGACCATAAAAAACTCCTACAATAGGAAAACTG  413

Query  64   TAAGATAAAGTCCTGAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGCAAA  123
            ||||||||||||| |||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  412  TAAGATAAAGTCCTAAGAATACCACTCATAGCATCCAGTTTAATAGAAAGCCTGGGCAAA  353

Query  124  GCTCAAGAGTGGATAACATCACCCGAAGTAATACAAAAACGAATATTAGTATCACAAGGA  183
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  352  GCTCAAGAGTGGATAACATCACCCGAAGTGATACAAAAACGAATATTAGTATCACAAGGA  293

Query  184  ACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCTAAA  243
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  292  ACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCTAAA  233

Query  244  GACTTCATATAAGAATCAAACTCCAAACCGGGGATATCTCTAAACTCATAACTTCAATAC  303
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  232  GACTTCATATAAGAATCAAACTCCAAACCGGGGATATCTCTAAACTCATAACTTCAATAC  173

Query  304  CATTGATGACCAGTAACCTTCACAGTTAAACTGCTATCAAGGTTTATTAAACCATAATAA  363
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  172  CATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAATAA  113

Query  364  TAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACACTA  423
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  112  TAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACACTA  53

Query  424  CACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  52   CACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (44): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Acanthobrama marmid*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii clone 4 cytochrome oxidase II (COII) gene, partial cds; mitochondrial

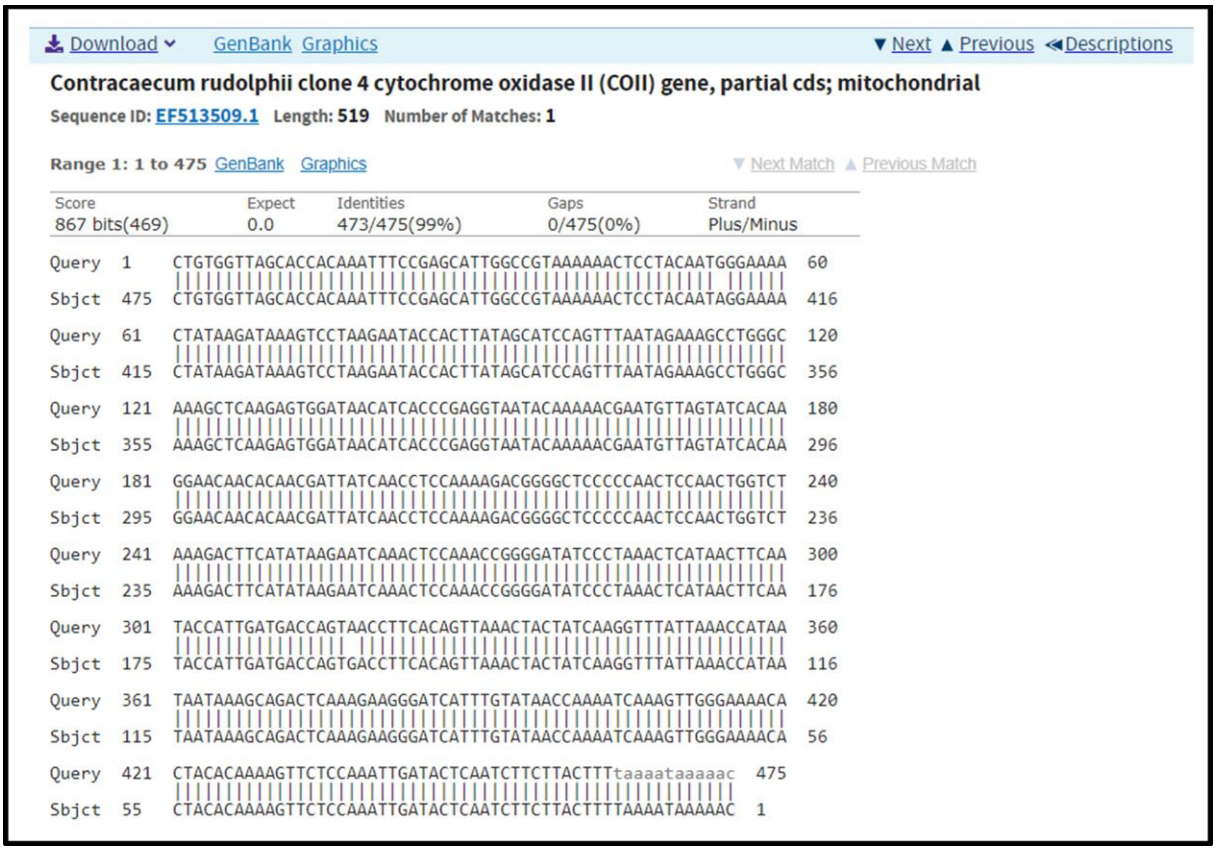
Sequence ID: EF513509.1 **Length:** 519 **Number of Matches:** 1

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
878 bits(475)	0.0	475/475(100%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (45): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Arabibarbus grypus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii clone 4 cytochrome oxidase II (COII) gene, partial cds; mitochondrial

Sequence ID: EF513509.1 Length: 519 Number of Matches: 1

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
867 bits(469)	0.0	473/475(99%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATGGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (46): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Capoeta trutta*, Query is the study or sample sequence and Subject is the GenBank sequence.

