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Molluscum Contagiosum genome: phylogenetic analysis

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Abstract

Molluscum Contagiosum virus (MCV), a double strand DNA virus that belongs to the Poxviridae family, MCV causes pearl disease. Seventy-five lesion samples were collected in Diyala province at dermatology clinics, from 30 September to 20 October 2019 from patients with Molluscum Contagiosum Virus after diagnosis by the specialist doctor, and further 25 samples were curreted frm skin as a control group collected from healthy. After viral genome extraction, specific primer was detected by conventional PCR then sequencing PCR then sequencing finaly registeration in NCBI and phylogenitic tree. Phylogenitic tree was generated to evlute the viral evalution and closest neighbour. The results of the PCR for control group (25 samples) were negative for all. From 75 samples only 7 samples were positive with specific bands at 393bp (9.3%) while 3 samples with specific bands at 393bp and nonspecific bands at 300bp (4%). Then samples with specific bands at 393bp that were divided into two groups according to similarity after sequencing. It was found that the matched rate was 100% when comparing each of these groups with the global isolates in NCBI. Two isolates were registered at NCBI with the accession number for the recorded isolates were LC520237 and LC520238.

Keywords: Molluscum virus, PCR, DNA sequence, MC021L gene.

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جينوم المليساء المعدية: تحليل الشجرة التطورية

سرور عبود محمد و زهراء جعفر جميل

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ألخلاصة

فيروس المليساء المعدية (MCV) ، وهو فيروس الحمض النووي المزدوج الشريط الذي ينتمي إلى عائلة Poxviridae ، يسبب مرض اللؤلؤ. تم جمع 75 عينة من الآفة في محافظة ديالى ، في العيادات الخارجية للأمراض الجلدية ، من 30 أيلول ولغاية 20 تشرين الاول 2019 من المرضى الذين يعانون من فيروس المليساء المعدية بعد التشخيص من قبل الطبيب المختص ، و 25 عينة التي قشطت من الجلد كمجموعة سيطرة تم جمعها من الأصحاء. بعد استخراج الجينوم ، تم الكشف عن البادئ المحدد بواسطة PCR التقليدي ثم التسلسل واخيرا التسجيل النهائي في NCBI والشجرة التطورية. تم إنشاء شجرة النشوء والتطور لتقييم التطور الفيروسي وأقرب السلالات. أظهرت نتائج تفاعل البلمرة المتسلسل ان جميع عينات السيطرة كانت سالبة بينما في 7 عينات مع حزم محددة للزوج قاعدي 393 وبنسبة (9.33 %) بينما 3 عينات مع حزم محددة عند الزوج القاعدي 393 و حزم غير محددة عند الزوج القاعدي 300 وبنسبة 4 %. تم تقسيم العينات مع الحزم المحددة للزوج قاعدي 393 إلى مجموعتين حسب التشابه بعد نتائج تحليل التسلسل. وقد وجد أن المعدل التطابق كان 100% عند مقارنة كل من هذه المجموعات بالعزلات العالمية في NCBI. تم تسجيل عزلتين في NCBI في رقم الانضمام للعزلات المسجلة LC520237، LC520238.

كلمات مفتاحية: المليساء المعدية، تفاعل انزيم البلمرة المتسلسل، تتابع الدنا، جين MC021L.

Introduction

Molluscum Contagiosum virus (MCV), a double strand DNA virus that belongs to the *Poxviridae* family [1], MCV causes pearl disease [2]. It was first described by Bateman in 1817, and Paterson demonstrated its contagious nature 1841 [3]. MCV has four different genotypes: MCV 1, MCV2, MCV 3 and MCV 4, respectively [4]. Virtually all pediatric cases are caused by MCV-1 [5]. MCV-2 affects adolescents and adults, and is mainly sexually transmitted [6].

