

First report on the morphological and molecular identification of *Lactocollybia variicystis* from Salahadin Governorate, North Central Iraq

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ABSTRACT

A rare agaric, *Lactocollybia variicystis* D.A. Reid and Eicker (Family: Marasmiaceae) is identified as a new record to Iraqi macromycota, based on morphological characteristics and molecular analysis. Descriptions, color photographs, comments and phylogenetic analysis based on ITS rDNA sequence data are given.

Key words: *Lactocollybia variicystis*, New record, Macromycota, Molecular analysis, Iraq

Introduction

Lactocollybia is a genus in the family Marasmiaceae (Agaricales). This worldwide genus includes 17-19 species (Kirk *et al.*, 2008; Hosen *et al.*, 2016). These species mainly occur in tropical and subtropical regions (Hausknecht and Krisai-Greilhuber, 2008; Hosen *et al.*, 2016). Species of *Lactocollybia* are classified according to several characters like habit, habitat, clamp-connections, presence or absence of the gelatinous film and types of cystidia (Singer, 1986; Hosen *et al.*, 2016). In this regard, most reports on *Lactocollybia* were based on morphological characteristics, thus its phylogenetic relationships remained unclear. Out of 19 species of *Lactocollybia*, only few sequences were deposited in Gen Bank (*L. variicystis* not included) (Hosen *et al.*, 2016; Desjardin and Perry, 2017). However, the family Marasmiaceae and its genus *Lactocollybia* are unknown in Iraq. In this study *L. variicystis* D.A. Reid and Eicker was identified as a new record to Iraq and bordering countries, based on morphological and molecular data.

Materials and Methods

Study area (Sampling and identification)

Fungal specimens were collected from decaying wood in burned site in Al-Alam province (Salahadin Governorate- north central Iraq) during September-October 2020. Macro and microscopic features were based on fresh specimens. The specimens were photographed in natural habitat and laboratory. Water and Melzer's reagent were used for microscopy. Identification of the fungal specimens were performed according to relevant sources (Singer *et al.*, 1990; Reid and Eicker, 1998; Salom and Siquir, 2014; Rommelaars, 2014; Hosen *et al.*, 2016; Senthilarasan and Kumaresan, 2016; Desjardin and Perry, 2017; Usman, 2018) and were confirmed by molecular analysis.

Molecular identification of *Lactocollybia variicystis*

Genomic DNA was isolated using a kit (ZR Plant/Seed DNA MiniPrep™) and PCR achieved by using a fragment of ITS as a target to amplify with a

forward primer (ITS1 F:5'-TCGTAAGGTGAACCTGCGG-3') and a reverse primer (ITS4 R:5'-TCCTCCGCTTATTGATATGC-3') (Primers set supplied by IDT (Integrated DNA Technologies company, Canada). The PCR amplification was performed in a total volume of 25 µl containing 1.5 µl DNA, 5 µl Taq PCR PreMix (Intron, Korea), 1 µl of each primer (10 pmol) then distilled water was added into tube to a total volume of 25 µl. The thermal cycling conditions were done as follows: Denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45s, 52 °C for 1 min and 72 °C for 1min with final incubation at 72 °C for 7 min using a thermal Cyclor (Gene Amp, PCR system 9700; Applied Biosystem). The PCR products were separated by 1.5% agarose gel electrophoresis, then the PCR product is sent to Macrogen Inc., South Korea to detect the nucleotides sequences. Identified sample was deposited in NCBI GenBank with accession number (MN221244.1) and in Biology Department, College of Education for Pure Sciences, Tikrit University, Iraq.

Results and Discussion

Macroscopic Features

Cap up to 1.5 cm in diameter, spherical, convex, convex to flattened in age, with or without central depression, hygrophanous, white, creamy, yellowish brown becoming creamy white, smooth, margin entire, creamy white, sometimes brown at age, gills white to creamy white, adnate, close to subdistant, narrow at the cap margin. Stipe 20-50 × 1-2mm, cylindrical, expanding at both the apex and the base, central to eccentric, creamy white, smooth or very finely pruinose, hollow, with scant white basal mycelium, context white (Fig. 1).

