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DNA Barcoding Identifies *Juniperus oxycedrus subsp. macrocarpa* in Derna Region, East Libya

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ABSTRACT

Many methods are accessible that apply diverse criteria for the reasons of identifying taxonomic specialization depending on DNA sequencing information. It is crucial for the studies of taxonomy and biodiversity using DNA barcode technology to fast and accurately make species identification in the forests. According to the Encyclopedia of Earth, many wonderful and rare plants are destroyed. In the tropics and subtropics, numerous evergreen conifers are jeopardized. These plants grow in remote places of our planet, which are inaccessible. It merits referring to that *Juniperus spp.* an imperative part of Mediterranean arid and semi-arid biological communities. *Juniperus oxycedrus subsp. macrocarpa* is a rare woody species found in Jebel Al-Akhdar, Libya in only one peripheral site north-west Derna. A robust analysis presented based on using morphological traits of needles, seeds and cones, and DNA technology. Along these lines, jeopardized plant populations could be recognized more effectively. This study universality of tree species DNA barcodes. such as the *rbcL* and *matK* plastid markers, and examined their abilities of species identification. The morphological and genetic results strongly support the recognition of *J. macrocarpa* at the subspecies *J. oxycedrus*.

Keywords: DNA barcoding; *Juniperus oxycedrus*; *matK*; *rbcl* and Sequencing.

INTRODUCTION

In the tropics and subtropics, numerous evergreen conifers are jeopardized. The comprehensiveness of primers is perceived as a vital criterion for assessing the suitability of DNA barcodes (Cowan *et.al*, 2006). DNA barcoding utilizes short DNA arrangements, ordinarily from a standard marker or markers, which might be utilized to address two unmistakable objectives: firstly, to identify unknown species and secondly, to discover new species (CBOP 2009).

DNA barcoding is a taxonomic method that uses a designated portion of specific genes (proposed to be analogous to a barcode) to identify an organism to species. Maturase K (*matK*) and Large subunit of Rubisco (*rbcL*) are plants plastids genes used for DNA barcoding of angiosperms.

El- Jabel El- Akhdar region (JAR) is located between longitude 32^{o} and 33^{o}N and 20^{0} to 23E. The region is about 360 km long and about 60 km in width from the seashore (Azzawam, 1995). JAR is a forest which well-stocked growing on fertile upland soil located in the northeastern part of Libya. The area has a distinctive environmental characteristic for being a permanent evergreen forested area. The genus *Juniperus* L. (*Cupressaceae*, gymnosperms) comprises of about 60 dioecious woody species. They are widely distributed throughout the northern hemisphere. It is naturally located from the Arctic regions to the south of tropical Africa and the mountains of Central America (Adams, 2011).

Juniperus oxycedrus L. has a place with *Oxycedrus* of *Juniperus* genus. It is a variable class with three subspecies: *J.oxycedrus subsp.oxycedrus, J. oxycedrus subsp. macrocarpa* and *J.oxycedrus subsp. badia* (Greuter *et al.* 1984). Which have differed inhabit, cone size and needle width (Lebreton and Mauracciole 1991).

Juniper was first described and named by Smith (1816) as *Juniperus macrocarpa.* Lately, it was classified as a subspecies of *Juniperus oxycedrus* L., by Ball (1878). However, the taxonomic status was supported by various authors (Amaral Francoet *al al.* 1993). Recent investigations, by Adams (2000) dependent on leaf fundamental oils and molecular data point to its delimitation as a species. This taxon distributed moderately in the Mediterranean region (Amaral Franco *et al.* 1993). The pressure in the human population inflated caused the halt of endangered species that were introduced to recovery programs (Blanca *et al.*, 1999).

J. oxycedrus supsp. macrocarpa is a rare woody species found in Jebel Al-Akhdar, Libya in only one peripheral site north-west Derna. The distribution of this population is physiographical dependent, where the individuals are restricted to the north-facing slope of the first rocky ridge close to the seashore.

