Studies on true morels (*Morchella*) from North Kashmir, India

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Jammu and Kashmir (J&K) India, harbours a rich diversity of mushrooms, but sporadic work has been carried out till date to explore the morel mushroom diversity. Besides, only a few morel species have been identified based on classical taxonomic studies, and taxonomic confusion exists regarding the differentiation and classification of morel mushrooms. Keeping this in mind, the present research aimed to analyse the geographical distribution and taxonomic revision of true morels from North Kashmir using modern phylogenetic tree analysis along with classical phenetic approaches. In this study, 11 collections of true morels, identified as *Morchella esculenta*, *Morchella crassipes*, *Morchella vulgaris*, *Morchella tridentina*, *Morchella elata* and *Morchella kaibabensis*, were found widely distributed in 16 sampling study sites. The true morels were found to be extensively distributed in the Kupwara district, followed by Baramulla, but were less common in the Bandipora district, J&K. To the best of our knowledge, the documented species *M. vulgaris*, *M. tridentina* and *M. kaibabensis* have not been recorded earlier from the study area.

Keywords: Geographical distribution, morels, mushroom flora, phenetic approaches, phylogenetic analysis, taxonomic revision.

FUNGI play a dynamic role in biomes as pathogens, decomposers and endophytes. Currently, the estimated number of fungal species on earth ranges between 1.5 and 12 million, of which only 140,000–150,000 have been described, while 41,000 are known to be macrofungi[1,2]. Since the introduction of DNA-based approaches for species identification, the number of newly described taxa has increased from approximately 1000 to around 2000 on a yearly basis[2]. Despite being a mega biodiverse country, only 2% of India's macrofungal wealth has been documented[3]. The exploration of mushroom diversity in Jammu and Kashmir (J&K), India, is still in the nascent stages[4,5]. So far, only 281 macrofungi have been reported by various investigators, of which ascomycetous mushrooms are only a few[6–11].

True morels (genus *Morchella*), a group of the world's popular and most expensive edible mushrooms, are of economic, medicinal, nutritional and scientific value[12,13]. A large population across the globe collects and sells true morels. Unfortunately, these anthropogenic activities cause vegetative destruction resulting in the disappearance of some *Morchella* species. Furthermore, delimitation of *Morchella* species, remains a complex problem because of their high morphological complexity, and plasticity of apothecium colour and shape. Hawksworth *et al.*[14] reported 28 species, while Kirk *et al.*[15] documented 36 species of morels. According to this Index Fungorum, as many as 315 species of *Morchella* from different countries of the world are reported to have been documented. In the literature, much confusion exists about the number of authentic *Morchella* species; some classical taxonomists recognize nearly 50 species, while others limit the number to 3–6 species only[16]. From India, only 12 species of *Morchella*, viz. *M. semilibera*, *M. elata*, *M. esculenta, M. conica*, *M. rotunda*, *M. crassipes*, *M. deliciosa*, *M. tomentosa*, *M. angusticeps*, *M. vulgaris* and *M. hybrid* are known so far[17–22].

The Kashmir Valley harbours a rich repository of mushrooms, but less exploratory work has been done on true morels primarily based on classical taxonomy[7,11,23–25]. In the present study, the *Morchella* samples collected from North Kashmir were examined using both classical and molecular taxonomic approaches. A single phylogenetic tree was constructed to understand the genetic diversity of the studied species of morels along with their morpho-anatomical characteristics.

Materials and methods

Sampling of mushrooms

Systematic field trips were undertaken to 16 different sampling sites of North Kashmir, viz. Lolab Valley, Handwara, Wadpora, Bungus Valley, Gulmarg, Tangmarg, Drung, Pattan, Warpora, Naid Khai, Pazalpora, Main Bandipora, Wular Lake, Malangam, Bankoot and Zalwan Bandipora for the collection of true morels during April 2019 to June 2021 (Figure 1). The studied samples were deposited in the KASH Herbarium, University of Kashmir, Srinagar, Jammu and Kashmir, India. During the survey, standard protocols and methods reported in the literature were followed to

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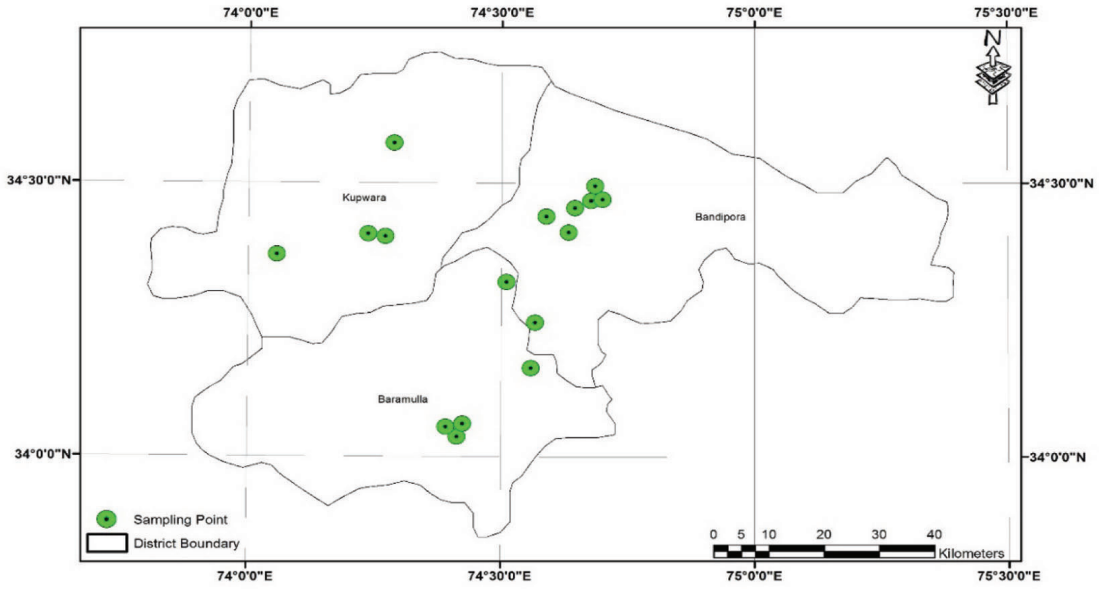

