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New long-season ecotype of *Morchella rufobrunnea* from northern Israel

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ABSTRACT

True morel (*Morchella* spp.) ascocarps commonly appear in the spring for only a few weeks in many regions worldwide. There has only been one report of a *M. esculenta* population from Israel, presumably mycorrhizal, persisting for several months at one site. The present study describes another species, presumably saprophytic, fruiting in northern Israel from early November to late May (winter and spring). This new long-season ecotype was identified as *M. rufobrunnea* (MS5-IL1) by sequencing of its nuclear ribosomal internal transcribed spacer region. It is reported for the first time in Israel, and for the first time outside of the American continent. Its productivity decline from 2001 to 2007 was correlated with concurrently collected rainfall and temperature data. Our data suggested that the number of morel species and ecotypes displaying long seasonality is greater than previously thought.

Key words: Ascocarp, ITS region, *Morchella rufobrunnea*, morel mushroom, phylogeny, seasonality.

INTRODUCTION

True morels (*Morchella* spp.) are edible wild mushrooms of economic importance belonging to the family *Morchellaceae*, order *Pezizales*, class *Pezizomycetes*, phylum *Ascomycota*. The ascocarp is characterized by a distinctive ridged cap. Morels are highly prized for their delicate taste. Although found in many regions all over

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the world, they are considered rare by mycophagists. Several studies on *Morchella* have been published, but the sum of our knowledge relating to ecology, biology and diversity of true morel species remains very limited[15,19].

Morels are difficult to study in nature due to their habit of sudden fruiting and their high variability with regard to ascocarp phenotype, habitat, and trophic state[19]. They are generally classified morphologically into black and yellow species, but the ascocarps within each species also differ in color nuances, shape, size, and ridge arrangement[24], complicating the task of systematizing them according to morphological traits. Globally, morels are found in a wide range of habitats and environmental conditions[3,4,6,7,9,10,14,21], and display different trophic stages[19]. Buscot and Roux[7] and Buscot[6] distinguished between two general ecological types of morel: pioneer and mycorrhizal.

With regard to seasonality, however, morels show less variability. All over the world, the different species of morels emerge in particular locations, generally in the spring, although several reports have described finding morels in the summer[27] or autumn[21], which vanish after a few weeks[2,3,10,14]. Ascocarp production of a particular species at a single site lasting for several months in a season has only been reported in one previous work by Goldway *et al.*[11]. This latter report was the first to describe a unique long-season *Morchella* ecotype, presumed to be mycorrhizal, from northern Israel (Dan ecotype). This ecotype continues to fruit for 8 months throughout the year, excluding the mid-winter months. Note that, although identified as *M. conica* in Goldway's report, molecular methods applied in our lab later revealed it to be *M. esculenta*. No second account of a long-season or winter-season morel has been published to date. The question thus arises: is long seasonality in *Morchella* species-related? or does it constitute an adaptive feature displayed by diverse morel species? In the present study, we report on a second species of morel that produces ascocarps for extended periods of time. This new population was discovered in the same nature reserve as the previously reported long-season Dan ecotype[11]. It was observed in early December 2001 in the first stages of emergence from the soil and persisted until May. The phenotype of its ascocarps differs from the earlier reported Dan ecotype, and from that of other species previously reported from Israel: *M. conica* and *M. esculenta*, according Binyamini[5]; or *M. elata* and *M. vulgaris* (= *M. conica*), according Barseghyan and Wasser[2]. The newly discovered mid-winter Israeli ecotype was identified using the internal transcribed spacer (ITS) region of its nuclear ribosomal DNA (nrDNA). Sequencing results for this ecotype and data on its ascocarp population dynamics during seasons 2001 through 2007 in relation to local seasonal climatic conditions are presented.

MATERIALS AND METHODS

Mushrooms. The morel mushrooms studied in this work were designated as Morel MS5-IL1 in the MIGAL Morel database. Specimens are preserved in a collection at MIGAL Institute (Kiryat Shmona, Israel).

