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ORIGINAL ARTICLE

INTRASPECIES GENETIC AND MORPHOLOGICAL ANALYSES OF THE NEW COLLECTION OF MEDICINAL TINDER *FOMES FOMENTARIUS* (L.) FR.1849 (AGARICOMYCETES, POLYPORALES, POLYPORACEAE) FROM IRAQ

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ABSTRACT

Fomes fomentarius (L.) Fr., 1849 (Agaricomycetes, Polyporales, Polyporaceae) is a significant fungal species widely used in traditional medicine. However, several studies have noted there is a genetic variation among strains collected from different geographic regions. The current study focused on identifying the genetic lineage of the first record of Iraqi strain *F. fomentarius* which was collected from the dead trunk *Ficus sycomorus* L., 1753 in Sulaylmaniyah Province. The study also and investigated the intraspecific genetic variability of the polypore. After multiple DNA sequence alignment analysis with international sequences, the study confirmed a genetic non-homogeneity of the presented species, and revealed the Iraqi strain has a unique 7 pb signature sequence and belonged to the lineage B. The Iraqi strain has only 2 base pairs differences from Slovak Southern European sequence and 4-8 base pairs from China, Asia (lineage B) and North American. Characterizing the genetic lineage of the current Iraqi strain is important to preserve its divers therapeutic prosperities and biotechnological applications of this species.

Keywords: Bioinformatic analyses, Intraspecific genetic variability, Lineages, *Polyporus plorans*, Rare species.

INTRODUCTION

The medicinal tinder polypore, *Fomes fomentarius* (L.) Fr., 1849 (Polyporaceae, Polyporales) is widely distributed across Africa and Asia and throughout North America and Europe. It flourishes in nearly all habitat zones in the forests of Eurasia and North America (Judova *et al.*, 2012). The species is popular in traditional Chinese medicine and still extensively used today. Extensively and involved globally as a source of bioactivity and metabolite production, medicinal complex materials, and therapeutic adjuvants (Chang and

Wasser, 2012; Dresch *et al*., 2015). Ecologically, this species is diversely active with secondary metabolites making the species one of the major decomposers of deciduous tree debris; consequently, it plays a key role as a CO2, CO2 emitters in forest ecosystems, and providies nesting cavities and shelter for animals and insects (Gilbertson, 1980; Judova *et al*., 2012; Mukhin *et al*., 2021).

The tinder polypore is characterized by binding hyphae or branched skeletal hyphae, which confer hardiness to the basidiomata that phylogenetically has been placed in a strongly supported clade (98%) with *Cryptoporus volvatus*, and *Datronia mollis* (Hibbett *et al*., 2014). Historically, the species strains also have been questioned their separation from isolates of *Fomes fasciatus* (Sw.) Cooke, 1885 due to their limited morphological features (Gilbertson and Ryvarden, 1986). However, with limited molecular and physiological data available, it has been confirmed that *F. fasciatus* and *fomentarius* are two distinctive morphological species with some cryptic species within this group of strains (Gáper *et al*., 2016).

The ITS rDNA sequence studies of *F. fomentarius* have shown that the species are genetically heterogenic and previously existed in two lineages (lineages A and B) within European strains (Judova *et al*., 2012) and recently in four sublineages (A1, A2, B1, B2) (Mukhin *et al*., 2018; 2021). Therefore, several studies have been concluded that *F. fomentarius* possibly likely contains cryptic species based on phylogenetic analyses using ITS or multiple genetic markers (Pristaš *et al*., 2013).

Therapeutically, *F. fomentarius* has a long history of therapeutic uses in Hungary, China, and India where it is regarded as a producer of several pharmacologically active compounds. Both *F. fomentarius* basidiomata and cultured mycelia have been recognized to be effective antitumor, glycemic, antiviral, and antimicrobial substances (Huang *et al*., 2012; Gáper *et al*., 2016). However, the results suggested that metabolic production and bioactivates are strongly triggered by fungal host or substrates and the geographic locations of isolates (Dresch *et al*., 2015).

In Iraq, several studies have reported the first records of medical macrofungi to date (Suliaman *et al*., 2022; Al Anbagi and Al-Khesraji, 2022; Marie *et al*., 2023). species of the genera *Populus* (poplars) along with *Salix* (willows), both related to the Malpighiales, Salicaceae, along with other subspecies are widely distributed across different geographic regions and are ecologically and economically important landscape trees in Iraq (FAO, 2009; Mustafa, 2018). Consequently, many Basidiomyceres species are expected to be associated with these plants or their microhabitats (Suliaman *et al*., 2017; Alshuwaili *et al*., 2021; Al Anbagi and AL-Khesraji, 2022). Recently, the polypore *F. fomentarius* was collected from the dead trunk *Ficus sycomorus* in Sulaylmaniyah Province (Marie, 2022). This important species requires genetic characterisation and detection or investigation of its antimicrobial activities. Thus, the aim of the present study is to analyse the intraspecific variability and determine the genetic lineage of *F. fomentarius* for the first time using phylogenetic species analyses based on available ITS sequences.

