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Morphological and Molecular Identification of New species of *Coprinopsis iraqicus* sp. nov. from Iraq

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Abstract: The wild macro fungi, commonly named mushroom, have been rarely studied in Iraq for many reasons. In this study macrofungal specimens were collected from a site in the Tikrit province, Salah ad-Din, that located in the mid-north of Iraq from December to January 2019-2020 for describing a cryptic species of *Coprinopsis* depending on morphological and molecular analyses. Description of fruiting bodies on their microhabitats was recorded and photographed in their natural microhabitats. Molecular and phylogenetic analyses based on the nuclear ribosomal internal transcribed spacer (ITS) region were performed. In this study, the results of Bayesian and Maximum Likelihood phylogenetic analysis based on ITS nucleotide sequences and morphological description revealed that *Coprinopsis iraqicus* sp. nov. was a cryptic species and formed distinct morphological features and a clade among its related species belonging to the genus *Coprinopsis.* More studies are needed to determine the distribution of this genus generally and the presented species specifically.

Keywords: Basidiomycota, Bioinformatic analysis, *Coprinopsis*, Iraqi mushrooms, Phylogenetic tree, ITS region

Coprinoid species are an interested group of macro fungi in the field due to undergoing autolysis to their deliquescent lamellae and basidiocarps with age. However, this group is highly divers phenotypically and poly-phyletically. Based on phenotype descriptions, this group stared by taxonomic classification into one broadly defined genus *Coprinus* Pers. (1797). Later based upon molecular phylogeny, the genus has been evident for cryptic speciation, and split into four main lineages appointed to four genera each of which has own sections (Nagy et al 2013a). These genera are *Coprinus* in Agaricaceae, *Coprinellus* P. Karst., 1879, *Coprinopsis* P. Karst., 1881, and *Parasola* Redhead, Vilgalys & Hopple, 2001 in the new family Psathyrellaceae (Hopple & Vilgalys 1999, Redhead et al 2001). The reclassification of coprinoid fungi was mainly depending on macro-micro morphological characters such as the cap texture, the presence or absence of the universal veil. The genus *Coprinopsis* contain species that have been mostly described based on the classical methods, morphological features as being grayish, deliquescent, and having a cuticular pileipellis with diverse structures of veil (Nagy et al 2012). However, this method shows slight discriminatory differentiation and/or significant overlap between several species (Nagy et al 2013a). The genus *Coprinopsis* has approximately 200 species around the world (Kirk et al 2008). Due to significant genetic diversity among the collections of its species, taxa are divided into twenty sections according to index fungorium (www.indexfungorum.org) based on veil anatomy and the presence or absence of cap pileocystidia. Functionally, basidiocarps of coprinoid species are terrestrial habitat and saprotrophs collected from distinctive microhabitats such as leafy and woody litter materials on the forest floor (Al Anbagi et al 2019), and woody chip and herbivore dung (Schafer 2010). The propagules of *Coprinopsis* or and Coprinoid species have also been dispersed widely across 10 plots (each 10 m x 10 m) on the leaf litter of the forest floor (Al Anbagi et al 2021).

The wild macrofungi, commonly named mushroom, have been rarely studied in Iraq due to several reasons. Scientifically, there are few specialists in this research area. That type of research needs researchers who not only have experience in field works but also experts in morphological descriptions with all advantages and disadvantages of traditional survey methods. In Iraq, the climate is another crucial cause. The weather is mainly a hot desert climate. It is externally hot and dry in summer with mild in winter. The temperature in the Baghdad could reach 50 C, making Baghdad one of the hottest capitals in the world with receiving only 150 mm of rainfall throughout the year. In the north-eastern part of Iraq, occupied by the mountains, it is the only part that receives substantial rainfall between October and April with annual precipitation 700 -1000 mm with temperature 8-25°C. However, the temperatures and precipitations with cooler nights due to the high altitude may