There are some striking features of MCV that make it distinctive as compared to the well-studied members of the genus *Orthopoxvirus*. First, MCV allows the infection to continue with little or no inflammation. In comparison, *Monkeypox* (MPX) and *Variola* (VAR) *viruses* cause acute diseases with morbidity and mortality rates substantially higher than MC. Second, infection with MCV remains confined to keratinocytes while viruses with VAR and *Vaccinia virus* (VAC) infect several different types of cells and tissues. Thirdly, MCV-encoded immune evasion molecules are distinct from those encoded by members of the genus *Orthopoxvirus*. The variation in molecules of immune evasion possibly reflects the different tropisms of these viruses in the tissues and the various types of diseases they cause. MCV is the sole *poxvirus* other than the VAR that is only pathogenic to humans [7]. The lesions are generally not painful, but they may itch or become irritated. Picking or scratching the bumps may lead to a spread of the viral infection responsible for molluscum contagiosum, an additional bacterial infection, and scarring [8]. The disease is endemic with a greater incidence in institutions and societies where overcrowding, poor sanitation, and poverty favor its spread. The incidence worldwide of disease is ranged from 2 % to 8 %. Due to the concurrent HIV infection, the incidence of infection has increased over the last three decades, primarily as a sexually transmitted disease; it has been estimated that between 5 and 20 per cent of HIV patients have MCV [9].

The aims of study is to detect a specific region in MC021L gene by conventional PCR then record variants among MCV strains by sequencing and Compared of MCV local strains with global strains by phylogenetic tree

Materials and Methods

The current study was conducted during the period from 30 September to 20 October /2019 in outpatient clinics of Dermatology in Baqubah city, Iraq**.** The study included 100 lesions samples from patients with ages ranged between 1-60 years old, the duration of their illness ranged between 5 days - 2 years. The samples were divided into 75 samples of patients with MCV, and 25 samples as a control. All infected patients were diagnosed on a clinical basis. The lesions

were curetted from each patient and placed in sterile tube contain 1ml of sterile phosphate buffer saline, pH 7.1 and the samples were stored at -30 °C until DNA extraction.

The DNA was extracted from the samples using DNA extraction kit ABIOpure Extraction (ABIOpure company/USA). The genes were amplified by PCR technique according to the special primers (Table 1).

Table 1 : Primer used in the study

MC021L	F 5`- GGCGCGTAGCCGAGCGG- 3` R 5`-GCTTCCGGGCTTGCCGCCGGGCAG-3`	393	10

The PCR technique was applied by adding 5µl from DNA extraction to the PCR tube containing 10 µl of the master mix and 1 µl of each Forword and Reverse of primers, 3 µl of the Nuclease Free Water were added to this tube to get 20 µl as a final size. The mixture then transferred to PCR system (BioRad/USA), the conditions of PCR reaction (program) are listed in (Table 2).

Table 2: PCR Program

Initial Denaturation	95	05:00	1
Denaturation Annealing	95	00:30	30
Annealing	65	00:30	
Extention	72	01:00	
Final Extention	72	07:00	1
Hold	4	10:00	

Sequencing and Phylogenetic tree

Sequencing of PCR product was carried out by sending the PCR DNA products with their specific primers by freezer bag to Macrogen company in Korea, **https//dna.macrogen com**. The sequencing study as designed between the sequence of standard gene BLAST program which is available at NCBI online at **http://www.ncbi.nlm.nih.goy**.macrogen com. and using BioEdit program. The evolutionary analysis was conducted using genious software.

Results and Discussion

Detection of region of MCV by specific primer

DNA was multiplied in the sample using the polymerase chain reaction technique using primer for MC021L. The presence of DNA was detected in the reaction products on the agarose gel. MC021L is the gene of the primer that 393 bp which started with the nucleotide in the location 26662 and ends with 27828, is homologs F13L[11], which encodes a major structural component of the virus and is frequently used for MCV genotyping purposes [12]. F13L is a major membrane component of extracellular vaccinia virions. It is the protein encoded by the vaccinia virus F13L open reading frame and is required for the wrapping of intracellular mature virions by cisternae derived from trans-Golgi or endosomal membranes and is an abundant, palmitylated component of the outer membrane of extracellular virions [13]. The F13L product, called p37K for its apparent mass determined by SDS– polyacrylamide gel electrophoresis (PAGE), is a 372 amino acid nonglycosylated polypeptide that is palmitylated at cysteine residues 185 and 186 and localizes in the Golgi network [14,15]. P37 is the most abundant protein in the envelope of the extracellular virus form of the prototype poxvirus, vaccinia virus (VV), is a crucial player in the process leading to acquisition of the envelope, virus egress and transmission [16]. The size of product was 393 bp in 7 samples with specific band only at 393bp (9.3%); while 3 samples with specific bands at 393bp and nonspecific bands at 300bp (4%) the rest samples were negative (86.7%) as shown in the figure 1.

