Kurdistan Regional Government
Ministry of Higher Education & Scientific Research
University of Sulaimani
College of Agricultural Engineering Sciences

MORPHOLOGICAL AND MOLECULARY STUDIES ON THE GENUS *Neoechinorhynchus* STILES AND HASSALL, 1905 FROM TWO FRESHWATER FISHES IN SULAIMANI PROVINCE

A Thesis

Submitted to the Council of the College of Agricultural Engineering Sciences at the University of Sulaimani in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in

Parasitology

Fish Parasitology

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بسم الله الرحمن الرحيم

وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ
وَكَانَ فَضْلُ اللهِ عَلَيْكَ
عَظِيمًا

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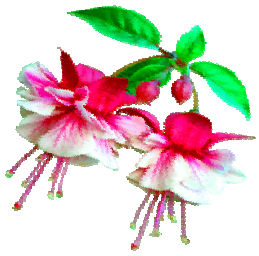


Dedication

This dissertation is dedicated to:

My beloved father, for his patient and continuous support during my work

My gracious mother …

To my lovely wife.

The candles lightening of my way …

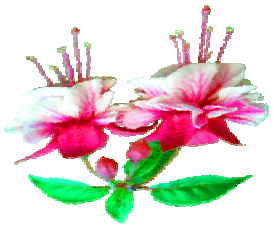
To my children

To my brothers

To my sisters

I express my heartfelt gratitude and admiration for their persistent encouragement.

Muqdad



Supervisor Certification

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Acknowledgements

I must bow to Almighty Allah, the most merciful and compassionate, the most gracious and beneficent, whose bounteous blessing enabled me to perceive and pursue higher ideals of life. I invoke peace for the Prophet Muhammad (peace is upon Him) who is an eternal source of guidance and knowledge for humanity as a whole.

I would deeply like to express my sincere appreciation to my supervisor, prof. Dr. Shamall Mohammad Amin Abdullah for his helpful advice, valuable guidance and moral support during the preparation of this dissertation.

My sincere thanks to the members of my committee: (Prof. Dr. Dr. Wijdan M. S. Mero, Prof. Dr. Nawroz A. Tahir, Asst. Prof. Dr. Fatin M. Nawwab Al-Deen, Asst. Prof. Dr. Bahzad H. S. Mustafa and Asst. Prof. Dr. Samir J. Bilal) for their time and vital contributions to this thesis, their guidance and assistance were greatly appreciated.

I would like to express my deepest appreciations and thanks to all members of the Dept. of Animal Science, and all my colleagues inside and outside the College of Agricultural Engineering Sciences.

My best thanks to Prof. Dr. Nawroz Abdulrazaq Tahir, Assist. prof. Dr. Samir Jawdat Bilal and Younis Sabir Abdullah . My best wishes to Hemin Raouf, Technical Officer, Kurdistan Institution for Strategic Studies and Scientific Research and for help through used Scanning Electron Microscope.

I want to extend large thanks to (Karwan M. Hamakhan, Sarwar M. Sadiq, Sarwar A. H. Amin, Saman M. Karim, Karzan A. Ahmad, Azad A. Muhamad, Ata M. Salih, Hemn N. Muhammad, Hemn G. Zahir, Mahdi Muhamad Abdullah).

I would like to express my sincerest thanks to my father, mother, wife, brothers and sisters for their prayers and moral support during whole of my life.

Muqdad

SUMMARY

This study was undertaken on two fish species during the period from October 2017 until February 2019. A total of 400 specimens of *Capoeta trutta* (Cyprinidae) from Dukan Lake and 400 specimens of *Planiliza abu* (Mugilidae) from Sirwan River were collected from northwestern and southeastern Sulaimani Governorate, respectively. Both fish species are native species in Kurdistan Region and whole Iraq. The PCR product of both fish species had been done for mitochondrial DNA cytochrome c oxidase subunit I (mtDNA COI) locus 625 bp and 61 cytochrome b (cytb) gene 521 bp. The DNA sequences were analyzed comparing to other stored genera and species of fish sequences from GenBank. The BLAST results showed 100% molecular based homology for both species.

Specimens of *C. trutta* were infected with the acanthocephalan *Neoechinorhynchus zabensis* at an overall prevalence of 86.96%, while specimens of *P. abu* were infected with *N. iraqensis* at an overall prevalence of 0.015%. *N. zabensis* was studied morphologically by compound light microscope and scanning electron microscope.

The PCR product of *Neoechinorhynchus zabensis* 622 bp. Phylogenetic tree analysis of DNA sequence of 18S rDNA gene was used to characterize *N. zabensis*. The BLAST result showed 95.28% identity with *N. buttnerae*. The genetic distance among *N. zabensis* and some *Neoechinorhyncus* species recorded in NCBI GneBank ranged from 0.08 to 0.14, the lowest genetic distance was recorded between *N. Zabensis* and *N. agilis* (MN148894.1) and the highest genetic distance was recorded between *N. zabensis* and *N.* sp. (MF784256.1). The Phylogenetic analysis demonstrated that *N. zabensis* occupies a separate position in the trees. The accession number MN621252 (GenBank) was taken for this parasite *N. zabensis* represent the first record in the NCBI GenBank.

In this study, numbers of 1220 *N. zabensis* parasites were collected from *C. trutta*. No significant differences were noticed in the infection rate between male and female fishes with this parasite. Prevalence, mean intensity and abundance of the infection with *N. zabensis* increased with increasing fish length. The infection with this parasite was high during spring - summer and low during autumn - winter.

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CHAPTER ONE

INTRODUCTION

Fish is important to human population in trade and economy. It is of importance in the diet of different countries especially in the tropics and subtropics areas where malnutrition is a major problem (Alune and Andrew, 1996). As the human population inevitably increases, the demand for fish as a source of protein also grows. There has been a tremendous increase in the development of fish farming and culture, attributed to the increased need for affordable animal protein especially in the tropics (Davies, *et al*., 2006). Fish is an excellent source of protein, containing all the ten essential amino acids in desirable concentrations for human beings with the cheaper rates (Agrawal, 1999). The white meat of fish contains 16-29% of protein and has a food value of 300- 1600 calories per pound (Shaukat, 2008).

Among the several families of freshwater fishes in the world the most diverse one is Cyprinidae having 220 genera including 2420 species, which belong to Cypriniformes order (Nelson, 2006). Most of Iraqi fish belong to Cyprinidae family, which involve 16 genera with 32 cyprinid species (Coad, 1996; Coad and Hussein, 2007). The distribution area of cyprinid genus *Capoeta* includes Western to Central Asia, such as Armenia, Azerbaijan, Afghanistan, Israel, Anatolia, Iraq, Uzbekistan, Georgia, and Iran (Banarescu, 1991). The genus *Capoeta* includes almost 10 species, 4 out of these occur in Iraq (Coad, 2010). The species commonly appear in streams and lakes, thus in both fast and slow flowing waters (Geldiay and Balik, 1996). *Capoeta trutta* is an economically important fish species with wide distribution in Turkey, Iran, Iraq and Syria (Gunduz *et al.*, 2014), which is dominantly thriving in both the Euphrates and Tigris river systems (Geldiay and Balik, 2007).

The grey mullets or mullets were discovered world-wide in a temperate to tropical coastal waters which is directly entering in estuaries and they are also resident in freshwaters. There are around 75 species and 20 genera in world (Nelson *et al*., 2016). In Iraq (*Liza abu*, *Liza klunzingeri*, *Liza oligolepis* and *Liza subviridis*) are exist (Coad, 2010). *Planiliza abu* is a mugilid species it is found in channels, drains, lakes, reservoirs ponds, canals, rivers, and streams on fish farms with entering estuaries. Ozdilek (2003) and Kuru (1979) stated that in Syria, Iraq, Pakistan, Turkey and Iran, mullet often occurs in inhabited places or schools. *Liza abu*, mugilid fish (Heckel, 1843), is locally known as khisni and it is distributed in all part of mid and south inland waters of Iraq (Al-Daham, 1984).

All living organisms, including fishes in nature or farms, can be exposed to the parasites. Fishes in nature are infected with a great variety of parasites, includes protozoans, monogeneans, trematodes, cestodes, nematodes, acanthocephalans and crustaceans (Price and Tom, 2005).

The phylum Acanthocephala consist proximately 1,150 species, with have allied small vermiform endoparasites, the adult feed on intestinal walls of vertebrates, especially in freshwater and marine fishes (Ruppert and Barnes, 1994). Generally, acanthocephalan parasites have been known to infect or potentially infect human beings in the countries that consume semi-raw seafood (Schmidt, 1971; Castro and Martinez, 2004).

Neoechinorhynchus (*Neoechinorhynchus*) *zabensis* Amin, Abdullah and Mhaisen, 2003 belonging to the Phylum Acanthocephala, Class Eoacanthocephala, Order Neoechinorhynchida, Family Neoechinorhynchidae. This acanthocephalan is mentioned in *Capoeta damascina* and *Capoeta trutta* in Greater and Lesser Zab Rivers in northern Iraq (Amin *et al*., 2003). These were believed just one since then, in the same two host kinds, in the Dokan Lake and Greater Zab River (Abdullah, 2009).

Determination of Acanthocephala depends on morphological differences which is difficult because of their great similarity. In addition, molecular systematic was clarified for many kinds of Acanthocephala actually complexes that include two or more sister species which are morphologically indistinguishable (Martinez-Aquino *et al*. 2009; Wayland, 2010). Molecular markers are used to make it easier to determine the variety inside and between the Acanthocephala types.

Molecular genetic marker represents the DNA sequence (nucleotide sequence) located at a particular site on the chromosome and possesses the characteristic of simple identification using molecular methods, and its inheritance which can be investigated. The usual method of genetic marking is the replication of a specific DNA fragment - usually a fragment of 18S rDNA or mitochondrial DNA (mtDNA) (Vardić Smrzlić, 2010).

The differences in environmental factors, physical conditions, seasonal abundance of fish parasites, level of infection and other macro- and microenvironment factors, as well as host age and sex, play an in important role determination the susceptibility of fish to the diseases (Meyer, 1970). Such information is important for disruption the parasite life cycle in its weakest points.

In general, the study of fish parasites is necessary to increase the productivity of pond farms, to improve the stocks of valuable commercial fisheries in the natural waters and to acclimatize fish in new sites or localities (Shul′man, 1961). Moreover, some freshwater fishes are known as intermediate hosts carrying the infective stages of some human parasites (Roberts and Janovy, 2009).

The main problems regarding the systematic study or identification of the freshwater fish of Iraq and Acanthocephala (*Neoechinorhynchus*) parasitic in freshwater fish of Iraq can be summarized as follows:

1- The morphological similarities of some fish species, which in some cases make the identification between some species, is difficult, such as between Cyprinidae and Mugilidae families.

2- Few molecular data on Iraqi fishes to support taxonomic conclusions implied from morphological data.

3- The description of *Neoechinorhynchus* species infecting freshwater fishes in Kurdistan and Iraq is very poor, the morphological classification of these species is not precise because they have a quite similar morphologically and descriptions which depend only on simple morphological characters without ultrastructural studying.

4- No molecular data on this parasite from Iraqi fishes are available to support taxonomic conclusions based on morphological data.

For these reasons, a multidisciplinary approach was applied in the present study.

1- Confirming molecular classification of the two fish species *Capoeta trutta* and *Planiliza abu* using mtDNA *COI* locus and cytochrome b (cytb) gene, partial cds, mitochondrial.

2- Studying the relationships the prevalence and mean intensity of the parasites, host sex, age and monthly variation of the isolated parasites.

3- Making morphological examination of the collected specimens, with the main aim to characterize individual taxa till the species level.

4- Using molecular studies for parasite description based on comparative analysis of nuclear genes 18s rDNA by amplification of the complete gene or a sequence of it.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Importance of Fish

Historically, the main focus of the people engaged in fisheries management has been to maximize sustainable returns from capture fisheries, and since capture fisheries have reached a plateau, to support aquaculture development (FAO 2011-2014). In many low-income countries who have water resources and fisheries, fish is important for the sustenance of income and food for people who are undernourished disproportionately, including micronutrient deficiencies (Thompson and Subasinghe, 2011).

Fishes, like many other forms of life, are immensely important for human. Today fishes form an important element in the economy of many nations and give incalculable recreational and psychological value to the naturalist, sports enthusiast, and home aquarist. Particular aspects of various species lend themselves to numerous studies such as behavior, ecology, evolution, genetics, and physiology. They are used as a general indicator for pollution, partly to the direct benefit of humans and partly to protect what people consider a valuable and necessary part of their heritage and life (Nelson *et al.*, 2016).

Fish and fish products have traditionally been considered a good source of protein, but now there are more and more emphasis in its role in supplying omega-3 acids and long-chain fatty, as well as being a rich source of vitamins and minerals that are lacking in many local diets (Toppe *et al.*, 2012 ; Weichselbaum *et al.*, 2013).

The inland fisheries resources are landed from various inland water bodies, which estimate the secret of about 1.5 million km^2. The major countries where these are discovered are Egypt, Sudan, Iraq and Syria. These lakes, rivers, marshes, swamps, ponds and natural and man-made lakes. Fisheries are mainly based in Iraq on the Euphrates and Tigris rivers, as well as some artificial reservoirs (Feidi, 1996).

The Tigris and Euphrates river system, lakes, seasonal flooding (with the submerged region of 15000-20 000 km^2), which they an important role in the country's economy. Tigris and Euphrates rivers and their branches are the main sources of inland freshwater in Iraq (Kitto and Tabish, 2004).

2.1.1 Fish distribution

The present database increases this list of taxa by providing occurrence data by drainage basin worldwide for the most diverse group of vertebrates (i.e. fishes), with more than 33500 species described to date (FishBase, 2017), from which 40% inhabit is permanently freshwater systems. The Eastern Mediterranean region covers all of Turkey and the Levant, the southern Caucasus, and Mesopotamia. It overlaps with three Biodiversity Hotspots (Myers *et al*. 2000, www. cepf.net): the Mediterranean Basin, Irano-Anatolia, and the Caucasus. This is incorporates 14 freshwater eco regions (Abell *et al*., 2008; WWF/TNC 2013), most of region them are only found within the region in terms of IUCN Red List assessments for freshwater fish. This study fills a large geographic gap between Europe, which has been assessed by Freyhof and Brooks (2011), Africa (Darwall *et al.*, 2011), Arabian Peninsula and the on-going assessment of the freshwater fishes of Iran. According to this assessment, there are 322 species of freshwater fishes present in the Eastern Mediterranean region, two thirds (66.8% / 215 species) of them are endemic to the region, with an additional 10 species that are near-endemics (i.e. with only small parts of their range outside the region). There are also at least 84 additional 'species' which more recognized from the area, but most of them currently remain un described and therefore have not been included in this assessment.

Biogeographical and hydrological factors are the major drivers of biodiversity patterns in freshwater fishes in the region. With 14 eco regions, each with its own set of endemic species, the Eastern Mediterranean region is biogeographically highly structured (Küçük *et al.*, 2009). There is a slow but continuous transition from the Mediterranean fauna in Greece and Western Turkey, to the (Euphrates and Tigris) fauna in the east. In Western Anatolia, most genera have close affinities to genera in Greece or to those of the northern Black Sea basin, however there are members of the cyprinid *Capoeta* present, a genus which is absent from adjacent Europe but widespread all over the Middle East except in the southern Arabian Peninsula (Levin *et al.* 2012). This is also the case in Central Anatolia, where most species belong to genera in common with Europe except the cyprinid genus *Pseudophoxinus*, which is almost endemic to the Eastern Mediterranean region and has its highest species diversity in Central Anatolia (Hrbek *et al.* 2004, Perea *et al.*, 2010; Küçük *et al*., 2013).

Freshwater fishes of the southern Caucasus mostly belong to the same genera as those from the northern Black Sea basin and the Caspian Sea basin, with the Kura-Aras River being mostly inhabited by widespread species of the Caspian Sea basin. However, all these rivers

have a considerable number of endemic species indicating to their long-lasting biogeographical isolation. The rivers of the Black and Caspian basin also have had recent connections to the upper Euphrates as several species of loaches are found in adjacent headwater streams in the Black Sea basin and in the upper Euphrates (for example *Oxynoemacheilus bergianus*). Another example is the presence of the Levantine cyprinid genus *Acanthobrama* in the Kura and Aras drainage (Perea *et al.*, 2010).

Mediterranean rivers such as the Seyhan, Asi and Jordan all have a fish fauna which is similar to the Euphrates including typical Mesopotamian species such as the cyprinids *Garra rufa* and *Capoeta damascina*, and the killifish *Aphanius mento* (Krupp, 1985). At the species level, a highly endemic fauna inhabits Mesopotamia itself but most species belong to genera that are also found in Europe and Anatolia. Several oriental genera are also represented, for example cyprinids of the genera *Barilius*, *Garra* and *Cyprinion* and a species of Mastacembelid spiny eel, several sisorid and one bagrid catfish, and loaches of the genera *Turcinoemacheilus* and *Paraschistura*. Several Mesopotamian species are more widespread in the Arabian / Persian Gulf basin and may occur south to the Gulf of Hormuz in Iran (Abdoli, 2000).

As in most parts of the world, ecological factors determine freshwater fish diversity within a given biogeographical unit. Species diversity increases with stream order, and in the Eastern Mediterranean region it is typically trouts of the genus *Salmo* that are found in the mountain streams (Turan *et al.*, 2009; 2012) , (Turan *et al.*, 2011). As these streams become slightly larger and warmer, several loaches of the genus *Oxynoemacheilus* occur together with cyprinids from the genera *Capoeta, Barbus,* and *Squalius*, and in larger streams additional cyprinid species and *Cobitis* loaches are also found. In the lower sections of streams euryhaline fishes from the families Clupeidae, Mugilidae, and Gobiidae are common. In the Shatt Al-Basrah canal, in the lower Euphrates drainage, the Bull Shark *Carcharhinus leucas* (NT) is found (Hussain *et al.*, 2012), however before river regulation, this shark occurred regularly upriver to Baghdad (Coad, 2010). Larger rivers in the region, including a number of Black and Mediterranean Sea catchments and the Euphrates/Tigris are (or were historically) visited by anadromous migratory species such as shads of the genera *Alosa* and *Tenualosa,* and sturgeons (*Huso huso*, *Acipenser* spp.) as well as several migratory cyprinids including *Rutilus frisii* (LC) and *Luciobarbus* species.

2.1.2 Cypriniformes

The order Cypriniformes is the most diverse order of freshwater fishes it is currently numbering over 4400 recognized species (Eschmeyer and Fong, 2017), and the species are of great interest in biology, economy, and in culture. It is occurring throughout North America, Africa, Europe, and Asia, cypriniforms are dominant members of freshwater habitats (Nelson, 2006), and some of them have even adapted to extreme habitats such as caves and acidic peat swamps (Romero and Paulson, 2001; Kottelat *et al.*, 2006). Many cypriniforms are important food and recreational fishes, and they are popular in the global ornamental pet trade.

Cypriniformes (minnows, carps, loaches, and suckers) is the largest group of freshwater fishes in the world. Diversity ranges from some of the smallest vertebrates in the world (Paedocypris, 7.9 mm in standard length) to members of Tor (almost 3 m SL) (Mayden and Chen, 2010). To place the Cypriniformes into perspective, about one third of freshwater fish species is a cypriniform and about 6 % of all vertebrate species is a cyprinform (Eschmeyer *et al.*, 2016). Species of Cypriniformes are distributed in freshwater habitats across Asia, Europe, Africa, and North America (Saitoh *et al.*, 2011).

2.1.2.1 *Capoeta trutta* (Heckel, 1843)

The genus *Capoeta* has a wide distribution in Southwest Asia and contains about 10 species of which 4 of them occur in Iraq. Its affinities are uncertain and may lie with the European *Barbus/Aulopyge* group or with Cyprinion and its southern and East Asian relatives. *Varicorhinus* Rüppell, 1836 (as used for Southwest Asian cyprinids) is a synonym of *Capoeta* Valenciennes in Cuvier and Valenciennes, 1842. *Capoeta* is distinguished from Varicorhinus of Africa since it has a denticulate last unbranched dorsal fin ray (as opposed to smooth), very small to medium-sized scales (large), lachrymal bone narrow and covering only a small part of the upper side of the rostrum (large and covering most of the rostrum), suborbital bones narrow and long (short and wide), posterior maxillary process not extending back to a level with the centre of the jugal (extends back to a level of the centre of the suborbitals), lower jaw long (short). Scaphiodon Heckel, 1843 has been used for *Capoeta* species in Southwest Asia. A general name for the members of this genus is twiny or touyeni. The name *Capoeta* is derived from the Armenian and Georgian name for female *Capoeta capoeta* packed with eggs “Kapwaeti”. The origin of *Capoeta* in Southwest Asia follows the same route as the genus *Barbus* (Coad, 2010).

Capoeta trutta is a fish species having economic importance with wide distribution in Turkey, Iran, Iraq and Syria (Gunduz *et al.*, 2014) which is dominantly thriving in both the Euphrates and Tigris river systems (Geldiay and Balik 2007). The combination of small scales, transverse mouth, dorsal and anal fin branched ray counts, the very strong last unbranched dorsal fin ray (longer than head length - usually strong but rarely weak), and the color pattern identifies this species (Coad, 2010).

2.1.3 Mugiliforms

A mullet is the popular name of fishes included in the Mugilidae, a species rich family that is the only representative of the order Mugiliformes. These fishes are distributed in several coastal aquatic habitats in tropical, subtropical and temperate regions of the world, where they are ecologically, recreationally and commercially important (Thomson, 1966). According to González-Castro and Ghasemzadeh (2016) and references herein, the family has approximately 26 genera, but Eschmeyer and Fong (2017) ascribe to Mugilidae 20 genera and 75 valid species.

This species formerly placed in the genus *Liza* but Durand *et al.* (2012) placed it in the genus *Planiliza* (Durand and Borsa, 2015; Jouladeh-Roudbar *et al.,* 2015; Eschmeyer *et al.*, 2016). *Planiliza abu* is a freshwater mullet, found in streams, rivers, drains, channels, canals, lakes, reservoirs and ponds, including fish farms (Coad, 2016). This species is found in rivers flowing to the northern and eastern Persian Gulf, and it is most common in Iran, Iraq, and Pakistan. It is found far upriver in Syria and Turkey, within the Tigris and Euphrates system (Coad, 2016).

2.1.3.1 *Planiliza abu* (Heckel, 1843)

This genus is characterized by thin to moderately thick, terminal upper lip without papillae, the lower lip is directed forwards with thin-edged, teeth are setiform, ciliiform or absent in the upper lip, ciliiform or absent in the lower lip, there is a symphysial knob to the lower jaw and the lower jaws meet at a 90º angle or more, the maxilla is bent down over the premaxilla and is either uniformly curved or is s-shaped, the maxilla end is visible when the mouth is closed, the anteroventral edge of the preorbital bone is serrate, weakly concave or kinked and ventrally it is broad and squarish, an adipose eyelid is present sometimes but is not well-developed being a narrow rim around the eye at all ages, pharyngobranchial organ with two

valves, pyloric caeca number 2-14, predorsal scales are unicaniculate, and the pectoral axillary scale is weak or absent (Coad, 2010).

Planiliza abu is a mugilid species discovered in channels, drains, lakes, reservoirs ponds, canals, rivers, and streams on fish farms with entering estuaries. Ozdilek (2003) state that in Syria, Iraq, Pakistan, Turkey and Iran, mullet often occurs in inhabited places or schools. *Liza abu*, mugilid fish, locally known as khisni, it is distributed in all part of mid and south inland waters of Iraq (Al-Daham, 1984). The high lateral scale count, long pectoral fins reaching almost level with the first dorsal fin origin when folded back (note fin tips often frayed, especially in preserved material, so not as apparent), short pectoral axillary scale, thin lips, 3 anal fin spines and 8 branched rays, relatively strong spines in the first dorsal and anal fins, and peg-like or set form teeth (not tricuspid) in the upper jaw only, distinguish this species from other species in the genus *Liza* and from other mullets (Coad, 2010).

2.1.4 Iraqi fish fauna

The history fish fauna study in Iraq started when the Sumerian, Babylonian and Assyrian people learnt to know fish species by names (Saggs, 1962). They succeeded in identifying and naming several freshwater and marine species, which were recorded on clay tablets (Landsberger, 1962). However, the real taxonomical works did not start until the 19th century, when Heckel (1843) described 17 freshwater fish species from the Tigris River at Mosul City, northern Iraq. Previous to that date, the works of Hasselquit (1722-1752) and Russell (1742-1753) on different parts of the Middle East were considered the early works on fish taxonomy in this part of the world. The authors of these works did not collect the specimens from Iraqi waters in spite of the fact that the species which they described were actually present in Iraq later on, thus they are considered to be out of the scope of their study. Several studies have focused on the classification of fresh water fishes in Iraq, which concerned with describing classifying drawing up a list of freshwater fish in Iraq in general or Tigris and Euphrates basin, starting with Khalaf (1961); Mahdi (1962); Mahdi and Georg (1969); Al-Nasiri and Hoda (1976); Al-Daham (1977); Banister (1980); Al-Daham (1982); Coad (1991; 1996; 2010).

Al-Daraji and Al-Salim (1990) identified five species of fishes (*Barbus luteus*, *B. sharpeyi*, *Aspius vorax*, *Liza abu* and *Silurus triostegus*) belonging to three families (Cyprinidae, Mugilidae and Siluridae) in Al-Hmmar Marsh northeast of Basrah city.

Al-Awadi (1997) identified 13 species of fishes in Bahr Al-Najaf Depression.

Rudaini *et al.* (1998) identified 17 species of fishes belonging to three families (Cyprinidae, Siluridae and Mugilidae) in Northern part of Saddam River.

Al- Rudainy *et al*. (2001) reported the most abundant fishes were *Liza abu* followed by *Cyprinus carpio* and *Barbus barbulus* among 17 species were recorded in the Haditha Dam.

Al-Tamimy (2004) recorded 28 species of fish belonging to five families (Cyprinidae, Bargridae, Mastacembeluidae, Mugilidae and Siluridae) near flowing warm thermal Al-Mussaib power plant built on the Euphrates River in Babel central Iraq.

Abd (2006) listed 40 species belonging to 26 families found in different area in southern marshes of Iraq and from the Shatt Al-Arab River.

Al-Nasiri and Mhaisen (2009) identified six species of fish (*Barbus grypus*, *B. luteus*, *B. xanthopterus*, *Cyprionion macrostomum*, *Cyprinus carpio* and *Liza abu*) from Tigris River passing through Salah Al-Deen Province.

Al-Saadi *et al*. (2009) identified seven species of fish (*Aspius vorax*, *Barbus grypus*, *B. luteus*, *B. sharpeyi*, *B. xanthopterus*, *Cyprinus carpio* and *Liza abu*) belonging to two families (Cyprinidae and Mugilidae) from Al-Husainia Creek north east of Karbala Province, mid Iraq.

Al-Rudainy (2010) identified 18 species of fish belonging to six families (Cyprindae, Bagridae, Mugilidae, Heteropneustidae, Mastacembelidae and Siluridae) from Al-Rathwania Lake, west of Baghdad.

Younis and AL-Shamary (2011) recorded 53 species of fish, 51 species were belonging to osteichthyes and two species to chondrichthyes from Shatt Al- Basrah, canal southern Iraq.

Younis *et al*. (2011) recorded 13 species of fish belonging to six families (Cyprinidae, Mugilidae, Siluridae, Heteropneustidae, Mastacembelidae and Bagridae) from Um Alnaaj, Al-Hawizah marsh, the east of the Tigris River southern of Iraq.

Al-Amari and Al-Taee (2012) recorded 20 species of fishes belonging to six families (Cyprindae, Mugilidae, Mastacembelidae,Bagridae, Siluridae and Cichilidae), *Liza abu*, *Barbus luteus* and *Carassius auratus* most abundant specie from Euphrates river at Al-Hindia city south of Baghdad, Iraq .

Mhaisen and Al-Nasiri (2012) identified 21 species of fishes belonging to four families (Cyprinidae, Mastacembelidae, Mugilidae and Siluridae) from Salah Al-Deen Province north Baghdad.

Mohamed *et al*. (2012a) collected 14 species of fishes from Chybayish Marsh delimited by triangle between Nasiriyah, Amarah and Qurna south of Baghdad as much as reported *liza abu* was the most abundant specie

Mohamed *et al*. (2012b) recorded 40 species belongs to 19 families all belonging0 to Osteichthyes in the Shatt Al-Arab River, *Carassius auratus* was most abundant species followed by *Tenualosa ilisha* and *Liza abu*.

Al-Jawda and Asmar (2013) recorded ten species of fishes (*Alburnus caeruleus*, *Aspius vorax*, *Carasobarbus luteus*, *Carassius auratus*, *C. carassius*, *Cyprinus carpio*, *Cyprinion macrostomum*, *Liza abu*, *Mystus pelusius* and *Silurus triostegus*) belonging to four families (Cyprinidae, Mugilidae, Bagridae and Siluridae) from Tigris River at North, Mid and South of Baghdad Province.

Mohamed *et al*. (2016) identified 47 species of fish belonging to 35 genera and 20 families, including 24 freshwater and 23 marine species from East Hammar Marsh south Iraq, *Planiliza abu* and *Carassius auratus* were the most abundant species.

Mohamed *et al*. (2016) identified four mullet species (*Planiliza subviridis, P. klunzingeri*, *P. carinata* and *Osteomugil speigleri*) by using morphometric and meristic characters and electrophoretic analysis of lateral muscle proteins by SDS-PAGE from Iraqi marine waters, Arabian Gulf.

2.1.5 Kurdistan Region Fish Fauna

The studies about fish fauna of water bodies in Kurdistan Region are yet limited. Overall, more studies are conducted on the parasites that infect the fishes. The following is a brief account on this topic.

Abdullah (1990) recorded 14 species of fishes (*Acanthbrama marmid*, *Barbus barbulus*, *B. grypus*, *B. kersin*, *B. subquincunciatus*, *B. xanthopterus*, *B. luteus*, *B.* esocinus, *Chondrostoma regium*, *Cyprinion macrostomum*, *Cyprinus carpio*, *Leuciscus cephalus*, *L. Lepidus* and *Liza abu*) belonging to two families (Cyprinidae and Mugilidae) from Dokan Lake.

Abdullah (2002) recorded 25 species belonging to seven families (Cyprinidae, Bargridae, Siluridae, Sisoridae, Heteropneustidae, Mugilidae and Mastacembelidae) from Greater Zab River at Aski-kalak and recorded 18 species belonging to four families (Cyprinidae, Siluridae, Mugilidae and Mastacembelidae) from Lesser Zab River at Alton kupri, 16 fish species were sympatric in both rivers.

Abdullah (2006) recorded 23 species of fish belonging to five families (Cyprinidae, Heteropneustidae, Mugilidae, Mastacembelidae and Sisoridae) from Dokan Lake, and recorded four species (*Leuciscus lepidus*, *Glyptothorax kurdistanicus*, *Heteropneustes fossilis* and *Mastacembelus mastacembelus*) for the first time in this lake.

Abdullah *et al.* (2007) identified 26 species of fish belonging to six families (Cyprinidae, Mugilidae, Heteropneustidae, Siluridae, Sisoridae and Mastacembelidae) from Darbandikhan Lake, *Capoeta damascina* was most abundant specie and recorded four species (*Barbus lacerta*, *C. damscina*, *C. trutta* and *G. kurdistanicus*) for the first time in this lake.