be appropriate for fungal growing and producing some fruiting bodies through the year in different parts of Iraq (https://www.climatestotravel.com/climate/iraq). These factors may lead to insufficient studies on macrofungi, but higher fungal diversity that needs to be investigated is also expected. From 2014 until today, many studies have been recorded various macrofungi using morphological descriptions and later molecular techniques. These were first-time recording and describing a wild macrofungal species of the discomycetous fungus*, Cheilymina theleboloides* from Babylon province, in the middle south of Iraq (Al anbagi 2014). There have been many efforts for first-time documentations and morphological classifications of macrofungi from Kurdistan region - Northern Iraq, Salah ad-Din province (north- central Iraq); Anbar province (western Iraq) (AL- Qaissi 2014, Muslat & Owaid, 2015, Al-Khesraji 2016, Suliaman et al 2017, AL-Khesraji 2018, AL-Khesraji et al 2018, Suliaman, 2019, Al-Khesraji & Suliaman, 2019). During these efforts, molecular approaches have been applied to confirm species identifications for some species (Suliaman, 2017, AL-Khesraji et al 2019, Aish et al 2020, Al-Khesraji et al 2021).

Nevertheless, the central and southern regions have been poorly studies. Due to geographic and environmental influences of Iraq, many genera may be expected to have new species. These have been evident by recording new species reported by molecular identification. In this study, we described a distinct species of *Coprinopsis* depending on morphological and molecular aanalyses. These were different from all other known species of the subsection Nivei of *Coprinus* (Citerin 1992, Ulje´ 2005) and from remaining recognized *Coprinopsis* species.

MATERIAL AND METHODS

Specimen sampling, processing, and morphological identifications : The specimens were collected from site in the Tikrit province that located in the mid-north of Iraq about 140 Km northwest of Baghdad (34° 43´ 51.1" N 43° 38´48.0" elevation 137m). The climate of Tikrit system is characterized by a hot desert with a mean annual temperature of 14.9-28.95°C (July, 25.8-43.6°C; January, 4-14.3°C) and precipitation of 15 mm (Jun – September 0 mm and December 37 mm that has the highest rainfall; https://www.weather-atlas.com). After rainy days, a series of visits were conducted for fruiting body inventories from December to January 2019- 2020. The site was dominated by native trees including *Populus* sp. and *Salix* sp. and fruit trees. The floor site was covered by mixed litter microhabitats. Fruiting bodies were collected directly from the carpeting mixed materials on the forest floor. Description of mycelia and fruiting bodies on their microhabitats were recorded and photographed in their natural microhabitats. Small pieces of fresh fruit bodies were put in paper bags and transfer to the lab. Samples were divided in two groups: one group for morphological and molecular analyses and another group for preserving. The preserved samples were oven-dried at 45–50 °C for 24 hours and conserved in paper bags based on relevant literature and field (Al Anbagi et al 2019). Later, specimens are deposited in Biology Department, College of Sciences, Tikrit University. For species identifications and classification, the macromorphological and micromorphological features were performed based on fresh samples. Light microscopy was used for describing microcharacters. Slides were prepared from fresh specimens in an aqueous solution. Observations and measurements were taken directly through the light microscope under an 40x. The gill tissue was described. Then, spore features were described and measured by random selecting of 20 well-formed spores. The morphological characters of basidia and cystidia were described, and their dimensions were presented after measuring 25 elements.

DNA extraction, PCR amplification and Sanger sequencing techniques : Genomic DNA was extracted from fresh materials, amplified, visualized and sequenced in the same manner in detail elsewhere (Al Anbagi et al 2019). The small portion of fruiting body was used to extract the genomic DNA using the Wizard Genomic DNA purification Kit (Promega, Madison, Wisconsin following the manufacturer's instructions. Later, the extracted DNA was amplified targeting the nuclear ribosomal DNA (rDNA) genes and using the universal primers ITS1(5´-TCCGTAGGTGAACCTTGCGG-3´) and ITS4 (5`-TCCTCCGCTTATTGATATGC-3`) (White et al 1990).