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Contracaecum rudolphii voucher DSSP-CRB6 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial

Sequence ID: EF558894.1 **Length:** 519 **Number of Matches:** 1

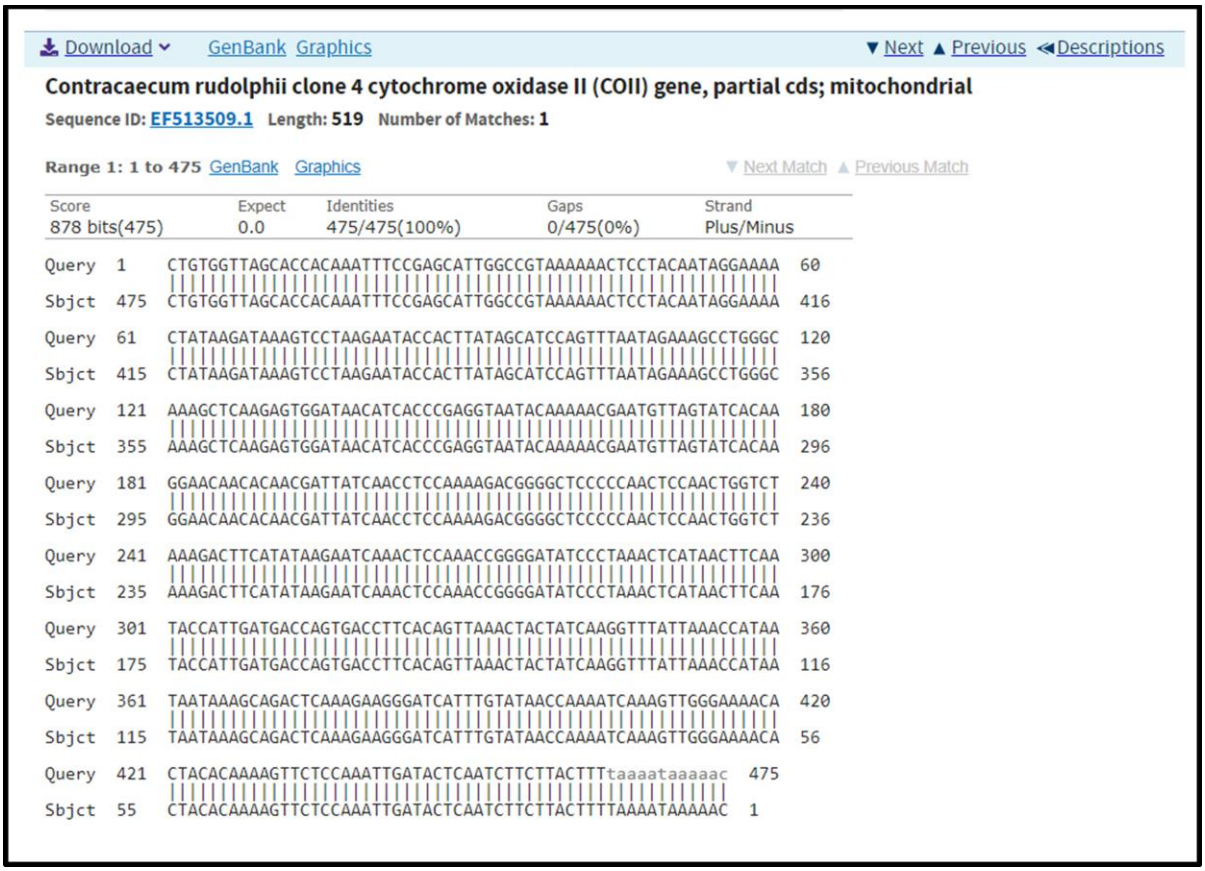
See 2 more title(s)

