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**18S rRNA GENE- AND COX1-GENE-BASED DIVERSITY
OF MONIEZIA SPP. AND AVITELLINA CENTRIPUNCTATA
IN RUMINANTS FROM AL-QADISIYAH PROVINCE, IRAQ**

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The present *18S rRNA* gene and *Cox1* gene-based work was proposed and performed to detect and study the history of evolution of tapeworms that could be recognized through the use of molecular techniques. For this investigation, 14 tapeworms were recovered from 50 intestinal-based content samples of 50 animals (sheep, goat, cattle, and buffalo). The collected adult tapeworms were first identified morphologically showing 6 *Moniezia expansa* and 7 *Moniezia benedeni*. Then, DNA from 14 worms (9 for the *18S rRNA* gene and 5 for the *Cox1* gene) was employed for polymerase chain reaction (PCR) and partial gene sequencing techniques. The results of the PCR recorded amplifications of all the sequences used for both genes. The sequencing indicated, via the use of *18S rRNA* gene, 5 *Moniezia benedeni*, 3 *Moniezia expansa*, and 1 *Avitellina centripunctata*. Moreover, it resulted, via the utilization of *Cox1* gene, 3 *Moniezia expansa* and 2 *Moniezia benedeni*. The isolates stood in close matching with global isolates. The current results provide important criteria for the reliable use of the techniques utilized in this study.

Keywords: *18S rRNA*, *Avitellina*, *Cox1*, *Moniezia*, PCR, sequencing.

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**ВЫЯВЛЕНИЕ РАЗНООБРАЗИЯ ЦЕСТОД *MONIEZIA* SPP. И *AVITELLINA*
CENTRIPUNCTATA ИЗ ЖВАЧНЫХ ЖИВОТНЫХ В ПРОВИНЦИИ АЛЬ-
КАДИСИЯ В ИРАКЕ С ИСПОЛЬЗОВАНИЕМ ГЕНОВ *18S PPHK* И *COX1***

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Молекулярно-генетическое исследование по генам *18S pPHK A* и *Cox1* предпринято для реконструкции эволюции цестод жвачных животных. Исследовано 14 экз. цестод из кишечника

50 животных (овцы, козы, коровы, буйволы). Вначале половозрелые цестоды были идентифицированы морфологически: 6 экз. *Moniezia expansa* и 7 экз. *Moniezia benedeni*. Затем ДНК 14 червей (9 для анализа *18SpPHK* и 5 для *Cox1*) были использованы для ПЦР и секвенирования участков генов. По результатам секвенирования участка гена *18SpPHK* выявлены 5 экз. *Moniezia benedeni*, 3 экз. *Moniezia expansa* и 1 экз. *Avitellina centripunctata*. С помощью анализа гена *Cox1* выявлены 3 экз. *Moniezia expansa* и 2 экз. *Moniezia benedeni*. Обнаружено близкое совпадение с последовательностями нуклеотидов генов в глобальном банке данных. Полученные результаты помогли установить критерии надежного использования молекулярных методик идентификации цестод.

Ключевые слова: *18S rRNA*, *Avitellina*, *Cox1*, *Moniezia*, ПЦР, секвенирование.