Separation is an empirical procedure for plant development. Marine archipelago provides an ideal temporal-spatial structure for the production of genotype variability (Whittaker and Fernández-Palacios, 2007). There has recently been considerable discussion about using DNA barcoding to identify plants (Chase and Fay, 2009).

Therefore, the main goal of this study was to clarify the taxonomy of the *Juniperus oxycedrus* by molecular DNA markers and sequences data. Besides, the morphometric analysis was performed on selected *J. oxycedrus subsp. macrocarba.*, to test the degree of morphological distinctiveness, and to highlight the most useful and significant diagnostic morphological traits.

MATERIALS AND METHODS

The study area

The study area is located on the second side of El-Jabal El-Akhadar Mountain in the eastern region of Libya (Derna), where the city lies between latitudes 22° 38′ 0 N and 32° 46′ 0″ E (Fig. 1). The climate of the study area is comparable to that of El-Jabal El-Akhadar with a mean temperature of about 20 C^{0}. The average rainfall ranges between 200-300 mm (El-Barasi and Saaed 2013).

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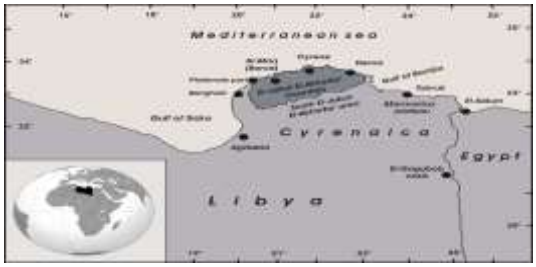

Figure 1. Location map of the study area(el-barasi *et al.* 2013).

Morphometric investigation

Twenty-five *J. oxycedrus subsp.macrocarpa* trees were selected randomly, which grows on dunes. Morphological direct traits individuals:, Cl cone length (mm); CD, cone diameter (mm); seeds number; SL, seed length (mm); ST, seed thicknesses (mm); NL and leave length (mm).Fifty con, fifty seeds and fifty leaves from trees were measured following the procedures described by Klimko *et al*. (2007).

Genetic analysis

Data collections

Ten trees of *J. oxycedrus subsp. macrocarpa*, were sampled. The leaves were collected randomly from each tree, well-isolated parts of their crowns at 1 m ground level.

DNA Isolation

DNA isolation was carried out from 1gm leaf tissues. Leaves were frozen in liquid nitrogen and homogenized using CTAB (Cetyl-tetramethyl ammonium bromide) method according to Doyle and Doyle (1990). The quantification of the total DNA amount was carried out by using Thermo Fisher Scientific Inc. NanoDrop 2000 Spectrophotometer Version 1.4.1.

Polymerase Chain Reaction (PCR) analysis:

The sequences of gene-specific primer pairs used are presented in Table (1) according to Güvendiren and Kaya (2015). PCR reactions were performed in 25 µL total volume contained the following components: 12.5 µL PCR master mix (Applied Biotech, Egypt), 8.5 µL distilled water, 1 µL of each primer and 2 µL cDNA as a template. Amplification was performed in Agilent technologies sure cycler-8800, USA. The optimized PCR cycles for the amplification parameters were given in Table (2). PCR products were separated on 1% agarose gels using 0.5x TBE buffer at 150 volts for 1hr. The gel was stained with ethidium bromide at a concentration of 0.5 mg/ml. 100 bp Plus Blue DNA ladder (Gene ON) was used as a molecular weight standard. Bands were visualized on a UV trans-illuminator and photographed using a gel documentation system (IN GENUS SYNGENE BIO Imaging, USA).

