

## THE AUTUMNAL OCCURRENCE OF THE VERNAL GENUS *MORCHELLA* (ASCOMYCOTA, FUNGI)

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World bioclimatic data on *Morchella* spp. are summarized and the autumnal occurrence of this principally vernal genus is discussed. A description of one Croatian collection based on living (fresh) material provides a template for detailed macro- and microscopic characterization of *Morchella* specimens. Ecological factors that influence morel fructification are also discussed with special emphasis on the *Morchella esculenta* species aggregate and the position of Mediterranean habitats in the framework of global bioclimates, while a collection from the Adriatic islet of Ilovik is studied in detail.

**Key words:** fructification season, bioclimates, species description template, Mediterranean

Matočec, N., Kušan, I., Mrvoš, D. & Raguzin, E.: Pojava proljetnog roda *Morchella* u jesen (Ascomycota, Fungi). *Nat. Croat.*, Vol. 23, No. 1., 163–177, 2014, Zagreb.

U ovom radu prikupljeni su svjetski podaci o bioklimatima koje naseljavaju vrste iz roda *Morchella* (smrčci) i raspravlja se o pojavi ovog primarno proljetnog roda u jesen. Opis jednog hrvatskog nalaza koji je temeljen na živom (svježem) materijalu predstavlja predložak za makro- i mikroskopsku karakterizaciju vrsta iz roda *Morchella*. Također se raspravlja o ekološkim čimbenicima koji utječu na fruktifikacijsku sezonu smrčaka. Poseban naglasak stavljen je na vrste iz agregata *Morchella esculenta* i položaj mediteranskih staništa u okviru globalnih bioklimata uz prikaz detaljno istraženog nalaza s jadranskog otočica Ilovika.

**Ključne riječi:** fruktifikacijska sezona, bioklimati, predložak za opis vrste, Mediteran

### INTRODUCTION

Species of the genus *Morchella* Dill. ex Pers. and the other members of the family *Morchellaceae* are all well known as typical widely distributed northern hemisphere vernal fungi. They normally develop their ascomata from March in the evergreen Mediterranean (cf. AGNELLO & LIVIERI, 2011) to July in the highest Alpine stands and the northernmost boreal areas (DISSING, 1980; DISSING & RAITVIIR, 1974; PETERSEN, 1980; WURTZ *et al.*, 2005). This applies to the broad range of temperate (cf. also MIHAIL *et al.*, 2007) and cold climates of the whole Northern Hemisphere. Few studies are known (e.g. MASAPHY *et al.*, 2009) that treat morels in the arid Mediterranean area as a transition zone between semi-humid Mediterranean and tropical areas, the authors demonstrating that the main morel fruiting season falls in the middle of the winter. However, morels in wider tropical areas without a four season successive cycle and rather slight temperature variations all year round, may occur irregularly, virtually throughout the year (GUZMÁN *et al.*, 1985; GUZMÁN

& TAPIA, 1998; HEIM, 1966; LE GAL, 1953; RIFAI, 1968). On the other hand, morels in temperate areas of the Southern Hemisphere are known to fruit typically during September/October, which corresponds with March/April in the Northern Hemisphere (e.g. RIFAI, 1968; GAMUNDÍ, 1975). Seemingly, the only well documented examples with the normal yellow morel fruiting pattern during autumn are those in GOLDWAY *et al.* (2000) in Israel and SINGH *et al.* (2004) in the SW Himalayan area, where these morels are apparently adapted to a specific climatic regime. The other sources on autumnal morel appearance are summarized by AGNELLO & LIVIERI (2011).

Most of the species groups are described from cooler areas of especially the Northern Hemisphere, while the wider tropical zone seem to house only taxa of the *Morchella esculenta* species aggregate and *M. rufobrunnea* Guzmán & F. Tapia, with the *M. elata* species aggregate and *M. semilibera* species aggregate without records (cf. GUZMÁN *et al.*, 1985; GUZMÁN & TAPIA, 1998; HEIM, 1966; KUO, 2008; LE GAL, 1953; RIFAI, 1968).