Moniezia spp are tapeworms that infest various ruminants such as sheep, goat, cattle, and buffalos. These worms induce some problems to the health of animals plus to industries leading to financial losses via the increase cost provided for the treatment and veterinary services (Kouam et al., 2018). Two species are well-known for their infestation effectiveness on animal industries; *Moniezia benedeni* and *Moniezia expansa*. For the morphological recognition of these tapeworms, characteristics features are presented on them such as scolex, neck and strobila. The scolex and neck are measured to be small in sizes with noticed-long chain of strobili. These interesting worms belong to the family of Anoplocephalidae and the order of Cyclophyllidea. Extra differences of *Moniezia* genus that are characterized by the presence of clear anterior, posterior, mature, and gravid segments of these worms. Another characteristic feature is that each proglottid has repeated-sexual parts. *Moniezia* spp. needs mites as an intermediate host for the lifecycle to be completed via the presence of grass feeding by the affected animals (Denegri et al., 1998; Aboma et al., 2015; Ohtori et al., 2015). *Avitellina centripunctata* is cestode that infests sheep, goats, cattle, and wide range of ruminants (Yildiz, 2007). The strobili of these worms are characterized by the presence of thousands of wide-very short proglottids. The lifecycle needs certain species of arthropods, oribatid mites and barklice, to be completed (Woodland, 1935; Yildiz, 2007). The present *18S rRNA* gene and *Cox1* gene based work was proposed and performed to detect and study the history of evolution of tapeworms that could be recognized through the use of molecular techniques that rely on *18S rRNA* and *Cox1* genes. For this investigation, 14 tapeworms were recovered from 50 intestinal-based content samples of 50 animals (sheep, goat, cattle, and buffalo). PCR and Partial gene sequencing were employed for this present work to fulfill the goal of the study.

MATERIALS AND METHODS

Sampling

For this work, 50 intestinal-based content samples were collected from 50 animals (sheep, goat, cattle, and buffalo). The collection work continued from August, 2016 to January, 2017. The locations of sampling were from different slaughter houses in Al-Qadisiyah province, Iraq. The tapeworms were placed separately in clean containers that contained PBS. At the Lab in the college of veterinary medicine, University of Al-Qadisiyah, adult worms were placed in containers that had 70 % ethanol and stored at -20°C until future work, starting with DNA extraction. Some of these tapeworms were subjected to morphological-based identification.

DNA extraction

A piece of the worm, 200mg, was first rinsed thoroughly with water to get rid of ethanol. KAPA Express Extract Kit (Cape Town, South Africa) or (Roche, Mannheim, Germany) was used to fulfill the extraction process relying on the protocol provided by the manufacturer. A NanoDrop was utilized to measure the resulted DNA for quality and quantity.

PCR and sequencing techniques

The technique followed the use of specific primers that were designed using PrimerQuest Tool (Integrated DNA Technologies, Inc., Belgium) and NCBI-based websites. The primers of the *18S rRNA* gene were F: ACGGTGAAACCGCGAATGG and R: GACATGACATGCAGTAGCAGTG that amplify a specific region in this gene at 841 bp. Moreover for the *Cox1* gene, the primers were F: TGTTGAGTATGTGGTTTGGTGC and R: AACTACCCACCATAACCACAGGATC that amplify a region at 684 bp. DNA from 14 worms (9 for the *18S rRNA* gene and 5 for the *Cox1* gene) was employed for the PCR. The kit that was used for the preparation of the mastermix was ordered from (Bioneer, Korea). The instructions of the kit were followed for this purpose. Regarding the conditions of the thermocycler used were 95°C for 5 min as for the primary denaturation, 35 cycles of (main denaturation at 94°C for 1 min, for the *18S rRNA* and *Cox1* 55°C and 53°C respectively for the annealing, for the *18S rRNA* and *Cox1* 55°C for 1 min and 72°C for 2.5 min respectively), and 72°C for 5 min regarding the finishing extension. the PCR products in the 1.25 % agarose gels were electrophoresed and visualized via the use of ethidium-bromide-based illumination screened by a UV imager. All PCR products were sent out for partial-gene sequencing using Sanger sequencing method (Macrohen Company, Korea). The phylogenetic tree was built up using maximum likelihood via the use of MEGA 7.0 (Saitou, Nei, 1987; Tamura et al., 2013).

RESULTS

According to the morphological characters, worms belonging to the genus *Moniezia* were recognized. The generic characters were as follows: genital pore, clear cirrus sac, vitelline gland, recognizable testes, and inter-proglottid-based glands. For the species identification, adult worm and egg features were used to separate between *M. expansa* and *M. benedeni* that were recovered in this study showing 6 *Moniezia expansa* and 7 *Moniezia benedeni* (figs. 1, 2). The results of the PCR recorded amplifications of all the sequences used for both genes (figs. 3, 4).

