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Research article

Morphological observation and biomass formation in different edible medicinal *Morchella* collections (Pezizomycetes, Ascomycota)

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Abstract
The ascomycetes in the genus *Morchella* (commonly called morels) have a considerable economic and biotechnological value for their culinary and medicinal proprieties. However, their biotechnological interest is not limited to the fruiting body cultivation, but also in mycelial production to obtain bioactive compounds and other biotech products. In order to better exploit the biotechnological potentialities of morels it is necessary to improve the knowledge on their biology and mycelial characteristics. In this paper morphological and growth characteristics of mycelia, as well as biomass formation of Italian collections of five edible medicinal *Morchella* species [*M. esculenta* (L.) Pers., *M. dunalii* Boud., *M. importuna* M. Kuo, O'Donnell & T.J. Volk, *M. disparilis* Loizides & P.-A. Moreau and *M. purpurascens* (Krombh. ex Boud.) Jacquet.] are presented.

Keywords
Morels, mycelia, morphology, biomass, *Morchella esculenta*, *Morchella dunalii*, *Morchella importuna*, *Morchella disparilis*, *Morchella purpurascens*

Introduction

Morchella species, the true morels (Morchellaceae, Pezizales and Pezizomycetes*)* belong of the phylum Ascomycota (Hibbett et al., 2007). They are mostly distributed in the northern hemisphere and develop ascomata for only a few weeks in early spring. Taxonomy, phylogeny and biogeography, ecological diversity and distribution, genomics and cultivation of *Morchella* spp. have been reviewed (Barseghyan et al., 2012; Du et al., 2015). Based on ascoma morphology, these macrofungi were placed into three groups: black, yellow and semi-free capped morels. However, according to recent molecular studies at least 80 species were recognized, mostly from China (Du et al., 2019; Loizides et al., 2021). Most of them are saprobic but some species seems to establish complex interactions with the roots of the plants which are far to be completely clarify (Ori et al., 2019). Morels have considerable economic and biotechnological values for their culinary and medicinal proprieties (Badalyan, 2012; Tietel and Masaphy, 2018; Badalyan and Zambonelli, 2019).

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Wild edible morels are also harvested and commercially exported from China, India, Turkey, Mexico, and the United States to European countries, such as France, Belgium and Switzerland, as fresh, frozen or dried organic food products (Pekşen and Akdeniz, 2012). The annual export of dried morels in China increased fivefold from 181,000 kg to 900,000 kg over the past five years, with average price 160 USD kg^{-1}.

True morels with their nutritional value, meaty texture, unique aroma and delicate flavor (bitter, umami, sour, sweet, salty and mouth-drying) are among the highly prized edible mushrooms (Beluhan and Ranogajec, 2011; Heleno et al., 2013; Vieira et al., 2016; Badalyan and Zambonelli, 2019). Several volatile and aromatic compounds, such as 1-octen-3-ol, 1-octadecanol, cyclooctylalcohol, silanediol, 2-methylaminoethanol, aldehydes, terpenoids, esters, phenolics, ketones and others have been detected in *Morchella* spp. (Taşkın, 2013; Tietel and Masaphy, 2018; Sunil and Xu, 2022).

Due to delicious flavour and short fruiting season, the worldwide demand for morels has stimulated their cultivation industry. Saprobic *Morchella* species (*Morchella sextelata* M. Kuo, *M. eximia* Boud., *M. exuberans* Clowez, Hugh Sm. & S. Sm., *M. importuna* M. Kuo, O'Donnell & T.J. Volk, *M. oweri* X.H. Du, *M. rufobrunnea* Guzmán & F. Tapia, *M. tomentosa* M. Kuo) are widely cultivated in China (Peng et al., 2015; He et al., 2019; Zhao et al., 2021; Xu et al., 2022). Total world production of the true morel *Morchella esculenta* (L.) Pers. is 150 tons dry weight which is about 1.5 million tons fresh weight with average price from 200 to 750 USD kg^{-1}.