Abbas and Sediq (2012) recorded 27 species of fishes belong to five families (Cyprinidae, Sisoridae, Mugilidae, Heteropneustidae and Mastacembelidae) from Dukan Lake.

Abdullah (2013) identified 17 species of fish belonging to four families (Cyprinidae, Bagridae, Siluridae and Mastacembelidae) in Darbandikhan Lake.

Gholami and Shapoori (2017) identified (*Cyprinus carpio*, *Ctenopharyngodon idella*, *Capoeta damascina*, *Chalcalburnus sp.*, *Hypophthalmichthys molitrix* and *Gambosia affinis*) from Cyprinidae and Poeciliidae families in Zarivar Lake, north Kurdistan Province, Marivan city, Iran.

2.1.6 Morphological (Traditional) Identification

Historically the morphology of fishes was the primary source of information for taxonomic and evolutionary studies. Despite the value and availability of genetic, physiological, behavioral, and ecological data for such study, systematic ichthyologists continue to heavily depend on the morphology for taxonomic characters. Species have characteristic shapes, sizes, pigmentation patterns, disposition of fins and other external features that assist recognition, identification, and classification fish species which identified relying on morphological characters (Strauss and Bond, 1990).

Information about the external morphology of fishes can be found in many standard references, including Beckman (1962), Strauss and Bond (1990), Coad (2010; 2017).

Morphological diversity of organisms as a tool in the exploitation of natural resources is always arised great curiosity, with a long historical and evolutionary context of correlations between the form of organisms and ecology (Cunico and Agostinho, 2006).

Meristic and morphometric characters are powerful tools for measuring discreteness and relationships among fish species. For this reason, analysis of morphometric and meristic characters has been widely used by ichthyologists to distinguish between different species and among different populations within a species (Guisande *et al*., 2010).

Historical methods of identifying, naming and classifying fishes are largely based on visible morphology. Although modern taxonomic study regularly employs many other traits, including internal anatomy, physiology, behavior, genes, isozymes and geography, morphological characters that remain the cornerstone of taxonomic treatments (Ward *et al*., 2009).

The information about identification of fishes around the world is very tremendous. For this reason, the present which review will be limited to cover the studies carried out in different locations of the world, which was published with approximately last fifteen years Table (2.1).

Table 2.1 Morphological of some fish species in different country

Fish species	**Location**	**Country**	**Source**
Cyprinidae	Upper Rivers of Crocker range national park Sabah	Malaysia	Rahim *et al.* (2002)
mugilidae	Southern Caribbean	Venezuela	Debrot (2003)
Cyprinidae	Camligoze Dam Lake, Sivas	Turkey	Dirican and Çilek (2012)
Cyprinidae	Narmada River	India	Bakawale and Kanhere (2013)
Capoeta capoeta	Kaboodval Stream	Iran	Ghorbani *et al.*

			(2013)
Liza abu	Tishreen Lake	Syria	Galiya *et al.* (2014)
Capoeta trutta, *Liza abu*	Karkheh River	Iran	Khoshnood (2014)
Capoeta oguzelii	Ezine Stream	Turkey	Elp *et al.* (2018)
Planiliza abu	Qarmat Ali River	Iraq	Mohamed *et al*. (2018)
Cyprinion fishes		Iran	Nasri *et al.* (2018)

2.1.7 DNA Sequence

Fishes show an astonishing diversity of shapes, sizes, and colors. The delimitation and recognition of fish species is not only of interest for taxonomy and systematic, but it is also a requirement for studying of natural history and ecology, fishery management, tracking the dispersal patterns of eggs and larvae, estimations of recruitment and spawn areas, and authentication of food products (Rasmussen *et al.*, 2009).

Fish identification is traditionally based on morphological features. However, due to high diversity and morphological plasticity, in many cases, fish and their diverse developmental stages are difficult to identify by using morphological characteristics alone (Victor *et al.,* 2009). Deoxyribonucleic acid (DNA) based identification techniques have been developed and proved to be analytically powerful (Teletchea, 2009). As a standardized and universal method, DNA barcoding identification systems have been widely advocated to identify species and uncover biological diversity in these years (Hebert *et al.,* 2004).

Currently, researchers started to apply new technologies based on the PCR technique such as recombinant DNA, polymorphic DNA markers and DNA sequence data to resolve the questions of fish taxonomy, phylogeny, population, genetic and evolutionary biology (Faddagh *et al*., 2012).

In general, the overall concordance between morphological and molecular studies is good; testing for congruence of relationships derived from independent data sets is a particularly robust approach to systematic problems (Miyamoto and Fitch, 1995).

Phylogenetic analysis based on morphology may result in misleading phylogenies since this character type increases the chance of homoplasy in phylogenetic tree reconstruction (Kocher and Stepien, 1997). A molecular systematic approach decreases the chance of using homoplasy (Nei and Kumar, 2000). Mitochondrial DNA analysis is a useful tool for molecular systematics because of its unique features (Meyer *et al.*, 1990; Normark*et al.*, 1991; Meyer 1992). These include patterns of maternal inheritance and rapid rates of evolutionary change in mtDNA compared to nuclear DNA making it a suitable tool for genetic studies among taxa of several fish groups at multiple taxonomic levels (Kocher and Stepien, 1997; Zardoya and Doadrio 1999; Durand *et al.*, 2002). The mitochondrial16S rDNA gene has proven a valuable evolutionary marker for fishes because it has produced robust phylogenies at various taxonomic levels (Brown *et al.*, 1982; Karaiskou *et al*., 2003; Perez *et al*., 2005).

Geometric morphometric and molecular techniques have become major tools for systematic ichthyologists and fish biologists for ratification of taxonomic problems at species and population levels (Çiftci and Okumus, 2002) Table (2.2).

Table 2.2 Molecular methods of some fish species in different country

Fish species	Method	Location	Country	Reference
Liza abu	Morphological and genetic	Orontes, Euphrates and Tigris	Turkey	Turan and Ergüden (2004)
Many fish species	DNA barcodes		Canada	Hubert *et al*. (2008)
Capoeta species	Mitochondrial 16S rDNA	Anatolia	Turkey	Turan (2008)
Many fish species	DNA barcoding		Cuba	Lara *et al*. (2010)
Mugilidae	Novel family- and genus-	central region	Taiwan	Lai *et al.* (2011)

	specific DNA markers			
Many fish species	DNA barcode	Tishreen Lake	North America	April *et al*. (2011)
Many fish species	DNA bar-coding		Negiria	Nwani *et al*. (2011)
Mugilidae	mitochondrial loci (16S rRNA		Turkey	Durand *et al.* (2012)
Mugillidae	PCR-sequencing method	Caspian Sea, Persian Gulf, Oman Sea and imported Egyptian species	Iran	Nematzadeh *et al*. (2013)
Many fish species	DNA barcodes		India	Chakrborty and Ghosh (2014)
Many fish species	DNA barcoding	Matang	Malaysia	Fogelström (2015)
Many fish species	DNA barcoding	Salween and Nujiang Rivers	China	Chen *et al*. (2015)
89 fish species	DNA barcode	Ribeira de lguape Basin and coastal rivers	Brazil	Henriques *et al*. (2015)
1218 fish species	DNA barcoding		Indonesia	Hubert *et al*. (2015)
Mugilidae	mitochondrial gene-based phylogeny		China	Xia *et al.* (2015)
genus *Capoeta*	cytochrome *b* (*cytb*) gene		Iran	Ghanavi *et al.* (2016)
grey mullets	*COI* sequence		Vietnam	Durand *et al.* (2017)
Capoeta trutta	mtDNA (COI)	Euphrates and Tigris	Turkey	Parmaksiz and Eksi (2017)

Mugilidae	(*CO1*) gene	Setiu Wetlands	Malaysia	Aaron *et al.* (2018)
Mugil hospes	Cytogenetic and (COI)	Barbones	Ecuador	Nirchio *et al.* (2018)
Chelon caeruleum	(COI)	Rashid coastal region	Egypt	Deef (2018)
Crenimugil crenilabis	mitochondrial DNA COI gene	southern coast of Jeju Island	Korea	Kwun and Myoung (2019)

2.1.8 DNA Sequence in Iraq

Faddagh *et al.* (2012a) used the DNA fingerprints of eight cyprindae fish species (*Luciobarbus kersin*, *Barbus barbulus*, *B. grypus*, *B. sharpeyi*, *B. luteus*, *B. xanthopterus*, *Aspius vorax*, *Cyprino carpio*) of Iraqi inland waters using RAPD-PCR technique with seven decamere primers to identify.

Faddagh *et al.* (2012b) used the mitochondrial 16S rRNA gene fragment of seven cyprinin fish species in Iraqi inland waters (*Barbus xanthopterus*, *B. kersin*, *B. barbulus*, *B. grypus*, *B. sharpeyi*, *B. luteus* and *Cyprinus carpio*) in Qurnah (Northern of Shatt Al-Arab River), Garmat Ali River and Abul-Khaseeb (Southern of Shatt Al-Arab) – Iraq.

Aziz (2015) studied the molecular diversity for nine species of Cyprinidae (*Barbus grypus*, *Carasobarbus luteus*, *Carassius carassius*, *Capoeta trutta*, *Chondrostoma regium*, *Cyprinion macrostomum*, *Cyprinus carpio*, *Luciobarbus esocinus* and *L. xanthopterus*) in Dukan Lake, Kurdistan Region.

Freyhof *et al.* (2016) recorded new species (*Eidiuemacheilus proudlovei*) from subterranean waters in the Little Zab River drainage in Iraqi Kurdistan, and studied the morphological and DNA barcode of this fish.

Abdulrahman *et al.* (2017) used RAPD-DNA markers to study genetic diversity between different types of common carps from (Dukan and Darbandikhan) Lakes in Sulaimani governorate-Iraq.

Agha (2017) identified *Acanthobrama marmid, Arbibarbus grypus, Barbus lacerta, Capoeta damascina, Capoeta trutta, Carassius auratus, Carasobarbus kosswigi,Carasobarbus luteus, Chandrostoma regium, Ctenopharyngodon idella, Cyprinion kais, Cyprinion macrostomum, Cyprinus carpio, Leuciscus vorax, Luciobarbus barbulus, Luciobarbus esocinus, Luciobarbus kersin, Luciobarbus subquincunciatus, Garra rufa, Squalius cephalus* (Family Cyprinidae) and *Pilaniliza abu* (Mugilidae) by using Morphological and Molecular method in Greater Zab River/Aski-Kalak in Kurdistan Region, Iraq.

2.2 Acanthocephala

Acanthocephala is a small group of obligate parasites that utilize arthropods and vertebrates in a conserved two-host life cycle. The name of the phylum refers to the thorny retractable proboscis which is a distinguishing feature used by anchor to the intestine of the vertebrate host (Dunagan and Miller, 1991).

Acanthocephala are without digestive tract so they and absorb nutrients directly from the lumen of the host intestine (Schmidt and Roberts, 2005).Their body wall consists of numerous pores, canals and several structurally distinct layers, which performs both a protective and absorptive function (Lee, 1966). Acanthocephalans are commonly considered as parasites with a low specificity to their intermediate, definitive or transport hosts (Taraschewski, 2000). Acanthocephalan parasites of fishes live either as adults in the intestine or as larvae (post-cystacanths) in fish tissues. All acanthocephalans utilize arthropods as intermediate hosts and vertebrates as definitive hosts.

It is believed that approximately 1150 species of Acanthocephalan parasites exist within the four orders: Neoechinorhynchidea, Echinorhynchidea, Aporhynchidea and Gigantorhynchidea. The occurrences of Acanthocephalan parasites in fishes have been studied extensively throughout the world. There are different species of Acanthocephalans belonging to different genera (Echinorhynchus, Neoechinorhynchus, Acanthocephalus, Corynosoma, Pallisentes, Rhabidorhynchus, Pseudorhadinorhynchus, Leptorhyncoides, Paragorgorhynchus, Acanthogyrus, etc). Which are commonly found in both marine and freshwater fishes throughout the world (Jithendran and Kannappan, 2010).

2.2.1 Systemic and evolution of Acanthocephala

Acanthocephalans are gonochoristic and invariably utilize arthropods as intermediate hosts and vertebrates as definitive hosts. Occasionally, vertebrates serve as paratenic hosts

harboring larval acanthocephalans that do not develop to adults unless ingested by the appropriate vertebrate definitive hosts (Nickol, 1985).

The evolution of parasitism is a tantalizing question in evolutionary biology, and in many respects acanthocephalans provide a potential model system to investigate adaptive processes associated with the evolution of parasitism. First, the diversity of acanthocephalans is limited to approximately 1150 described species (Amin, 1985). Second, the basic life cycle is highly conserved among all acanthocephalans. Third, substantial phylogenetic evidence from both morphology and molecular data indicates that acanthocephalans have a close evolutionary relationship with Rotifera (Clement, 1985; Lorenzen, 1985; Winnepenninckx *et al.,* 1995; Garey *et al.,* 1998; Mark Welch, 2000).

The identification of a free-living sister taxon to the entirely parasitic Acanthocephala offers an unprecedented opportunity to study the evolution of obligate parasitism in terms of character innovations versus character loss, evolutionary trends in host and habitat specificity, and adaptive radiation with regard to morphological and ecological diversification (Brooks and McLennan, 1993). The taxonomic groups in Acanthocephala have been identified based on morphological features and host characteristics (Bullock, 1969).

2.2.2 Classification of the Acanthocephala

Amin presented a new system for the classification of the Acanthocephala in 1985in Crompton and Nickol's (1985) book 'Biology of the Acanthocephala and recognized the concepts of Van Cleave (1952). Many changes have taken place and many new genera and species, as well as higher taxa, have been described since. An updated version of the 1985 scheme incorporating new concepts in molecular taxonomy, gene sequencing and phylogenetic studies is presented.

The hierarchy was undergone a total face lift with Amin (1987) through addition of a new class, Polyacanthocephala (and a new order and family) to remove inconsistencies in the class Palaeacanthocephala. Amin and Ha (2008) added a third order (and a new family) to the Palaeacanthocephala, Heteramorphida, which combines features from the palaeacanthocephalan families Polymorphidae and Heteracanthocephalidae. Other families and subfamilies have been added but some have been eliminated, e.g. the three subfamilies of Arythmacanthidae: Arhythmacanthinae Yamaguti, 1935; Neoacanthocephaloidinae Golvan, 1960; and Paracanthocephaloidinae Golvan, 1969. Amin (1985) listed 22 families,

122 genera and 903 species (4, 4 and 14 families; 13, 28 and 81 genera; 167, 167 and569 species in Archiacanthocephala, Eoacanthocephala and Palaeacanthocephala, respectively).

Amin (1985) included a detailed historical account of the Acanthocephala since the first recognizable reference of worms having proboscides was made by Redi (1684).The first name of the acanthocephala was described by Rudolphi (1802) and gave an ordinal rank with one genus, Echinorhynchus. The diversity of this group of worms and fragmented the old genus Echinorhynchusinto three families (Echinorhynchidae, Gigantorhynchidae,Neorhynchidae), which formed the basis of the more recent classification of the Acanthocephala was recognized by Hamann (1892).

2.2.3 Morphological characteristics of Acanthocephala

The acanthocephalans are easily recognized because of their proboscis, which bears chitinoid hooks. The proboscis may become withdrawn when the worm is removed from the host (Hoffman, 1999). These elongated worms with non segmented bodies have neither alimentary canal nor circulatory system. They are of separated sexes, male being shorter than females and characterized by their cement glands and copulatory bursa (Duijn, 1973). Detailed account on acanthocephalan morphology is demonstrated by Nickol (2006). The adult acanthocephalans absorb host digested food directly through their teguments, may block host intestine in cases of heavy infection (Khamees, 1983) and cause diverse pathological changes in the intestine of their hosts (Hasan,2004; Lefebvre *et al.*, 2012; Amin *et al.*, 2015).

Acanthocephalans have a long proboscis, this parasite is very dangerous and cause death of their hosts by perforation of the intestine (Amlacher, 1970; Dujin, 1973).The body of an acanthocephalan consists of a proboscis and a trunk. The hooks themselves can damage the host intestine, and can affect overall fish health. In some cases, hooks can actually penetrate through the intestinal wall, leading to perforations, which can be fatal. It is unclear how often this happens in nature. Most of the acanthocephalan trunk consists of reproductive organs. The sexes are separate, and mating takes place in the vertebrate host intestine. Acanthocephalans are considered pseudocoelomates, i.e., their mesoderm does not line their entire body cavity. Acanthocephala lack a digestive tract. Instead, they absorb nutrients directly from the lumen of the host intestine, absorption occurs across the tegument of the parasite (Schmidt and Roberts, 2005).

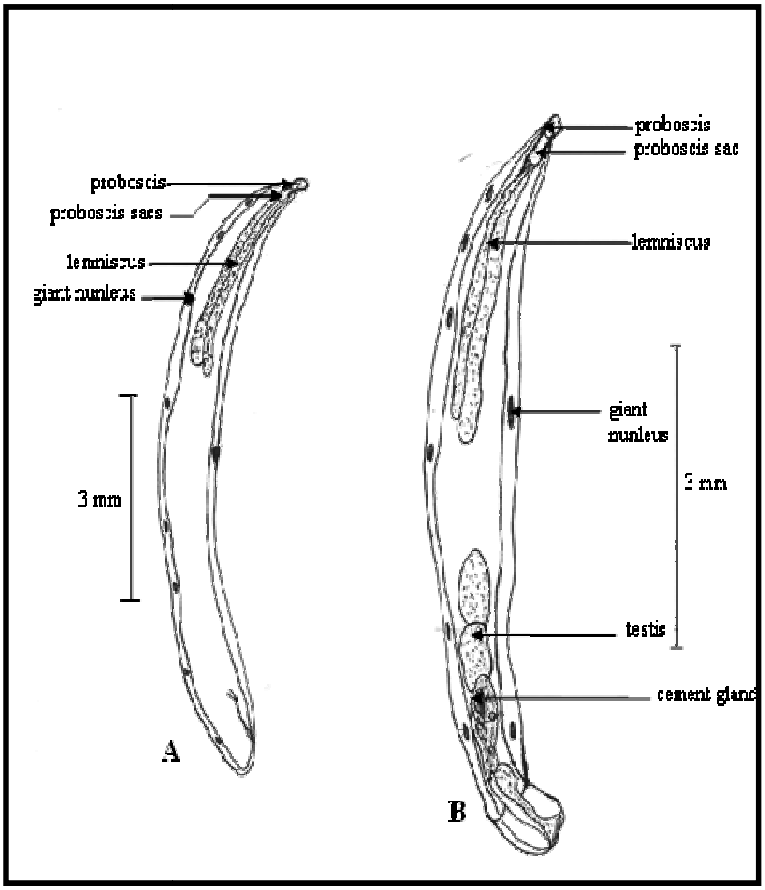

Figure 2.1 ***Neoechinorhynchus sp.***
A: Female of ***Neoechinorhynchus zabensis***
B: Male of ***Neoechinorhynchus zabensis*** **(Amin *et al*., 2003)**

2.2.4. Life cycle of Acanthocephala

Due to involvement of number of hosts, acanthocephalans have complex life cycles, for both developmental and resting stages. Only in 25 species, complete life cycles have been worked out. For the development to occur, (Fig. 2. 2) when the eggs are released from the female containing the acanthor are ingested by an arthropod, usually a crustacean. Inside the intermediate host, the acanthor is released from the egg and transforms into an acanthella. Acanthella then penetrates the gut wall and transforms into the infective cystacanth stage (cyst) in the body cavity. This stage after is eaten by a suitable final host develops into a mature adult, or by a paratenic host, in which the parasite again forms a cyst. When consumed by a suitable final host, a fish, the cycstacanth removes its cyst wall, everts its proboscis, pierces the gut wall and then feeds, grows and develops its sexual organs. After mating, adult male uses the excretions of its cement glands to plug the vagina of the female, to prevent subsequent mating from occurring. Embryos develop inside the female, and the life cycle repeats (Nabi *et al.* 2015).

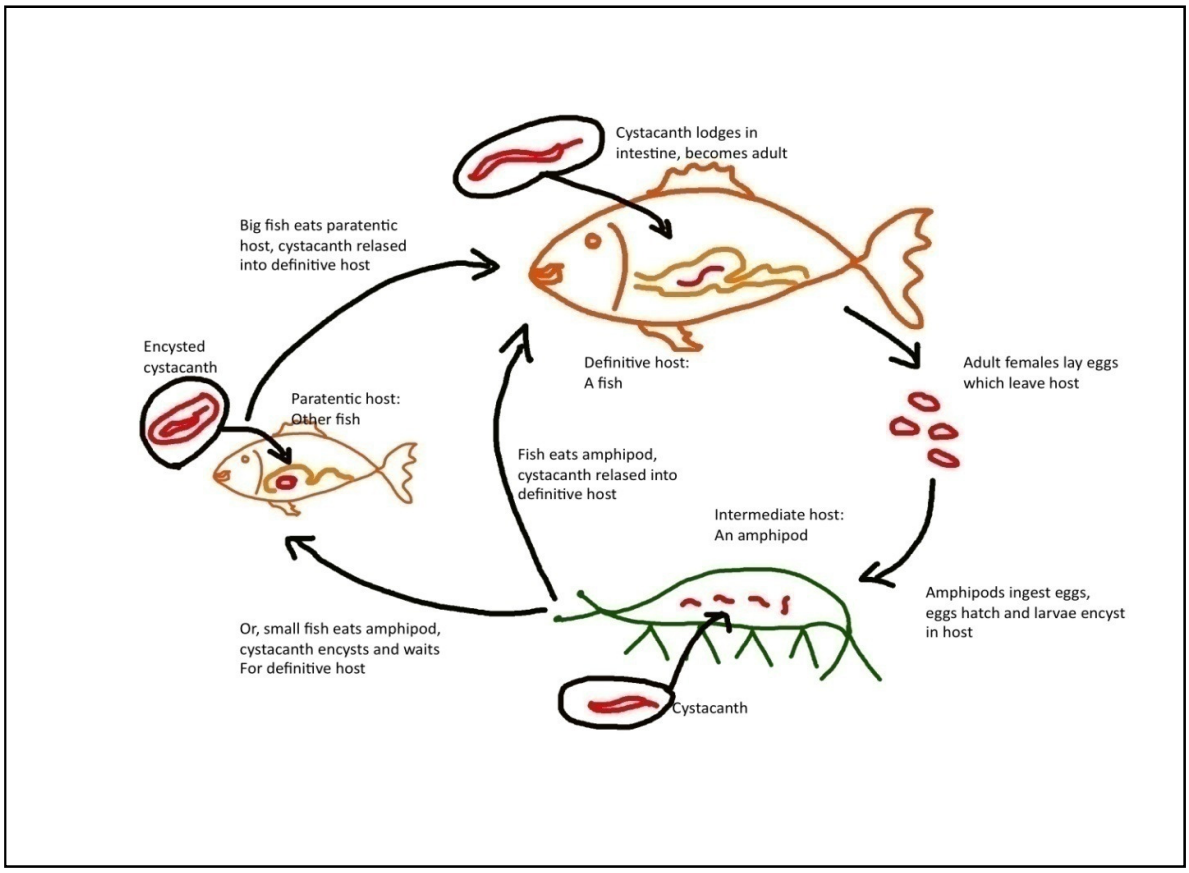

Figure 2.2 Life cycle of Acanthocephala (Nabi *et al*., 2015)

2.2.5 Pathology

Numerous reports are available on the pathological conditions caused by Acanthocephalans in fishes. Usually in acanthocephalan infections, pathology appears to be negligible when parasites are attached to the epithelial mucosa only but deeply embedded forms like *Pomphorhynchus spp.* can cause serious pathological conditions that result in extensive granuloma and subsequent fibrosis (McDonough and Gleason, 1981). The hooked proboscis of acanthocephalans which is used to anchor the worm to the intestinal wall of the fish, can damage the host intestine, and can affect overall fish health (Schmidt and Roberts, 2005). In some cases, hooks have been found to penetrate through the intestinal wall which lead to perforations, extensive inflammation, peritonitis and systemic clinical changes (Bullock, 1963) that can be fatal (Schmidt and Roberts, 2005). Extensive inflammation of the worm infested fish is dominated by granulocytes and macrophages, depending on the host species, and the structure of the proboscis hooks and tegument of the parasite (Reddy and Benarjee,

2011). Acanthocephalan parasites have been found to cause occlusion of the gut and invasion/migration of the parasites into uncommon locations have also been reported (Nickol, 2006). De Buron and Nickol (1994), reported occlusion of gut in *M. cephalus* infected with the acanthocephalan *Neoechinorhynchus sp*. Also absorption of valuable nutrients, involvement of toxins and localized toxaemia in the host fish due to acanthocephalan infestation has also been reported by some authors (Holloway, 1966) .The pathology of acanthocephalan parasites *Pomphorhynchus laevis* in fishes has been reported by (Wanstall *et al*., 1986; Dezfuli, 1991). Larval stages (cystacanths) of acanthocephalans leads to local changes in low to moderate infections in visceral organs (liver, spleen) while heavy infection, in juvenile fish in particular, led to extensive granuloma, fibrosis and ultimately atrophy of either a portion of or the entire organ (Paperna and Zwerner, 1976). According to Taraschewski (2000) and Kabata (1985) density of worms and depth of parasite penetration into the host tissues are the two main factors that determine the pathogenicity of acanthocephalans. Severe damage to the intestinal villi will hamper the normal digestive and absorptive functions of the animal by reducing the absorptive area while the damages associated with the tissue reactions in the wall of the intestine will alter the nature of the tissues, affecting its functional efficiency and the overall health status of the fish. Also absence of intestinal folds, loss of columnar appearance of epithelial cells and formation of yellowish white fibrous nodules in the intestine is predominant in acanthocephalan infection. (Khurshid and Ahmed, 2012). It has also been found by various workers that the number of acanthocephalans increases with the increase in the size of the host fish. (Jithendran and Kannappan , 2010).

2.2.5.1 Diagnosis

The diagnosis of acanthocephalan parasites infecting fish could be made possible by dissecting the individual fish and then stretching its intestine in normal saline and carefully opening by using needles. Adults can be identified based on the pattern of hooks on the proboscis, thus it is important that this portion of the worm is preserved and visible. If free floating forms of adult worms are not present, then the worms attached to the intestine should be carefully removed from its attachment site and placed in water which creates an osmotic turgor that detaches their proboscis from the intestine. Feacal sedimentation techniques utilizing formalinethyl acetate are considered superior to flotation techniques for identifying acanthocephalan eggs as their eggs are large and heavy. The eggs of this group are also elongated with a thick outer wall and thin inner walls, often appearing to have 3

layers that cover the acanthor larva. A positive identification of acanthocephalan can be made, if the spines at one end of the larva are visible. Eggs of acanthocephalans are usually clear but eggs of some species are brown due to fecal staining as they pass along of the intestinal tract of the host. Also feacal samples from the fish may be stained and viewed under objective x10 and x40 of the microscope for the detection of the larvae (Nabi *et al.*, 2015).

2.2.6. Some Acanthocephala records world- wide

Occurrence of disease conditions particularly due to parasites has become a major constraint in aquaculture (Bondad-Reantaso *et al*., 2005). Besides the direct losses caused by mortality, parasites have considerable impact on growth, resistance to other stressing factors, susceptibility to predation, marketability and pave way for secondary infections (Woo, 2006). Parasitic infections in fishes are common, especially in wild populations where ecological requirements for intermediate hosts and parasite transmission are met (Feist and Longshaw, 2008). Management of parasitic problems is the major limiting factor in fin fish aquaculture in terms of profitability and environmental health (Costello, 2009; Burridge *et al.*, 2010). Acanthocephalans are a group of endoparasitic helminths commonly found in both marine and freshwater fishes worldwide (Jithendran and Kannappan, 2010). The worldwide data on fish parasitology is very tremendous. For this reason, this review will be restricted to cover only those studies that carried out about parasite species of acanthocephalans in different locations of the world over the present decade (from 2004) Table (2.3).

Table 2.3 Some acanthocephal with hosts in different country

Species	Host	Country	Reference
Neoechinorhynchus golvani	*Herichthys cyanoguttatum*	Mexico	Salgado-Maldonado *et al.* (2004)
Neoechinorhynchus rutili	*Barbus capito*in	Iran	Pazooki *et al.* (2007)
Pomphorhynchus moyanoi	*Percilia gillissi*	Chile	Olmos and Habit (2007)
Acanthogyrus tilapiae	*Cyprinus carpio*	Mozombiqe	Boane *et al*.(2008)

Acanthocephalus anguillae, *A. lucii* and *Acanthocephalus sp.*	*Gymnocephalus cernuus*in	Poland	Dzika *et al.* (2008)
Leptorhynchoides thecatus, *Neoechinorhyncus rutili* and *N. cristatus*	*Lepomis gibbosus, Lepomis macrochirus* and *Micropterus salmoides*	United states of America	Hendricks and Reyda (2009)
Acanthocephalus amini	*Cichlasoma urophthalmus*	Mexico	Salgado-Maldonado and Novelo-Turcotte (2009)
Pomphorhynchus omarsegundoi	*Gymnotus carapo*	Argentina	Arredondo and Gil de Pertierra (2010)
Neoechinorhynchus indicus	*Leptomelanosoma indicum*in	India	Gudivada *et al.* (2010)
Neoechinorhynchus (Neoechinorhynchus) pimelodi	*Pimelodus maculates, Bergiaria westermanni*, *Schizodon knerii* and *Brycon orbignyanus*	Brazil	Lopes *et al.* (2011)
Neoechinorhynchus brentnickoli	*Dormitator latifrons*	Mexico	Monks *et al.* (2011)
Echinorhynchus salmonis, *Acanthocephalus dirus* and *Neoechinorhynchus pungitius*	*Cottus cognatus* and *pungitius pungitius*fishes	United states of America	Muzzall *et al.* (2012)
Neoechinorhynchus zabensis	*Capoeta trutta* and *Capoeta barroisi*	Turkey	Oğuz *et al.* (2012)
Neoechinorhynchus mamesi	Dormitator *latifrons*	Mexico	Pinacho-Pinacho *et al.* (2012)
Neoechinorhynchus prolixum and *Acanthella sp*	*Synodontis batensoda*	Nigeria	Eyo *et al.* (2013)
Neoechinorhynchus veropesoi	*Plagioscion squamosissimus*	Brazil	Melo *et al.* (2013)

Neoechinorhynchus agilis	*Mugil cephalus*	Guyana	Rajeshkumar *et al.* (2013)
Neoechinorhynchus vittiformis and *Neoechinorhynchus bryanti*	*Eleutheronema tetradactylum* and *Liza subviridis*	Australia	Smales (2013)
Neoechinorhynchus mexicoensis	*Dormitator maculates*	Mexico	Pinacho-Pinacho*et al.* (2014)
Acanthosentis cheni	*Coilia nasus*	China	Song *et al.* (2014)
Neoechinorhynchus zabensis	*Capoeta trutta*	Turkey	TavakoL *et al.* (2015)
Echinorhynchus baeri	*Salmo trutta*	Turkey	Amin *et al*. (2016)
Acanthocephala dirus	*Caranx ignobilis*	India	Sakthivel *et al.* (2016)
Neoechinorhynchus zabenesi	*Capoeta barroisi*	Iran	Borazjani *et al.* (2017)
Neoechinorhynchus inermis	*Ageneiosus inermis*	Brazil	Brito-Porto *et al.* (2017)
Neoechinorhynchus villoldoi	*Austrolebias bellottii*	Argentina	Montes *et al.* (2017)
Neoechinorhynchus buttnerae	*Colossoma macropomum*	Brazil	Costa *et al.* (2018)
Neoechinorhynchus beringianus	*Pungitius pungitius*	Russia	Mikhailova and Kusenko (2018)
Neoechinorhynchus buttnerae	*Colossoma macropomum*	Brazil	Pereira and Morey (2018)

2.2.7. Some Acanthocephala records of Iraq

Hrezog (1969) recorded *Neoechinrhynchus rutili* for first time from *Barbus xanthopterus* and *Liza abu* in Faluja.