The sequencing indicated, via the use of *18S rRNA* gene, 5 *Moniezia benedeni*, 3 *Moniezia expansa*, and 1 *Avitellina centripunctata*. Moreover, it resulted, via the utilization of *Cox1* gene, 3 *Moniezia expansa* and 2 *Moniezia benedeni*. The current isolates, MH173843.1, MH173844.1, MH173845.1, MH173846.1, MH173847.1, MH173848.1, MH201211.1, MH201212.1, MH201213.1, and MH201214.1 stood in close matching with global isolates (figs. 5, 6). Tables 1 and 2 provide statistics about the isolates regarding their sampled animals.

DISCUSSION

Moniezia spp. are tapeworms that infest various ruminants such as sheep, goat, cattle, and buffalos. These worms induce some problems to the health of animals plus to industries leading to financial losses via the increase costs provided for the treatment and veterinary services (Diop et al., 2015a; Kouam et al., 2018). Two species are well-known

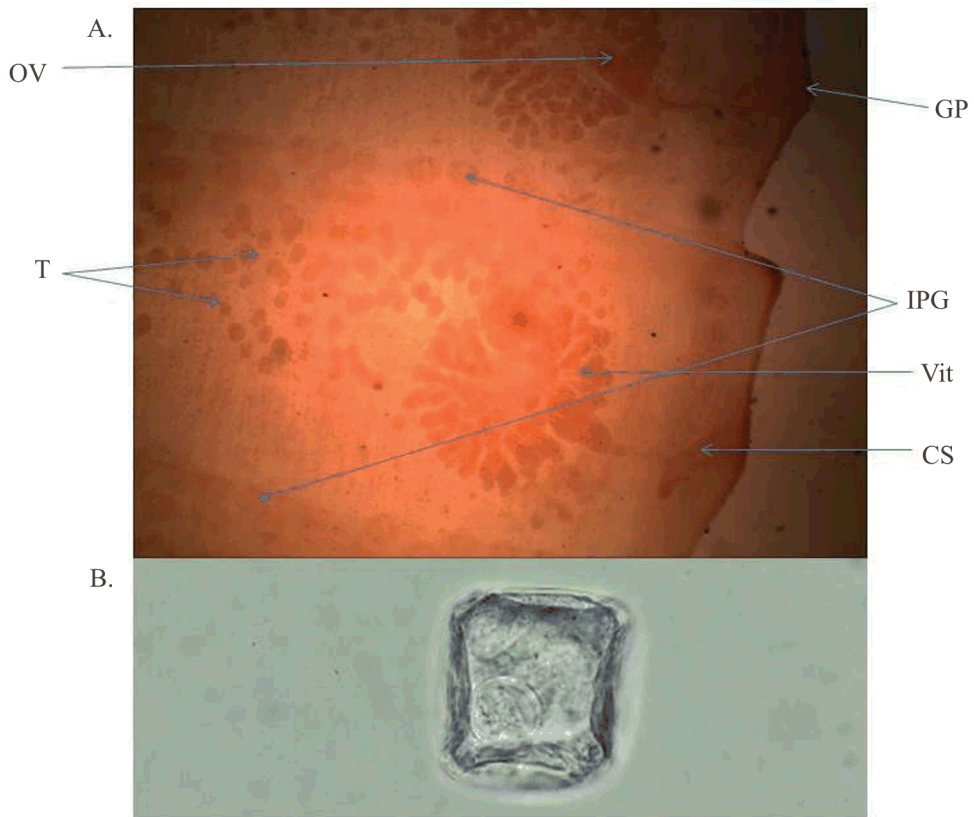


Fig. 1. A. *Moniezia expansa*. IPG = inter-proglottidal glands, T = Testes, Vit = Vittline gland, CS = Cirrus sac, GP = Genital pore, OV = Ovary. (X10) (We can see the IPG because they reach to the margin of segments. B. *Moniezia expansa* egg (Quadral form). (X10).