MATERIALS AND METHODES

Specimens' information: The basidiocarps were collected from the dead tree trunk *Ficus sycomorus* using a clean knife during a mushroom survey in 2021 in selected parts of Sulaymaniyah Province, North Iraq. The phenotypic characteristics of the fruiting body were described including macro and microscopic characteristics, as documented by AL-Obaidy (2023). The current scientific name and taxonomic position of species follow the recent edition of Authors of fungal names available in the international Index Fungorum website www.indexfungorum.org.

The extracted DNA of *F. fomentarius* was amplified and sequenced using ITS1 and ITS4 primers (White *et al*., 1990) with a Thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem, the newly generated sequence was later submitted into the GenBank database under the genetic code OQ109278.

Phylogenetic and statistical analysis: The submitted sequence was reverified and reanalyzed using the Basic Local Alignment Search Tool (BLAST) accessible through the National Center for Biotechnology Information (NCBI) Gen Bank (www.ncbi.nlm.nih.gov/genbank/). The reiterating of sequence similarity searching was applied to detect the closest sequences to the Iraqi strain for subsequent genetic analyses (Tab. 1).

Phylogenetic analyses were achieved using 15 rDNA ITS sequences obtained. These included the Iraqi strain of *F. fomentarius* and selected sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/, Tab. 2). According to Gáper *et al*. (2016) and Mukhin *et al*., (2018), the selected sequences served as references for sublineages A1, A2, B1and B2. These sequences were added and multiple aligned using MAFFT Algorithm with manual verification of the obtained results. The 7 bp signature sequence TCGTTTG. From the aligned sequences was detected to discriminate *F. fomentarius* genotypes using Genoius program (Judova *et al*., 2012). The sequence of *F. fasciatus* was selected as an outgroup (Badalyan *et al*., 2022). The RAxML analyses were performed after determining the best ML model and a tree was constructed using RAxML 7.2.8. Bootstrap values were calculated using Maximum Parsimony analyses with 1000 bootstrap replicates.

Table (1): The blast results of the compared Iraqi sequence *Fomes fomentarius* with NCBI isolates.

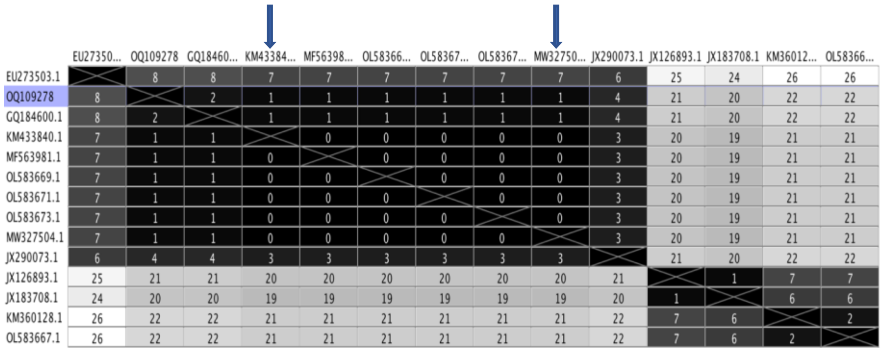
Species	**Identification %**	**Host tree**	**Country**	**GenBank code**
Fomes fomentarius	99.69	*Fagus sylvatica*	Serbia	MW327504
F. fomentarius	99.53	*Acer platanoides*	Asian part of Russia	MF563981
F. fomentarius	99.53	*Carpinus* sp.	Armenia	OL583673
F. fomentarius	99.53	*Fagus* sp.	Armenia	OL583671
F. fomentarius	99.53	*Salix alba*	Armenia	OL583669

Table (2): Overview of polypore strains with isolates in Table (1) used for phylogenetic analyses including GenBank accession number, natural substrate (host), and provenance.

