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Morchella galilaea – Expanded phylogeography and relaxed seasonality of a globally distributed autumnal morel

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ABSTRACT

Morchella galilaea is unique among morels by its autumnal fruiting and a worldwide but disjunct distribution, strongly biased towards islands. The drivers of this reversed seasonality and transcontinental dispersal remain poorly understood. New records of *M. galilaea* from several islands of the Mediterranean, and from the Reunion Island in the Indian Ocean, all unveiled here, invited us to revisit and discuss the biogeography, phenology and ecology of this intriguing morel. Our ecological and molecular findings do not support recent anthropogenic introductions as causal for the tropism of *M. galilaea* for islands. Instead, we postulate a tentative model of evolution in which the paleotropical origins of the species may be responsible for the release of strict seasonality constraints inherited early during the genus' evolutionary history.

Keywords:

Ascomycota
Biogeography
Climatic refugia
Malta
Gozo
Olea europaea
Phenology
Tropics

1. Introduction

Morchella Dill. ex Pers. species (Pezizales, Ascomycota), commonly known as morels, are globally distributed macrofungi of culinary interest. Since 2010, the emergence of molecular tools propelled a resurgence of interest in their taxonomy and provided new insights into the evolutionary history of the genus (Tas,kin et al. 2010, 2012; O'Donnell et al. 2011; Du et al. 2012; Richard et al. 2015; Loizides et al. 2015, 2016, 2021, 2022). Phylogenetic studies showed morel species to belong to three main clades: the ancestral/Rufobrunnea clade (white morels, section *Rufobrunnea*), and two sister clades – the/Elata clade (black morels, section *Distantes*), and the/Esculenta clade (yellow morels, section *Morchella*) (Tas,kin et al. 2010, 2012; O'Donnell et al. 2011; Du et al. 2012; Richard et al. 2015). Taxonomic work followed shortly after, resulting in several newly described species from Spain (Clowez et al. 2014, 2015, 2020); Australia (Elliott et al. 2014); Cyprus (Loizides et al.

2016); Turkey (Tas,kin et al. 2016); Canada (Voitk et al. 2016); South, Central and North America (Kuo et al. 2012; Baroni et al. 2018; Machuca et al. 2021), and China (Du et al. 2019). So far, 58 phylogenetic clades are tied to classical or recently proposed binomials, while the number of morel species worldwide delineated by molecular phylogenetics exceeds 80 (Loizides et al. 2022).

Among the most intriguing species described in recent years is *M. galilaea* S. Masaphy & Clowez (2012), which, prior to its formal description, had been assigned the informal designation “*Mes-16*” (O'Donnell et al. 2011). *Morchella galilaea* is unique within the genus by its autumnal fruiting. Initially described from specimens collected under *Fraxinus syriaca* Boiss. (*Oleaceae*) occurring at the Upper Galilee region of Israel (Clowez 2012), the species has since been reported from numerous tropical and subtropical localities around the globe, but so far excluding the New World (Degreef et al. 2009; O'Donnell et al. 2011; Kanwal et al. 2011; Du et al. 2012, 2020, 2023; Tas,kin et al. 2012, 2015;

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Matošćec et al. 2014 (as “*M. esculenta* aggr.”); Richard et al. 2015; El-Gharabawy et al. 2019; Clowez and Moreau, 2020; Marcos et al. 2021; Loizides et al. 2022). In addition to these biogeographical and phenological peculiar features, *M. galilaea* has recently been shown to display a unique cytology of its ascospores, potentially impacting its dispersal abilities (Du et al. 2023). The whole genome of this species is now available (<https://mycocosm.jgi.doe.gov/Morgal1/Morgal1.home.html>).

In recent years, several occurrences of morels fruiting in autumn in both hemispheres have been reported in a number of publications (Masaphy et al. 2009; Loizides et al. 2015), but also informally in regional bulletins, mycological forums and social networks (Mifsud 2017, Johansson and Staiger, 2019 and unpubl. data), potentially extending the distribution range of *M. galilaea*. Here we present nine new records of *M. galilaea* from the Mediterranean region and the Reunion island in the Indian ocean confirmed by molecular sequencing, and then discuss the potential drivers for its autumnal fruiting and proliferation on islands. Of the newly reported populations, four were found on the small island of Gozo (Malta). Integrating these local findings into the existing literature, we further comment on the ecology of *M. galilaea*, the current hypothesis of its anthropogenic dispersal, and propose a tentative synthetic model linking the unique biogeography and seasonality of the species, to an evolutionary perspective.

2. Materials & methods

Field work and studied material: Putative plant associations surrounding *Morchella* populations were described from forays carried out between December 2021 and December 2022. Study sites included fields in Gozo (Maltese archipelago, following the first observation by Mifsud (2017)), combined with past findings of autumnal morels in mainland Italy (2009), Croatia (2012), Reunion Island, France (2017), Sicily, Italy (2021) and Majorca Island, Spain (2021). The characterization of each collection’s habitat included the identification of nearby trees and dominant shrubs based on above-ground observations. Abiotic parameters including the soil type, opening of the habitat were also noted. The location of the historical records from Gozo was revisited to detect further specimens and characterize the habitat in greater detail.

