Study on the Cestode *Postgangesia inarmata* from the Silurid Fish *Silurus glanis* from Kurdistan Region, Iraq

Samir J. Bilal and Shamall M.A. Abdullah

Department of Biology, College of Education, University of Salahaddin, Erbil, Iraq
e-mail: shamall_m_a_abdullah@yahoo.com

Abstract. A total of 48 specimens of the catfish *Silurus glanis* were collected from Greater Zab River as well as 36 specimens from the Lesser Zab River, Kurdistan Region, north of Iraq. The examination of fishes cleared the presence of the cestode *Postgangesia inermata* which was identified by using compound light microscope and scanning ultrastructure microscopy. Also histological sections were prepared and a molecular study was performed by amplification and sequencing of 1srDNA.

Keywords: *Postgangesia inermata*, *Silurus glanis*, Ultrastructure, Histology, Molecular study.

Introduction

Tapeworms of the genus *Postgangesia* Akhmerov, 1969 (Cestoda: Proteocephalidea) are parasitic in freshwater fishes, especially in Siluridae from Russia and Iraq (5). Members of this genus are characterized by having an apical organ, scolex and neck with spines, ovary is bilobed and massive, uterus with lateral diverticula, outgrowths begin anteriorly, vitellaria are lateral in cortex, testes, ovary and uterus medullary and genital pores are median (8).

In Iraq, only one species was recorded namely *P. inermata* de Chambrier, Al-Kallak & Mariaux, 2003 which was described as a new species from *Silurus glanis* from Tigris River near Mosul city (11).

The present study was planned to investigate *P. inermata* from *S. glanis* from Greater Zab and Lesser Zab rivers. The investigation includes its morphology, surface ultrastructure and histological structure.

Materials and Methods

A total of 48 specimens of the catfish *Silurus glanis* were collected from Greater Zab River as well as 36 specimens from the Lesser Zab River by fisherman by using cast nets and gill nets, during the period from December 2010 until the end of December 2011. Fishes were kept in a cool box with river water and transferred alive to the laboratory, and identified according to Coad (4). The fishes were opened from the ventral side. The gastrointestinal tract was dissected out from the rectum to the esophagus and opened longitudinally and examined carefully for cestodes (2).

A- **Light Microscopy (LM):** The samples of cestodes for light microscopy were handled according to Scholz & Hanzelová (16), as follows: Specimens were stained with Mayer's hydrochlorid carmine, destained in 70% acid ethanol (i.e. ethanol with

several drops of HCl), dehydrated through a graded ethanol series, cleared in clove oil and mounted in Canada balsam as permanent preparations.

B- **Histological Examination:** According to Scholz & Hanzelová (16), the specimens were prepared for histological studies as follows: Pieces of strobila were embedded in paraffin wax, sectioned at 8-10 µm (longitudinal sections of strobila), stained with hematoxylin-eosin dye and counterstained with 1% acidic eosin B solution. Illustrations were made using a drawing attachment for an Olympus BX51 microscope with the use of Nomarski differential interference contrast. Measurements were taken with the aid of analysis B v.5.0 software.

C- **Surface Ultra Structure, Scanning Electrone Microscopy (SEM):** Samples were prepared following Scholz & Hanzelová (16). Specimens were fixed and preserved like that used for L.M., specimens transfered from 70% ethanol to 80%, 96% and 100% ethanol (twice) for at least 20 minutes for each one. Chemical method was used for drying of tapeworms by using Hexamethyldisilazane, HMDS. Specimens were covered with this material for 5-10 min. Finally, when the specimens were totally dried, were embbeded on the target and sputter-coated with 20-25 nm of gold, in embbeding chamber of gold plating (7). Specimens were examined by using a JEOL JSM-7401F scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 4 kV GB low linked to an external computer system. Each specimen was observed with focus on the morphology of the scolex.

D- Molecular Study (DNA Sequencing)

DNA Extraction

In order to assess DNA sequences of cestodes collected, a total of eight specimens, fixed alive in 99% ethanol, were collected from *S. glanis* of the two studied rivers and analyzed molecularly. The genomic DNA was isolated by using Phenol-Chloroform protocol, according to Posada & Crandal (14).