Habash and Daoud (1979) recorded new species of *Neoechinrhynchus agilis* from Mugli hishni fish in Shatt al-Arab, Basarah, Iraq.

Ali and Shaaban (1984) recorded Ergaesilus species, Trichodina species and *Neoechinrhynchus agilis* from *Liza abu* in Al-Latifyia artificial pond.

Mhaisen (1986) discovered *Neoechinrhynchus agilis* in *Liza abu* from Shatt al-Arab River and the North West of the Arab Gulf.

Ali *et al.* (1987) identified two acanthocephalan, *Neoechinrhynchus rutili* from *Barbus belayewi* and *Neoechinrhyncus agilis* from *Liza abu* in Diyala River, Iraq.

Mhaisen *et al.* (1988) recorded *Neoechinrhynchus agilis* in *Liza abu* from Mehaijeran Creek, a western brunch of Shatt al-Arab River south of Basrah.

Ali *et al.* (1989) examined *Liza abu* fish and description of *Neoechinrhynchus agilis* from Babylon fish farm in Hilla city.

Rahemo and Ami (1991) identified *Neoechinrhynchus sp.* and *Neoechinrhynhcus rutili* from two families of fish (Cyprinidae and Siluridae) in Tigris River in three regions in Neinava Governorate.

Balasem *et al.* (1993) found *Neoechinrhynchus agilis* in *Liza abu*from Tigris River at Al-Zaafaraniya , South of Baghdad.

Mhaisen *et al.* (1993) recorded of *Neoechinrhynchus agilis* in *Liza abu* in Basarah province.

Rahemo and Al-Abbadie (1994) recorded *Neoechinrhynchus rutili*in *Liza abu* from Al-garaf River in shatra Town.

Mhaisen (1995) identified *Neoechinrhynchus agilis* in *Liza abu* in Basarah province marshy area.

Asmar *et al.* (1999) found *Neoechinrhynchus agilis* in *Liza abu* from Al-Qadisiya Dam Lake on the Euphrates River, Anbar province.

Mhaisen *et al.* (1999) reported *Neoechinrhynchus agilis* from *Liza abu*in Al-Majara and Al-Anjoor at Habbaniya Lake.

Amin *et al.* (2001) described new species of *Neoechinrhynchus iraqensis* from *Liza abu* in the Euphrates River.

Balasem *et al.* (2001) found *Neoechinrhynchus rutili* in *Liza abu* from Diyala River.

Al-Nasiri *et al.* (2003) discovered *Neoechinrhynchus iraqensis* from *Liza abu* in a man-made Lake at Baghdad region.

Al-Sady *et al.* (2003) examined *Liza abu* with the identified of *Neoechinrhynchus iraqensis* from the Euphrates River, Al-Anbar province.

Asmar *et al.* (2003) studied on *Liza abu* they found *Neoechinrhynchus iraqensis* and *Neoechinrhynchus rutili* in Tigris River.

Balasem *et al.* (2003) identified two Acanthocephala (*Neoechinrhynchus iraqensis* and *Neoechinrhynchus cristatus*) from *Liza abu*in Qadisya Dam Lake, Euphrates River.

Mhaisen *et al.* (2003) recorded *Neoechinrhynchus iraqensis* from the *Liza abu*in the drainage network system at Al-Madaen, south of Baghdad.

Asmar *et al.* (2004) identified *Neoechinrhynchus iraqensis* from *Liza abu* in Al-Zaafaraniya farm, south Baghdad.

Mustafa *et al.* (2006) recorded *Neoechinrhynchus iraqensis* in *Liza abu* from Tigris River in Mosul city.

Al-Abadi (2006) examined *Liza abu, Aspius vorax* and *Barbus luteus* was found *Neoechinrhynchus rutili* from Gharraf River in AL-Shatra & AL-Gharraf in Thi-Qar provience.

Al-Jadua (2008) identified *Neoechinrhynchus iraqensis* in *Liza abu* from a local drainage net, north of Al-Diwaniya province.

Rahemo and Nawwab Al-Deen (2008) recorded *Neoechinorhynchus rutili* and *Neoechinorhynchus iraqinensis* from (*Cyprinus carpio, Barbus luteus, Chondrostoma regius* and *Varicorhinus trutta*) and *Liza abu* respectively, was found in different urban area of Al-tamim governorate.

Taher *et al.* (2009) identified *Neoechinorhynchus iraqinensis* from *Liza abu* in Al-Najaf province.

Al-Awadi *et al.* (2010) recorded *Neoechinorhynchus iraqinensis* in *Barbus xanthopterus* and *Liza abu* from Bahr Al-Najaf depression, mid Iraq.

Yassin (2010) identified of *Philometra intestinalis*, *Contracaecum sp.* and *Neoechinorhyncus rutili* from *Liza abu* and *Lernaea cyprinacea* from *Cyprinus carpio* in Al-Shenafya River.

Al-Saadi *et al.* (2011) found eight types of external parasites (*Ichthyophthirius multifiliis*, *Trichodina domerguei*, *Microcotyle donovani*, *Clinostomum complanatum*, *Dermoergasilus varicoleus*, *Ergasilus barbi*, *E. mosulensis* and *E. sieboldi*) and six type of internal parasites (*Contracaecum sp., Cucullanuscyprini, Neoechinrhynchus cristatus*, *Neoechinrhynchus iraqinesis*, *Neoechinorhynchus rutili* and *Paulisentis fractus*) from *Liza abu* in Al-Husainia creek, Karbala province.

Al-Asadiy *et al.* (2012) studied on *Liza abu* obtained from Euphrates River-Al-Syaagh region there were noticed *Neoechinorhynchus iraqinesis.*

Mansor *et al.* (2012) isolated *Neoechinorhynchus cristatus* and *Neoechinorhynchus iraqinesis* from *Liza abu* in three stations Tigris River namely (Al-Zaafaraniya, Al-Tagei and Al-Shawaka) at Baghdad city.

Mhaisen and Al-Nasiri (2012) literature reviewed on the parasites (*Neoechinorhynchus cristatus*, *N. iraqensis*, *N. rutili*, *N. zabensis* and *Paulisentis fractus*) from *V. trutta*, *Liza abu*, *V. trutta*, (*B. belayewi*, *C. auratus*, *Liza abu* and *S. triostegus)* and *B. barbulus* respectively in Salah Al-Deen province.

Mhaisen *et al.* (2012) literature reviewed on the parasites *Neoechinorhynchus iraqensis* from *Cyprinus carpio* and *Liza abu* and *Neoechinorhyncus rutili* from *Cyprinus carpio*, *Ctenopharyngodon idella* and *Hypophthamichthys molitrix* in Al-Furat fish farm, Babylon province.

Mhaisen*et al.* (2015) identified of *Neoechinorhynchus iraqensis* and *Paulisentis fractus* from *Liza abu* in Euphrates River at Al-Musaib city.

Mhaisen and Al-Rubaie (2016) check listed of parasites *Neoechinorhynchus iraqensis from cyprinus carpio* and *Liza abu* and *Neoechinorhynchus rutili from C. idella*, *C. carpio*, and *H. molitrix* in Babylon Province.

Mhaisen and Al-Rubaie (2018) check listed of parasites *Neoechinorhynchus iraqensis* and *Paulisentis fractus* from *planiliza abu* in Babylon province.

Taha *et al.* (2018) described *Neoechinorhynchus iraqensis* from *Planiliza abu* (Mugilidae) by scanning electron microscopic in Tigris River.

Mhaisen (2019) check listed of parasites *Neoechinorhynchus iraqensis* from *Planiliza abu* (Mugilidae) and *Neoechinorhynchus rutili* from *L. xanthopterus* (reported as *B. xanthopterus*) and *planiliza abu* in Thi-Qar province.

2.2.8. Some Acanthocephala records of Kurdistan region

Rashid and Hussain (1988) recorded *Neoechinrhynchus rutili* for first time frpm *Barbus esocinus* in Greater Zab in northern Iraq.

Rashid *et al.* (1989) reported *Neoechinrhynchus rutili* from *Barbus esocinus* in Lessser Zab in northern Iraq.

Abdullah (1990) found *Neoechinorhynchus rutili* and *Pomphorhyncus laevis* for first time from *Barbus esocinus* and *Barbus barbulus* respectively in Dukan Lake.

Abdullah (1997) found *Pomforhyncus laevis* from *Barbus barbulus and Barbus xanthopterus* in Dukan Lake, Surdash and Iski-kalik.

Abdullah (2000) reported *Neoechinorhynchus rutili* from *Barbus esocinus* in Erbil markets.

Abdullah (2002) recorded new species of *Pomphorhyncus spindletruncatus* from *Barbus xanthopterus* and *Aspius vorax*in in Lesser Zab and Greater Zab respectively and showed *Neoechinorhynchus iraqensis* from *Liza abu* in Greater and Lesser Zab and.

Amin *et al.* (2003a) discovered new species of *Neoechinorhynchus zabensis* from *Capoeta damascina* and *Capoeta trutta* in the Greater and Lesser Zab Rivers.

Amin *et al.* (2003b) found new species of *Pomphorhynchus spindletruncatus* from *Aspius vorax* and *Barbus xanthopterus* in the Lesser Zab River near Alton Kupri and from the Greater Zab River near Iski-kalik.

Abdullah and Rasheed (2004) examined *Barbus esocinus* it was recorded *Neoechinorhynchus rutili* and *Pomphorhyncus laevis* from *Barbus barbulus* in Dokan Lake.

Abdullah and Mhaisen (2007) found *Pomphorhyncus spindletruncatus* from *Barbus xanthopterus* in Lesser Zab River.

Abdullah (2009) recorded *Neoechinorhynchus zabensis* in *Capoeta damascina* and *Capoeta trutta* from Dokan Lake and Greater Zab River.

Bilal and Abdullah (2009) recorded a new host (*Varicorhinus umbla*) for *Neoechinorhynchus zabensis* and found same parasite in *Capoeta trutta* from Bahdinan River, southeast of Greater Zab River, west of Erbil city.

Abdullah (2013) recorded *Neoechinorhynchus zabensis* from *Capoeta trutta* and *Pomphorhyncus spindletruncatus* from *Squalius lepidus* and *Silurus triostegus* in Darbandikhan Lake.

Abdullah and Abdullah (2015) found *Pomphorhyncus spindletruncatus* from *Squalius Lepidus* and *Silurus triostegus* and *Neoechinorhynchus zabensis* from *Capoeta trutta* in Darbandikhan Lake.

Hashim *et al.* (2015) showed *Neoechinorhynchus iraqensis* from *Liza abu* and *Silurus triostegus* and *Neoechinorhynchus zabensis* from *Capoeta damascina* in higher zab river at Aski kalak.

Mhaisen and Abdullah (2017) check listed of parasites reported *Neoechinorhynchus iraqensis* from *Planiliza abu* and *Silurus triostegus* in Greater Zab and Lesser Zab River and *Neoechinorhynchus rutili* from *Luciobarbus esocinus* in Greater Zab River, Lesser Zab River, Dokan Lake and Erbil's fish market and *Neoechinorhynchus zabensis* from *Capoeta damascina* in Greater Zab River, Lesser Zab River, Dokan Lake; from *Capoeta trutta* in Greater Zab River, Bahdinan river and Darbandikhan lake and from *C. umbla* in Bahdinan river; *Pomphorhynchus laevis* from *Luciobarbus barbulus* in Dokan lake, Greater Zab River and Surdash stream of Sulaimania, from *Luciobarbus xanthopterus* in Greater Zab River and Surdash stream of Sulaimania and *Pomphorhynchus spindletruncatus* from *Leuciscus vorax* in Greater Zab River, *Luciobarbus xanthopterus* in Lesser Zab River, *Silurus triostegus*, *Squalius lepidus* in Darbandikhan lake.

2.3 Molecular markers

Determination of Acanthocephala on the basis of morphological feature is difficult to identify because of their great similarity. In addition, molecular systematic was shown to have complexity of since many species of Acanthocephala have two or more of sister species that are morphologically indistinguishable. Molecular markers are used to make it easier to determine the variety inside and in between the Acanthocephala species (Martinez-Aquino *et al*. 2009; Wayland, 2010).

Molecular genetic marker represents the DNA sequence (nucleotide sequence) located at a particular site on the chromosome and possesses the characteristic of simple identification using molecular methods, and its inheritance that facilitate its investigation (Vardić Smrzlić, 2010).

Such markers have extensive application in the identification of parasite, characterization of the cryptic species type, and for the researching of the host selection model (Anderson, 2001). The usual method of genetic marking is the replication of a specific DNA fragment - usually a fragment of 18S rDNA or mitochondrial DNA (mtDNA) (Vardić Smrzlić, 2010).

2.3.1 18S rDNA

18S rRNA gene is located on the core DNA and encodes for ribosomal RNA which is a ribosome component. Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5,8S, 5S and 28S. The rRNA genes are most conserved (at least variables) in the cell. For this reason, genes encoding rRNA are determined by the nucleotide sequences for the identification of the taxonomic group, determining the mutual relationship between different groups and determining the rate of separation of species (Dlugosz and Wisniewski, 2006).

In genetic research, the data on the nucleotide sequences for the 18S rRNA gene were used to prove the hypothesis that the suckers were independent and monofilament knee was grouped into three classes, and to determine kinship with Rotifera (García Varela *et al.,* 2000). With the help of the 18S rDNA nucleotide sequences was carried out a method of identifying of Acanthocephala (Perrot Minnot, 2004) Table (2.4).

Table 2.4 Molecular methods of some *Neoechinorhynchus* species in different country

Species	Method	Country	Reference
Neoechinorhynchus golvani	ITSs and LSU rDNA gene	Mexico and Costa Rica	Martı´nez-Aquino *et al.* (2009)
Neoechinorhynchus mamesi	(cox 1) and domains D2 and D3	Mexico	Pinacho-Pinacho *et al.* (2012)
Echinorhynchus gadi	PCR-RFLP	Iceland	Sobecka *et al.* (2012)
Neoechinorhynchus species	(cox 1) and (18S rRNA)	North-East Asia	Malyarchuk *et al.* (2014)
Neoechinorhynchus (Neoechinorhynchus) mexicoensis	ITS and LSU rDNA gene	Mexico	Pinacho-Pinacho *et al.* (2014)
Neoechinorhynchus species	28S rDNA	Middle-America	Pinacho-Pinacho *et al.* (2015)
Neoechinorhynchus iraqensi and *Neoechinorhynchus zabensis*	5.8S rDNA	Iraq	Hassan *et al.* (2016)
Neoechinorhynchus species	cox 1, ITS and D2+D3 domains	Middle-America	Pinacho-Pinacho *et al.* (2018)
Neoechinorhynchus (Neoechinorhynchus) johnii	18S rRNA and ITS1-5.8S-ITS2 region	Vietnam	Amin *et al.* (2019)

2.4 Effect of some ecological factors on Acanthocephala

Kennedy (1975) noted that many workers showed the absence of any differences in the infection of both sexes of fishes with most parasites.

Moravec and Scholz (1994) recorded *N. rutili* on *B. barbus* from the Jihlava River, Czech Republic, and noted positive significant correlations between host total lengths.

Martins *et al* (2001) described the prevalence of *Neoechinorhynchus curemai* from *Prochilodus lineatus* in the Volta Grande Reservoir, MG, Brazil, and noted prevalence of 83.3%. However, the higher mean intensity was observed in August (66.5) and no relation between number of parasite and fish size.

Öztürk (2002) observed that the increased infection rate with increasing the length of *Tincatinca* with *Acanthocephalus lucii* from Lake Uluabat, Turkey.

Koyun (2012) recorded *N. zabensis* from *Capoeta umbla* from Murat River, Turkey, and noted that the infection increased with increasing length of host and reported the infection were rather low during summer and winter but no detected during spring and autumn.

Aydoğdu *et al.* (2015) observed *Neoechinorhynchus agilis* from *Chelon labrosus* in Beymelek Lagoon Lake in Antalya, Turkey, and reported there were no significant differences between host sexes, showed the highest prevalence and mean intensity in smaller fish and noticed increase in infection with the fish length and showed was rather low in spring, and this species was also not detected in summer.

Violante-Gonzaleza *et al.* (2016) identified biotic and abiotic factors that influence the temporal abundance of *Neoechinorhynchus brentnickoli* from *Dormitator latifrons* in Tres Palos Lagoon, Guerrero, Mexico.

Borazjani *et al.* (2017) recorded *Neoechinorhynchus zabensis* from *Capoeta barroisi* in Dalaki River, Boushehr province, Iran, and reported there were significant differences between spring and summer season and autumn and winter seasons.

Chagas *et al* (2019) evaluated the occurrence of *Neoechinorhynchus buttnerae* in tambaqui farming (*Colossoma macropomum*) and the parasite-host relationship in the town of Rio Preto da Eva, Amazonas (AM) state, Brazil.

Özcan *et al.* (2019) determined *Neoechinorhynchus rutili* from *Cyprinus carpio*, *Barbus rajanorum*, *Alburnus sp.*, *Capoeta angorae*, *Capoeta barroisi, Leuciscus cephalus* and *Luciobarbus pectoralis* in Menzelet Dam Lake in Kahramanmaraş, Turkey, and distributed according to ecological terms.

In Iraq, some ecological information about Acanthocephala (*Neoechinorhynchus*) parasites was published such as:

Khamees and Mhaisen (1988) recorded *Neoechinorhynchus agilis* {which was later identified as *N. iraqensis* (Mhaisen, 2002)} from *Liza abu* from Mehaijeran creek, in Basrah

city, showed the absence of differences between both sexes of fishes and infections were high during Summer and low during Autumn.

Ali (1989) showed no significant differences in the infection of both sexes of *B. esocinus* with *N. rutili* from Greater Zab River, Kurdistan region, Iraq.

Abdullah and Ali (1999) showed positive relation between infection with *N. rutili* and length of *B. esocinus* and noted that the infection was high during summer and low during autumn.

Al-Sady (2000) found *N. iraqensis* from *Liza abu* in Al-Faluja region, Al-Anbar province, and noticed increase in infection with the fish length.

Abdullah (2002); Abdullah and Mhaisen (2007) indicated that there were no significant differences between males and females of *B. xanthopterus* in infection with *Pomphorhynchus spindletruncats* from Lesser Zab River, and noted that the infection with *P. spindletruncats* increased with increasing length of host, and showed infection rate was high during Spring and Summer, and low during Autumn and Winter.

Abdullah (2009) recorded *N. zabensis* from *C. damascina* and *C. trutta* in Dokan Lake and Greater zab River, and showed increase in infection with the fish length and noticed the infection with this worm was high during spring and summer and low in autumn and winter.

Jasim (2019) recorded *Neoechinorhynchus iraqensis* from *Cyprinus carpio* in Tikrit city, with percentage of infection 1.6%.

CHAPTER THREE

MATERIALS AND METHODS

3.1Materials

3.1.1 Instruments and utilities

In the present study the following instruments were used which they shown in Table (3.1).

Table 3.1 Instruments which they used in the practical part.

No.	Instruments	Note
1	Camera Lucida (Drawing tube)	
2	Dissection Instruments	
3	Dissecting Microscope	
4	Optical Microscope	Motic
5	Digital Camera	
6	Scanning Electron Microscope	CamScan 3200 LV
7	Thermo Cycler (MultiGene)	
8	Agarose Gel Electrophoresis	
9	Gel Documentation	
10	Nanodrop Spectrophotometer	
11	Genetic analyzer 3500	
12	Micro Centrifuge	
13	Cooling Centrifuge	
14	Ware Bath	

3.1.2 Chemicals and stains

In the present study the following chemicals and stains were used which they shown in Table (3.2).

Table 3.2 Chemical materials and stains which they used in the practical part.

No.	Chemicals	Stains
1	Canada balsam	Mayer's acid carmine
2	Ethanol	Ethidium bromide
3	Formalin	Loading dye buffer
4	Glycerin gelatin (Jelly glycerin)	
5	Normal saline	
6	HCl	
7	Terpineol	
8	Kit for extraction DNA	
9	Agarose	
10	TBE buffer	
11	NaOH	
12	Tris-HCl	
13	Triton X-100	
14	DNA ladder	
15	Primers	
16	PCR master mix	
17	DNA ladder	

3.1.3 Description of Study Sites

A- Dukan Lake is the largest lake in the Iraqi Kurdistan region, which lies in the northwestern of Sulaimani, about 76 km from the city center (Shaban, 1980).The lake has a full-pool operating altitude of 511m and unregulated spillway at 515m above sea level (Toma, 2000).The boundaries of the lake extend between latitude of 34˚17'N - 36˚33'N and a longitude of 43˚17'E - 46˚24'E (Fig. 3.1). It was constructed in 1954-1959 by Damez-Bullot Dam (a French company) on lesser Zab River near Dukan gorge to prevent flooding, irrigation, electric generation, fishery and recreation (Karfin and Shahrawan).

B- Sirwan River is known in Kurdish as the Sirwan and in Arabic as the Diyala, which flows from its headwaters in the Zagros Mountains of western Iran to its ultimate confluence with the Tigris River just south of Baghdad. The latitude of Sirwan River is 33° 13' 14.88" N and longitude is 44° 30' 23.04" E (Fig. 3.1).

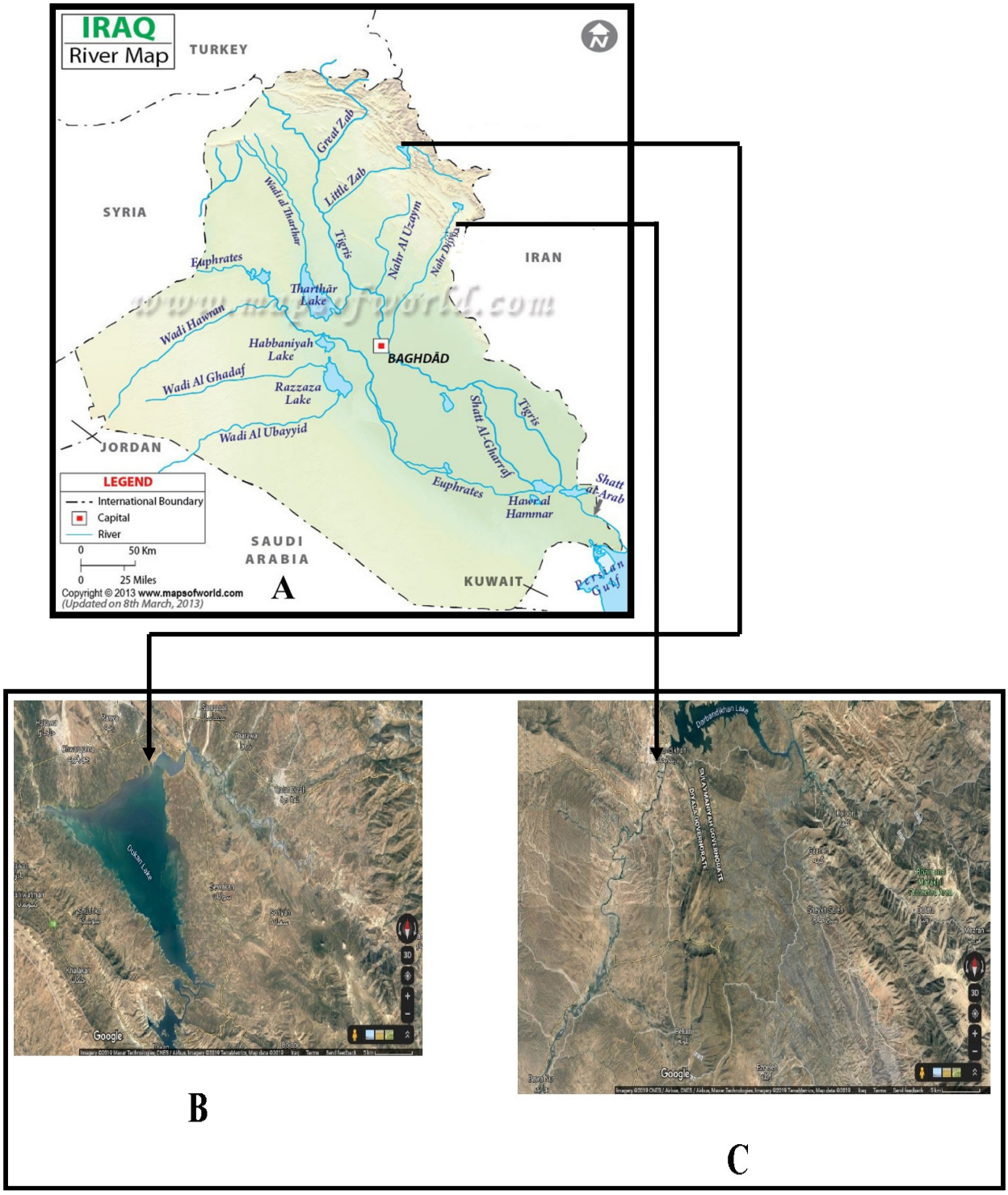

Figure 3.1 A- Map of Iraq showing Dukan Lake and Sirwan River
B- Google-Earth Satellite map showing Dukan Lake
C- Google-Earth Satellite map showing Sirwan River

3.1.4 Sample collections

3.1.4.1 Fishes

A total of 800 fishes (400 *Capoeta trutta* from Dukan Lake and 400 *Planiliza abu* from Sirwan River), were collected weekly by fishermen using gill nets, during the period from October 2017 until the end of February 2019. Fishes were kept in a cool box with river water and transferred to the laboratory in the Department of Animal Science, College of Agricultural Engineering Sciences, University of Sulaimani.

3.1.4.2 Parasites

Acanthocephalans revealed were first washed in saline solution, fixed in absolute ethanol for DNA extraction and refrigerated in cold water for 12 h, and then fixed in 70% ethanol. They were stained in Mayer's acid carmine, destained in 4% HCl in 70% ethanol, dehydrated in ascending concentrations of ethanol, cleared in graduated (increasing) concentrations of terpineol in 100% ethanol to 100% terpineol, then placed in 50% terpineol and 50% Canada balsam, and finally mounted in Canada balsam (Amin *et al.*, 2003a).

3.2 Methods

3.2.1 Morphometric and meristic measurements

The specimens were taken out of the cool box and the body length was measured using a one-meter measuring board graduated in millimeters (mm). The morphometric and parameters were measured from left side of each specimen. According to Beckman (1962), Coad (2010; 2017) the morphometric and meristic characters were studied as shown in the following Table (3.3).

Table 3.3 Morphological characters and meristic abbreviations and description

Abbreviations	Description
TL	Total length
SL	Standard length
HL	Head length
BD	Body depth
ED	Eye diameter
SnL	Snout length
Pre-O	Pre orbital distance
PrD	Pre dorsal fin distance
LD	Length of the dorsal-fin ray
Pre-Pectoral	Pre pectoral fin distance
Pre-Pelv	Pre pelvic fin distance
Pre-ans	Pre – anal distance
LA	Length of the anal-fin ray
ALL	Above lateral line scales
BLL	Below lateral line scales
PrD1	First pre dorsal fin distance
PrD2	Second pre dorsal fin distance

3.2.2 Description and systematic position of the species

The fishes were identified according to Beckman (1962), Coad (2010; 2017), and the scientific names of the fishes have been named according to Froese and Pauly (2017).

3.2.3 Molecular study of the fishes

3.2.3.1 Extraction of DNA

Samples were taken from the liver of two fish species with the debate concerning their identification by morphological characters only. The liver tissue samples (20 mg) were digested and homogenized with liquid nitrogen. Genomic DNA was extracted according the protocol of AccuPrep® Genomic DNA extraction Kit (Bioneer Corporation Cat. No.: K-3032 Korea) and as follows:

1. The sample was homogenized (20 mg) with a mortar and pestle, placed in a clean 1.5 ml tube (see "Additional required materials"), then 200 µl of Tissue Lysis buffer (TL) was

added. The weighted, fresh or frozen tissue was immediately placed in liquid nitrogen and grind to a fine powder with mortar and pestle under liquid nitrogen. Incomplete disruption will lead to significantly reduced yield and can cause clogging of the binding column tube.

2. Twenty µl of Proteinase K were mixed by vortex mixer, and incubated at 60 ℃ for 1 hr, or until the tissue is completely lysed. The sample was changed in clarity from turbid to clear, indicating that protein digestion has occurred. The time required for lysis will vary depending on the type of used tissue. Lysis will usually take 1~3 hr, and for efficient lysis, a shaking water bath or rocking platform was used. In the case of unavailability of these, vortex was used by 2~3 times, every 30 min during the incubation.

3. The tube briefly spin down to remove drops from inside the lid and 200 µl add of Binding buffer (GC), was added and immediately mixed by vortex mixer. The sample must completely resuspend to achieve maximum lysis efficiency.

4. Incubated at 60 ℃ for 10 min.

5. 100 µl of Isopropanol was added, mixed well by pippetting. After this step, briefly spined down to get the drops clinging under the lid.

6. Carefully transferred the lysate into the upper reservoir of the Binding column tube (fit in a 2 ml tube) without wetting the rim.

7. Close the tube and centrifuge at 8,000 rpm for 1 min. You must close each Binding column tube to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (>10,000 rpm) until the binding column tube is empty.

8. Opened the tube and transferred the Binding column tube to a new 2 ml tube for filtration (supplied).

9. 500 µl of Washing buffer 1 (W1) was added without wetting the rim, closed the tube, and centrifuged at 8,000 rpm for 1 min.

10. Opened the tube and poured the solution from the 2 ml tube into a disposal bottle.

11. Carefully added 500 µl of Washing buffer 2 (W2) without wetting the rim, closed the tube, and centrifuged at 8,000 rpm for 1 min.

12. Centrifuged once more at ca. 12,000 rpm for 1 min to completely removed ethanol, and check that there is no droplet clinging to the bottom of Binding column tube. Residual W2 in the Binding column tube may cause problems in later applications.

13. Transferred the Binding column tube to a new 1.5 ml tube for elution (supplied), add 200 µl of Elution buffer (EL, or nuclease-free water) onto Binding column tube, and wait for at least 1 min at RT (15~25 ℃) until EL is completely absorbed into the glass fiber of Binding

column tube. To increase DNA yield, you should wait for 5 min after adding Elution buffer (EL). The volume added EL can be adjusted from 50 µl to 100 µl. A smaller volume will result in a more concentrated solution, but total yield may be reduced.

14. Centrifuged at 8,000 rpm for 1 min to elute. About 180 µl ~ 200 µl of eluent can be obtained when using 200 µl of Elution buffer (or nuclease-free water). For an improved yield, elute the sample twice and use after concentration process. The eluted genomic DNA is stable and can be used directly, or stored at 4 ℃ for later analysis. For long-term DNA storage, you should elute with Elution buffer (EL) and store at -20 ℃, because DNA stored in water is subject to acid hydrolysis.