Range 1: 3 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
869 bits(470)	0.0	472/473(99%)	0/473(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTAGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTAGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCGCAA  180
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaa  473
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAA  3
```

Fig. (47): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Carasobarbus luteus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii clone 4 cytochrome oxidase II (COII) gene, partial cds; mitochondrial

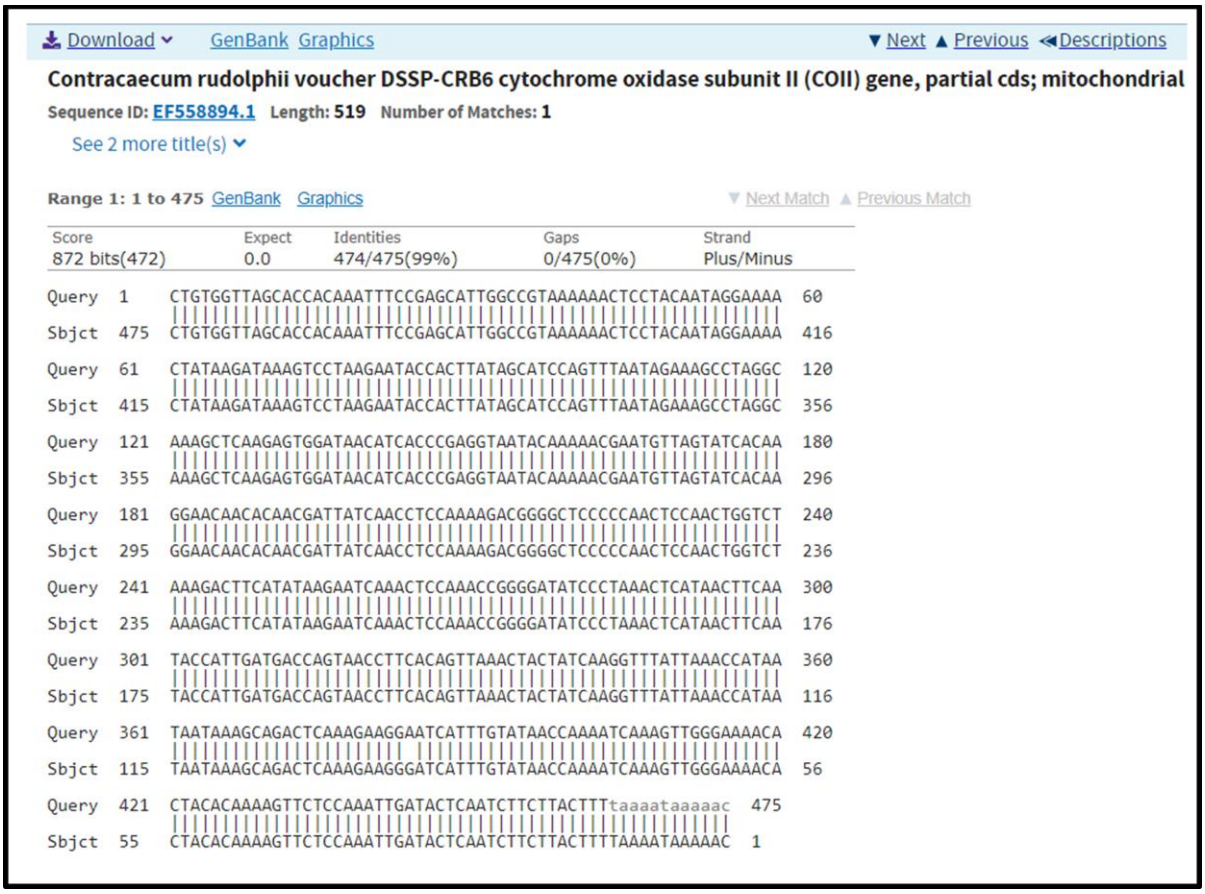
Sequence ID: EF513509.1 Length: 519 Number of Matches: 1

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
878 bits(475)	0.0	475/475(100%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (48): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Chondrostoma regium*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii voucher DSSP-CRB6 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial

Sequence ID: EF558894.1 **Length:** 519 **Number of Matches:** 1

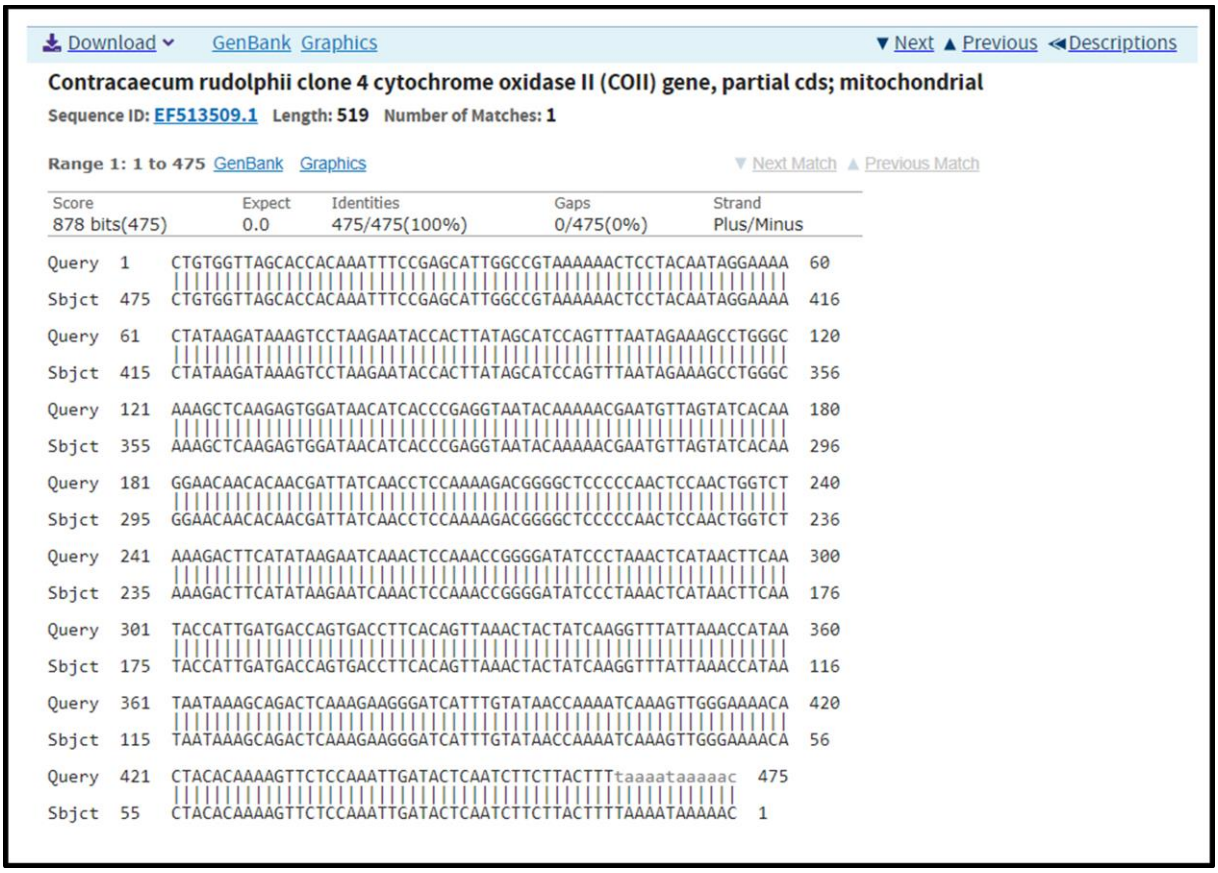
See 2 more title(s)

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