Field studies. The field of study was the Dan Nature Reserve in northern Israel (31 30 N, 34 45 E). The geographical zone is characterized by a Mediterranean climate and fauna, with a sunny and dry summer and a rainy season that extends from

October to May, with mid-season temperatures of 0-16 C. The census zone, an area of 0.5 km^2 in the Nature Reserve, was divided into quadrants of about 40 m^2 in size that were monitored for morel ascocarps. Ascocarps of the new morel ecotype were found in only one quadrant, and further inspections of the studied mushrooms were limited to this sample unit. Once the mushroom patch was located, the site was visited twice a week, and all of the ascocarps found at the site were counted from their first emergence, when they were at least 0.5 cm in height. The number of total monthly and seasonally counted ascocarps was determined for the first season (December 2001-June 2002), and subsequently from October to June of each year from 2002 to 2007, in order to study the persistence of the population in this particular site. Daily climatic data were obtained during the ascocarp production periods from a meteorological station located 10 km from the study site. Total annual precipitation and total average daily temperature (sum of average daily temperatures) during the rainy season (October to May) and the hot season (June to September) were determined for each season, and correlation analyses (r^2) between those climatic parameters and mushroom production from 2001-2007 were determined.

Molecular analyses. The ITS region was used for phylogenetic analyses of the MS5-IL1 ecotypes according to Buscot *et al.*[8] and Wipf *et al.*[26]. Freeze-dried tissues from three different ascocarps derived from ascocarp-production events in January and April 2002 were used for molecular analyses. DNA was extracted by the phenol/chloroform-extraction procedure according to Henrion *et al.*[13] from freeze-dried tissue crushed in liquid nitrogen. The regions ITS1 and ITS2, together with the 5.8S rRNA gene, were amplified according to White *et al.*[25]. PCR amplifications were carried out using a Flexigene thermocycler (Techne, U.K.) under conditions described by Wipf *et al.*[26]. Amplification products were cleaned using the Accuprep PCR purification kit (HyLabs, Israel) and sequenced by HyLabs, Israel.

Alignments and phylogenetic analyses. The sequence of the ITS region, including the 5.8S nrDNA of the Israeli ecotype MS5-IL1, as well as a sequence obtained from Dr. O'Donnell (NCAUR, ARS, USDA) for comparison, were submitted to the GenBank (accession numbers DQ355921 and DQ355922, respectively). Sequences were assembled and edited by SeqMan program. Multiple alignments and phylogenetic trees were constructed using the programs Lasergene MegAlign (DNASTAR, U.S.A.) and MEGA 4[22]. To examine the relationship between the MS5-IL1 ecotype sequence and those of other *Morchella* species, the ITS region sequences including the 5.8S gene were compared with other sequences retrieved from the GenBank database (NCBI: www.ncbi.nlm.nih.gov) [**Table 1**]. Members of the *Morchellaceae* (representative species of *Verpa* and *Disciotis*), other than the genus *Morchella* were used as out-group candidates (**Table 1**).

The phylogenetic tree was based on multiple sequence alignments and cluster analyses. A dendrogram comparing the ITS regions was created along with 2,000 bootstrapping repeats test of phylogeny using the Neighbor Joining algorithm of Saitou and Nei[20]. Sequence analysis characteristics for the whole ITS region are presented in **Table 2**. The phylogenetic tree based on the ITS region was submitted to the TreeBase database (Accession number S2401).

Table 1. Sequences retrieved from the GenBank (NCBI: www.ncbi.nlm.nih.gov).

Accession no.	Species	GenBank source
DQ355921	*M. rufobrunnea* (MS5-IL1)	
DQ355922	*M. rufobrunnea* Guzman & Tapia	Guzman and Tapia, 1998*
AJ544194	*M. conica* Pers.	Kellner *et al*., 2007
AJ544203	*M. angusticeps* Peck	Kellner *et al*., 2007
AJ544200	*M. elata* Fr.	Kellner *et al*., 2007
AJ539476	*M. spongiola* Boud.	Kellner *et al*., 2007
AJ539480	*M. crassipes* (Vent.) Pers.	Kellner *et al*., 2007
AJ543741	*M. esculenta* (L.) Pers.	Kellner *et al*., 2007
AY544667	*Disciotis venosa* (Pers.) Arnould	Schoch C. (Direct submission, 2004)**
FJ176853	*Verpa bohemica* (Krombh.) J. Schröt.	Schoch C. (Direct submission, 2008)**

* The sequence was provided by Dr. O'Donnell, USDA, U.S.A.
** Department of Botany and Plant Pathology, Oregon University, OR, U.S.A.