Molecular studies: Nine collections of morels (see sequenced material below) were sequenced at the internal transcribed spacers and 5.8S rDNA locus (ITS) to ascertain their identification. Briefly, DNA extraction and PCR amplification were conducted with the REDEExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturer’s instructions. The ITS was amplified from each collection, with primers ITS-1F and ITS-4 (White et al. 1990; Gardes and Bruns 1993), as described in Richard et al. (2015). Amplicons were purified and sequenced in both directions by Eurofins Genomics (Ebersberg, Germany). Raw sequence data were edited and assembled with Codon Code Aligner 9.0.2 (CodonCode Corp., Centerville, MA, USA) and deposited in GenBank under the accession numbers indicated in the next section. Phylogenetic analyses were all performed online at www.phylogeny.lirmm.fr (Dereeper et al. 2008). Multiple sequence alignment was carried out with MUSCLE 3.7 (Edgar 2004). Maximum likelihood (ML) phylogenetic analyses were performed with PhyML 3.0 (Guindon et al. 2010), using the GTR model of evolution. Branch support was assessed using the non-parametric, Shimodaira–Hasegawa, version of the approximate likelihood-ratio test (SH-aLRT), implemented in the latest release of PhyML. Trees were built online using Newick Display 1.6 on NGPhylogeny.fr (<https://ngphylogeny.fr/>) and edited with Inkscape 0.91 (<https://inkscape.org/fr/>). Haplotypes were analysed in MEGA11 (Tamura et al. 2021) and clustered with Popart 1.7 (Leigh et al. 2015), using the Median Joining method.

Sequenced material: **Croatia:** Hrvatsko Primorje region, islet of Ilovik, degraded thermo-Mediterranean, fully evergreen, sclerophyllous garrigue on shallow calcareous soil, dominated by *Quercus ilex*, accompanied with *Pistacia lentiscus*, *Myrtus communis* and *Arbutus unedo*, 15 m asl,

31 Oct 2012, E. Raguzin, CNF-2/9281, GenBank ITS [OR778595](https://www.ncbi.nlm.nih.gov/nuclot/OR778595). **France:** Réunion, Cirque de Mafate, Ilet trois Roches, basaltic erosion sludge with no tree/plant around, May 2017, J. Legros via P. Clowez, PhC304, GenBank ITS [OR778594](https://www.ncbi.nlm.nih.gov/nuclot/OR778594). **Italy (Fig. 1a):** Apulia, Brindisi, Bosco dei Lucci, 49 m asl., in a Mediterranean maquis including typical trees such as *Quercus suber*, *Pistacia lentiscus*, *Phillyrea latifolia* and *Myrtus communis*, 6 Nov 2009, C. Agnello & A. Livieri, LIP0400228, GenBank ITS [OR778592](https://www.ncbi.nlm.nih.gov/nuclot/OR778592); Sicily, Messina, Rometta, 100 m asl, abandoned *Citrus* spp grove with presence of *Quercus* sp. in calcareous, clayey soil, 15 Nov 2021, M. Calanni Rindina, herb. Calanni Rindina N° C1, GenBank ITS [OR196838](https://www.ncbi.nlm.nih.gov/nuclot/OR196838). **Spain (Fig. 1b):** Balearic Islands, Mallorca, Llubí, Son Prim, Mediterranean maquis with *Pinus halepensis*, *Pistacia lentiscus*, *Olea europaea* & *Cistus* spp., 110 m asl, 27 Nov 2021, J. Cladera via J.C. Salom, JCS-269A, GenBank ITS [OR778596](https://www.ncbi.nlm.nih.gov/nuclot/OR778596). **The Maltese Islands (Fig. 2):** Gozo, Xewkija, G’ nien Blankas, under *Olea europaea* on calcareous, loamy, and slightly clayey soil, 80 m asl, 18 Dec 2016, S. Mifsud, SM146, GenBank ITS [OR778593](https://www.ncbi.nlm.nih.gov/nuclot/OR778593); ibid, Munxar, Wied tal-Munxar/Wied tax-Xlendi valley system, valley bed composed of calcareous, clayey and rocky ground, 40 m asl, 1 Dec 2022, SM310, GenBank ITS [OR778597](https://www.ncbi.nlm.nih.gov/nuclot/OR778597); ibid, Kerc’em Wied il-Lunzjata, cultivated area on a loamy and slightly clayey, calcareous soil, 60 m asl, 4 Dec 2021, SM673, GenBank ITS [ON179774](https://www.ncbi.nlm.nih.gov/nuclot/ON179774); ibid, Nadur, Wied Bing’emma/Wied San Blas valley system, under *Olea europaea* on a loamy and slightly clayey, calcareous soil, 100 m asl, 12 Dec 2021, SM679, GenBank ITS [ON179780](https://www.ncbi.nlm.nih.gov/nuclot/ON179780).

3. Results

Nine collections of *M. galilaea* were sequenced from the Mediterranean islands of Gozo (Malta, n = 4), Ilovik (Croatia, n = 1), Sicily (Italy,



Figure 1. *Morchella galilaea*: a. Italy, Brindisi, Bosco dei Lucci, LIP0400228; b. Spain, Mallorca, Llubí, JCS-269A. Photos: a, C. Agnello; b, J.C. Salom.



Figure 2. *Morchella galilaea*: a. Malta, Gozo, SM078; b. Malta, Gozo, SM310; c. Malta, Gozo, SM673; Malta, Gozo, d. SM679. Photos: S. Mifsud.

$n = 1$) and Balears (Spain, $n = 1$), as well as from continental Italy ($n = 1$) and the Reunion island in the Indian Ocean (France, $n = 1$). To further investigate the widespread distribution and ecology of *M. galilaea*, we assembled and phylogenetically analysed an ITS dataset including all available sequences of this species from GenBank, together with the nine novel ones reported here (Fig. 3).