872 bits(472)	0.0	474/475(99%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTAGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTAGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTAACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGAATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (49): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Cyprinus carpio*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii clone 4 cytochrome oxidase II (COII) gene, partial cds; mitochondrial

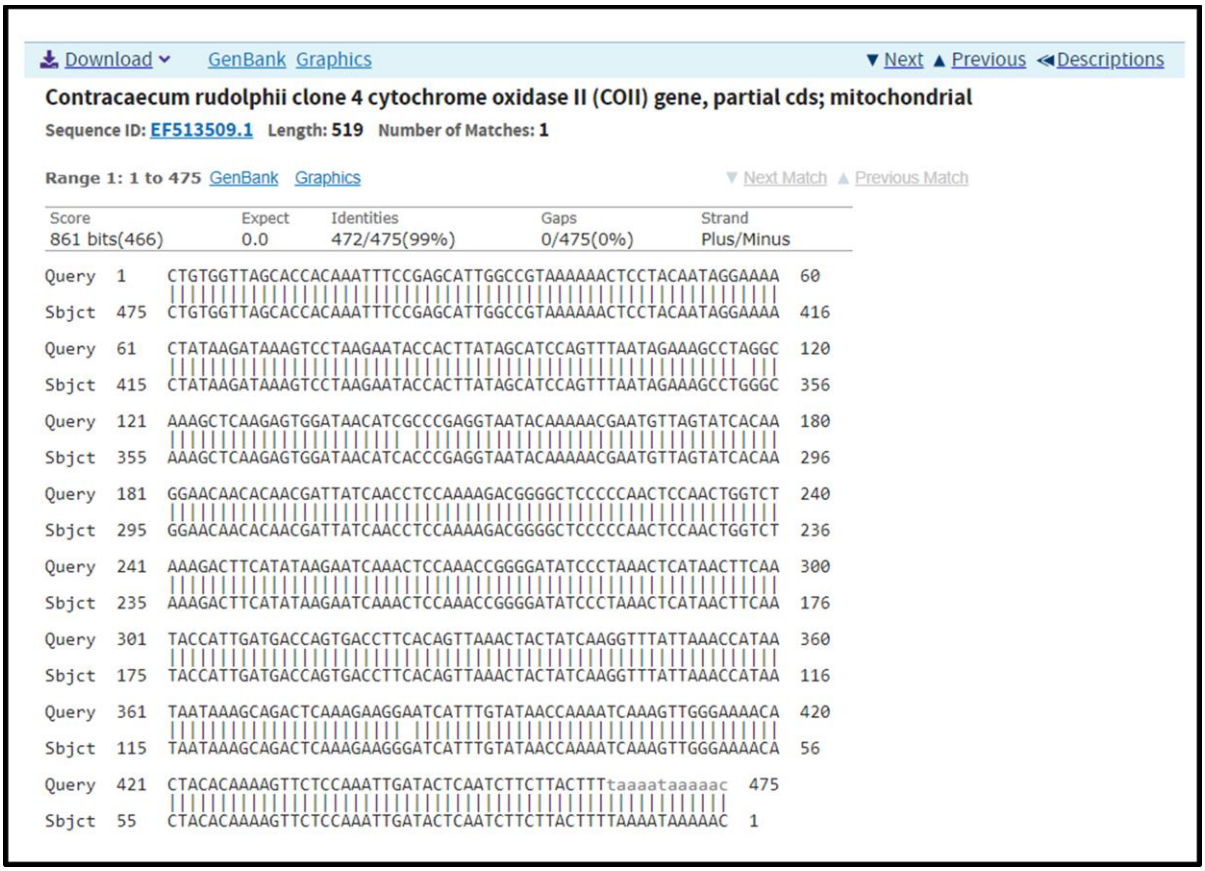
Sequence ID: EF513509.1 Length: 519 Number of Matches: 1

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
878 bits(475)	0.0	475/475(100%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (50): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Luciobarbus barbulus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii clone 4 cytochrome oxidase II (COII) gene, partial cds; mitochondrial

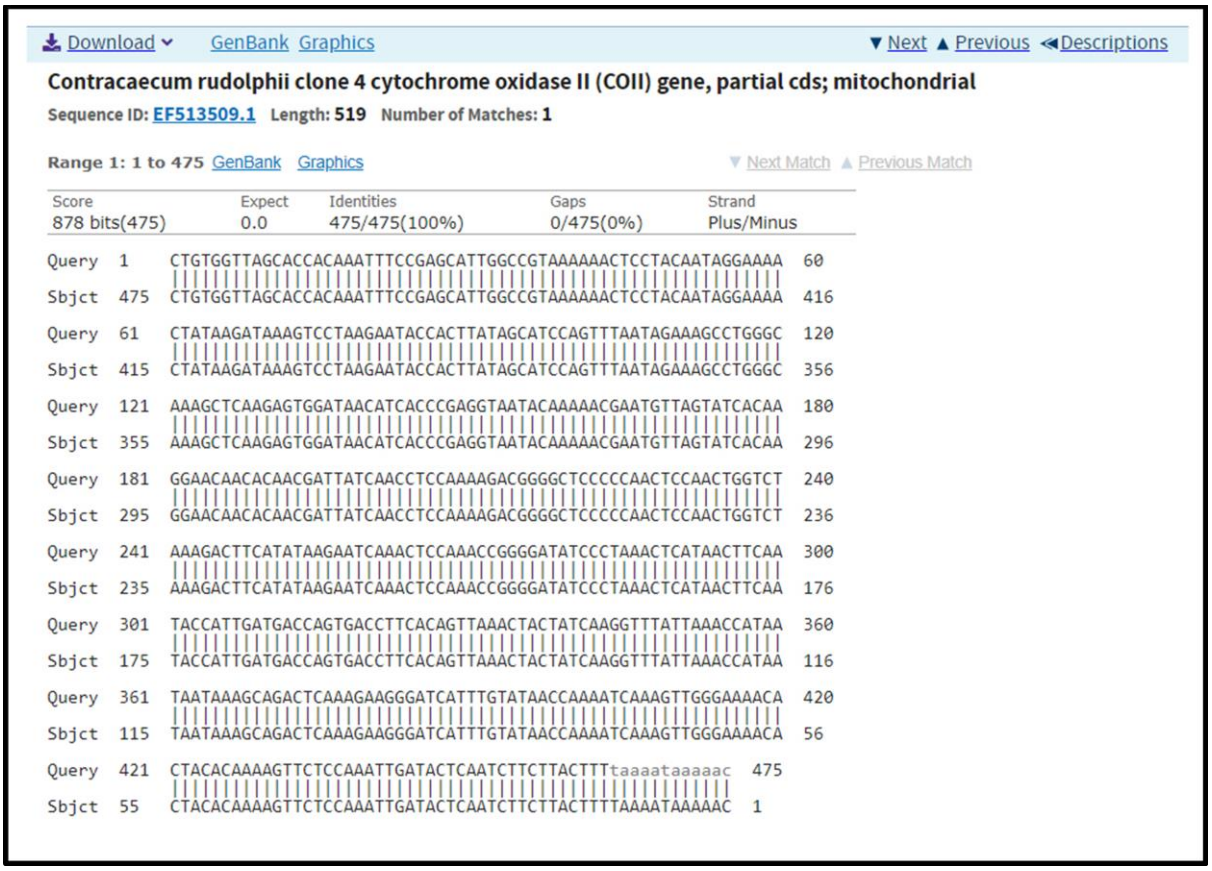
Sequence ID: EF513509.1 **Length:** 519 **Number of Matches:** 1

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
861 bits(466)	0.0	472/475(99%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTAGGC  120
