

Morphological and molecular characterization of *Solostamenides mugilis* (Vogt, 1879) (Monogenea: Microcotylidae) parasitizing long spine Scrapper Fish, *Capoeta trutta*

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Abstract

This study aimed to investigate the morphometric and molecular characterizations of *Solostamenides Mugilis* in *Capoeta trutta* in Iraq. *Capoeta trutta* was captured from Lesser Zab River, Erbil Province, Kurdistan Region, Iraq. The specimens were studied morphologically with a dissecting microscope, with fixation in 5% formaldehyde. For molecular analysis specimens were preserved in absolute ethanol. Following DNA extraction, the region of 28S rRNA was amplified by Polymerase Chain Reaction (PCR), and the order of the nucleotides was determined by the genetic analyzer. Morphological properties, as well as DNA analyses of collected specimens, showed that the collected specimens belong to *Microcotylidae*, namely *Solostamenides mugilis*, with the prevalence of infection and mean intensity (9.37% and 4.1) respectively. During this examination, the monogenetic species *S. mugilis* was documented on the cyprinid fish (*Capoeta trutta*) as the first occurrence in Iraq. Therefore, *C. trutta* is regarded as a new host for this genus within the Cyprinid family.

Keywords: *Capoeta trutta*, *Cyprinidae*, 28S rRNA, *Microcotylidae*, *Solostamenides*

Introduction

The most widespread and diverse freshwater fish family is *Cyprinidae*. There are 1727 species in this family. It comprises approximately 8.5% of all fish species in the world and lives naturally in all kinds of habitats. The most numerous species in Iraq's freshwaters belong to this family, where native fish make up 72% of the total fish populations (Coad, 2010; Fricke et al., 2018).

The investigation of fish parasites is necessary to enhance the stocks of important commercial fisheries in natural waters, in order to raise pond fish farms' productivity, and enhance the chances of fish adaptability in new locations (Al-Jawda, 2020; Shulman, 1961). The majority of monogeneans (Platyhelminthes) are monoxenous ectoparasites of fishes that are host-specific (Ono et al., 2020; Whittington, 1998). They are the most common freshwater fish gill parasites in the world (Woo et al., 2006).

Microcotylidae Taschenberg, 1879 is one of the most controversial families within *Monogenoidea* Bychowsky, 1937, in which several genera and subgenera have been erected (fifty genera and more than 160 species). It has gotten a lot of attention. Yet, the specific structure and position of numerous genera remain uncertain (Bouguerche et al., 2019, Mamaev, 1986). *Solostamenides* is a genus within *Microcotylidae*, that was described and named by Unnithan in the light of the following features: Its penis has spines that resemble hooks, whereas the atrial margin is muscular and armless with one vaginal pore located mid-dorsally (Unnithan, 1971). Identification of monogeneans needs rigorous morphological analysis and taxonomic competence as in other *helminths* (Brooks, 2000).

Although traditional morphology-based approaches are still often used to classify species, they have some limitations. Furthermore, the use of molecular markers, despite their increasing popularity, appears to be not entirely error-free (Patwardhan et al., 2014). But in recent times, molecular analysis has been used in combination with morphological descriptions, enabling researchers to explain species status (Verma et al., 2018). Identifying and characterization of microorganisms in environmental samples could be a valuable interest to study their effect. Molecular characterization can assist to detect biodiversity of different species (Suliman et al., 2022). In some cases, it is of vital importance to explore effective methods such as molecular approach for characterization and early diagnoses of some species (Ma et al., 2022).

In fact, over the past ten years, molecular tools have been used in the detection of *Microcotyle* species (Syn: *Solostamenides*) (Ayadi et al., 2017), even when only a few DNA have been

sequenced so far (Muñoz, 2019). During this examination, the monogenetic species *S. mugilis* was documented on the cyprinid fish (*Capoeta trutta*) as the first occurrence in Iraq, so the objectives of the current article are to study the morphometric and molecular characterizations of *Solostamenides Mugilis* in *Capoeta trutta* in Iraq, and to show the importance of fish monogenean is directly related to the importance of the fishes.