DNA Amplification

For phylogenetic studies, the D1-D3 large subunit nuclear ribosomal RNA gene (lsrDNA) or (28S rDNA) region was amplified by PCR 1550-1570 bp with (LSU5) forward primer (TAGGTCGACCCGCTGAAYTTAAGC) and (1500R) reverse primer (GCTATCCTGAGGGAAACTTCG), gene was amplified by using the following PCR conditions: denaturation for 5 minutes at 94 C°, followed by 35 cycles of 30 s at 94 C°, 30 s at 55 C°, 2 minutes at 72 C° and completed by 7 minutes at 72 C°(3, 9, 13).

Gel Electrophoresis

All products that came out from PCR machine were verified on a 1% agarose gel which was prepared as follows: The procedure for electrophoreses DNA on a 1% agarose horizontal slab gel was performed as follows: in the present study 80 V for 30 minutes were used (12, 20).

DNA Sequencing

1- Purified PCR products were BigDye® Terminator v3.1 cycle sequencing kit and PRISM 3130xl automatic sequencer (Applied Biosystems) were used for bidirectional sequencing of the PCR products using the set of PCR and internal sequencing primers (3).

2- Sequences were assembled and inspected for errors in Geneious Pro 5.3.6, according to Drummond *et al.* (6), aligned using the E-INS-i algorithm of the program MAFFT (7) and the ambiguously aligned positions were manually excluded from resulting alignments in MacClade 4.08 as shown in Maddison & Maddison (10).

3- The phylogenetic relationships were evaluated under the maximum likelihood (ML) criteria in the program RAxML ver. 7.2.8-ALPHA (18, 19), employing the GTR+Γ substitution model. All model parameters and boot strap nodal support values (1000 repetitions) were estimated using RaxML (21). The resulted sequences were blusted with sequences of Gene Bank online at Clustal W. The sequences of studies specimens were named Query and the Gene Bank sequences were named Subject.

Results and Discussion

S. glanis were surveyed for cestodes during the period of the present study. The survey showed the occurrence of *P. inarmata* in their intestine with 41.7% prevalence of infection and 1.63 mean intensity.

Description: Medium sized worms, dorsoventrally flattened, body 38.34-82.21 mm long, 2.49-2.54 mm wide. Strobila slightly craspedote with superficial transverse folds. Mature proglottids, 0.213-0.310 mm long and 1.276-1.514 mm wide (Fig. 1C, 2B). Gravid proglottids, 0.395-0.517 mm long and 2.040-2.223 mm wide (Fig. 1D, 2C)**.** Scolex small, 0.406-0.432 mm long and 0.390-0.398 mm wide, with four uniloculated suckers (Fig. 1A, 2A), covered entirely with spiniform microtriches, 0.0026-0.0033 mm long, extending beyond level of posterior margin of suckers (Fig. 4B). Suckers 0.129-0.184 mm long and 0.132-0.168 mm wide. Rostellum-like apical organ, 0.158-0.207 mm in diameter, scolex pierced basally by numerous microscopic openings in several rows (Fig. 1A, 2A). Proliferation zone long, up to 3 mm (Fig. 1A). Internal longitudinal musculature dense, forming anastomosed longitudinal bundles, uninterrupted laterally at level of vitelline follicles. Secondary muscle layers in cortex not clearly delimited (Fig. 3A, 3B).

Testes medullary, spherical to ovoid, 118-147 in number, arranged in two dorso-lateral fields united anteriorly, separated from vitelline fields by osmoregulatory ducts (Fig. 1C, 2B). Genital pore irregularly alternated. Genital atrium absent, cirrus pore and vaginal pore separated and mostly the second (about 80% of proglottids) located anterior to the first (Fig. 1C, 1D). Cirrus pouch ovoid to sub-spherical, 0.152-0.232 mm long, thick-walled at its base. Cirrus without spines. Ejaculatory duct coiled, thin. Vas deferens coiled, between base of cirrus pouch and median part of proglottd (Fig. 1C, 2B, 3A).