Figure 1. Map of North Kashmir, India showing the 16 sampling sites surveyed during the present study.

collect the true morels[22,26,27]. While visiting different sites and areas for the collection, local mushroom hunters and older adults from tribal communities, including Bakarwals, were consulted and also engaged as guides.

Methodology for classical taxonomic study

The ascocarps were photographed from the study sites using a camera (Nikon DSLR, D-500, and 24-megapixel). They were carefully dug out with the help of a knife and placed in collection bags. Various morphological characters such as shape, size, colour and dimensions of the fruiting bodies, which helped identify the collected morels, were noted under natural conditions before preservation. Likewise, the diverse microscopic characteristics were examined from rehydrated sections, mounted in 3% KOH and stained with cotton blue or Melzer's reagent. These sections were examined under a trinocular microscope. Observations of micro- and macroscopic characteristics were done according to the procedures reported in the literature[17,18,27]. After proper micro-morphological studies the collected morel mushrooms were finally identified by consulting different field guides, the relevant literature and referring to recent monographs and keys[18,24,28,12,22]. Websites like www.mycokey.com, www.mushroomobserver and www.mushroomexpert.com were also used for identification and related information.

Methodology for molecular systematics

The total genomic DNA was extracted from the dried fruiting bodies using the NucleoSpin® Plant II Kit (Macherey-Nagel). PCR amplification using specific primers (ITS1 and ITS4) was done according to White *et al.*[29] with some modifications, using a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, USA). The PCR amplification profile was maintained at initial denaturation of 98°C for 10–15 min, followed by 40 cycles at 98°C for 5 sec, 58°C for 10 sec (annealing), 72°C for 15 sec (elongation) and final elongation at 72°C for 60 sec. PCR products obtained were observed in agarose gel (1.2%) taking a 2-log DNA ladder (NEB) as the molecular standard, and images of the UV-illuminated gel were captured using a gel documentation system (Bio-Rad).

Sequencing

Following the manufacturer's protocol, the sequencing reaction was performed in the PCR thermal cycler using the Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). The quality of these isolated sequences was checked with the aid of Sequence Scanner Software v1 (Applied Biosystems). The isolated ITS sequences had ORFs in the range of 300–1200 bp. These sequences were then submitted to GenBank to obtain the accession numbers.

Phylogenetic analysis

Phylogenetic analysis of the true morels was carried out using MEGA-X software. The nucleotide sequences were aligned employing the ClustalW alignment tool under the full processing mode. The phylogenetic tree analysis was done using the maximum composite likelihood method given by Kumar *et al.*[30], and the Tamura–Nei method as suggested by Tamura and Nei[31] with 1000 bootstrap values. About 20–25 nucleic acid sequences were taken from the NCBI

database as suggested by various researchers[32–34] as shown in Table 1 and the phylogenetic tree was constructed.

Cord and two-way cluster analysis

Cord and cluster analysis was done to reveal the linkages or relationships and similarities between collection sites and reported species of morels. To compute Euclidian's two-way cluster analysis, PAST (Version 4.03) was used. Origin (version 2021b) was used to prepare the chord diagram.

Results

In the present study, during an extensive survey of different areas of North Kashmir for exploration and documentation of true morel mushrooms, six species namely *M. esculenta* (L.) Pers., *M. crassipes* (Vent.) Pers., *M. vulgaris* (Pers.) Gray, *M. tridentina* Bres., *M. elata* Fr. and *M. kaibabensis* Beuj, T. A. Clem. & T. J. Baroni were collected and identified using classical and molecular taxonomic techniques.

Cord and two-way cluster analysis of true morels

Cord analysis revealed that the documented *Morchella* species were widely distributed in the sampling sites surveyed during the present study (Figure 2). It inferred the linkage between the reported true morels and surveyed sampling sites and evinced that among the 16 sites surveyed, Gulmarg and Handwara harboured most of the documented morel species.

The two-way cluster analysis revealed three clusters corresponding to the surveyed sites and distribution of the reported morels (Figure 3). Majority of the surveyed sites revealed the presence of similar mushroom species. However, no *Morchella* species was reported from Warpora, Malangam, Bankoot, Lolab Valley, Bungus Valley and main Bandipora.

Table 1. Database materials used for molecular phylogenetic studies

Species	Acession number	Reference
Morchella kaibabensis	MW479971	GenBank
Morchella tridentina	MW479972	GenBank
Morchella elata	OL504954	GenBank
Morchella crassipes	OL654279	GenBank
Morchella esculenta	OL654278	GenBank
M. kaibabensis	NR_168766	GenBank
M. tridentina	NR_158851	GenBank
M. elata	MW881501	GenBank
M. crassipes	GQ228465.	GenBank
M. esculenta	MN752421	GenBank
Morchella vulgaris	OM049833	GenBank
M. vulgaris	KM588018	GenBank
Verpa conica	MZ919245	GenBank
V. conica	MW322802	GenBank

Classical taxonomy of true morels

In the present study, the micro-morphological and other related details of all the collected *Morchella* mushrooms were recorded. Considerable variations in shape, colour and size of ascocarps, ascospores, pileus and stipe were observed in the collected morel species. A detailed description of the collected and identified *Morchella* spp. from the surveyed areas of North Kashmir during the present study is given below.