Material and methods

Lesser Zab river is the largest branch of the Tigris River (400 km). It is located between 34°-36° North latitude and 43 °-46 ° East longitudes (Fig. 1) (Abdullah, 2015). In the present study, 64 specimens of *Capoeta trutta* (*longspine scraper*) were caught from the Lesser Zab River in Taqtaq District by local fishermen using gill nets twice a month. Taxonomic descriptors were used to assess the fish's species composition according to Froese (Froese, 2021). Fish's gills were checked for parasites under a dissecting microscope. After counting live worms, each worm was fixed and stored in 70% alcohol and placed in ammonium picrate-glycerin or glycerin jelly. After that, Olympus BX53 microscope was used to study the parasites.

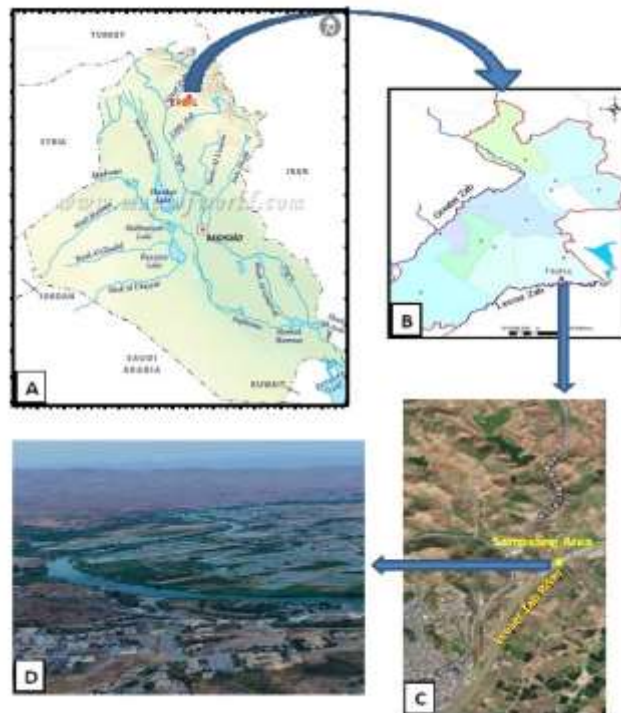


Figure 1. A- Map of Iraq showing Kurdistan Region. B- Map of Erbil Province showing Taqtaq district, C- Google satellite Map of Taqtaq district showing Lesser Zab River (location of sampling area), D- Photograph showing sampling area

Some preserved specimens were treated with essential oils, stained with acetic carmine, parched in graduated ethanol serial (70, 96, and 100%), and mounted in Canada balsam to investigate the morphology of the genital atrial and attachment organs (Al-Helli et al., 2019, Palm, 2004). The mean intensity and prevalence of the parasites were measured according to (Bush et al., 1997; Muñoz, 2019). After microscopic inspection, molecular analysis was carried out utilizing 28S rRNA as a gene marker.

The DNA was extracted according to Whittington (1998). Genomic using a DNA extraction kit (GeNet Bio, KOREA) and following the manufacturer's instructions with minor modification. The polymerase chain reaction (PCR) was used to amplify a region of 28S rDNA. Using universal primers C1 (F=5' ACCCGCTGAATTTAAGCA 3') and C3 (R=CTCTTCAGAGTACTTTTCAAC). (Mollaret et al., 2000).

PCR reaction and conditions were performed using MJ Research, Applied Biosystem (AB) thermal cycler. 50 µL reaction mixture was prepared in PCR tubes containing 2.5 µL of DNA templates, 25 µL of OnePCRTM master mix (Genedirex, Korea), 1 µL of each primer and 20.5 µL of double deionized water (ddH₂O). Cycling conditions included initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 51°C for 45 s and extension at 72°C for 45 s, and final extension at 72°C for 5 min. Agarose gel electrophoresis was employed to check the efficiency of PCR reactions. The samples were prepared and run in 2% gel of agarose then stained with SYBR green that makes the DNA visible under UV light. The ABI 3130X nucleotide sequence analyzer (SINGAPORE) was used to find nucleotides order of 28S rDNA from the specimens. The PCR fragments of the specimens were excised from the agarose gel and used as a source of DNA template for sequence specific PCR amplification (Quiazon et al., 2008). PCR-amplified 28S rDNA gene specimens were automatically sequenced using Applied Biosystems (Genedirex, Korea), then sequences of the helminthes was deposited to GeneBank (AF 131722.1).

