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First Morphological and Molecular (28S rDNA) Characterizations of *Eudiplozoon nipponicum* (Monogenea, Diplozoidae) parasitizing *Cyprinus carpio* in Kurdistan Region, Iraq

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Abstract: The present study is the first morphological and molecular characterization of the monogenean *Eudiplozoon nipponicum* (Goto, 1891) parasitizing gill filaments of common carps (*Cyprinus carpio*) obtained from the Lesser Zab River in northeastern Iraq in the subdistrict of Altun Kopru from July to October 2022 and transported to the Laboratory of Zoology Research, Salahaddin University-Erbil, Iraq. Most previous studies regarding the considered parasite have targeted morphological data analysis. However, DNA sequence outline is typically supportive in systematics, since it contains aspects that are absent from morphological research. The main goal was to molecularly identifying *E. nipponicum* by utilizing the nuclear 28S rDNA region by PCR and nucleotide sequencing approach. The sequences were obtained and compared to the accessible GenBank sequences. The results justify the validation of *E. nipponicum* in Iraq by using traditional (morphology-based) and modern (molecular-based) parasitological techniques. The latter one showed 99.11% (identity percentage) of *E. nipponicum* in comparison to the registered sequences of NCBI. Additionally, this was considered as the first wide-ranging morphological and molecular study in the studied region. The phylogenetic relationship was concluded using Maximum Likelihood (ML) method. It was concluded that besides of the morphological characterization of *E. nipponicum*, PCR sequencing was considered as a suitable molecular method for recognizing monogenean diplozoid species on the fishes.

Keywords: common carps, *Eudiplozoon nipponicum*, Gill, Iraq, Molecular, Morphology.

Introduction

The freshwater fish family Cyprinidae is the most diverse fish family, which comprised 3023 available species (1782 valid species) belonging to 285 genera (Fricke *et al.*, 2023). It makes up roughly 4.91% of all fish species in the world and lives naturally in many kinds of locations. The most endemic species of Iraq's freshwaters, where native fish make up 52 valid species from 11 fish families, which are found in three dominant fish families including Cyprinidae (Freyhof *et al.*, 2021). Monoecious blood-feeding flatworms of the family Diplozoidae

(Monogenea: Polyopisthocotylea) are described as mandatory ectoparasites of the declared fish family. Diplozoids, such as *Eudiplozoon nipponicum* (Chmurciakova *et al.*, 2020), be conspicuous by their unusual coupling approach where two larval individuals (diporpae) throughout development mate and mature into a cross-like assembly (Valigurová *et al.*, 2011).

Concerning to the Japanese strain of *Diplozoon*, the preliminary explanation was completed by Goto (1891) through fish host species, namely *Carassius vulgaris* in Japan, at that moment the investigator nominated neither of the grouping of the sample nor the examined area. After that Khotenovsky (1981) emended this species to a novel genus, *Sindiplozoon* Khotenovsky, 1981., but currently, the genus *Eudiplozoon* contains only one species, which is namely as *E. nipponicum* (Nishihira & Urabe, 2020).

The morphological traits of a species are the most crucial factor in defining and classifying it. In specific, the size and shape of the attachment organ's sclerotized hard components are particularly significant for identifying species (Khang *et al.*, 2016), where the most common morphological feature of sclerotized parts are the dimensions of the median hooks and the four couples of clamps. Even more particularly, it has been established that the measurement of the central hook sickle length, the form of the frontal end of the middle plate, and the frontal joining sclerites of the clamps are the aspects that are most important for identifying species (Civáňová *et al.*, 2013).

The clamps progressively grow, and a substantial helpful association between the size and shape of the sclerites and the length of the host fish has been revealed. Unfortunately, these properties are unstable (Matejusová *et al.*, 2002). They also reside as ectoparasites on the common carp's gills, their mucosal surface is one of the primary fish immune defense systems, can be infested by the monogenean *E. nipponicum* in both larval and adult stages (Ilgova *et al.*, 2020). The parasite has a sophisticated digestive system that is suitable for hematophagous nourishing. It has the mouth aperture within conspicuous oral suckers, an eversible pharynx through surrounding glandular tissues, and a blind-ending intestine lined with caecum. The glandulo-muscular organs situated at the top of the organism, that open through the mouth angle which regarded as being a part of the digestive tract (Valigurová *et al.*, 2011).

Diplozoids are difficult to identify and then classify as species, therefore integrative techniques have recently been used more and more frequently (Koskova *et al.*, 2010; Hodová *et al.*, 2018). Both ribosomal nuclear DNA (rDNA) region and restriction fragment length polymorphism (RFLP) analysis have been used frequently to conduct the molecular investigation and the other aspects of an integrative strategy, on diplozoid monogeneans (Matejusová *et al.*, 2002; Matejusová *et al.*, 2004; Gao *et al.*, 2007; Civáňová *et al.*, 2013; Nishihira & Urabe, 2020). However, only a few species have been exposed to molecular comparisons up until now, which is why further methods would considerably progress the precise explanation of the systematics and lineages of diplozoids.