Morels have also been appreciated by humans for their medicinal values (Hobbs, 1995; Badalyan, 2012; Ajmal et al., 2015; Badalyan and Zambonelli, 2019; Sunil and Xu, 2022). They have been used in traditional medicine in China, India, and Pakistan to treat excessive sputum, gastrointestinal disorders, and other pathological conditions (Paul et al., 2018). Recent studies suggested that morels possess antimicrobial, anti-inflammatory and antioxidant (Nitha et al., 2007; Jander-Shagug and Masaphy, 2010; Fu et al., 2013; Dissanayake et al., 2021), hepatoprotective (Nitha et al., 2013), antitumor and immunomodulatory (Liu et al., 2018), antiviral, anti-leishmanial (Peretz et al., 2018), neuroprotective and neuritogenic (Xiong et al., 2016; 2017), purgative, emollient, tonic, wound-healing (Sunil and Xu, 2022) and other medicinal properties.

The health-promoting effects and bioactivity of morel mushrooms have been mainly attributed to a wide range of bioactive molecules (polysaccharides, tocopherols, ascorbic acid and vitamin D, minerals, carotenoids, flavonoids, triglycerides, free fatty acids, sterols, organic and phenolic acids) present in their wild and cultivated ascomata, as well as mycelia (Heleno et al., 2013; Tietel and Masaphy, 2017; Dissanayake et al., 2021).

Morels, used as nutraceuticals and nutriceuticals (functional food) are mostly gathered from the wild due to difficulties to cultivate them especially outside of China. The increasing demands of morels worldwide the further progress in fundamental fungal biology research and innovative cultivation technologies are required (Arkan and Güler, 1992; Güler and Arkan, 2000). Cultivation of fermented mycelium will expand production of *Morchella* as functional food, food flavoring and other biotech products (Bulam et al., 2018). However, the high level of morphological variability of mycelial characteristics of morels during different developmental stages of the life cycle, including asexual reproduction modes, creates several problems for large scale biotechnological exploitation of these mushrooms (Yuan et al., 2021). Further studies of morphological characteristics and growth rate of vegetative mycelia, ecology and genomics of morels need to be addressed to protect their natural resources.

In this paper, morphological and growth characteristics of mycelia, as well as biomass formation of Italian collections of five edible medicinal *Morchella* species [*M. esculenta*, *M. dunalii* Boud., *M. importuna*, *M. disparilis* Loizides & P.-A. Moreau, *M. purpurascens* (Krombh. ex Boud.) Jacquet.] are presented.

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Material and methods

Fungal cultures

Ten strains of *M. esculenta, M. dunalii, M. importuna, M. disparilis,* and *M. purpurascens* with different origin isolated in central-north Italy have been studies (Table 1). They were isolated on potato dextrose agar 25 g L^{-1} (PDA, Difco) by a portion of the sterile tissue of the stalk or by ascospores. Ascospores were obtained by pipetting 100 µL of sterile water with 100 ppm of streptomycin within alveola containing mature asci. After one or two ten-fold dilutions, 100 µL of ascospore suspension was spread into Petri dishes containing PDA added with 100 ppm of streptomycin. After 12-18 h, the germinating ascospores were detected under a stereomicroscope and transferred into new PDA Petri dishes by a sterile needle.

The identity of each isolated strain was confirmed by amplifying and sequencing their ITS nrDNA region using the primer pair ITS1f-ITS4 (Gardes and Bruns, 1993) following the protocol of Bonuso et al. (2006). Purification and sequencing reactions were performed by Microsynth seqlab (Balgach, Switzerland) using both forward (ITS5) and reverse (ITS5i) primers. The obtained sequences were deposited in Genebank under the accession numbers (Table 1).

Table 1 - Metadata of Italian collections of *Morchella* spp. used in this study

Species	Strain	Origin	Region	Locality	Accession number
M. disparilis	MdiSU1-1	Spore	Sardinia	Domusnovas	OQ116872
M. dunalii	MduNU2-4	Spore	Sardinia	Porto Ferro	OQ116874
	MduGR3-4	Spore	Tuscany	Castiglione della Pescaia	OQ116873
M. esculenta	MesAQ2-C	Spore	Abruzzi	L'Aquila	OQ116875
	MesAQ6–2	Context	Abruzzi	Castel del Monte	OQ116876
	MesFI2-3	Spore	Tuscany	Palazzolo sul Senio	OQ116877
M. importuna	MimBL1-1	Spore	Veneto	Auronzo di cadore	OQ116878
	MimMO1-1	Spore	Emilia-Romagna	Vignola	OQ116879
M. purpurascens	MpuAQ1	Context	Abruzzi	Castel del Monte	OQ116880
	MpuTN2	Spore	Trentino Alto Adige	Madonna di Campiglio	OQ116881

After molecular identification, the isolated strains were morphologically screen on PDA and liquid malt-extract (ME: 200 mL 7° malt-extract obtained from the beer factory, 800 mL distilled water, pH 6.0) media.