Results and discussion

In the present study, *Solostamenides mugilis* (Syn: *Microcotyle mugilis*), a microcotylid monogenean, was described (Fig 2 and Fig 3). Prevalence and mean intensity were reported as 9.37% and 4.1, respectively, on the gill filaments of *Capoeta trutta* from the Lesser Zab River near to Taqtaq District. The description is based on six specimens (stained and unstained). The body was fusiform and elongated, the total length was 9,000 (8,000–10,000), and widest point on

ovary level was 1,250 (1,000-1,500). Clamps set in two rows ranging from 60 to 80, with typical *Microcotyle*-type. The size of clamp was 52×67 (50×60) – (55×75) with the largest at the haptor's center. The diameter of buccal organs was 65 to 70 of buccal sucker. The size of pharynx oval was 50-75. The esophagus was long and wide with obvious diverticula, and the gut was divided at the level of vaginal atrium. Testes were relatively large, numbering between 85 and 100, following ovarian, which were interracial in the dorsal portion of the body. The vas deferens was prominent and coils anteriorly in the midline.

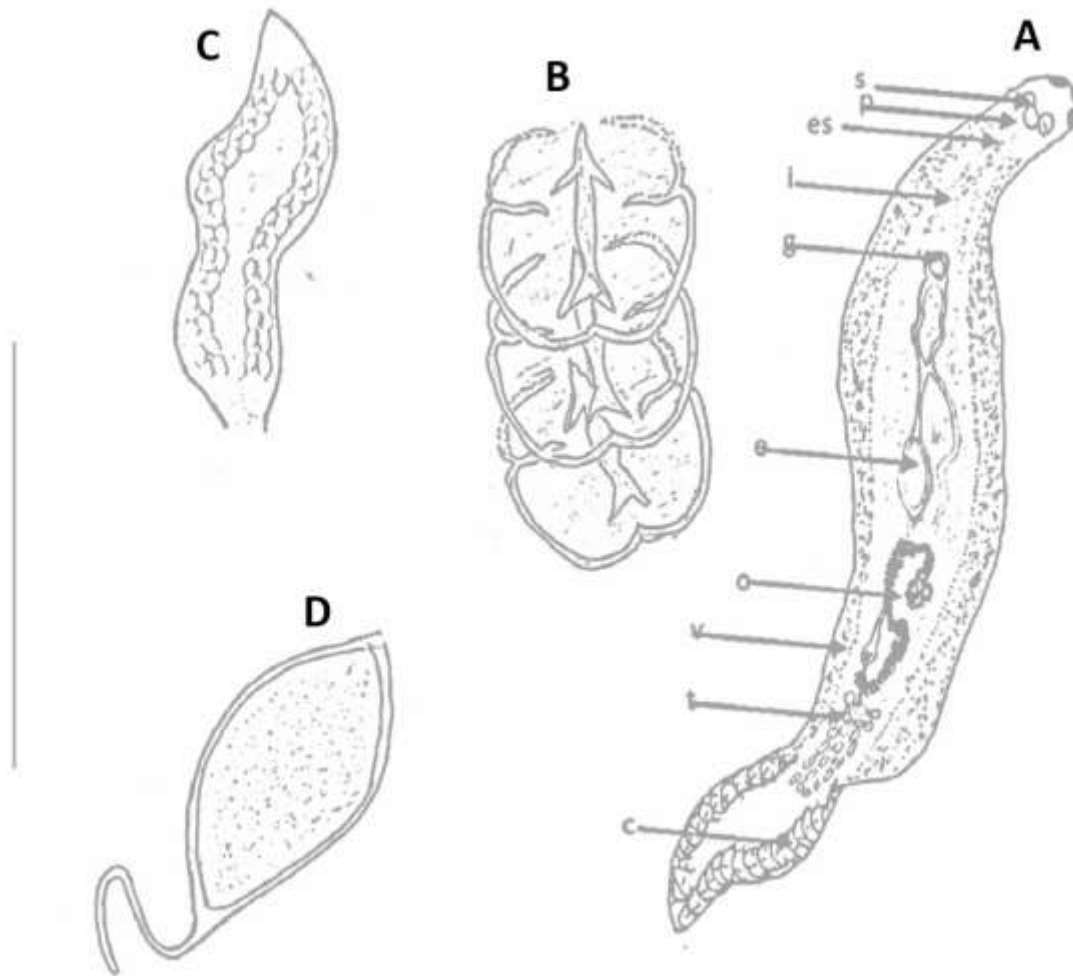


Figure 2. Camera Lucida drawings of *Solostamenides mugilis* from *Capoeta trutta*: A- Whole mount B- Clamps C- Haptor D- Egg. Abbreviations- c: clamp, e: egg, es: esophagus, g: genital atrium, i: intestinal caecum, o: ovary, p: pharynx, s: sucker, t: testis, v: vetillaria. (Scale bar: A= 2.1 mm, B=67 μ m, C= 381 μ m, and D=142.9 μ m.)

The ovary was in the shape of a question mark located in front of the testicles. The right ovarian lobe gives rise to ovarian canal. Uterus extends cranially and medially toward the gonopore. Vaginal hole located after genital atrium. Mehlis gland was visible. Vitellaria had great number of follicles close to the intestinal branches and extended from the intestinal fork to the haptor. The vitello-vaginal storage was formed just at levels of the ovaries by the expansion and medial extension of the vitelline channels. The egg was oval in shape, 120 to 225 in length and with short filament. Gonopores enter the vaginal chamber in ventral side to the intestinal fork.