Ovary medullary, massive, bilobate, 0.351-0.548 mm long and 0.706-1.237 mm wide, with few dorsal and ventral lobules. Vitelline follicles paramuscular, arranged in a pair of longitudinal rows on each side of internal longitudinal musculature, it locks slightly dorsally in transverse sections, elongated nearly the entire proglottid length (Fig. 1C, 2B, 3A, 3B). Vagina 0.663-0.820 mm long, with thickened terminal portion composed of dense, non-muscular chromophil cells (Fig. 1C, 2B, 3B). Vaginal pore funnel-shaped in gravid proglottids. Uterus medullary, 0.219-0.288 mm long, preformed in immature proglottids.

Eggs shed through 3-4 pore-like uterine structures (Fig. 1C, 2B, 3B). Oncospheres spherical, 0.0237-0.0267 mm long and 0.0129-0.145 mm wide. Ventral and dorsal osmoregulatory ducts without anastomoses, situated between vitelline follicles and testes, sometimes overlapping latter. Ventral osmoregulatory ducts up to twice the width of dorsal ducts.

During the examination of transverse sections of mature proglottid, the rectator muscles appear very good developed but internal longitudinal musculature appear to be lost or interrupted (Fig. 3A, 3B).Testes medullary, vitelline follicles paramuscular, uterus central, vagina thick walled at the vaginal opening due the presence of chromophore cells (Fig. 3A). Cirrus pouch with thick wall at the base and surrounded with well developed musculature (Fig. 3B). In fully gravid segment, the entire space is filled with uterus that harbors the onchospheres (Fig. 3C).

From scanning electron microscopy of the scolex of this species, microtriches were noticed clearly covering the entire scolex including suckers (Fig. 4A). The microtriches of the present cestode are spine-like in shape with acute distal end (Fig. 4B). These features were also noticed by de Chambrier *et al.* (5).

According to molecular examination of this parasite it shows 99% resemblance with the sequences of *P. inarmata* from the gen bank (Appendix 1, Plates 1 & 2), even there are some differences between the present specimens with that of de Chambrier *et al.* (5), like the shape of cirrus pouch which is ovoid to sub-spherical in specimens of the present study, whereas, its ovoid to elongated in the original paper.

This parasite was described as a new species by de Chambrier *et al.* (5) in *S. glanis* from Tigris River in Mosul city. The present study represents the first recording in Kurdistan Region.

Rahemo & Al-Niaeemi (15) described *Proteocephalus hemispherous* from the intestine of *S. glanis* in the Tigris River at Mosul, Iraq. They distinguished it from *P. osculatus* in possessing a large and well-developed apical organ, nearly square mature proglottids and the shape of the ovary. Conspecific tapeworms were misidentified as *Silurotaenia siluri* from *S. triostegus* from Diyala River by Ali *et al.* (1), despite the absence of any spines on the apical organ. *P. hemispherous* is undoubtedly a member of the Gangesiinae, based on the morphology of the scolex and strobila. It may well

be conspecific with *Postgangesia inarmata* described from the same fish host (*S. glanis*) in Iraq.

However, there is a marked difference in the number of testes between these taxa (70-80 in *P. hemispherous* versus 115-151 in *P. inarmata*) and the apical organ of *P. hemispherous* appears to be much deeper than that of *P. inarmata*, in which it is flattened (5, 15). Therefore, according to Scholz *et al.* (17), *P. hemispherous* is transferred to *Postgangesia* as *Postgangesia hemispherous*. Its possible conspecificity with *P. inarmata* should be verified on the basis of comparison of the type or voucher specimens of both the taxa.