Table 1. The sequence of specific primers employed in this study:

Primer	Designation	Oligo sequence from 5´to3´	Source
MatK (J1)	Forward	5′ TTC CAA CTA GAT CGC ACC AT 3′	Güvendiren and Kaya (2015)
MatK (J1)	Reverse	5′ ATT CCA AAG GAA CAG GGA GA 3′	
MatK (J2)	Forward	5′ CTA CTC AAT TCA TCC GGA AA 3′	
MatK (J2)	Reverse	5′ CCT AAT TGT TCT CGA ACT ACA C 3′	
RbcL	Forward	5' ATGTCACCACAAACAGAGACTAAAGC 3'	
RbcL	Reverse	5' GTAAAATCAAGTCCACCRCG 3'	

Table 2. The amplification protocol of Polymerase Chain Reaction (PCR):

Primer	Step	Temperature (in °C)	Time	Cycles
MatK (J1) and *MatK* (J2)	Initial activation	94	5 min	1
	Denaturation	94	1min	30
	Annealing	60	1min	
	Extension	72	2min	
	Final extension	72	3min	1
RbcL	Initial activation	98	45 sec	1
	Denaturation	98	10 sec	35
	Annealing	55	30 sec	
	Extension	72	40 sec	
	Final extension	72	10 min	1

Purification and sequencing

The amplified DNA product for both forward and reverse primers were excised from the gel and purified using a BIO BASIC INC.EZ-10 Spin Column PCR Products purification kit. The automated sequencer of the Sanger method was used for the sequencing of purified selected genes by Macrogene Company (Korea). Sequenced data from the forward and reverse primers were checked, carried out with the National Center for Biotechnology Information (NCBI) databases and aligned using the basic local alignment search tool (BLAST) network service (Assel et al., 2019).

RESULTS AND DISCUSSION

Results

Morphological analysis:

Juniperus species under study were naturally grown in Derna in Northeast Libya. They were considered as endangered species by Farjon (2013). Since the numbers of trees were not large, most of them were far from each other.

The results showed that morphological traits of Juniperus species under study have an average of 2.6 m in height, which had large cones, 15.38 mm Cone length and 15.72 mm Cone diameter. The cone's colors were light brown to dark brown, slightly purplish and pruinose. The average of seed length (SL) was 6.82mm while the thickness was (4.79) (Fig. 2). The average number of seeds per cone was 3 seeds. Its leave are up to 2.5 mm wide (Table, 3).

Table 3. Mean of morphological traits:

Traits	Mean
Cone length	15.38
Cone diameter	15.72
Seed length	6.82
Seed thickness	4.79
Leave length	2.5

Figure 2. Morphology of cones and seeds of *Juniperus oxycedrus subsp. Macrocarpa.*

Genetic analysis

Results showed the success of PCR amplification for *rbcL* and *matK*. The rates of DNA sequencing were 97.76%, 100.00, 99.54, 99.39, 99.77 and 99.43%, respectively, suggesting that both *rbcL* and *matK* were universal for tree species, where consolidating two markers improves the exactness of species distinguishing.

Three different bands were detected from the PCR product of these primers,. The purified DNA was sequenced using the automated sequencer of the Sanger method by Macrogene Company (Korea). DNA homology searches were carried out with the National Center for Biotechnology Information (NCBI) databases, using the basic local alignment search tool (BLAST) network service (www.ncbi.nlm.nih.gov/ BLAST). However, the nucleotide sequences were illustrated in Table (4). Results revealed that, with using *rbcl* primer, the sample under study is similar to *Juniperus oxycedrus Subsp. macrocarpa* by 99%. While, using *matK* primer the similarity is to *Juniperus oxycedrus* by 99%.

Table 4. PCR products sequence results and the similarity genes.