Macromorphological observation

Mycelial inocula (5 mm^3) of each strain were transferred to the centre of PDA plates (Ø 90 mm, three plates per isolate) and incubated at 23 °C in the dark, except daily checks. Morphological characteristics of colonies (form, texture, mycelial density, pigmentation of aerial mycelia and agar, etc.) were described according to Stalpers's scales (Stalpers, 1978). After 10 days of incubation in the dark, observation of cultures, including sclerotia development was continued under a day/night regime during 21 days.

Growth rate

The daily growth rate (GR, mm d^{-1}) of mycelia on PDA was measured during 3-4 days until it completely covered the agar plates. It was calculated by the following formula: $GR = R_1\text{-}R_0/T$, where

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R is radius of colony, *T* is the incubation time. Average growth rates (GR_{avr}) were calculated from daily GR values of each measurement. Each strain was repeated three times with inocula from the same mother cultures. Data from six measurements from each strain were expressed as mean ± standard deviation (SD).

Micromorphological observation

For micromorphological studies, including development of asexual spores and vegetative sclerotia, the strains were grown on sterile microscope cover slips (2 × 2 cm, 2 - 3 for each plate) placed onto PDA plates next to growing mycelia (Badalyan et al., 2011). The plates were incubated at 23 °C and from the 3rd day when mycelia were starting to overgrow the cover slips they were removed for observation under a microscope (Omano OM157T, USA) equipped with digital camera (× 0.5, OptixCam, Summit Series, 10.0MP, USA) connected to a computer software program OptixCam OCView v. 7.1. Micromorphological studies were carried out without staining. Two magnifications, × 300 and × 1,200, have been applied. The dishes and preparations made from the growing mycelia were observed during 3-4 weeks.

The Erlenmeyer flasks with 100 mL ME medium were inoculated by 10 plugs (5 mm^3 each) and incubated at 23 °C under static conditions during 10 and 21 days. The micro- and macromorphological growth characteristics of mycelia have been described (Supplementary Fig. S1).

Biomass formation

After 10 and 21 days of growth in static culture, the biomass samples were separated from cultural liquid by filter paper (FILTRAK, FN 18), washed three times by distilled water, dried at 50–60 °C and weighted. The weight of obtained dry biomass samples were recalculated in g L^{-1}.

The taxonomic significance of observed mycelial characteristics of studied collections, such as morphological, GR value, development of sclerotia and other asexual structures, as well as formation of biomass were evaluated. The cultures were incorporated into Culture Collection of the Laboratory of Fungal Biology and Biotechnology, Yerevan State University, as well as the culture collection of the L'Aquila University.

Results and Discussion

Morphological observation

Mycelial colonies of studied *Morchella* strains on PDA were at first pale-creamy, later beige, yellow-rusty or cinnamon brown. The pigmentation was starting from the centre of colonies and tended to increase during the cultivation times. Aerial mycelia were well-developed, flappy-cottony, later woolly-felt. The density of colonies increased during the growth, advancing from the surface of the agar towards the plate cover (Supplementary Fig. S2). Agar was light or dark brown, rusty-brown to cinnamon-brown (Table 2).

After 5–7 days of growth, development of spherical, rusty-brown or beige sclerotia from highly proliferating intertwined hyphae of thickened and pigmented outer layer of colonies have been described in all species/strains, except *M. purpurascens* MpuAQ1 (Supplementary Fig. S2).

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Table 2 - Morphological observation of mycelia of *Morchella* collections on PDA during 21 days of growth at 23 °C.

