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# MOLECULAR IDENTIFICATION AND PHYLOGENETIC-TREE ANALYSIS OF *MONIEZIA* SPECIES FROM SHEEP IN AL-DIWANIYAH CITY

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## ABSTRACT

The present study was performed to detect the molecular and the phylogenetic identification of species that belonging to the genus of *Moniezia* Blanchard, 1891 which affected intestines of sheep in Al-Diwaniyah city, Iraq; fifty intestine samples were sought for the infestation of *Moniezia* spp. from the city slaughterhouse from 1 October to 30 November 2017, this tapeworm was found to infest the intestines of 13 sheep.

For morphological identify the genus of this tapeworm, eggs from one gravid proglottid of the thirteen worms were examined, polymerase chain reaction (PCR) and the PCR-product-based sequencing were applied on 4 *Moniezia* tapeworms targeting a specific region of the 18S rRNA gene.

The sequencing has shown 2 species of *Moniezia*, SP1 and SP2 ,these two species revealed close matching on the phylogenetic tree to an according to the current study findings, *Moniezia* spp. affect on sheep in the city of Al-Diwaniyah, Iraq, these findings give interesting information about the evolution history of this worm in the studied city.
Keywords: Cestoda, *Moniezia*, PCR, Phylogeny, Sheep.

## INTRODUCTION

The genus of *Moniezia* are considered as high prevalent worms that infest sheep intestines, the disease conditions by these worms lead to risky-economic crises around the world (Soulsby, 1982; Mazyad and El-Nemr, 2002). The characteristic scolex, neck, and strobili are the highly recognized parts of the worms. Cyclophyllidea and Anoplocephalidae are the order and the family of this genus respectively, each proglottid has repeated sexual parts for better differentiation of these worms; mites are considered the main intermediate hosts for *Moniezia* species that provide a source of infestation via feeding on grass (Denegri *et al*., 1998).

Monieziasis is the term of illness that is caused by species of *Moniezia*, for this genus as a tapeworm has limited species such as *M. expansa* (Rudolphi, 1810), *M. benedeni* (Moniez 1879) and *M. monarda* (Ohtori *et al*., 2015).*M. expansa* affects sheep (high incidence), cattle, goats, swine, and very rarely human (El-Shazly *et al*., 2004; Gómez-Puerta, 2008). Young animals appear to be the main targets for the infestation by *M. expansa* (Wymann, 2008);

several proglottids that have sensory-organ-based anterior-scolexes, neck, and the strobilus are the main parts of this species. According to Brusca and Brusca, (1990), the sensory parts are present along the body of the worm and used for tactile stimulation; metrics of the parasite could be expanded as 8-10 meters in length and 1.5 centimeters in width (Chilton *et al*., 2007).

To study the evolution history of *Moniezia* species in the city of Diwaniyah, Iraq, the present study was initiated to evaluate the identity matching or mismatching of the city species with global species that belonging to this genus.

## MATERIALS AND METHODS

Intestines from 50 sheep (22 male, 28 female; 20 with age <6 months, 18 with age 6 to <12 months and 12 with age > 12 months) were examined for the infestation of *Moniezia* spp. from the city slaughterhouse.

To identify the genus of this tapeworm morphologically, eggs from one gravid proglottid of the thirteen worms were examined (Rahif, 1998); sequencing of the polymerase chain reaction (PCR) products were applied on four *Moniezia* tapeworms targeting a specific region of the 18S rRNA gene (743bp).The protocol of gSYAN DNA Extraction Kit (Gene aid, USA) was followed to extract the genomic DNA from the mature proglottids of the worms. Accu Power TMPCR Pre Mix (Bioneer, Korea) was performed to prepare the master mix using the manufacturer's instructions. The primers (AY752651.1),F: TGCTACCCGCATGATGTTGT and R: ACACAGTTGGCTGCACTCTT were used in this study. (Wickström *et al.*, 2005).

The thermocycler reaction-based conditions were 1 cycle of initial denaturation at 95℃ for 5min, 30 cycles of (denaturation at 95℃ for 30sec, annealing at 58℃ for 30sec, and extension at 72℃ for 1min), and 1 cycle of final extension at 72℃ for 5min. We had optimized these conditions previously to fulfill the amplification requirements for this study.

Electrophoresis was used to separate the PCR products on a 1.5% agarose gel at 100 volts and 80 amp for 1hour; a UV-light-based imager was used to identify these products in the gel.

Sequencing was applied on the positive-PCR products (Macrogen Company, Korea) employing AB DNA sequencing system. NCBI Websites and MEGA 6.0 software were utilized to analyze the evolutionary history of the species included in this study. The phylogenetic tree was generated via the use of the Maximum Composite Likelihood method by phylogenetic tree UPGMA method (Saitou and Nei, 1987; Tamura *et al*., 2013).