Microscopic features

Basidia 25- 30 × 5- 7.5 µm, 4- spored .basidiospores 5 -8.75 × 5- 6.5 µm, smooth, hyaline, each with one large oil droplet, ellipsoid to amygdaliform, thin

walled, inamyloid. pleurocystidia and cheilocystidia similar, cylindrical, subcylindrical, lageniform, hyaline, 30-50 × 6.5-10 µm, cheilocystidia more numerous than pleurocystidia, gloeocystidia, yellow or golden in color, variable in shape, fusiform, claviform, rounded, embedded in gelatinous film, reaching 80-150 × 10-15 µm (Fig. 1). Habit and habitat: saprotrophic, solitary or gregarious on the *Prunus armeniaca* dead wood in burned site. September – October. Al-Alam Township, Salahadin Governorate - Iraq. *L. variicystis* was rarely observed in the study area. This species was found in Spain (Salom and Siquier, 2014), West Africa (Desjardin and Perry, 2017), South Africa (Reid and Eicker, 1998), Germany (Glaser, 2016) and Pakistan (Usman, 2018). However, reports on *L. variicystis* are not available from the middle east, India (Farook *et al.*, 2013, Debnath *et al.*, 2020) and China (Hosen *et al.*, 2016). Morphologically, *L. epia* and *L. subvariicystis* are very close to *L. variicystis*. However, *L. epia* (Berk and Broome) Pegler differs from *L. variicystis* in having larger cap and stipe, more elongate basidiospores and in forming cylindrical – clavate cheilocystidia (Senthilarasu and Kumaresan, 2016; Desjardin and Perry, 2017) whereas *L. variicystis* differs from *L. subvariicystis*. Iqbal and T. H. Li in forming shorter but wider basidiospores (Hosen *et al.*, 2016).

Molecular Identification of Macrofungi

The DNA was extracted with high purity and concentration (Fig. 2A). Also, a perfect PCR was obtained that was confirmed by obtaining the target band, which is 550 bp (Fig. 2B), *Lactocollybia variicystis* clone TaAh3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence in GenBank: MW221244.1., and the main conclusion of DNA sequencing revealed the presence of a nucleotide substitution at site 63 of the base cytosine to become thymine, and at site 239 the substitution of the base of guanine to become thymine (Fig. 2C), and it has an identical percentage of

Table 1. Matching conclusion shows that there are two mutations from the standard strain.

No. of sample	Type of substitution	Location	Nucleotide	Sequence ID with compare	Identities	Source
1	Transition	63	T>C	ID: MN251097.1	99%	<i>Lactocollybia variicystis</i>
	Transversion	239	T>G			