Primer	Sequences	Similarity	Species
*RbcL*a (F)	TGGATTACAGATTAACTTATTATACTCCGGAATATCAACCAAAGATACTGATATCTTGGCAGC ATTTTCCGAGTCACTTCCTCCAACCTTGGAGTGCCCCCCGAAGAAGCGGGAGCAGCGGTAGCT AGCCGAATCTTCCACTTGGTACGTGGACCACTGTTTGGACCGATGGCCTTACCAGTCTTGATC GCTACAAGGGGCGATGCTTTGATTTGAACCCGTTCCTGGAGAAGAAACTCAATTTATTGCCTA TGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGCTCTGTGACTAACCTGTTTACTTCTATTGTA GGTAATGTATTTGGATTCAAAGCTTTACGGGCTCTACGTCTGGAAGATTTACGAATTCCTCCT GCTTATTCAAAAACTTTTCAAGGCCCACCACATGGTATTCAAGTAGAAAGAGATAAATTAAAC AAATATGGTCGTCCTTTATTGGGGATGTACTATCAAACCAAAATTGGGTCTATCTGCCAAGAA TTATGGTTAGAGCGGTTTATGAATGTCTCCGCGGGATTTTTTTTTTTTTTTAAAAAAAAAAAAA GAAAAAATTAAAAAATCAAAATACAAAATCTCAATGGCTATTTTACAATTACCCATACTGAC GCCTGGCGTGTGGTTACAGGCTTTTAGGCT	Identical 97.76% Accession FR831949.1	*Juniperus oxycedrus subsp. macrocarpa*
*RbcL*a (R)	TCATAATTCTTGGCAGATAGACCCAATTTTGGTTTGATAGTACATCCCAATAAAGGACGACCA TATTTGTTTAATTTATCTCTTTCTACTTGAATACCATGTGGTGGGCCTTGAAAAGTTTTTGAAT AAGCAGGAGGAATTCGTAAATCTTCCAGACGTAGAGCCCGTAAAGCTTTGAATCCAAATACA TTACCTACAATAGAAGTAAACAGGTTAGTCACAGAGCCTTCTTCAAAAAGGTCTAAAGGGTA AGCTACATAGGCAATAAATTGAGTTTCTTCTCCAGGAACGGGTTCAATATCATAGCATCGCCC CTTGTAGCGATCAAGACTGGTAAGGCCATCGGTCCAAACAGTGGTCCACGTACCAGTGGAAG ATTCGGCAGCTACCGCTGCTCCCGCTTCTTCGGGGGGCACTCCAGGTTGAGGAGTGACTCGGA ATGCTGCCAAGATATCAGTATCTTTGGTCTGATATTCCGGAGTATAATAAGTTAATCTGTAAT CTTTAACACCCGCTTTGAATCCGACACTTGCTTTAGTCTCTTGGGGGGGGGAAAATTAAAAAT AACAACCCCTAAACAACAAAGCACCCCTAACCAGGCTAGTTTCCCCAAGGGGGGGGGTTTTA CCAAGTACATTCCCCCACTGGACCCGCCTGATTTTTTTTGGGTTGGCCCTGGCTTTTTAAATGC CAGGTTCGGTCCTAGCCACAGACCATGCCCGGGGATTTACCGGGCTTTTAAGCCATTCCTTGA TATTCCGGCAAAGGCACGGGCCCAAAAGTGACGGTACATGGGCAGAATTTCCAGGGAAGCCC TGTTCCAGTAAATTCGTCGCAGAGCCAAAATTCGGTACATGCGGAGGAGAGT	Identical 100.00% Accession FR831949.1	*Juniperus oxycedrus subsp. macrocarpa*