Morchella esculenta (L.) Pers. In *Syn. meth. fung.* (*Gottingen*), 2: 618. (1801). (Figure 4 *a* and *b*).
Synonym (s): *Morchella rotunda* (Fr.) Boud., *Helvella esculenta* (L.) Sowerby, *Phallus esculentus* L.
Common name(s): Common morel, yellow morel, sponge mushroom, or true morel.
Local name(s): Gucchi or Batta guech or Khazer kann guech.
Voucher specimen no.: 4333-KASH Herbarium.

Description

Pileus: Pale brownish to greyish-brown in colour, the ridge borders are typically not darker than those of the pits, and the shape is oval, rarely sharply cone-shaped with a rounded top or more prolonged. Pileus: Empty, with a bottom edge linked to the stipe, and measures 2–7 cm wide and 2–10 cm in height; Stipe: White to light yellow, empty, upright, bulbous base, generally about 2–9 long and 2–3 cm wide. Flesh: Fragile or brittle. Ascospores: Ellipsoidal, smooth, and translucent, measuring about 16.5–21.0 × 8.0–11.0 µm.

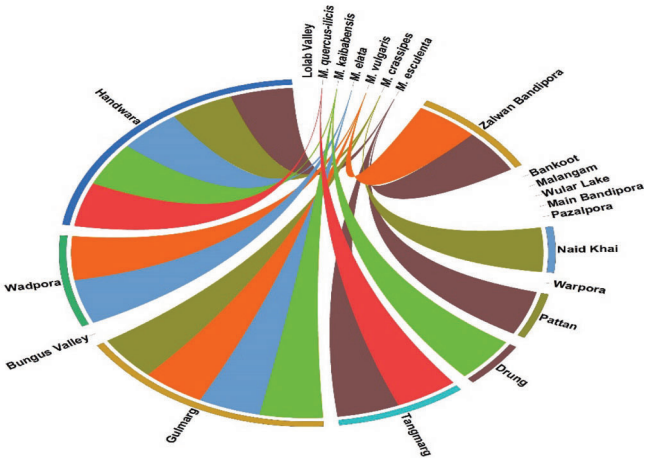

Figure 2. Linkage between the collected *Morchella* species and the study sites. The chord diagram shows the distribution of various reported morel mushrooms from different study sites in North Kashmir. The size of a circle section corresponds to the spectral counts of the surveyed sampling sites, while as the curves connecting them shows the amount of spectra shared by two entities like morel mushroom species and study sites.

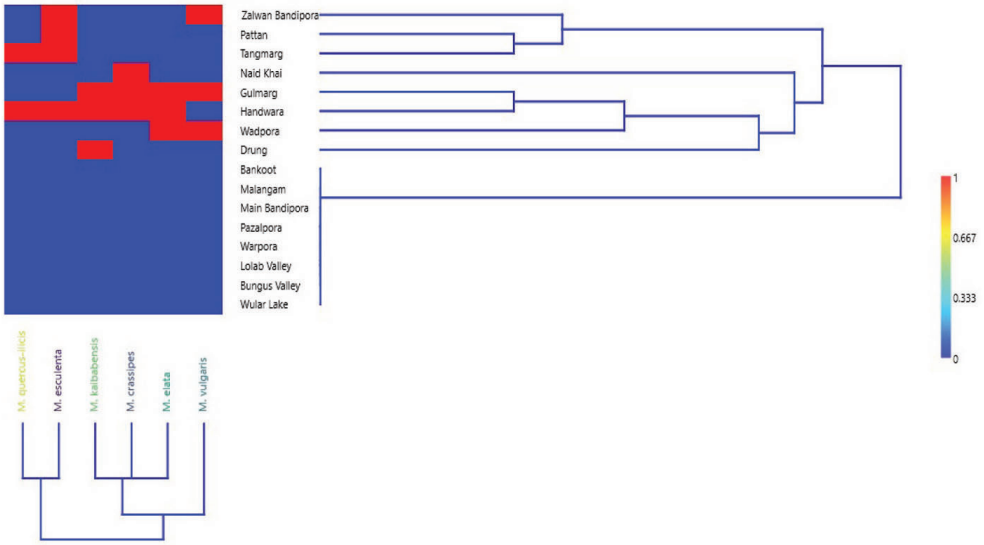

Figure 3. Two-way cluster analysis using Euclidian's cluster method showing the presence of different true morel mushroom species among the sampled sites in North Kashmir.