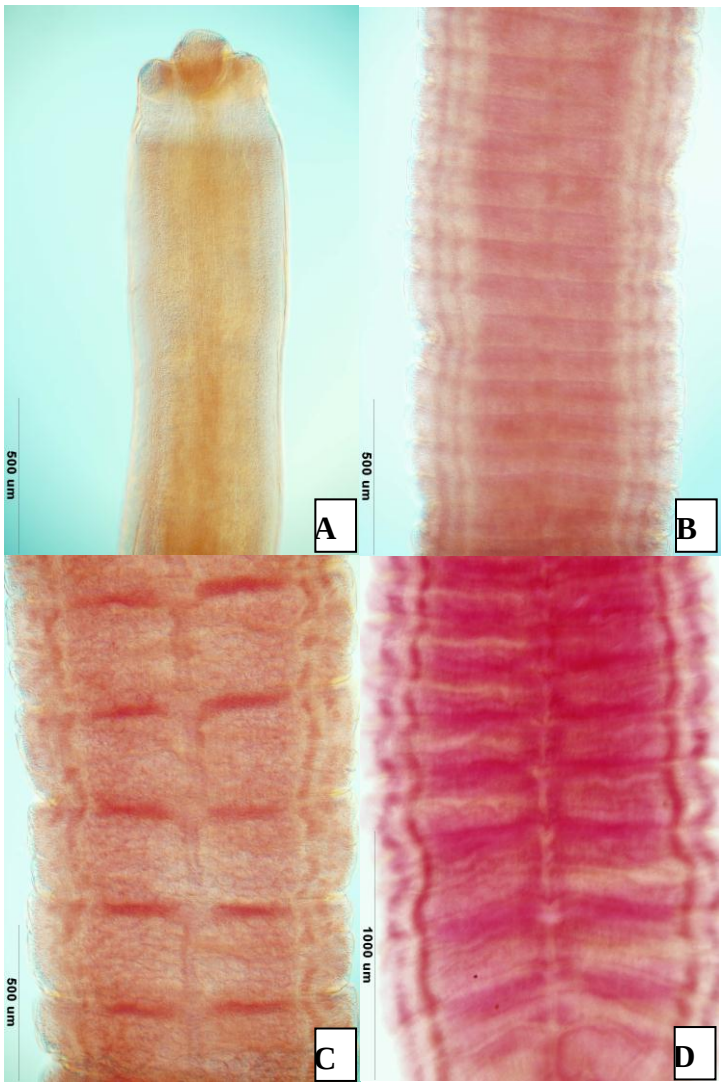


Fig. 1: Photomicrograph of *Postgangesia inarmata*. A- Scolex, B- Immature proglottid, C- Mature proglottid, D- Gravid proglottid.