RESULTS AND DISCUSSION

The ascocarps of MS5-IL1 were first detected in early December of the 2001-2002 season, at which time they appeared as small, 0.5-1.0 cm high stalk-like primordia, and were followed until maturity. Ascocarps appeared singly or in small clusters, each with up to five mushrooms. The individuals were observed growing on relatively bare gravel soil at the edge of a natural grove, under diverse species of trees (*Rhamnus alaternus*, *Platanus orientalis*, *Populus euphratica*, *Laurus nobilis*, *Fraxinus syriacus*), and on recently disrupted soil near a newly paved path. No association was detected between the roots of any of these trees and the morel mushrooms.

New ascocarps continued to emerge from first sighting in early December until the end of May 2002 (**Fig. 1**). In the following season, 2002-2003, fruiting began in mid-November and lasted until mid-May, but fewer ascocarps were produced (**Fig. 1**). A similarly prolonged fruiting season encompassing the winter months was recorded in the third season of the study, 2003-2004 (**Fig. 1**), again with less ascocarps than in the previous season. The 2004-2005, 2005-2006, and 2006-2007 seasons showed further decreases in ascocarp production.

The patch of MS5-IL1 mushrooms persisted in the sample unit area for a period of more than 5 months per season, spanning the winter season (**Fig. 1**). The ecotype differed phenologically from ecotypes found elsewhere in the world, where morel ascocarps appear for a short period of time in the spring and summer. In the U.S.A., for instance, morels fruit mainly in April and May in different parts of the country, while in Himachal Pradesh, India, black morels appear in spring (March-May) and yellow morels at the end of the summer (August-September)[21].

The long ascocarp-production season could be related to the local climatic conditions. Temperature and rainfall are considered to be the main factors affecting ascocarp development in fungi[1], and they affected the development of the ecotype under investigation as well. The MS5-IL1 mushrooms appeared at the site only during the Israeli rainy season (from October to May), which is accompanied by low to moderate temperatures (ranging from 15-28 C during the day, and 5-15 C at night, with the lowest temperatures recorded in mid-winter, *i.e.*, January and February). The period of moderate temperatures and rainy days lasted for several months, enabling the MS5-IL1 mushroom to fruit throughout that period. No ascocarps were recorded in the Israeli dry summer, in which monthly averages can reach 35 C. Elsewhere in the world, in places where snow covers the ground, morels appear in the spring or summer after the snow thaws[23].

Fruiting of a single species of *Morchella* in a certain site for several months per season has been previously reported only for *M. esculenta*, an ecotype known as the "Dan morel", also discovered in the Dan Nature Reserve[11]. However, the "Dan morel" differs from the MS5-IL1 ecotype in its ecological characteristics. Unlike the MS5-IL1 ecotype, the previously reported Dan ecotype was considered to be mycorrhizal and to have developed in association with the roots of the Syrian ash tree (*F. syriacus*). It emerged on the bank of a river with a year-round flow of cool water, while the MS5-IL1 ecotype appeared on soil surfaces that are only wetted by rain. Moreover, the Dan ecotype ascocarps were not observed during the mid-winter months, *i.e.*, January, February