The lack of DNA sequence information for many species, often only one sequence for each species, and the predominance with one non-translating region of ribosomal marker (ITS2 rDNA), which is challenging to accurately alignment and indicates extreme divergence

among taxa, are barriers that have been reported. Correct systematic and DNA-based genetic analysis of the assemblage is problematic limited in species recognition (Hebert & Gregory, 2005). However, little evidence regarding diplozoid monogeneans abundances, and the variety of these species are there. A more common documentation of species identification, including genotypes, sequence structures, standard assortments in various sources, and aligned with the use of novel marker are more rigorous morphological characterization, and more systematic barcoding approaches, are thus recommended. The problems described here are not restricted to the study of the Diplozoidae members and may be useful for other groups as well (Dos Santos & Avenant-Oldewage, 2020).

E. nipponicum was recognized for the first time in Iraq by Al-Nasiri (2003) as *Diplozoon nipponicum* on the gills of *C. carpio*. The main goal of the current study is to molecularly characterize *E. nipponicum*, which parasitizes *C. carpio* in the Kurdistan region of Iraq, in order to distinguish it from other parasites that have been identified morphologically alone.

Materials & Methods

Description of study area

The study location is situated in the Altun Kopri subdistrict of northeastern Iraq on the edge of the Lesser Zab River (Prde). It originates from Iran and is placed between 34° to 36° north latitude and 43° to 46° east longitudes, 40 km northwest of Kirkuk City and 50 km from Erbil City as shown in Fig. 1 (Abdullah & Nasraddin, 2015).

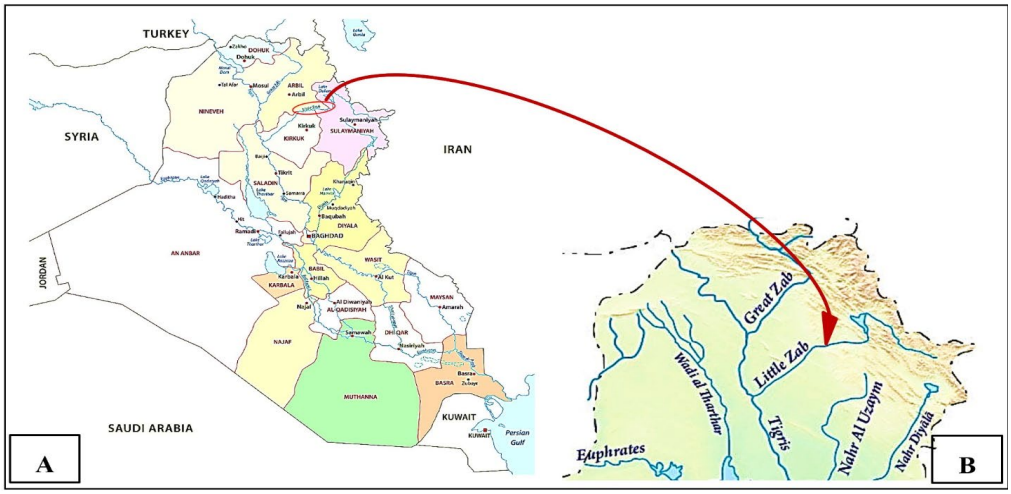

Fig. (1): A- Map of Iraq, display the parts of the country. B- Sample collecting zone, viewing Lesser Zab River at Altun-Kopri (34°-36° N Latitude and 43°-46° E Longitude)

https://www.worldatlas.com/maps/iraq, https://www.mapsofworld.com/iraq/river-map.html

Fish host and parasite collection

Throughout July to October 2022, a total of 138 common carps (*Cyprinus carpio*) were collected by local fishermen using gill nets then transported by means of cork container to the Laboratory of Zoology Research., Salahaddin University-Erbil, Iraq, at Science College, Department of Biology, within 24 to 48 hours of being caught, fish samples were dissected out. According to Coad (2010), all fish samples were classified as *C. carpio*, Family: Cyprinidae, and

the scientific names were taken from FishBase (Froese & Pauly, 2022).

The gills that had been removed from the examined fish were separated and put in a Petri dish with a little amount of tap water. Then they were rested microscopically to isolate the parasite. Gene marker 28S rDNA was used as marker gene for molecular analysis. With the assistance of a fine disposable pipette, at least five living *E. nipponicum* were taken from the water and retained for subsequent DNA extraction in an Eppendorf microcentrifuge tube containing nearly absolute (99%) ethanol.

Photos and measurements

For capturing images under a stereo- and light microscope, a Sony Xperia Camera Phone Version Z 2.0 with 21Mega Pixels was used. An ocular-stage micrometer in parallel with the software Image-J was used to measure the size of isolated parasites.