MatK (J1) (F)	AATCAACAGGTTATTCTTGGGTCCGGGGGTTTAAAAAATGGATAACTTCCAAAGAAATTCAA ATAAACATCGATCTTGGCAACAATTCTTTTTATATCCGCTTTTTTTTCAGGAAGATCTTTACGC AATTGCTCATGATCATCATTTAGATAGATCTGGTTCCGAGGAACCGACGGAAATTTTAGTTTC TCATTTTTTGAGTTTCCTGACTGTAAAACGTTCAATACGTCGAATACGTAAACAGAATAATTC AATTAGTTTATTCAGGAATCCCGATTCAAATAAATTCATTGAATACAATAAGAATTCTTTTAA ATCGATACTAAAAGGGTTTACAATTGTCTTGGAAGTTTCGTTCGCAATGCGATCAAAACATTT CATAAAAGGAATGGATGGATGGAATAGTCTCCGATCCATCCATTGCATATTTCCTTTTATGGA GGATAAATTACCACATTCCAATTATATATCAGATATACGAGTGCCCTACTCAATTCATCCGGA AATTTTGGTTCGACTTTTTCGTCGCTGGATCCTAGATACTCCTTCTTTGCATTTATTACGATGGA TTCTCCATGAATGTAGTTTTAGTCGAGAAAATTTGCAGAAATCTCTGATTACACAGAGAGAAA ATACGTTCTCCCTGTCCCCTTGGGAAAAAAAAAAAAAAACCCGTTTGCCCTTTTTTCAAATAC CCCCCGGTGCCACCCCCCCCGCAAAAAAACGCGCCACTCTTTTTAACGCCCTAGGTACATTGT TACTTATTCCCCTCCATCCTACCCCCCCCTCTCCCTTTCCTCCAGATCTACCCCCCCCCCGGGTA CCCCCCAAAGTTTCCTTCTCGACCCTGGCCTTCCCCCATACTTTGCGCCCCAATTTCCCCCCTTT TCCCCCCCTCCCCCCCCCCGTTACCTTACAAATAAAACCCGCCAATAAGGAACCTTTCACCCC CTTTTTTGCCCGAAGTTTTAAACAGCTTGGGTAGCCGAGCTTCAACGAGCATCTCAATCAGTG GGCCACCAGCCCCGTTTGTTTGTGAATGCGGTAGCACAAGATGGCCCACTATCTATCTAAAGT CATCATCCGCCGGTCCGTTGCAAATCCTGTCAAAGGT	Identical 99.54% Accession HM024041.1	*Juniperus oxycedrus*
MatK (J1) (R)	TTTCCCTGGGTATCAGAGATTTCTGCAATTTTCTCGACTAAACTACATTCATGGAGAATCCATC GTAATAAATGCAAAGAAGGAGTATCTAGGATCCAGCGACGAAAAAGTCGAACCAAAATTTCC GGATGAATTGAGTAGGGCACTCGTATATCTGATATATAATTGGAATGTGGTAATTTATCCTCC ATAAAAGGAAATATGCAATGGATGGATCGGAGACTATTCCATCCATCCATTCCTTTTATGAAA TGTTTTGATCGCATTGCGAACGAAACTTCCAAGACAATTGTAAACCCTTTTAGTATCGATTTA AAAGAATTCTTATTGTATTCAATGAATTTATTTGAATCGGGATTCCTGAATAAACTAATTGAAT TATTCTGTTTACGTATTCGACGTATTGAACGTTTTACAGTCAGGAAACTCAAAAAATGAGAAA CTAAAATTTCCGTCGGTTCCTCGGAACCAGATCTATCTAAATGATGATCATGAGCAATTGCGT AAAGATCTTCCTGAAAAAAAAGCGGATATAAAAAGAATTGTTGCCAAGATCGATGTTTATTT GAATTTCTTTGGAAGTTATCCATTTTTTAAACCCCCGGACCCAAGAATAACCTGTTGGATTATT AAAGATAATACATGGTGCGATCTCAAATTGGGAAAAAAAAAAAAAAAAACAACACACAAAC AAAAAAAAAAAAAAAAAAAAAAAAAAAA	Identical 99.39% Accession HM024041.1	*Juniperus oxycedrus*