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCGCCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            |||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGAATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            |||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (51): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Luciobarbus esocinus*, Query is the study or sample sequence and Subject is the GenBank sequence.



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Contracaecum rudolphii clone 4 cytochrome oxidase II (COII) gene, partial cds; mitochondrial

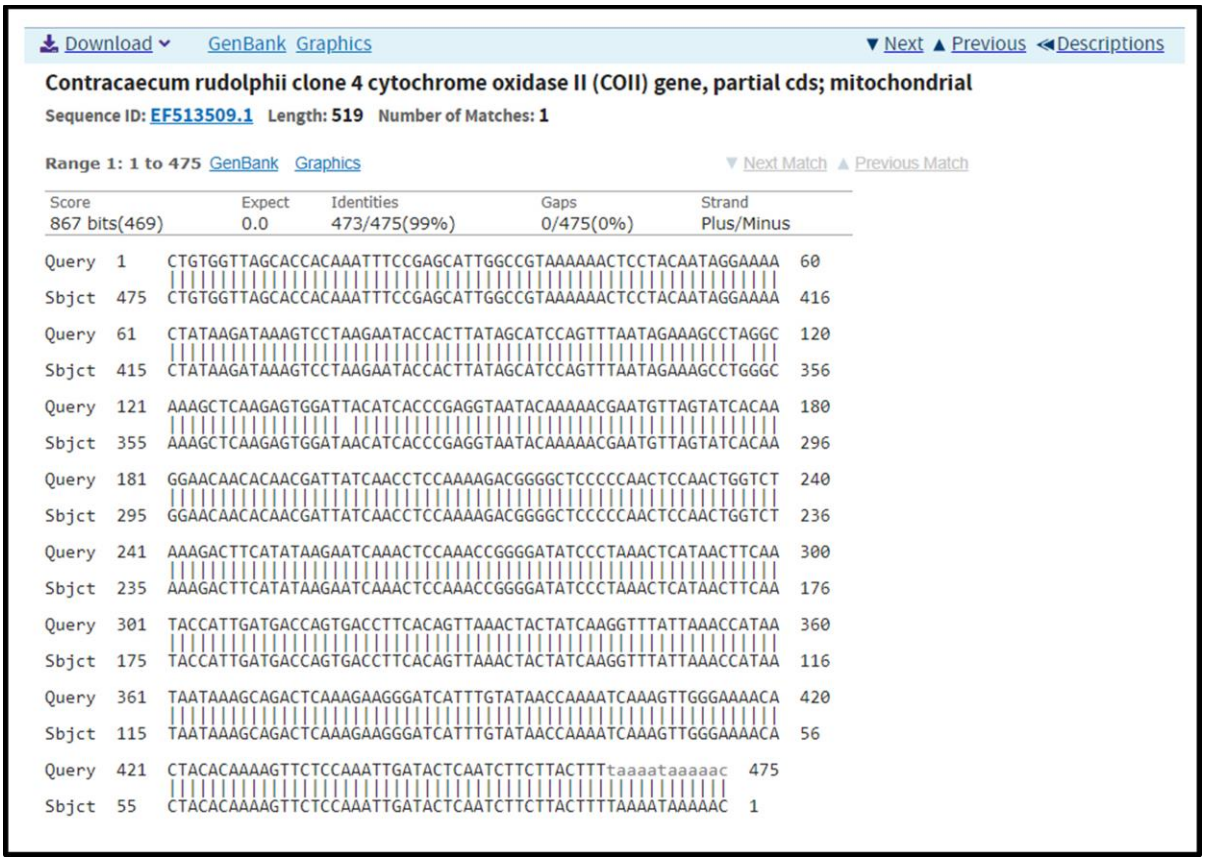
Sequence ID: EF513509.1 Length: 519 Number of Matches: 1

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
878 bits(475)	0.0	475/475(100%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  356

Query  121  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (52): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Luciobarbus xanthopterus*, Query is the study or sample sequence and Subject is the GenBank sequence.



Download GenBank Graphics ▼ Next ▲ Previous ◀◀ Descriptions

Contracaecum rudolphii clone 4 cytochrome oxidase II (COII) gene, partial cds; mitochondrial

Sequence ID: EF513509.1 **Length:** 519 **Number of Matches:** 1

Range 1: 1 to 475 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
867 bits(469)	0.0	473/475(99%)	0/475(0%)	Plus/Minus