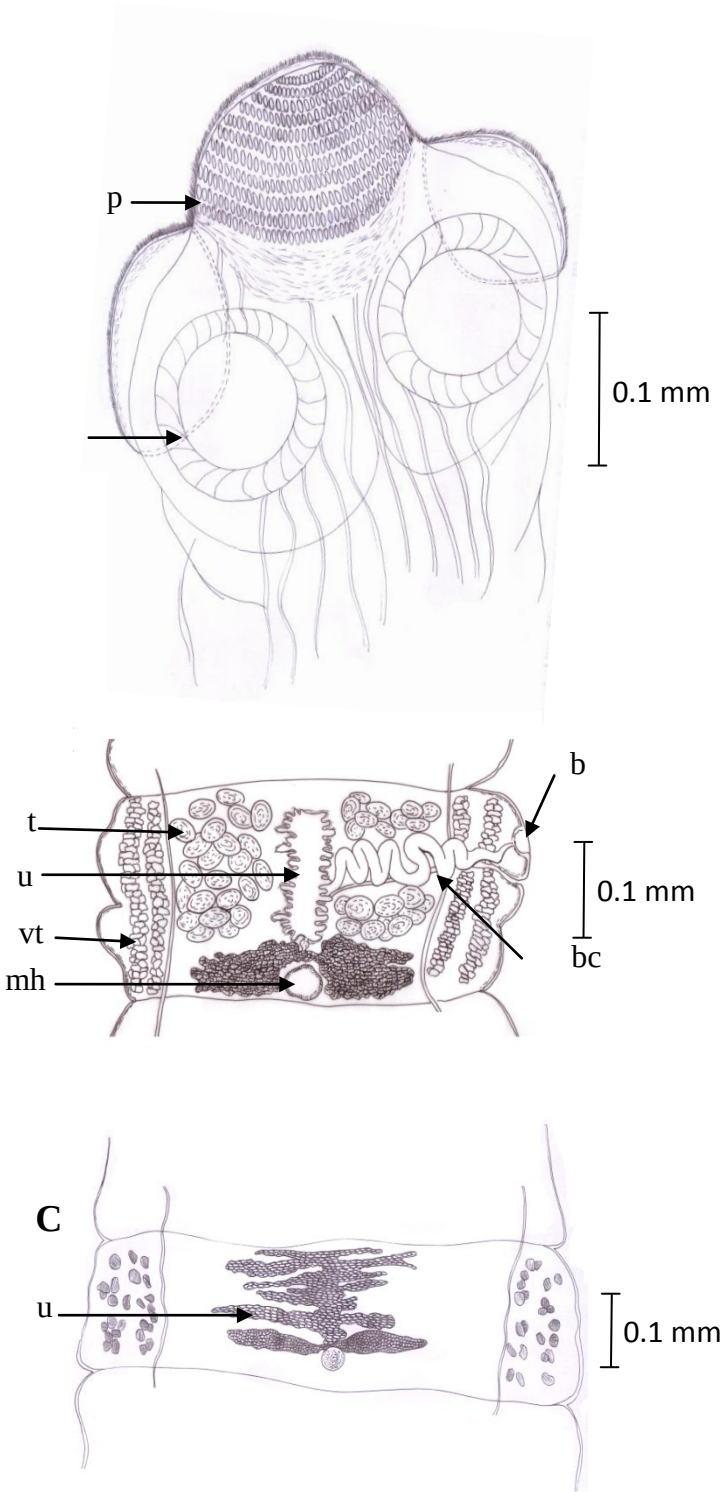


Fig. 2: Lucida drawings of *Postgangesia inarmata*. A- Scolex, B- Mature proglottid, C- Gravid proglottid.
Abbreviations: bc= birth canal, bp= birth pore, m= Mehlis' gland, p= probocics, s= sucker, t= testes, u= uterus, vt= vitellaria.

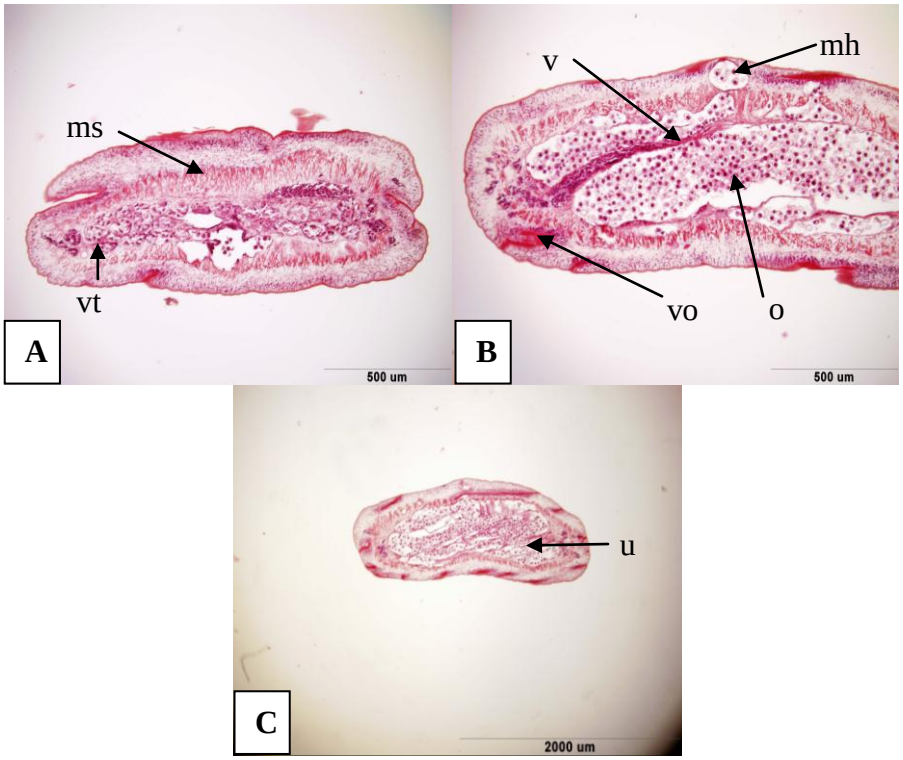


Fig. 3: Photomicrograph of *Postgangesia inarmata* sections. A- Cross section of mature proglotid through ovarian region, B- Cross section of mature proglottid through vaginal region, and a part of cirrus sac appeared, C- Cross section of gravid proglotid.
Abbreviations: mh= Mehlis' gland, ms= muscles, o=ovary, p= probocics, v= vagina, vtvo=vaginal opening. u=uterus, vt= vitellaria.