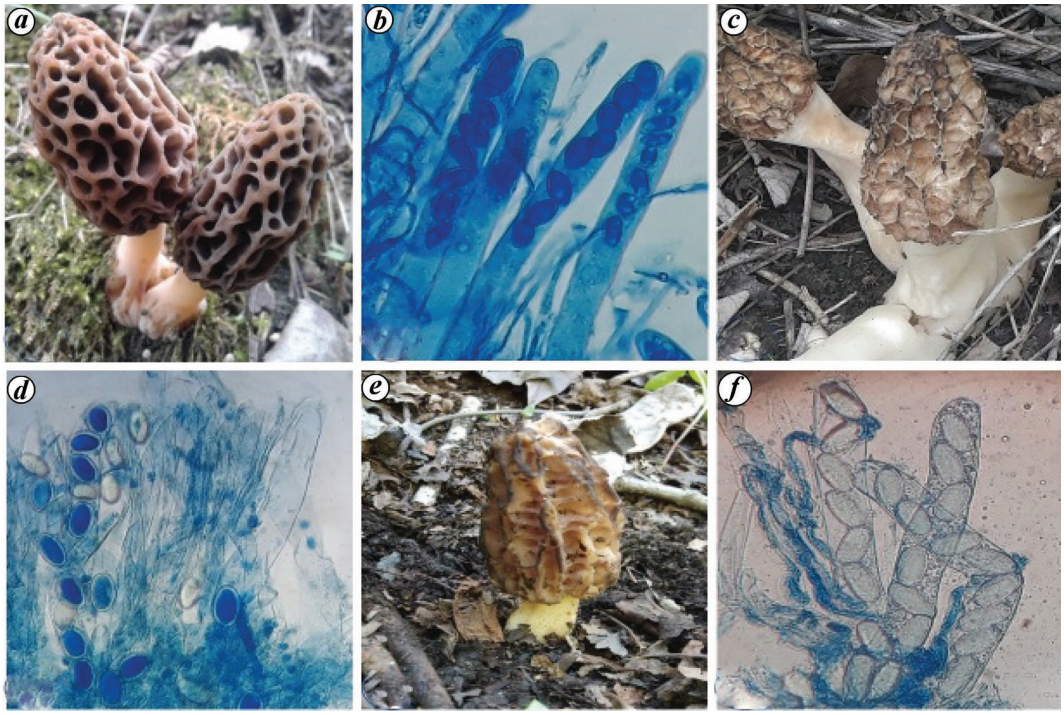

Figure 4. ***a***, ***b***, *Morchella esculenta* (L.) Pers.: ***a***, Pale brownish to greyish-brown fruiting body showing pileus and stipe. ***b***, Asci with ellipsoidal ascospores (400×). ***c***, ***d***, *Morchella crassipes* (Vent.) Pers.: ***c***, Fruiting body showing pileus and stipe. ***d***, Asci with eight ellipsoidal ascospores (400×). ***e***, ***f***, *Morchella vulgaris* (Pers.) Gray: ***e***, Fruiting body showing pileus and stipe. ***f***, Asci with large ellipsoidal ascospores (400×).

Asci: Eight-spored, cylindrical and translucent. Paraphyses: Cylindrical, filamentous and translucent like asci.

Habit and habitat: Saprotrophic or mycorrhizal found in forests, orchards, disturbed grounds and burnt areas. They occur singly or in groups of 3–7 members.

Season: Early spring. Edibility: Edible, highly prized and delicious mushroom.

Site of collection(s): Pattan, Tangmarg, Handwara and Bandipora.

Remarks: Edges of the ridges are usually not darker than the pits. The size of the pileus is 2–7 × 2–10 cm, whereas the stipe is 2–9 × 2–3 cm in size. *Ascospores* are thin walled, measuring about 16.5–21.0 × 8.0–11.0 µm. They occur singly or in groups of 3–7 members.

Morchella crassipes (Vent.) Pers. In *Syn. meth. fung. (Gottingen)*, 2: 621 (1801). (Figure 4 *c* and *d*).
Synonym(s): *Phallus crassipes* Vent., *Morchella esculenta* var. *crassipes* (Vent.) Kreisel.
Common name(s): Morel mushroom.
Local name(s): Kan guech or Gucchi.
Voucher specimen no.: 4334-KASH Herbarium.

Description

Pileus: Yellow to brownish-yellow, sub-globose to elongate, rarely conical, honeycomb-like, measuring about 5.5–8.5 cm across and 6–14 cm in height, comprising irregularly fertile pits separated by narrow ridges, unlike *Morchella esculenta* ridges which are very thin. Stipe: Smooth, hollow, tapering towards the apex with bulbous base, white to pale creamish, about 5–13 cm long and 3–4.5 cm across. Flesh: Thin, white, and brittle. Ascospores: Elliptical, smooth, translucent, without oil droplets, measuring about 17.5–22.0 × 10.0–13.0 µm. Asci: Eight-spored, cylindrical and translucent. Paraphyses: Cylindrical, filamentous, and hyaline.
Habit and habitat: Saprotrophic, found in coniferous forests, orchards and grows scattered or in small groups of 3–5 fruiting bodies.
Season: Spring. Edibility: Edible and highly prized mushroom.
Site of collection (s): Gulmarg, Naid Khai, Handwara and Bandipora.
Remarks: Fruiting bodies comprise irregularly fertile pits separated by narrow ridges, unlike *Morchella esculenta*, which has very thin ridges. The pileus is honeycomb-like, measuring about 5.5–8.5 × 6–14 cm, whereas stipe is smooth and hollow with dimensions of 5–13 and 3–4.5 cm. Ascospores are smooth, hyaline and without oil droplets, measuring about 17.5–22.0 × 10.0–13.0 µm. They occur in groups of 3–5 members.

Morchella vulgaris (Pers.) Gray. In *Nat. Arr. Brit. Pl. (London)* 1: 662. (1821). (Figure 4 *e* and *f*).
Synonym(s): *Morchella conica* Pers., *Morchella esculenta* var. *vulgaris* Pers.
Common name(s): Yellow morel or grey morel.
Local name(s): Gucchi, guech or Khazer kann guech.
Voucher specimen no.: 4335-KASH Herbarium.