The 45 *M. galilaea* sequences constitute a strongly supported monophyletic lineage (0.99 support) within the/Esculenta clade of “yellow morels”, now taxonomically treated as sect. *Morchella* (Richard et al. 2015). A remarkable feature of this phylogeny is its “bushy” topology, unusual in *Morchella* and reflecting considerable genetic polymorphism of this species at the ITS locus. The underlying multiple sequence alignment identifies no less than 37 variable sites along the 1094-nt long consensus sequence, 16 of which are parsimony-informative. Twenty-one single-nucleotide polymorphisms are unique to a single sequence (singletons or private polymorphisms).

The corresponding populations originate from all continents, with the notable exception of continental Americas, and striking positive bias (15/45, 33%) towards islands (Fig. 4). Based on currently available data from sequenced material, *M. galilaea* fruits mostly in autumn, i.e. between September and December in the northern hemisphere (blue dots)

and between March and July in the southern hemisphere (green dots). However, vernal fruiting of the species has also been reported from Israel and Yunnan, China (Du et al. 2012).

To gain further insight into the biogeography of this species, we built a haplotypic network of the available sequences (Fig. 5). The resulting pattern delineates 22 distinct ITS haplotypes, 18 of which are represented by a unique sequence, one by two sequences, one by three sequences, one by four sequences and a major one by 14 sequences. Topologically, the network consists of 26 nodes representing current (black nodes) and inferred (white nodes) haplotypes, and 29 edges relying them by a minimal number of nucleotide changes. Most nodes are of low degree ($d = 1$ or 2), reflecting the numerous aforementioned private polymorphisms and possibly supporting an equal number of independent evolutionary routes. Two nodes of higher degree ($d = 8$ and 9 , red nodes) constitute hubs that organize the network in two modules, in which they may represent supported parent haplotypes at the origin of all other included sequences. These two haplotypes are linked by a single nucleotide change (red edge), but also by three additional, indirect paths (green edges), reflecting porosity between the two modules, i. e. extended or repeated gene flow between the two parent haplotypes. The first parent haplotype is currently present in the sub-Himalayan

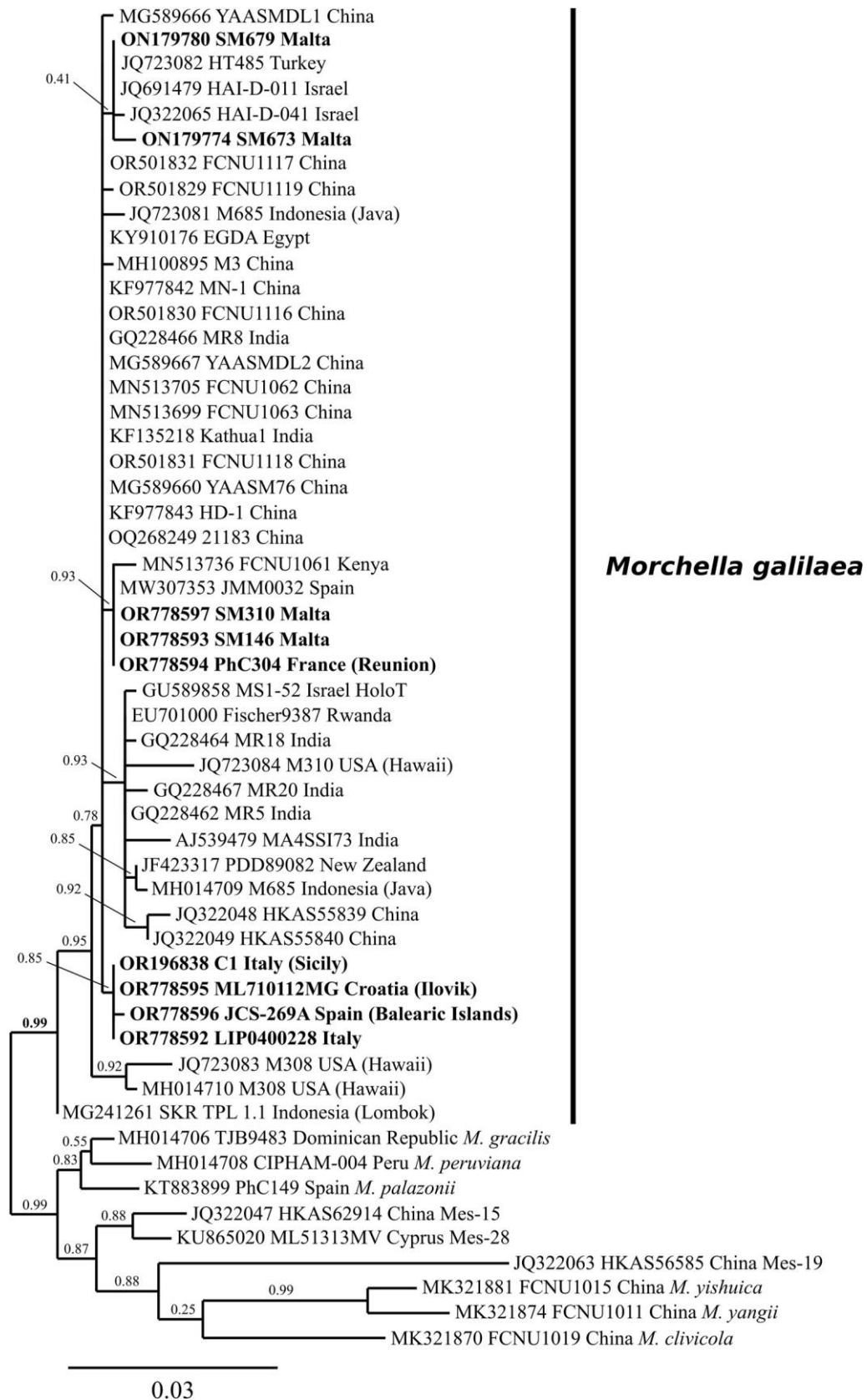


Figure 3. ITS Maximum Likelihood phylogeny of *Morchella* sect. *Morchella* pro parte. Sequences newly generated for the present study are highlighted in Bold.