Species	Strain	Macro- and micromorphological characteristics
M. disparilis	MdiSU1-1	Colony initially creamy, light brown in the centre, cobweb-woolly, with even edge, later rusty-brown, cinnamon-brown, woolly-felt with well-developed fluffy aerial mycelium, agar cinnamon-brown, large sclerotia light to dark rusty-brown, brown over the entire surface of agar. Hyphae well-septate, brownish, granulate by large fatty inclusions anastomosed, vertically, later double and triple branched, arthroconidia are present.
M. dunalii	MduNU2-4	Colony creamy, light brown in the centre, cobweb, later felt, edge even, agar from light to dark brown. Rare, small, whitish-creamy, later large, orcha-rusty sclerotia along the edge of the colony on dense mycelial clusters. Hyphae septate, anastomosed, rusty-brown, granulate by large fatty inclusions, vertically later triple branched, clusters of budding cells, octahedral and cubic crystals and arthroconidia present.
	MduGR3–4	Colony whitish-creamy, later light brown in the centre, cobweb, felt, edge even, agar light brown, brown, rare sclerotia light rusty. Hyphae well-septate, anastomosed, hyaline, later brownish-cinnamon, granulate by large fatty inclusions, vertically, double and triple branched.
M. esculenta	MesAQ2-C	Colony whitish-creamy, cobweb-woolly, edge even, mycelium and agar rusty-brown, numerous rusty-brown sclerotia at the edges of colony. Hyphae well-septate, granulate by large fatty inclusions, vertically branched, with large drops of exudate; sclerotia composed of budding cells with typical out-growing spiny hyphae, arthroconidia present.
	MesAQ6-2	Colony whitish-creamy, light brown in the centre, rusty-brown, cobweb-woolly-felt, edge even, agar rusty-brown, small sclerotia rusty-brown at the edges of colony. Hyphae well-septate, brown, initially branched vertically, granulate by large fatty inclusions, anastomosed. Brown sclerotia formed of budding cells with typical out-growing spiny hyphae, arthroconidia present.
	MesFI2-3	Colony whitish-creamy, rusty-brown, brown, cobweb, woolly-felt, edge even, agar rusty-brown, brown; small rusty-brown sclerotia at the edge, later in the centre of colony. Hyphae well-septate, granulate by large fatty inclusions, initially branched vertically, sclerotia formed of budding cells with typical out-growing spiny hyphae.
M. importuna	MimBL1-1	Colony milky-creamy, beige, cobweb-woolly-felt, edge even, agar beige, light reddish-brown, beige-rusty to dark brown sclerotia along the edge of the colony. Hyphae well-septate, intensively granulate by fatty inclusions, vertically and dichotomously branched, with large drops of exudate, dark brown sclerotia composed of oval to round-shaped cells.
	MimMO1-1	Colony milky-creamy, beige, fluffy, later cobweb-woolly-felt, edge even, agar light beige, dark brown sclerotia over the entire surface of colony. Hyphae well-septate, initially vertically, later dichotomously branched, intensively granulate with large fatty inclusions, with drops of exudate, clusters of ochre-brown hyphae and sclerotia composed of oval to round-shaped budding cells.
M. purpurascens	MpuAQ1	Colony creamy, fluffy-felt, later brown, dark cinnamon-brown, woolly-felt, denser in the centre and light brown along even edges, agar brown, dark cinnamon-brown; sclerotia absent. Hyphae well-septate, hyaline, vertically branched, finely granulated, anastomosed; horn-like branched hyphae, octahedral and cuboid crystals, arthroconodia present, sclerotia not observed
	MpuTN2	Colony whitish, light brown in the centre, felt, woolly-felt, edge even, agar light brown, yellowish-rusty sclerotia over the entire surface. Hyphae well-septate, hyaline, vertically branched, anastomosed, finely granulated, horn-like hyphae, octahedral and cuboid crystals, yellowish-rusty sclerotia present.

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Table 3 - Morphological observation of *Morchella* collections growing on ME medium during 21 days at 23°C.