Species	Host tree	Country	GenBank code
F. fomentarius	*Salix* spp.	Iran	KM433840
F. fomentarius	*Populus tremula*	Slovakia	GQ184600
F. fomentarius	*Fagus* sp.	Armenia	OL583667
F. fomentarius	*Picea abies*	Austria	KM360128
F. fomentarius	*Betula papyrifer*	USA	JX126893
F. fomentarius	*Betula papyrifera*	USA	JX183708
F. fomentarius	Unspecified	China	JX290073
F. fomentarius	Unspecified	China	EU273503
F. fasciatus	*Platanus occidenttalis*	USA	JX126900

RESULTS AND DISCUSSION

The newly submitted sequence was 723 continuous base pairs with 48% of GC content. The molecular identification of the Iraqi query sequence confirmed the morphological species affinity and identified it as *F. fomentarius.* The BLAST results in NCBI databases showed a sequence similarity, the value of the newly sequence 99.69% and query coverage of 100% with the *F. fomentarius* strain TMF2 which was isolated from the deciduous forest located on mountain Avala, Serbia, from a beech *Fagus sylvatica* tree that has accession number MW327504. This newly generated ITS sequence also showed 99.53% similarity with other isolates highlighted with blue arrows in Diagram (1). The ITS regions in particular are useful for taxonomic reasons due to being highly variables between species and their conserved with intraspecific similarities usually higher than 99% (White *et al.*, 1990). The currently generated ITS sequence is the first deposited sequences in Iraq and the third sequence from Asia, the world's largest continent, submitted to the GenBank. According to our current knowledge, the species has been isolated from only a few strains in China and two strains in Iran representing the Asia isolates sublineage.

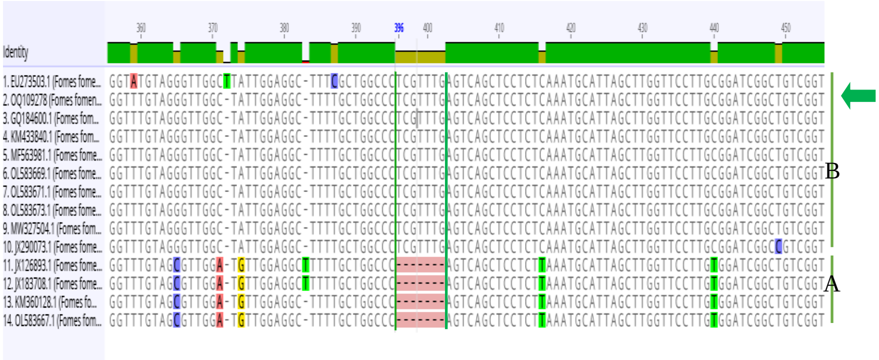


	EU27350...	OQ109278	GQ18460...	KM43384...	MF56398...	OL58366...	OL58367...	OL58367...	MW32750...	JX290073.1	JX126893.1	JX183708.1	KM36012...	OL58366...
EU273503.1		8	8	7	7	7	7	7	7	6	25	24	26	26
OQ109278	8		2	1	1	1	1	1	1	4	21	20	22	22
GQ184600.1	8	2		1	1	1	1	1	1	4	21	20	22	22
KM433840.1	7	1	1		0	0	0	0	0	3	20	19	21	21
MF563981.1	7	1	1	0		0	0	0	0	3	20	19	21	21
OL583669.1	7	1	1	0	0		0	0	0	3	20	19	21	21
OL583671.1	7	1	1	0	0	0		0	0	3	20	19	21	21
OL583673.1	7	1	1	0	0	0	0		0	3	20	19	21	21
MW327504.1	7	1	1	0	0	0	0	0		3	20	19	21	21
JX290073.1	6	4	4	3	3	3	3	3	3		21	20	22	22
JX126893.1	25	21	21	20	20	20	20	20	20	21		1	7	7
JX183708.1	24	20	20	19	19	19	19	19	19	20	1		6	6
KM360128.1	26	22	22	21	21	21	21	21	21	22	7	6		2
OL583667.1	26	22	22	21	21	21	21	21	21	22	7	6	2	

Diagram (1): The heatmap and number of bases/ residues which are not identical of the *Fomes fomentarius* Iraq strain (violate color) compared with some international strains in the DNA alignment of partial internal transcribed spacer (ITS) sequences. The Iraqi strain was different in a single nitrogen base from the bases found in DNA isolates in the column 4 (KM433840) to the column 9 (MW327504).

The current results from the heatmap differences displayed that sequence divergence between the Iraqi strain of *F. fomentarius* and the outgroup *F. fasciatus* was 100 base pairs in agreement with the findings of Gáper *et al.* (2016). The sequence of the Iraqi strain is highly homologous with only one base difference per sequence due to the insertion of a single adenine (A) base into its sequence in the site 23 Compared to the closest international sequences (Diag. 1). The number of base differences between the Iraqi sequence (OQ109278) and the Slovak Southern European sequence, (GQ184600) was 2 base pairs while differences with other sequences from China, Asia (lineage B), North American, and Northern European (both lineage A) strains were 4-8 and 20-22 base pairs respectively. These results concurred with previous results which revealed there were base sequence between lineages A and B of *F. fomentarius* nearly 20 times higher (Gáper *et al*., 2016).