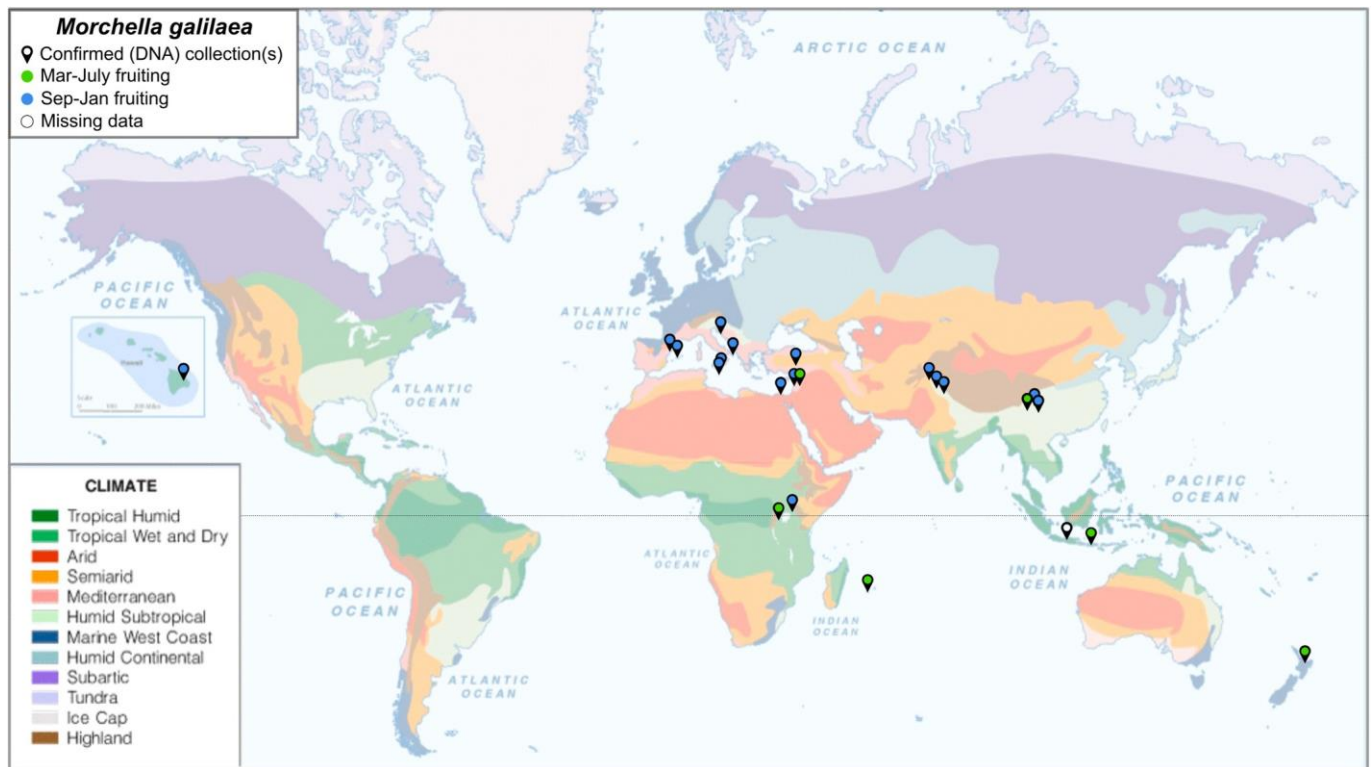


Figure 4. Updated global biogeography and phenology of *M. galilaea*.

region of north-western India and in Rwanda. The second one, which is the major haplotype, is present in the same region of India, in the Sino-Himalayan region of China, in the Reunion island, in Egypt, as well as in Malta and Spain. Seven out of twelve (58%) known sequences from China display the latter haplotype and three are predicted to have derived directly from it by one to two nucleotide changes.

4. Discussion

4.1. Collections of *Morchella galilaea* in Malta

The present study updates our knowledge on the diversity of Maltese morels and confirms four populations of *M. galilaea* on Gozo Island. All collections were found fruiting in autumn (between the end of November and the beginning of December) in damp and shaded places sheltered under trees, receiving partial sunlight. They were present in areas with high soil humidity, while SM310, SM673 and SM679 were present in the vicinity of a small watercourse. Fruiting occurred a few days after heavy rain, when the atmospheric moisture was on average > 80% and the temperature range between 15 °C (night) and 21 °C (daylight). In a morphological point of view, ascospores from Gozo were not markedly different from those from Mallorca or South Italy (Fig. 1 and 2).

Notably, all populations of *M. galilaea* in Gozo were found under *Olea europaea*. In the case of SM310 and SM679, these trees are archaeophytic, autochthonous and of old age (estimated to be more than 400 years old from the huge trunks for the latter locality). The orchard was afforested in the 1960s in the station of SM146 and, in the case of SM673, presumably recently cultivated. The occurrence of this morel with ancient olive trees supports a long-established and likely indigenous status of *M. galilaea* in the Maltese Islands. The possibility that mycelium was introduced from recently imported trees (following e.g. Tas,kin et al. 2010, 2012; O'Donnell et al. 2011) and then spread into natural sites could be considered, but seems unlikely given that ascospores have mostly been collected under olive trees of old age and no

collections of this species are known to us in the vicinity of alien or introduced trees. Therefore, it appears that this morel is fruiting naturally in Mediterranean maquis, whereas in Gozo, it is predominantly found in old copses of olive trees and was possibly able to spread to nearby afforested sites located in humid areas such as rocky valleys.