## MATERIALS & METHODS

Autumnal morel material examined was collected on 31 Oct 2012 by the fourth author on the islet of Ilovik in the northern part of the Adriatic Sea, Croatia (Plate 4a). The recorded local climatologic elements are compared with the other cases of autumnal morel occurrence. Our own well documented morel fruiting patterns are compared with others representing various bioclimatic schemes. Vital macroscopic and microscopic characters were recorded by the application of vital taxonomy methods as described in BARAL (1992) and on freshly fixed sections. The mounting media was tap water unless otherwise stated, i.e. 5% weight water solution of potassium hydroxide (KOH); IKI (type of Lugol's solution after BARAL, 1987); water solution of Brilliant Cresyl Blue (CRB) after BARAL (1992); water solution of Congo Red (CR) after PFISTER *et al.* (2009); lactic acid solution of Cotton Blue (CB) after ERB & MATHEIS (1983); Acetocarmine (AC) after HARMAJA (1974) and Melzer reagent (MLZ) after HUHTINEN (1990). Species identification and determination of the variation range of qualitative and quantitative characters were based on ascotal anatomy and hymenial elements on three apothecia out of total six fruitbodies of a single collection. Colour descriptions are based on KORNERUP & WANSCHER (1967) with a colour code in parentheses. Drawings were made freehand and microphotographs were taken with a Nikon Coolpix 4500 camera mounted directly on the microscope. The fresh collection was photographed both *in situ* and *ex situ*. Dried material with a spore deposit is kept in the Croatian National Fungarium (CNF).

In this paper we use RIFAI (1968) and WEBER (1995) for the species concept in the *Morchella esculenta* species aggregate until integrated (polyphasic) world-wide taxonomic study on morels is conducted. Molecular datasets should be combined with various non-molecular methods supplemented with exhaustive revisions of available type material. KUO *et al.* (2012) provided too scanty microscopic data for considerably enlarged species diversity, and they were ineffective in differentiating the treated species. Additionally, both the descriptions and the key are inapplicable to Europe because most *Morchella* species showed clear provincialism and endemism (O'DONNELL *et al.*, 2011).

## Abbreviations

\* = living state of cell/tissue; † = dead state of cell/tissue; MC = metacromatic corpuscles; Q = ascospore length/width ratio; WBs = Woronin bodies