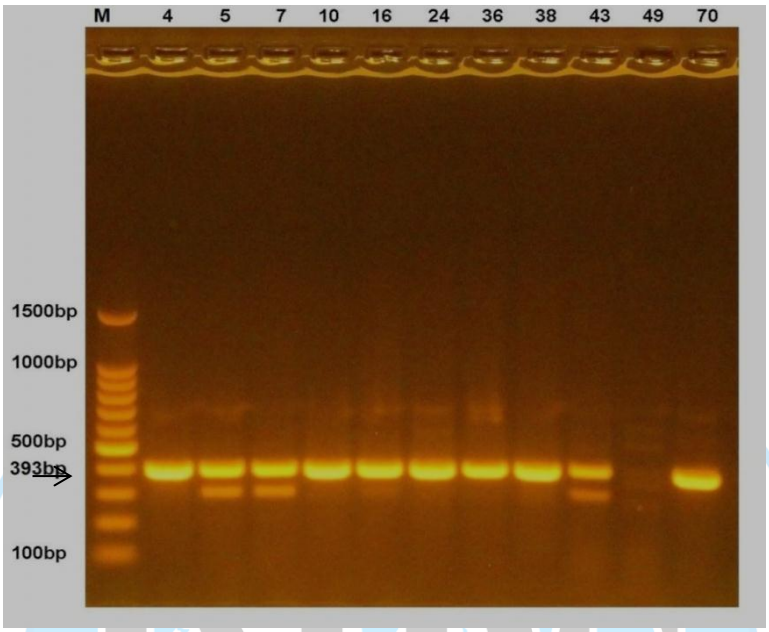

Figure 1: Gel electrophoresis for detection of region of MCV by specific Primer 1. PCR product of MCV that showed 7 samples with specific at 393 bp in lanes 4, 10, 16, 24, 36, 38, while 3 samples with specific bands at 393bp and nonspecific bands at 300bp in lanes 5, 7, 43 using 1.5% agarose gel at 100 volt for 90.0 minute stained with Eth.Br. Lane M:100pb (DNA marker)

The result of the current study in contrast with the result study that done in Diyala by Al-Azawy, showed that 85% of samples cotain this this primer [17]. Another study different with study done in Basra by Gatea, showed that all samples (102 samples) contain this primer at percentage 100% [10]. Also study disagree with study conducted in Turkish by Saral, which showed all samples contain this primer at percentage 100% [18]. This discrepancies may be due to mutation happened in this region or may be appeared as a new strain and also might have stemmed from the methodologies employed by different authers and may be different genotypes

Determination of nucleotides sequence in amplified pieces of the MC021L gene

The sequence of the multiplexed piece of the MC021L gene for the MCV was analyzed using a genious software program and the results were compared with the sequence of genes in the database using Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) website to confirm the molecular diagnosis resulting from

Conventional PCR reaction. The results of the current study revealed that the primer with a 393 bp which includes 7 samples (4,10,16,24,36,38, 70). After the sequencing process, the isolates were compared and found that 3 isolates are completely identical (24,36,70) and this isolates were gathered as Group 1 (Figure 2). while other isolates (4,10,16,38) were named group 2 as in figure 3. The comparison of Group 1 with global isolates, presented that the identical ratio 100 % as in the figures 4 and 5. When comparing group 2 also with global isolates, it was found that identical ratio 100 % as in figures 6 and 7.