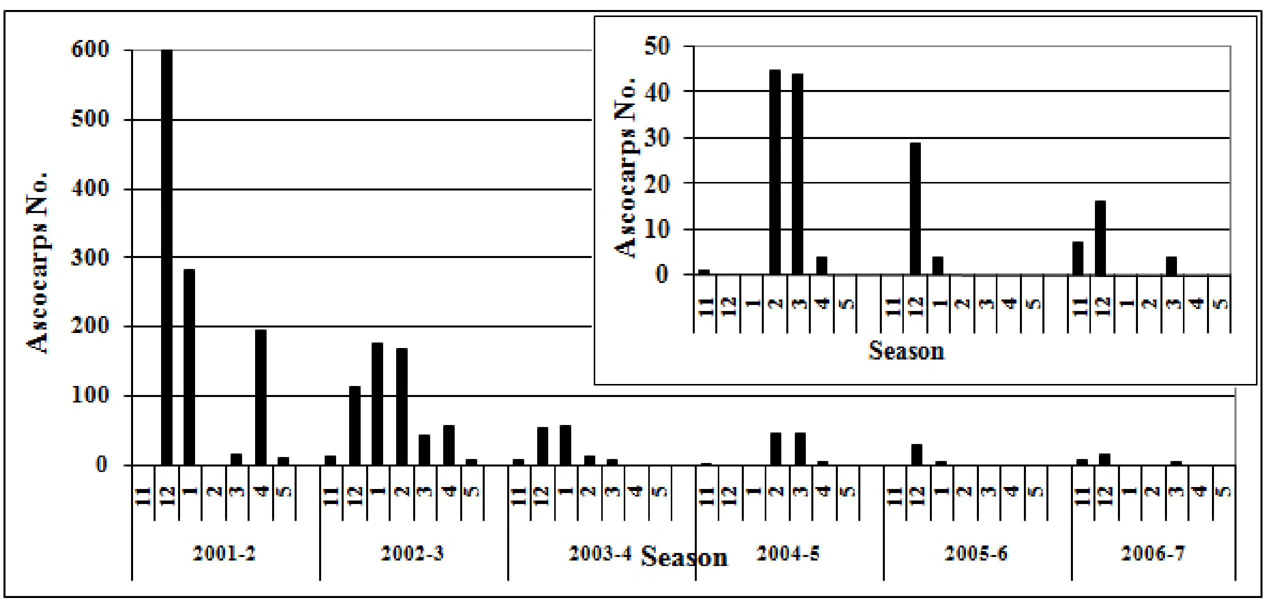

Fig. 1. Seasonality of MS5-IL1 ecotype ascocarp production of *Morchella rufobrunnea* in seasons 2001-2007. The number of mushrooms represents a cumulative monthly count. Inserted graph: magnified presentation of seasons 2004-2007 data.

and March[11], as were the ascocarps of the studied MS5-IL1 ecotype.

The MS5-IL1 ecotype also differs from the Dan ecotype with regards to its persistence over the years. While the Dan ecotype was considered stable, with a stable population over the course of years, the new ecotype's population exhibited a gradual decline in the years following the first season of this study, from 2001-2007 (**Fig. 1**). No mushrooms could be detected at all at the site in 2007-2008.

Correlation of ascocarp productivity over the years with climatic conditions yielded different relationships, depending on whether the first season (2001-2002), with the highest population, was included in the analysis. The reduction in productivity from the second season of the study (2002-2003) to the last season (2006-2007) showed a high correlation with the reduced amount of seasonal rainfall during that period (r^2= 0.91) [**Fig. 2A**], and with increasing summer temperature (r^2= 0.61), but not with winter temperature (**Fig. 2B-C**). However, when the first season (2001-2002) with its high ascocarp production was included in the analysis, there was no correlation (r^2= 0.137 for rain; r^2= 0.46 for summer temperatures). As mentioned, season 2001-2002, with the highest mushroom production, had lower precipitation, while the following season 2002-3, had the highest precipitation but lower mushroom production.

The high productivity in 2001-2002 could not be explained by rain or temperature. Other environmental factors, including soil parameters, such as nutrient boosting or depletion, and a decrease or increase in competitors due to soil disruption, could be responsible for the mushroom's productivity, as it has been suggested in order to explain the decline