The PCR products were sized and visualized on 1% agarose gel via electrophoresis stained with Red Safe. The amplified DNA was sent for Sanger sequencing (Microgen, Seoul, Korea). The represented sequencing was manually edited and then spontaneously assembled in the Geneious program version 9.1.8 (Biomatters Ltd., Newark, New Jersey). Similar to Al Anbagi et al (2019) the sequence identity was verified using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov/genbank/). The species sequence was compared with fungal nucleotide sequences using query coverage of > 80% and > 97–100% sequence similarity. The current taxonomic level of the investigated species was based on Index Fungorum (www.indexfungorum.org). Later, the sequences were deposited in the NCBI's GenBank

database under the accession number MZ265188 and MZ265189. The interesting species was putatively assigned to a particular ecological functional group based on the information available in the relevant literature (Rinaldi et al 2008, Tedersoo et al 2014).

Bioinformatic and phylogenetic analyses: The ITS forwarded and reversed sequences of two fungal isolates were trimmed, and low-quality edges were removed. Then, individual sequences of each isolate were assembled to contig with the Geneious program (Kearse et al 2012). The sequences of these isolates were blasted against the ITS database. Later, ninety-nine close related sequences to the investigated sequences were obtained from the GenBank. All sequences were aligned using ClustalW (Thompson et al 1994). Preliminary trees were created using Neighhor-joining. For the final tree, sequences of isolates were aligned with covering all available GenBank sequences of *Coprinopsis* species (81 species), reduced to one sequence per species, and included sequences generated by experts of *Coprinopsis* taxonomy (Keirle et al 2004, Navarro-Gonza´lez et al 2006, Nagy et al 2010a,b, 2011, 2013a, b Geml et al 2012, Osmundson et al 2013, Ruiz et al 2013, O¨ rstadius et al 2015, Elwess et al 2016)and some unpublished sequences available in GenBank. Some species from close related genera (5 species) were chosen as the outgroups (Table 1) for the preliminary hypothesis on the phylogenetic placement of *Coprinopsis*. These trees were used default settings of MAFFT v7.309 (Katoh & Standley 2013), and the alignment was manually adjusted as necessary (data not shown). The outgroup (*Parasola*) was chosen based on related references (Nagy et al 2013b). Bayesian analyses were completed using Metropolis Coupled Markov Chain Monte Carlo (MCMC) methods as fulfilled in MrBayes V3.2.6 to infer phylogenetic trees (Huelsenbeck & Ronquist 2001) in Geneious version 9.1.8 under a general time- reversible (GTR) model of sequence evolution (Darriba et al 2012). These analyses run for 1,100,000 generations and begun with random starting trees. Those trees were sampled every 200 generations with the heating chain temperature set at 0.2. The first 100,000 trees were discarded, and the remaining trees were used to calculate posterior probabilities (PP) of the individual clades in the majority rule consensus tree. The unconstrained branch lengths and uninformative topology priors were set based on default settings of MrBayes. For maximum likelihood (ML) analyses, RAxML V7.2.8 were performed (Stamatakis 2014) and run with rapid bootstrap for 1000 replicates in Geneious version 9.1.8. The general time- reversible GTR model of nucleotide substitution with the additional options of modeling gamma rate heterogeneity (G) and proportion invariable sites (I) was accommodated. The resulting trees were visualized in Geneious version 9.1.8.

RESULTS AND DISCUSSION

Classification Position of *Coprinopsis iraqicus* sp. nov.

Kingdom: Fungi

Phylum: Basidiomycota.

Class: Agaricomycetes

Order: Agaricales.

Family: Psathyrellaceae (the family formerly treated under the name Coprinaceae).

Ecology: Terrestrial habitat, Saprotrophic nutrition (lignocellulosic substrates).

Habit and Habitats: Gregarious often fruiting in dense clusters within various substrates including wood chips, plant litter materials, and dung manured soil. It has been also found in both mixed straw- dung, and in the burned plant straw on wet, muddy soils.

Phenology: Specimens found from November to January in 2019 -2020.

Edibility: Unknown.

Distribution: The species was observed in the village in Tikrit province, Salah ad-Din in mid-north of Iraq. It could be a local species till now. However, few studies have been investigated macrofungi generally in Iraq, thus, it could be suggested to be collected from other areas in Iraq. The current species is the fourth species related to the genus that has been published. The previous three species mentioned were also collected from Erbil, Salah ad-Din, Dukan and Sulaymaniyah areas.