Table 1. Shows statistics about the isolates regarding their sampled animals via the use of 18S rRNA gene

Animal	ID	Cestode species
Cattle	DIQ2	<i>M. benedeni</i>
Cattle	DIQ3	<i>M. benedeni</i>
Cattle	DIQ4	<i>M. benedeni</i>
Sheep	DIQ5	<i>M. benedeni</i>
Sheep	DIQ9	<i>Avitellina centripunctata</i>
Buffalo	DIQ6	<i>M. benedeni</i>
Buffalo	DIQ10	<i>M. expansa</i>
Goat	DIQ11	<i>M. expansa</i>
Goat	DIQ12	<i>M. benedeni</i>

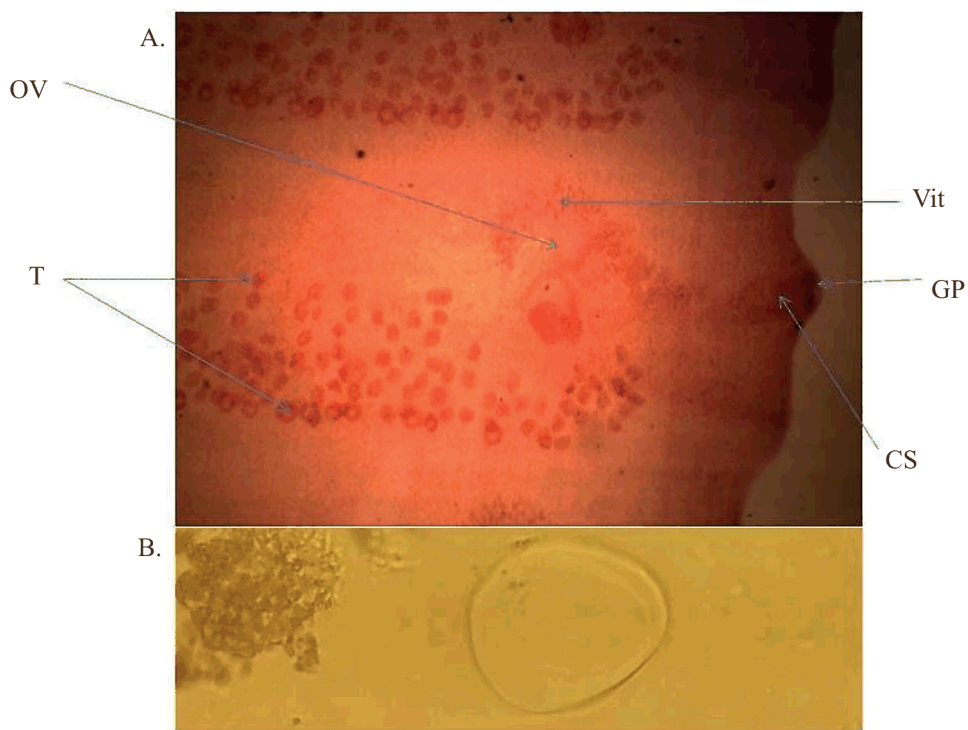


Fig. 2. A. *Moniezia benedeni*, T = Testes, Vit = Vittline gland, CS = Cirrus sac, GP = Genital pore, OV = Ovary. (X10) (The inter-proglottidal glands do not reach to the margin of segments). B. *Moniezia benedeni* egg (Triangle). (X40).

for their infestation effectiveness on animal industries. The present study was successful in determining the presence of *Moniezia* spp. via the morphological identification. This step is important in the diagnosis any parasites especially tapeworms that have their external characteristic features. The use of the PCR technique confirm that worms are identical to the genus *Moniezia*, thus confirming the previous studies (Mehlhorn, 2016). These results agree with (Nguyen et al., 2012) who detected tapeworms via the use of a PCR method. The 18S rRNA gene sequencing analysis revealed 5 *Moniezia benedeni*, 3 *Moniezia expansa*, and 1 *Avitellina centripunctata*. This technique is proved to be reliable in identifying these tapeworms, and it is useful in detecting more species than that in the other method, that