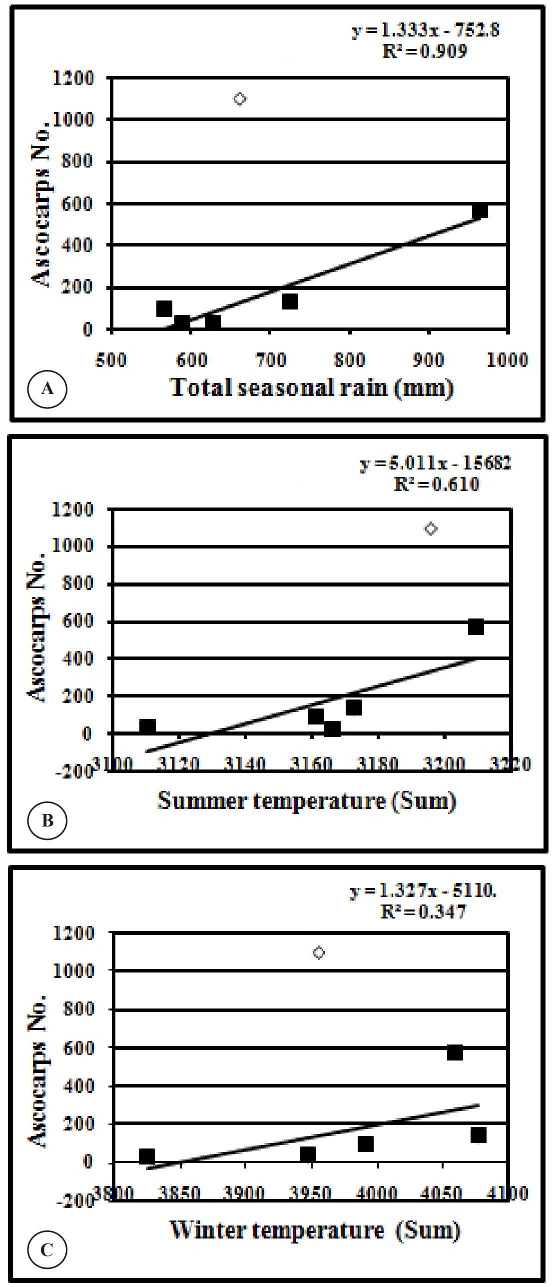

Fig. 2. Relationship between morel productivity (*Morchella rufobrunnea*) and climatic conditions over the years of the period studied. A: Total seasonal amounts of precipitation. B: Total summer temperatures (sum of average daily temperatures, June to September). C: Total winter temperatures (sum of average daily temperatures, October to May). Regression correlation coefficient was determined for seasons 2002-2007 only (■), *i.e.*, excluding 2001-2002 season (◊).

of other pioneer ecotypes of morel[7]. MS5-IL1 appeared in recently disrupted soil, and this disruption may have resulted in a sharp increase in nutrients and a decreased level of competition, which would enable the observed high mushroom production in the first season. In the subsequent years, although correlation analysis of the 2002-2007 seasons showed high correlation (r^2) values with rain and summer temperature, other factors, such as food depletion and increasing competition, may have also had an effect on the gradual decline of the mushroom population at this particular site. As mentioned, the mushrooms studied here were found under different tree species with no obvious connection to their root systems, suggesting a non-mycorrhizal nature, which also confirms this ecotype as a pioneer. Moreover, their ascocarp-production pattern, consisting of seasonal reappearance over a brief span of a few years, also supports the suggestion that MS5-IL1 is a pioneer ecotype according to the definition of Buscot and Roux[7]. The sudden appearance of morel mushrooms at a particular site for a few years is a known phenomenon, generally observed in after-fire forests and in other disrupted soils[6,7,9,18,24].

Molecular identification of the MS5-IL1 morel ecotype. The MS5-IL1 ecotype displayed several morphological and ecological characteristics that distinguished it from the Israeli morels *M. conica* and *M. esculenta*[5], as well as *M. vulgaris* and *M. elata*[2]. These characteristics are as follows: its conical head exhibits dark pits and white ridges when young, but turns an overall brownish color when mature (**Fig. 3**); it blushes in irregular spots of brown to orange, when injured; and the mature ascocarp can reach a height of 5 to 10 cm. In view of the distinctive morphology of the MS5-IL1 morel ecotype as compared to *M. conica* and *M. esculenta*, together with its unique phenology, *i.e.*, winter-growing long season, the question arose: was MS5-IL1 a different ecotype? or did it constitute a new species differing from all previously studied morels? Analysis of the ITS region from the nrDNA was used, a method frequently followed to classify morels[8,15,26]. A fragment of 859 kb was obtained from this mushroom by PCR, using the ITS1 and ITS4 primer pairs to amplify the ITS region. Juxtaposition of the MS5-IL1 ITS region with those of other morel sequences retrieved from the GenBank database revealed no matches. Multiple sequence alignment of several morel ITS sequences and cluster analyses placed the MS5-IL1 morel on a separate branch of the dendrogram in relation to all other morel species in that database. It differed from the known morel species *M. esculenta*, *M. conica*, *M. elata*, *M. angusticeps*, *M.*

Fig. 3. *Morchella rufobrunnea* MS5-IL1 in its natural habitat growing at the edge of a paved path. Bar= 1 cm.