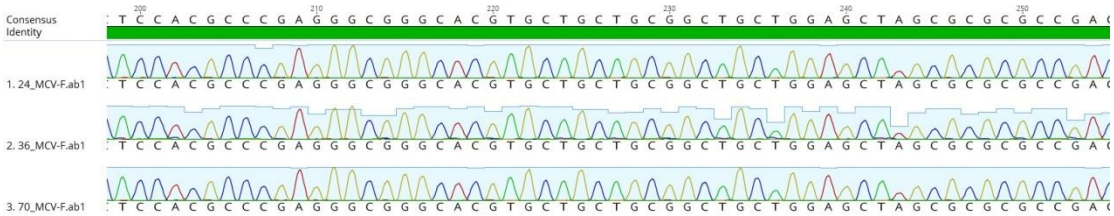

Figure 2: Paradigm for results of sequence analysis for samples (24, 36, 70) of MC021L (group 1)

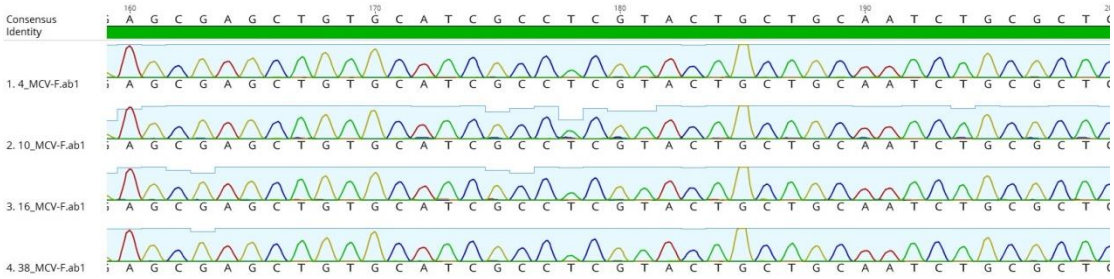

Figure 3: Paradigm for results of sequence analysis for samples (4, 10, 16, 38) of MC002L (group 2).

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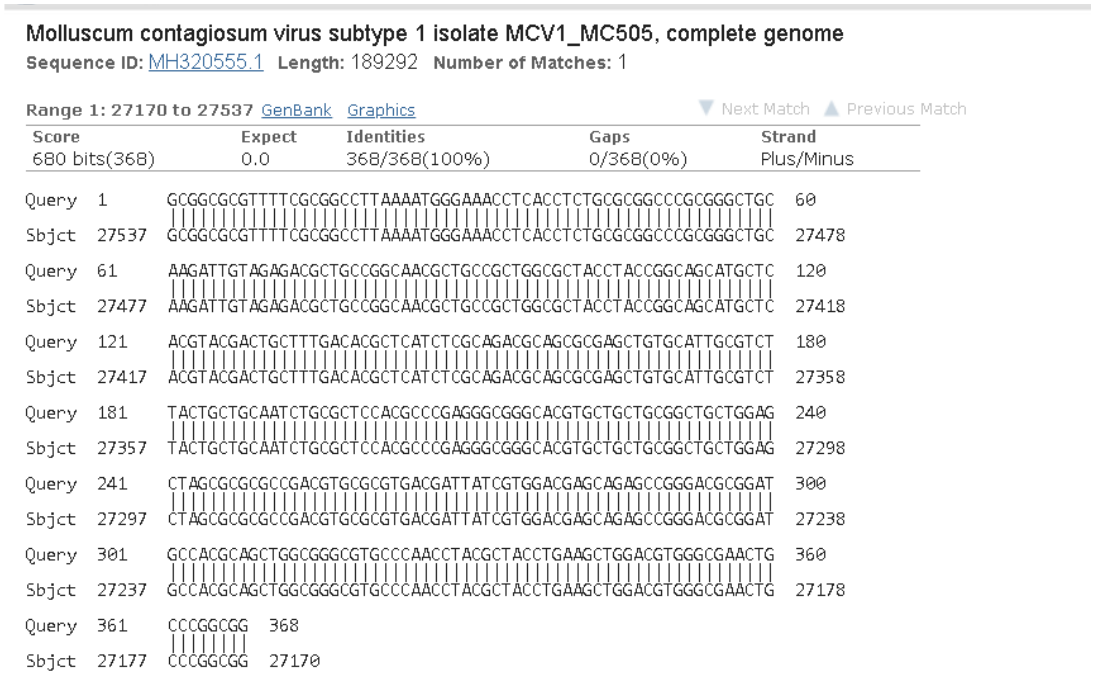

Figure 4: Identity with a nucleotide sequence with the Refseq reference gene (MC021Lgroup 1) located in the NCBI database.