Description

Pileus: Predominantly greyish in colour and unlike *Morchella esculenta*, the ridges and grooves are extremely irregular and brightly coloured, elongate to somewhat conical, measuring about 4.5–6.0 cm across and 6.5–9.0 cm in height. Stipe: Smooth, hollow internally like a cap, base is slightly enlarged and grooved, with creamish dust-like particles on the surface, white to creamish, measuring about 3.0–5.5 cm long and 2.5–4.0 cm across. Flesh: Brittle, white and thin. Ascospores: Ellipsoidal, smooth, hyaline, without oil droplets, measuring about 18.5–25.0 × 9.0–12.5 µm. Asci: Eight-spored, cylindrical, broad, and translucent.
Habit and habitat: Found in coniferous forests, orchards and grows singly or in small groups.
Season: Late spring to early summer. Edibility: Edible and highly prized mushroom.
Site of collection (s): Gulmarg, Wadpora and Zalwan Bandipora.
Remarks: The ridges and grooves are extremely irregular and brightly coloured. The size of the pileus is 4.5–6.0 × 6.5–9.0 cm, whereas the stipe is 3.0–5.5 × 2.5–4.0 cm, smooth, with creamish dust-like particles on the surface. Ascospores are ellipsoidal and 18.5–25.0 × 9.0–12.5 µm in size.
Morchella elata Fr. In *Syst. mycol. (Lundae)*, 2(1): 8. (1822). (Figure 5 *a* and *b*).
Synonym(s): *Helvella esculenta* (L.) Sowerby, *Morchella rotunda* (Fr.) Boud.
Common name(s): Black morel or fire morel.
Local name(s): Dum guech, Kan guech.
Voucher specimen no.: 4336-KASH Herbarium.

Description

Pileus: 3.5–8 cm in diameter and 5–7.5 cm in height, hollow and egg-shaped inside with distinctive honeycomb surface comprising dark or tan, black ridges and shady brown pits, blackening or fading with maturity or age. Stipe: Attached to the bottom of pileus, smooth at the top and grooved at base, white and hollow, measuring about 1.0–2.5 cm wide and 4–9 cm tall. Ascospores: Smooth, with polar oil droplet, elliptical and translucent with dimensions of 17–24 × 10.5–14 µm. Asci: Cylindrical, measuring about 350–300 × 15–18 µm and each ascus is eight-spored.
Habit and habitat: Grows individually or in clusters on the ground, usually near conifer trees.
Season: Early spring. Edibility: Edible and highly prized.
Site of collection(s): Gulmarg, Tangmarg, Wadpora and Handwara.
Remarks: The ridges are black or tan, whereas the pits are shady brown in colour and fade with age. Pileus is egg-shaped with dimensions of 3.5–8 × 5–7.5 cm, whereas the stipe is smooth at the top and grooved at base with dimensions 1.0–2.5 × 4–9 cm. Ascospores are smooth, with polar oil droplet, and measuring about 17–24 × 10.5–14 µm.

Morchella kaibabensis Beug, T. A. Clem. & T. J. Baroni. In Baroni, Beug, Cantrell, Clements, Iturriaga, Laessoe, Holgado Rojas, Aguilar, Quispe, Lodge & O'Donnell, *Mycologia*, 110(6): 1208. (2018). (Figure 5 *c* and *d*).
Common name(s): Grey or black morel.
Local name(s): Guech, kann guech.
Voucher specimen no.: 4337-KASH Herbarium.

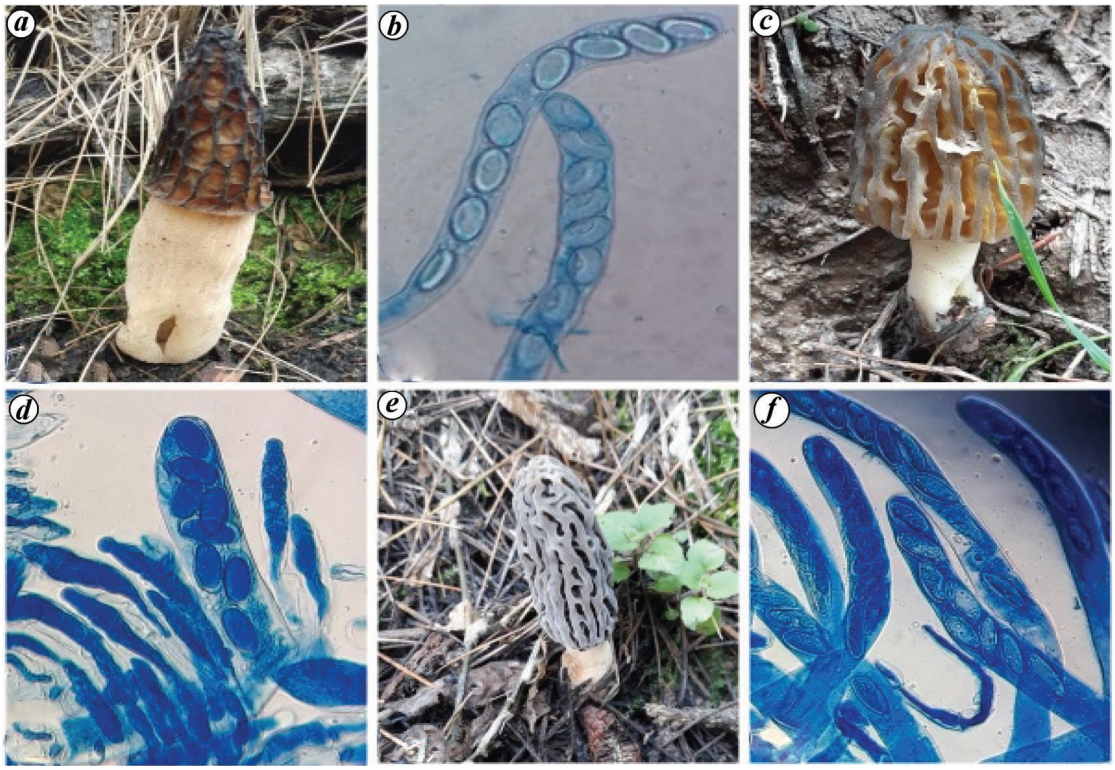

Figure 5. ***a***, ***b***, *Morchella elata* Fr.: ***a***, Honeycomb-shaped black to brown fruiting body showing pileus and long stipe. ***b***, Asci with ascospores (400×). ***c***, ***d***, *Morchella kaibabensis* Beug, T. A. Clem. & T. J. Baroni: ***c***, Fruiting body showing pileus having long pits and creamish stipe. ***d***, Asci with eight ascospores (400×). ***e***, ***f***, *Morchella tridentina* Bres.: ***e***, Conical ascocarp showing pileus and short stipe. ***f***, Asci with eight ellipsoidal ascospores (400×).