The quantity of DNA was checked and quantification was done by Nanodrop spectrophotometer the quantity for *Capoeta trutta* and *Planiliza abu* was 1.74 and 1.76 respectively. The quality of the extracted DNA was ssessed by agarose gel (1%) electrophoresis.

3.2.3.2 PCR amplification

In the present study, two primers which were obtained from (Macrogen company) in South Korea was used**.** The descriptions of primers regarding their names, primer sequences are given in Table (3.4). Amplifications of DNA were performed using a thermal cycler (MultiGene OptiMax Thermal Cycler TC9610 /TC9610-230, with the final reaction volume of 25 µl. Each reaction contained of prime *taq* premix (2X) Genet Bio PCR master mix (*Taq* DNA Polymerase 1 unit/10 µl, 20 mM Tris-HCl, 80 mM KCl, 4 mM MgCl2, enzyme stabilizer, sediment, loading dye, pH 9.0, 0.5 mM of each dATP, dCTP, dGTP, dTTP), primers (10 pmoles/ µl), DNA template (40 ng) and 7.5 µl of water free DNase.

In this study two PCR protocols were used to check amplification of DNA for two fish species *Capoeta trutta* and *Planiliza abu*. Protocols are as follows in Table (3.5).

Table 3.4 Primers name, sequence and GenBank accession number for two fish species

Fish species	Primer name	Primer sequence	GenBank accession number	Reference
Capoeta trutta	mtDNA COI	F: 5'-TCAACCAACCACAAAGACATTGGCAC-3' R: 5'-GACTTCTGGGTGGCCAAA-GAATCA-3'		Darabi (2014)
Planiliza abu	(cytb) gene	F: CTGCATTCGTAGGCTATGTC R: CTGCATTCGTAGGCTATGTC	JQ060190.1	

Table 3.5 PCR profiles for two fish species

Protocol 1	Step	PCR temp. (°C)	Time	Cycles
Capoeta trutta	Initial denaturation	95	3 min	1x
	1. Denaturation	95	30 sec	35x
	2. Annealing	62	30 sec	
	3. Extension	72	45 sec	
	Final extension	72	10 min	1x
Protocol 2	**Step**	**PCR temp. (°C)**	**Time**	**Cycles**
Planiliza abu	Initial denaturation	95	5 min	1x
	1. Denaturation	95	30 sec	40x
	2. Annealing	61	30 sec	
	3. Extension	72	30 sec	
	Final extension	72	10 min	1x

3.2.3.3 Agarose gel electrophoresis separation

A volume of 10 µl PCR product was electrophoresed on 2% agarose gel. Ethidium bromide were used to stain bands and visualized on a gel documentation (ENDURO™ GDS Touch Gel Documentation System) by using 100 bp DNA ladder (gene direx) the ladder was supplied in a ready for using format having fluorescent tracking dyes and DNA stain, expected size of the PCR amplicon was 625 bp for *Capoeta trutta* and 521bp for *Planiliza abu*.

3.2.3.4 DNA sequencing

A mitochondrial DNA cytochrome c oxidase subunit I (mtDNA *COI*) locus and cytochrome b (cytb) gene was amplified by PCR conventional or other type mention. In the present study, Genetic analyzer 3500, Applied Bio systems (USA) was used to find the nucleotides order of mtDNA *COI* and cytb for *C. trutta* and *P. abu* fish samples respectively. The PCR product of the fish samples were used for sequence specific PCR amplification and sent to the Macrogen Company in South Korea for nucleotide sequence analyses.

3.2.3.5 Photographs and measurements of the parasites

Photos were taken with Sony Cyber-Shot Digital camera model DSC-W570, 16.1 mega pixels. The figures were drawn by using a Camera Lucida (Drawing tube). Measurements of the parasite were made with Motic digital microscopy 111, 2-4 magnification, Motic educator, China.

3.2.3.6 Parasitic identification

The detected parasites were identified according to their morphology. Parasites were identified according to Bykhovskaya-Pavlovskaya *et al*. (1962), Gussev (1985) and Pugachev *et al.* (2010).

3.2.3.7 Scanning Electron Microscope (SEM)

For SEM, a few male and female specimens of *N. zabensis* previously were fixed in 70% ethanol and placed in critical point dryer baskets and dehydrated using the ETOH series of 95% and 3 N 100% for at least 10 min per soak followed by critical point drying (Lee, 1992). Samples were then mounted on SEM sample mounts, gold coated, and observed with a scanning electron microscope (CamScan 3200 LV). Digital images of the structures were obtained using computer-based digital imaging soft ware.

3.2.3.8 Molecular Study of the Parasite

3.2.3.8.1 Extraction of DNA

For DNA analysis of *Neoechinirhynchus zabensis* which isolated from *Capoeta trutta* fish species collected from the Dukan Lake. The fixed parasites in 99% ethanol were identified on the base of morphological characteristic. Then the genomic DNA was extracted according to

Beltran *et al*. (2008). 240 µl of NaOH (250 mM) was added to each tube. After a 15 min incubation period at 25°C, the tubes were heated at 99°C for 2 min. Then, 15 µl HCl (250 mM), 80 µl of Tris-HCl (500 mM) and 80 µl Triton X-100 (2%) were added and a second heat shock at 99°C for 2 min was performed and then centrifuged at 14000 rpm for 5 minute, The upper layer of fluid was transferred to a new tube.

The quantity of DNA was checked and quantification was done by Nanodrop spectrophotometer the quantity for *Neoechinirhynchus zabensis* wa 1.72. Agarose gel (1%) electrophoresis was used to assess and identify the quality of the extracted DNA.

3.2.3.8.2 DNA amplification

For phylogenetic study the 18S rDNA gene was amplified by PCR for (*Neoechinorhynchus zabensis*). The descriptions of primers regarding their names, primer sequences are given in Table (3.6). Amplification of DNA was performed using a thermal cycler (MultiGene OptiMax Thermal Cycler TC9610 /TC9610-230), with the final reaction volume of 25 µl. Each reaction volume contained prime taq premix (2X) Genet Bio PCR master mix (Taq DNA Polymerase 1 unit/10 µl, 20 mM Tris-HCl, 80 mM KCl, 4 mM MgCl2, enzyme stabilizer, sediment, loading dye, pH 9.0, 0.5 mM of each dATP, dCTP, dGTP, dTTP), primers (10 pmoles/ µl), DNA template (40 ng) and water free DNase.

Tale 3.6 Primers name, sequence and GenBank accession number for two fish species

Species	Primer name	Primer sequence	Reference
Neoechinorhynchus zabensis	18S rDNA gene	F: 5'- GCCGCGGTAATTCCAGCTC-3' R: 5'- CTGGTGTGCCCCTCCGTC-3'	Mujakić, 2014

Table 3.7 PCR profiles for *Neoechinorhynchus zabensis*

Species	Step	PCR temp. (°C)	Time	Cycles
Neoechinorhynchus zabensis	Initial denaturation	94	3 min	1x
	1. Denaturation	94	30 sec	35x
	2. Annealing	56	45 sec	
	3. Extension	72	1 min	
	Final extension	72	12 min	1x

3.2.3.8.3 Agarose gel electrophoresis separation

A volume of 10 µl PCR product on 2% agarose gel was electrophoresed. Ethidium bromide was used to stain bands and visualized on a gel documentation (ENDURO™ GDS Touch Gel Documentation System) by using 100 bp DNA ladder (gene direx) the ladder was supplied in a ready for using format having fluorescent tracking dyes and DNA stain, the size of the PCR amplicon was 622 bp for *Neoechinorhynchus zabensis*.

3.2.3.8.4 DNA sequencing

The 18S rDNA gene was amplified by PCR. In the present study, Genetic analyzer 3500, Applied Bio systems (USA) was used to find the nucleotide order of 18S rDNA gene. The PCR product of the parasite sample were used for sequencing specific PCR amplification and sent to the Macrogen Company in South Korea for nucleotide sequence analyses.

3.3 Ecology of fish parasites

Acanthocephalans species isolated from male and female fish during each month were grouped into four groups on basis of fish length (18–21.5, 22–25.5, 26-29.5 and 30-33.5) cm.

3.3.1 Criteria of infection

The use of ecological terms is in accordance with Margolis *et al*. (1982). For testing the differences in prevalence, mean intensity and abundance of infection between fish sexes, length groups and monthly fluctuations, t- test was conducted and the data were analyzed by XLSTAT (2016). All statistical analysis was performed at the significant level of 0.05.

1- Prevalence of infection (Percentage, Frequency and Incidence)**:** The percentage of individual number of host species infected with particular parasite species per total number of host was examined during a certain period.

2- Mean intensity of infection: Mean number of particular parasite species per infected host in a sample during a certain period.

3- (Relative density of infection): Total number of individuals of a particular parasite species in a sample of hosts - Total number of individuals of the host species (infected + uninfected) in the sample.

3.4 Statistical Analysis

For testing the differences in prevalence, mean intensity and abundance of infection between fish sexes and monthly fluctuations, t- test (One-tailed and Two-tailed) and for the length groups, Kruskal-Wallist test (Two-tailed) was conducted; data were analyzed by XLSTAT (2016). All statistical analysis was performed at the significant level of 0.05.

3.5 Genetic Analysis

Multiple sequences alignment was performed using ClustalW as implemented in MEGAX together with other species of acanthocephalans. Phylogenetic analyses were conducted using MEGAX with 1000 bootstrap replicates for prior testing of reliability. For nucleotide alignment of 18S rDNA region, a phylogenetic tree was constructed using the Neighbor-joining tree method in MEGAX. Pairwise distance analyses were carried out using the Maximum Composite Likelihood model Kumar *et al*. (2018).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Fishes

A total of 800 fishes (400 *Capoeta trutta* from Dukan Lake and 400 *Planiliza abu* from Sirwan River) were collected during the period from October 2017 until February 2018.

4.1.1 Morphology

The following is an account on morphometric and meristic of the fishes recorded in the present study.

1- Family Cyprinidae

Capoeta trutta (Heckel, 1843) (Fig. 4.1)

Common names: Touyeni, Qur xora.

Synonym: *Scaphiodon trutta* Heckel, 1843.

According to the diagnostic characteristics of the seven specimens:

Morphology: The mouth is inferior and transverse, small scales, there are denticles or teeth on the last dorsal fin ray.

Color: brownish to yellowish or olive-green on the back with silvery-white flanks and the belly lighter, white with silvery tints. The head, the body and the dorsal fin are covered with small, distinctive black spots, often c- or x-shaped.

Total length (TL): 18-32 (25) cm.

Standard length (SL):15 -27.5 (21.25) cm.

Head length (HL): 4-5 (4.5) cm.

Body depth (BD): 4.5-7 (5.75) cm.

Eye diameter (ED): 0.7 – 1.3 (1) cm.

Snout length (SnL): 0.9 – 1.5 (1.2) cm

Pre orbital distance (Pre-O): 1.5 – 2 (1.75) cm

There are one pair barbels on the upper jaw.

Pre dorsal fin distance (PrD): 8 – 12.5 (10.25).

Length of the dorsal-fin ray (LD): 3.2 – 5.5 (4.35) cm.

Pre pectoral fin distance (Pre-Pectoral): 4 – 5.5 (4.75) cm

The pectoral fin ray length: 3 – 3.8 (3.4) cm

Pre pelvic fin distance (Pre-Pelv): 9.5 – 13 (11.25) cm

The pelvic fin ray distance: 2.8 – 3.5 (3.15)

Pre – anal distance (Pre-ans): 14 – 20 (17) cm

Length of the anal-fin ray (LA): 3 – 5 (4) cm.

Above lateral line scales (ALL): 14 – 17.

Below lateral line scales (BLL):11 – 14.

Figure 4.1 ***Capoeta trutta***

2- Family Mugilidae

Planiliza abu (Heckel, 1843) (Fig. 4.2)

Common names: Khishni, Dra masi, Raqa masi, Zibra.

Synonyms: *Liza abu* (Heckel, 1843).

According to the diagnostic characteristics of the four specimens:

Morphology: Lips are thin, long pectoral fins reaching almost level with the first dorsal fin origin when folded back, relatively strong spines in the first dorsal and anal fins, Scales are strongly ctenoid on the exposed part and the fish feels rough to touch when rubbed from tail to head.

Color: The back is dark to light green or greyish-green, the flanks silvery to white and the belly white.

Total length (TL): 14 - 21 (17.5) cm.

Standard length (SL): 12 -18 (15) cm.

Head length (HL): 3 – 3.5 (3.25) cm.

Body depth (BD): 4 – 5.5(4.75) cm.

Eye diameter (ED): 0.8 – 0.9(0.85) cm.

Snout length (SnL): 1 cm

Pre orbital distance (Pre-O): 1 – 1.5 (1.25) cm.

No barbels.

First pre dorsal fin distance (PrD1): 7 – 8 (7.5) cm.

Second pre dorsal fin distance (PrD2): 11.5 – 13 (12.25) cm.

Length of the dorsal-fin ray (LD): 2.5 – 3.5 (3) cm.

Pre pectoral fin distance (Pre-Pectoral): 4 – 4.5 (4.25) cm.

The pectoral fin ray length: 2 – 2.8 (2.4) cm.

Pre pelvic fin distance (Pre-Pelv): 6 – 7 (6.5) cm.

The pelvic fin ray distance: 2.5 – 3.5 (3) cm.

Pre - anus distance (Pre-ans): 11.5 – 13 (12.25) cm.

Length of the anal-fin ray (LA): 2 – 3 (2.5) cm.

Above lateral line scales (ALL): 7.

Below lateral line scales (BLL): 6.

Figure 4.2 ***Planiliza abu***

The morphological characters and meristic of *C. trutta* and *P. abu* are indicated in Table (4.1).

Table 4.1 The morphological characters and meristic for *Capoeta trutta* and *Planiliza abu*.

Characteristics	*Capoeta trutta*	*Planiliza abu*
Total length (TL)	18-32 (25) cm.	14 - 21 (17.5) cm.
Standard length (SL)	15 -27.5 (21.25) cm.	12 -18 (15) cm.
Head length (HL)	4-5 (4.5) cm	3 – 3.5 (3.25) cm
Body depth (BD)	4.5-7 (5.75) cm	4–5.5 (4.75) cm
Eye diameter (ED)	0.7 – 1.3(1) cm	0.8 – 0.9 (0.85) cm
Snout length (SnL)	0.9 – 1.5 (1.2) cm	1 cm
Pre orbital distance (Pre-O)	1.5-2 (1.75) cm	1 – 1.5 (1.25) cm
Pre dorsal fin distance (PrD)	8 – 12.5 (10.25)	-
Length of the dorsal-fin ray (LD)	3.2 – 5.5 (4.35) cm	2.5–3.5 (3) cm
Pre pectoral fin distance (Pre-Pectoral)	4 – 5.5 (4.75) cm	4 – 4.5 (4.25) cm
Pre pelvic fin distance (Pre-Pelv)	9.5 – 13 (11.25) cm	6 – 7 (6.5) cm
Pre – anal distance (Pre-anl)	14 – 20 (17) cm	11.5 – 13 (12.25) cm
Length of the anal-fin ray (LA)	3-5 (4) cm	2 – 3 (2.5) cm
Above lateral line scales (ALL)	14-17	7
Below lateral line scales (BLL)	11-14	6
Number of barbels	One pair barbels on the upper jaw	No barbels
The pectoral fin ray length	3 – 3.8 (3.4) cm	2 – 2.8 (2.4) cm
First pre dorsal fin distance (PrD1)	-	7 – 8 (7.5) cm
Second pre dorsal fin distance (PrD2)	-	11.5 – 13 (12.25)

The two fish species in this study are belonging to the family Cyprinidae and Mugilidae. The description and measurement of the present samples of these fishes are similar to those indentified by Beckman (1962) and Coad (2010).

As a comparison between some characteristics of *C. trutta* in this study such as standard length, total length, body depth, head length, eye diameter, length of the dorsal-fin ray, snout length, length of the anal-fin ray, above lateral line scales and below lateral line scales are 25, 21.25, 4.5, 5.75, 1, 1.2, 4.35, 4, (14-17) and (11-14), respectively are in agreement with the results of Agha (2017), which are 28.18, 23.74, 4.86, 5.7, 0.76, 1.5, 4.98, 3.6, (15-16) and (10-11).

Standard length, total length, body depth, head length, eye diameter, length of the dorsal-fin ray, snout length, length of the anal-fin ray, above lateral line scales and below lateral line scales of *P. abu* in this study are 17.5, 15, 3.25, 4.75, 0.85, 1, 7 and 6, respectively which are in agreement with the results of Agha (2017), which are 18.38, 16, 3.53, 4.45, 0.75, 1, 7, 6.

The morphological results of *P. abu* in this study are similar to the results of Khayyami *et al.* (2014) on morphological variability of Liza abu and with Mohamed *et al.* (2016) on comparative taxonomical for *Planiliza subviridis*, *P. klunzingeri*, *P. Carinata* and *Osteomugil speigleri*. Standard length of *Planiliza abu* in this study is in agreement with the results of Mohamed *et al.* (2018).

Traditionally, in the freshwaters of Iraq, four species (*Capoeta aculeata*, *Capoeta barroisi*, *Capoeta damascina* and *Capoeta trutta*) represent the genus *Capoeta* and four species (*Liza abu*, *Liza klunzingeri*, *Liza oligolepis* and *Liza subviridis*) represent the genus *Liza* (Coad, 2010).

4.1.2 DNA sequence

DNA extraction performed on 150 and 120 specimens for *C. trutta* and *P. abu*, respectively were successfully generated DNA contained products (These numbers of fish were used for DNA extraction).

The PCR product of mtDNA COI locus was 625 bp for *Capoeta trutta* and cytochrome b (cytb) gene was 521 bp for *Planiliza abu* Fig. (4.3).The result of the present study about DNA sequencing in two species of fish, putted to BLAST and 617 bp and 446 for *Capoeta trutta* and *Planiliza abu* respectively, were alignments with sequences of fish species stored in GenBank. The molecular study showed the presence of the two species belonging to *Capoeta trutta* and *Planiliza abu*. BLAST results are indicated in Table (4.2).

Table 4.2 The BLAST results of fish species.

No. Samples	Genus and species	Molecular based homology (%)
1	*Capoeta trutta*	100% identified homology
2	*Planiliza abu*	100% identified homology

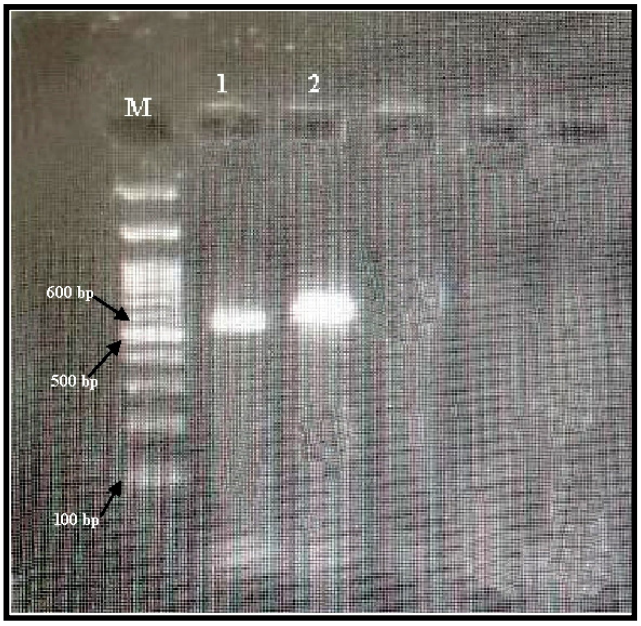

Figure 4.3 PCR amplification of mtDNA COI locus for *Capoeta trutta and* cytochrome b (cytb) gene for *Planiliza abu* fish species. Lane M= DNA ladder 100 bp, lane1 = *Planiliza abu* (521 bp) and lane 2= *Capoeta trutta* (625 bp).

Partial cds, Cytochrome oxidase subunit I (*COI*) gene mitochondrial and partial cds 61 cytochrome b (cytb) genes mitochondrial are compatible with the same sequence fragment marker, which is available at the GeneBank in the National Centre for Biotechnology Information (NCBI). Figures (4.4.; 4.5; 4.6; 4.7) showed pair wise analysis and partial sequence of the two fish specimens.

The current study represents the first molecular study for *C. trutta* and *P. abu* in Iraq. The results of *C. trutta* in family Cyprinidae in this study are in agreement with the results of Faddagh *et al.* (2012a) who identified eight cyprinid fish species, and found high similarity between species, from 84.4% between *Luciobarbus kersin* (as *B. kersin*) and *L. xanthopherus* (as *B. xanthopherus*) to 52% between *Mesopotamichthys sharpeyi* (as *B. sharpeyi*) and *L. barbulus* (as *B. barbulus*) to 86.9% between *Leuciscus vorax* (as *A. vorax*) and *Arbibarbus grypus* (as *B. grypus*). Faddagh *et al.* (2012b) also used the mitochondrial 16S rRNA gene fragment as a molecular marker to study taxonomical status of seven cyprinin fish species in Iraqi inland waters: *Barbus kersin*, *B. xanthopterus*, *B. barbulus*, *B. sharpeyi*, *B. grypus*, *Cyprinus carpio* and *C. luteus,* the results assured that the six *Barbus* species genetically belong to sub-family Cyprininae which belong to family Cyprinidae. Aziz (2015) examined nine species of Cyprinidae family, the result of DNA sequencing showed that all species belong to family Cyprinidae the phylogenetic relationship degree with this family for *C. luteus*

which was a BP of 87%, for *C. regium*, *C. carpio* and *C. Carassius* was a BP of 75%, for *C. macrostomum*, *L. esocinus*, *C. trutta* and *L. xanthopterus*a was a BP of 90% and for *Barbus grypus* was a BP of 76%.

In this study the results are in agreement with Parmaksiz and Eksi (2017) who used mtDNA *COI* 625 loci to study the genetic diversity in populations from 47 samples of *Capoeta trutta.* The result of sequence analysis showed six polymorphic sites and seven haplotypes on that locus, which is also in agreement with Turan (2008) who determined the subspecies of *Capoeta* corresponding to taxonomic entities and defined species using traditional gene sequencing of mitochondrial 16S rDNA. The database included 124 variable sites, parsimony informative was 103 sites. The results in this study are similar to the results of Zareian *et al.* (2016) who used mitochondrial cytochrome *b* gene sequences for phylogenetic relationship of *Capoeta* species, and it was found that three major groups were detected: Clade I: *Capoeta trutta* group which is the Mesopotamian *Capoeta* group having very close related taxa (*barroisi*, *trutta and turani).* Clade II: *Capoeta damascina* complex group (*capoeta* group small scale) including the Anatolian-Iranian groups such us (*buhsei*, *saadii*, *banarescui* and *damascina*) and widespread highly diversified groups. Clade III comprises closely related taxa; *Capoeta capoeta* complex group (the Aralo-Caspian group, large scale *capoeta* group).

The results in this study are in agreement with Nematzadeh *et al.* (2013) using PCRsequencing method to establish phylogenetic relationships among six mugilidae species (*M. capito*, *Valamugil buchanani*, *Mugil cephalus*, *Liza subviridis*, *L. saliens* and *L.aurata)* and genetic differences were determined. The results demonstrate that in the mitochondrial 16s rRNA genome number of bases was approximated 600 base pairs. Also (Lai *et al.*, 2011) (80) random primers for random amplified polymorphic DNA (RAPD) were used for the examination of 15 fish families. Results clarify that in the Mugilidae family a novel specific PCR product was found, OPAV04 primer was employed also in the *Liza* genus, by using OPAV10 primer other novel specific PCR product was found.

The results of the present study are not in agreement with Faddagh *et al*. (2012b) who showed that the *Liza abu* and *Liza klunzingeri* did not respond to the modified primer in mitochondrial 16S rRNA gene but in this study *Planiliza abu* responded to the cytochrome b (cytb) gene, partial cds; mitochondrial.

This taxonomic position has changed and most researchers in the field now agree that DNA coding is a useful tool in the process of identifying and indexing species. There are still researchers who doubt that one can distinguish the gene of all species and refers to the fact that taxonomists who evaluate their findings on morphological basis have a range of many

different characters, not one, to help them, for this the present study used a molecular tool for identification. Molecular techniques such as PCR and DNA sequencing were proven to be very specific and highly sensitive to detect species of fish. However, using them in diagnostic laboratories are very rare. Moreover, DNA amplification is not cheap and it is tedious, also samples can face cross contamination which is dangerous, fortunately nowadays by developed methods these issues are decreased (Agha, 2017).

In the present study, 617 and 446 bp was aligned for *Capoeta trutta* and *Planiliza abu* respectively; the two specimens were morphologically identified by using Coad keys. The sequences compared with sequences of other genera and fish species segments stored in Gen Bank. The results showed that the morphometric data and molecular methods were successful in identifying of *C. trutta* and *P. abu*.

Samples of *Capoeta trutta* and *Planiliza abu* have been morphologically identified. DNA sequencing results showed that the studied two fish species belong to *Capoeta trutta* and *Planiliza abu.* Gen Bank analysis indicated that the two sequenced species were correctly identified.

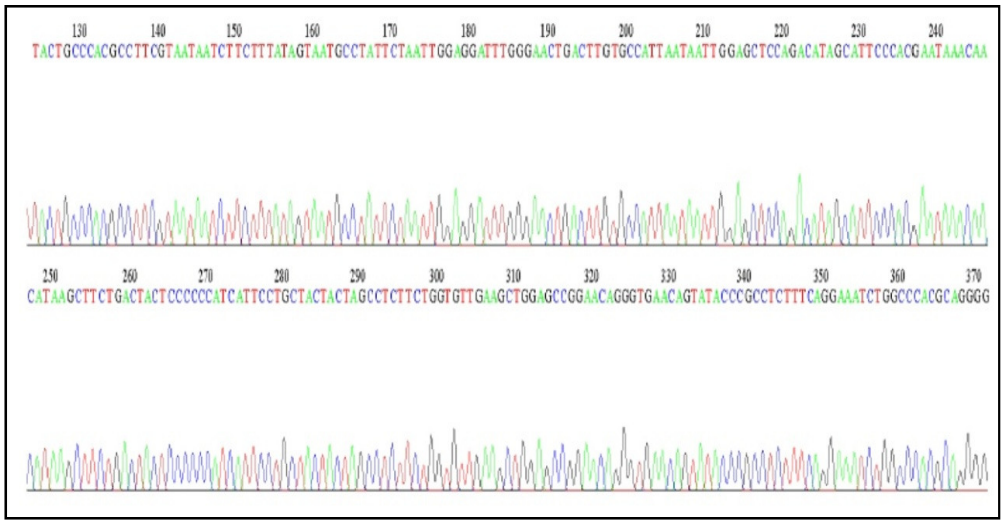

Figure 4.4 The partial sequencing result of partial cds, Cytochrome oxidase subunit I (COI) gene; of *Capoeta trutta*.

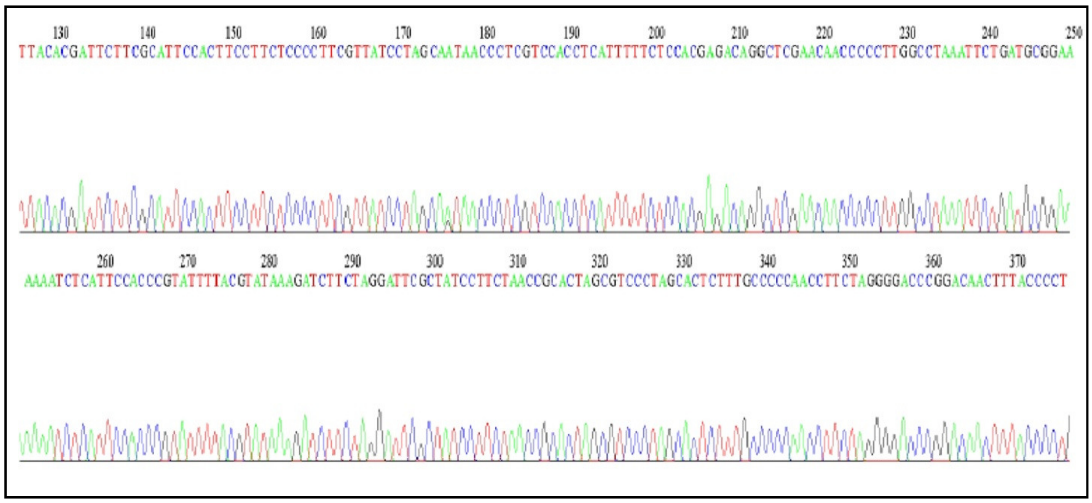

Figure 4.5 The partial sequencing result of partial cds 61 cytochrome b (cytb) genes mitochondrial, of *Planiliza abu*.

Capoeta trutta cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: MH592463.1 Length: 652 Number of Matches: 1

Range 1: 36 to 652 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1140 bits(617)	0.0	617/617(100%)	0/617(0%)	Plus/Plus

```
Query  37   TGGGCACTGCTTTAAGCCTTCTCATTCGAGCCGAATTAAGCCAACCCGGATCACTTCTAG  96
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           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  157  TAGTAATGCCTATTCTAATTGGAGGATTTGGGAACTGACTTGTGCCATTAATAATTGGAG  216
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Query  217  CTCCAGACATAGCATTCCCACGAATAAACAACATAAGCTTCTGACTACTCCCCCCATCAT  276
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Query  277  TCCTGCTACTACTAGCCTCTTCTGGTGTTGAAGCTGGAGCCGGAACAGGGTGAACAGTAT  336
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Sbjct  576  TCCTAACAGACCGAAACCTCAACACCACATTCTTTGACCCCGCCGGAGGAGGAGACCCAA  635

Query  637  TCCTCTACCAACACCTA  653
           |||||||||||||||||
Sbjct  636  TCCTCTACCAACACCTA  652
```

Figure 4.6 Pair wise alignment partial cds, Cytochrome oxidase subunit I (COI) gene of *Capoeta trutta*. Query is the study or sample sequence and sbjct is the GenBank sequence.