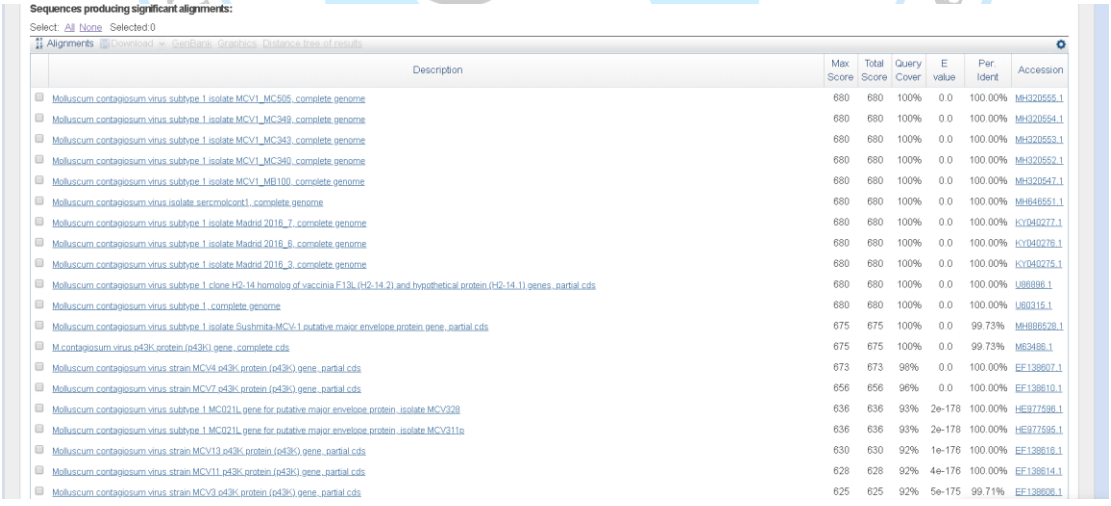

Figure 5: Comparison with international isolates of MCV(group 1 of MC021L).

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Molluscum contagiosum virus subtype 2 isolate MCV2_MC515, complete genome
Sequence ID: MH320556.1 **Length:** 189257 **Number of Matches:** 1