Description

Pileus: Conical to somewhat spherical, 30–40 cm in height and 2–5 cm wide, ridges are grey and become black at maturity, pits are vertically elongated, measuring up to 2 cm in length and 0.5–1.0 cm in breath with a depression of about 0.6 cm. Cap has free edges and shows distant attachment compared to the other true morels. Stipe: Cylindrical to clavate showing depressions at the base, white to creamish-white with rough surface, 20–30 cm in height and 0.5–1.5 cm wide. Ascospores: Elliptical, smooth and translucent, measuring about 20–25 × 12–17 µm. Asci: Cylindrical, translucent, eight-spored and thin-walled.

Habit and habitat: Grows usually singly and not in clusters like other morels on the ground, usually near pine trees.

Season: Late summer. Edibility: Edible.

Site of collection(s): Gulmarg, Handwara and Drung.

Remarks: The ridges are grey and become black at maturity; pits are vertically elongated with a depression of about 0.6 cm. Pileus has free edges, measuring about 30–40 × 2–5 cm and shows distant attachment compared to the other true morels. The stipe is 20–30 × 0.5–1.5 cm with a rough surface which differentiates it from *M. brunnea*, which is similar. Ascospores are ellipsoidal and 20–25 × 12–17 µm in size. It usually grows singly and not in clusters like the other true morels such as *M. snyderi*.

Morchella tridentina Bres. In *Fung. trident.* 2(11–13): 65. (1892). (Figure 5 *e* and *f*).

Synonym(s): *Morchella conica* var. pseudoeximia Clowez, *Morchella quercus-ilicis* Clowez, L. Ballester and L. Romero, *Morchella frustrate* M. Kuo.

Common name(s): Black morel.

Local name (s): Kann guech.

Voucher specimen no.: 4338-KASH Herbarium.

Description

Pileus: Conical to sub-spherical, 4–6 cm in height and 2–4 cm wide, light black to grayish-black and later changes to pinkish-brown, longitudinal ridges are dense and more or less parallel having the same colour as the pits, but gradually become rusty brown, while transverse ridges are abundant and narrow, forming an irregular ladder-like pattern. Pits are shallow and somewhat deep. Stipe: Whitish or creamish, usually enlarged at the base with ridges on either side, hollow, soft, with dimensions of 2–4 cm and 1–2 cm. Ascospores: Ellipsoidal, smooth, thin-walled and measuring about 13–17.5 × 7.5–10 µm. Asci: Cylindrical, curved and eight-spored.

Habit and habitat: Grows separately or in groups on the soil, usually near conifer (pine) trees.

Season: Late summer. Edibility: Edible.
Site of collection (s): Handwara and Tangmarg.
Remarks: Longitudinal ridges are dense and less, whereas transverse ridges are abundant and narrow, forming an irregular ladder-like pattern. Pits are shallow than *M. kaibabensis* and other black morels. The pileus is conical with dimensions of 4–6 × 2–4 cm, whereas the stipe is creamish, soft and 2–4 × 1–2 cm in size. Ascospores are 13–17.5 × 7.5–10 µm in size, whereas asci are cylindrical and curved.

Identification of morels based on molecular characterization

In the present study, nine ascocarps/fruiting bodies belonging to *Morchella* (M1, MA1, M2, MA2, M3, M4, M5, MA5 and M6) were analysed at the molecular level using various molecular tools and techniques to confirm their identity further and also to analyse genetic variation among these species. The following molecular methods or tools were employed.

PCR amplification of the ITS region

The results of PCR amplification of the ITS region revealed that MA2, M3, MA5 and M6 showed ITS sizes of ~700 kb, while as M1 showed the ITS size of ~800 kb. However, MA1, M2 and M4 showed ITS size of ~500 kb, whereas M5 showed ITS size of ~1179 kb (Figures 6 and 7).

Nucleotide BLAST analysis

The results of nucleotide BLAST analysis revealed that the sequences of M1 and MA1 presented close similarity (97% and 98%) with *M. esculenta*. The nucleotide sequences of M2 and MA2 on BLAST analysis exhibited a 100% and 86.12% match to *M. tridentina* (*Morchella quercus-ilicis*), while M3 sequences displayed a close resemblance (97–98%) with *M. elata.* Similarly, the sequences of M4 exhibited a 99.44% match to *M. kaibabensis*. Sequence analysis of M5 and MA5 showed a 94% and 95.44% similarity to *M. crassipes*. Likewise, M6 sequences revealed 95.44% similarity with *M. vulgaris*. In order to obtain GenBank accession numbers, these *Morchella* nucleotide sequences were deposited to the National Center for Biotechnology Information (NCBI) database (Table 2).