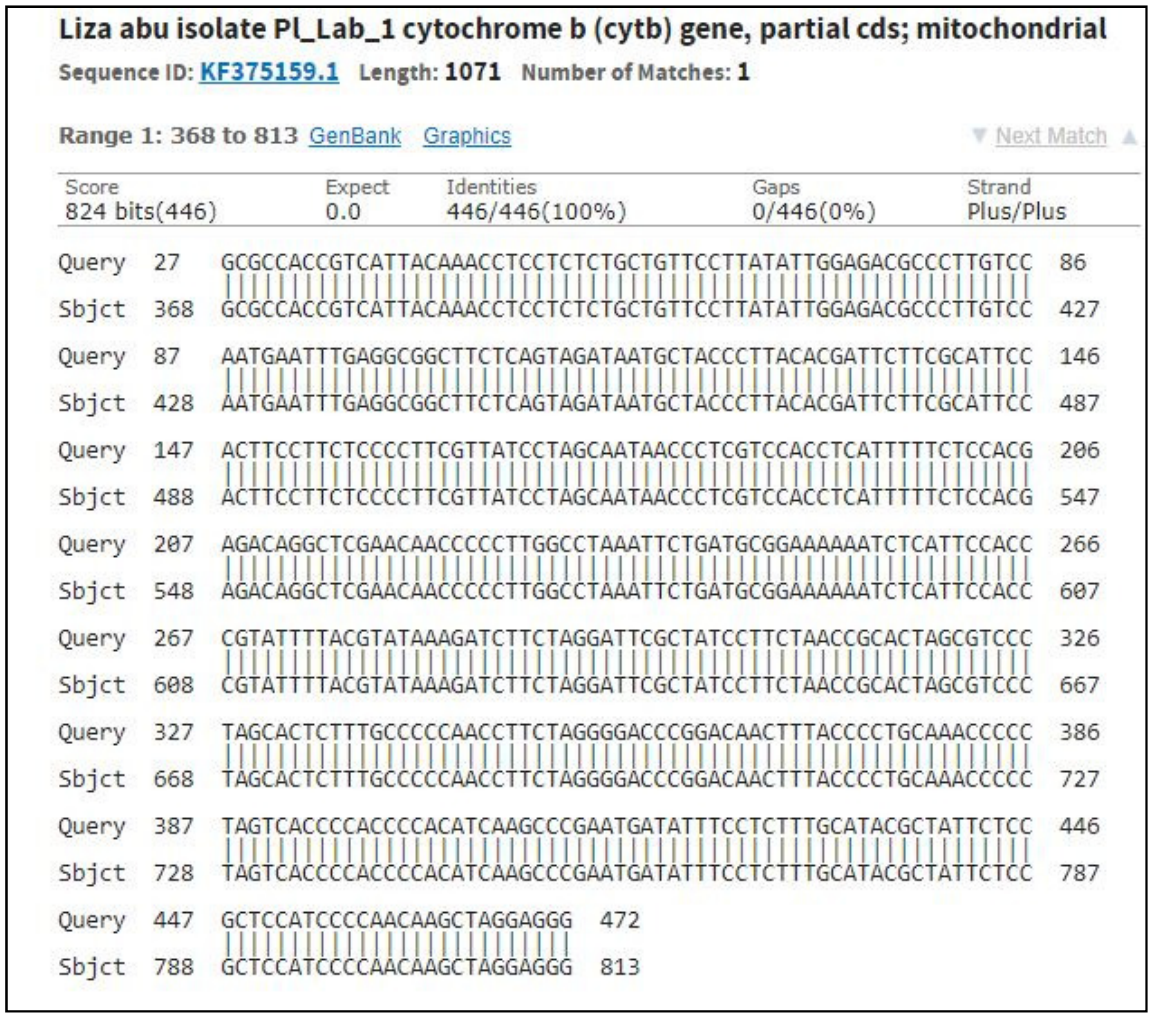

Figure 4.7 Pair wise alignment of partial cds 61 cytochrome b (cytb) genes mitochondrial of *Planiliza abu*. Query is the study or sample sequence and sbjct is the GenBank sequence.

4.2 Parasites

A total of 400 *C. trutta* and 400 *P. abu* were surveyed for parasitic acanthocephalans during the period of the present study. The classifications of these parasites are shown in (Table4.3). The survey showed the occurrence of *N. zabensis* in the intestine of *C. trutta*, with an overall prevalence % 86.96; mean intensity 3.49 and abundance 3.04. The occurrence of *N. iraqensis* in the intestine of *P. abu*, with an overall prevalence 0.015; mean intensity 1.1 and abundance % 0.015 (Table 4.4).

Table 4.3 Parasite species recorded in Dukan Lake and Sirwan River according to their classification status.

Kingdom: Animalia
Subkingdom: Bilateria
Infrakingdom: protostomia
Superphylum: Platyzoa
Phylum: Acanthocephala Rudolphi, 1802- spiny-headed worm
Class: Eoacanthocephla Van cleave,1936
Order: Neoechinorhynchida Southwell and Macfie,1925
Family: Neoechinorhynchidae Ward, 1917
Subfamily: Neoechinorhynchinae Ward, 1917
Genus: *Neoechinorhynchus* Stiles and Hassall, 1905
Species: ***Neoechinorhynchus zabensis*** Amin, Abdullah and Mhaisen, 2003
Neoechinorhynchus iraqensis Amin, Al-Sady, Mhaisen and Bassat, 2001

Table 4.4 The prevalence, mean intensity abundance for *Neoechinorhynchus zabensis* and *Neoechinorhynchus iraqensis*

Fishes	Parasites	No. of Fishes		Prevalence %	Mean intensity (Range)	Abundance
		Examined	Infected			
C. trutta	*N. zabensis*	400	348	86.96	3.49 (5-60)	3.04
P. abu	*N. iraqensis*	400	8	0.015	1.1 (1-2)	0.015

4.2.1 General description of *Neoechinorhynchus zabensis*

The body is cylindrical and usually medium; proboscis is short with six longitudinal rows of hooks, each row contains three hooks, anterior hooks are longer and stouter than others (Fig. 4.8; 4.9 A). Neck unremarkable, proboscis receptacle about six times as long as proboscis. Lemnisci sub equal, ribbon shaped.

Male: Length of trunk 5.09-10.11 mm, width 0.85-1.50 mm. Proboscis hooks in anterior circle is 37 - 45 long; in middle circle 30 - 35 long; in posterior circle 30–35 long. Proboscis receptacle is about 426–728 (566) long by 125–187 (150) wide. Longer lemniscus is 2.0-3.75 mm long, shorter lemniscus is about 1.54-3.3 mm long. Reproductive system in posterior half of trunk and extends to posterior end of bursa. Cement gland is large, tapering posteriorly, 0.36–1.20 (0.73) mm long by 0.28–0.64 (0.40) mm wide anteriorly, with 8 vesicular giant nuclei, contiguous to cement gland reservoir 175–400 (278) long by 125–250 (179) wide (Fig. 4.9 B).

Female: Length of trunk 8.81-14.88 mm, width 1.01 - 2.11 mm. Proboscis hooks in anterior circle 37 - 46 long; in middle circle 30 - 40 long, in posterior circle 30 – 40 long. Total length of proboscis is between 100 - 125 long while the width is between 100-123 long. Proboscis receptacle is 510–728 (593) long by 135–198 (170) wide. Longer lemniscus is between 3.10-4.35 mm long, shorter lemniscus is 2.90-4.13 mm long by 0.10–0.31 (0.23) mm wide. Reproductive system is sinuate membranes, distal vaginal swelling, markedly sub terminal gonopore, uterus and uterine bell is as long as vagina, and one paired muscular para-vaginal appendage (Fig. 4.9 C).

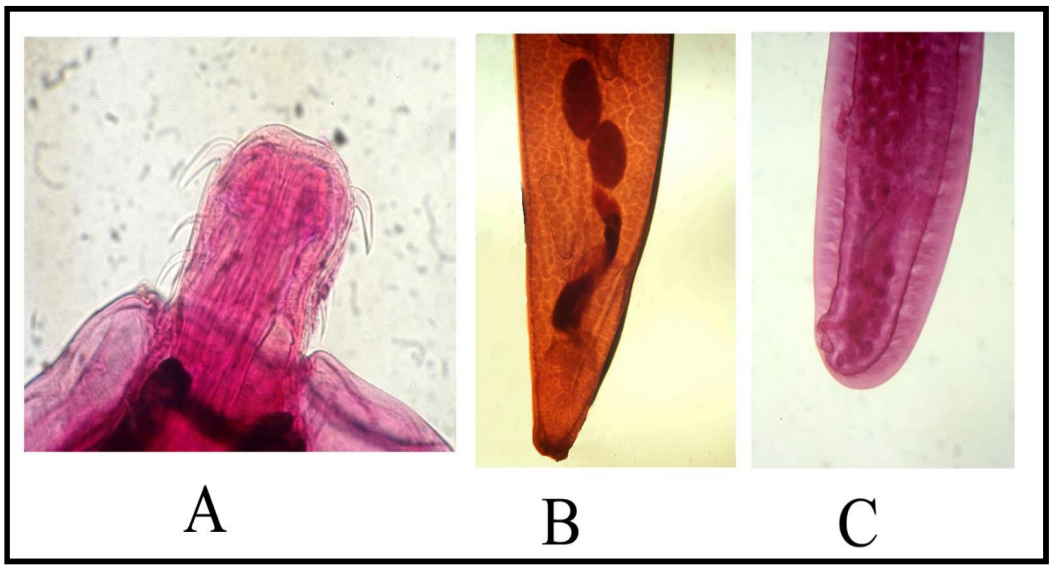

Figure 4.8 ***Neoechinorhynchus zabensis***
A- Proboscis of worm (32X)
B- Male worm (32X)
C- Female worm (32X)

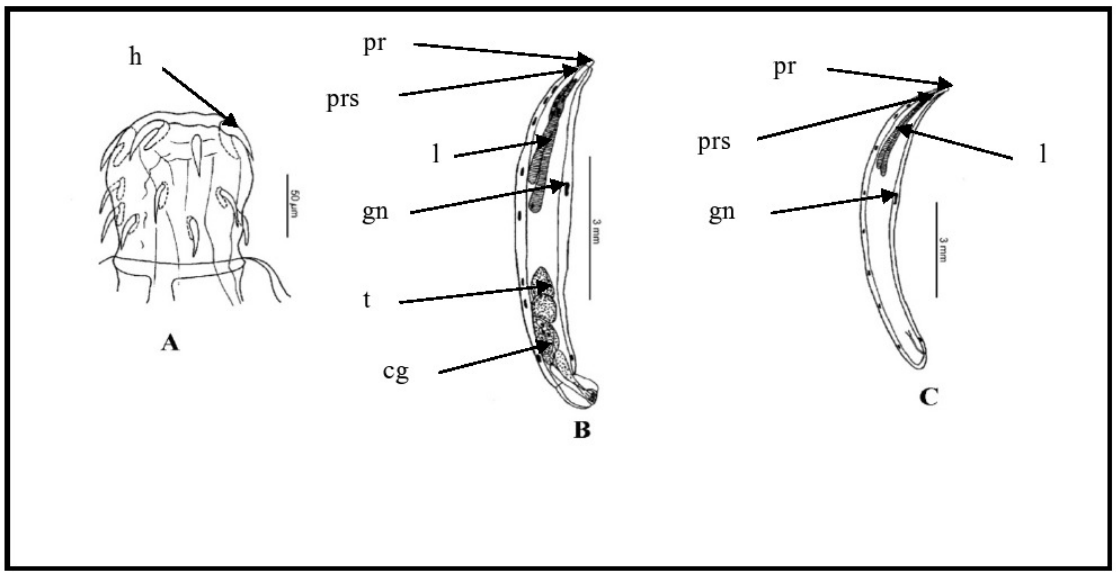

Figure 4.9 ***Neoechinorhynchus zabensis***

A- Camera lucida drawing of proboscis worm
B- Camera lucida drawing of male worm
C- Camera lucida drawing of female worm
cg= cement gland; gn= giant nucleus; h= hook; l= lemniscus; prs= proboscis sac; pr= proboscis; t= testis

Neoechinorhynchus zabensis was identified for the first time in Iraq as a new species by Amin *et al.* (2003a) from *Capoeta damascina* and *Capoeta trutta* in Greater Zab and Lesser Zab rivers. Later on, it was recorded from *C. trutta* and *C. umbla* from Bahdinan River by Bilal and Abdullah (2009). According to Mhaisen (2019), a total of eight fish host species (*Arabibarbus grypus*, *Capoeta damascina*, *Capoeta trutta* (*Varicorhinus trutta*), *Capoeta umbla*, *Carasobarbus luteus*, *Carassiu sauratus*, *Planiliza abu* (as *Liza abu*) and *Silurus triostegus*) were reported for this parasite in Iraq.

Morphometric characteristics of the specimens of *N. zabensis* described in this study were similar to those reported in the original description by Amin *et al.* (2003a) and Abdullah (2013).

As shown in the results of the present study, it was noticed that the prevalence of infection with *N. zabensis* in *C. trutta* was high %86.96. The result of this study was higher than the one reported by Amin *et al.* (2003a) about Greater Zab River and Lesser Zab River with 33.3% and 47.7% prevalence respectively and Hashim *et al.* (2015) the prevalence was 34.6% in *C. damascina*. In this study the results of prevalence were lower than reported by Amin *et al.* (2003a) who recorded *N. zabensis* from *C. damascina* in Greater Zab River and Lesser Zab River with 93.3 and 93.2% prevalence, respectively and Abdullah (2009) recorded *N. zabensis*

from *C. trutta* in Dokan Lake 98.51% prevalence, but higher than from Greater Zab River with 72% prevalence.

In the study, the range of mean intensity of *N. zabensis* (5-60) parasite was higher than that reported by Amin *et al.* (2003a) which recorded *N. zabensis* from *C. trutta* in Greater Zab River and Lesser Zab River with 0-30 parasite in both River, also were higher than Abdullah (2009) who recorded *N. zabensis* from *C. trutta* in Dokan Lake and Greater Zab River with 4-35 and 2-32 parasite respectively. In this study mean intensity of *N. zabensis* was higher than that reported by Hashim *et al.* (2015) was recorded 1.55 parasites in *C. damascina.*

4.2.2 General description of *Neoechinorhynchus iraqensis*

Long slender worm, proboscis bulbous anteriorly where first two circles of hooks are found. Posterior part of proboscis supporting third circle of hooks narrowest anteriorly but gradually and slightly expanding posteriorly into neck. Proboscis hooks in anterior circle alternating at two levels; hooks in anterior level smaller than hooks in posterior level. Lemnisci large, markedly unequal; larger lemniscus, with three giant nuclei (proximal, middle and distal), more than twice size of smaller lemniscus, which has only one proximal giant nuclei Fig. (4.10; 4.11 A)

Male: trunk 15-26.8 (19.55) mm length by (0.56-0.79) (0.70) wide mm. Proboscis 99-129 (111) long by 87-119 (100) wide at base. Anterior bulbous part of proboscis is about 79-109 (89) long by 87-119 (100) wide. Posterior part of proboscis, following constriction and neck 54-86 (69) long by 79-124 (97) wide. Larger proboscis hooks in anterior circle is between 29-36 (32) long; smaller hooks in same circle 21-29 (25) long. Hooks in middle and posterior circles are about 11-14 (13) and 14-17 (16) long, respectively. Larger lemniscus is between 4.24-8.40 (6.75) mm long by 0.13-0.30 (0.23) mm wide. Syncytial cement gland 0.74-1.97 (1.26) mm long by 0.32-0.61 (0.46) mm wide. Common sperm duct 0.81-1.30 (1.04) mm long by 0.16-0.43 (0.31) wide. Bursa 622-997 (764) long by 363-914 (555) wide. Fig (4.11 B).

Female: trunk is about 26- 73.6 (53.2) mm long by 0.52-1.25 (0.86) mm wide. Proboscis is about 99-131 (109) long by 106-136 (120) wide at base. Neck is around 29-54 (38) long by 101-134 (119) at base. Anterior bulbous part of proboscis 61-99 (78) long by 104-136 (116) wide. Larger proboscis hooks in anterior circle 26-41 (33) long; smaller hooks in same circle 21-31 (26). Hooks in middle and posterior circles 11-19 (15) and 14-21 (18) long, respectively. Larger lemniscus 5.40- 10.2 (7.74) mm long by 0.18-0.35 (0.29) mm wide. Smaller lemniscus 2.37- 4.66 (3.22) mm long by 0.08-0.20 (0.12) mm wide. Reproductive

system 0.95-1.39 (1.21) mm long. Uterus bell about as long as vagina and uterus. Gonopore is sub-terminal position. (Fig 4.11 C).

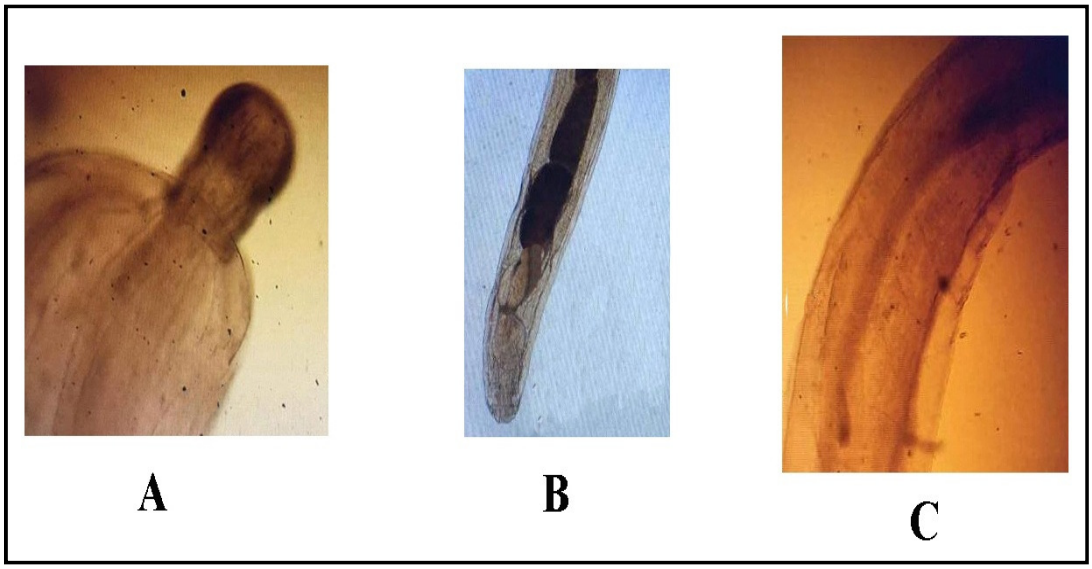

Figure 4.10 ***Neoechinorhynchus iraqensis***
A- Proboscis of worm (20X)
B- Male worm (20X)
C- Female worm (20X)

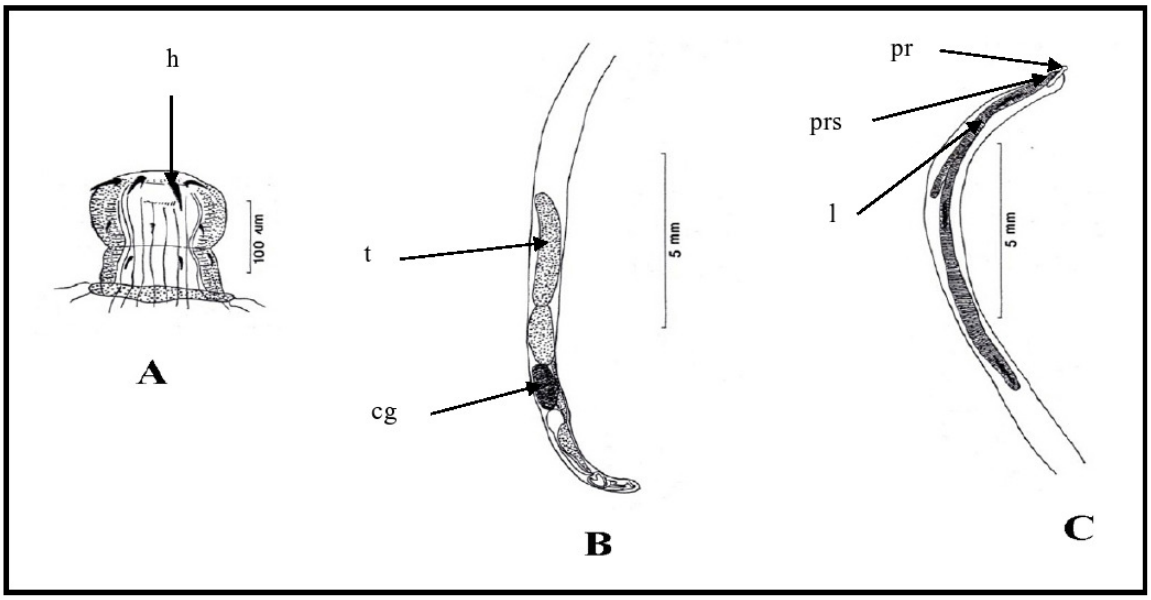

Figure 4.11 ***Neoechinorhynchus iraqensis***
A- Camera lucida drawing of proboscis worm
B- Camera lucida drawing of male worm
C- Camera lucida drawing of female worm
cg= cement gland; h= hook; l= lemniscus; prs= proboscis sac;
pr= proboscis; t= testis

Neoechinorhynchus iraqensis was identified for the first time in Iraq as a new species by Amin *et al.* (2001) from *Liza abu* in the Euphrates River. Next, it was recorded from *L. abu* from different markets at Baghdad by Hasan *et al.* (2009); by Hashim *et al.* (2015) from *Silurus triostegus* and *Liza abu* in higher Zab River in Aski kalak Erbil; by Taha *et al.* (2018) from *Planiliza abu* in Tigris River and by Jassim, (2019) from *Cyprinus carpio* in Tikrit city. Morphometric characteristics of the specimens of *N. iraqensis* described in this study were similar to those reported in the original description by Amin *et al.* (2001).

As shown in the results of the present study, it was noticed that the prevalence of infection with *N. iraqensis* in *P.abu* was low. This result was lower than the reported by Jassim, (2019) recorded *N. iraqensis* in *Cyprinus carpio* from Tikrit city with 1.6%.

4.2.3 Scanning electron microscope (SEM)

In the description of *N. zabensis*, Amin *et al.* (2003a) noted features characteristic of the species that distinguished it from other species of *Neoechinorhynchus* Hamann, 1892 in Stiles and Hassall, 1905. These features are shared by the specimen reported previously which include: proboscis length and width were the same, 2 levels of anterior hooks, middle and posterior hooks of equal length (Fig. 4.12).

Scanning electron microscope examination in this study revealed not different between morphometric characteristics of the specimens of *N. zabensis* described in this study and the original description by Amin *et al.* (2003). In Iraq, there has not been any description by Scanning electron microscope for *Neoechinorhynchus zabensis*, while Taha *et al.* (2018) studied *Neoechinorhynchus iraqensis*.

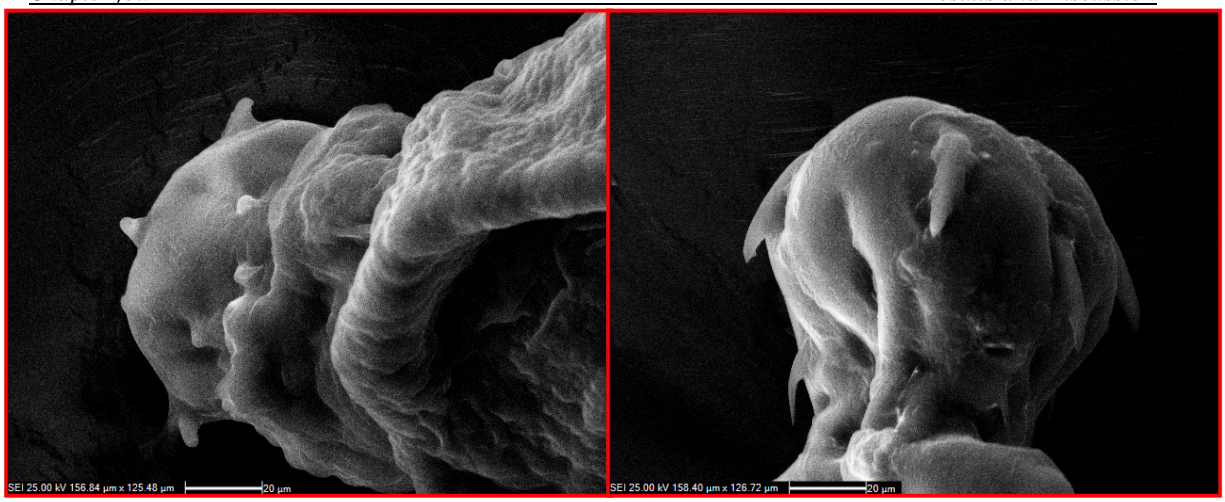

Figure 4.12 SEM of *Neoechinorhynchus zabensis* specimen from *Capoeta trutta*

4.2.4 Genetic Determination

The PCR product of 18S rDNA was 622 bp for *Neoechinorhynchus zabensis* Fig. (4.13). The partial sequencing result of the *N. zaensis* showed in Fig. (4.14). The nucleotide sequences of 18S rDNA after editing the total length is 581pb were analyzed. The nucleotide composition was as follows: 25.99% T, 19.44% C, 27.54% A and 27.02% G are shown in Table (4.5).

The genetic distance of *Neoechinorhynchus zabensis* was compared with sequences of the closely related species of the same genus, *Neoechinorhynchus*, and other acanthocephalans retrieved from GenBank Table (4.6). The genetic distance estimated among *Neoechinorhynchus zabensis* and other species of *Neoechinorhynchus* ranged from 0.08 to 0.14.

Fig. (4.15) show the phylogenetic tree relationship of *Neoechinorhynchus zabensis* with other *Neoechinorhynchus* species recorded in GenBank based on 18S rDNA.

Table 4.5 Nucleotide compositions of *Neoechinorhynchus_zabensis* and some *Neoechinorhynchus* sp. recorded in NCBI GenBank.

***Neoechinorhynchus* sp.**	**Nucleotide compositions**				**Total**
	T(U	**C**	**A**	**G**	
Neoechinorhynchus_zabensis	25.99	19.45	27.54	27.02	581
Neoechinorhynchus_sp.	25.83	20.05	27.25	26.85	1765
Neoechinorhynchus_crassus	26.76	19.54	27.15	26.53	1760
Neoechinorhynchus_pseudemydis	26.44	19.37	27.62	26.55	1770
Neoechinorhynchus_sp.	25.95	19.9	27.55	26.55	998
Neoechinorhynchus_saginata	25.84	19.54	27.90	26.70	1745
Neoechinorhynchus_crassus	26.62	19.51	27.24	26.62	1773
Hebesoma_violentum	25.77	20.19	25.88	28.14	931
Neoechinorhynchus_beringianus	26.31	19.26	26.31	28.10	893
Neoechinorhynchus_tumidus_isolate	25.78	19.53	26.67	28.01	896
Neoechinorhynchus_salmonis_isolate	26.37	19.26	26.48	27.87	929
Neoechinorhynchus_cylindratus	25.91	19.98	26.84	27.24	1501
Neoechinorhynchus_simansularis	26.45	19.13	26.66	27.74	930
Neoechinorhynchus_buttnerae	25.88	19.85	27.58	26.67	1773
Neoechinorhynchus_yamagutii	27.62	18.81	27.62	25.93	590
Neoechinorhynchus_yamagutii	27.48	19.05	27.48	25.96	593
Neoechinorhynchus_sp.	26.54	19.61	26.81	27.02	1861
Neoechinorhynchus_dimorphospinus	25.82	19.96	26.89	27.31	1673
Neoechinorhynchus_personatus	26.84	18.79	28.35	26.00	596
Neoechinorhynchus_agilis	26.66	18.86	28.94	25.52	615
Neoechinorhynchus_agilis	25.90	18.82	28.76	26.50	664
Neoechinorhynchus_agilis	26.26	18.74	28.72	26.26	651
Neoechinorhynchus_agilis	26.26	18.74	28.87	26.11	651
Overall mean	26.25	19.52	27.32	26.89	1136.47

Table 4.6 Pairwise distance of *N. zabensis* with some *Neoechinorhynchus* recorded in GenBank.

N. zabensis																						
N. sp.	0.12																					
N. crassus	0.12	0.04																				
N. pseudemydis	0.12	0.04	0.02																			
N. sp.	0.14	0.11	0.11	0.11																		
N. saginata	0.11	0.04	0.02	0.01	0.11																	
N. crassus	0.12	0.04	0.00	0.02	0.11	0.02																
H. violentum	0.12	0.04	0.04	0.04	0.09	0.05	0.04															
N. beringianus	0.11	0.04	0.02	0.01	0.10	0.01	0.02	0.04														
N. tumidus	0.11	0.04	0.01	0.01	0.09	0.00	0.01	0.04	0.00													
N. salmonis	0.11	0.04	0.01	0.01	0.09	0.01	0.01	0.04	0.00	0.00												
N. cylindratus	0.11	0.03	0.01	0.01	0.10	0.01	0.01	0.04	0.00	0.00	0.00											
N. simansularis	0.10	0.04	0.01	0.01	0.09	0.00	0.01	0.04	0.00	0.00	0.00	0.00										
N. buttnerae	0.10	0.03	0.03	0.03	0.10	0.04	0.03	0.03	0.04	0.04	0.04	0.03	0.04									
N. yamagutii	0.09	0.10	0.10	0.10	0.14	0.10	0.10	0.12	0.10	0.10	0.10	0.09	0.10	0.09								
N. yamagutii	0.09	0.10	0.10	0.10	0.14	0.10	0.10	0.12	0.10	0.10	0.10	0.09	0.10	0.09	0.00							
N. sp.	0.10	0.07	0.06	0.06	0.11	0.07	0.06	0.08	0.07	0.07	0.07	0.06	0.07	0.06	0.02	0.02						
N. dimorphospinus	0.11	0.07	0.07	0.06	0.10	0.07	0.07	0.08	0.08	0.08	0.08	0.06	0.07	0.06	0.04	0.04	0.03					
N. personatus	0.08	0.11	0.11	0.10	0.15	0.10	0.10	0.12	0.11	0.11	0.11	0.10	0.11	0.10	0.04	0.04	0.04	0.06				
N. agilis	0.08	0.10	0.11	0.10	0.13	0.10	0.11	0.12	0.11	0.11	0.11	0.10	0.10	0.10	0.04	0.05	0.04	0.06	0.01			
N. agilis	0.09	0.10	0.10	0.10	0.12	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.05	0.05	0.04	0.06	0.01	0.00		
N. agilis	0.09	0.10	0.10	0.10	0.12	0.10	0.10	0.11	0.10	0.10	0.10	0.09	0.10	0.09	0.04	0.04	0.04	0.06	0.01	0.00	0.00	
N. agilis	0.09	0.10	0.10	0.10	0.13	0.10	0.10	0.11	0.10	0.10	0.10	0.09	0.10	0.09	0.04	0.04	0.04	0.06	0.01	0.00	0.00	0.00

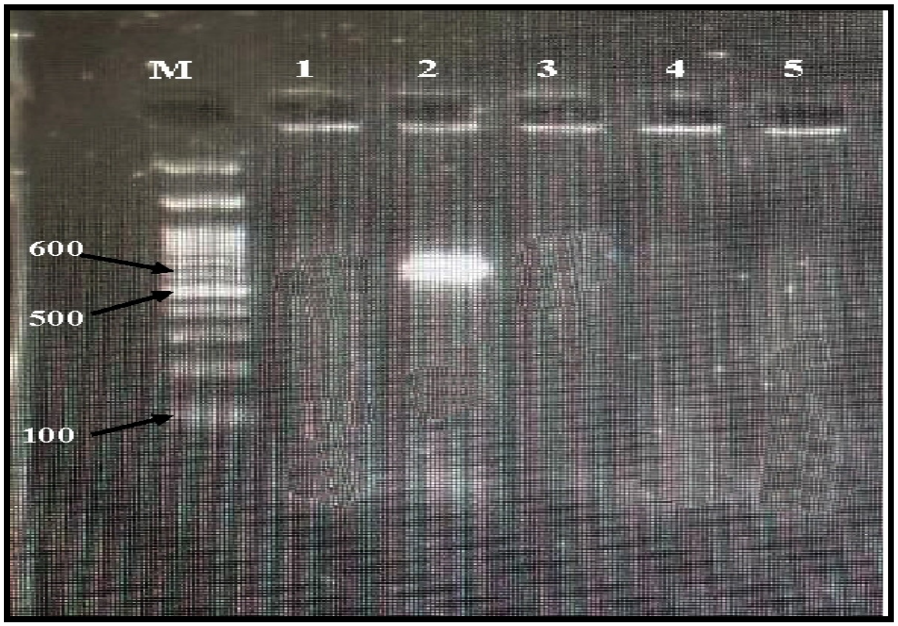

Figure 4.13 PCR amplification of *Neoechinorhynchus zabensis* in *Capoeta trutta* fish species. Lane M= DNA ladder 100 bp, lane 2= amplified nuclear 18S rDNA gene product size 622 bp.