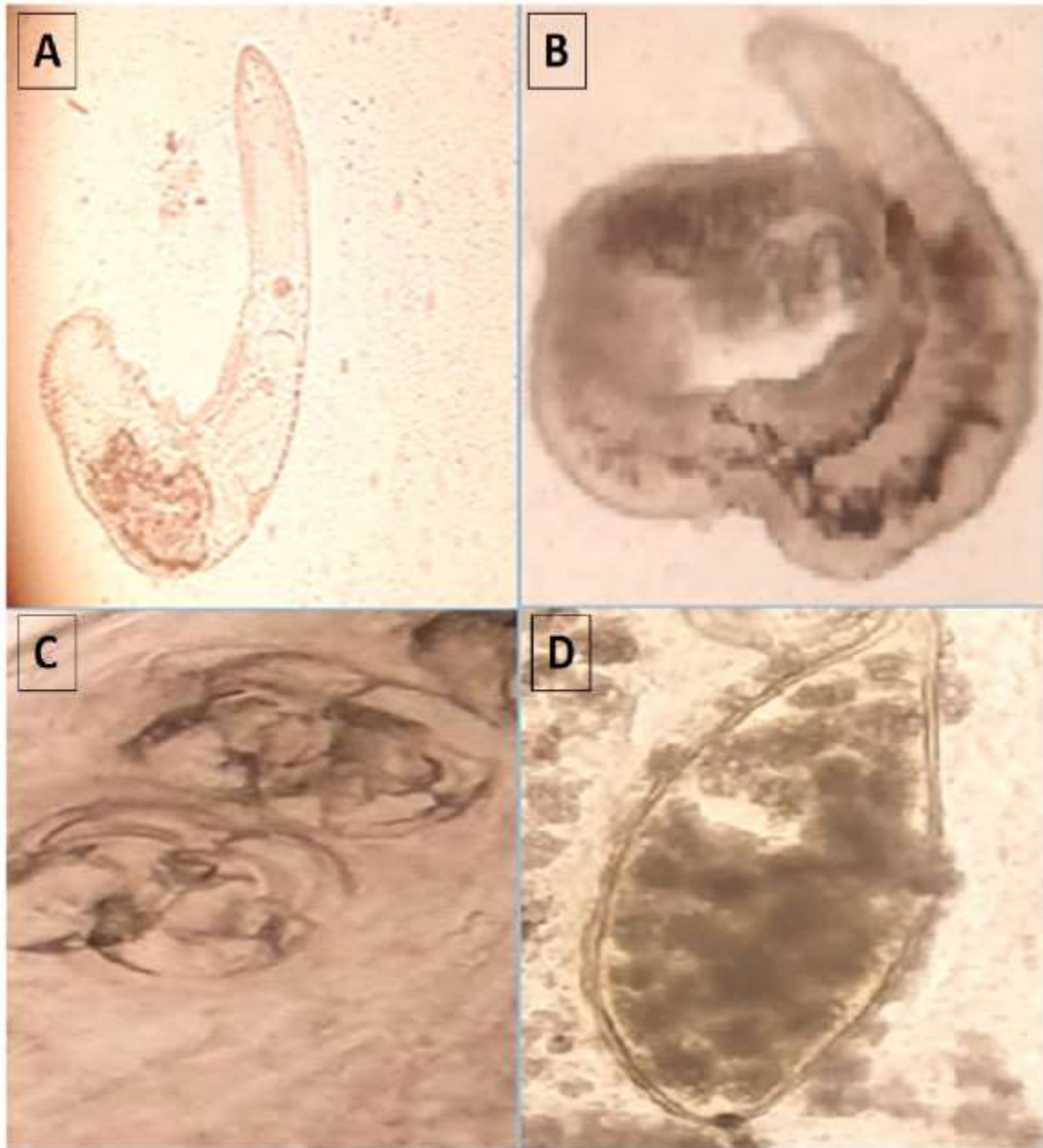


Figure 3. Photomicrograph of *Solostamenides mugilis* from *Capoeta trutta* : A: Larval stage (10X), B: Adult stage (10X), C: Clamps (40X) , D: Egg (40X)

The current specimen' measurements and descriptions matched with the other described specimen of same species from *Mugil cephalus* in Mediterranean (Euzet et al., 1993, Euzet and Combes, 1969). Furthermore, this description corresponded with another study, that was reported from *Siganus rivulatus* in AL-Sinn fish farm, Syria (Layka, 2018).

After molecular analysis of the specimens, we showed that the sequence of 28S rDNA of monogenean specimens was made of 326 bp (the amplified fragment was 352bp). After

sequencing, 26 missing nucleotides (related to quality of sequencing analysis) were removed, then the sequence was processed through BLAST and then compare with other sequences of *Solostamenides* species in the Gene bank database. The BLAST findings showed that its query sequence was remarkably similar to *S. mugillis* with more than 98% as (Fig. 4). To date, the genus *Solostamenides* reportedly contains five designated species: *Solostamenides mugilis* (Vogt, 1879) (Unnithan, 1971); *Solostamenides pseudomugilis* (Hargis, 1957) (Unnithan, 1971); *Solostamenides platyorchis* (Zhang and Yang (2001); *S. paucitesticulatus* (Kritsky and Öktener, 2015) and *Solostamenides iraqensis* (Al-Nasiri and Babuelna, 2018).