Morphological Characters

Macromorphological characters : *Basidiocarps* thin and soft when fresh, small-sized, fragile and short-lived. *Pileus* 4-25 × 5-10 mm; first sub-globular nearly conical and mostly bill-shaped then expanding to become broadly convex; margin softly striated, splitting in age; white when young, then change to white to pale cream, tan and becoming grayish at old specimens, the changing of pileus color starting from the end towards the top of the pileus. The surface dry radially lined and splitting in old specimens; covered with a massive white powdery granular to scaly leading to snowy pileus initially; the centers of surface pilus whiteish turned to yellowish tan and eventually light brown, sometimes fading to tan, with flattened-scaly. The fruiting body types non-deliquescent when being seen in the field and after being transported to the lab. *Lamellae* attached to the stipe, white, then change to pale cream, dark brown, and grayish becoming black with age; one series lamellae, distant to mostly sub-distant spaced. Spore print dark brown. Odor and test not distinctive. *Stipes* 5-10 ×18-60 mm; white to cream

Table 1. Species sequences obtained from Genbank used for construction of phylogentic tree

Taxa of phylogentic trees	Accession number
Coprinellussclerocystidiosus CBS 195.52 from TYPE material	NR_164277.1
Coprinopsis acuminata voucher SZMC-NL-0958	JX118698.1
C.aesontiensis voucher LZ P-7614	KY554753.1
C.afrocinerea MJ-2017a voucher CNF 1/5838	MG662162.1
Coprinopsisafronivea PB-2016a	KX017208.1
Coprinopsis ammophilae strain WAT 24982	HQ847008.1
Coprinopsis annulopora strain Enderle 30.71987	HQ847017.1
Coprinopsis argentea strain SZMC-NL-1678 i	HQ847040.1
Coprinopsis atramentaria voucher Seq_MP15	MW553148.1
Coprinopsis babosiae voucher SZMC-NL-0871	JX118685.1
Coprinopsis bicornis voucher Ulje 1216	JX118690.1
Coprinopsis brunneistragulata voucher SZMC-NL-FVBD 3821	JX118724.1
Coprinopsis brunneofibrillosa voucher Pegler 2704	JX118664.1
Coprinopsis calospora strain CBS 612.91	MH862284.1
Coprinopsis candidolanata voucher 794	JF907837.1
Coprinopsis canoceps voucher MushroomObserver.org/352292	MK346221.1
Coprinopsis cerkezii voucher CNF 1/7253	KX869912.1
Coprinopsis cf.*atramentaria* voucher JLF7063	MK874613.1
Coprinopsis cf. *cinerea* voucher Mushroom Observer # 316138	MH497221.1
Coprinopsis cf. *uliginicola*voucher JLFNSCFS51	MK874610.1
Coprinopsis clastophylla CBS 473.70	NR_154756.1
Coprinopsis cothurnata strain CBS 174.49	MH856479.1
Coprinopsis depressiceps voucher WTU-F-018322	MK169334.1
Coprinopsis episcopalis voucher ASIS25879	KP004959.1
Coprinopsis filamentifera voucher HB20171117A	MK069600.1
Coprinopsis fluvialis strain SZMC-NL-0840	HQ847011.1
Coprinopsis friesii voucher AM954	MK072829.1
Coprinopsis geesterani voucher HB20100810A	MK063784.1
Coprinopsis gonophylla strain CBS 144.47	MH856190.1
Coprinopsis insignis voucher HMAS 281305	MK966570.1
Coprinopsis jonesii voucher SZMC-NL-0154	JX118726.1
Coprinopsis krieglsteineri voucher SZMC-NL-3413	JX118701.1
Coprinopsis kubickae voucher CNF 1/6614	MH422562.1
Coprinopsis laanii strain CBS 476.70	MH859802.1
Coprinopsis lagopides voucher S.D. Russell iNaturalist #8536159	MN892574.1
Coprinopsis luteocephala strain SZMC-NL-2754	HQ847012.1
Coprinopsis macrocephala strain VKT-1	EU591956.1
Coprinopsis marcescibilis strain CBS 165.47	MH856199.1
Coprinopsis marcida voucher WTU-F-018311	MK169335.1
Coprinopsis martinii strain O50524	GU234126.1
Coprinopsis melanthina voucher WU19918	KC992961.1
Coprinopsis musae C J. Vesterholt 06-179	NR_148070.1
Coprinopsis narcotica strain CBS 171.39	MH855976.1

Cont...