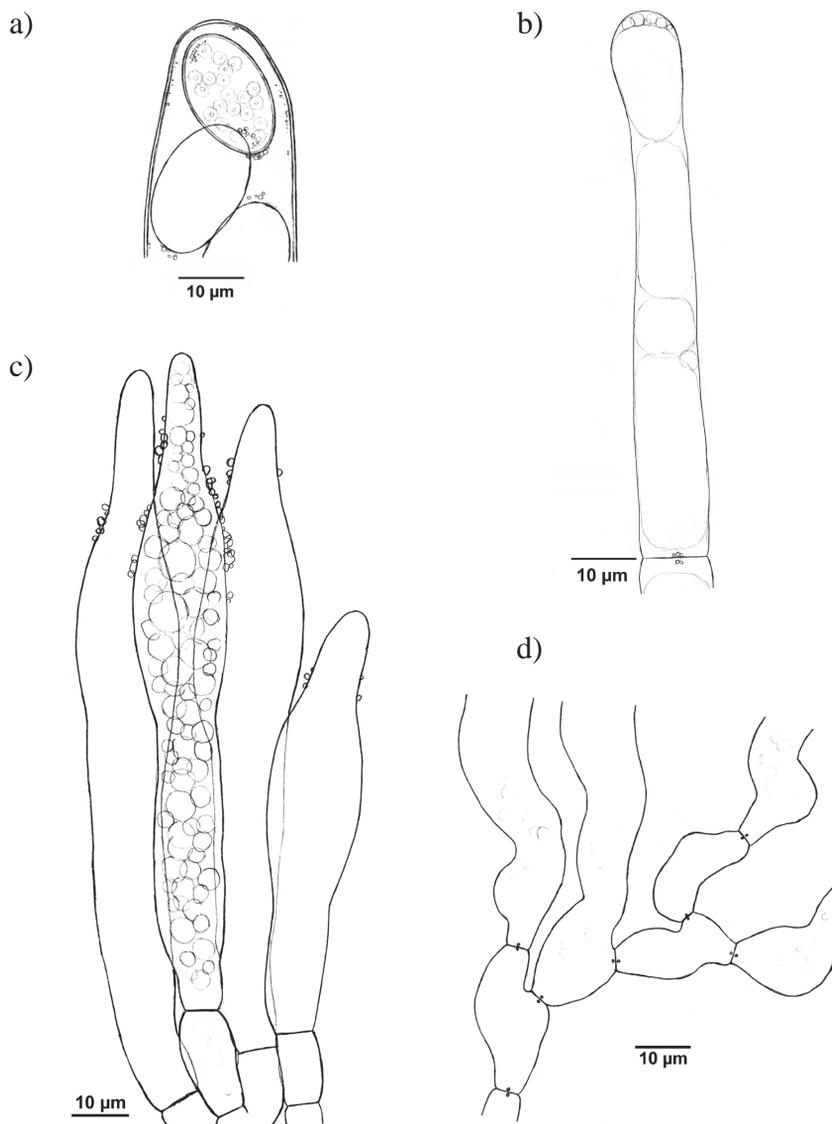
## RESULTS

*Morchella esculenta* (L.) Pers. s. lat.**Macroscopy (Plates 1a-d)**

**ASCOMATA** in total \*86–115 mm high; **pileus** ovoid to conical, \*52–59 mm high and \*31–52 mm wide, primary alveoli orange yellow (4A6), greyish yellow (4B6) to greyish orange (5B6), irregular, 18–22 per side, predominantly vertically oriented with transverse secondary ribs, primary ribs more conspicuous, \*0.6–1.5 mm wide, at first white (1A1), when mature becoming rusty orange (6B6–6B7) to brownish orange (6C7) with irregular micro-pustulate pattern caused by drying off and degeneration of terminal cells (Plate 2b); **stipe** ± cylindrical, hollow, thickened and furrowed at the base, \*34–52 mm high, \*9.4–10.5 mm wide at apical part and \*17–20 mm wide at the base, whitish (1A1), finely pustulate; odour typically morel-like; spore deposit yellowish white (1A2, 2A2, 3A2) depending on spore mass thickness; under UV light surface of fresh ascomata internal excipulum and stipal flesh at  $\lambda=254$  nm purplish red (13A8, 14A8) and at  $\lambda=366$  nm cinnamon-ochre (5B7), stipal internal excipulum surface at  $\lambda=254$  nm violet (17A8), other structures without fluorescence.

**Microscopy (Plate 1 e-n; 2a-f; Fig.1a-d)**

**PILEUS - Asci** \*324–378 × 22.4–26.1  $\mu\text{m}$ , *pars sporifera* \*97–128  $\mu\text{m}$ , n=10; 8-spored, all normally developed, with no significant heteromorphy seen, uni- to biseriate, mature \*asci protrude 46.6–94.2  $\mu\text{m}$  above the paraphyses, subcylindric obtuse, thickest in the middle part, gradually tapered towards apex, base mostly bulbous, arising from simple septa, some giving rise to another ascogenous cell at the other septum; in \*IKI completely inamyloid; in \*CRB walls not stained, ascoplasm bluish violet (18A6–18A7) to violet blue (19A6–19A7); in \*CR walls not stained; in \*CB walls greenish blue (24A6). **Ascospores** \*(17.5–)19.2–24.7(–25.5) × 11.9–14.4(–16)  $\mu\text{m}$ , \*Q=(1.41–)1.50–1.87(–2.0), n=150; in \*CB (19.6–)20.4–23.3(–25.2) × (11.9–)12.3–14.2  $\mu\text{m}$ , Q=(1.43–)1.52–1.83(–1.96), n=50, in \*H<sub>2</sub>O ellipsoid to narrowly ellipsoid, 1-celled, containing most often 16–20 nuclei when fully mature, homopolar, thin-walled, smooth, eguttulate, hyaline, around 10% of all measured with polar groups of greyish-yellow (4B6) to orange yellow (4B7–4B8) low refractive globules, polar globose exudate rapidly detaching after ejection, sheath absent; in \*IKI internal wall layer with greyish ruby (12C7) staining, nuclei and nucleoli highly contrasted but due to the other numerous small vacuoles they are hardly discernable except for individual ones disposed ± near the spore wall; sporoplasm generally rusty orange (6B7–6B8) without localised glycogen accumulations; in \*CRB completely unstained but nuclei and nucleoli best contrasted among all applied media; after addition of KOH to \*CRB extra-nuclear sporoplasm become bluish grey (23B3) to greyish blue (23B4), polar MCs quickly formed and stable for a longer period, violet blue (19A6, 19B6–19B7); in \*CR without any staining, nuclei and nucleoli equally visible as in H<sub>2</sub>O; in \*CB wall stable, sporoplasm without de Bary bubbles, mature ascospores without any cyanophilous reaction, submature ones with light blue (23A5) sporoplasm; in \*AC nuclei not carminophilic, sporoplasm pink (12A5). **Paraphyses** with cylindrical obtuse to tapered subclavate, subcapitate, finger-like or tapered subclavate apical cell in deeper interascal region while subulate to sublanceolate near the rib edges, apical cells \*(51–)74–118 × 10.3–18  $\mu\text{m}$ , rather thin-walled, wall hyaline to pale yellowish grey (3B2), at first highly vacuolated, soon vacuoles coalesce into larger globose and very large oblong ones, WBs hexagonal, \*0.5–1.3  $\mu\text{m}$  wide; in \*IKI wall unstained, cytoplasm highly granulated, some granules