Phylogenetic analysis

The genetic diversity of true morels from North Kashmir was studied by constructing a phylogenetic or evolutionary tree. The dendrogram or phylogenetic tree was constructed utilizing several *Morchella* species reported during the study, as well as a closely related species of *Verpa conica* as an outgroup clade or taxon. Phylogenetic tree analysis using maximum composite likelihood method with 1000 bootstraps was performed to study the genetic diversity of morels from North Kashmir. The phylogenetic re-establishments grouped all *Morchella* sequences into two (*elata* and *esculenta*) clades separating the black true morels and yellow true morels. In order to root the tree, sequences of *V. conica* (MZ919245 and MW322802) were utilized as the outgroup genus. The yellow morel clade was additionally alienated into diverse groups such as *esculenta*, *crassipes* and *vulgaris* clusters. Similarly, the black true morels were alienated into *kaibabensis*, *quercus* and *elata* assemblages. The phylogenetic tree revealed that yellow morels, namely *M. esculenta*, *M. vulgaris* and *M. crassipes* formed a single assemblage. All these groupings were aided by high bootstrap values (Figure 8). Some sequences like M1

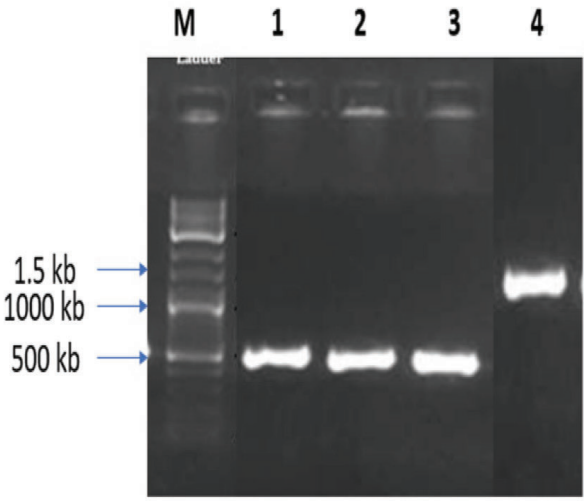

Figure 6. Gel electrophoresis images of ITS–PCR products of true morels amplified with ITS1 and ITS4 primers. Lane M, DNA ladder; lanes 1–4, Species of morels MA1, M4, M2 and M5 respectively. This is a composite figure from different gels.

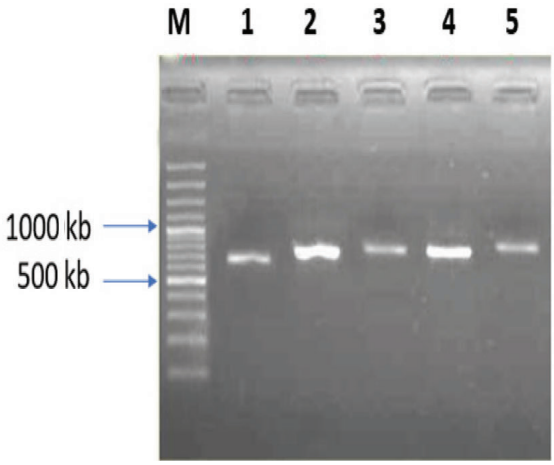

Figure 7. Gel electrophoresis images of ITS–PCR products of some true morels amplified with ITS1 and ITS4 primers. Lane M, DNA ladder; lanes 1–5, Species of morels M6, MA2, M3, MA5 and M1 respectively. This is a composite figure from different gels.

Table 2. ITS sequences of *Morchella* submitted to NCBI along with GenBank accession numbers, sequence ID, release date and names of species

Accession no.	Sequence	Release date	Species
MW479971	SR1765-AN-CAITSR_C02.ab1	20 January 2021	*M. kaibabensis*
MW479972	SR1765-M2-CAITSR_E01.ab1	20 January 2021	*M. tridentina*
MW479973	SR1664-MW1-ITSR_B08.ab1	20 January 2021	*M. tridentina*
OL504954	SR1779-M13-CAITSR_C14.ab1	22 November 2021	*M. elata*
OL654279	SR1791-MA15-ITSR_D02.ab1	1 December 2021	*M. crassipes*
OL504953	SR1778-M15-CAITSR_C13.ab1	22 November 2021	*M. crassipes*
OL504956	SR1781-M5-CAITSR_E16.ab1	22 November 2021	*M. esculenta*
OL654278	SR1790-MA5-AITSR_E01.ab1	1 December 2021	*M. esculenta*
OMO49833	SR1792-M6-CAITSR_E02.ab1	3 January 2022	*M. vulgaris*