Table 1. Species sequences obtained from Genbank used for construction of phylogentic tree

Taxa of phylogentic trees	Accession number
*Coprinopsis neolagopus*AB097564.1	AB097564.1
Coprinopsis neophlyctidospora CBM FB-37998	NR_137526.1
Coprinopsis nevillei GG08090401	HM126488.1
Coprinopsis nivea voucher 4585	JF907848.1
*Coprinopsis novorugosobispora*AB978534.1	AB978534.1
Coprinopsis ochraceolanata voucher SZMC-NL-0192	JX118697.1
Coprinopsis pachyderma voucher FVDB 3237	JX118731.1
Coprinopsis pannucioides voucher LO143-03	DQ389727.1
Coprinopsis phlyctidospora voucher 15575	JF907842.1
Coprinopsis picacea strain SZMC-NL-0174	JN943110.1
Coprinopsis pinguispora voucher UBC:F33455	MN954725.1
Coprinopsis poliomalla voucher HB20151219A	MK072612.1
Coprinopsis psammophila voucher CNF 1/6401	MK491274.1
Coprinopsis pseudofriesii strain SZMC-NL-2631	HQ847016.1
Coprinopsis pseudomarcescibilis AH 33711	NR_158341.1
Coprinopsis pseudonivea specimen voucher SZMC:NL:2340	FM163181.1
Coprinopsis pseudoradiata voucher SZMC-NL-0956	JX118687.1 1
*Coprinopsis rugosobispora*specimen_voucher: BR-44338-09	AB983245.1
Coprinopsis rugosomacrospora KRAM F-58717	NR_148112.1 1
Coprinopsis sclerotiger strain CBS596.80	GQ249277.1
Coprinopsis sclerotiorum strain SZMC-NL-0564	HQ847039.1
Coprinopsis scobicola strain Orton964	HQ847021.1
Coprinopsis semitalis strain CBS291.77	GQ249278.1
Coprinopsis spelaiophila voucher WU 14574	JX118674.1
Coprinopsis spilospora voucher JLF8953	MW555596.1
Coprinopsis stangliana strain SZMC-NL-2153	FM878027.1
Coprinopsis striata voucher HMAS 290163	MK966572.1
Coprinopsis strossmayeri voucher JU16585	MG981027.1
*Coprinopsis sylvicola*MN809536.1	MN809536.1
Coprinopsis tectispora voucher Schafer20090720001	JX118666.1
Coprinopsis trispora voucher MR180722	MN227299.1
Coprinopsis udicola GB A. Melzer 1240	NR_148071.1
Coprinopsis undulata voucher WTU-F-041708	MK169349.1
Coprinopsis urticicola strain ZMGR16	MT446068.1
Coprinopsis utrifer strain SZMC-NL-0591	FN396140.1
Coprinopsis variegata voucher SDR-MM5698	MG748581.1
Coprinopsis vermiculifer strain CBS132.46	GQ249279.1
Coprinopsis verticillata strain CBS 254.	MH873439.1
Coprinopsis xenobia voucher G. Mu oz	KF178383.1
Parasolaochracea BP NL-3621	NR_158793.1

color, central, smooth, lined, hollow, surface dull, dry; slender shape without seeing volva or annual, subglobose base when young and clavate to slightly enlarged at mature, sometimes equal (Fig. 1).