DNA extraction

An extraction kit was used to pick out the genomic DNA of isolated species (BIONEER, KOREA) with making slight adjustments in accordance with the company's guidelines (the lysis time period for the tissue was extended to 3 hours with 99% ethanol for DNA pelleting instead of isopropanol). The harvested diplozoids were ordered and softened by dipping them in Eppendorf tubes and transported to buffer tubes comprising 200 µL tissue lysis solution, then maintained in an incubator for 3 hours. Using NanoDrop (ND- 1000, USA), the DNA concentration quantity and quality were achieved. The amount of genomic DNA produced was larger than 0.5 g, and the ratio of (A260-320)/(A280-320) was greater than 1.5.

DNA amplification and sequencing

In order to use PCR to amplify a portion of the 28S rDNA sequence, universal primers were designed. Forward primer sequence C1 (5′-ACCCGCTGAATTTAAGCAT-3′) at location 25, and reverse primer sequence C3 (5′-CTCTTCAGAGTACTTTTCAAC-3′) at location 390 were selected by Mollaret *et al.* (2000) and expectable to be precise to flatworms (including monogeneans). Applied Biosystem (AB) MJ Research was used to achieve the thermal cycler PCR reaction and parameters. The volume of reaction mixtures was finalized as 50 µL prepared in a PCR tube with 2 µL extracted genetic materials (DNA), 25 µL OnePCR™ master mixes (GENEDIREX, KOREA), 1 µL for each primer (Forward and Reverse) and 21 µL double distilled water (ddH_2O). The conditions were used to conduct the thermal cycling: the primary denaturation was set as 94˚C for 5 min, followed by denaturation of 35 cycles at 94˚C for 45 sec, then annealing at 51˚C for 45 sec, after that prolonged at 72˚C for 45 sec, and the las extension was at 72˚C for 5 min. The PCR products were examined on 2% agarose gel electrophoresis, using observed under UV light and ethidium bromide to detect the bands. The predicted size of the PCR products was 365 bps. PCR product of 28S rDNA were sequenced via ABI 3130X nucleotide sequence analyzer (SINGAPORE). From the agarose gel, the parasite PCR fragments that served as the DNA template were removed and subjected to sequence-specific PCR amplification.

Phylogenetic study

The confirmed nucleotide sequence result of *E. nipponicum* 28S rDNA sequences were connected into the MEGA 11 software system

package (Tamura *et al.*, 2021) by Maximum Likelihood (ML) method. They were aligned with the available diplozoid sequences of BLAST by Clustal W alignment for building the evolutionary tree of development. The tree concerning the analysis of 15 monogenean taxa (13 diplozoid nucleotide sequences taken from BLAST alignment, one was the present sequence of the Iraqi isolate of *E. nipponicum* and the last one was *Gyroductylus carassii* used as an out group) from freshwater cyprinid fish. The relevant taxa's frequency of clustered trees was displayed next to the branches. The p-distance model, which measures distances in relations to base number changes per region, was used to compute the evolutionary relationship. The tree was automatically generated by using partial 28S rDNA, ITS-1, and COX-1 nucleotide sequences. Various techniques were applied to a matrix of pairwise distances calculated by means of the Tamura-Nei model, and the configuration with the highest log probability rate was then chosen. The final dataset contained 1984 positions altogether. The lengths of the branches match the predicted number of replacements per site. The tree matched the numbers along the branches, which stand for bootstrap values (1000 bootstraps). Above the clades, the rate of matching denotes on the trees in which the related taxa were clustered in the bootstrap values was displayed. To validate the validity of the inferred tree, bootstrap values were added, and Tamura's estimation of evolutionary divergence between sequences was computed according to Tamura *et al.* (2021).

Results & Discussion

The current study focuses on individuals that have permanently matching and considered the juvenile/adult forms under the name *Eudiplozoon nipponicum*. As in the other members of the family Diplozoidae, the frame of the *E. nipponicum* in the full-sized stage stereotypically appeared as x-symbol. This x-shaped stage is involved of two anterior bodies (fore-bodies), which are represented as the oral end and two posterior bodies (hind-bodies) along with the posterior haptors of the exhibition of two fused individuals (Fig. 2). On the other hand, the sucked blood accumulated as a red swelling and appeared from the hind bodies specially during feeding of the parasite on the gills of the host. The typical bilobular swelling appeared on both sides of the hind bodies, which is represented as a unique taxonomic character for the isolated species (Fig. 2A-C). The urinated region between the two permanently fused individuals is shown in Fig. (2 D). The most prominent regions from the hind bodies appeared as the intestinal canal, folding the region and four pairs of haptoral sections are shown in fig. (2E, and 2F). The detailed structural components of the buccal cavity are viewed from the forebody anterior region with emphasis on glandular constructions, in the region of the buccal space, localization of coupled oral suckers are shown (Fig. 2 G). The comprehensive magnified *E. nipponicum* hindbody with four pairs of clamp unit structure showing in fig. (2E and 2F).