```
Query  1    CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  CTGTGGTTAGCACCACAAATTTCCGAGCATTGGCCGTAAAAAACTCCTACAATAGGAAAA  416

Query  61   CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTAGGC  120
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  415  CTATAAGATAAAGTCCTAAGAATACCACTTATAGCATCCAGTTTAATAGAAAGCCTGGGC  356

Query  121  AAAGCTCAAGAGTGGATTACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  180
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  AAAGCTCAAGAGTGGATAACATCACCCGAGGTAATACAAAAACGAATGTTAGTATCACAA  296

Query  181  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  GGAACAACACAACGATTATCAACCTCCAAAAGACGGGGCTCCCCCAACTCCAACTGGTCT  236

Query  241  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  AAAGACTTCATATAAGAATCAAACTCCAAACCGGGGATATCCCTAAACTCATAACTTCAA  176

Query  301  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175  TACCATTGATGACCAGTGACCTTCACAGTTAAACTACTATCAAGGTTTATTAAACCATAA  116

Query  361  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  115  TAATAAAGCAGACTCAAAGAAGGGATCATTTGTATAACCAAAATCAAAGTTGGGAAAACA  56

Query  421  CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTtaaaataaaaac  475
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55   CTACACAAAAGTTCTCCAAATTGATACTCAATCTTCTTACTTTTAAAATAAAAAC  1
```

Fig. (53): Pair wise alignment COX-2 sequence of *Contracaecum* larva collected from *Mactacembelus mastacembelus*, Query is the study or sample sequence and Subject is the GenBank sequence.

الخلاصة

خلال الدراسة الحالية، تم أخذ عينات عشوائية من الأسماك من 26 منطقة أغلبها من روافد نهر الزاب الصغير ونهر سيروان في محافظة السليمانية، إقليم كوردستان- العراق. حيث تمّ جمع 2122 سمكة، تابعة إلى 36 نوعاً، 26 أجناس وثمانية عوائل، خلال الفترة المحصورة بين شهر كانون الثاني وحتى نهاية شهر كانون الأول 2018. تم فحص الأسماك للكشف عن يرقات الديدان الخيطية من جنس كونتراسيكام (*Contracaecum*) المتطفيلة عليها.

أظهرت الدراسة أن العائلة الأكثر تنوعاً كانت Cyprinidae بواقع 14 نوعاً (38.88٪) تليها Leuciscidae بواقع ثمانية انواع (22.22٪)، تليها Nemacheilidae بواقع ستة انواع (16.66٪)، ثم Xenocyprididae بواقع نوعين (5.55٪)، ونوعاً واحداً لكل من Bagridae، Heteropneustidae، Mastacembelidae، Mugilidae، Siluridae و Sisoridae بنسبة (2.77 ٪).

كشفت الدراسة الحالية أن اكثر الأنواع انتشاراً كانت سمكة البني كبير الفم (*Cyprinion macrostomum*) بنسبة 15.17٪، تليها التيلة المرقطة (*Capoeta trutta*) بنسبة 10.46٪، ثم الكارب الأعتيادي (*Cyprinus carpio*) في المرتبة الثالثة (9.18 ٪). بينما كانت أقل الأسماك انتشاراً سمكة الشلق (*Leuciscus vorax*) (0.047٪).

تمّ تسجيل سمكة *Alburnoides velioglui* لأول مرة في العراق. وتمّ إجراء التوصيف المورفومتري والجذري لهذه الأسماك باستخدام السيتوكروم-ج أوكسيديز الميتوكوندريا الوحدة الفرعية (COX-1) كعلامة الباركود الحمض النووي لتوضيح الوضع التصنيفي لهذه السمكة. التوصيف الوراثي لـ *A. velioglui* في الدراسة الحالية موجودة في قاعدة بيانات GenBank تحت رقم الانضمام (MN893770).