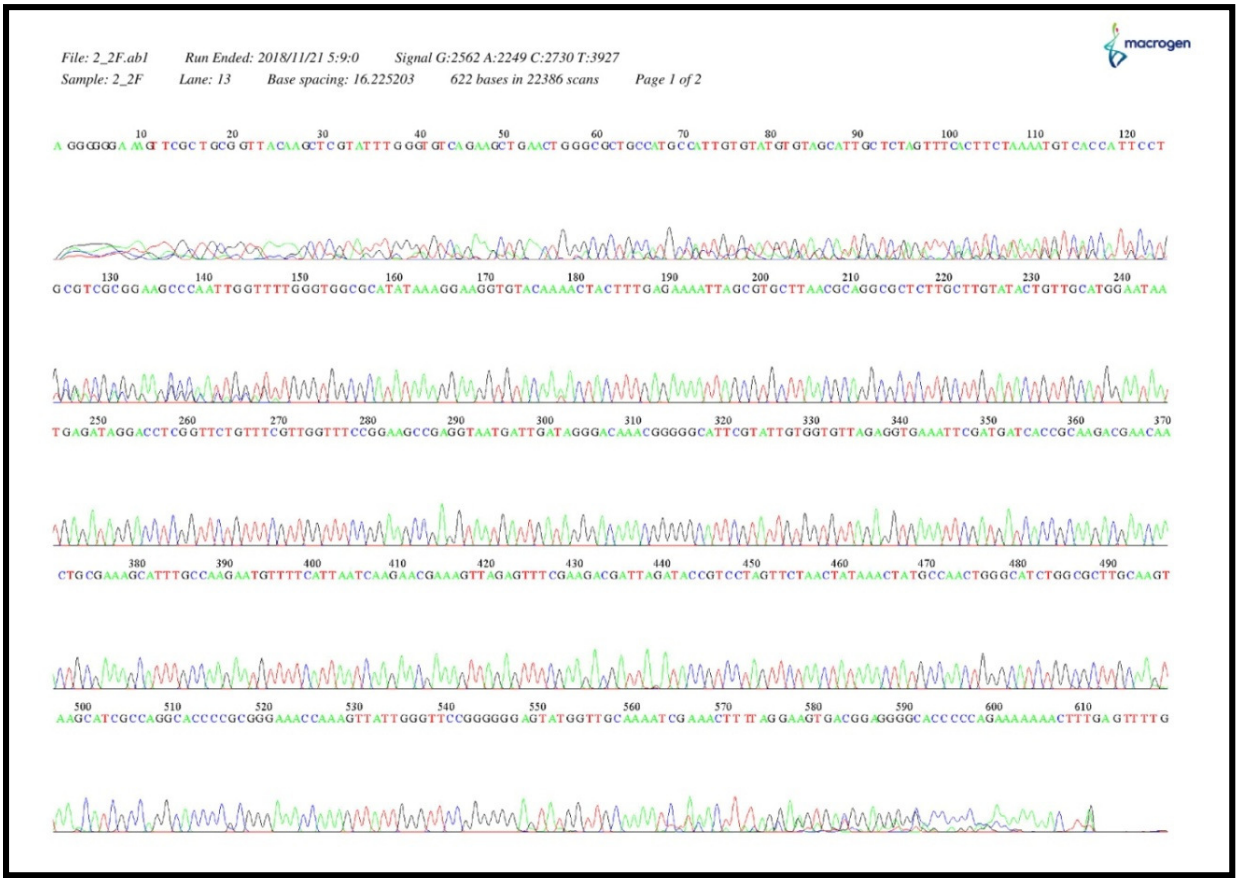

Figure 4.14 The partial sequencing result of 18S rDNA of *Neoechinorhynchus zabensis*

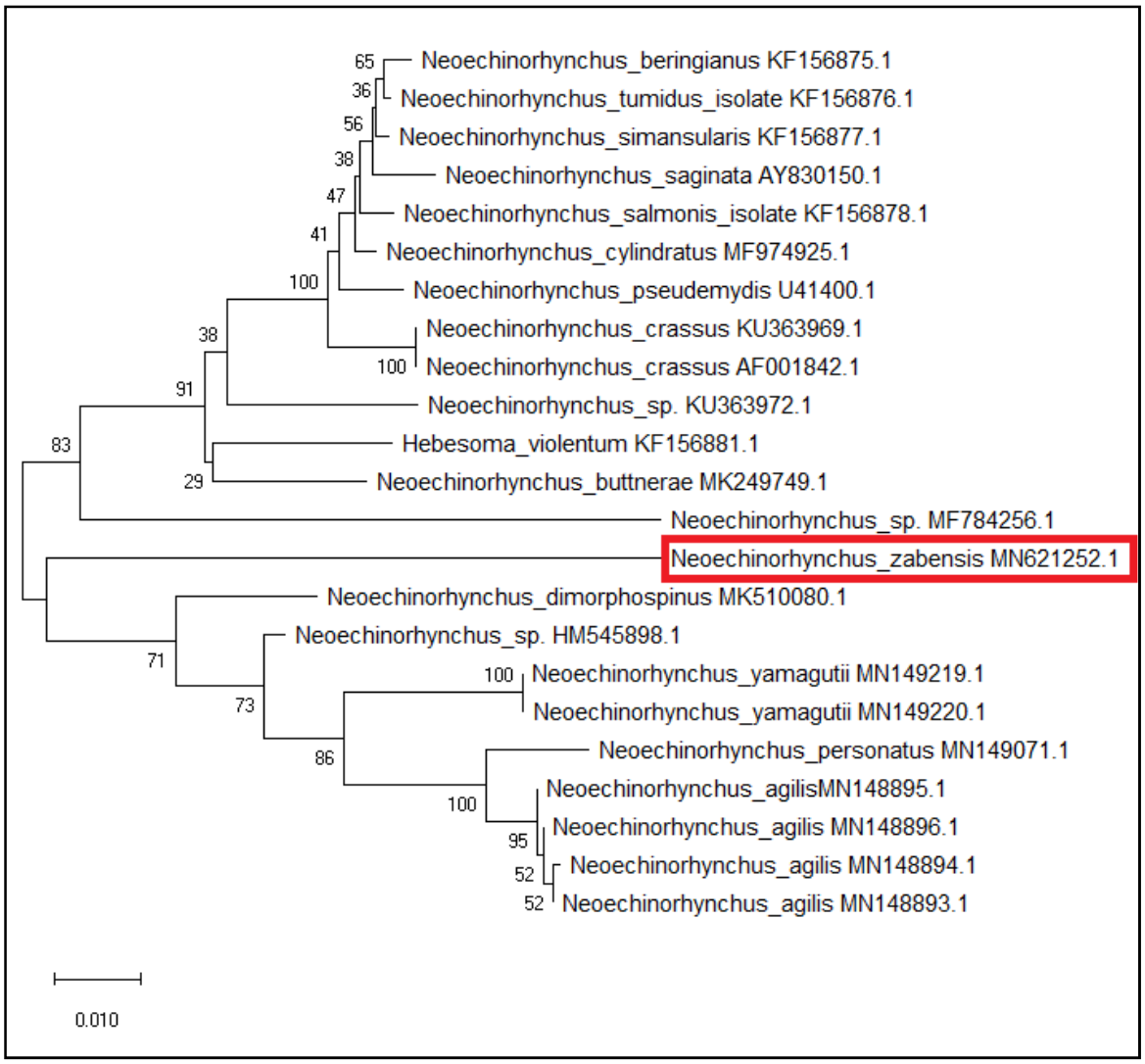

Figure 4.15 Phylogenetic analysis of the 18S rDNA region of *Neoechinorhynchus zabensis* using the Neighbor-joining tree method. Numbers at nodes indicate ML bootstrap values (1000 replications) with GenBank accession numbers listed alongside the species names.

PCR reaction was successfully amplified by sequencing of 18S rDNA. The lowest genetic distance recorded between *Neoechinorhynchus zabensis* and *Neoechinorhynchus agilis* (MN148894.1) is 0.08 and the highest genetic distance recorded between *Neoechinorhynchus zabensis* and *Neoechinorhynchus* sp. (MF784256.1) is 0.14. The alligments sequence showed 95.28% identity with *Neoechinorhynchus buttnerae* under accession number (MK249749) in the GenBank database Appendixes (1-11). Phylogenetic tree analysis demonstrated that *N. zabensis* occupies a separate position in the trees.

In Iraq, there have not been any studies on molecular characterization for this parasite to classify it. It was only classified on morphological characterization by (Amin *et al.*, 2003 and Abdullah,

2013). However one study was done by Hassan *et al.* (2016) who studied *N. zabensis* using 5.8S rDNA. In this study the region 18s rDNA was duplicated which is an important marker.

Phylogenetic analysis was undertaken here to resolve the relationship between *Neoechinorhynchus zabensis* and other species from GenBank, and this can be recognized using 18S rDNA molecular marker. A few species of the genus *Neoechinorhynchus* were analyzed using molecular data by García-Varela and Pinacho-Pinacho (2018). To compare this species with others that has been recorded in NCBI GenBank on genus basis. Previous studies of the genus *Neoechinorhynchus* for cox 1 ranged from 0.23 to 2.06% in *N. mamesi* and from 0.23 to 3.21% in *N. brentnickoli* (Pinacho-Pinacho *et al.*, 2012). The genetic divergence estimated among the *Neoechinorhynchus* species ranged from 0 to 2.5% for the 18S rRNA gene (Malyarchuk *et al*., 2014).The result of this study agreement with Pinacho-Pinacho *et al*. (2014, 2015, 2017, and 2018) the genetic divergences within *Neoechinorhynchus*, ranging between 2.3 and 46.8% for LSU, 4.7 and 61.9% for ITS and 9.7 and 59.2% for cox 1 and it differ from the genetic divergences between *N. (N) cylindratus*, *N. (N) panucensis* and *N. (N) emyditoides* which was higher (García-Varela and Pinacho-Pinacho, 2018).

The result of the present study indicated that this species occupies a separate position in the trees. The accession number MN621252 (GenBank) was taken for this parasite *N. zabensis* and for the first time is registered in the NCBI GenBank.

4.2.5 Some environmental factor affecting the parasites infection in fish

Before discussing the effect of some environmental factors on parasitic infection in fish, is worthwhile, to mention that these factors were studied only in *C. trutta* which was selected with *N. zabensis* repeated for most of the months and with suitable prevalence and intensity.

4.2.5.1The effect of host sex on parasitic infection

Male and female of *C. trutta* did not show any statistically significant difference in their infection rate with *N. zabensis* t- value for prevalence, mean intensity and abundance were about t observation = 1.247, t = 0.921 and t = 1.117 respectively at (p value > alpha 0.05) (Table 4.7). For this reason, data for both sexes were pooled for further analysis.

As indicated from the results of the present study, non-significant differences were noted in the infection rate of *C. trutta* male and female with *N. zabensis*. The present result agrees with Kennedy (1975) who stated that many research did not found any differences in the infection rate

for both sexes of fishes with most parasites. However, the same author gave some examples on the presence of such differences.

There is many research who confirmed the effect of sex on the infection rates such as Khamees and Mhaisen (1988) who noted *N. agilis* which was later identified by (Mhaisen, 2002) as *N. iraqensis* in *Liza abu* from Mehaijeran creek, in Basrah city, by Ali (1989) on *N.rutili* of *B. esocinus* in Greater Zab River, by Abdullah (2002); Abdullah and Mhaisen (2007) on *Pomphorhynchus spindletruncats* of *B. xanthopterus* in Lesser Zab River and Aydoğdu *et al.* (2015) on *Neoechinorhynchus agilis* of *Chelon labrosus* in Beymelek Lagoon Lake in Antalya, Turkey.

The similarity of the food and feeding habits of male and female fishes and their occupancy of the same habitat which was explained by (Dogiel, 1961) may give evidence on the absence of any differences in the parasitic fauna acquired with food of both sexes. Living in the same habitat with the absence of morphological differences between both sexes of fish's provides evidence on the similarity of the infection rate with parasites. However, some authors gave examples on the presence of such infection rate differences between males and females (Amin, 1984; Measures, 1988; Abdullah and Ali, 1999; Koyun, 2012) due to the feeding behavior and morphological differences between both sexes which make one gender more a predator than the other.

Table 4.7 The prevalence, mean intensity and abundance of *N. zabensis* in the male and female infection of *C. trutta* from Dukan Lake.

Fish sex	No. of fish Examined	No. of fish Infected	Prevalence ± SD.	No. of parasites	Mean intensity ± SD.	Abundance ± SD.
♂	210	184	87.61±1.643	670	3.64±0.837	3.19±0.733
♀	190	164	86.31±3.202	550	3.35±0.709	2.89±0.590
Both	400	348	86.96	610	3.49	3.04
t (Observed value)			1.247		0.921	1.117
t (Critical value)			2.074		2.074	2.074

Prevalence: p-value (Two- tailed) 0.225 > alpha 0.05

Mean intensity: p-value (Two- tailed) 0.367 > alpha 0.05

Abundance: p-value (Two- tailed) 0.276 > alpha 0.05

4.2.5.2 Effect of host length (age) on parasitic infection

The present results generally showed that the infection with *N. zabensis* parasites occurred in all length groups of *C. trutta*, but it increased by increasing fish length. The statistical analysis showed significant differences (p value < alpha 0.05) in prevalence, mean intensity and abundance of infection rate between different length groups of the fishes. The prevalence in various size groups of *C. trutta* ranged between 68.96-97.26% (Table 4.8). The present results showed that the infection with *N. zabensis* in *C. trutta* from Dokan Lake increased by increasing fish length. The prevalence, mean intensity and abundance of infection was high 97.26±6.537, 4.1±0.721and 4.06±0.599 respectively in the largest fish group (30-33.5 cm).

The increase in the infection rate with *N. zabensis* accompanied with the increase in fish length can be attributed to the accumulation of the infective stages consumed with the intermediate host, as these parasites attach to the intestine of fish by their proboscis and stay there.

The present study results agrees with the results recorded by numerous studies in Iraq about *N. rutili* infection in *B. esocinus* (Abdullah and Ali, 1999), for *N. iraqensis* infection in *Liza abu* (Al-Sady, 2000), for *P. spindletruncats* in *B. xanthopterus* infection in Lesser Zab River (Abdullah, 2002; Abdullah and Mhaisen, 2007) and for *N. zabensis* from *C. damascina* and *C. trutta* in Dokan Lake and Greater zab River (Abdullah, 2009).

The result of the present study also agrees with the results obtained for *N. rutili* from *B. barbus* in Jihlava River, Czech Republic (Moravec and Scholz, 1994), for *Acanthocephalus lucii* in *Tincatinca* from Lake Uluabat, Turkey (Öztürk, 2002), for *N. zabensis* in Murat River, Turkey (Koyun, 2012) and for *N. agilis* in *Chelon labrosus* from Beymelek Lagoon Lake in Antalya, Turkey Aydoğdu *et al.* (2015). Amin (1985) referred to an increase in parasitic abundance with the host age (size) which may result from a relatively stable host feeding behavior. Generally, to put the above findings into perspective, four patterns of parasite abundance *versus* host age are recognized: (1) abundance increasing with age, (2) abundance independent of age, (3) abundance maximal in middle age and (4) abundance decreasing with age (Dogiel, 1961).

Table 4.8 The prevalence, mean intensity and abundance of different age's groups as reflected by length of *Capoeta trutta* with *Neoechinorhynchus zabensis* from Dukan Lake.

Fish length group (cm)	No. of fish Examined	No. of fish Infected	Prevalence (%) ± SD.	No. of parasites	Mean intensity (range) ± SD.	Abundance ± SD.
18-21.5	87	60	68.96±13.755	155	2.5±0.161	1.78±0.415
22-25.5	122	107	87.70±2.903	342	3.1±0.281	2.80±0.237
26-29.5	118	110	93.22±5.266	426	3.8±0.597	3.60±0.520
30-33.5	73	71	97.26±6.537	297	4.1±0.721	4.06±0.599
k (Observed value)			32.569		34.226	39.086
k (Critical value)			7.815		7.815	7.815

p-value (Two- tailed) 0.0001 < alpha 0.05

4.2.5.3 Effect of seasonal variation on parasitic infection

A survey of prevalence, mean intensity and abundance of *N. zabensis* infection in *C. trutta* in individual months is given in Table (4.9). It is apparent that this parasite occurred in this fish throughout the year, with a prevalence of 66.66-95.5%, mean intensity (2.9-4) and abundance of infection (1.9-3.8) in Dukan Lake. The infection rate of *C. trutta* with *N. zabensis* showed a significant difference (p value < alpha 0.05). The mean intensity was highest during August about 4 and lowest during October was 2.9 (Table 4.8). The prevalence of infection was high in August about 95.5 and low in October was 66.66. The abundance of infection was high during August about 3.8 and low during October was 1.9.

The increase in infection rate by *N. zabensis* during spring and summer seasons can be attributed to the abundance of a large number of intermediate hosts in the water and the increase in the feeding activity of the fishes (Ginetsinskaya, 1961). The low infection during the autumn and winter can be attributed to both lower feeding activity of the fishes and the rarity of larval stages that infect fishes as well as lower number of intermediate hosts (Moravec *et al.*, 1997). Similar fluctuations in prevalence were noted in case of *N. agilis* (= *N. iraqensis*) from *L. abu* in Mehaijeran creek, in Basrahcity (Khamees and Mhaisen, 1988), *N. rutili* from *Barbusesocinus* in Dokan lake (Abdullah and Ali, 1999), *P. spindletruncats* from *B. xanthopterus* in Lesser Zab

River (Abdullah, 2002; Abdullah and Mhaisen,2007), *N. zabensis* from *C. damascina* and *C. trutta* in Dokan Lake and Greater Zab River (Abdullah, 2009) and *N. zbensis* from *Capoeta umbla* in Murat River, Turkey Koyun (2012).

The findings of this study also confirm the suggestion of Granath and Esch (1983) that the seasonal changes abundance of fish parasites are affected by various factors such as temperature and food consumption. The high infection rate with *N. zabensis* in *C. trutta* from Dukan Lake can be attributed to the reason that Dukan Lake has a closed ecosystem which leads to the accumulation of intermediate hosts containing parasite life stages, which results at the end spreading of these intermediate hosts in wider areas. Amin (1986a; 1986b) demonstrated that fishes in closed systems are affected by few of parasites group with a high prevalence. Dogiel (1961) indicated that the relationship between the parasite fauna and the geographical position of the hosts habitat is governed not by a single factor, but by a different factors such as climatic conditions, presence or absence of intermediate hosts, water, the type of the bottom and current velocity, … etc.

Table 4.9 Monthly fluctuations of the prevalence, mean intensity and abundance of *Capoeta trutta* with *Neoechinorhynchus zabensis* from Dukan Lake.

Month	No. of fish Examined	No. of fish Infected	Prevalence (%)	No. of parasites	Mean intensity	Abundance
Jan.	30	26	86.66	84	3.2	2.8
Feb.	32	28	87.5	85	3	3
Mar.	31	28	90.3	91	3.2	2.6
Apr.	36	32	88.88	113	3.5	3.1
May	27	25	85.18	92	3.6	3.4
June	29	26	89.65	100	3.8	3.4
July	33	30	90.9	114	3.8	3.4
Aug.	45	43	95.5	174	4	3.8
Sept.	40	37	92.5	145	3.9	3.6
Oct.	33	22	66.66	65	2.9	1.9
Nov.	34	24	70.58	75	3.1	2.2
Dec.	30	27	90	82	3	2.7
Overall mean	33.33	29	86.19	101.66	3.41	2.99
t (Observed value)			34.422		29.960	18.042
t (Critical value)			1.796		1.796	1.796

p-value (One- tailed) 0.0001 < alpha 0.05

CONCLUSIONS

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

1. During this study two species of fishes belonging to the family Cyprinidae and Mugilidae were identified in Dukan Lake and Sirwan River respectively.
2. DNA sequence analysis revealed and confirmed the validity of the two fish species *Capoeta trutta* and *Planiliza abu*.
3. During this study two species of parasites were recorded *Neoechinorhynchus zabensis* in *Capoeta trutta* in Dukan Lake and *Neoechinorhynchus iraqensis* in *Planiliza abu* in Sirwan River.
4. No significant differences were noticed in the infection rate between males and females of *C. trutta* infected with *N. zabensis*
5. The infection rate *N. zabensis* was higher in longer fishes.
6. Infection of *N. zabensis* in *Capoeta trutta* showed significant monthly fluctuation ($p<0.05$) with high prevalence, mean intensity and abundance during August and lowest during October.
7. The overall prevalence of *N. iraqensis* in *P.abu* was very low (%0.015).
8. Phylogenetic tree analysis demonstrated that the *N. zabensis* occupies a separate position in the trees.
9. The sequence 18S rDNA of *N. zabensis* was referenced in the GenBank under accession number (MN621252).

Recommendations

1. Kurdistan Region is rich with water bodies containing variable fish fauna. Limited studies have been performed on these fishes. Therefore, it is recommended to direct researchers to focus on project of economic importance.
2. Improving fish production by encouraging the establishment of fish farms nearby the rivers which will play a significant role in enhancing the production of high quality proteins in addition it will be a key role in creating job oppertunities among the community.
3. The study of molecular characterazation and the determination of phyllogenetic relationships between fish parasitic fauna in Iraq especially *N. iraqensis* is very important to understand the phyllogenetic tree of each group.
4. Using new techniques such as electron microscope and molecular to do more studies on Iraqi fish parasite fauna.

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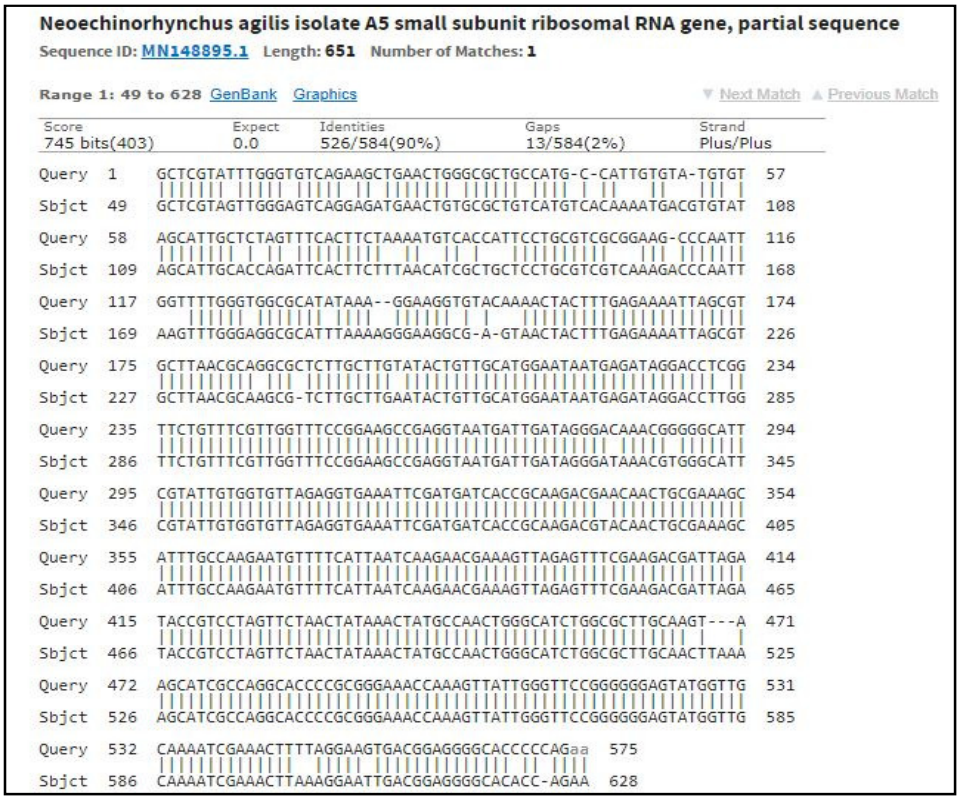

Neoechinorhynchus agilis isolate A5 small subunit ribosomal RNA gene, partial sequence

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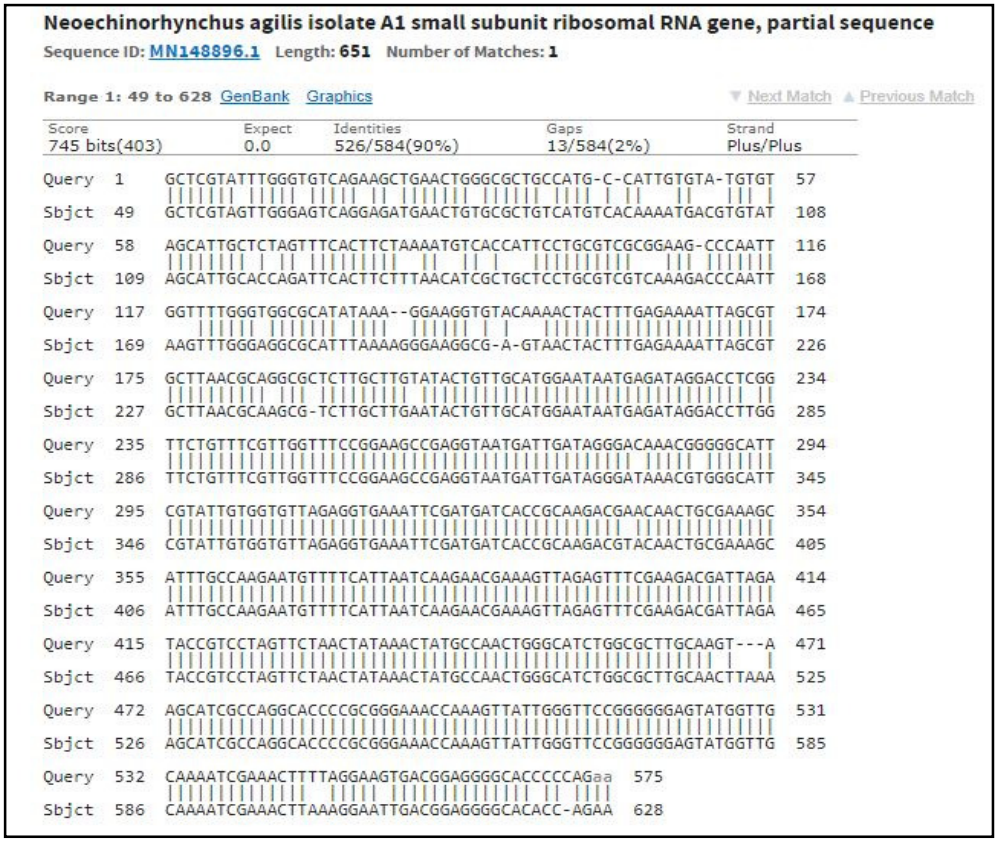

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Sbjct  227  GCTTAACGCAAGCG-TCTTGCTTGAATACTGTTGCATGGAATAATGAGATAGGACCTTGG  285

Query  235  TTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGACAAACGGGGGCATT  294
            |||||||||||||||||||||||||||||||||||||||||||||||||| |||||| |||||||
Sbjct  286  TTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGATAAACGTGGGCATT  345

Query  295  CGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGAACAACTGCGAAAGC  354
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  346  CGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGTACAACTGCGAAAGC  405

Query  355  ATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGA  414
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406  ATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGA  465

Query  415  TACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAAGT---A  471
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||| |   |
Sbjct  466  TACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAACTTAAA  525

Query  472  AGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGGAGTATGGTTG  531
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526  AGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGGAGTATGGTTG  585

Query  532  CAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCAGaa  575
            ||||||||||||||  ||||| ||||||||||||||| || ||||
Sbjct  586  CAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-AGAA  628
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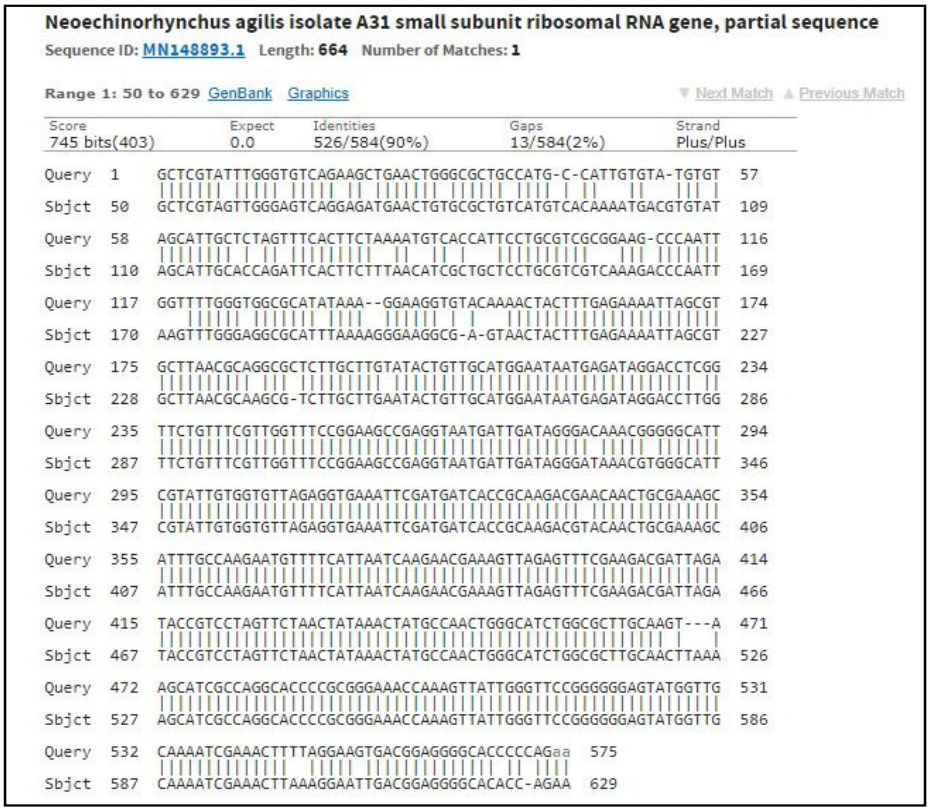
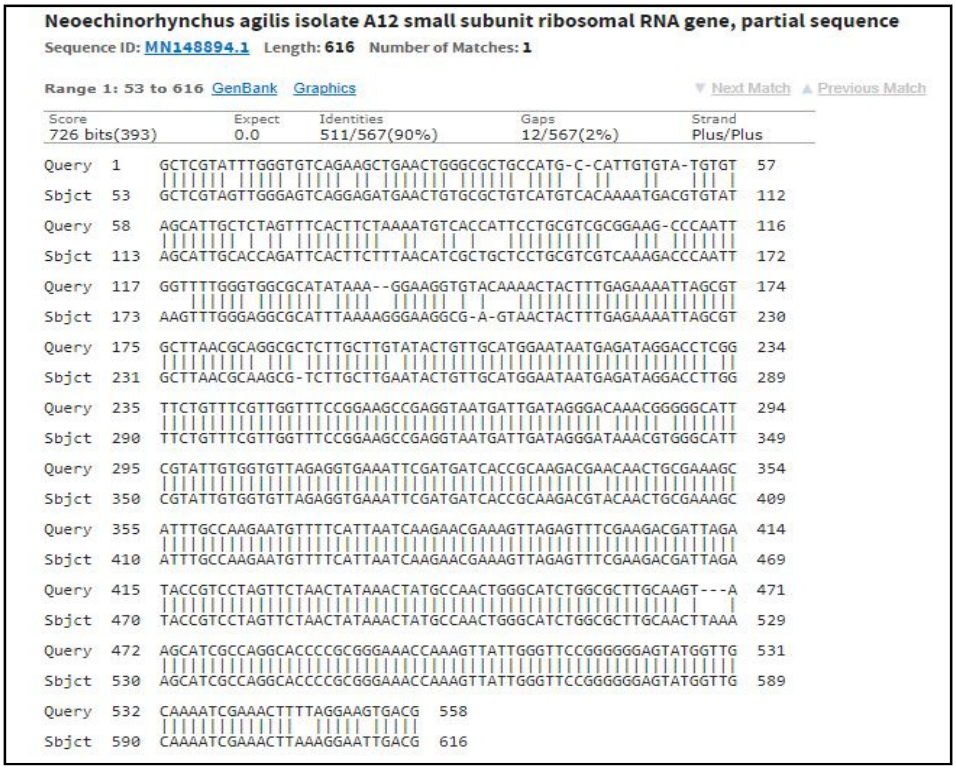
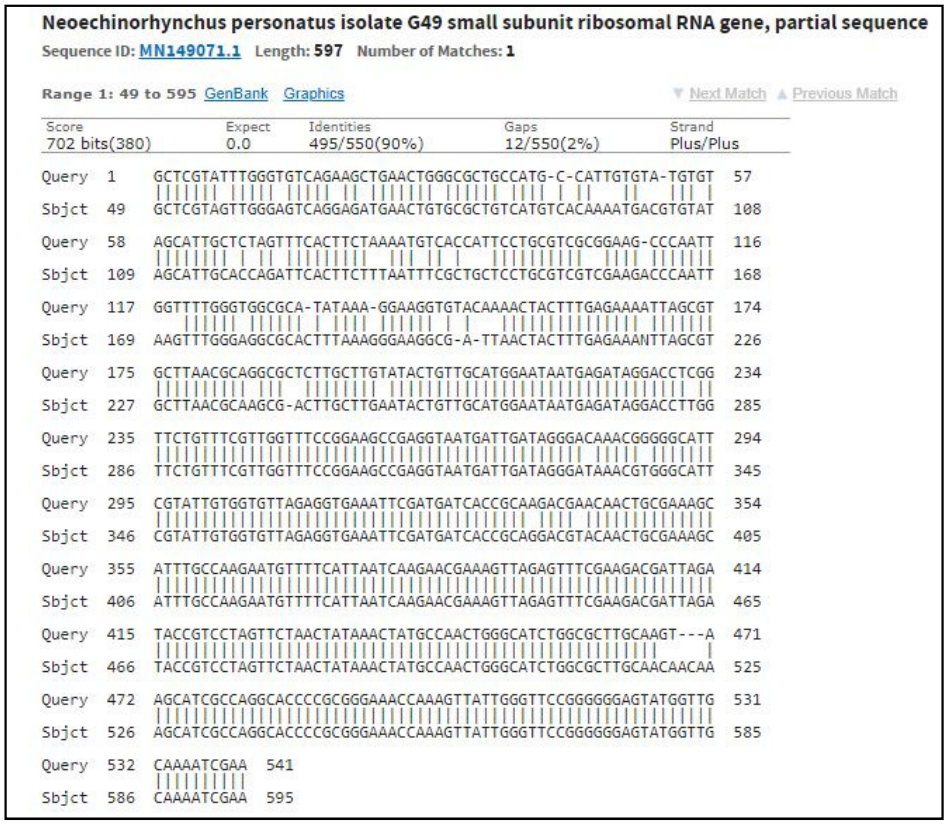
Appendix 1 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. agilis* MN148895.1 and MN148895.1.