Solostamenides mugilis was originally identified by Vogt (1878) as *Microcotyle mugilis* on *Mugil cephalus*. Afterward Euzet et al. (1969) re-described the species and after all, Unnithan (1971) renamed it to *Solostamenides mugilis* which was chosen by the author to be the genus' type species (Euzet, et al. 1969; Unnithan, 1971).

Unnithan in 1971 also renamed *Microcotyle pseudomugilis* to *Solostamenides pseudomugilis* that was initially recognized by Hargis (1956) in the west of the Atlantic in *M. cephalus* (Woo et al., 2006). Subsequently, in 1991, the species was redescribed by Williams from the same host species in west of Australia (Williams, 1991). Afterwards, Zhang and Yang (2001) identified *S. platyorchis* in *M. cephalus* in south of China and assigned it to *Solostamenides* because it had spines on the copulatory organ. In addition, Kritsky and Oktener (2015) reported *S. paucitesticulatus* in 2015 as new species by noticing the spines on its copulatory organ. Thus, it appears that occurrence of spines on copulatory organ is a critical characteristic for distinguishing *Solostamenides* spp. (Bouguerche et al., 2019; Unnithan, 1971). Recently, the fifth species of *Solostamenides* was recorded as *S. iraqensis* by Al-Nasiri and Babuelna in 2018 (Al-Helli et al., 2019; Al-Nasiri and Balbuena, 2018).

