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ORIGINAL ARTICLE

MORCHELLA CONICA PERS., 1818 (PEZIZALES, MORCHELLACEAE): A NEW RECORD FROM IRAQ

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ABSTRACT

The present study reports *Morchella conica* Pers.1818, which belongs to the family, Morchellaceae as a new record of Iraqi macromycota based on the morphological and molecular methods. During their short and often sporadic fruiting season, this fungal species was found in mixed forest unburned areas in Branan ranges (Suliamaniya Province, Northeast Iraq). Currently, *M. conica* is the second *Morchella* species reported from Iraq. The current study aimed to introduce this new record, which is poorly studied in the Middle East. *M. conica* is morphologically described and phylogenetically confirmed. The relationship between this species and other species within the genus was studied using the nrDNA ITS sequences from different species and diverse geographical regions. Maximum likelihood (ML) analyses were also conducted to build the molecular phylogeny of this species. The results of the presented species are essential for assessing the genus geographic distribution and developing information about species of this highly prized edible, industrial medicinal fungus.

Keywords: Bioinformatic, Iraq, *Morchella conica*, Morchellaceae, Phylogenetics, rRNA.

INTRODUCTION

True morels (species of Morchella Dill. ex Pers.: Fr.) are edible ascomycetous fungi belonging to the Pezizales Morchellaceae (Hibbett *et al*., 2007). The wild mushroom fruits sometimes exist prolifically in various forest types throughout western North America. Morels are amongst the most highly prized and valuable edible fungi in the world (Olfati *et al*., 2009; Nitha *et al*., 2017; Tietel and Masaphy, 2018). Morels have considerably gained attention and are widely in demand due to their nutritional and medicinal values as well as the diversity of bioactive ingredients as being radioprotective for mitochondria and DNA and anti-inflammatory, immunostimulants (Tietel and Masaphy, 2018; Yang *et al*., 2019; Nitha *et al*., 2020). True morels are also known for their broad ecological plasticity due to being colonized a wide range of habitats (burned

areas, coastal dunes, orchards, grasslands, and forests) with a wide spectrum of trophic strategies. Through secreting enzymes into the external substrates and absorbing the released nutrients, the taxon obtains its nourishment mostly as saprotrophs, slight parasitism and mycorrhiza-like interactions have also been reported (Du *et al*., 2012).

Although *Morchella* is an imperative genus, the genus still has large gaps in our knowledge about its taxonomy, biology, ecology, and diversity. Taxonomically, *Morchella* is a complex genus and its taxonomic classification system of fungi is still unsolved (Wurtz *et al*., 2005; Masaphy, 2011; Richard *et al*., 2014; Loizides *et al*., 2016). It has been proposed the genus needs in-depth morphological studies and considers species with unique habitats for moving more research into an ecosystem perspective. The species of this genus have been mostly reported and morphologically described from Europe, with only few species described in Asia and USA (Du *et al*., 2012). Based on the classical morphological taxonomy and molecular phylogenetic and biogeographic studies, the genus has been divided into three main distinct groups or clades, Esculenta clade (yellow morels, e.g., *M. esculenta*, *M. deliciosa*, and *M. crassipes* (Vent.) Pers., Elata clade (black morels, e.g., *M. angusticeps* Peck, *M. conica* and *M. elata* Fr.), including semifree capped morels deeply nested. The third group was blushing morels presented an early diverging basal lineage containing *M. rufobrunnea*, and *M. rigidoide*, distributed in the tropics or subtropics (O'Donnell *et al*., 2011; Du *et al*., 2012).

The recent molecular phylogenetic studies revealed that the occurrence of at least 60 species of *Morchella* worldwide (Kuo *et al*., 2012; Du *et al*., 2012; Baroni *et al*., 2018; Petrzelova and Sochor, 2019; Clowez *et al*., 2020). Only a part of the total macrofangal wealth has been subjected to scientific investigation and, mycologists remain to unravel the unexplored and hidden Iraqi macrofungi (e.g. Abdulla *et al*., 1989; Al-Khesraji, 2016). Despite its biogeographic significance, Iraq is still unexplored from macrofungal point of view (Suliaman *et al*., 2017). Reports on Pezizales including Morchellaceae from the country are very limited (Al Anbagi, 2014; Al-Khesraji, 2016, 2018; Al-Khesraji and Al-Hayawi, 2019; Al-Khesraji and Suliaman, 2019). With increasing interest in the genus *Morchella* worldwide, only *M. esculenta* has so far been reported from northeast Iraq (Al-Khesraji, 2016).