The sequence genotype of the Iraqi strain was classified as genotype B based on the presence of the 7 bp signature sequence TCGTTTG in both the ITS1 region of the isolate (data not shown) and alignment sequences of the investigated strains showed in Diagram (2). According to Judova *et al.* (2012), the presence of 7 bp signature sequence in the ITS1 region is used to distinguish *F. fomentarius* genotypes in which all strains are classified as genotype B when that sequence presence while strains are classified as genotype A, when strains lacking sequence as being shown in Diagram (2). Other researchers have confirmed this signature and use it to differentiate isolates in into lineages A and B or A1, A2 and B for strains collected from (Gáper *et al*., 2016; Náplavová *et al*., 2019). This evidence suggests that *F. fomentarius* may include several sympatric cryptic species and multifaceted genetic structure of *F. fomentarius* population presenting different host preferences (Judova *et al*., 2012; Gáper *et al*., 2016).



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1. EU273503.1 (Fomes fome...   GGTATGTAGGGTTGGCTTATTGGAGGC-TTTCGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
2. OQ109278 (Fomes fomen...    GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
3. GQ184600.1 (Fomes fom...    GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
4. KM433840.1 (Fomes fom...    GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
5. MF563981.1 (Fomes fome...   GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
6. OL583669.1 (Fomes fome...   GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
7. OL583671.1 (Fomes fome...   GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
8. OL583673.1 (Fomes fome...   GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
9. MW327504.1 (Fomes fom...    GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTGTCGGT
10. JX290073.1 (Fomes fome...  GGTTTGTAGGGTTGGC-TATTGGAGGC-TTTTGCTGGCCCTCGTTTGAGTCAGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGCGGATCGGCCGTCGGT
11. JX126893.1 (Fomes fome...  GGTTTGTAGCGTTGGA-TGTTGGAGGCTTTTTGCTGGCCC-------AGTCAGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGTGGATCGGCTGTCGGT
12. JX183708.1 (Fomes fome...  GGTTTGTAGCGTTGGA-TGTTGGAGGCTTTTTGCTGGCCC-------AGTCAGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGTGGATCGGCTGTCGGT
13. KM360128.1 (Fomes fo...    GGTTTGTAGCGTTGGA-TGTTGGAGGC-TTTTGCTGGCCC-------AGTCAGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGTGGATCGGCTGTCGGT
14. OL583667.1 (Fomes fom...   GGTTTGTAGCGTTGGA-TGTTGGAGGC-TTTTGCTGGCCC-------AGTCAGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGTGGATCGGCTGTCGGT
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Diagram (2): The DNA alignment of partial internal transcribed spacer (ITS) sequences of *Fomes fomentarius* Iraq strain (pointed in the green arrow) with some international strains. The strains were divided into two variable lineages: lineage A strains (GenBank accession nos. starting with JX126893 and ending with OL583667) and lineage B strains (GenBank accession nos. starting with EU273503 and ending with MW327504) based on Judova *et al*. (2012). Lineage specific residue is surrounding by two green lines. Unidentical nucleotide residues are colored by different.

Intraspecies genetic and morphological analyses

The phylogeny tree of the current results supports the separation of *F. fomentarius* from Iraq and other international species sequences from the sequence of *F. fasciatus* as distinct phylogenetic species (Diag. 3) in agreement with the previous studies (McCormick and Grand, 2013; Badalyan *et al*., 2022). The investigated isolates in the presented tree clearly indicate the presence of four clades within *F. fomentarius* with Bootstrap values >70%. The tree separated clades based on the geographic origin and the substrate. Both these features are essential factors driving speciation in this genus (Dresch *et al*., 2015). The topology of the tree in the current study aligns with Dresch *et al*. (2015) who inferred four clades of *F. fomentarius* corresponding to North European, North American and East Asian, Chinese, and South European clades using the Maximum likelihood method. The strains of these distinct lineages grow on different host tree species North European (Austria), and Chinese isolates and another clade compressing a mixture of isolates from Iraq (the current strain), Iran, Sothern European (Slovakia) and Asia parts of Russia (Southern Urals). The Iraqi sequence clustered with the Iranian and Southern European strains (sequences) which were sister clade to Chinese strains.