## RESULTS AND DISCUSSION

Fifty intestines were examined for the infestation of *Moniezia* spp. in the city slaughterhouse; this tapeworm was found to infest the intestines of 13 sheep. Genital pore, cirrus sac, vitelline gland, testes, and inter-proglottid gland were noticed on the mature segments of the tapeworm (Pl.1).

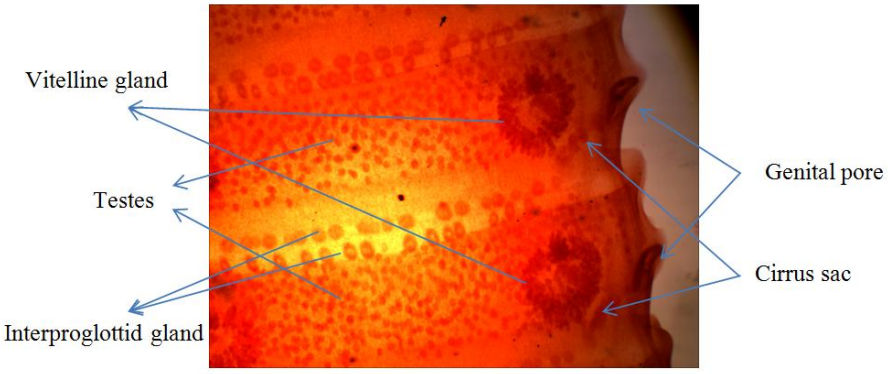


**Plate (1):** Mature segments of *Moniezia* spp. (Genital pore, Cirrus sac, Vitelline gland, Testes, Inter-proglottid gland)

Polymerase chain reaction (PCR) showed the product amplification at 743bp of the 18S rRNA gene (Pl.2); the PCR-product-based sequencing was applied on 4 *Moniezia* tapeworms targeting a specific region of the 18S rRNA gene.

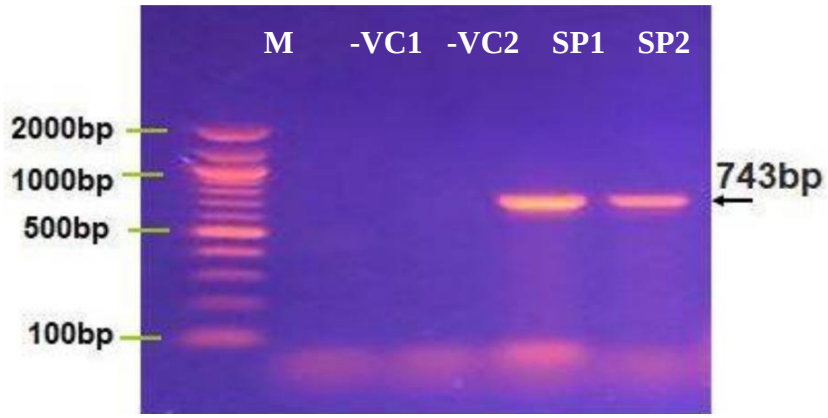


**Plate (2):** Agarose-gel-based electrophoresis. (SP 1 and 2 are positive for *Moniezia* spp. VC 1 and 2 are negative controls, M is the ladder (2000-100bp))

The sequencing has shown 2 species of *Moniezia*, SP1 (MH298620.1) and SP2 (MH298621.1), these species revealed close matching on the phylogenetic tree to an isolate from China (GU817405.1) (Diag.1).