4.2. *Morchella galilaea*'s trophism – A mixed lifestyle?

The trophic status of morels has been hotly debated. Although early authors (e.g., Robbins and Hervey 1959; Dennis 1978) had speculated that morels are strictly saprotrophic, more complex associations between some species of *Morchella* and vascular plants have been proposed and in some cases been histocytologically and molecularly verified (Buscot and Kottke 1990; Buscot 1994; Dahlstrom et al. 2000; Stark et al. 2009; Baynes et al. 2012). Still, the precise mechanisms of such associations remain poorly understood, and it is possible that trophic niches among different species of *Morchella* vary. Buscot (1992) postulated a secondary mycorrhizal association established through mycorrhizae formed by other fungi, while Dahlstrom et al. (2000) argued for a complex symbiosis, having observed features of both ectomycorrhizal and ectendomycorrhizal colonization between morels and Pinaceae *in vitro*. In addition, Baynes et al. (2012) molecularly confirmed an endophytic association between *M. sextelata*, a post-fire species, and *M. snyderi*, with cheatgrass (*Bromus tectorum*). Many authors nowadays view the majority of morel species as facultative biotrophs, perhaps alternating between an endophytic and a saprotrophic lifestyle during different stages of their life cycle (Tedersoo et al. 2010; Kuo et al. 2012; Loizides et al. 2015, 2016, 2021; Smith et al. 2017; Baroni et al. 2018).

Our newly sequenced collection of *M. galilaea* from the Reunion island in the Indian Ocean was fruiting in basaltic erosion sludges, within or near Filaos (*Casuarina equisetifolia* L.) litter, but without the presence of any tree, supporting at least a facultatively saprobic lifestyle of this species under some circumstances. In contrast, all collections from Gozo and that from the Balearic Islands were found in close vicinity to *Olea europaea*, suggesting a possible trophic interaction of *M. galilaea* with the

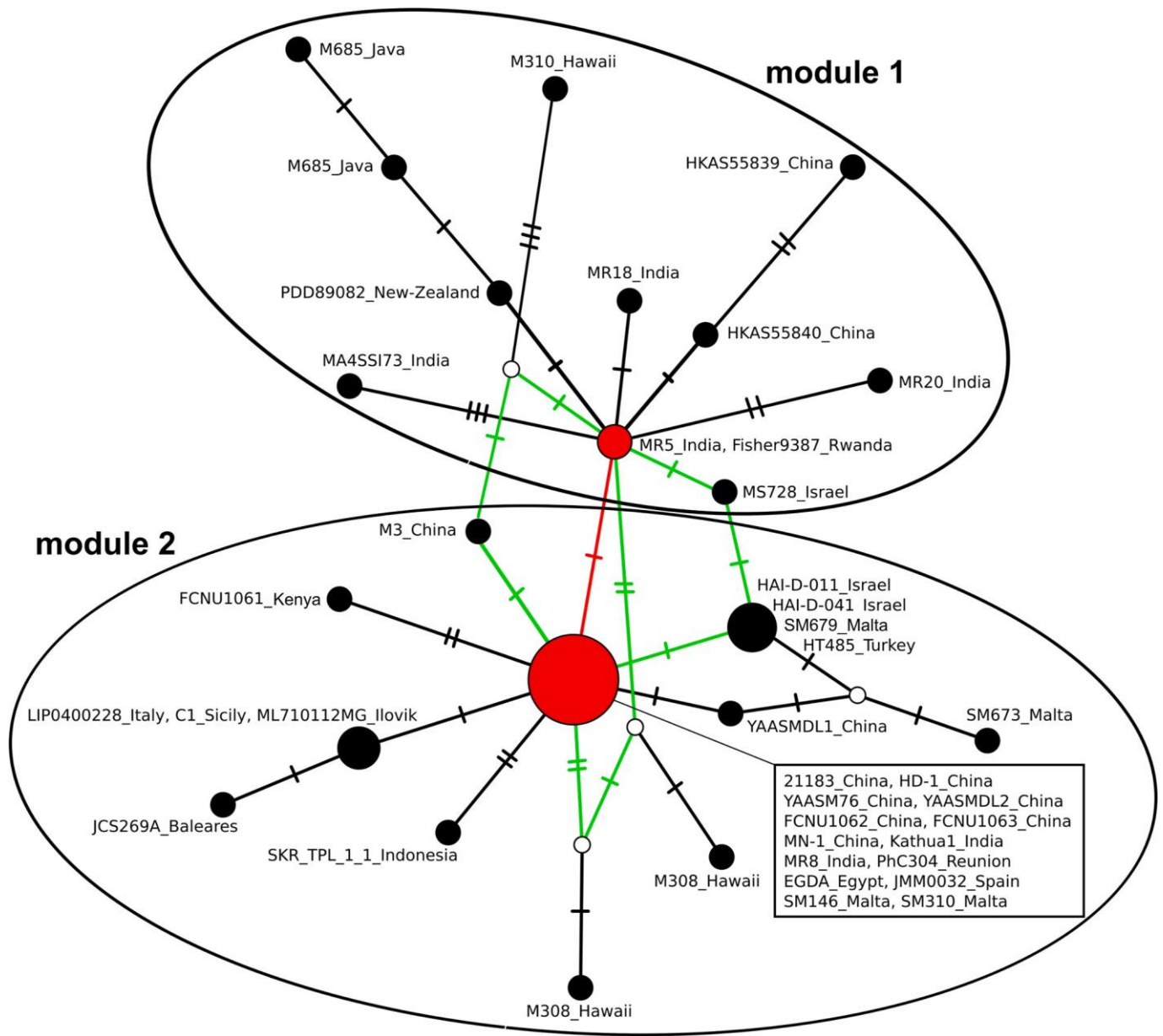


Figure 5. ITS haplotype network of *Morchella galilaea*.