According to the authors, the last two species *S. paucitesticulatus* and *S. iraqensis* are identical in several features like: body length, clamps number, number of spines in the male copulatory organ (MCO), and egg size. However they provided some characteristics to distinguish these species “(that have already been described from the same host, Pallniza abu)” such as the presence or lack of muscle bands in its oral sucker, diameter of testicular, MCO spine length, the nature of egg filaments, and clamp's shape in the median part (X or Y- like) (Al-Nasiri and Balbuena, 2018; Kritsky and Öktener, 2015). But all of the above mentioned features variable even within the same species may be related to intraspecific variances in fixation and staining techniques and in

these cases they take taxonomic importance. In addition of above statements, there is a fact that both species were investigated from the same fish host and shared a similar geographic distribution (Mesopotamia, Turkey and Iraq). All above states led the authors to assume that *S. iraqensis* was a synonym of *S. paucitesticulatus* (Al-Helli et al., 2019), due to the strong host specificity of monogenean species and the common knowledge that each species of *Solostamenides* only infects one kind of host (Kritsky, Öktener, 2015). This argument that mentioned above supports the results of the present investigation.

Both species of *S. mugilis* (Vogt, 1878), and *S. pseudomugilis* (Hargis, 1956) were originally identified on *Mugil cephalus*. These two species were distinguished by Hargis (1956) depended on location of the spines copulatory organ, which were found in vaginal atrium instead of a "cirrus.". According to Hargis, *S. pseudomugilis* might a synonymous of *S. mugilis* of Parona, Perugia (1890) based on the same distinction (Hargis, 1956; Unnithan, 1971). However, Sproston (1946) believed that the genital armament described by Parona, Perugia (1890) and Vogt (1878) were the same (Sproston, 1946). As a result, there has been misunderstanding concerned the classification of the two species that make up the genus (Jianyini and Tingbao, 2001), and it also supporting our finding in the host-specificity of *Monogenoides*.

By recording *S. mugilis* in this survey, three species of *Solostamenides* have been identified from two kinds of fish in Iraq (Al-Helli et al., 2019; Al-Nasiri and Balbuena, 2018). Among those, only these species was noted in the Kurdistan Region on gills of cyprinid fish. *Solostamenides mugilis* resembles other species morphologically, but differs from them in having more testes, the arrangement of the rows of the testes, the form of each testis, the number of haptorial clamps (more Clamps), the number of spines, number of copulatory organ, and other characteristics as table 1 (Al-Nasiri and Balbuena; 2018, Jianyini and Tingbao, 2001).

Solostamenides mugilis (syn. *Microcotyle mugilis*), has been identified in a number of mugilid species from a variety of geographical localities, includes: Syria, Turkey, France, Italy, Greece, and Tunisia (Caillot et al., 1999; Derbel et al., 2022; Layka and Bardrn, 2018; Merella and Garippa, 2001; Ragias et al., 2005; Sezen and Price, 1967) respectively. Based on the fish habitats, studies that revealed *S. mugilis* infections from fishes may be divided into two main types. Reports on *S. mugilis* of marine fishes included those from marine Aquaculture waters (AL-Sinn fish farm) in Syria, Italian marine fish, Sarikum Lagoon Lake and Black Sea coast in Turkey, (Layka and Bardrn, 2018; Özer and Acar, 2022; Öztürk, 2013; Strona et al., 2010) respectively.

The parasite in fresh water fish was also investigated in several studies. Among them: (Caillot et al., 1999; Öztürk and Özer, 2014; Ragias et al., 2005; Sezen and Price, 1967; Yemmen et al., 2011). All previously described *Solostamenides* have been discovered from Mugilid family, in which three of them (*S. mugilis*, *S. pseudomugilis*, and *S. platyorchis*) in *M. cephalus* and two others (*S. paucitesticulatus* and *S. iraqensis*) being found in Palliniza abu (Al-Nasiri and Balbuena, 2018; Hargis, 1956; Jianyin and Tingbao, 2001; Kritsky and Öktener, 2015; Unnithan, 1971).