Appendix 2 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. agilis* MN148893.1 and MN148894.1.



Neoechinorhynchus personatus isolate G49 small subunit ribosomal RNA gene, partial sequence

Sequence ID: MN149071.1 Length: 597 Number of Matches: 1

Range 1: 49 to 595 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
702 bits(380)	0.0	495/550(90%)	12/550(2%)	Plus/Plus

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Query  1    GCTCGTATTTGGGTGTCAGAAGCTGAACTGGGCGCTGCCATG-C-CATTGTGTA-TGTGT  57
            ||||||| ||||| ||||| || ||||||| |||||| |||| | ||   ||   ||| |
Sbjct  49   GCTCGTAGTTGGGAGTCAGGAGATGAACTGTGCGCTGTCATGTCACAAAATGACGTGTAT  108

Query  58   AGCATTGCTCTAGTTTCACTTCTAAAATGTCACCATTCCTGCGTCGCGGAAG-CCCAATT  116
            |||||||| | || |||||||||  ||| || |   ||||||||||  |||| |||||||
Sbjct  109  AGCATTGCACCAGATTCACTTCTTTAATTTCGCTGCTCCTGCGTCGTCGAAGACCCAATT  168

Query  117  GGTTTTGGGTGGCGCA-TATAAA-GGAAGGTGTACAAAACTACTTTGAGAAAATTAGCGT  174
             |||||| |||||| | |||| |||||| | |   |||||||||||||| |||||||
Sbjct  169  AAGTTTGGGAGGCGCACTTTAAAGGGAAGGCG-A-TTAACTACTTTGAGAAAATTAGCGT  226

Query  175  GCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATGGAATAATGAGATAGGACCTCGG  234
            |||||||||| |||  |||||||| ||||||||||||||||||||||||||||||||| ||
Sbjct  227  GCTTAACGCAAGCG-ACTTGCTTGAATACTGTTGCATGGAATAATGAGATAGGACCTTGG  285

Query  235  TTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGACAAACGGGGGCATT  294
            ||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  286  TTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGATAAACGTGGGCATT  345

Query  295  CGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGAACAACTGCGAAAGC  354
            |||||||||||||||||||||||||||||||||||||||| |||| ||||||||||||||
Sbjct  346  CGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAGGACGTACAACTGCGAAAGC  405

Query  355  ATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGA  414
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406  ATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGA  465

Query  415  TACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAAGT---A  471
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||     |
Sbjct  466  TACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAACAACAA  525

Query  472  AGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTG  531
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526  AGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTG  585

Query  532  CAAAATCGAA  541
            ||||||||||
Sbjct  586  CAAAATCGAA  595
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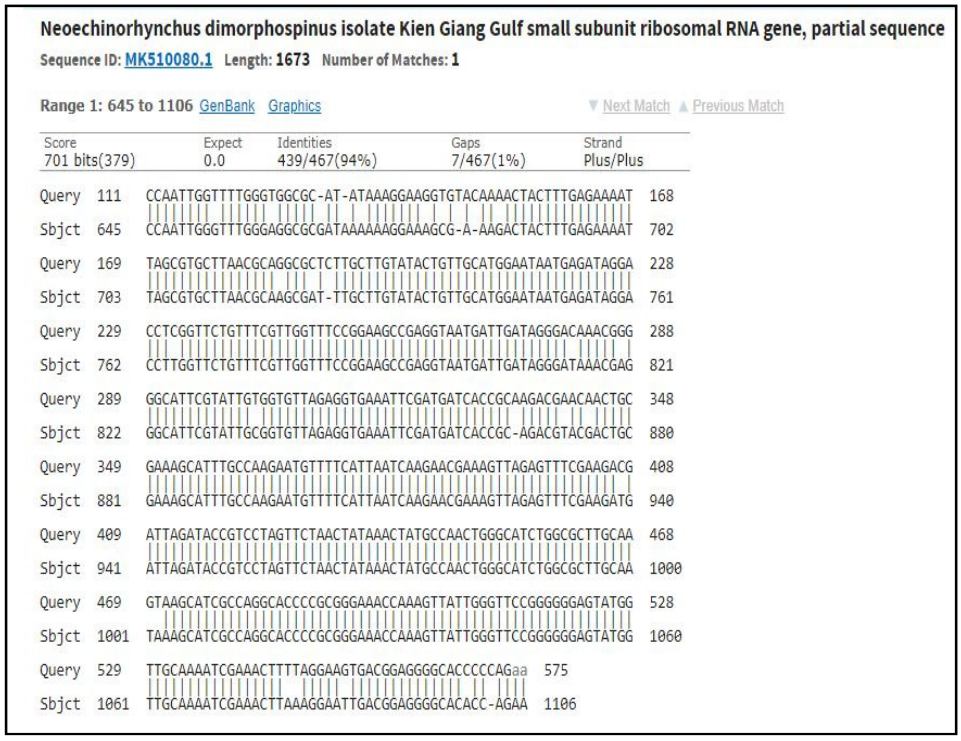

Neoechinorhynchus dimorphospinus isolate Kien Giang Gulf small subunit ribosomal RNA gene, partial sequence

Sequence ID: MK510080.1 Length: 1673 Number of Matches: 1

Range 1: 645 to 1106 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
701 bits(379)	0.0	439/467(94%)	7/467(1%)	Plus/Plus

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Query  111  CCAATTGGTTTTGGGTGGCGC-AT-ATAAAGGAAGGTGTACAAAACTACTTTGAGAAAAT  168
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Sbjct  645  CCAATTGGGTTTGGGAGGCGCGATAAAAAAGGAAAGCG-A-AAGACTACTTTGAGAAAAT  702

Query  169  TAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATGGAATAATGAGATAGGA  228
            |||||||||||||| ||| | ||||||||||||||||||||||||||||||||||||||
Sbjct  703  TAGCGTGCTTAACGCAAGCGAT-TTGCTTGTATACTGTTGCATGGAATAATGAGATAGGA  761

Query  229  CCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGACAAACGGG  288
            ||| |||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  762  CCTTGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGATAAACGAG  821

Query  289  GGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGAACAACTGC  348
            |||||||||||| |||||||||||||||||||||||||||| |||| || |||||
Sbjct  822  GGCATTCGTATTGCGGTGTTAGAGGTGAAATTCGATGATCACCGC-AGACGTACGACTGC  880

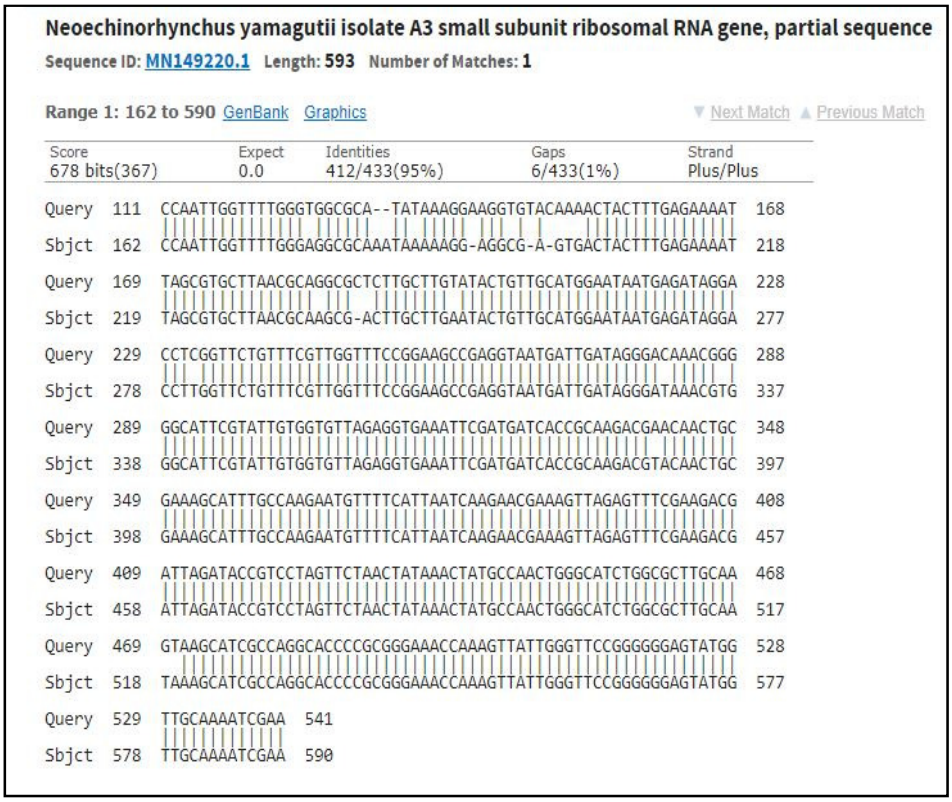
Query  349  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACG  408
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  881  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGATG  940

Query  409  ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  468
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  941  ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  1000

Query  469  GTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  528
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1001 TAAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  1060

Query  529  TTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCAGaa  575
            |||||||||||||||  ||||| |||||||||||||| || ||||
Sbjct  1061 TTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-AGAA  1106
```

Appendix 3 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. personatus* MN149071.1 and *N. dimorphospinus* MN510080.1.



Neoechinorhynchus yamagutii isolate A3 small subunit ribosomal RNA gene, partial sequence

Sequence ID: MN149220.1 Length: 593 Number of Matches: 1

Range 1: 162 to 590 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
678 bits(367)	0.0	412/433(95%)	6/433(1%)	Plus/Plus

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Query  111  CCAATTGGTTTTGGGTGGCGCA--TATAAAGGAAGGTGTACAAAACTACTTTGAGAAAAT  168
            ||||||||||||||| ||||||  || |||||  ||| | |    ||||||||||||||||
Sbjct  162  CCAATTGGTTTTGGGAGGCGCAAATAAAAAGG-AGGCG-A-GTGACTACTTTGAGAAAAT  218

Query  169  TAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATGGAATAATGAGATAGGA  228
            ||||||||||||||| |||  |||||||| ||||||||||||||||||||||||||||||
Sbjct  219  TAGCGTGCTTAACGCAAGCG-ACTTGCTTGAATACTGTTGCATGGAATAATGAGATAGGA  277

Query  229  CCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGACAAACGGG  288
            ||| |||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  278  CCTTGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGATAAACGTG  337

Query  289  GGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGAACAACTGC  348
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  338  GGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGTACAACTGC  397

Query  349  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACG  408
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  398  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACG  457

Query  409  ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  468
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  458  ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  517

Query  469  GTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  528
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  518  TAAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  577

Query  529  TTGCAAAATCGAA  541
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Sbjct  578  TTGCAAAATCGAA  590
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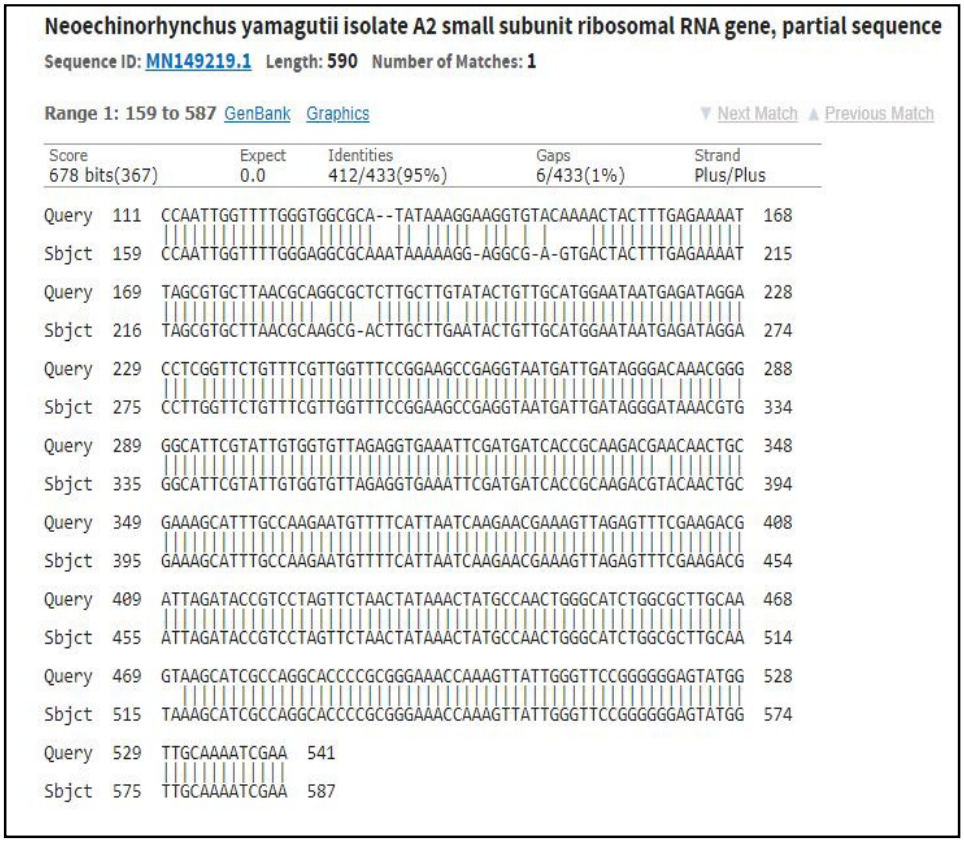

Neoechinorhynchus yamagutii isolate A2 small subunit ribosomal RNA gene, partial sequence

Sequence ID: MN149219.1 Length: 590 Number of Matches: 1

Range 1: 159 to 587 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
678 bits(367)	0.0	412/433(95%)	6/433(1%)	Plus/Plus

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Query  111  CCAATTGGTTTTGGGTGGCGCA--TATAAAGGAAGGTGTACAAAACTACTTTGAGAAAAT  168
            ||||||||||||||| ||||||  || |||||  ||| | |    ||||||||||||||||
Sbjct  159  CCAATTGGTTTTGGGAGGCGCAAATAAAAAGG-AGGCG-A-GTGACTACTTTGAGAAAAT  215

Query  169  TAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATGGAATAATGAGATAGGA  228
            ||||||||||||||| |||  |||||||| ||||||||||||||||||||||||||||||
Sbjct  216  TAGCGTGCTTAACGCAAGCG-ACTTGCTTGAATACTGTTGCATGGAATAATGAGATAGGA  274

Query  229  CCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGACAAACGGG  288
            ||| |||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  275  CCTTGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGATAAACGTG  334

Query  289  GGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGAACAACTGC  348
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  335  GGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGTACAACTGC  394

Query  349  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACG  408
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACG  454

Query  409  ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  468
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Sbjct  455  ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  514

Query  469  GTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  528
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  515  TAAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  574

Query  529  TTGCAAAATCGAA  541
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Sbjct  575  TTGCAAAATCGAA  587
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Appendix 4 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. yamagutii* MN149220.1 and *N. yamagutii* MN149219.1.

Neoechinorhynchus sp. JYW-2010 18S ribosomal RNA gene, complete sequence

Sequence ID: HM545898.1 Length: 1861 Number of Matches: 1

Range 1: 747 to 1208 GenBank Graphics ▼ Next Match ▲

Score	Expect	Identities	Gaps	Strand
695 bits(376)	0.0	438/467(94%)	7/467(1%)	Plus/Plus

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Query  111  CCAATTGGTTTTGGGTGGCGC-A-TATAAAGGAAGGTGTACAAAACTACTTTGAGAAAAT  168
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Sbjct  747  CCAATTGGGTTTGGGAGACGCGATTAGAGAGGAAGGCG-A-GTGACTACTTTGAGAAAAT  804

Query  169  TAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATGGAATAATGAGATAGGA  228
            |||||||||||||||| |||  |||||||| ||||||||||||||||||||||||||||||
Sbjct  805  TAGCGTGCTTAACGCAAGCG-ACTTGCTTGAATACTGTTGCATGGAATAATGAGATAGGA  863

Query  229  CCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGACAAACGGG  288
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||| | |
Sbjct  864  CCTTGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGATAGGGATAAACGTG  923

Query  289  GGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCAAGACGAACAACTGC  348
            |||||||||||||||||||||||||||||||||||||||||||| ||||| ||||||||
Sbjct  924  GGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGC-AGACGTACAACTGC  982

Query  349  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACG  408
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Sbjct  983  GAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACG  1042

Query  409  ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  468
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1043 ATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAA  1102

Query  469  GTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  528
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1103 CAAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGG  1162

Query  529  TTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCAGaa  575
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Sbjct  1163 TTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-AGAA  1208
```

Neoechinorhynchus buttnerae small subunit ribosomal RNA gene, partial sequence

Sequence ID: MK249749.1 Length: 1773 Number of Matches: 1

Range 1: 713 to 1133 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
669 bits(362)	0.0	404/424(95%)	4/424(0%)	Plus/Plus

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Query  153  ACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATG  212
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Sbjct  713  ACTACTTTGAGAAAATTAGCGTGCTCAACGCAGGCAAT-TTGCTTGTATACTGTTGCATG  771

Query  213  GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
            |||||||||| ||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  772  GAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCTGAGGTAATGATT  831

Query  273  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
            |||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  832  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCTGTGATCACCG  891

Query  333  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  392
            |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  892  CAAGACGAACTACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  951

Query  393  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  452
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  952  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  1011

Query  453  CATCTGGCGCTTGCAAGTAA-GCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGG  511
            ||||||||| |||||  ||| ||||||| |||||||| ||||||||||||||||||||||
Sbjct  1012 CATCTGGCGATTGCAT-TAATGCATCGGCAGGCACCCTGCGGGAAACCAAAGTTATTGGG  1070

Query  512  TTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCC  571
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Sbjct  1071 TTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-  1129

Query  572  AGaa  575
            ||||
Sbjct  1130 AGAA  1133
```

Appendix 5 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N.* sp. HM545895.1 and *N. buttnerae* MK249749.1.

Neoechinorhynchus simansularis isolate E1 18S ribosomal RNA gene, partial sequence

Sequence ID: KF156877.1 **Length:** 930 **Number of Matches:** 1

Range 1: 84 to 503 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
656 bits(355)	0.0	401/423(95%)	3/423(0%)	Plus/Plus

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Sbjct  84   ACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCG-AC-TGCTTGTATACTTATGCATG  141

Query  213  GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
            |||||||||| ||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  142  GAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCTGAGGTAATGATT  201

Query  273  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
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Sbjct  202  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCTGTGATCACCG  261

Query  333  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  392
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  321

Query  393  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  452
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  381

Query  453  CATCTGGCGCTTGCAAGTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGT  512
            ||||||||| |||||  || |||||| |||||||| |||||||||||||||||||||||||
Sbjct  382  CATCTGGCGATTGCATTTATGCATCGGCAGGCACCCTGCGGGAAACCAAAGTTATTGGGT  441

Query  513  TCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCA  572
            |||||||||||||||||||||||||||||||||  ||||| ||||||||||||||| || |
Sbjct  442  TCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-A  500

Query  573  Gaa  575
            |||
Sbjct  501  GAA  503
```

Neoechinorhynchus salmonis isolate E4a 18S ribosomal RNA gene, partial sequence

Sequence ID: KF156878.1 **Length:** 929 **Number of Matches:** 1

Range 1: 84 to 503 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
651 bits(352)	0.0	400/423(95%)	3/423(0%)	Plus/Plus

```
Query  153  ACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATG  212
            ||||||||||||||||||||||||| || |||||||  | ||||||||||||  ||||||
Sbjct  84   ACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCG-AC-TGCTTGTATACTTATGCATG  141

Query  213  GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
            |||||||||| ||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  142  GAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCTGAGGTAATGATT  201

Query  273  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
            ||||||||||||||||||||||||||||||||||||||||||||||||||  |||||||||
Sbjct  202  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCTGTGATCACCG  261

Query  333  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  392
            |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262  CAAGACGAACTACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  321

Query  393  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  452
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  381

Query  453  CATCTGGCGCTTGCAAGTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGT  512
            ||||||||| |||||  || |||||| |||||||| |||||||||||||||||||||||||
Sbjct  382  CATCTGGCGATTGCATCTATGCATCGGCAGGCACCCTGCGGGAAACCAAAGTTATTGGGT  441

Query  513  TCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCA  572
            |||||||||||||||||||||||||||||||||  ||||| ||||||||||||||| || |
Sbjct  442  TCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-A  500

Query  573  Gaa  575
            |||
Sbjct  501  GAA  503
```

Appendix 6 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. simansularis* KF156877.1 and *N. salmonis* KF156878.1.

Neoechinorhynchus cylindratus 18S ribosomal RNA gene, partial sequence

Sequence ID: MF974925.1 **Length:** 1501 **Number of Matches:** 1

Range 1: 562 to 981 GenBank Graphics Next Match

Score	Expect	Identities	Gaps	Strand
651 bits(352)	0.0	401/424(95%)	5/424(1%)	Plus/Plus

```
Query  153  ACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATG  212
            ||||||||||||||||||||||||| || ||||||| | ||||||||||||  ||||||
Sbjct  562  ACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCG-AC-TGCTTGTATACTTATGCATG  619

Query  213  GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
            |||||||||| ||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  620  GAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCTGAGGTAATGATT  679

Query  273  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
            ||||||||||||||||||||||||||||||||||||||||||||||||  ||||||||||
Sbjct  680  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCTGTGATCACCG  739

Query  333  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  392
            |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  740  CAAGACGAACTACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  799

Query  393  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  452
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  800  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  859

Query  453  CATCTGGCGCTTGCAAGTAA-GCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGG  511
            ||||||||| |||||  ||| |||||| |||||||| ||||||||||||||||||||||
Sbjct  860  CATCTGGCGATTGCAT-TAATGCATCGGCAGGCACCCTGCGGGAAACCAAAGTTATTGGG  918

Query  512  TTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCC  571
            |||||||||||||||||||||||||||||||||  ||||| |||||||||||||| ||
Sbjct  919  TTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-  977

Query  572  AGaa  575
            ||||
Sbjct  978  AGAA  981
```

Hebesoma violentum isolate I1 18S ribosomal RNA gene, partial sequence

Sequence ID: KF156881.1 **Length:** 931 **Number of Matches:** 1

Range 1: 87 to 505 GenBank Graphics Next Match

Score	Expect	Identities	Gaps	Strand
638 bits(345)	1e-178	396/421(94%)	2/421(0%)	Plus/Plus

```
Query  155  TACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATGGA  214
            |||||||||||||||||||||||| |||||||| | ||||||||||||||||||||||
Sbjct  87   TACTTTGAGAAAATTAGCGTGCTCAACGCAGGCGAT-ATGCTTGTATACTGTTGCATGGA  145

Query  215  ATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATTGA  274
            ||||||||| |||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  146  ATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCTGAGGTAATGATTGA  205

Query  275  TAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCGCA  334
            |||||||||||||||||||||||| |||||||||||||||||| |||||||||| |
Sbjct  206  TAGGGACAAACGGGGGCATTCGTATTGCGGTGTTAGAGGTGAAATTCTGTGATCACCGTA  265

Query  335  AGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTA  394
            |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  266  AGACGAACTACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTA  325

Query  395  GAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCA  454
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  326  GAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGGCA  385

Query  455  TCTGGCGCTTGCAAGTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGTTC  514
            |||||||  ||||      |||||||||||||||  |||||||||||||||||||||||
Sbjct  386  TCTGGCGACTGCACTCGTGCATCGCCAGGCACCCTCCGGGAAACCAAAGTTATTGGGTTC  445

Query  515  CGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCAGa  574
            |||||||||||||||||||||||||||||||  ||||| |||||||||||||| || |||
Sbjct  446  CGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-AGA  504

Query  575  a  575
            |
Sbjct  505  A  505
```

Appendix 7 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. cylindratus* MF974925.1 and *Hebesoma violentum* KF156881.1.

Neoechinorhynchus beringianus isolate B1 18S ribosomal RNA gene, partial sequence

Sequence ID: KF156875.1 **Length:** 893 **Number of Matches:** 1

Range 1: 84 to 503 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
645 bits(349)	0.0	399/423(94%)	3/423(0%)	Plus/Plus

```
Query  153  ACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATG  212
            ||||||||||||||||||||||||||| || |||||||  | ||||||||||||  ||||||
Sbjct  84   ACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCG-AC-TGCTTGTATACTTATGCATG  141

Query  213  GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
            |||||||||| ||||||||||||||||| ||||||||||||||| ||||||||||||
Sbjct  142  GAATAATGAGAAAGGACCTCGGTTCTGTTTTGTTGGTTTCCGGAAGCTGAGGTAATGATT  201

Query  273  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
            ||||||||||||||||||||||||||||||||||||||||||||||||  ||||||| |
Sbjct  202  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCTGTGATCACTG  261

Query  333  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  392
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  321

Query  393  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  452
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  381

Query  453  CATCTGGCGCTTGCAAGTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGT  512
            ||||||||| ||||| || |||||| |||||||| ||||||||||||||||||||||||
Sbjct  382  CATCTGGCGATTGCATTTATGCATCGGCAGGCACCCTGCGGGAAACCAAAGTTATTGGGT  441

Query  513  TCCGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCA  572
            |||||||||||||||||||||||||||||||||  ||||| |||||||||||||| || |
Sbjct  442  TCCGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-A  500

Query  573  Gaa  575
            |||
Sbjct  501  GAA  503
```

Neoechinorhynchus tumidus isolate C1 18S ribosomal RNA gene, partial sequence

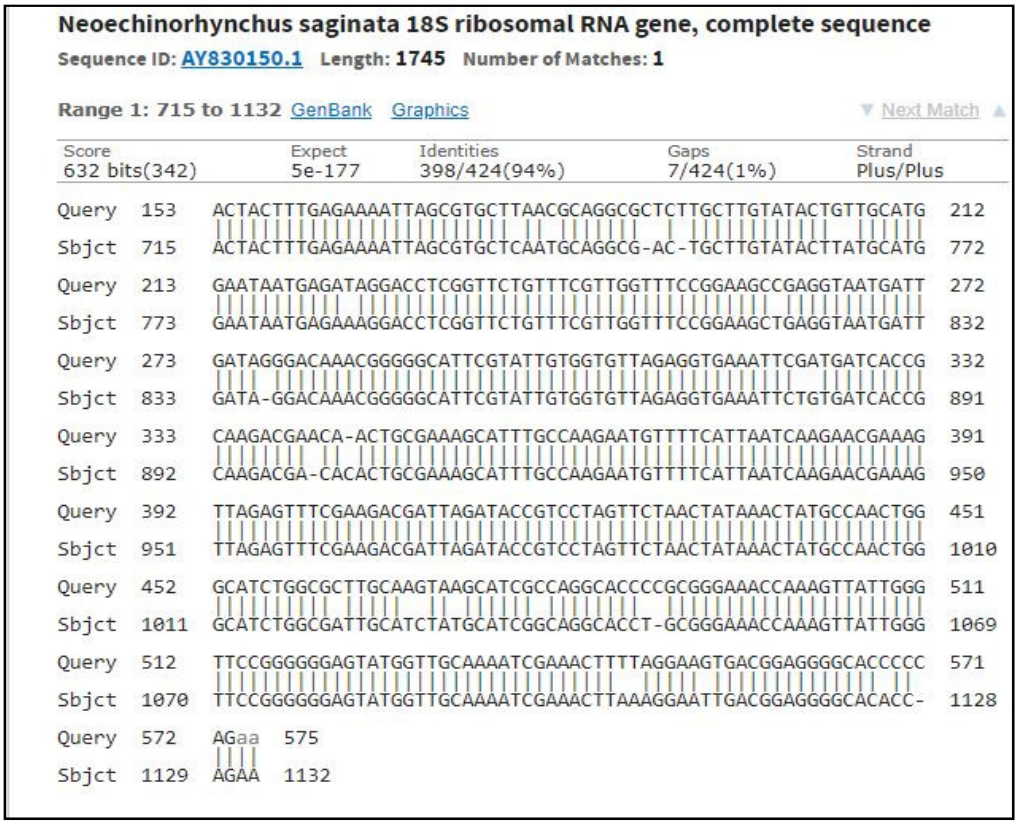
Sequence ID: KF156876.1 **Length:** 896 **Number of Matches:** 1

Range 1: 84 to 503 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
651 bits(352)	0.0	400/423(95%)	3/423(0%)	Plus/Plus

```
Query  153  ACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATG  212
            ||||||||||||||||||||||||||| || |||||||  | ||||||||||||  ||||||
Sbjct  84   ACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCG-AC-TGCTTGTATACTTATGCATG  141

Query  213  GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
            |||||||||| ||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  142  GAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCTGAGGTAATGATT  201

Query  273  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
            ||||||||||||||||||||||||||||||||||||||||||||||||  |||||||||
Sbjct  202  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCTGTGATCACCG  261

Query  333  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  392
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262  CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  321

Query  393  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  452
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322  TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  381

Query  453  CATCTGGCGCTTGCAAGTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGT  512
            ||||||||| |||||   | ||||||| |||||||| ||||||||||||||||||||||||
Sbjct  382  CATCTGGCGATTGCATTCATGCATCGGCAGGCACCCTGCGGGAAACCAAAGTTATTGGGT  441

Query  513  TCCGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCA  572
            |||||||||||||||||||||||||||||||||  ||||| |||||||||||||| || |
Sbjct  442  TCCGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-A  500

Query  573  Gaa  575
            |||
Sbjct  501  GAA  503
```

Appendix 8 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. beringianus* KF156875.1 and *N. tumidus* KF156876.1.