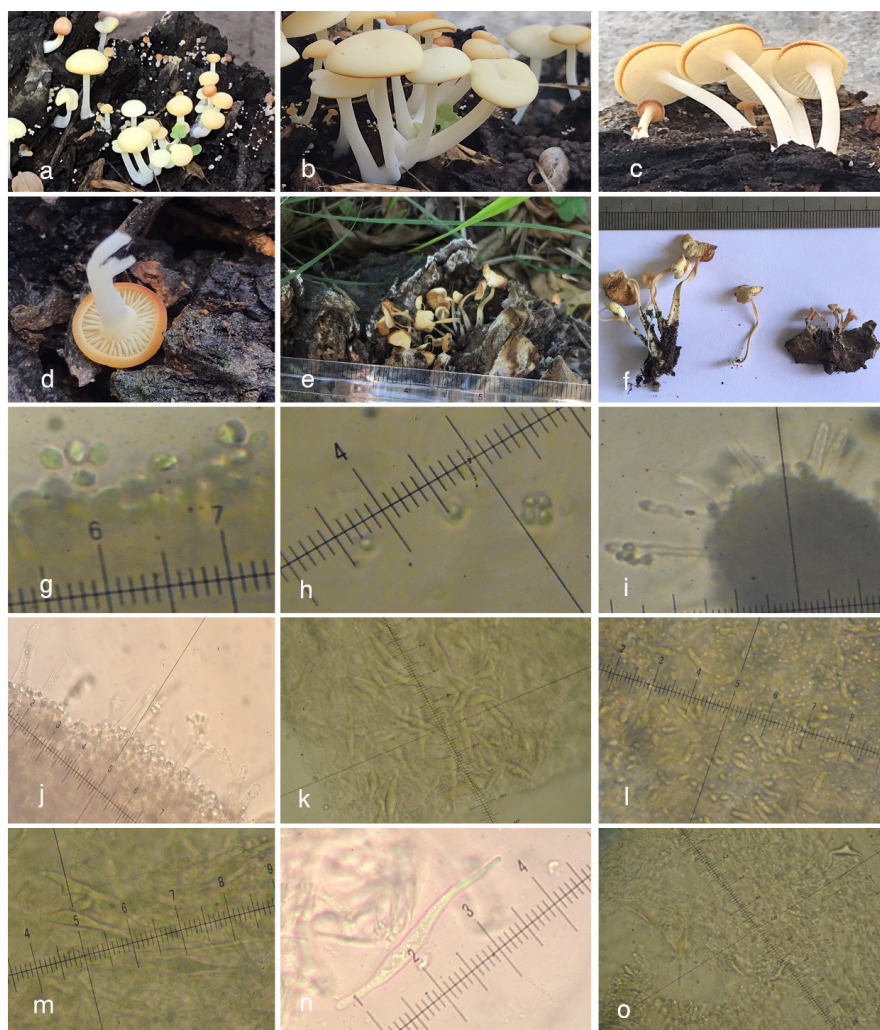


Fig. 1. *L. variicystis*. a-e : in natural habitat, f : in lab., g : basidium, h : basidiospores, i : cheilocystidia , j : pleurocystidia, k-o : galeocystidia.

99% with standard strain (Table 1). The Comparison those with the global species recorded in the NCBI the percentage of match between them varied from 88% to 100% (Table 2).

It was revealed that this type had no counterpart in the environment of Iraq, in other words that the discovery was made for the first time in Iraq and when using this data to build the phylogenetic tree the following emerged: Results of comparison with global samples registered with the NCBI are also shown; the space occupied by the Iraqi specimen that is recorded with a number MN221244.1 TaAh3 IRAQ in the phylogenetic tree was closest to the sample MN250288 from Pakistan (Fig.3A).

The molecular analysis showed the location of the Iraqi specimen MN221244.1 TaAh3 IRAQ within the

phylogenetic tree, which consisted of 17 similar species that formed seven main groups, three of which branched into subgroups, according to their origin and evolution. The most recent group included the species MN221244.1 TaAh3 IRAQ with *Lactocollybia variicystis* MN250288.1 Pakistan, *Lactocollybia variicystis* MN250289.1 Pakistan, *Lactocollybia variicystis* MN270925. 1 Pakistan and *Lactocollybia variicystis* MN251097.1 Pakistan, Where the match rate between them was from 99%, and the closest strain to this group is the *Lactocolly biavariicystis* MT304650.1 from South Africa in the same rate. As for the rest of the strains, they diverged from the Iraqi sample and its group in different proportions (Fig. 3B). This means that the Iraqi sample collected from Al-Alam township may somewhat resemble



Fig. 2. A-Gel electrophoresis of genomic DNA, 1% agarose gel. B- PCR product of primer 550 bp, the product was electrophoresis on 2% agarose, DNA ladder (100). C- Complete sequence for PCR product.

Table 2. The molecular identity of the Iraqi macrofungi species used in this study with the worldwide *Lactocollybia variicystis* samples.