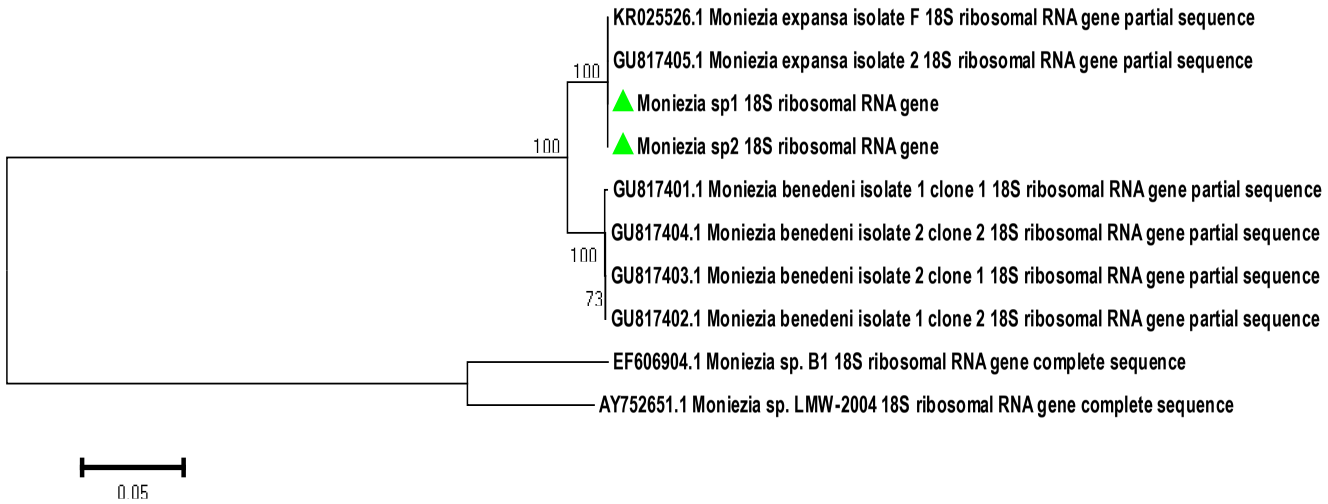




**Diagram (1):** Phylogenetic-tree analysis relied on18S-rRNA-gene specific-region sequencing. The sequencing has shown 2 species of *Moniezia* spp., SP1 (MH298620.1) and SP2 (MH298621.1). These two species revealed close matching on the phylogenetic tree to an isolate from China (GU817405.1). The comparison was performed using NCBI-based nucleotide-nucleotide website.

According to the present study, the *Moniezia* spp. were found to be wide-prevalent and caused the infestation in sheep intestine, the morbidity of *Moniezia* infestation in the current study was 26%, which indicates a risky situation in which the disease caused by these tapeworms may lead to economic crises in Al-Diwaniyah city (Diop *et al*., 2015).

In 2012, the species of this genus were detected in the intestines of camels, and that was according to a study performed by Anisimova (2012), this study was estimated the rate of infestation to be as 32.35% and 15.38% in Al-Diwaniyah and Al-Najaf cities respectively. The present study gives information that agrees partially with Fadl *et al*. (2011) who showed that the infestation of this tapeworm was 0.9% in sheep of Baghdad sampled regions; the infestation prevalence of these tapeworms may go high during spring and summertime, especially when having high numbers of mites.

Identifying the morphology of the five *Moniezia* tapeworms were performed using a modified Carmen stain in which genital pore, cirrus sac, vitelline gland, testes, and inter-proglottid gland were noticed on the mature segments of the tapeworms, and these results agree with Melhorn (2001).

The PCR results showed the amplification of the specific region of the 18S rRNA gene (743bp) in these tapeworms, and this agrees with (Nguyen *et al*., 2012) who used the same technique; the results of the sequencing identified these tapeworms in the intestine of the tested sheep in the city, and the phylogenetic tree provided information that our species were matched up with a Chinese strain; this matching may indicate a certain relation between our strain and the Chinese one which could be as a result to have come from the same ancestor. According to the current study findings, *Moniezia* spp. affect sheep in the city of Al-Diwaniyah, Iraq; these findings give interesting information about the evolution history of this worm in the studied city.

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# التحديد الجزيئي وتحليل شجرة النشوء لأنواع الجنس *Moniezia* من الاغنام في مدينة الديوانية

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الخلاصة

اجريت الدراسة للكشف عن التحديد النشوئي والجزيئي لديدان الانواع العائدة للجنس *Moniezia* Blanchard, 1891 التي تؤثر على امعاء الاغنام في مدينة الديوانية، العراق.

تم استخراج 50 معي اغنام للبحث عن الاصابة بأنواع هذا الجنس في مجزرة المدينة، اذ وجدت هذه الديدان الشريطية في امعاء 13فرداً من الاغنام لغرض التشخيص المظهري لجنس هذه الديدان . صبغت خمسة قطع جسمية ناضجة بصبغة الكارمن مبينة الاشكال التناسلية البالغة لهذه الدودة.

عند تطبيق تفاعل انزيم البلمرة المتسلسل ودراسة تعاقب القواعد النتروجينية لاربعة ديدان من جنس *Moniezia* لاستهداف منطقة خاصة من جين *16S rRNA* ، حيث اظهرت دراسة التعاقب نوعان من هذا الجنس، SP1 و SP2؛ كما اظهر هذان النوعان تطابقا متقاربا في شجرة النشوء من عزلة من الصين، استنادا لنتائج الدراسة الحالية، فأن انواع *Moniezia* spp. تصيب الاغنام في مدينة الديوانية، العراق. تعطي هذه النتائج معلومات ملفتة للانتباه عن تأريخ التطور لهذه الدودة في منطقة الدراسة.