The current study presents the results of morphological and molecular identifications which were confirmed using a molecular phylogeny of a newly recorded black morel, *Morchella conica* Pers., 1818 from Iraq.

MATERIALS AND METHODS

Specimens' collection and morphological features

Fresh specimens of morels were collected during the investigation on macrofungi in April-May 2019 on Baranan Mountain (elevation 1200-1400 m) north Darbandikhan City (35.116258°N 45.686245°E) of Suliamaniya Province, Eastern North of Iraq, as a part of Iraqi Kurdistan Region. Data related to a natural habitat, site coordinates, a soil type, and vegetation were recorded at the field. The specimens were photographed in

their natural habitats as well as in the laboratory after being somewhat dried, and transferred to the laboratory for morphological and molecular analyses. Later, the fruit bodies were air-dried and deposited in the Biology Department, College of Education for Pure Sciences, Tikrit University, Iraq.

The macromorphological features were described for fresh fruiting bodies. Measurements of the microscopic features were also performed on the fresh materials; these specimens were described and photographed under a light microscope. The classical technique for fungal identification was concluded according to the relevant literature and keys (Negi, 2006; Watanabe, 2010; Lakhanpal *et al*., 2010; Kuo *et al*., 2012). Later, the morphological identification was confirmed based on amplification of the ribosomal DNA internal transcribed spacer (rDNA-ITS) for two isolates and phylogenetic analyses (White *et al*., 1990).

Molecular and DNA sequencing techniques

The identity of isolated strains was confirmed using DNA amplifications and sequencings of the fungal gene ITS region. Genomic DNA was extracted from fresh fruiting body specimens using the ZR Plant/Seed DNA MiniPrep kit (Zymo Research), following the manufacturer's instructions. The extracted DNA was stored at -20 °C for further analysis. The rDNA-ITS was amplified using the ITS1 and ITS4 primers (White *et al*., 1990). The PCR amplifications were performed in a total volume of 25 µl. This consisted of 1.5 µl DNA, 5 µl Taq PCR PreMix (Intron, Korea), and 1µl of each primer (10 pmol). The deionized distilled water was finally added to complete the total volume. The thermal cycling conditions were as follows: 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 52°C for 1 min, and 72 °C for 1min, followed by 72 °C for 7 min using a Thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). Amplicons were loaded and visualized on 2 % agarose gels stained with Red safe (Intron Korea) under ultraviolet light (309 nm) for quantity determination (Al- Khesraji *et al*., 2021). The successful DNA amplifications were sent to Macrogen Inc./ South Korea for sequencing .

Bioinformatic and molecular phylogenetic analyses

Obtained sequence data of fungal isolates were trimmed, and low-quality edges were removed. The individual sequences were assembled to contigs with the Geneious program (Kearse *et al*., 2012). These sequences were blasted against the ITS database in the NCBI's Gene Bank database for species identification based on the query sequence similarity. The generated sequences in the present study were deposited in GenBank under the accession number MW291450. The results of BLASTN search with the sequences having 99% and above similarity were used for further analyses. Phylogenetic analyses were performed to assess the fungal sequence based on the result of the BLAST search in GenBank and sequences of close related *Morchella* species as recommended (Du *et al*., 2012). The DNA sequences were aligned using MAFFT v7.309 (Katoh and Standley, 2013), and the aligned sequences were manually modified as necessary. Maximum likelihood (ML) analyses were conducted using RAxML V7.2.8 (Stamatakis, 2014). Bootstrap algorithm was realized on the dataset for 1000 replicates in Geneious

version 9.1.8. All characters were preserved as unordered and equally weighted. The alignment gaps were treated as missing data. The resulting tree was visualized in Geneious version 9.1.8.