	Accession	Country	Source	Compatibility
1.	ID: MN251097.1	Pakistan	<i>Lactocollybia variicystis</i>	99%
2.	ID: MN270925.1	Pakistan	<i>Lactocollybia variicystis</i>	99%
3.	ID: MN250289.1	Pakistan	<i>Lactocollybia variicystis</i>	99%
4.	ID: MN250288.1	Pakistan	<i>Lactocollybia variicystis</i>	99%
5.	ID: MF100971.1	USA	<i>Lactocollybia variicystis</i>	98%
6.	ID: MT304650.1	South Africa	<i>Lactocollybia</i> sp.	96%
7.	ID: MN337886.1	India	<i>Lactocollybia variicystis</i>	97%
8.	ID: KP840552.1	Italy: Sicily	<i>Lactocollybia epia</i>	92%
9.	ID: MT304643.1	South Africa	<i>Lactocollybia</i> sp.	96%
10.	ID: MW018892.1	USA: Hawaii	<i>Lactocollybia</i> sp.	90%
11.	ID: NR_154134.1	China	<i>Lactocollybia subvariicystis</i>	90%
12.	ID: KY042116.1	China	<i>Lactocollybia subvariicystis</i>	90%
13.	ID: MN523272.1	China	<i>Lactocollybia epia</i>	89%
14.	ID: MK770323.1	China	Uncultured <i>Lactocollybia</i>	86%
15.	ID: MH166807.1	Mexico: Jalisco	<i>Lactocollybia</i> sp.	86%
16.	ID: KU320581.1	India	<i>Lactocollybia epia</i>	85%
17.				

the Pakistani environment, which supports this convergence and makes the possibility that the Iraqi species evolved in the same way as the Pakistani species and diversified.

Conclusion

The present study described *Lactocollybia variicystis* (Family :Marasmiaceae) as a new record to Iraq ,

based on morphological characteristics and molecular analysis. This species is one of the rare agaric fungi in the study area (i.e. Al-Alam Township / Salahadin Governorate/ Iraq) as well as in other parts of the world.

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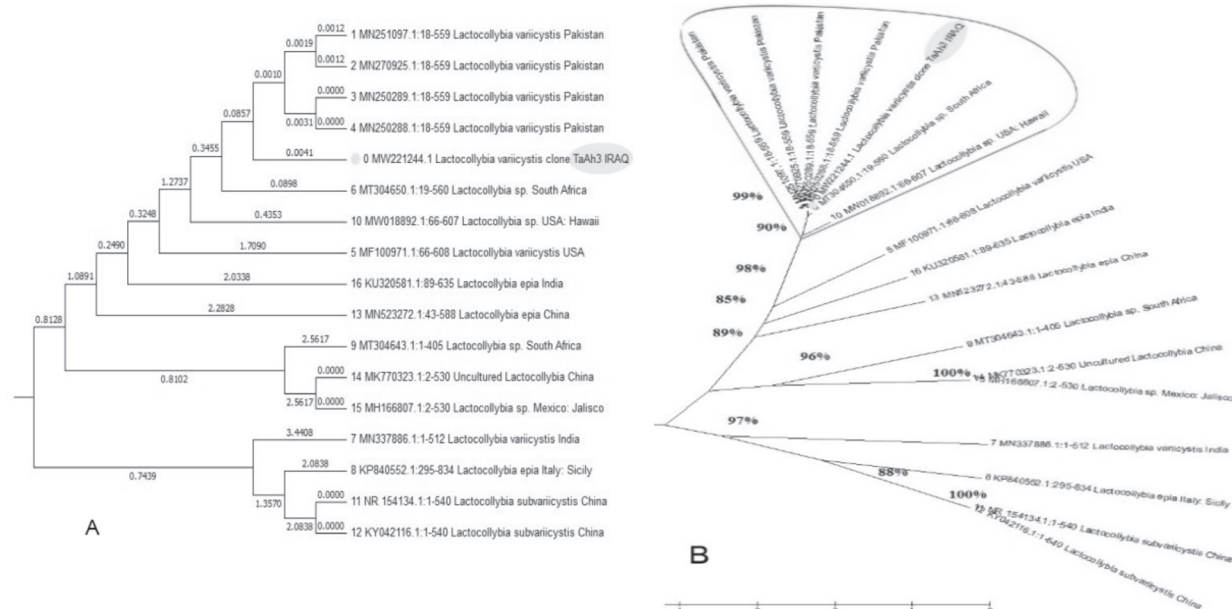


Fig. (3). A- Phylogenetic tree of *L. variicystis* that were matched with the MN221244.1 TaAh3 IRAQ, Bootstrap values were obtained from matching degree between them according to the sequencing data. B- Phylogenetic tree according to development and evolution. The group marked in red is the most recent evolutionary group among the others.

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