Micromorphological characters : *Pileus* cuticle, lamella tissue composed of interwoven or parallel, thin-walled, hyaline hyphae, *Basidiospores* 5-7.5 × 7.5-12.5 micrometer (µm), smooth, thick-walled, light brown and dark reddish-brown, ellipsoid to ovoid and narrowly amygdaloid to limoniform with some rounded base with prominent apiculus and with central, papillate germ pore, but few eccentric pores, mostly 1 µm wide germ-pore (Fig. 2). *Basidia* hyaline; clavate, 7.5-10 × 27.5-37.5 µm (measured without sterigmata)**,** come out from the terminal cells of hyphal tissues; developing 4 Sterigmata 1 µm long on which 4-spored generated. *Cheilocystidia* found on the edge of the lamella**,** scattered, broadly ellipsoid to clavate 10-12.5 × 27-35 µm, thin-walled, hyaline. *Pleurocystidia* found on the face of the lamella, broadly ellipsoid to clavate shape, thin-walled, hyaline, similar to cheilocystidia, 10-12.5 × 25-37.5 µm.

The wild macrofungi, commonly named mushroom, have been rarely studied in Iraq. The *Coprinellus disseminates*, *C. radians*, *C. flocculosus*, *C. comatus*, *Coprinopsis atramentaria*, *C. strossmayrei*, *C. romagnesiana*, *Parasola plicatilis*, *Psathyrella candolleana*, and *P. spadiceogrisea* were the first coprinoid fungi described from Salah ad-Din governorate in north central Iraq (Al-Khesraji et al 2017, Suliaman 2017, Al-Khesraji 2018, AL-Khesraji et al 2018). All the previous taxa have been characterized mainly based on the morphological techniques, except *C. strossmayrei*. and *P. candollena*, that were conformed to their classification using a molecular technique (Suliaman 2017, AL-Khesraji et al 2019). However, several studies confirmed that coprinoid fungi are complex taxa and have cryptic species with polyphyletic groups as being recognized for species of *Coprinus* and *Coprinopsis*. Furthermore, taxonomic changes happened for some species of both genera before they gained their current name. Therefore, it is difficult to accurately identify species of the current *Coprinopsis* with only morphological features and limited studies. In the current study, the new species of *Coprinopsis* is the first new record and described based on morphological and molecular characterizations from Iraq. That diverges from all known species of *Coprinopsis* species as well as the subsection Nivei of *Coprinus* s.l. (Cite´rin 1992, Ulje´ 2005) later included in the genus *Coprinopsis* (Hopple & Vilgalys 1999, Moncalvo et al, 2002). Its macromorphological descriptions are similar to species related to the subsection Nivei by having small fruiting bodies, pilus white to grey, fragile snowy white caps when young with pale yellow-brown in the center of pileus and expended pileus, and radially grooved and splitting in old specimens with limoniform, dark pigmented spores (Uljé & Noordeloos 1993). However, this species has a unique and distinct pattern of morphological characters (Figs. 1& 2). According to the taxa key, the species would be classified into *C. utrifer* depending on the expended pileus and the average length of spores or species with spores shorter than 9 µm (Uljé & Noordeloos1993, Nagy et al 2012). However, a phylogenetic tree based on rDNA in the present study provided another suggestion. The species has small dimensions of basidiocarps and average lengths of basidiospores that overlapped between the *Coprinopsis cerkezii* with very small pileus 3-11mm diameter and with an average length of spores 3-3.4 µm and *C. utrifer* with expended pileus up to 25 mm and average spore lengths more than 9 µm (Ulje´& Noordeloos 1993, Jayasiri et al

Fig. 1. Basidiocarps of *Coprinopsis* sp. from Iraq include different ages of basidiocarps (A & B); old specimen and lamellae color (C); young basidiocarps in cluster on natural habitats in burned plant materials (D & E); specimens covered with white snowy mass (F)