Certain species belonging to diplozoids which were described previously in Iraq, all of them were identified according to their morphological characteristics and there is inadequate description. However, all subsequently researchers reported them as *E. nipponicum*.

E. nipponicum was recognized for the first time in Iraq by Al-Nasiri (2003) as *Diplozoon nipponicum* on the gills of *C. carpio* from a synthetic lake located adjacent Baghdad City.

After that *E. nipponicum* was reported from three different fish hosts in Iraq (Mhaisen, 2023). These were *Aspius vorax*, (=*Leuciscus vorax*) from the Tigris River passing through Tikrit City (Al-Jubori & Al-Nasiri, 2014), *Barbas sharpeyi* (= *Mesopotamicthys sharpeyi*) from Al-Husainia creek, north east of Karbala Province (Al-Saadi *et al.*, 2010) and *Planiliza abu* from Diyala River in Diyala Province (Mohammed, 2017). The results of the present study represent as the first recorded of *E. nipponicum* in the Kurdistan region of Iraq.

Epidemiologically, in addition to misidentification of the *Diplozoon* spp. from fishes in Iraqi literature review showed that, a total of 15 recognized diplozoids species from the genera *Diplozoon*, *Eudiplozoon*, and *Paradiplozoon* are now known from fishes of Iraq (Mhaisen & Abdul-Ameer, 2014). Since the identification of the first diplozoid species in Iraqi fishes was demonstrated by Rahemo (1980), several studies have been carried out in Iraq, which have supported to record more diplozoid species there. Although, According to Mhaisen (2023), the family Diplozoidae is represented from fishes of Iraq with one species of *Diplozoon*, two species of *Eudiplozoon* and 21 species of *Paradiplozoon* in addition to some unidentified species of *Diplozoon*. Although existing information on the DNA-based phylogeny of these taxa are insufficient, the 28S rDNA has been successfully employed to identify differences in DNA sequence among monogenean species (Singh & Chaudhary, 2010; Ahmadi *et al.*, 2017).

Regarding to the consequences of the existing study, beside of the morphological study, the findings of the research outcome is represented as a first molecular identification and phylogenetic characterization of isolated *E. nipponicum* in Iraq. On the other hand, this was joined to detect possible species polymorphism, and make accessible a "checkpoint" for upcoming molecular researches on the isolated species. Information on the isolated species, and the classification of them reported in the country were only depended on the basic structural traits. Consequently, the submissions of molecular depictions for the examined species are required, which will be such the first study in Iraq regarding to diplozoid monogeneans. The results also designated that the nuclear 28S rDNA unit is extremely well-preserved and then useful in systematic parasitic flatworm researches, including diplozoid monogeneans.

The chromatograms originate to release and evaluate the troubleshooting of DNA nucleotide sequences. The chromatogram displays separate four-colours, sharp and evenly set apart peaks that are exact-defined for very sensitive nucleotide sequencing results. Released sequence lengths were all about 333 bp long (Fig. 3). The harvested DNA nucleotide sequences of isolated parasite were placed to Basic Local Alignment Search Tool (the BLAST), and then linked with existing GenBank DNA sequences. The BLAST consequences exhibited 99.40% of identity percentage with the obtainable *E. nipponicum* DNA sequences at the National Center for Biotechnology Information (NCBI) as demonstrated in fig. 4).

In Platyhelminthes taxonomic identification, PCR-based techniques have shown that the sequences of 28S rDNA are a reliable way for recognizing the monogenean taxa and their phylogenetic origins (Koyee *et al.*, 2016; Koyee & Abdullah, 2019). Considering the structure and size of the attachment apparatus among taxa of diplozoids, there are merely minimal architectural variations which do not provide taxonomists with plenty information (Dos Santos & Avenant-Oldewage, 2020).