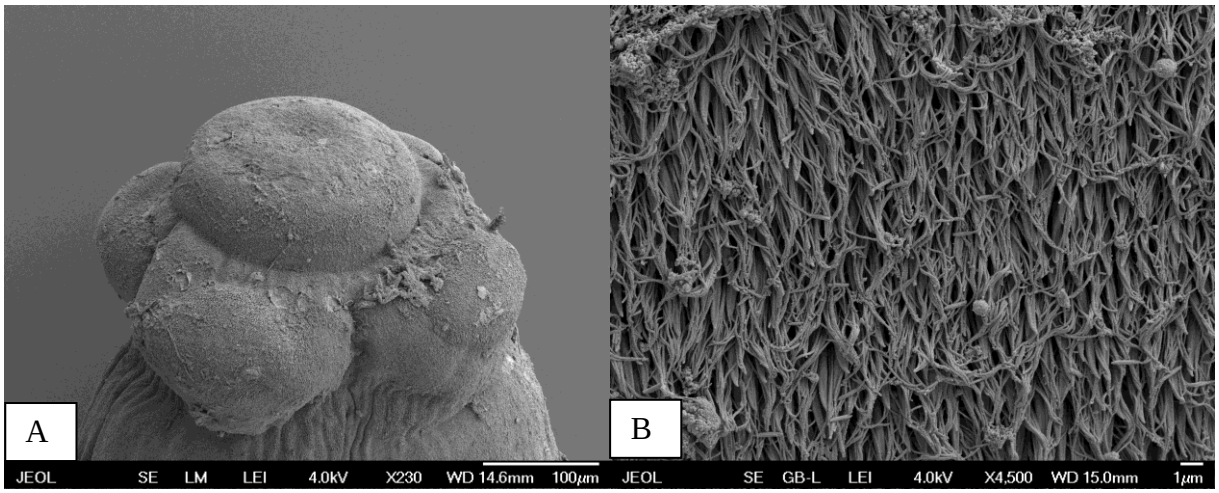
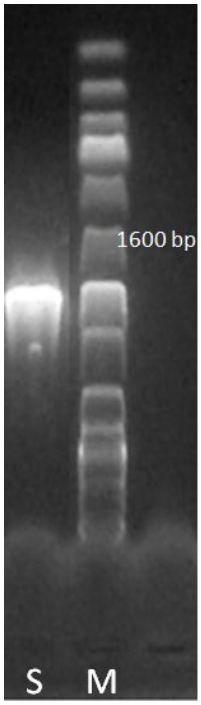


Fig. 4: Scanning electron micrograph of *P. inarmata*. A- Scolex, B- Michrotriches from scolex surface.