Table 2. Shows statistics about the isolates regarding their sampled animals via the use of *Cox1* gene

Animal	ID	Cestode species
Cattle	DIQ14	<i>M. benedeni</i>
Cattle	DIQ15	<i>M. benedeni</i>
Sheep	DIQ13	<i>M. expansa</i>
Sheep	DIQ12	<i>M. expansa</i>
Buffalo	DIQ11	<i>M. expansa</i>

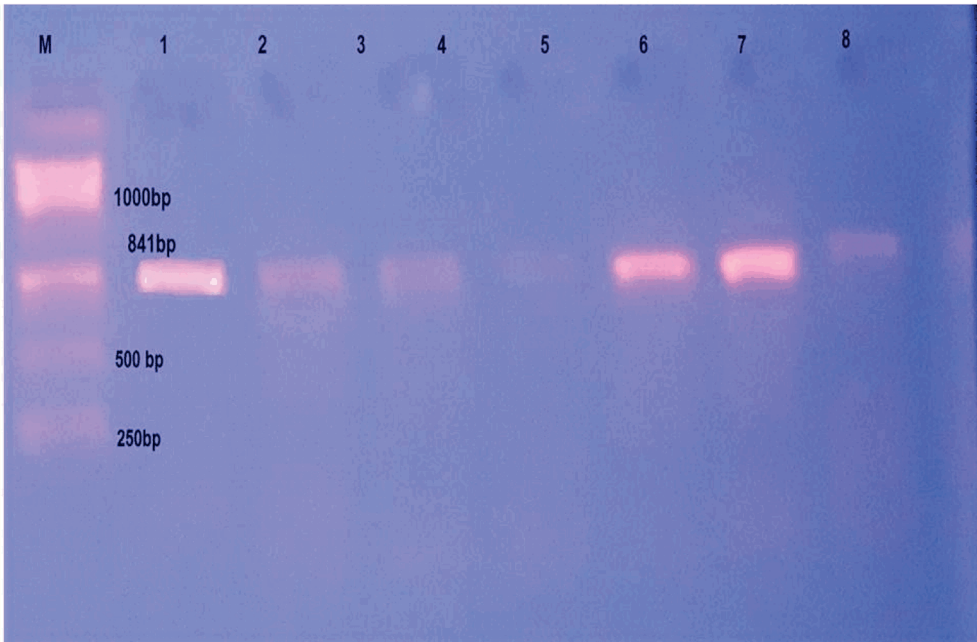


Fig. 3. Image of the PCR products via gel-based electrophoresis of the *18S rRNA* gene.
Positive lanes are 1–8. Ladder is M lane.