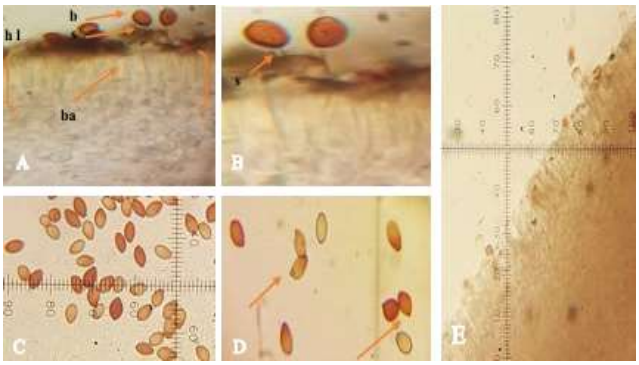

Fig. 2. Micromorphological characters of *Coprinopsis* sp. (A), illustrated hymenium layer (hl) with short clavate basidia (ba) bearing spores (b) on sterigmata (s), basidia with four sterigmata and 2 attached basidiospores (B); centric and eccentric germ pore of basidios-pores (C & D); lamellae tissue with basidia and cheilocystidia in the hymenium layer(E)

2015). The investigated species has larger pileus with smaller Cheilocystidia compared to *C. afronivea,* 19-18 mm and 12–17 µm. On the other hand, its pileus and Cheilocystidia are smaller than *C. pseudonivea* 28-57 mm and 28–57 µm and *C. nivea* 20-54 mm 29–71 m respectively. The phylogenetic tree has presented these differences clearly, as shown below.

Phylogenetic analyses: The results of blast and preliminary trees (neighbor joining tree built with 99 close related sequences) revealed that isolates of a species collected in this study have low identical similarity to close related sequences (<91.2%) and clustered in a distinct clade belong to the genus *Coprinopsis* (data not shown). Furthermore, Bayesian and Maximum phylogenetic analysis based on ITS nucleotide sequences revealed that *Coprinopsis iraqicus* nov. in this study was a cryptic species and formed a distinct clade among its related species belong to the genus *Coprinopsis* (Fig. 3). The new Iraqi *Coprinopsis* with other members of the genus *Coprinopsis* in the present tree split into several smaller clades. Its position nested in an isolated position as a sister clade and basal to the assemblage of *Coprinopsis* species belong to subsection known as pseudoneviea such as the *C. marcibilies, C. utrifer, C. pseudoneviea*, *C. afronivea*, and *C. cerkezii* some of them known as pseudoneviea. The topology of Bayesian and Maximum Likelihood phylogenetic trees were similar and strongly supported by Maximum Likelihood bootstrap value (100) and the Bayesian posterior probability (1.00) for the clade of this cryptic species, indicating that *Coprinopsis iraqicus* sp. nov. is distinct taxon (Fig. 3). Although *Coprinopsis iraqicus* sp. nov. had morphologically some similarity with other coprinoid species, a phylogenetic tree based on rDNA successfully discriminated against the present species of *Coprinopsis* (Fig. 3). The position of new Iraqi *Coprinopsis* in the present phylogenetic tree takes an isolated position. Its position with other *Coprinopsis* members split into several smaller clades (Fig. 3). In the present clade, the *Coprinopsis* sp. is placement close to