Range 1: 26734 to 27101 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
680 bits(368)	0.0	368/368(100%)	0/368(0%)	Plus/Minus

```
Query  1      GCGGTGCGTTTTCGCGGCCTTAAAATGGGAAACCTCACCTCTGCGCAGCCCGCGGGCTGC  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27101  GCGGTGCGTTTTCGCGGCCTTAAAATGGGAAACCTCACCTCTGCGCAGCCCGCGGGCTGC  27042

Query  61     AAGATTGTCGAGACGCTGCCGGCGACGCTGCCGCTGGCGCTACCTGCCGGCAGCATGCTC  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  27041  AAGATTGTCGAGACGCTGCCGGCGACGCTGCCGCTGGCGCTACCTGCCGGCAGCATGCTC  26982

Query  121    ACGTACGACTGCTTCGACACGCTCATCTCGCAGACGCAGAGCGAGCTGTGCATCGCCTCG  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26981  ACGTACGACTGCTTCGACACGCTCATCTCGCAGACGCAGAGCGAGCTGTGCATCGCCTCG  26922

Query  181    TACTGCTGCAATCTGCGCTCCACGCCCGAGGGCGGGCACGTGCTGCTGCGGCTGCTAGAA  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26921  TACTGCTGCAATCTGCGCTCCACGCCCGAGGGCGGGCACGTGCTGCTGCGGCTGCTAGAA  26862

Query  241    CTAGCGCGCGCCAACGTGCGCGTGACTATTATCGTGGACGAGCAGAGCCGGGACGCGGAC  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26861  CTAGCGCGCGCCAACGTGCGCGTGACTATTATCGTGGACGAGCAGAGCCGGGACGCGGAC  26802