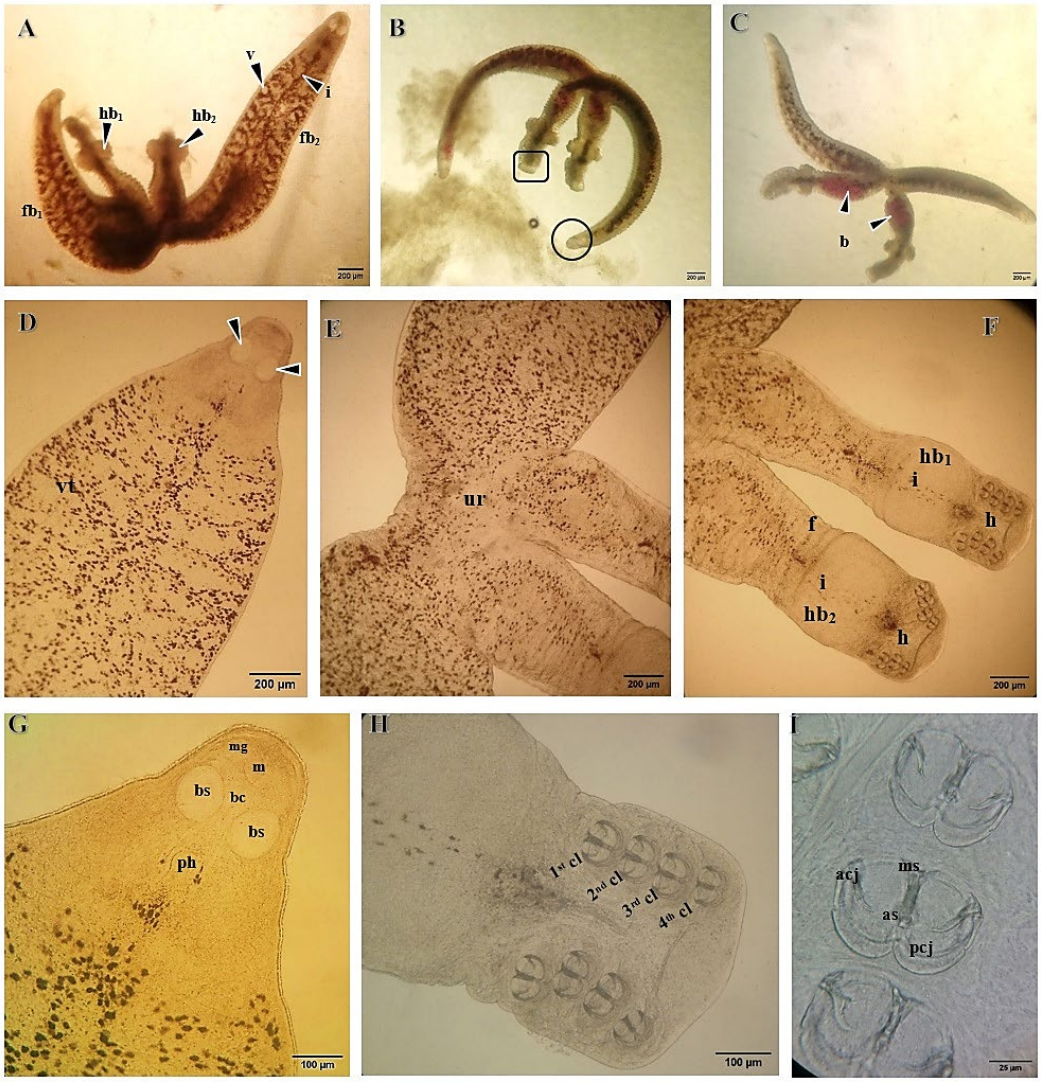

Fig. (2): Photomicrograph of the general and specific body part view in paired adults of the *Eudiplozoon nipponicum*

A- The hindbody (hb_1 and hb_2) bearing typical bilobular enlargement (arrowheads), with the two forebodies (fb_1 and fb_2), beside of intestine (i) and Vitellaria (vt).

B- Black circle indicates the zone of the anterior end with a mouth and the black square, the zone of the haptor.

C- Two arrows showed the accumulation of the sucked blood (b) within the internal organs of the hind bodies (hb).

D- Forebody anterior region microphotograph ventral view of the *E. nipponicum*, the two-arrows showed oral suckers.

E- Microphotograph of twin adult united region (ur) of *E. nipponicum*.

F- Microphotograph of hind bodies (hb_1 and hb_2) showed intestinal tract (i), folding region (f), and haptoral region (h) structure of *E. nipponicum*.

G- Specific organization microphotograph view of the forebody anterior region in *E. nipponicum*, with emphasis on glandular structures, in the area of the mouth cavity, showing localization of paired buccal suckers mg: musculo-glandular organs, m: mouth, bc: buccal cavity, bs: buccal sucker; ph: pharynx

H- Microphotograph of magnified *E. nipponicum* hindbody with four pairs of clamps (1^{st} – 4^{th} cl). Microphotograph of the clamp unit detailed structure of *E. nipponicum*, showing three units of clamps. acj: anterior clamp jaw; as: additional sclerite (posterior and anterior), ms: median sclerite; pcj and posterior clamp jaw