MatK (J2) (F)	TTCATTTTTCGTCGCTGGATCCTAGATACTCCTTCTTTGCATTTATTACGATGGATTCTCCATGA ATGTAGTTTTAGTCGAGAAAATTTGCAGAAATCTCTGATTACACAGAGAGAAAATACGTTCTC CCTGTTCCTTTGGAATTTTTATGTGTATGAATGCGAATCGTTTTTAATTCCTCTTATTAAACGAT TTTTTAATTCACAATCATTGTTATATGAATCTTTTCCGGATCGAACTCATTTTGAGAAAAAGAT CAAAGATATTGTTCTATTTCCTCCTCAAAAAATTTCAACAAAAAAGATCTGGTTGTTGAAGAA TTCTTTCATCCATTATGTGAGATATGGAGAAAGATCCCTTATAGCTCTAAAGGGTACGCATCT TCAAGTGAAAAAATGTAGATATCATCTGTTTCATTTTTGGCAATACTATTTTCATCTTTGGTTT CAACCGTATAGGATATGCAGTCTTGAATTATCCAAGATTTCTTTTTCTTTTTTAGGTTTTTTTAT GCATGTTAAAATGAGACCTCTCGTGGTTAGAGCCAAAATGCTAGATGATTTATTCATTACCGA TCTTATTACCAATGAATTAAACTCAACAGCTCCGATTAAACCAATTCTTTTTTCTTTAGCTAAA GAAAAGTTTTGTGACATCTCAGGGTGGCCAATTAGTAAATTGTCTTGGACCAGTCTATCAGAT GATGATATTCTCGATCGATTCGATCGAATTTGGATAAATCTTTTTGATTACTACAGTGGATCCA TCAATCAAGATGGTTTATATCATATAAAGTATATACTTTTAATTTCATGTGCTAAAACTTTGGC CTGTAAACATAAAAGTACTATACGTGTAGTTCGAAAACAAATTAAGGGAAAATGGGGAAAAA TAAAAACAAAATTTTAAGAATAATTAATATAAAAATTGAATAAAAGGAGGGATATATGGGTA AGGTAGAGTGATATGTAGGGATAATTGTTGGTAAAGGAAATGTGAGTGGGTGTTTGGGTTTG GAGAGGATAGGAAGGGAGTAAGTGAAAATTTTATTGATGTTGATAATTAATTATGGGGGAAG GGAGGGAAATTAATTAAGATGGGTTT	Identical 99.77% Accession HM024041.1	*Juniperus oxycedrus*
MatK (J2) (R)	GAATTTTTTTTGCTTAAGGCCAAGTTTTAGCACATGAAATTAAAAGTATATACTTTATATGATA TAAACCATCTTGATTGATGGATCCACTGTAGTAATCAAAAAGATTTATCCAAATTCGATCGAA TCGATCGAGAATATCATCATCTGATAGACTGGTCCAAGACAATTTACTAATTGGCCACCCTGA GATGTCACAAAACTTTTCTTTAGCTAAAGAAAAAAGAATTGGTTTAATCGGAGCTGTTGAGTT TAATTCATTGGTAATAAGATCGGTAATGAATAAATCATCTAGCATTTTGGCTCTAACCACGAG AGGTCTCATTTTAACATGCATAAAAAAACCTAAAAAAGAAAAAGAAATCTTGGATAATTCAA GACTGCATATCCTATACGGTTGAAACCAAAGATGAAAATAGTATTGCCAAAAATGAAACAGA TGATATCTACATTTTTTCACTTGAAGATGCGTACCCTTTAGAGCTATAAGGGATCTTTCTCCAT ATCTCACATAATGGATGAAAGAATTCTTCAACAACCAGATCTTTTTTGTTGAAATTTTTTGAGG AGGAAATAGAACAATATCTTTGATCTTTTTCTCAAAATGAGTTCGATCCGGAAAAGATTCATA TAACAATGATTGTGAATTAAAAAATCGTTTAATAAGAGGAATTAAAAACGATTCGCATTCATA CACATAAAAATTCCAAAGGAACAGGGAGAACGTATTTTCTCTCTGTGTAATCAGAGATTTCTG CAAATTTTCTCGACTAAAACTACATTCATGGAGAATCCATCGTAATAAATGCAAAGAAGGAG TATCTAGGATCCAGCGACGAAAAAGTCGAACCAAAATTTCCGGATGAAATTGAGTAGGAATT TGGAAAGGGGAAGGGGGGGGAGGAAGGAGGGAGAGAGAAGGAAGAAAGAAGGGGGGAAG AGAAGGAGGGGGGAAGAGGAGAGAAGAAGAAGAAAGAGAGGAAGGAGAGAGAAGAGAGA GGAGAGAGGAAGAAGAAAGGAGAGAGAAAGGAAA	Identical 99.43% Accession HM024041.1	*Juniperus oxycedrus*