Query  301    GCCACGCAGCTGGCAGGTGTGCCCAACCTACGCTACCTGAAGATGGACGTGGGCGAGCTG  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26801  GCCACGCAGCTGGCAGGTGTGCCCAACCTACGCTACCTGAAGATGGACGTGGGCGAGCTG  26742

Query  361    CCCGGCGG  368
              ||||||||
Sbjct  26741  CCCGGCGG  26734
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Figure 6: Identity with a nucleotide sequence with the Refseq reference gene (MC021Lgroup 2) located in the NCBI database.

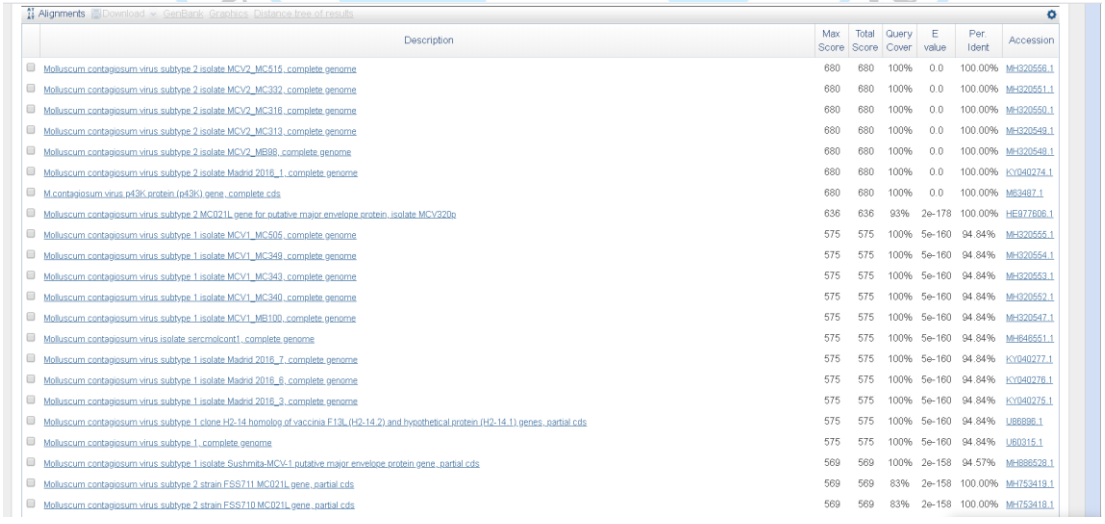

Figure 7: Comparison with international isolates of MCV (group 2 of MC021L)

Genetic analysis of MCV sequence

A segment of the MC021L gene of the MCV was amplified by PCR technique, Electrophoresis images showed that the amplified piece appeared in all samples of the MC021L gene with the size of 393 bp. The sequence for these pieces was analyzed by using Sequence Analyzer in the Macrogen company of Korea and the sequence analysis model is showen in isolates Figure (2,3) respectively. Results of sequential analysis of isolates were compared against NCBI database for the purpose of detecting mutations in isolates. Results revealed a sequence match of 100% of the MC021L gene. Two isolates were recorded in the NCBI and the serial number for the registered isolates are LC520237 and LC520238.