Species	Strain	Macro- and micromorphological characteristics
M. disparilis	MdiSU1-1	Well-developed substrate and poorly developed beige, later dark beige aerial mycelia with dark beige or dark brown sclerotia. Hyphae well-septate, brownish, granulate, vertically, double and triple branched, anastomosed.
M. dunalii	MduNU2-4	Well-developed substrate mycelium, dark beige aerial mycelium, numerous brown sclerotia in aerial and substrate mycelia. Hyphae well-septate, brown, granulate, brown sclerotia composed of budding oval-cylindrical cells.
	MduGR3–4	Well-developed substrate mycelium, small brown sclerotia in aerial and substrate mycelia. Hyphae well-septate, brown, granulate, sclerotia composed of budding cylindrical cells.
M. esculenta	MesAQ2-C	Well-developed substrate and poorly developed aerial mycelia, large dark beige, yellowish sclerotia in surface and substrate mycelia. Hyphae aseptate or well-septate, granulate, vertically and dichotomously branched.
	MesAQ6–2	Well-developed substrate and greyish-brownish aerial mycelia, dark beige sclerotia in surface, later substrate mycelia. Hyphae aseptate or well-septate, granulate, vertically and dichotomously branched.
	MesFI2-3	Well-developed substrate and poorly developed aerial mycelia, large yellowish, dark beige, brwon sclerotia in aerial and substrate mycelia. Hyphae well-septate or aseptate, granulate, vertically and dichotomously branched, rare octahedral crystals in cultural liquid.
M. importuna	MimBL1-1	Well-developed substrate and poorly developed aerial mycelia, brown sclerotia in aerial and substrate mycelia. Hyphae mainly aseptate, intensively granulate, sclerotia composed of oval-round shaped budding cells.
	MimMO1-1	Well-developed substrate and poorly developed aerial mycelia, brown sclerotia in surface and substrate mycelia. Hyphae well-septate, granulate, vertically and dichotomously branched with large drops of exudate, clusters of ochre-brown hyphae and sclerotia with chain of compact oval budding cells.
M. purpurascens	MpuAQ1	Poorly developed substrate mycelium, aerial mycelium fluffy, woolly-felt, greyish-beige-brown, sclerotia absent. Hyphae septate, hyaline, brownish, poorly branched.
	MpuTN2	Well-developed substrate mycelium, beige sclerotia in surface and substrate mycelia. Hyphae mostly aseptate, hyaline, brownish, brown sclerotia composed of round- and oval-shaped budding cells, horn-like hyphae present.

The sclerotia differed in size, abundance and colour. In *M. esculenta*, *M. disparilis* and *M. importuna* cultures, they were smaller, rusty-brown, beige-rusty and dark brown, respectively, while in *M. dunalii* larger in size and ochre-rusty (Supplementary Figs 3a–c). The sclerotia in *M. importuna*, *M. disparilis*, and *M. esculenta* were more abundant over the surface of the agar, while in *M. dunalii* they were developed only at the edge of the plates. Small, yellow-rusty sclerotia over the surface of colony were rarely observed in *M. purpurascens* MpuTN2 strain isolated from stalk of ascomata (Table 2 and Supplementary Fig. S2).

Budding (oval, elongated, rounded, and cylindrical) cells (Figs 1a–e) and spiny hyphae out-growing from the sclerotia (Fig. 1f) have been reported as characteristic feature in observed *Morchella* strains. Elongated-cylindrical to oval budding cells or chlamydospores were described in *M. disparilis* MdiSU1-1 (Fig. 1a), cylindrical to rounded-oval cells in *M. dunalii* MduNU2-4 (Fig. 1b,c), and rounded to ovoid cells in *M. importuna* MimBl1-1 (Figs 1d,e) strains. The spiny hyphae

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out-growing from the sclerotia and budding rounded cells were typical for *M. esculenta* strains (Fig. 1f). Development, pigmentation, structure and abundance of sclerotia are considered as morphological traits in studied *Morchella* spp.