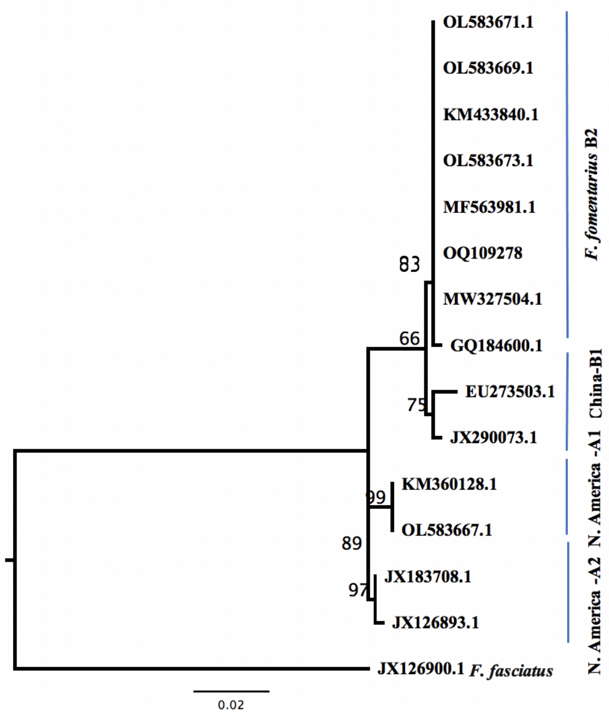

Diagram (3): Phylogenetic placement of the *F. fomentarius* Iraqi strain and some international strains from ITS data inferred by using the Maximum Likelihood method.

Unlike the findings of Badalyan *et al*. (2022), the current results separated the Iranian and Chinese strains from each other into different groups. The presented results support the recent proposal that *F. fomentarius* is a taxonomically complex species (Badalyan *et al.*, 2022). Within a single lineage, species strains isolated from distinctive substrates exhibited significant variation in their optimal growth temperatures, secondary metabolites, and bioactivities of fruit body extracts. Therefore, understanding the intraspecies genetic relations between strains connecting with other data such as species host, geographic location, and physiological properties along with other related factors provides valuable insights into the medicinal *F. fomentarius* lineages/sublineages (Dresch *et al.*, 2015).

CONCLUSIONS

In conclusion, the current results reveal the interspecies genetic variation of *Fomes fomentarius* from the north part of Iraq (a new record) in comparison to strains from other parts of the world. Further studies are needed to collect isolates of the species from other parts of Iraq to detect whether there are other lineages/sublineages and/or even other species related to the genus. Species delimitation is a significant issue in medicinal fungi. Therefore, detecting intraspecies genetic variability of *F. fomentarius* strains is essential due to their diverse therapeutic prosperities. That is vital for improving biological features of species strains as enhanced bioactive materials leading to improve the medicinal value or biotechnological applications of this species.

CONFLICT OF INTEREST STATEMENT

"The authors declare no conflict of interest to declare".

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التحاليل الوراثية و المظهرية لداخل النوع للتسجيل الجديد للفطر الطبي

Fomes fomentarius (L.) Fr., 1849

(Agaricomycetes, Polyporales, Polyporaceae)

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الخلاصة

يعد الفطر *Fomes fomentarius* (L.) Fr., 1849 العائد لصنف Agaricomycetes ، رتبة Polyporales و عائلة Polyporaceae من الانواع المهمة التي تستخدم على نطاق واسع في الطب التقليدي. مع ذلك، فقد لوحظ أن هناك تباينا وراثيا بين سلالات الأنواع التي تم جمعها من مناطق جغرافية مختلفة في العديد من الدراسات. ركزت الدراسة الحالية على تحديد النسب الجيني Genetic lineage للتسجيل الأول للسلالة العراقية *F. fomentarius* التي جمعت من الجذع الميت للنوع *Ficus sycomorus* L., 1753 في محافظه السليمانية والتحري عن التباين الوراثي داخل النوع لهذا البوليبور Polypore. بعد تحليل محاذاة التسلسل الحامض النووي منقوص الاوكسجين المتعدد مع التسلسلات الدولية، اكدت الدراسة عدم التجانس الجيني للنوع المدروس واظهرت أن السلالة العراقية لها بصمة القواعد النيتروجينية السبعة 7 pb وتنتمي إلى السلالة B. اختلفت السلالة العراقية فقط بزوجين قاعديين عن تسلسل جنوب أوروبا السلوفاكية و4-8 أزواج قاعدية من الصين وآسيا (السلالة B) وأمريكا الشمالية. يعد توصيف السلالة الوراثية لسلالة العراقية الحالية أمرا مهما للحفاظ على التنوع العلاجي والتطبيقات التكنولوجية الحيوية لهذا النوع.