Table 2. Sequence analyses of ITS region including 5.8S gene of *Morchella rufobrunnea* MS5-IL1 and other species of the *Morchellaceae* (genera *Morchella*, *Verpa*, *Disciotis*).

Putative species	GenBank accession number	Sequence size	Matched sequence length	Nucleotide overlap	Degree of similarity*
M. rufobrunnea (MS5-IL1)	DQ355921	859			
M. rufobrunnea (O'Donnell)	DQ355922	872	859	856	99.7
M. conica	AJ544194	737	776	476	62.3
M. angusticeps	AJ544203	740	780	467	59.9
M. elata	AJ544200	737	798	477	59.8
M. spongiola	AJ539476	1186	945	557	58.9
M. crassipes	AJ539480	1226	937	552	58.9
M. esculenta	AJ543741	1138	949	549	57.9
Disciotis venosa	AY544667	1390	885	306	34.6
Verpa bohemica	FJ176853	886	873	300	34.4

* Degree of similarity is given as percentage of base pair identity with the best match.

crassipes, and *M. spongiola* (**Fig. 4**, **Table 2**). However, the sequence did match one sample found at a database (USDA), revealing it to be virtually identical (99.7%) to the ITS region of the Mexican *M. rufobrunnea*[12,17] (Dr. K. O'Donnell, personal communication and permission). This sequence was also loaded into the GenBank database (accession number DQ355922). In a comparison of ITS regions, the MS5-IL1 sequence showed 62.3% to 57.9% similarity with the other *Morchella* species sequences, being more closely related to the black morel mushroom (*M. angusticeps*, *M. conica*, *M. elata,*), and least closely related to the yellow morel (*M. crassipes*, *M. esculenta*, *M. spongiola*) [**Table 2**]. Lower similarity (34.6% and 34.4%, respectively) was obtained with the ITS sequences of *Disciotis venosa* and *Verpa bohemica*, fungi belonging to other genera of the *Morchellaceae*. The comparison between the sequence of only the conserved 5.8S region (based on Wipf *et al.*[26]) of the new ecotype and those from the other *Morchella* species listed in **Table 1**, showed 100% similarity with *M. rufobrunnea* sequence, while 99.4% with the *M. conica* sequence and 98.8% similarity with other *Morchella* species. Only 41.2% and 37.4% similarity was obtained comparing with *D. venosa* and *V. bohemica*, showing that the new ecotype is closely related to the other members of the genus *Morchella.*

The ITS region of the new ecotype was identical in both winter (January) and spring (April) ascocarps, confirming that the same morel species was fruiting from December until the end of May at the same site. These data suggested that adaptation of the MS5-IL1 morel to winter conditions