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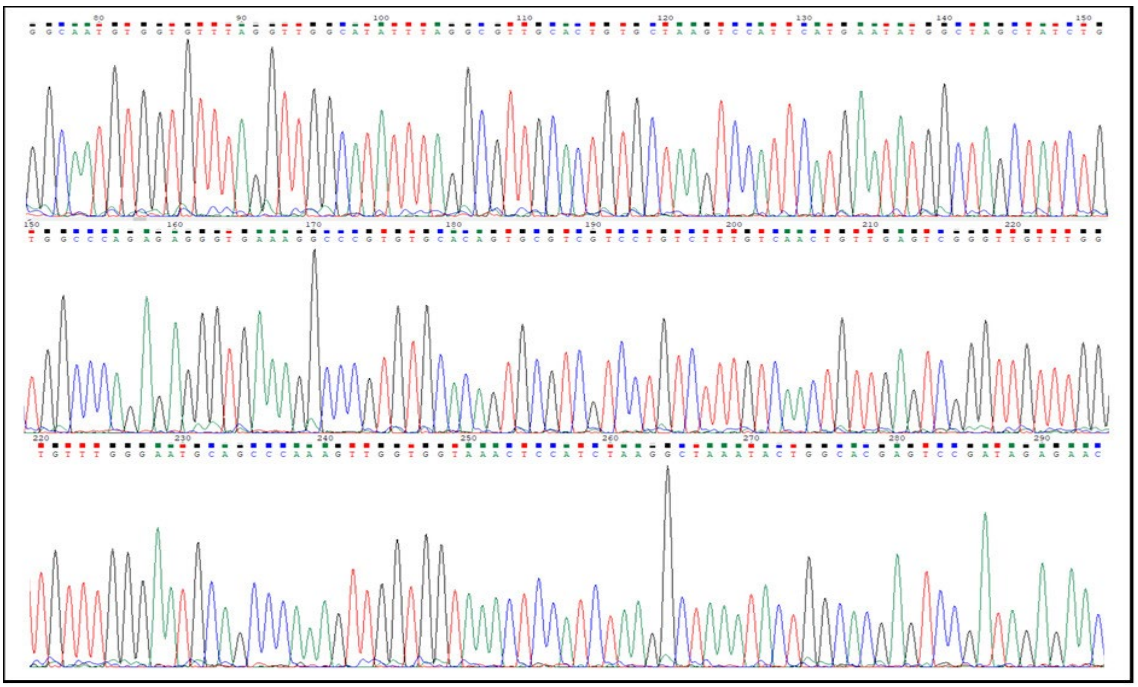

Fig. (3): The chromatogram of PCR products, sequence result from 28S rDNA extracts from *E. nipponicum*. Note the evenly- spaced peaks and the lack of "noise" (the baseline), represented as well-defined colour peaks

Score	Expect	Identities	Gaps	Strand
606 bits(328)	6e-169	333/335(99%)	2/335(0%)	Plus/Plus

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Query  1    TAACCAGG-TT-CCTTAGTAACGGCGAGTGAACAGGGATTAGCCCAGCACCGAAGCCTGC  58
            ||||||||  ||  ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  23   TAACCAGGATTCCCTTAGTAACGGCGAGTGAACAGGGATTAGCCCAGCACCGAAGCCTGC  82

Query  59   GGTCGTTTGGTCGTTCGGCAATGTGGTGTTTAGGTTGGCATATTTAGGCGTTGCACTGTG  118
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  83   GGTCGTTTGGTCGTTCGGCAATGTGGTGTTTAGGTTGGCATATTTAGGCGTTGCACTGTG  142

Query  119  CTAAGTCCATTCATGAATATGGCTAGCTATCTGGCCCAGAGAGGGTGAAAGGCCCGTGTG  178
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  143  CTAAGTCCATTCATGAATATGGCTAGCTATCTGGCCCAGAGAGGGTGAAAGGCCCGTGTG  202

Query  179  CACAGTGCGTCGTCCTGTCTTTGTCAACTGTTGAGTCGGGTTGTTTGGGAATGCAGCCCA  238
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  203  CACAGTGCGTCGTCCTGTCTTTGTCAACTGTTGAGTCGGGTTGTTTGGGAATGCAGCCCA  262

Query  239  AAGTTGGTGGTAAACTCCATCTAAGGCTAAATACTGGCACGAGTCCGATAGAGAACAAGT  298
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  263  AAGTTGGTGGTAAACTCCATCTAAGGCTAAATACTGGCACGAGTCCGATAGAGAACAAGT  322

Query  299  ACCGTGAGGGAAAGTTGAAAAGTACTCTGAAGAGA  333
            ||||||||||||||||||||||||||||||||||
Sbjct  323  ACCGTGAGGGAAAGTTGAAAAGTACTCTGAAGAGA  357
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Fig. (4): Pairwise alignment of *E. nipponicum* 28S rDNA sequence. Query is the sequence sample and Sbjct is the sequence that taken from GenBank. The identity percentage was shown as 99% with the sequenced ID AF382037.1. The only two deletions noticed from total 333 amplified bp

Relating molecular diagnostic techniques to phenotypic taxonomy, the former one has advantages over the later. They can use dependable methods which include a variety of well-studied molecular diagnostic aspects, including different rates of point mutation, or polymorphism (Dwivedi *et al.*, 2017). Concerning this, the abovementioned model approaches exclude morphological analysis. Investigations on 28S rDNA regions demonstrated a high degree of specific homology (Rana & Das, 2016; Dwivedi *et al.*, 2017).