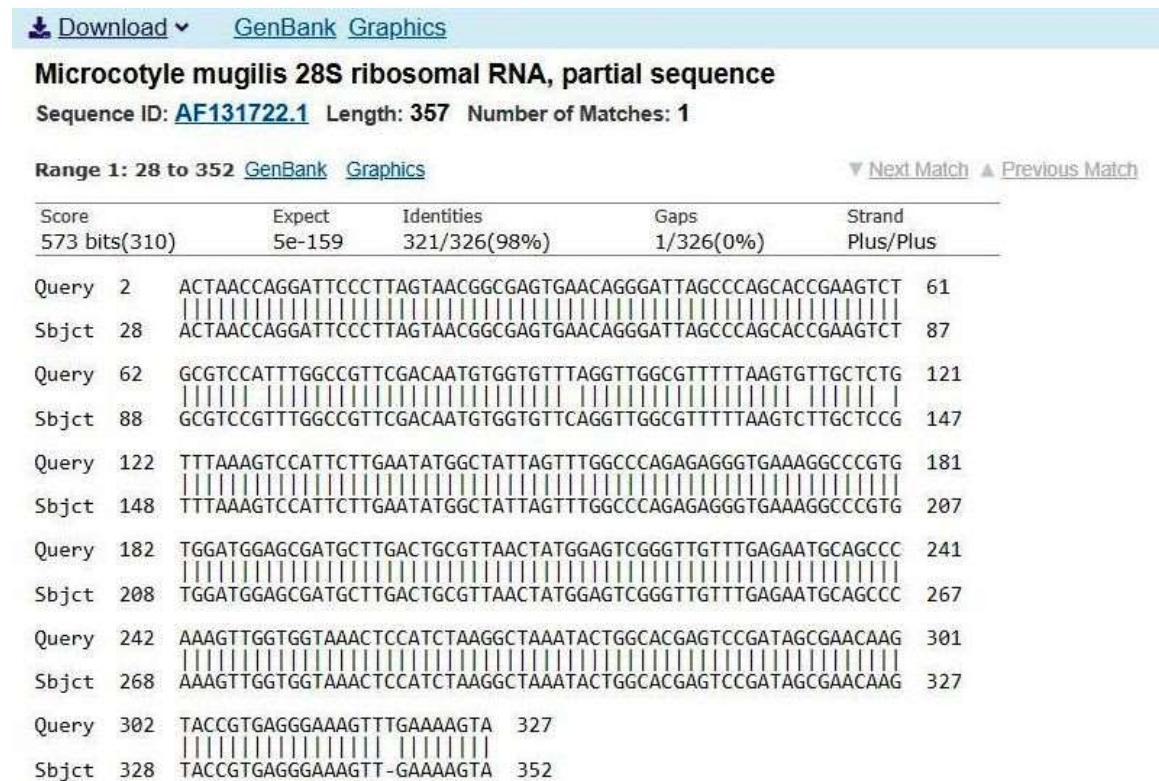


Figure 4. Alignment of the 28rDNA sequence in pairs for *Solostamenides mugilis*, query sequence and Sbjct in the GenBank sequence.

The present investigation reveals additional species of the genus in fresh water habitats in Iraq. It also added another host of the genus, and it shows that *Solostamenides* spp. may have some degree of host specificity. It is correct to state that the discovery of *Solostamenides* species in cyprinid hosts in the current study was regarded as an unexpected discovery since this genus generally parasitizes mugilid family. And according to the previous study the genus has not additional host

(El Hafidi et al., 1998; Layka and Bardrn, 2018; Öztürk and Özer, 2014; Radujkovic, 1982; Ragias et al., 2005; Yemmen et al., 2011). Thus, the new host of the genus was introduced within cyprinid family by the current investigation.

Due to the morphological uniformity of the species, the morphological identification of *Solostamenides* species is not simple and not striated forward (Lablack et al., 2022). As a result, more dependable techniques, such as molecular analysis, are required to identify species that belong to the genus *Solostamenides* (Özer and Acar, 2022). *S. mugillis* would be easily recognized from the other species of its genus at the molecular level.

In the present study by universal primers, the primary sequence analysis of the examined specimens demonstrated that the monogenean from northern Iraq corresponds to a species *S. mugillis*. Its rDNA corresponds with the identical rDNA sequence fragment marker that is found at the GeneBank in the National Center for Biotechnology Information (NCBI) (AF 131722.1). During this examination, the monogenetic species *S. mugillis* was documented on the cyprinid fish (*Capoeta trutta*) as the first occurrence in Iraq. It also shows that *Solostamenides* spp. may have some degree of host specificity. The morphological characters and DNA sequence-based examination of the specimens allowed for the identification of *S. mugillis*. We might learn more about the range and specificity of these monogeneans also find other species of the genus by conducting more studies of fish hosts in fresh, brackish, and marine waters in diverse geographical areas.

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