olive tree. The former is essentially an endomycorrhizal host, therefore such an association could be facultatively endophytic (following Tedersoo et al. 2010; Baynes et al. 2012) or even parasitic, although intracellular colonization (as observed by Dahlstrom et al. 2000) cannot be excluded. In our field visits in Malta, specimen SM146 was dug out and found directly attached to a dead lignified root of *Olea europaea* (Fig. 6), with only herbaceous weeds (*Oxalis pes-caprae* L., *Lobularia maritima* (L.) Desv. *Asparagus aphyllus* L.) present nearby. In this ecoregion, *M. galilaea* has also been reported under *Fraxinus syriaca*, *Pinus halepensis* and various *Quercus* species (e.g. Marcos et al. 2021), which, taken together with its sporadic sightings in treeless localities, strongly suggests a dual biotrophic/saprobic lifestyle.

The link between morels and plants of the *Oleaceae* family is not new and was first reported by Robert (1865), after morel mycelia were seen to be attached to the roots of olive trees. The currently inferred evolutionary history of the genus further suggests that interactions of morels with this tree family are ancient, as the two species belonging to the ancestral/Rufobrunnea clade - *M. anatolica* Is, Ilog'lu, Spooner, Allı & Solak and *M. rufobrunnea* Guzman' n & F. Tapia, are both commonly found

with *O. europaea* in the Mediterranean region (Loizides et al. 2015, 2016, 2021), and sometimes with *Fraxinus angustifolia* (Palazo'n Pema'n et al. 2017). Another basal species in sect. *Distantes*, *Morchella tridentina*, is also frequently found with olive trees (Loizides et al. 2015; Loizides 2017). In North America, several morel species are found in the vicinity of *Fraxinus americana* (Kuo et al. 2012), while a number of European morels are reported with *F. excelsior* (Richard et al. 2015; Petr'zelov'a and Sochor 2019). All these suggest an old and worldwide association of morels with *Oleaceae* and by extension an indigenous presence of *M. galilaea* in the Mediterranean rather than a recent anthropogenic introduction associated with horticulture, silviculture and alien flora.

4.3. A paleotropical climatic relict?

The updated distribution of *M. galilaea* presented here based on phylogenetically confirmed collections, includes the sub-Himalayan region of India and Pakistan, the Sino-Himalayan China, Indonesia, East-Central Africa, the Mediterranean Basin, and the remote islands of Hawaii, Reunion and New Zealand (Fig. 2). This expansion range

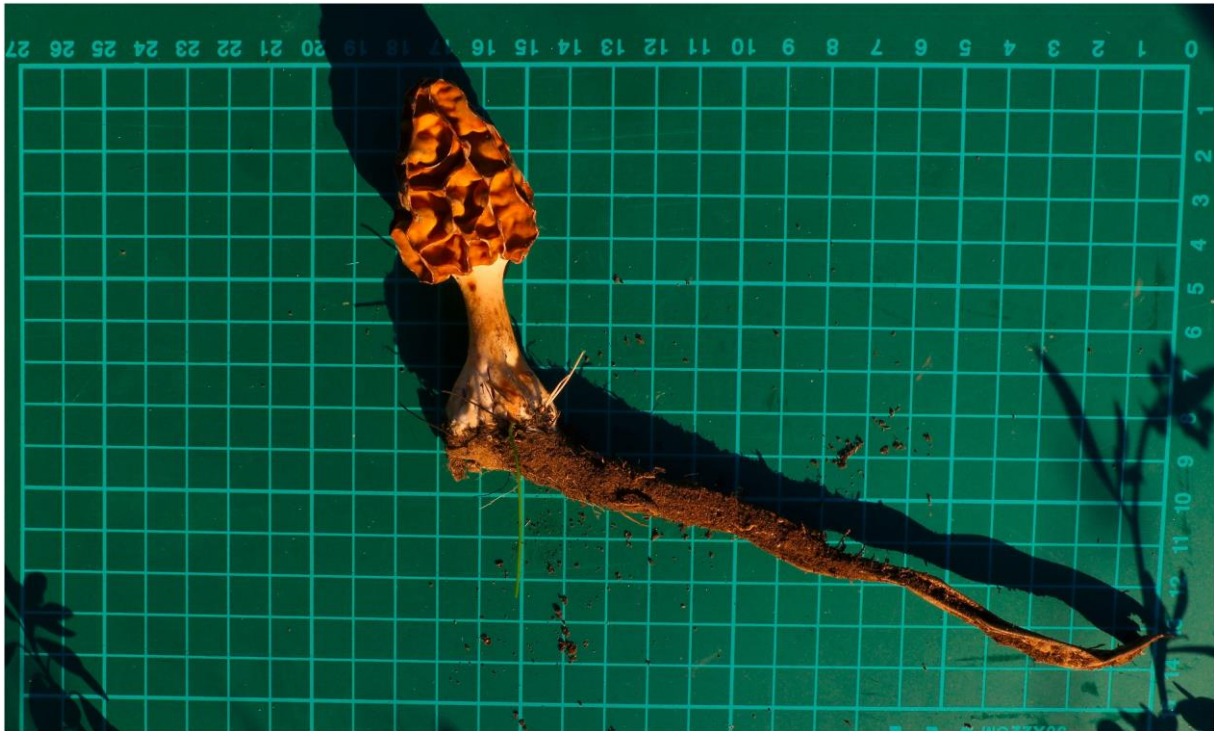


Figure 6. Specimen of *Morchella galilaea* attached to a dead root, presumably of *Olea europaea* excavated *in situ*.