RESULTS AND DISCUSSION

Habitat and distribution

Solitary on soil under mixed forest unburned sites during April and May in 2019. The fruiting bodies were found in the wet ground between rocky and brown soil covered with decayed plant litter and woody debris. Samples were collected under diverse tree species including *Quercus* sp., *Populus* sp., and *Nerium* sp. However, some specimens were found away from the trees.

Several fruiting bodies of *M. conica* so far are known only from northern Iraq in the current study. The fungus was collected from various localities on the Baranan Mountain in Darbandikhan area (10 Km north Darbandikhan Lake), in Suliamaniya.

Morphological features

The Cap of *M. conica* has 2-6 cm high and 1.5-3 cm wide, conical with acute apex, attached to the stipe, yellowish when young and brownish or black at age, with vertical ribs connected by transverse ribs, forming a series of rectangular or square pits. Stipe 3-8 cm long and 1-3 cm wide, cylindrical with swollen base, hollow, white to yellowish-white, surface with fine granules, rarely bald (Pl. 1A-C). The Asci was cylindrical, hyaline, with a base rounded, usually 8-spored 300-350 × 18-22 µm (Pl. 1D). Ascospores were elliptical, smooth-walled, hyaline, homogenous with no oil droplets, inside sacs, some external oil droplets occur adjacent to each end of the spores 20-22 × 10-13 µm (Pl. 1E). Paraphyses cylindrical with rounded apex, septate, hyaline, 220-300 × 8 -10 µm.

In the present paper, *M. conica* was reported as a new addition to Iraqi macrofungi and a second *Morchella* species described from the country. Macro-and microscopic features of *M. conica* are in agreement with previously related descriptions (Negi, 2006; Watanabe, 2010; Lakhanpal *et al*., 2010). However, some morphological variations within and between *Morchella* species may be expected due to the plasticity of macro- and micromorphological traits (Phanpadith *et al*., 2019; Ali *et al*., 2021). In the study area, morphological differences were not observed between fruiting bodies of *M. conica* collected from different substrates/ hosts in unburned sites. The fruiting bodies of current species were found under diverse tree species including *Quercus* sp., *Populus* sp. and *Nerium* sp. presumably to survive using diverse niches. However, it has been reported that 70% of the Elata clade species were found in a coniferous forest with a few species found within temperate deciduous forests (Du *et al*., 2012).

Regarding the trophic status, *M. conica* and other true morels were considered saprotrophic, mycorrhizal, or even facultative (Keefer, 2005; Du *et al*., 2012; Kuo, 2012). In this study, the presence of *M. conica* under decomposed plant debris in places away from trees may suggest that the fungus acts as a saprotroph rather than mycorrhizal. True

morels including *M. conica* are known to fruit after a forest fire (Wurtz *et al*., 2005; Negi, 2006; Masaphy, 2011; Du *et al*., 2012; Larson *et al*., 2016; Miller *et al*., 2017) presumably supporting their saprotrophic mode. Li *et al* (2013) mentioned that black morels like *M. conica* were saprotrophic and those with yellow caps like *M. esculenta* were mycorrhizal. However, switching lifestyles, for example, from symbiotic to saprotrophic interactions and vice versa in macrofungal species have been suggested and recorded to access organically restricted nutrients (Bahnmann *et al*., 2018; Al Anbagi, 2020). This aspect in *Morchella* has not been resolved yet and needs further attention in the future studies.

Molecular identification and phylogenetic analysis

Amplification of the ITS rRNA region of morels resulted in size product 663 bp. A BLASTN query of investigated sequence in GenBank revealed that they had more than 99% similarity with *M. conica* (accession numbers AJ544195 and EF080999). However, the Iraqi morel sequence had more than 99% sequence similarity with *M. elata* Fr. (accession numbers MN462953 and MN462952), *M. importuna* M. Kuo, O'Donnell & T.J. Volk, and *M. esculenta* (L.) Pers. (accession numbers MF170632 and GU373504 respectively) and *Morchella* sp. (MK955411, Diag. 1). The size of the cap and stipe of *M. elata* and *M. importuna* were wider. However, compared with their asci, the size of asci of *M. conica* were wider with a broadly rounded base containing smaller ascospores with taller and narrower paraphyses (Ali *et al*., 2021). The insufficiently identified sequences and detected named sequences in GenBank have been reported.