Neoechinorhynchus saginata 18S ribosomal RNA gene, complete sequence

Sequence ID: AY830150.1 Length: 1745 Number of Matches: 1

Range 1: 715 to 1132 GenBank Graphics Next Match

Score	Expect	Identities	Gaps	Strand
632 bits(342)	5e-177	398/424(94%)	7/424(1%)	Plus/Plus

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Query  153  ACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATG  212
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Sbjct  715  ACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCG-AC-TGCTTGTATACTTATGCATG  772

Query  213  GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
            |||||||||| ||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  773  GAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCTGAGGTAATGATT  832

Query  273  GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
            |||| ||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  833  GATA-GGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCTGTGATCACCG  891

Query  333  CAAGACGAACA-ACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAG  391
            |||||||| || |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  892  CAAGACGA-CACACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAG  950

Query  392  TTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGG  451
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  951  TTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGG  1010

Query  452  GCATCTGGCGCTTGCAAGTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGG  511
            |||||||||| |||||  || |||||| |||||||| ||||||||||||||||||||||
Sbjct  1011 GCATCTGGCGATTGCATCTATGCATCGGCAGGCACCT-GCGGGAAACCAAAGTTATTGGG  1069

Query  512  TTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCC  571
            |||||||||||||||||||||||||||||||||||| ||||| ||||||||||||||| ||
Sbjct  1070 TTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGACGGAGGGGCACACC-  1128

Query  572  AGaa  575
            ||||
Sbjct  1129 AGAA  1132
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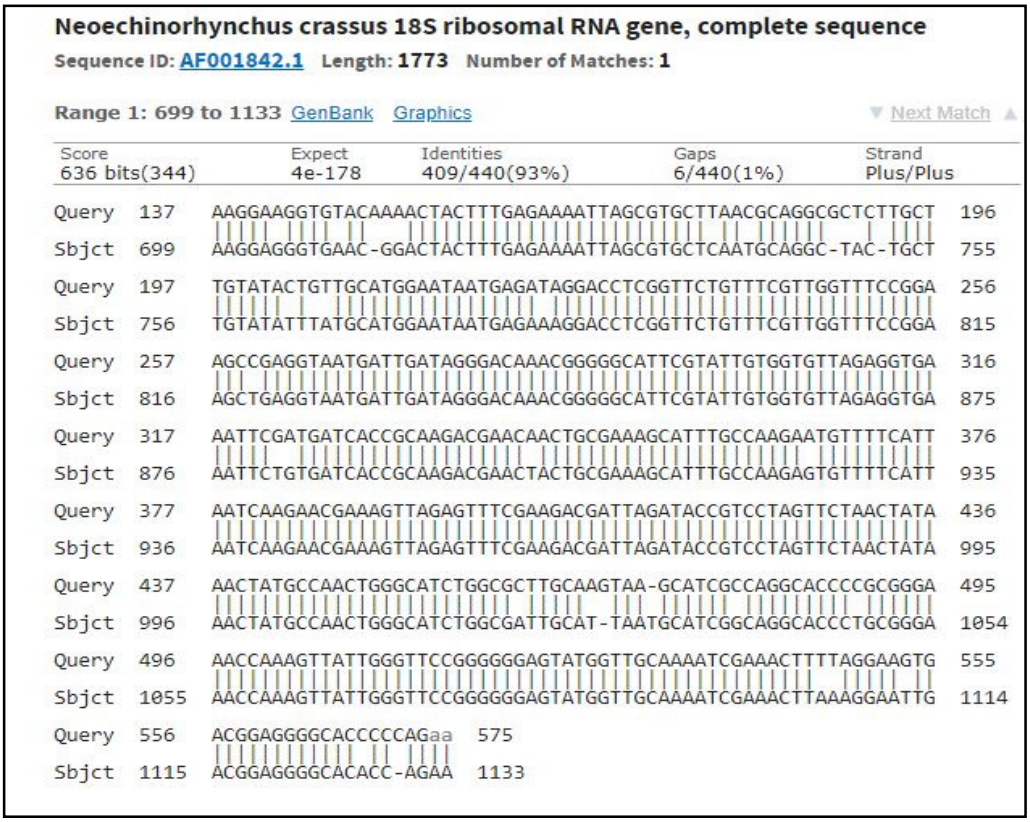

Neoechinorhynchus crassus 18S ribosomal RNA gene, complete sequence

Sequence ID: AF001842.1 Length: 1773 Number of Matches: 1

Range 1: 699 to 1133 GenBank Graphics Next Match

Score	Expect	Identities	Gaps	Strand
636 bits(344)	4e-178	409/440(93%)	6/440(1%)	Plus/Plus

```
Query  137  AAGGAAGGTGTACAAAACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCT  196
            ||||| |||| ||   |||||||||||||||||||||||||| || ||||||   | ||||
Sbjct  699  AAGGAGGGTGAAC-GGACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGC-TAC-TGCT  755

Query  197  TGTATACTGTTGCATGGAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGA  256
            ||||||  |  |||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  756  TGTATATTTATGCATGGAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGA  815

Query  257  AGCCGAGGTAATGATTGATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGA  316
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  816  AGCTGAGGTAATGATTGATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGA  875

Query  317  AATTCGATGATCACCGCAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATT  376
            |||||  |||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  876  AATTCTGTGATCACCGCAAGACGAACTACTGCGAAAGCATTTGCCAAGAGTGTTTTCATT  935

Query  377  AATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATA  436
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  936  AATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATA  995

Query  437  AACTATGCCAACTGGGCATCTGGCGCTTGCAAGTAA-GCATCGCCAGGCACCCCGCGGGA  495
            |||||||||||||||||||||||| |||||  ||| |||||| |||||||| ||||||
Sbjct  996  AACTATGCCAACTGGGCATCTGGCGATTGCAT-TAATGCATCGGCAGGCACCCTGCGGGA  1054

Query  496  AACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTG  555
            |||||||||||||||||||||||||||||||||||||||||||||||||  |||||  ||
Sbjct  1055 AACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTG  1114

Query  556  ACGGAGGGGCACCCCCAGaa  575
            |||||||||||  ||  ||||
Sbjct  1115 ACGGAGGGGCACACC-AGAA  1133
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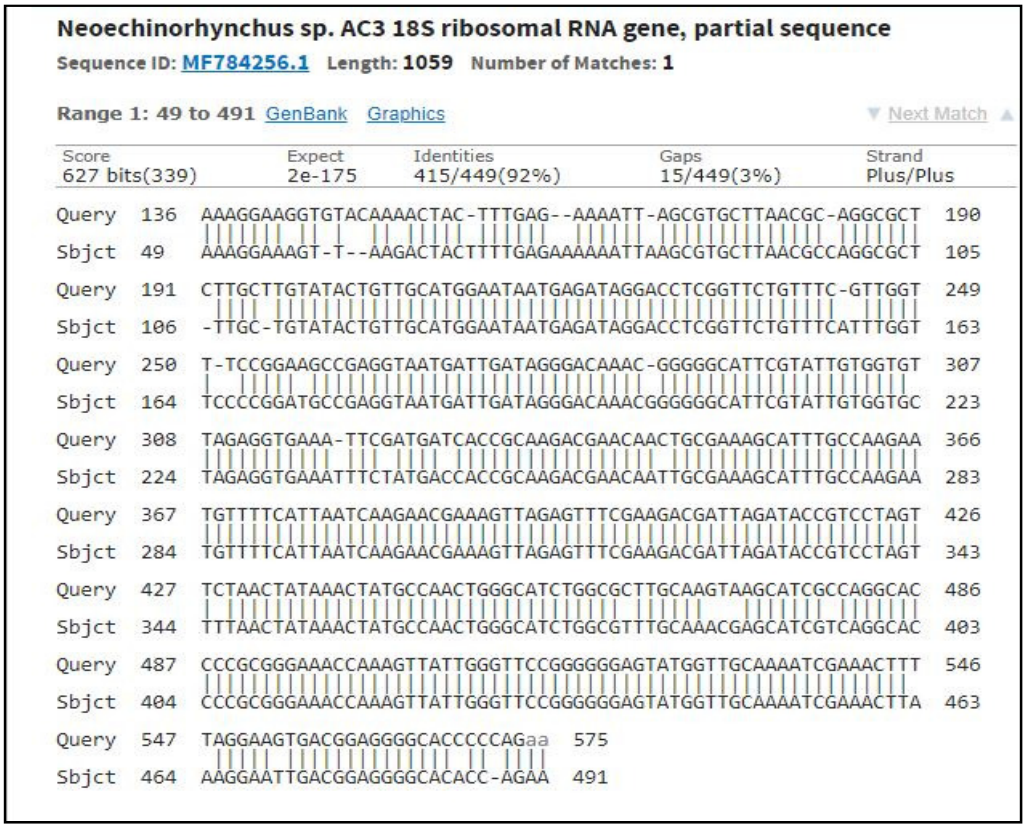
Appendix 9 Sequence alignment of *Neoechibiorhynchus zabensis* of 18S rRNA gene with *N. saginata* AY830150.1 and *N. crassus* AF001842.1.



Neoechinorhynchus sp. AC3 18S ribosomal RNA gene, partial sequence

Sequence ID: MF784256.1 Length: 1059 Number of Matches: 1

Range 1: 49 to 491 GenBank Graphics Next Match

Score	Expect	Identities	Gaps	Strand
627 bits(339)	2e-175	415/449(92%)	15/449(3%)	Plus/Plus

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Sbjct  49   AAAGGAAAGT-T--AAGACTACTTTTGAGAAAAAATTAAGCGTGCTTAACGCCAGGCGCT  105

Query  191  CTTGCTTGTATACTGTTGCATGGAATAATGAGATAGGACCTCGGTTCTGTTTC-GTTGGT  249
Sbjct  106  -TTGC-TGTATACTGTTGCATGGAATAATGAGATAGGACCTCGGTTCTGTTTCATTTGGT  163

Query  250  T-TCCGGAAGCCGAGGTAATGATTGATAGGGACAAAC-GGGGGCATTCGTATTGTGGTGT  307
Sbjct  164  TCCCCGGATGCCGAGGTAATGATTGATAGGGACAAACGGGGGGCATTCGTATTGTGGTGC  223

Query  308  TAGAGGTGAAA-TTCGATGATCACCGCAAGACGAACAACTGCGAAAGCATTTGCCAAGAA  366
Sbjct  224  TAGAGGTGAAATTTCTATGACCACCGCAAGACGAACAATTGCGAAAGCATTTGCCAAGAA  283

Query  367  TGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGT  426
Sbjct  284  TGTTTTCATTAATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGT  343

Query  427  TCTAACTATAAACTATGCCAACTGGGCATCTGGCGCTTGCAAGTAAGCATCGCCAGGCAC  486
Sbjct  344  TTTAACTATAAACTATGCCAACTGGGCATCTGGCGTTTGCAAACGAGCATCGTCAGGCAC  403

Query  487  CCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTT  546
Sbjct  404  CCCGCGGGAAACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTA  463

Query  547  TAGGAAGTGACGGAGGGGCACCCCCAGaa  575
Sbjct  464  AAGGAATTGACGGAGGGGCACACC-AGAA  491
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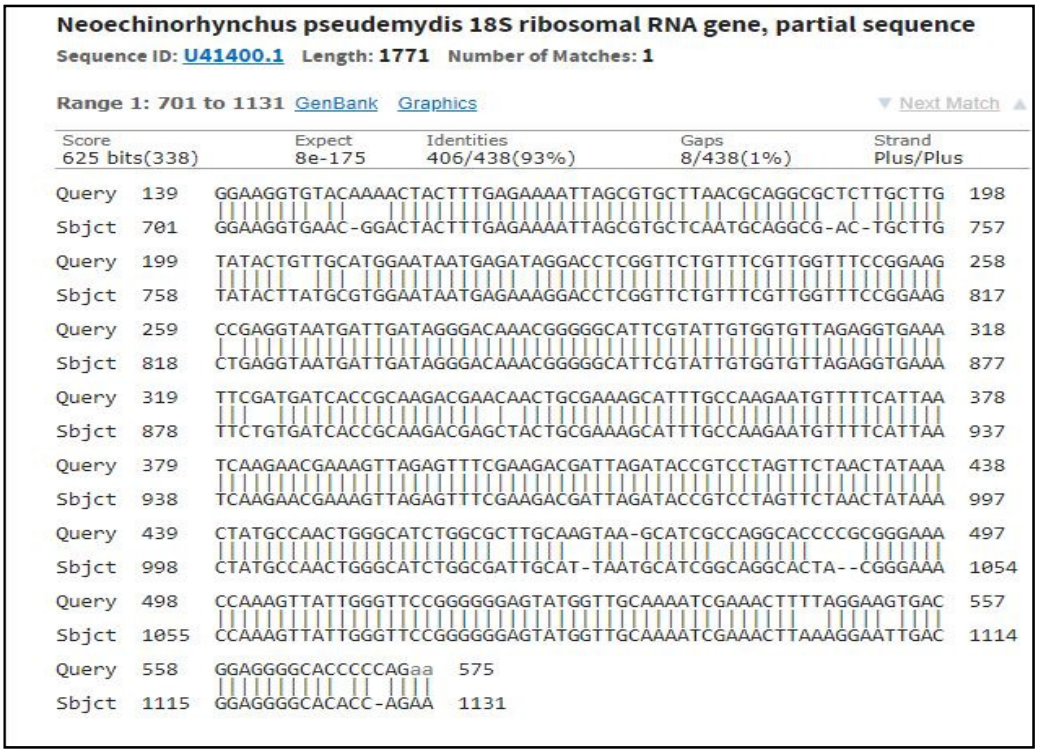

Neoechinorhynchus pseudemydis 18S ribosomal RNA gene, partial sequence

Sequence ID: U41400.1 Length: 1771 Number of Matches: 1

Range 1: 701 to 1131 GenBank Graphics Next Match

Score	Expect	Identities	Gaps	Strand
625 bits(338)	8e-175	406/438(93%)	8/438(1%)	Plus/Plus

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Query  139  GGAAGGTGTACAAAACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTG  198
Sbjct  701  GGAAGGTGAAC-GGACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCG-AC-TGCTTG  757

Query  199  TATACTGTTGCATGGAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAG  258
Sbjct  758  TATACTTATGCGTGGAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAG  817

Query  259  CCGAGGTAATGATTGATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAA  318
Sbjct  818  CTGAGGTAATGATTGATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAA  877

Query  319  TTCGATGATCACCGCAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAA  378
Sbjct  878  TTCTGTGATCACCGCAAGACGAGCTACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAA  937

Query  379  TCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAA  438
Sbjct  938  TCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAA  997

Query  439  CTATGCCAACTGGGCATCTGGCGCTTGCAAGTAA-GCATCGCCAGGCACCCCGCGGGAAA  497
Sbjct  998  CTATGCCAACTGGGCATCTGGCGATTGCAT-TAATGCATCGGCAGGCACTA--CGGGAAA  1054

Query  498  CCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGAC  557
Sbjct  1055  CCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGAC  1114

Query  558  GGAGGGGCACCCCCAGaa  575
Sbjct  1115  GGAGGGGCACACC-AGAA  1131
```

Appendix 10 Sequence alignments of *Neoechinorhynchus zabensis* of 18S rRNA gene with *N.* sp. MF784256.1 and *N. pseudemydis* U41400.1

Neoechinorhynchus crassus isolate NC-IR-Gandoman1 18S ribosomal RNA gene, partial sequence

Sequence ID: KU363969.1 **Length:** 1761 **Number of Matches:** 1

Range 1: 699 to 1131 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
623 bits(337)	3e-174	407/440(93%)	8/440(1%)	Plus/Plus

```
Query  137   AAGGAAGGTGTACAAAACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCT  196
             ||||| |||| ||   |||||||||||||||||||||||| || ||||||   | ||||
Sbjct  699   AAGGAGGGTGAAC-GGACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGC-TAC-TGCT  755

Query  197   TGTATACTGTTGCATGGAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGA  256
             |||||| |  ||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  756   TGTATATTTATGCATGGAATAATGAGAAAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGA  815

Query  257   AGCCGAGGTAATGATTGATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGA  316
             ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  816   AGCTGAGGTAATGATTGATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGA  875

Query  317   AATTCGATGATCACCGCAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATT  376
             |||||  |||||||||||||||||| ||||||||||||||||||| ||||||||||||
Sbjct  876   AATTCTGTGATCACCGCAAGACGAACTACTGCGAAAGCATTTGCCAAGAGTGTTTTCATT  935

Query  377   AATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATA  436
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  936   AATCAAGAACGAAAGTTAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATA  995

Query  437   AACTATGCCAACTGGGCATCTGGCGCTTGCAAGTAA-GCATCGCCAGGCACCCCGCGGGA  495
             |||||||||||||||||||| |||||  ||| |||||| |||||||   ||||||
Sbjct  996   AACTATGCCAACTGGGCATCTGGCGATTGCAT-TAATGCATCGGCAGGCACT--GCGGGA  1052

Query  496   AACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTG  555
             ||||||||||||||||||||||||||||||||||||||||||||||||||  |||||| ||
Sbjct  1053  AACCAAAGTTATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTG  1112

Query  556   ACGGAGGGGCACCCCCAGaa  575
             |||||||||||| || ||||
Sbjct  1113  ACGGAGGGGCACACC-AGAA  1131
```

Neoechinorhynchus sp. GL-2015 18S ribosomal RNA gene, partial sequence

Sequence ID: KU363972.1 **Length:** 1769 **Number of Matches:** 1

Range 1: 719 to 1139 GenBank Graphics ▼ Next Match ▲

Score	Expect	Identities	Gaps	Strand
619 bits(335)	4e-173	394/423(93%)	2/423(0%)	Plus/Plus

```
Query  153   ACTACTTTGAGAAAATTAGCGTGCTTAACGCAGGCGCTCTTGCTTGTATACTGTTGCATG  212
             |||||||||||||||||||||||||| || |||||||| | | ||||||||||||||||||
Sbjct  719   ACTACTTTGAGAAAATTAGCGTGCTCAATGCAGGCGAT-TCGCTTGTATACTGTTGCATG  777

Query  213   GAATAATGAGATAGGACCTCGGTTCTGTTTCGTTGGTTTCCGGAAGCCGAGGTAATGATT  272
             |||||||||||| |||||||||||||| |||||||||||||||||| ||||||||||||
Sbjct  778   GAATAATGAGAAAGGACCTCGGTTCTGATTCGTTGGTTTCCGGAAGCTGAGGTAATGATT  837

Query  273   GATAGGGACAAACGGGGGCATTCGTATTGTGGTGTTAGAGGTGAAATTCGATGATCACCG  332
             |||||||||||||||||||||||||||||||   |||||||||||||||||||  ||||| |
Sbjct  838   GATAGGGACAAACGGGGGCATTCGTATTGCAGTGTTAGAGGTGAAATTCTGTGATCACTG  897

Query  333   CAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  392
               |||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  898   TGAGACGAACGACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGT  957

Query  393   TAGAGTTTCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  452
             |||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  958   TAGAGTATCGAAGACGATTAGATACCGTCCTAGTTCTAACTATAAACTATGCCAACTGGG  1017

Query  453   CATCTGGCGCTTGCAAGTAAGCATCGCCAGGCACCCCGCGGGAAACCAAAGTTATTGGGT  512
             ||||||||| |||||  || |||||| |||||||||| ||||||||||||||||||||||
Sbjct  1018  CATCTGGCGATTGCATTTATGCATCGGCAGGCACCCTGCGGGAAACCAAAGTTATTGGGT  1077

Query  513   TCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTTTAGGAAGTGACGGAGGGGCACCCCCA  572
             |||||||||||||||||||||||||||||||||||  |||||| || |||||||||||| || |
Sbjct  1078  TCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAATTGGCGGAGGGGCACACC-A  1136

Query  573   Gaa  575
             |||
Sbjct  1137  GAA  1139
```

Appendix 11 Sequence alignments of *Neoechinorhynchus zabensis* of 18S rRNA gene with *N. crassus* KU363969.1 and *N.* sp. KU363972.1.

الخلاصة

أجريت هذه الدراسة على نوعين من الاسماك خلال الفترة من تشرين الأول 2017 الى شباط 2019. تم جمع 400 سمكة من التيلة المرقطة *Capoeta trutta* من بحيرة دوكان و 400 سمكة من (الخشني *Planiliza abu*) من نهر سيروان في شمال غرب و جنوب شرق محا فظة السليمانية على التوالي. وهما من أنواع الأسماك المحلية في أقليم كوردستان و العراق عموما . كان تسلسل الحمض النووي لكلا النوعين هما mitochondrial DNA cytochrome c oxidase subunit I (mtDNA COI) locus and 61 cytochrome b (cytb) gene, partial cds; mitochondrial على التوالي. كان تسلسل الحمض النووي للنوعين هما موضع COI mtDNA بحوالي 625bpوالجين cytb من حوالي 521bpعلى التوالي. بعد تحليل التسلسلات و مقارنتين مع الأنواع الأخرى المخزونة في GenBank، أعطت تطابقا بنسبة % 100 على أساس تماثل الجزيئي.

كانت أسماك *C. trutta* مصابة بالدودة شوكية الرأس *Neoechinorhynchus zabensis* وبنسبة الانتشار الكلي 86.96 % ، بينما *P. abu* مصابة ب *N. iraqensis* و بنسبة % 0.015. تمت دراسة *N. zabensis* شكليا بواسطة المجهر الضوئي المركب و المجهر الإلكتروني الماسح ، وكذلك جزيئيا.

المنتج PCR من *Neoechinorhynchus zabensis* بحوالي 622bp. من تم استخدام التحليل الوراثي لتسلسل الحمض النووي لجين 18S rDNA لتوصيف *N. zabensis*. و أظهرت تطابقا بنسبة % 95.28 مع *N. buttnerae*. تراوحت المسافة الوراثية بين *N. zabensis* وبعض أنواع *Neoechinorhynchus* من 0.08 إلى 0.14 ، سجلت أدنى مسافة وراثية بين *N. Zabensis* و *N. agilis*، في حين كانت أعلى مسافة وراثية بين *N. zabensis* و .*N* sp. يتضح من نتائج شجرة الأصول بأن *N. zabensis* تحتل مكانة منفصلة في شجرة الآصول. و قد تم تسجيلها في البنك الجيني NCBI GenBank تحت الرقم (MN621252) لهذه الانواع من الطفيلي *N. zabensis* و يتعد هذا التسجيل أول تسجيل للطفيلي في البنك الجيني NCBI GenBank.

في هذه الدراسة، تم جمع 1220من الديدان *N. zabensis* من سمكة *C. trutta*. لم تظهر فروق معنوية بين ذكورالاسماك المصابة وإناثها المصابة بهذا الطفيلي. كما ان نسبة انتشار، متوسط شدة و وفرة الاصابة ب *N. zabensis* زاد بزيادة طول السمكة. سجلت أعلى نسبة الاصابة خلال الشهر الربيع و الصيف، بينما كانت أدنى الاصابة خلال الشهر الخريف و الشتاء.

حكومة أقليم كوردستان
وزارة التعليم العالي و البحث العلمي
جامعة السليمانية
كلية علوم الهندسة الزراعية

دراسة مظهرية و جزيئية على جنس *Neoechinorhynchus* STILES AND HASSALL, 1905 من نوعين من أسماك المياه العزبة في محافظة السليمانية

رسالة

مقدمة الى مجلس كلية علوم الهندسة الزراعية في جامعة السليمانية

كجزء من متطلبات نيل شهادة دكتوراه في علم الطفيليات

علم الطفيليات الأسماك

من قبل

مقداد كمال علي

ماجستير (2013)، جامعة السليمانية

باشراف

د. شەمالّ محمد امين عبدالله

أستاذ

2020 م

1441ه

پوختە

ئەم توێژینەوەیە ئەنجامدرا لە سەر دوو جۆر ماسی لە ماوەی تشرینی یەکەم 2017 تا کانونی دووەمی 2019. هەروەها جەخت لەم پۆلێن کردنە کرایەوە لە ڕێگەی تاقیکردنەوەی گەردی. کۆی 450 نموونەی ماسی لە جۆری (مشارە *Capoeta trutta*) کۆکرایەوە لە دەریاچەی دوکان وە 400 نموونەی ماسی لە جۆری (زبرە *Planiliza abu*) لە ڕوباری سیروان لە باکوری ڕۆژئاوا و باشوری ڕۆژهەلاتی پارێزگای سلێمانی، یەك بە دوای یەك. ئەم دوو جۆرە لە ماسی تۆمارکراون وەکو جۆری ناوخۆیی. ڕیزبوونی شریتی DNA بۆ هەردوو جۆری ماسی (*C. trutta* و *P. abu*) لە پارچەی mitochondrial DNA cytochrome c oxidase subunit I (mtDNA COI) locus and 61 cytochrome b (cytb) gene, partial cds; mitochondrial, یەك بە دوای یەکدا. زنجیرەی DNA هەردوو جۆری ماسی *C. trutta* and *P. abu* کە پارچەی mtDNA 625bp COI وە پارچەی cytb gene 521bp دواتر بلاست کران لە گەڵ زنجیرەی توخم و جۆرەکانی کە هەلگیراون لە بانکی بۆهێڵەکان. ئەنجامی بلاستەکە 100% لێکچوونی ناوەکی دەرخست بۆ هەردوو جۆری ماسیەکە.

ماسی *C. trutta* تووش بوو بە acanthocephalan *Neoechinorhynchus zabensis* وە ڕێژەی بلاوبونەوەکەی % 86.96 ، هەروەها ماسی *P. abu* تووش بوو بە *N. iraqensis* وە ڕێژەی بلاوبوونەوەکەی % 0.015. لێکۆڵینەوەی شێوەیی بە مایکرۆسکوبی ڕووناکی ئالۆز و مایکرۆسکۆبی ئەلیکترۆنی ڕووکەش بۆ کرمی *N. zabensis* ئەنجام درا، لەگەڵ لێکۆڵینەوە لەسەر ئاستی گەردیی DNA.

بەرهەمی PCR بۆ *Neoechinorhynchus zabensis* بریتی بوو لە 622bp شیکاری ڕەچەڵەك بۆ ڕیزبوونی ترشە ناووکی دی ئێن ئەی لە پارچەی 18S rDNA بەکار هێنرا بۆ وەسفی *N. zabensis*. ئەنجامی بلاستەکە دەریخست کە ئەم کرمە 95.28% لەیەك چوونی بۆماوەیی هەیە لە گەڵ *N. buttnerae* وە دووری بۆماوەیی لە نێوان *N. zabensis* و هەندێ جۆری *Neoechinorhynchus* لە نێوان 0.08 بۆ 0.14دایە، بە جۆرێك کەمترین دووری بۆماوەیی تۆمار کراوە لە نێوان *N. zabensis* و *N. agilis* وە

زۆرترین دووری بۆماوەیی تۆمار کراوە لە نێوان *N. zabensis* و *N.* sp. . لەسەر بنچینەی ئەنجامی دەختی ڕەچەڵەك دەردەكەوێت كە *N. zabensis* شوێنێكی جیاكراوەی گرتووە. ژمارەی جین بانكی (accession number MN621252) وەرگیراوە بۆ ئەم مشەخۆرە كە بۆ یەكەم جارە تۆمار بكرێت لە بانكی بۆهێڵەكان (NCBI).

لەم توێژینەوەیەدا 1220 مشەخۆر لە جۆری *Neoechinorhynchus zabensis* لە ماسی *C. trutta* كۆكرایەوە . ئەنجامی توێژینەوەكە دەریخست كە هیچ جۆرە جیاوازیەك نیە لە نێوان ڕەگەزی نێر و مێی ماسی *C. trutta* لە ڕێژەی توشبوونی بە مشەخۆری *N. zabensis* ، بەڵام ڕێژەی توشبوون زیادی كردووە بە زیاد بوونی درێژی ماسیەكە وە بە گوێرەی ئەنجامەكان بەرزترین ڕێژەی توشبوون لە وەرزەكانی بەهار و هاویندایە وە كەمترین لە وەرزەكانی پایز و زستاندایە.

حکومەتی هەرێمی کوردستان
وەزارەتی خوێندنی باڵا و توێژینەوەی زانستی
زانکۆی سلێمانی
کۆلێجی زانستە ئەندازیاریە کشتوکاڵییەکان

لێکۆڵینەوەی شێوەیی و گەردیی لەسەر توخمی *Neoechinorhynchus* STILES AND HASSALL, 1905

لە دوو جۆر لە ماسی ئاوی سازگار لە پارێزگای سلێمانی

نامەیەکە

پێشکەش کراوە بە ئەنجومەنی کۆلێجی زانستە ئەندازیاریە کشتوکاڵییەکان لە زانکۆی سلێمانی

وەک بەشێک لە پێداویستیەکانی بەدەستهێنانی بڕوانامەی دکتۆرا لە مشەخۆرزانی

مشەخۆرزانی ماسی

لە لایەن

مقداد کمال علی

ماجستێر (2013)، زانکۆی سلێمانی

بەسەرپەرشتی

د. شەمانّ محمد ئەمین عبدالله

پرۆفیسۆر

2020 ز 2719 ك