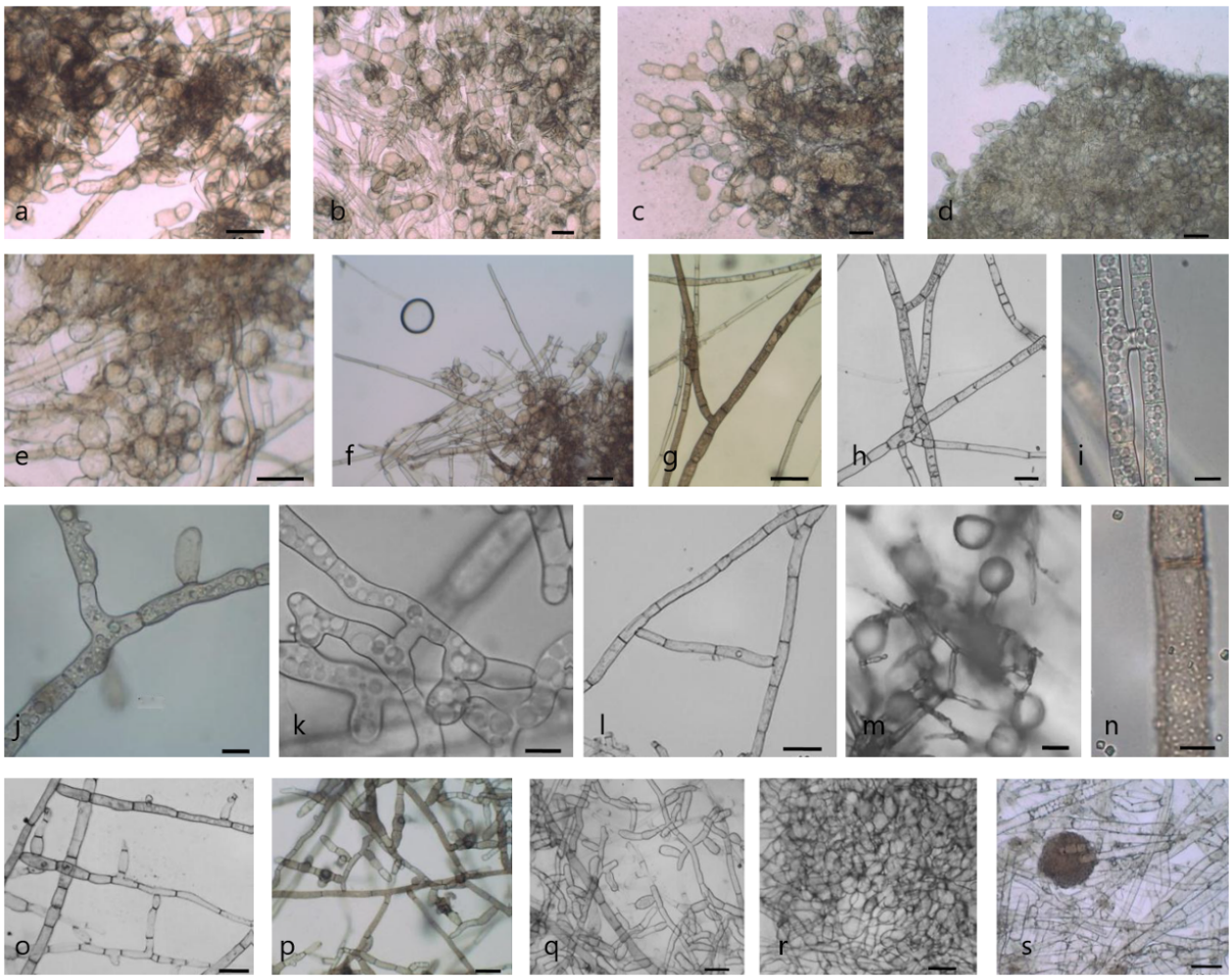

Fig. 1 - Micromorphology of studied *Morchella* cultures: (a) sclerotia and budding cells in MdiSU1-1, (b,c) MduNU2-4, (d,e) MimBl1-1, (f) sclerotium with out-growing spiny hyphae and budding cells in MesFI2-3, (g,h) double and triple hyphal branching in MdiSU1-1, (i–k) hyphal granulation in MdiSU1-1, MesAQ2-C and MimMO1-1, (l) hyphal anastomoses in MdiSU1-1, (m) droplets of exudate in MesAQ2-C, (n) crystals in MpuTN2, (o) arthroconidia in MdiSU1-1, (p) MesAQ2-C, (q) MpuAQ1 on PDA, (r) budding hyphae and (s) sclerotia in liquid cultures of MpuTN2 and MesFI2-3, respectively. Bars = 10 µm.