لقد تمّ جمع اليرقات من جنس *Contracaecum* (n=140) من 30 سمكة مصابة تعود إلى 10 أنواع مختلفة من الأسماك (السّمنان *Acanthobrama marmid*، الشبوط الأعتيادي *Arabibarbus grypus*، تيلة المرقطة *Capoeta trutta*، الحمري *Carasobarbus luteus*، البلّوط الملوكي *Chondrostoma regium*، الكارب الأعتيادي *Cyprinus carpio*، ابو براطم *Luciobarbus barbuls*، البزّ *L. esocinus*، الكطّان *L. xanthopterus* و المرمريج *Mastacembelus mastacembelus*) وبنسبة الأصابة 35٪، 0.81٪، 0.90٪، 4.49٪، 5.76٪، 2.05٪، 0.92٪، 1.92٪، 19.35٪ و 1.06٪ على التوالي.

تمّت الدراسة الشكل الخارجي لهذه اليرقات بواسطة المجهر الضوئي والمجهر الإلكتروني الماسح (Scanning electron microscope). بالإضافة إلى ذلك، أجريت دراسة التحليلات الجزيئية عن طريق التضخيم والتسلسل ومقارنة مواقع جينية مختلفة (ITS-1، ITS-2 و COX-2) لمختلف يرقات *Contracaecum* المعزولة. وهذه التسلسلات الجينية تم مقارنتها ايضاً مع تسلسلات أنواع من الديدان الخيطية القريبة في قاعدة البيانات الجينية (GenBank). تمّ الحصول على ثلاثين تسلسلًا لهذه اليرقة التي تمّ جمعها. تمّ تضخيم ITS-1، ITS-2 و COX-2 عن طريق تفاعل سلسلة البلمرة (Polymer chain reaction) وتسلسلها. وكشفت أن عينات يرقات *Contracaecum* التي تمّ جمعها من عشرة أنواع الأسماك تعود لنوع واحد وهو *Contracaecum rudolphii* B أستنادًا إلى نسبة الهوية في قاعدة بيانات بنك الجينات. وقد تمّ وصف التحليل الوراثي للنمط الوراثي. التوصيف الوراثي لهذه اليرقات في هذه الدراسة متاح في قاعدة بيانات بنك الجينات. تمّ إيداع تسلسلات ITS-1، ITS-2 و COX-2 التي تمّ الحصول عليها في قاعدة البيانات الجينية (بنك الجيني) وأظهرت أرقام انضمامها.

التركيب المظهري-الدقيق والتوصيف الجزيئي ليرقات *Contracaecum* (الخيطيات) المتطفلة على بعض الأسماك في محافظة السليمانية، أقليم كوردستان-العراق

أطروحة
مقدمة الى مجلس كلية العلوم
في جامعة السليمانية كجزء من متطلبات نيل شهادة
درجة دكتوراه فلسفة في علوم الحياة
(علم الطفيليات)

من قبل

يونس صابير عبدالله

بكالوريوس في علوم الحياة (٢٠٠٣)، جامعة السليمانية
ماجستير في علوم الحياة (٢٠١٣) ، جامعة السليمانية

باشراف

د. شمال محمد أمين عبدالله

استاذ

د. رضا حسن حسين

استاذ المساعد

جماد الأول, ١٤٤٢(هجري)
١٤٤٢\٥\٢

كانون الأول, ٢٠٢٠(ميلادي)
٢٠٢٠\١٢\١٧

پوختە

لەماوەی ئەنجامدامی ئەم توێژینەوەیە، نموونەی ماسیەکانی پارێزگای سلێمانی وەگیران لە 26 شوێنی جیاواز لەو ئاوانەی کە دەچنەوە سەر هەردوو رووباری زێی بجوك و رووباری سیروان لە هەرێمی کوردستانی عێراق. کۆی 2122 ماسی ئاوی سازگار کۆکرانەوە، کە بۆ 36 جۆر دەگەڕێتەوە لە 26 رەگەزی ماسی کەسەر بە 10 خێزانی ماسییەکانن، وە پشکنینیان بۆکرا بۆ هەبوونی کرمۆکەی کرمی دەزوویی مشەخۆر لە جۆری کۆنتراسیکەم (*Contracaecum*) لە ماوەی نێوان مانگی کانونی دووەم تا کۆتایی مانگی کانوونی یەکەمی 2018.

توێژینەوەکە دەرخستووە کە زۆرترین خێزانی ماسی کە بلاّوە لەم پارێزگایەدا بریتیە لە خێزانی ماسییە شەبوتیەکان (Cyprinidae) کە 14 جۆر ماسی لەخۆدەگرێت بە رێژەی 38.88٪، پاشان خێزانی قەشاش (Leuciscidae) دێت کە 8 جۆر ماسی لەخۆدەگرێت بە رێژەی 22.22٪، پاش ئەوانیش خێزانی مارمێلکە ماسییەکان (Nemacheilidae) دێت کە 6 جۆر ماسی لەخۆ دەکرێت بە رێژەی 16.66٪، پاشان خێزانی شەبوتیە نامۆکان (Xenocyprididae) دێت کە دوو جۆر ماسی لەخۆدەگرێت بە رێژەی 5.55٪، پاشان خێزانی زیکە (Bagridae)، نقەی پێوەدەر (Heteropneustidae)، مارماسی (Mastacembeliade)، زبرە (Mugilidae)، نقە (Siluridae) و گورگە ماسییەکان (Sisoridae) دێن کە تەنها یەك جۆر ماسی لەخۆدەگرن بۆ هەریەکەیان بە رێژەی 2.77٪.