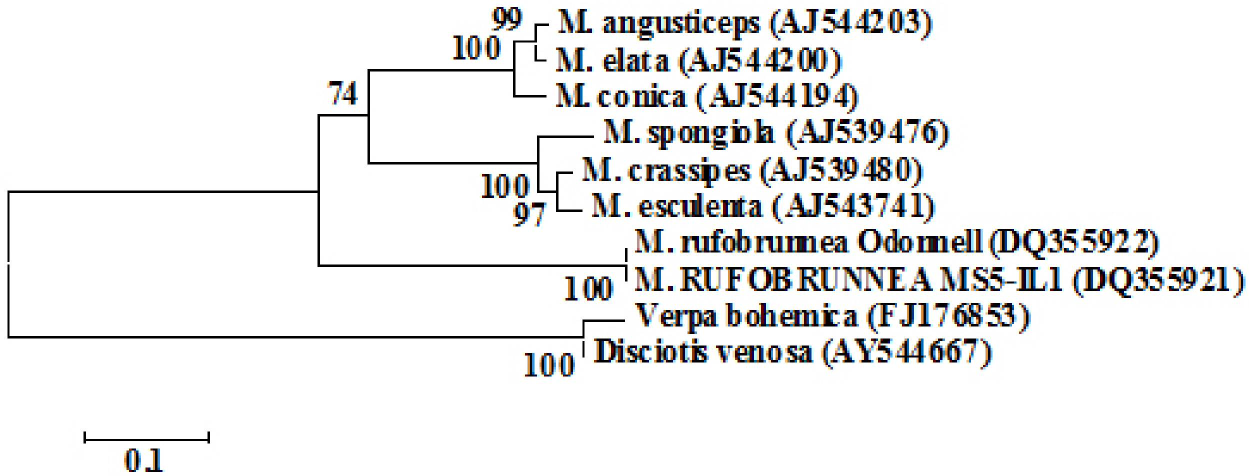

Fig. 4. Dendrogram showing the relationships and degrees of similarity among several representative ITS sequences from morels, and outgroup species belonging to the genera *Verpa* and *Disciotis*. The dendrogram was created along with 2,000 bootstrapping repeats test. The bootstrapping results are shown in Table 2. Numbers in parentheses are the GenBank accession numbers for the sequences.

in Israel and its long fruiting season are species-related. In addition to Israel and Mexico, *M. rufobrunnea* has recently been discovered growing in February in the U.S.A., in California[16]. Both Mexico and California have Mediterranean-climate regions, similar to Israel's climate, which could explain the dispersal of *M. rufobrunnea* in those locations.

The findings described show that the long-season phenomenon in *Morchella* species is more widely distributed than previously thought. The previously reported *M. esculenta* (Dan ecotype)[11] and the currently reported *M. rufobrunnea*, reported in Israel for the first time, both exhibit several months of ascocarp production per season, although they differ in their ecological characteristics. The first is presumably mycorrhizal, while the second is a pioneer type. *M. esculenta* does not fruit in Israel in mid-winter, while *M. rufobrunnea* does. *M. esculenta* produces only few ascocarrps, persist over the course of years, while *M. rufobrunnea* shows a decline in ascocarp production. Long seasonality of *M. rufobrunnea* (MS5-IL1) was found to be related to the moderate climate of the Israeli winter, while the persistence over the years was related partially to hot summer and rainy winter season. Israel, being in a transitional geographical zone, can support a wide range of species and ecotypes with long seasonality. *M. rufobrunnea* (MS5-IL1) growth in Israel is reported here for the first time. It is also the first time that this species has been found outside the American continent. It is suggested that climatic similarities between regions support the appearance of similar species. Further studies are needed to support our observations, particularly in other countries that share similar climatic conditions with Israel.