Several stages during sclerotia development in *M. esculenta* and *M. conica* Pers. strains have been described: growth and branching of hyphae of primary mycelia, interweaving of hyphae of secondary mycelia to form compact masses and formation, growth and maturation of sclerotia (Arkan and Güler, 1992). According to authors the vegetative chlamydospores and asexual conidia were also developed during sclerotia formation suggesting that *Morchella* fungi display diverse reproduction and survival strategies under different environmental conditions. Moreover, morphological development of conidia and nuclear distribution during their asexual reproduction are little known and deserved further studies (Yuan et al., 2021).

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Hyaline or brown, well-septate and anastomosed hyphae (Fig. 1l) were typical for all studied cultures (Table 2). Perpendicular initial hyphal branching was observed in all collections, while dichotomous and triple branching in *M. disparilis* (Figs 1g,h), *M. dunalii* and *M. importuna* strains. Extensively or finely granulated by fatty inclusions anastomosed hyphae were characteristic feature for studied *Morchella* collections (Figs 1i–l). Drops of exudate were observed in mycelia of *M. esculenta* (Fig. 1m) while cuboid, cylindrical and octahedral crystals in *M. dunalii* and *M. purpurascens* (Fig. 1n) cultures. Horn-like hyphae were typical for *M. purpurascens* strains (Table 2 and 3). In studied strains asexual arthroconidia were described in *M. esculenta* (MesAQ2-C and MesAQ6-2), *M. disparilis* (MdiSU1-1), *M. dunalii* (MduNU2-4) and *M. purpurascens* (MpuAQ1) strains (Figs 1o–q).

Many authors referred asexual stage in the life cycle of several *Morchella* spp. (*M. angusticeps* Peck, *M. conica*, *M. semilibera* DC., *M. spongiola* Boud., *M. steppicola* Zerova*, M. esculenta*) to *Costantinella*-like morphotype (Paden, 1972; Volk and Leonard, 1990; Buchalo et al., 2009; Carris et al., 2015). *Costantinella* is a genus of anamorphic fungi, such as *Costantinella terrestris* (Link) S. Hughes, *Costantinella cristata* Matr., *Costantinella clavata* Matr. etc. *Costantinella* spp. which are growing on soil, moss and woody debris. Phylogenetic linkage between both *Morchella* and *Costantinella* morphotypes was confirmed by several cultural and molecular studies (Volk and Leonard, 1990; Carris et al., 2015). *Costantinella* was also described in the related genera, such as *Disciotis* Boud., *Gyromitra* Fr. and *Hydnotrya* Berk. & Broome (Carris et al., 2015). The widespread occurrence of anamorphic morphotypes in morels suggests that the life cycles of these pleomorphic and polymorphic fungi are more complex than previously recognized (Carris et al., 2015). Since formation of mitospores in the life cycle of morels is well documented in culture, their role under natural conditions is not clearly defined. However, their wide geographic occurrence suggests that asexual conidia could play a role as fertilizing agents (Carris et al., 2015).

The substrate mycelium during static (surface) growth was well-developed in all strains, except *M. esculenta* MesAQ6-2 and *M. purpurescens* MpuAQ1 in which the aerial mycelia were developed (Supplementary Fig. S1, Table 3). Budding and intensively granulated hyphae, beige, dark beige or brown sclerotia were observed in aerial and substrate mycelia of all strains, except *M. purpurescens* MpuAQ1 (Figs 1r,s). Horn-like hyphae were described in static culture of *M. purpurescens*. No arthroconidia were seen during observation.

Thus, the studied cultures by micromorphological characters on agar and liquid media were not differed significantly (Tables 2 and 3).

Growth rate and biomass formation

Mycelia of morels are the fastest growing among all taxonomic groups of mushrooms. In studied *Morchella* collections the range of average growth rate (GR_{avr}) was from 11.55 mm d^{-1} to 18.41 mm d^{-1} (Table 4). Mycelia, growing from the centre of the Petri dish, almost covers the entire plate within 2-3 days. The highest GR_{avr} was detected in *M. importuna* (18.41 and 16.25 mm d^{-1})*,* then *M. esculenta* (13.91-14.08 mm d^{-1}), *M. pururanscens* MpuAQ1 (13.80 mm d^{-1}), *M. dunalii* MduNU2-4 (13.40 mm d^{-1}) and *M. disparilis* (13.30 mm d^{-1}) cultures. Two strains of *M. dunalii* MduGR3–4 and *M. pururanscens* MpuAQ1, showed relatively lower average growth rate values (11.70 mm d^{-1} and 11.55 mm d^{-1}), respectively (Table 4).