combines a wide paleotropical and Mediterranean distribution and, conversely, excludes regions that have histories of glaciation. From a climatic perspective, these two areas display some similarities, with the Mediterranean climate being also known as the “dry summer subtropical climate”. A common feature of all these areas is their mild and wet winters without frost, and warm to hot summers.

The unusually broad sequence polymorphism of *M. galilaea* at the ITS locus, including high ratio of private polymorphisms (21 out of 37, i.e. 57%), supports several independent and relatively ancient, isolated populations that had time to imprint their own phylogenetic history into DNA, as neutral mutations. Recently introduced populations are expected to share most of their SNPs and indels with the populations they originate from, which is not what the multiple sequence alignment of *M. galilaea* shows. In this respect, the case of Maltese populations is interesting, since the ITS sequences of the four populations from Gozo differ from each other at six positions, with two polymorphisms not represented outside Maltese sequences within the clade (Fig. 1 and data not shown).

Although it is difficult to infer strong evolutionary scenarios from haplotype networks built from a single genetic locus, some geographic signal emerges as, e.g. the supported common origins of populations from, respectively, Java + New Zealand; Israel + Turkey and the Balears + Croatia + Italy. Conversely, our network does not support a single origin for the four Maltese populations nor for the three Hawaiian haplotypes, suggesting several independent introductions into these two islands.

Although broad and disjunct distributions have in the past been attributed to recent anthropogenic introductions (O’Donnell et al. 2011; Du et al. 2012, 2015; Tas, kin et al., 2010; 2012; Richard et al. 2015), this hypothesis has been contested for the majority of morel species (Loizides et al. 2015, 2016, 2021). Taken together, our phylogenetic findings support an ancient origin for most populations of *M. galilaea* across the species’ range. They do not exclude recent, secondary introductions through human activities, but such anthropogenic propagation dating back, in the best case, to a few centuries ago, cannot explain the observed phylogenetic structure of the species.

Moreover, as stated above for Gozo and other Mediterranean localities, the close affinity of *M. galilaea* to olive trees, themselves paleo-endemic in this region, suggests an ancient presence of *M. galilaea* and other Oleaceae-associated morels in the Mediterranean basin. The absence of *M. galilaea* from the New World and regions of the Northern Hemisphere that remained glaciated during the Pleistocene is indeed more consistent with the hypothesis of climatic refugia (Loizides et al. 2021) rather than that of recent anthropogenic introductions. This hypothesis, already proposed to explain the disjunct or restricted distribution of other archaic morels (*M. anatolica*, *M. rufobrunnea* and *M. tridentina*), can better account for the widespread yet disjunct distribution of *M. galilaea* unravelled here, especially since its extant populations appear to be concentrated in refugia hotspots of biodiversity that remained unglaciated during the last Ice Age (see Fig. 4 and e.g. Harrison & Noss, 2017).

The broad and disjunct distribution of *M. galilaea* combined with its tropism for islands, are indicative of high colonization skills for this species. This trend undoubtedly benefits from the unusually high number of heterokaryotic ascospores produced by *M. galilaea*, which increases the genetic diversity and survival of propagules, according to the so-called heterokaryotic advantage (Du et al. 2023). In addition, the species is unique within the genus by producing numerous conidia directly from ascospores, protecting the latter from adverse environmental conditions or further enhancing the dissemination of diversified genotypes (Du et al. 2023). This dual strategy combining sex-like mechanisms promoting genetic polymorphism (high number of heterokaryotic ascospores, heterothallism), with asexual mechanisms promoting amplification and higher dissemination of these genotypes, is somehow reminiscent of what Wang et al. (2023) have recently uncovered in invasive Californian populations of *Amanita phalloides*, homokaryotic individuals intermixed and able to mate with regular heterokaryotic individuals. This species is then both unisexual and bisexual, and combines these two modes of reproduction to maintain high levels of genetic diversity, while benefitting from mating-free sporulation for increased spreading of the species.