Being has a high percentage similarity with other species in the current study; it may be potentially suggested misidentifications of *Morchella* sequences in GenBank. The ITS gene alone was able to identify 77.4% of the known phylospecies in *Morchella* using ITS1 and ITS2. At least 66% of the named *Morchella* sequences in GenBank have been found misidentified such as those with binomials (Du et al., 2012; Petrželová and Sochor, 2019). Some species of this genus such as *M. esculenta*, *M. crassipes*, and *M. elata* included different phylogenetic species. That is because of the near absence of type studies and informational databases, including references to voucher specimens and/or cultures (Kanwal *et al*., 2011; Du *et al*., 2012) for aiding DNA sequence-based identifications of true morels. Additionally, predominant cryptic speciation has been discovered between macrofungi including true morels. With only 2.6 and 4.5% of the estimated fungal species discovered (Hyde *et al*., 2020), detecting a new species in *Morchella* is highly raised especially with reporting the most novel species in North America, Turkey, and Asia as well as Europe (Du *et al*., 2012; Loizides *et al*., 2016).

In the present study, the phylogenetic analyses of *Morchella* sequences confirmed the species identification after being a BLAST query and reconstructed some related black *Morchella* sequences similar to other researchers who have used a molecular phylogeny to confirm the species identification (Du *et al*., 2012). The phylogenetic tree of black morels was divided into conica groups as well as other clustered species. *M. conica* from Iraq interestingly clustered into one clade with *M. conica* from Germany while the

specimens belong to this species from different countries such as China were separated into other clades. These relations may suggest that the disjunct distributions of species have been produced through distinct processes during their evolutionary relationships. The current results agree with other findings that geographically isolated populations of the *M. conica* may have more variation than those between two putatively different species (Bunyard *et al*., 1994; Li *et al*., 2013; Ali *et al*., 2021). The sequences of *M. conica* grouped nearest from *M. costata*, *M. esculenta*, and *M. elata* similar to the outcomes of other scientists (Kanwal *et al*., 2011; El-Wakil and Al-Gifri, 2020). However, some species within the Elata clade still have unsolved evolutionary relationships (Due *et al*., 2012).

Plate (1): *Morchella conica*; (A) Fruiting body at the natural habitat, (B) Fruiting bodies in different age of the development, (C) Air-dried fruiting bodies, (D) Ascus with 8 ascospores, (E) Ascospores.