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Table 4 - Mycelial average growth rate ($GR_{avr,}$ mm d^{-1}) and biomass formation (g, g L^{-1}) of *Morchella* collections on PDA at 23 °C

Species	Strain	GR_{avr} ± SD	Biomass (g) 10th day ± SD		Biomass (g) 21st day ± SD	
			g	g L^{-1}	g	g L^{-1}
M. disparilis	MdiSU1-1	13.30 ± 0.42	0.08 ± 0.02	0.8 ± 0.02	0.76 ± 0.02	7.6 ± 0.24
M. dunalii	MduNU2-4	13.40 ± 0.42	0.11 ± 0.01	1.1 ± 0.01	0.78 ± 0.02	7.8 ± 0.17
	MduGR3-4	11.70 ± 0.32	0.07 ± 0.01	0.7 ± 0.01	0.47 ± 0.01	4.7 ± 0.05
M. esculenta	MesAQ2-c	14.08 ± 0.83	0.06 ± 0.03	0.6 ± 0.03	0.74 ± 0.02	7.4 ± 0.17
	MesAQ6-2	13.91 ± 0.97	0.06 ± 0.01	0.6 ± 0.01	0.74 ± 0.03	7.4 ± 0.26
	MesFI2-3	13.91 ± 0.53	0.06 ± 0.01	0.6 ± 0.01	0.77 ± 0.03	7.7 ± 0.28
M. importuna	MimBL1-1	18.41 ± 0.44	0.15 ± 0.02	1.5 ± 0.02	0.92 ± 0.03	9.2 ± 0.25
	MimMO1-1	16.25 ± 0.80	0.14 ± 0.01	1.4 ± 0.01	0.86 ± 0.04	8.6 ± 0.43
M. purpurascens	MpuAQ1	11.55 ± 0.11	0.09 ± 0.01	0.9 ± 0.01	0.45 ± 0.03	4.5 ± 0.34
	MpuTN2	13.80 ± 0.35	0.14 ± 0.01	1.4 ± 0.01	0.67 ± 0.03	6.7 ± 0.28

In our experimental conditions, *Morchella* collections formed around 0.9-1.5 g L^{-1} of dry biomass in average during 10 days of surface cultivation (Table 4, Supplementary Fig. S4). During 21 days of growth the cultures formed from 4.5 to 9.2 g L^{-1} of dry biomass. More than 6.0 g L^{-1} biomass was accumulated all strains, except *M. purpurascens* MpuAQ1 (4.5 g L^{-1}), which did not form sclerotia, and *M. dunalii* MduGR3-4 (4.7 g L^{-1}), possibly due to well-developed aerial mycelia. The strains which developed substrate mycelia were accumulated from 6.7 to 8.6 g L^{-1} dry biomass. The highest amount of biomass 1.5 and 9.2 g L^{-1} on 10th and 21th days of growth, respectively was accumulated by *M. importuna* MimBL1-1 strain (Table 4, Supplementary Fig. S4).

Thus, a similar growth trend between biomass and GR_{avr} was found (Supplementary Fig. S4). Mycelial GR_{avr}, formation of biomass and development of sclerotia may indicate the aging cultures. It is essential for commercial evaluation of mycelial strains to avoid usage of aging spawn in morel cultivation.

Conclusion

Screening of cultural characteristics of five edible medicinal *Morchella* collections (*M. esculenta*, *M. dunalii*, *M. importuna*, *M. disparilis*, *M. purpurascens*), such as colony morphology and pigmentation, GR_{avr}, development of vegetative (sclerotia) and asexual (arthroconidia) structures in the life cycle, as well as biomass formation in liquid medium revealed their taxonomic significance. Mycelial characteristics are required for proper taxonomic identification and commercial evaluation of *Morchella* cultures during their biotechnological cultivation.

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