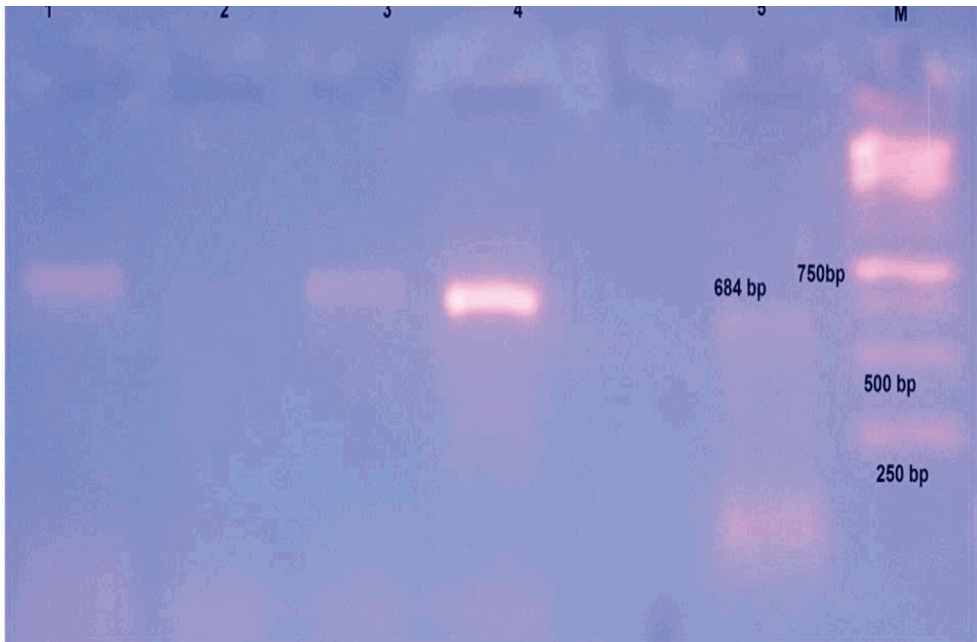


Fig. 4. Image of the PCR products via gel-based electrophoresis of the *CoxI* gene.
Positive lanes are 1, 3, 4, and 5. Ladder is M lane.

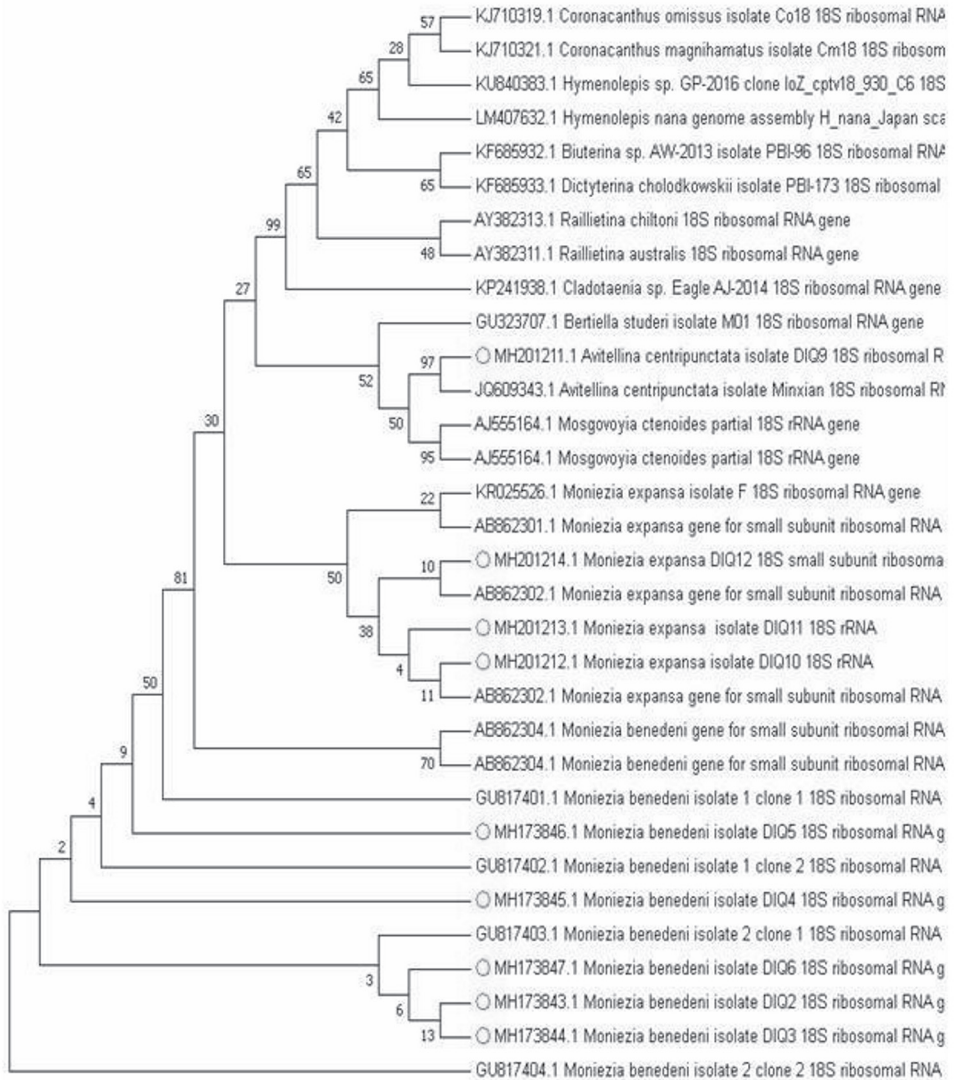


Fig. 5. Phylogenetic tree based on the 18S rRNA gene partial sequencing.