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LITERATURE CITED

1. Arnolds, E. 1981. Ecology and coenology of macrofungi in grasslands and moist heathlands in Drenthe, the Netherlands. Part 1. Introduction and synecology. *Bibliotheca Mycologica* 83: 1-407.
2. Barseghyan, G. and S. P. Wasser. 2008. Species diversity of the genera *Morchella* St. Amans and *Helvella* L. ex St. Amans (Ascomycota, Pezizales) in Israeli mycobiota. *Nova Hedwigia* 87: 315-336.
3. Bartelli. I. 1990. May is morel month in Michigan. Extension Bulletin E-614, Michigan State University, East Lansing. 22 pp.
4. Batra, L. R. 1983. Edible Discomycetes and Gasteromycetes of Afghanistan, Pakistan and North India. *Biologia* 29: 293-304.
5. Binyamini, N. 1984. *Larger Fungi of Israel. Ascomycotina, Basidiomycotina*. Ramot Publishing Co., Tel Aviv. 172 pp.
6. Buscot, F. 1989. Field observations on growth and development of *Morchella rotunda* and *Mitrophora semilibera* in relation to forest soil temperature. *Canadian Journal of Botany* 67: 589-593.
7. Buscot, F. and J. Roux. 1987. Association between living roots and fruit bodies of *Morchella rotunda*. *Transactions of the British Mycological Society* 89: 249-252.
8. Buscot, F., D. Wipf, C. Di Battista, J. C. Munch, B. Botton and F. Martin. 1996. DNA polymorphism in morels, I. PCR/RFLP analysis of the ribosomal DNA spacers and microsatellites-primed PCR. *Mycological Research* 100: 63-71.
9. Carpenter, S. E., J. M. Trappe and J. Ammirati Jr. 1987. Observation of fungal succession in Mount St. Helens devastation zone, 1980-1983. *Canadian Journal of Botany* 65: 716-728.
10. Friedman, S.A. 1986. Morels, May apple and the meaning of life. *Mushroom, the Journal of Wild Mushrooming* 4: 5-9.
11. Goldway, M., R. Amir, D. Goldberg, Y. Hadar and D. Levanon. 2000. *Morchella conica* exhibiting a long fruiting season. *Mycological Research* 104: 1000-1004.
12. Guzman, G. and F. Tapia. 1998. The known morels in Mexico, a description of a new blushing species, *Morchella rufobrunnea*, and new data on *M. guatemalensis*. *Mycologia* 90: 705-714.
13. Henrion, B., G. Chevalier and F. Martin. 1994. Typing truffle species by PCR amplification of the ribosomal DNA spacers. *Mycological Research* 98: 37-43.
14. Kaul, T. N., M. L. Kachroo, J. L. Kachroo, A. Krishna and C. K. Atal. 1981. Myco-ecological studies on morel bearing in Kashmir. *Mushroom Science* 11: 789-795.
15. Kellner, H., C. Renker and F. Buscot. 2005. Species diversity within *Morchella esculenta* group (Ascomycota: Morchellaceae) in Germany and France. *Organisms Diversity & Evolution* 5: 101-107.
16. Kuo, M. 2005. *Morels*. The University of Michigan Press, Ann Arbor. 205 pp.
17. O'Donnell, K., N. Weber, S. Rehner and G. Mills. 2003. Phylogeny and biogeography of *Morchella*. Proceedings of the 22nd Fungal Genetics Conference, Asilomar. *Fungal Genetics Newsletter* 50 (Suppl.). Abstract 443. (www.fgsc.net/asil2003).
18. Pilz, D., N. S. Weber, M. C. Carter, C. G. Parks and R. Molina. 2004. Productivity and diversity of morel mushrooms in healthy, burned, and insect-damaged forests of northeastern Oregon. *Forest Ecology and Management* 198: 367-386.
19. Pilz, D., R. McLain, S. Alexander, L. Villarreal-Ruiz, S. Berch, T. L. Wurtz, C. G. Parks, E. McFarlane, B. Baker, R. Molina and J. E. Smith. 2007. *Ecology and management of morels harvested from the forests of western North America*. USDA General Technical Report PNW-GTR-710, Portland, OR, U.S.A. 161 pp. (www.fs.fed.us/pnw/publications).
20. Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.
21. Singh, S. K., S. Kamal, M. Tiwari, R. D. Rai and R. C. Upadhyay. 2004. Myco-ecological studies of natural morel bearing sites in Shivalik hills of Himachal Pradesh, India. *Micologia Aplicada International* 16:1-6.
22. Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007.

MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.

23. Volk, T. J. and T. J. Leonard. 1990. Cytology of the life-cycle of *Morchella*. *Mycological Research* 94: 399-406.
24. Weber, N. S. 1995. *A Morel Hunter's Companion*. Thunder Bay Press, Lansing. 208 pp.
25. White, T. J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322. *In: PCR Protocols, a Guide to Methods and Applications*. Eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White. Academic Press, San Diego.
26. Wipf, D., J.-C. Munch, B. Botton and F. Buscot. 1996. DNA polymorphism in morels: complete sequences of the internal transcribed spacer of genes coding for rRNA in *Morchella esculenta* (yellow morel) and *Morchella conica* (black morel). *Applied and Environmental Microbiology* 62: 3541-3543.
27. Wurtz, T., A. Wiita, N. Weber and D. Pilz. 2005. Harvesting morels after wildfire in Alaska. Research Note PNW-RN-546. USDA, Forest Service, Pacific Northwest Research Station, Portland, OR, U.S.A.