The phylogenetic analysis via the Maximum Likelihood (ML)-technique established on the Tamura-Nei model and shows the pattern of branching with significant bootstrap support for the branches. Analysis of phylogenetic relationship stated the validation, and the systematic situation of isolated monogenean *E. nipponicum* belongs to the Diplozoidae, Fig. (5) designated the Maximum-Likelihood (ML) method and its phylogenetic position in comparison to other the phylogenetic position of it in comparison to the other diplozoids species. The above-mentioned (ML) technique showed a sister group form and closely comparable bootstrapped values (100%) between the isolated species of the current study and a partial 28S rDNA sequence of *E. nipponicum* (AF382037.1) on the gills of common carp of the Czech Republic (Olson & Littlewood, 2002).

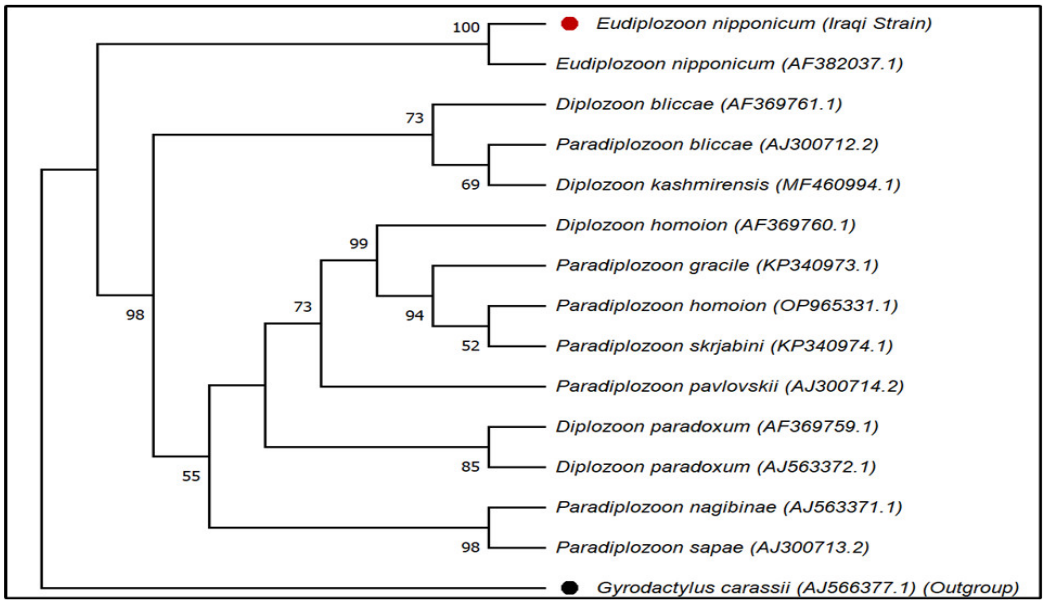

Fig. (5): Maximum Likelihood (ML) phylogram concerning the analysis of 15 monogenean taxa (13 diplozoid nucleotide sequences taken from BLAST alignment, one was the present sequence of the Iraqi isolate of *E. nipponicum* denoted as ared spot and the last one which was *Gyroductylus carassii* used as an outgroup represented as a black spot) from freshwater cyprinid fish. The corresponding taxa's percentage of clustered trees is displayed close to the clades. The tree was created automatically using partial 28S rDNA, ITS-1, and COX-1 sequence data. Different techniques were applied to a matrix of pairwise distances calculated using the Tamura-Nei model, and the configuration with the highest log likelihood value was then selected. The final dataset contained 1984 positions collectively. The lengths of the branches match the predicted amount of substitution per location. The tree was matching to the numbers along clades denote bootstrap values (1000 bootstraps). Evolutionary evaluates were directed in MEGA11 (Tamura *et al.*, 2021).

Different clades of diplozoids species have been seen in the tree topology of the species cluster. There were just two strain species in the first group (Iraqi isolated of *E. nipponicum* and those isolated from the Czech Republic) being bootstrapped 100%. Three species were grouped in the second category (*Diplozoon bliccae*, *D. kashmirensis* and *Paradiplozoon bliccae*) having bootstrap values between 69-73%. The third group included four species (*D. homion*, *Paradiplozoon homion*, *P. gracili* and *P. skrjabini*) having bootstrap values 52-99%. On the other hand, the combination bootstrap values regarding to the third and the fourth monotypic clade of *P. pavlovskii* was 73%. The fifth clade denoted the presence of two closely sister strains of *D. paradoxum* in the light of the ML phylogenetic tree and they have the identical situation in the clades with a totally and closely associated strains having 85% bootstrap value. As opposed to that, *P. negibinae* and *P. sapae* were constructed phylogenetically in the sixth clade with 98% bootstrap value.

Due to the fact that different researchers have sequenced the ribosomal region's domains, which are not completely equivalent, the 28S rDNA of diplozoids has distinct "marker segments" in the sequences that are now accessible. It is difficult to say if this indicator would demonstrate adequate determination to investigate monogenean diplozoids variety because of the variable 28S coverage of sequences from various species and researchers, the irregular length of the sequence (351-1133 bp), and the restricted number of species for which 28S rDNA sequence available (Dos Santos & Avenant-Oldewage, 2020).