4.4. Could *Morchella galilaea* be a phenologically “reset” species rather than strictly autumnal?

The most noteworthy – and largely diagnostic – characteristic of *M. galilaea*, is its unusual fruiting in autumn and sometimes in early winter (mostly between October and December in the Northern Hemisphere), contrary to all other morels which typically fruit between late winter and late spring. Only *M. rufobrunnea* is reported to occasionally fruit in autumn in some of its stations (Masaphy et al. 2009). For example, a collection from Hal-Far area in Z'urrieq, Malta, confirmed by one of us (GenBank ITS [OR573494](#), collection SM682) was found in mid-December 2021, almost concurrent with the fruiting of *M. galilaea*. However, *M. rufobrunnea* rather displays persisting fruiting periods spanning late fall to early spring rather than a predominantly autumnal phenology as *M. galilaea* typically displays (Masaphy et al. 2009; Loizides et al. 2015). One such “long-season” ecotype of *M. rufobrunnea* has been identified and studied in the Dan Nature Reserve of northern Israel, not far from the type locality of *M. galilaea* (Masaphy et al. 2010). Prior to that, a similar case had been reported from the same Reserve, and referred to as the “Dan ecotype”. Although the identity of the species in question, originally misidentified as “*M. conica*” by Goldway et al. (2000), remains uncertain (a sequence has been generated but not published), it is possible that this collection represents *M. galilaea*. Consistently, Du et al. (2012) published three sequenced vernal collections of *M. galilaea*, one of which originated from Israel (HAI-D-041/[JO322065](#)), the two others from Yunnan, China. It should be noted here that autumnal fruiting of a morel is so unusual that it is unlikely to be ignored and will often be reported or subjected to DNA sequencing. Conversely, vernal (including bimodal) occurrences of *M. galilaea* may easily be overlooked because of the simultaneous fruiting of other yellow morel species with which it can be morphologically confused. Therefore, *M. galilaea* may not be viewed as a strict autumnal or “reversed” species, but rather as a phenologically plastic one that can potentially fruit in both seasons if the right conditions occur.

What precedes raises two questions that need to be addressed: 1) by which mechanism did *M. galilaea* break loose from the strict vernal fruiting programming that governs the entire genus (and indeed many Ascomycota lineages); and 2) why does the species preferentially fruit in autumn once seasonality constraints have been released? Little is known about the origins and long-term stability of seasonality in mushroom fruiting. Considering how strict and conserved this process is in most Dikarya lineages, however, it seems reasonable to infer that sensor genes are involved and are under positive selection. Fungi are able to detect the ratio of different wavelengths of light to adapt their reproduction, morphogenesis, virulence and metabolism, similar to phototropin-mediated signalling in plants, and also possess circadian clocks (Naranjo-Ortiz and Gabaldón 2020). The combination of wavelength-ratio sensing and circadian clocks constitutes the molecular bases of seasonality in plants physiology (Johansson and Staiger 2015). Thus, seasonal cycles are likely in Fungi, mediated by a set of candidate genes that should ensure, e.g., vernal fruiting of *Morchella* and other Ascomycota lineages on one hand, and autumnal fruiting of most Basidiomycota lineages on the other. This underlying complex mechanism bears a cost from an evolutionary perspective, so if involved genes have no other function than the control of seasonality, they are likely to be lost or inactivated upon release of selective pressure in lineages occurring near the equator, where no spring and autumn seasons can be defined. In this respect, the tropics may thus act as an “eraser” of genetic seasonality programs. Depending on various factors (nature and number of involved genes, duration of the tropical “exposure”), such loss may be irreversible, as is in the case of ballistosporium in gasteroid Basidiomycota (Sánchez-García et al., 2020), or just express a relaxation of the genetic control of seasonality.

The current distribution of *M. galilaea* on the worldwide scale strongly suggests a paleotropical origin of this lineage. When radiating

away from the tropics into higher latitudes, the ancestral lineage may have yielded phenologically “reset” species that adapted according to their own ecological skills and local constraints, but free from the strict vernal fruiting habit apomorphic for the genus. In most cases, autumn may dominate because of e.g., more favourable temperatures and humidity patterns, or perhaps reduced competition with other *Morchella* species, but vernal fruiting would, if this hypothesis is correct, still be possible locally. Such evolutionary scenario is admittedly speculative, but offers testable predictions. One is that other species sharing tropical ancestry with *M. galilaea* may display a similar relaxed seasonality in their fruiting pattern. The latest lineage encompassing *M. galilaea* includes also *M. clivicola*, *M. gracilis*, *M. palazonii*, *M. peruviana*, *M. yangii*, *M. yishuica*, *Mes-13*, *Mes-15*, *Mes-19* and potentially also *Mes-28* (Loizides et al. 2021, 2022). Some of these species are restricted to the equator or lack associated phenological data to be investigated but strikingly, the holotype of *M. gracilis* was found fruiting in the centre of the Dominican Republic (19° of latitude N) in early January, which is winter there (Baroni et al. 2018). From available sequence data from Spain and China, *M. palazonii* is a vernal species, but one harvest from Pakistan fruited in mid-August (HUP-11550/[OQ566762](#), Ishaq et al., unpublished). The yet-to-be-named *Mes-15* lineage, so far mostly reported from China, also displays a vernal phenology. However, a collection of this species (erroneously identified as “*M. crassipes*”), has recently been confirmed in Pakistan, where it was fruiting in early February (Morch 01/[KY402197](#), Azhar et al., unpublished).

Although more phenological data on *M. galilaea*'s closest species is needed to further support our model, these preliminary findings are intriguing and suggest that the default program for a strict vernal fruiting of morels may have been lost or relaxed in at least three lineages sharing the same late ancestry with *M. galilaea*. Future studies addressing more broadly the genetic control of seasonality traits in other organisms, may also indirectly address the role of the tropics in such a process.

CRedit authorship contribution statement

Stephen Mifsud: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Data curation, Conceptualization, Project administration, Resources, Validation. **Carlo Agnello:** Data curation, Investigation, Writing – original draft, Writing – review & editing, Resources. **Mirko Calanni Rindina:** Data curation, Investigation, Writing – original draft, Writing – review & editing, Resources. **Joan Carles Salom:** Data curation, Investigation, Writing – original draft, Writing – review & editing, Resources. **Michael Loizides:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing, Validation. **Franck Richard:** Conceptualization, Data curation, Supervision, Validation, Writing – original draft, Writing – review & editing, Visualization. **Jean-Michel Bellanger:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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