Appendix 1 (Plate 1): Sequences of *P. inarmata* from *S. glanis*. >IRQ33

TAGGTCGACCCGCTGAATTTAAGCATATCACTAAGCGGAGGAAAAGAAACTAACCAGGATT
CCCCTAGTAACGGCGAGTGAAGAGGGAAGAGCCCAGCACCGAAGCCTGCGGCAGTTTTGCT
GCTAGGCAATGTGGTGTTTGGGTCGGCTCGTGGGACCGCCACTCCACTCGAAGTCCAGCAT
TGAGTATGGTTACTGGATTTGGCCCAGAGAGGGTGAAAGGCCCGTACGGGTGGAGGTTCAG
ACATGTAAGGCGGTTCACCAGGTCGGCCTTAGAGTCGGGTTGTTTGGGAATGCAGCCCAAA
GTGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGAGTCCGATAGCGAACAAGTACC
GTGAGGGAAAGTTGAAAAGTACTCTGAARARAGAGTAAACAGTACGTGAAACCGCATGCA
GGTAAACGGGTGGCGTCAAGCTGCAAGCCCGGAGGATTCAGCCAGCTAGGATGTTGTGTAT
GCGCCTGGCGCATCTATCAGTCGGAGTATGATTGGATAGTCCACCGGGAGACGGTGGGTCT
GGCCGCAAGGTCAGGATATGTGTACCGGGTGGGTGCCGGAGCATGCTATTCGTCTGGGGGC
TGTCTAGCTGGTGCACTTTCTCCGTGGTGAACACCACGACCGGTGGAATTGCCAGTCTGCTG
TGTCCAAGTCGTGTTTGGTTGGTCCTTGTGGCTAATTGGGTGCGATCACAGGCAAACTTCTC
AGTAAACGGCGTAGAGGTGTTTCGGCATCTTTGCGTGTCATCGGCTACTGGTTGTCAACGGG
CCTGCTCAGTGTTTGTTGTAAATGCTGCCGACATTGAGTGGTCTGGTGGGGCATGGTGGTAA
GAAAAACTGTGCAAGGCACCGGGGTTATCGGCCTCAAATGTTGCATCACGCGCCCATGTTA
CAAATGGCTTWGTGGCGGTGCTATTGCTGTTTGCCCGATGTTGAGTGTGATTGTCGTGTCGC
CTGCAAAAAGTAGGTCCGGCGGTGGCTTAATTCGGGATGAACAGTGGATGGTGTTGTCAGT
GTGGGATGTGGTGGGCCAAATAGTCAGTGGTGTAGTGGTAGACGAGCTACCCGACCCGTCT
TGAAACACGGACCAAGGAGTTTAACATGTATGCGAGTCAATGGGCCTTACGAAACCCAAAG
GCGCAGTGAAAGTGAAGCTTCGACTCGTCTCGAAGTGTGGTGAGATCCTGCTGTTACTCGC
ATCAGTCTGTGTGTCAGCATCAGGCTACTAAGAGCAGTGGGCGCATCACCGGCCCGTCCCA
TGATGTGGTCATTGGATATTGTCTTCTGTGGTTAGTCCTGCTCTAGCAGTGGTGGCTGCCAT
GGTGGTGCCAGTGCGTCATCGGGGCGGTGCATGAGCATACACGTTGAGACCCGAAAGATGG
TGAACTATGCTTGCGTAGGTTGAAGCCAGAGGAAACTCTGGTGGAGGACCGCAGCGATTCT
GACGTGCAAATCGATCGTCAAACGTGAGCATAGGGGCGAAAGACTAATCGAACCATCTAGT
AGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGC



Appendix 1, (Plate 2): The D1-D3 large subunit nuclear ribosomal RNA gene (lsrDNA) or (28S rDNA) region was amplified by PCR (1550-1570 bp) with (LSU5) forward primer (TAGGTCGACCCGCTGAAYTTAAGC) and (1500R) reverse primer (GCTATCCTGAGGGAAACTTCG), amplified DNA of *Postgangesia inarmata* from *Silurus glanis*.

References

1- Ali, N.M.; Al-Jafery, A.R. & Abdul-Ameer, K.N. (1987). Parasitic fauna of freshwater fishes in Diyala River, Iraq. J. Biol. Sci. Res., 18(1): 163-181.

2- Amlacher, E. (1970). Textbook of fish diseases (Engl. Transl.). T.F.H. Publ., Jersey City: 302pp.

3- Brabec, J.; Scholz, T.; Králová-Hromadová, I.; Bazsalovicsová, E. & Olson, P.D. (2012). Substitution saturation and nuclear paralogs of commonly employed phylogenetic markers in the Caryophyllidea, an unusual group of non-segmented tapeworms (Platyhelminthes). Int. J. Parasitol., 42: 259-267.

4- Coad, B. W. (2010). Freshwater fishes of Iraq. Pensoft Publisher, Sofia: 274pp + 16pls.

5- de Chambrier, A; Al-Kallak, S.N.H & Mariaux, J. (2003). A new tapeworm, *Postgangesia inarmata* n. sp. (Eucestoda: Proteocephalidea: Gangesiinae), parasitic in *Silurus glanis* (Siluriformes) from Iraq and some comments on the Gangesiinae Mola, 1929. Syst. Parasitol., 55: 199-209.