Discussion

DNA barcoding is a useful tool for species identification, it enables the researcher to distinguish species and find new ones. However, DNA barcodes tags can't generally recognize firmly related species, and the size and culmination of standardized identification databases are key parameters for their fruitful application. Thus this study tested the ability of *rbcL*, *matK* plastid markers to identify the samples under research.

Besides clarifying phyletic connections, DNA sequence data is helpful for the elucidation of the scientific categorization choices. For instance, the taxonomic of *Juniperus* has been disputable for quite a long time. A large number of the taxa are profoundly variable and characterized based on morphological trait.

The dioecious species, *J.oxycedrus subsp. macrocarpa (Cupressaceae)*, is 1– 5 m high, fanning, with huge shelter. Cone improvement begins in summer with the fertilization of female cones and finishes in the following summer through embryo development. Female cones can be found at various phases of development on a similar plant while fruit aging and dispersal are conveyed from October till January.

One of these the traits ordinarily utilized systematically is the span of ready cones. Especially in the family *Juniperus* it delimits some taxa, and in *J. oxycedrus* it recognizes the subspecies *oxycedrus* and *macrocarpa*. Another valuable biometric trait in this family is the number of seeds per cone. Beforehand, Gauquelin *et al* (1988) isolated two subspecies in *J. thurifera* as indicated by this trait and biochemical traits. PCR product sequences also revealed that the sample under study is analysis techniques that were used to determine and recognized the selected species using *matK* and *rbcl* primers.

Int his study, the authors had to update the condition of learning on the scientific categorization of the *J. oxycedrus subso. macrocarpa*, in view of thisr study and on research by different authors, which has once in a while been the wellspring of discussion. Molecular analyses and different morphological investigations kept up the rank of *J. macrocarpa*. This study, according to Roma-Marzio *et al* (2017) and Cano *et al* (2018) likewise affirm that for a decent separation among species of groups with troublesome interpretation, morphometric molecular analysis approaches are helpful to illuminate the rank of to the taxa.

Thus, these results further prove that *rbcL* and *matK* as a plant core barcode can viably recognize plant species. These findings indicated that both morphological and genetical analysis accentuated that the sample of El-Jabal El-Akhadar Mountain in the Derna region east Libya is most probably *Juniperus oxycedrus subsp. macrocarpa.*

Conflict of interest

The authors declare that they have no conflict of interest.

Data Availability Statement

Data openly available in a public repository that issues datasets with DOIs.

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تحديد الشفرة الوراثية للعرعر الشوكي *Juniperus oxycedrus subsp. macrocarpa* في منطقة درنة ، شرق ليبيا

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هناك العديد من الطرق التي تطبق معايير متنوعة لتصنيف وتحديد الهوية بناءاً على تسلسل الحمض النووي DNA ، وتكمن اهمية استخدام تقنية الشفرة الوراثية في دراسات التصنيف والتنوع البيولوجي لتسريع تحديد هوية النواع بدقة في الغابات وطبقا لموسوعة الارض، تتعرض العديد من النباتات معراة البذور في المناطق الإستوائية وشبه الإستوائية للخطر. والجدير بالإشارة هنا أن جنس العرعر يعتبر جزء لا يتجزأ من المجتمعات البيولوجية الجافة وشبه الجافه في البحر المتوسط يعتبر العرعر الشوكي ***Juniperus oxycedrus subsp. macrocarpa*** من الأنواع الخشبية النادرة والتي تنمو طبيعيا شمال غرب درنه بالجبل الأخضر – ليبيا. تم تحليل الخصائص المورفولوجية للأوراق والثمار والبذور واستخدام تقنية الحمض النووي عن طريق استخدام تحديد العلامات الوراثية البلاستيدية rbcl و matk لقدرتها على تحديد النوع. واثبتت النتائج الوراثية والمورفولوجية بقوة أن النوع ***Juniperus macrocap*** هو ***Juniperus oxycedrus subsp. macrocarpa*** .

الكلمات الدليلية: العرعر الشوكي ; ليبيا; الشفرة الوراثية;matK; rbcl .