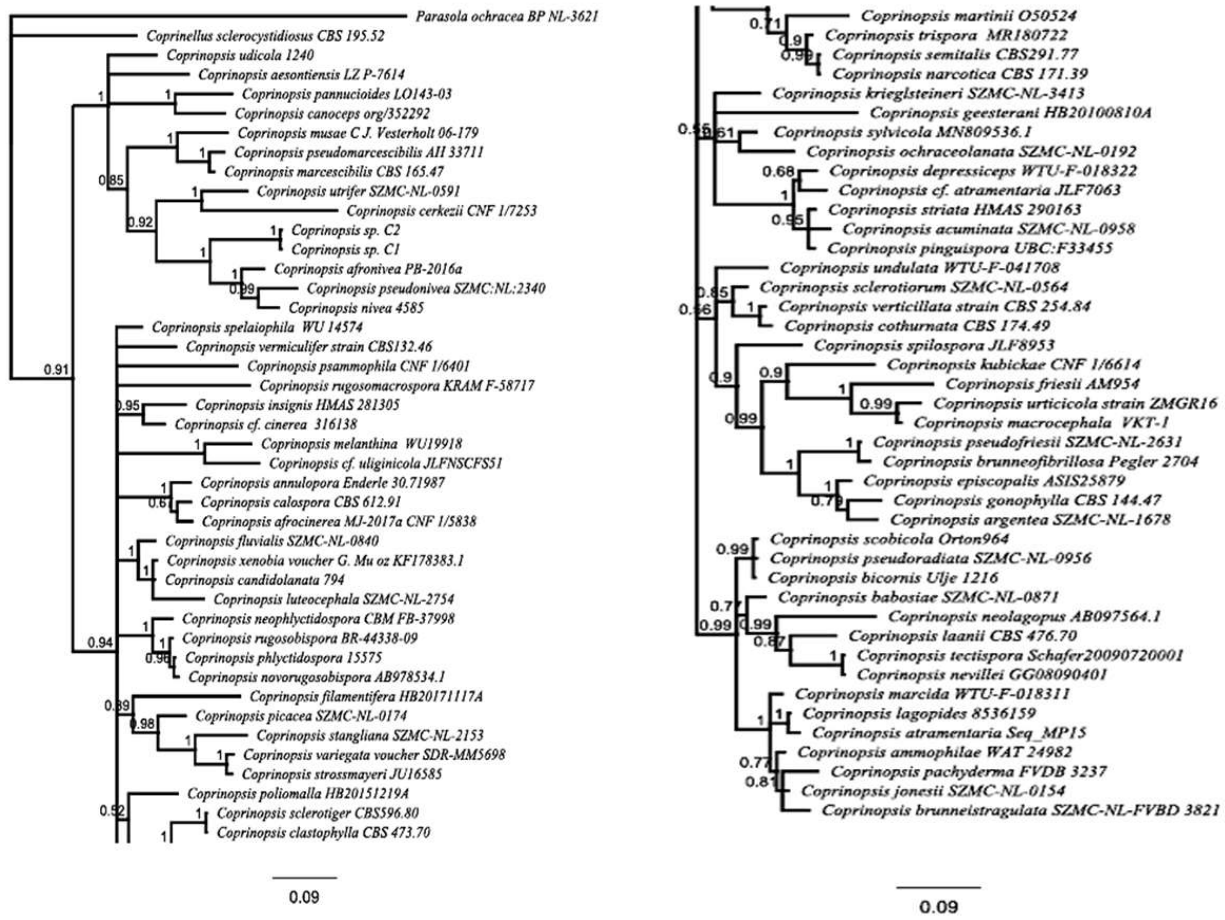

Fig. 3. Bayesian phylogenetic tree inferred from the dataset of ITS region's sequences from *Coprinopsis iraqicus* sp. nov. and related species. The isolates of new species are shown in bolt. Bayesian posterior probability values are indicated at the nodes. The tree is rooted with two outgroup species, *Coprinellus sclera cystidiosus,* and *Parasola ochracea*. The number of nucleotide substitutions per site is indicated as the bar

species known as pseudoneviea. *Pseudoneviea* species as defined by Nagy et al (2012) are characteristic by usually having pure white or whitish basidiomes with cuticular pileipellis when the fruiting body is deliquescent. With adding more sequencing from identified species and new species into databases, and with uncertainties in the placement of some clades. However, it may be difficult to certain the current species clad. The morphological features are no longer evidence for describing new species or recording the identified species because difficulties in recognizing homologous traits and molecular techniques have evident there are complex species within a species. These could be even spilt into new genera as being suggested for coprinoid fungi (Nagy et al 2013b), although the larger clades of phylogenetic analyses can be distinguished by morphological traits clearly.

CONCLUSION

The study revealed the presence of new *Coprinopsis* sp. and fourth recorded species in Iraq after those isolated from the north part of the country. The current species record indicated that genus distribution extends to Iraq. More studies are needed to determine the distribution of this genus generally and the presented species specifically. The high temperature and few precipitations are important factors preventing macrofungi from producing the sexual form, fruiting bodies in Iraq. Therefore, more efforts are needed to inventory macrofungi under suitable environmental conditions in other regions of the country.

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