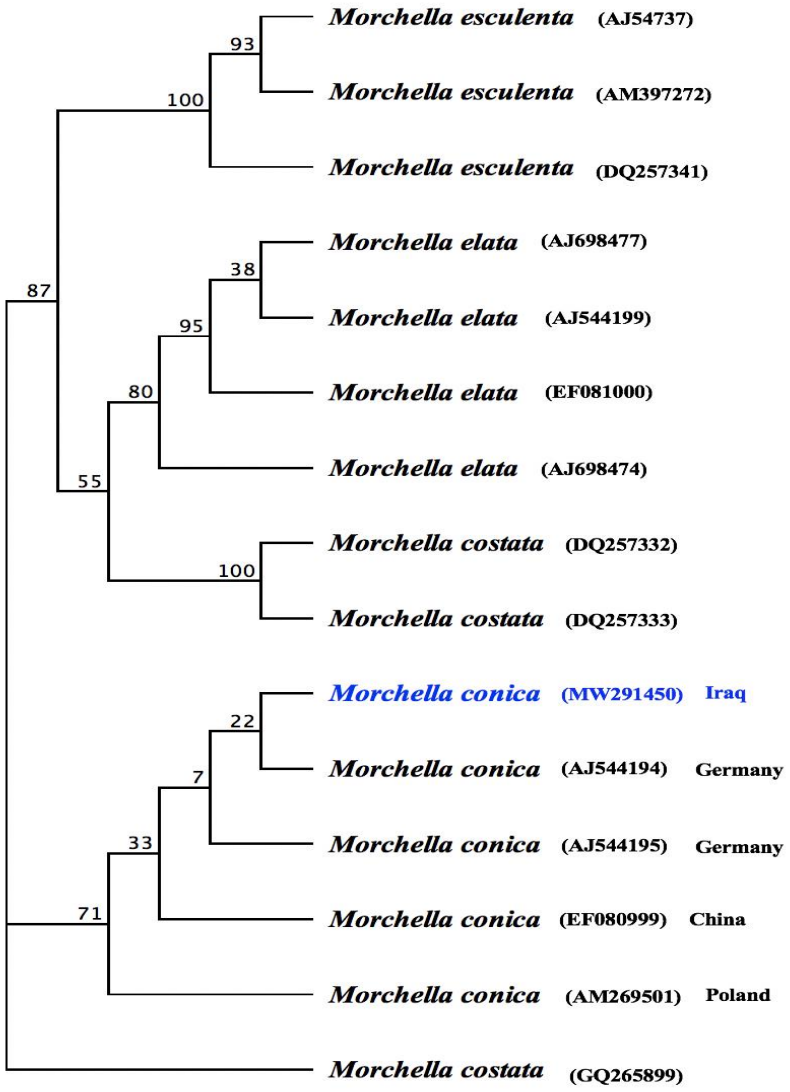

Diagram (1): The maximum likelihood tree based on ITS/5.8S rRNA gene sequences represented the position of *M. conica* from Iraq, resulting from Rapid Bootstrapping algorithm and a ML search in RAxML. The number within parentheses indicates the GenBank accession number. The bootstrap probability value is shown at the nodes.

CONCLUSIONS

The present study reported the first black morel *M. conica*; this species was found in mixed forest unburned area in Branan ranges, northeast Iraq. Previously, yellow morel, *M. esculenta*, has been collected from northern Iraq, but the identification was entirely

based on the morphological features. The current results were based on the morphological description and molecular analyses. The phylogenetic analysis clustered the Iraqi *M. conica* sequence closest to other specimens from different geological regions. Further surveys on this group of fungi are urgently needed to detect the distribution of this genus as well as other macrofungi. Detecting, describing, and identifying the first record of fruiting bodies of *M. conica*, Elata clade, in Iraq with yearly high temperature and low rainfall, and complex topography conceivably offered useful information about environmental tolerance, especially with being considered Asia or China as the center of species diversification and distribution of the modern *Morchella*.

CONFLICT OF INTEREST STATEMENT

We are the authors of the submitting manuscript, declare and verify there are no significant financial supporters for the current research from any providers.

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تسجيل جديد للفطر *Morchella Conica* Pers., 1818 من العراق (Pezizales, Morchellaceae)

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الخلاصة

سجلت الدراسة الحاليه النوع *Morchella conica* Pers.1818 العائد للعائلة Morchellaceae كتسجيل جديد ضمن الفطريات الكبيرة العراقية، اعتمادا على الطرق المورفولوجية والجزيئية. خلال موسم الإثمار القصير والمتقطع في كثير من الأحيان، عثر على هذا النوع الفطري في مناطق الغابات المختلطة غير المحترقة ضمن منطقه بروانه (محافظة السليمانية، شمال شرق العراق). كان الهدف من الدراسة هو تقديم التسجيل الجديد لهذا النوع، الذي قلما درس في الشرق الأوسط. حاليا، النوع المدروس هو ثاني نوع من أنواع الجنس *Morchella* تم تسجيله من العراق.

وصف *M. conica* مظهرياً و اكد الوصف من الناحية الجزيئية، كما درست العلاقة بين هذا النوع والأنواع الأخرى ضمن الجنس باستخدام تسلسلات المنطقةnrDNA ITS من أنواع مختلفة ومناطق جغرافية متنوعة. أيضا، تم أجراء تحليلات الاحتمال الأقصى (ML) لبناء الأصل التطوري الجزيئي لهذا النوع.

تعتبر نتائج النوع المسجل الحالي اساسيه لتقييم التوزيع الجغرافي للجنس وتوفير المعلومات حول أنواع هذا الفطر عالي القيمة الغذائية، الصناعية والطبية.