ئەم لێکۆڵینەوەیە زۆرترین و بلاّوترین جۆری ماسی لەم پارێزگایە دەستنیشان کردووە کە ئەویش ماسییە پانکەیە (*Cyprinion macrostomum*) بە رێژەی 15.17٪، پاشان ماسی مشارەیە (*Capoeta trutta*) بە رێژەی 10.46٪، پاشان لەسەر ئاستی سێیەم ماسی کارپی ئاسایی (*Cyprinus carpio*) دێت بە رێژەی 9.18٪. ئەوەش روون بۆتەوە کە دەگمەنترین ماسی لەجۆری ماسی قەشاش (*Leuciscus vorax*) بوو بە رێژەی 0.047٪.

لەم بەدواداچوونە زانستیەدا ماسیەکی تازە بەدیکرا کە یەکەم جارە تۆماربکرێن لەسەر ئاستی عێراق کە ئەویش ماسی ئەلبورنۆیدس ڤیلیۆگلویە (*Alburnoides velioglui*). لێکۆڵینەوە لە سیفاتی روکەشی و پێوانەییەکانی بۆ ئەنجامدرا. هەروەها لێکۆڵینەوەی بۆهێڵی مایتۆکۆندریای (COX-1) بۆ ئەجامدرا بەمەبەستی پۆلێنکردنەکەی. سیفاتە بۆهێڵییەکەی ئەم ماسییە لە بانکی بۆهێڵ بەردەستە لەژێر ژمارەی تۆماری بانکی MN893770 .

کرمۆکەی کرمی دەزوویی لە رەگەزی کۆنتراسیکەم (140 دانە) کۆکرانەوە لە 30 ماسی توشبوو کە دەگەڕێنەوە بۆ 10 جۆری جیاواز لە ماسییەکان ئەوانیش (تەنکە ماسی *Acanthobrama marmid*، سورە *Arabibarbus grypus*، مشارە *Capoeta trutta*، خڕە سورە *Carasobarbus luteus*، کلك رەشە *Chondrostoma regium*، کارب ئاسایی *Cyprinus carpio*، لوتوو *Luciobarbus barbuls*، بزە *L. esocinus*، سمێڵە

L. xanthopterus و ماماسی *Mastacembelus mastacembelus*). ئەم توێژینەوەیە ڕێژەی توشبووان بە مشەخۆری کرمۆکەی کۆنتراسیکەم 35٪، 0.81٪، 0.90٪، 4.49٪، 5.76٪، 2.05٪، 0.92٪، 1.92٪، 19.35٪ وە 1.06٪ دەرخست لە ماسییەکان یەک بەدوای یەک.

لێکۆڵینەوە لە شێوەی کرمۆکەکان کرا بە هۆی وردبینی ئاسایی (Optical microscope) و ووردبینی ئەلکترۆنی ڕووکەشی (Scanning electron microscope). لەگەڵ ئەوەشدا لێکۆڵینەوەی گەردی بۆهێڵی بۆ کرمۆکەکان ئەنجامدرا بە زیادکردن و خوێندنەوەی زنجیرەی نیوکلۆتایدەکان و بەراووردکردنی چەند شوێنێکی دیاریکراوی بۆهێڵەکان (ITS-1، ITS-2 وە COX-2) بۆ هەر کرمۆکەیەک لە هەر ماسییەکی توشبوو. لەم توێژینەوەیەدا 30 زنجیرەی بۆهێڵی لە کرمۆکە کۆکراوەکان دەستکەوت. بۆهێڵەکانی ITS-1، ITS-2 وە COX-2 زیادکرانەوە بە کارلێکی پەلمەڕێنی زنجیرەیی (PCR) پاشان خوێندنەوەی زنجیرەی نیوکلۆتایدەکانی بۆ کرا. وە دەرکەوت کە کرمۆکە کۆکراوەکان لەهەر 10 جۆر ماسییەکاندا دەگەڕێنەوە بۆ یەک جۆر کە ئەویش کۆنتراسیکەم ڕۆدۆلفی Bیە (*Contracaecum rudolphii* B)، بە پشت بەستن بە ڕێژەی لەیەکچوون لە ئاماری زانیاری بانکی بۆهێڵی. سیفاتە بۆهێڵەکانی ئەم مشەخۆرە کە لێکۆڵینەوەی لەسەرکراوە لە بانکی بۆهێڵ (GeneBank) بەردەستە، وە ژمارەی تۆماری بانکی بۆهێڵەکانیان لە لێکۆڵینەوەکەدا ئاماژە پێکراوە.

پێکهاتەی شێوەیی- وردو خەسڵەتی گەردی کرمۆکەکانی *Contracaecum* (دەزوولەییەکان) مشەخۆر لەسەر هەندێک جۆری ماسی لە پارێزگای سلێمانی، هەرێمی کوردستان-عێراق

تێزێکە
پێشکەش کراوە بە ئەنجومەنی کۆلێجی زانست
لەزانکۆی سلێمانی وەک بەشێک لە پێداویستیەکانی بەدەستهێنانی
بڕوانامەی دکتۆرا لە زانستی بایۆلۆجی
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