utilized *Cox1* as a target gene. However, it was consistent, via the utilization of *Cox1* gene, in detecting 3 *Moniezia expansa* and 2 *Moniezia benedeni*. The current isolates were placed in close matching with global isolates on the phylogenetic tree. However, our isolates have distinct mutations that may occur in the past and let them to branch out from the global isolates (Yan et al., 2013; Ohtori et al., 2015). The results of the phylogenetic tree may indicate that these isolates of the sampled animals were from different ancestors (Diop et al., 2015b; Ohtori et al., 2015; Guo, 2016). In a recent published work by (Haukisalmi et al., 2018), 6 *Moniezia* species were identified in 2 clades. Although their results showed *M. expansa* and *M. benedeni* in Clade1 of their phylogenetic tree, some of the 6 species were

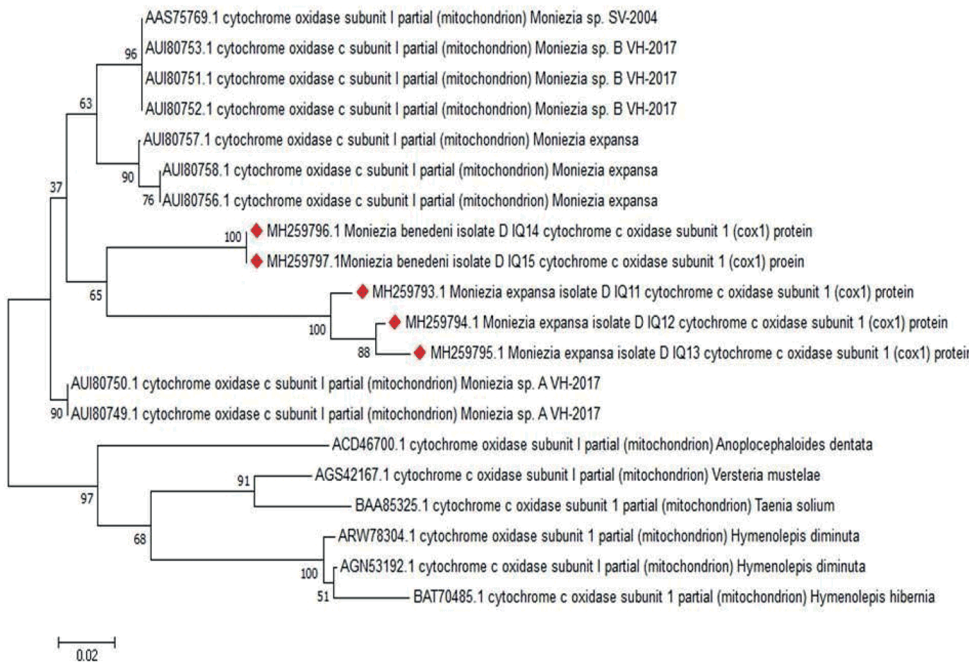


Fig. 6. Phylogenetic tree based on the Cox I gene partial sequencing.

organized in the Clade2. Our results agree to some extent with this work especially when look at our tree that placed these both species in different clades. These variations could have been initiated because the geographical differences between Iraq and Finland and Alaska. In Iraq, the disease control systems may not be as good as the ones in those places, and this may introduce certain impacts on generating modifications in the nucleotide sequences leading to the appearance of new strains. The current results provide important criteria for the reliable use of the techniques utilized in this study.

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