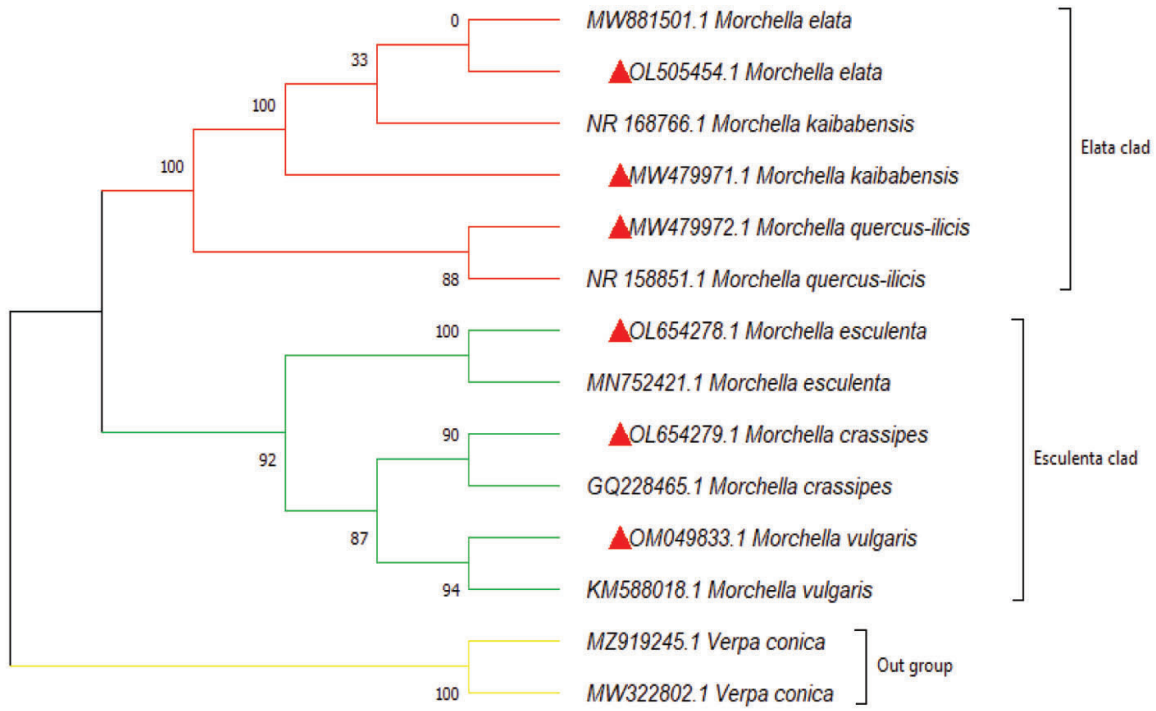

Figure 8. Maximum composite likelihood tree for phylogenetic analysis of *Morchella* spp. based on the analysis of ITS sequences, resulting from a rapid bootstrapping algorithm of 1000 replicates. The sequences of *Verpa conica* have been used as outgroup taxon. The GenBank accession numbers are included. Different clades such as *elata* and *esculenta* with various groups have been marked with bootstrap values shown at the nodes. The *Morchella* species identified in the present study are highlighted by a red triangle.

(OL504956) and MA1 (OL654278) belonged to the *esculenta* group and were identified as *M. esculenta*, whereas the sequences of M5 (OL504953) and MA5 (OL654279) clustered with the *crassipes* group and were hence regarded as *M. crassipes*. Similarly, the sequences of M6 (OM049833) clustered with the *vulgaris* group of yellow morels and were regarded as *M. vulgaris*. However, black morels such as *M. kaibabensis*, *M. elata* and *M. tridentina* grouped into a common clade known as the *elata* clade. The sequences of M2 (MW479972) showed cluster formation with *M. tridentina*, whereas the sequences of M3 (OL504954) were grouped with *M. elata*. Besides, the sequences of M4 (MW479971) isolate exhibited cluster formation with *M. kaibabensis*. All of these groups and clusters were strongly supported by high bootstrap values (Figure 8).

It is quite evident from the present study that both classical and molecular taxonomy reveal the presence of mainly two clades of true morels from North Kashmir. The yellow morels include *M. esculenta*, *M. vulgaris* and *M. crassipes*, while the black morels include *M. elata*, *M. tridentina* and *M. kaibabensis*.

Discussion

In the present study, six species of *Morchella* have been identified based on classical and molecular characterization. It is clear from the study in morels that classical fungal taxonomy helps identify various species. However, ambiguity in the identification of morel mushrooms cannot be

resolved purely based on traditional taxonomy, due to the plastic and polymorphic nature of their ascocarps. Therefore, molecular characterization using phylogenetic analysis was carried out in the present study and the identity of the collected species of morels was confirmed. In similar studies from elsewhere, new insights have been gained in solving the species differentiation is true as well as pseudomorels[18,28,33,35]. In earlier studies from Kashmir, the true morels were generally identified and classified using only conventional methods, which may have resulted in ambiguity in their identification due to variations in their morphological characters depending on varied soil and climatic conditions[24]. In view of this, along with classical taxonomic study we also undertook phylogenetic analysis. The results conform with those of earlier studies on morels from J&K[18,22]. We identified and documented six different species of *Morchella*, namely *M. esculenta*, *M. crassipes*, *M. vulgaris*, *M. tridentina*, *M. elata* and *M. kaibabensis* from North Kashmir. Among these, to the best of our knowledge, *M. tridentina*, *M. vulgaris* and *M. kaibabensis* have not been reported earlier from Kashmir. *M. crassipes*, *M. esculenta* and *M. elata* have been reported earlier from other regions of J&K[18,21,22,24].

As has been done in the present study, a large number of researchers across the world have undertaken taxonomic studies on morels using classical, phenetic and phylogenetic approach[28,36,37]. While working on the taxonomy of true morels, Baroni *et al.*[38] and Ali *et al.*[39] using micro-morphological and phylogenetic techniques reported *M. hispaniolensis*, *M. kaibabensis*, *M. peruviana*, *M. gracilis*, *M. crassipes*, *M. elata* and *M. spongiola* from North America and Pakistan respectively. Likewise, Loizides *et al.*[27] have given a detailed account of 11 species of true morels based on morphological and molecular taxonomy.

Conclusion and future prospective

The present taxonomic study on true morels from North Kashmir has resulted in the documentation of three species, viz. *M. vulgaris*, *M. tridentina* and *M. kaibabensis*, which have not been reported earlier from the study region. The forested areas of J&K are rich in mushroom species. Extensive research using classical and molecular taxonomic techniques is required to unearth the hidden mushroom biota, including morel fungi, and may also be used to assess the nutritional and neutraceutical components for use in promoting human welfare.

Conflict of interest. The authors declare that they have no conflict of interest.

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