Phylogenetic tree

The obtained results showed that the isolates in cluster 1 were closer to the isolate KY040276 from Spain in 2016, and the isolate EF138619 from Thailand in 2016, then the remaining isolates EF138616, EF138609, EF138610 also were close to that classified in Thailand in 2016 figure10. The isolates in cluster 2 were closer to the isolate KY040274 from Spain in 2016, and the isolate KT289516 from Iran in 2015, then the remaining isolates KT 289440, KT289408, KT289413 all from Iran in 2012 figure 11.

Conclusions

Molecular detection of Molluscum contagiosum virus was best way to explain the genotype properties of virus. The occurrence of genetic diversity in MC021L, as isolates within this gene were conforming to global isolates in Spain, Thailand at 2016 and isolates also in Iran at 2015.

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***Molluscum Contagiosum* genome: phylogenetic analysis**

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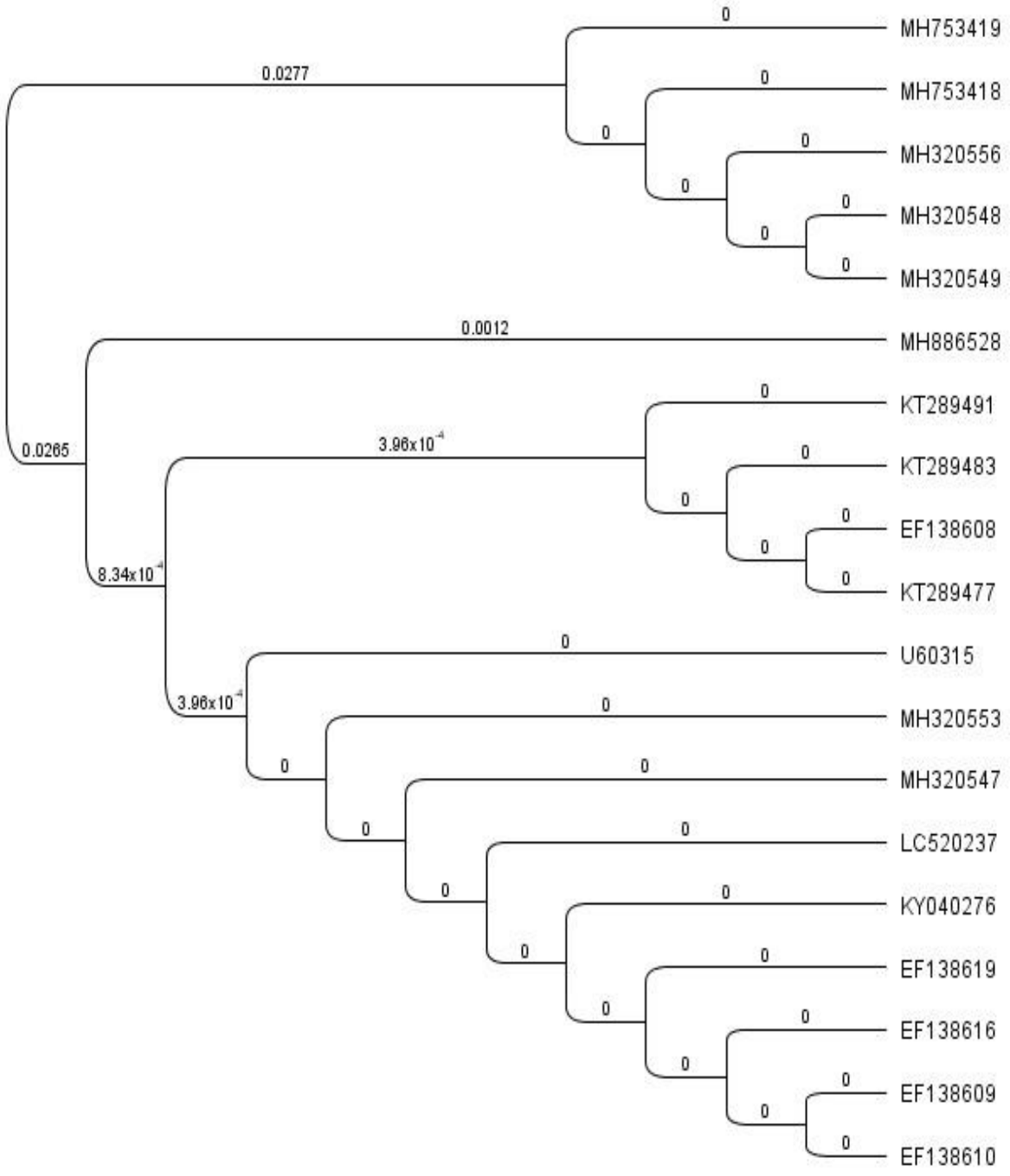

Figure 10: Phylogenitic tree of MCV (group 1 MC021L gene)

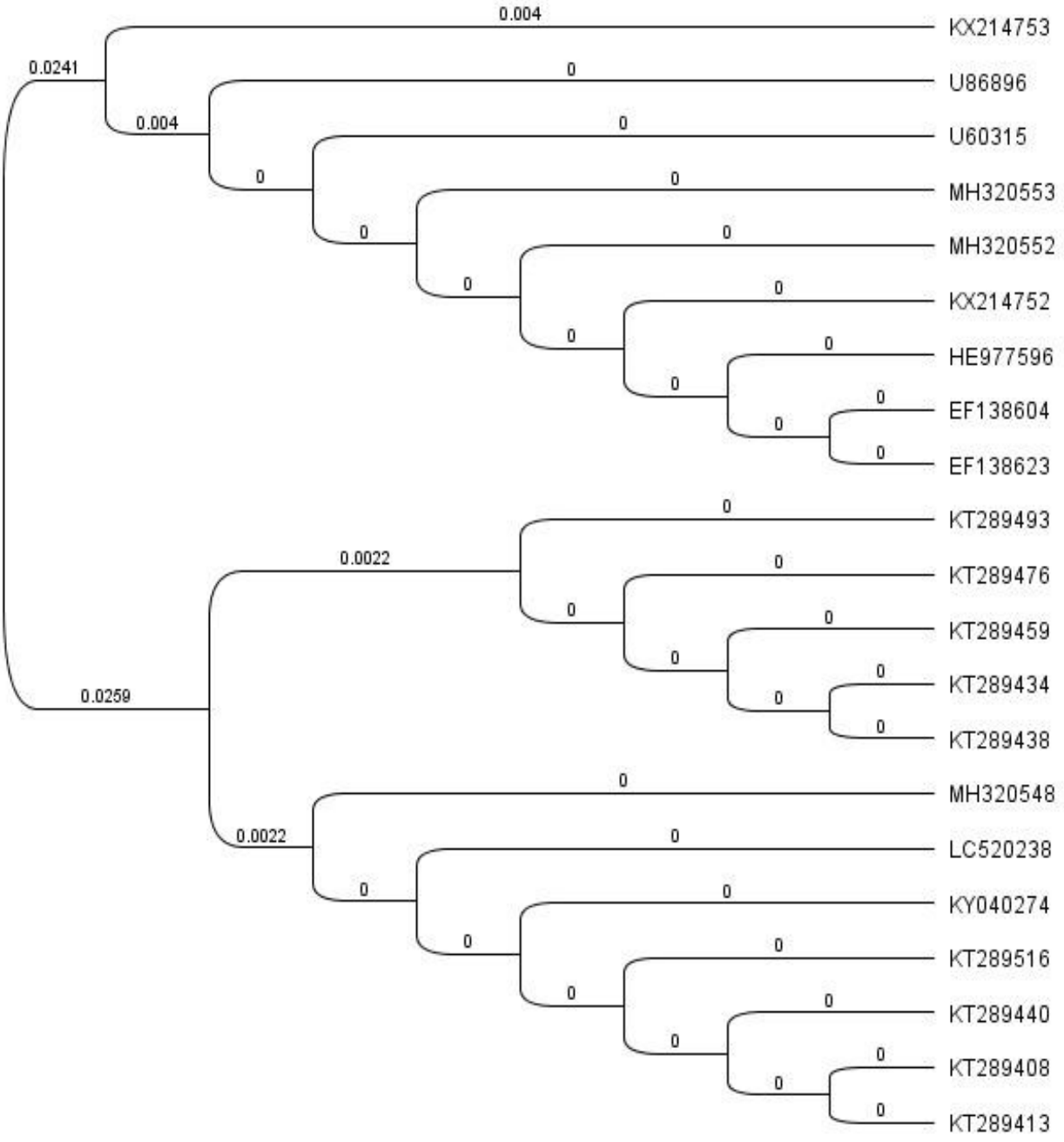

Figure 11: Phylogenitic tree of MCV (group 2 of MC021L gene)

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