6- Drummond, A.J.; Ashton, B.; Buxton, S.; Cheung, M.; Cooper, A.; Heled, J.; Kearse, M.; Moir, R.; Stones-Havas, S.; Sturrock, S.; Thierer, T. & Wilson, A. (2010). Geneious V5.3. Available from http://www.geneious.com.

7- Katoh, K.; Kuma, K.; Toh, H. & Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res., 33: 511-518.

8- Khalil, L.F.; Jones, A. & Bray, R.A. (1994). Keys to the cestode parasites of vertebrates. CAB Intr., Wallingford: 751pp.

9- Littlewood, D.T.J.; Curini-Galletti, M. & Herniou, E.A. (2000). The interrelationships of Proseriata (Platyhelminthes: Seriata) flatworms tested with molecules and morphology. Mol. Phylogenet. Evol., 16: 449-466.

10- Maddison, D.R. & Maddison, W.P. (2005). MacClade 4: Analysis of phylogeny and character evolution. Version 4.08a. http://macclade.org.

11- Mhaisen, F.T. (2013). Index-catalogue of parasites and disease agents of fishes of Iraq, (Unpublished: mhaisenft@yahoo.co.uk).

12- Murray, C. & Murray, S. (1975). Genome DNA gell elecrtophoresis. J. Mol. Biol., 98: 551.

13- Olson, P.D.; Cribb, T.H.; Tkach, V.V.; Bray, R.A. & Littlewood, D.T.J. (2003). Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int. J. Parasitol., 33: 733-755.

14- Posada, D. & Crandal, K.A. (1998). Model test: testing the model of DNA substitution. Bioinformatics, 14: 817-818.

15- Rahemo, Z.I.F. & Al-Niaeemi, B.H.S. (2001). A new cestode species from a freshwater catfish. Riv. Parasitol., 18(62) No. 1: 71-74.

16- Scholz, T. & Hanzelová, V. (1998). Tapeworms of the genus *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae), parasites of fishes in Europe. Studie AV ČR, Academia Praha 2/98: 119pp.

17- Scholz, T.; Hanzelova´, V.; Škeříkova´, A.; Shimazu, T. & Rolbiecki, L. (2007). An annotated list of species of the *Proteocephalus* Weinland, 1858 aggregate sensu de Chambrier *et al.* (2004) (Cestoda: Proteocephalidea), parasites of fishes in the Palaearctic Region, their phylogenetic relationships and a key to their identification. Syst. Parasitol., 67: 139-156.

18- Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22: 2688-2690.

19- Stamatakis, A.; Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol., 57: 758-771.

20- Thomas, M. & Davis, H. (1975). Agarose gel electrophoresis of DNA horizontal and vertical. J. Mol. Biol., 91: 315.

21- Wicht, B.; Yanagida, T.; Scholz, T.; Ito, A.; Jiménez, J. & Brbec, J. (2010). Multiplex PCR for differential identification of broad tapeworms (Cestoda: *Diphyllobothrium*) infecting humans. J. Clin. Microbiol., 48(9): 3111-3116.

دراسة على الدودة الشريطية *Postgangesia inarmata* من أسماك الجري الأوربي *Silurus glanis* في إقليم كوردستان، العراق

سمير جودت بلال وشمال محمد أمين عبدالله

قسم علوم الحياة، كلية التربية، جامعة صلاح الدين، أربيل، إقليم كردستان، العراق

الخلاصة. تم جمع 48 سمكة من نوع الجري الأوربي *Silurus glanis* من نهر الزاب الكبير و 36 نموذجا من نهر الزاب الصغير في إقليم كوردستان، شمال العراق. أوضح فحص هذه الأسماك وجود الدودة الشريطية *Postgangesia inermata* والتي شُخصت باستخدام المجهر الضوئي المركب والمجهر الإلكتروني الماسح، كما تم أيضا تحضير المقاطع النسيجية من الديدان وإجراء الدراسة الجزيئية بتضخيم ودراسة تسلسل الجين 1srDNA.