Additionally, it has been noted that *Paradiplozoon kashmirense* is a synonym of *Eudiplozoon nipponicum* (Pandey, 2010) and *E. kamegaii* (Nishihira & Urabe, 2020). Given the specificity of the *P. kashmirense* sequences that are currently accessible and their significant evolutionary distance from those of *E. nipponicum*, it seems possible that either the documentation or the synonymizing with *E. nipponicum* were inaccurate. This specifies that there is still require several works to be done in order to adequately represent diplozoids species from the subcontinent in systematic and scientific studies (Dos Santos & Avenant-Oldewage, 2020).

It was established that the ribosomal DNA constituent is the most valid technique for parasitic identification and classification since it occurs frequently throughout many eukaryotes' genetic material as a multi-sequence repeating group. It probably includes regions that are extremely polymorphic and protected (Susurluk *et al.*, 2007). However, taxonomy has certain limitations with conventional morphology-based identification. Additionally, even though the number of studies using DNA markers has increased, it is not completely out of error (Patwardhan *et al.*, 2014; Dos Santos & Avenant-Oldewage, 2020). On the other hand, in addition to accurate validation of species identification, molecular techniques are used in phylogeny. It is important to mention that nuclear rDNA is used to resolve helminthic parasite, taxonomic complications (Dodangeh *et al.*, 2017; Dutra Vieira *et al.*, 2017; Mohanta & Itagaki, 2017).

Conclusion

According to the recent findings, the study is recognized as the first extensive morphological and genomic-based assessment in the considered area for characterizations of monogenean parasites in accordance with the 28S rDNA sequences, which demonstrate to be an effective indicator for distinguishing *E. nipponicum*. The conventional morphological

studies and validation of such parasites are especially problematic; hence the DNA-based method was applied in combination to morphological-based classification as a valued model to discriminate novel species of monogenean parasites.

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Contributions of authors

Q.M.K.: Sample collection, laboratory techniques, manuscript writing and revising.

S.M.A.: Suggestion the title of the research, manuscript writing and revising, with morphological identification of the parasite.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical approval

All ethical guidelines related to fish and care issued by national and international organizations were implemented in this study.

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أول توصيف مظهري وجزيئي (**28S rDNA**) للنوع ***Eudiplizoon nipponicum*** (أحادية المنشأ، عائلة دبلوزويدي) المتطفل على الكارب الإعتيادي، إقليم كردستان، العراق

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المستخلص: تعد الدراسة الحالية هي أول وصف مظهري وجزيئي لأحادي المنشأ *Eudiplozoon nipponicum* المتطفل على خياشيم (غلاصم) سمكة الكارب الاعتيادي *Cyprinus carpio* والتي جمعت من نهر الزاب الصغير عند مدينة التون كوبري في شمال شرق العراق، خلال المدة من شهر تموز الى شهر تشرين الاول ٢٠٢٢. نقلت الاسماك إلى مختبر أبحاث علم الحيوان، جامعة صلاح الدين- أربيل، العراق. استهدفت معظم الدراسات السابقة المتعلقة بالطفيلي المعني بتحليل البيانات المظهرية. ومع ذلك ، فإن دراسة تسلسل الحمض النووي عادة ما يكون داعماً لهذه الدراسات، لأنها تحتوي على جوانب غائبة عن البحث الصرفي. كان الهدف الرئيسي هو التعرف على التصنيف الجزيئي للطفيلي *E. nipponicum* باستخدام منطقة 28S rDNA النووية من خلال فحص تسلسل النوكليوتيدات وتسلسل تفاعل البوليمير المتسلسل. تم الحصول على التسلسلات ومقارنتها بتسلسلات بنك الجينات المتاح عبر الموقع الالكتروني التي يمكن الوصول إليها. تبرر النتائج التحقق من ان *E. nipponicum* في العراق باستخدام تقنيات الطفيليات التقليدية (الفحص المظهري) والحديثة (الجزيئية). بينت النتائج وجود تطابقا بنسبة ٩٩ % مع سلسلة DNA لطفيلي *E. nipponicum* من بنك الجينات. تعتبر الدراسة الحالية هي أول الدراسات المظهرية والجزيئية في المنطقة لطفيلي قيد الدراسة. ايضا تمت دراسة العلاقة التطورية باستخدام طريقة الاحتمالية القصوى (ML). تم الاستنتاج أنه بجانب التوصيف المظهري لهذا الطفيلي ان تسلسل PCR يعد كطريقة جزيئية مناسبة للتعرف على انواع أحادية المنشأ على خياشيم الأسماك.

الكلمات المفتاحية: سمكة الكارب الاعتيادي ، *Eudiplozoon nipponicum*، غلاصم، العراق، جزيئي، مظهري.