**Fig. 1.** a) ascus apex, b) paraphysis apical cell, c) terminal cells on primary rib, d) ascogenous system; del. N. Matočec

light orange (5A5); in <sup>\*</sup>CRB walls unstained, cytoplasm with scattered violet blue (19A6, 19B6-19B7) MCs; in <sup>\*</sup>CR walls unstained, cytoplasm unstained or with very sparse pink (12A5) granules inside; in <sup>†</sup>CB wall not stained, cytoplasm of apical cells greyish blue (23B6-23C6); in <sup>†</sup>AC nuclei not carminophilic.

**Interhymenial trama** consists of medullar part and terminal cells on primary ribs: (1) medullar part composed of subhymenium and underlying tissue which are mutually not

differentiated, the texture  $^{\circ}255\text{--}580\ \mu\text{m}$  thick, composed of hyaline, tightly woven *textura intricata-epidermoidea*, greyish in mass, hyphae  $^{\circ}4.5\text{--}28.5\ \mu\text{m}$  wide; in  $^{\circ}\text{IKI}$  texture unstained; in  $^{\circ}\text{CRB}$  some cylindrical cells with violet blue (19A6, 19B6-19B7) flocculated globules; in  $^{\circ}\text{CB}$  walls turquoise white (24A2) to pale turquoise (24A3), cytoplasm pastel blue (23A5); in  $^{\circ}\text{MLZ}$  without dextrinoid reaction; (2) primary ribs covered with pustulate pattern composed of fascicles of terminal protruding cells, pustules reddish brown (8D6-8D7, 8E6-8E7) on whitish surface in maturity, individual terminal cells lanceolate,  $^{\circ}74\text{--}147 \times 15.5\text{--}27.8\ \mu\text{m}$ , mostly with  $\pm$  thin hyaline walls on upper areas, and slightly thicker reddish grey (7B2) to greyish red (7B3) walls on their bases continuing to a few basal prismatic cells, cytoplasm highly vacuolated for a longer period, wall mostly covered in part with hyaline small crystalloid granules; in  $^{\circ}\text{IKI}$  wall unstained, cytoplasm highly granulated, some granules light orange (5A5); in  $^{\circ}\text{CRB}$  walls unstained, cytoplasm with both unstained medium refractive  $\pm$  angular bodies and deep greyish blue (22D7, 22E7) or violet blue (19A6, 19B6-19B7) granules and globules; in  $^{\circ}\text{CR}$  walls unstained, cytoplasm at least partly red (10A8, 10B8) granulated; in  $^{\circ}\text{CB}$  walls turquoise white (24A2) to pale turquoise (24A3), cytoplasm pastel blue (23A4). **Pileal ectal excipulum** none. **Pileal internal excipulum** consists of three layers: (1) pileal medulla composed of subhymenium and medullary layer which are mutually not differentiated, the texture  $^{\circ}232\text{--}436\ \mu\text{m}$  thick, composed of hyaline tightly woven *textura intricata*, greyish in mass, hyphae  $^{\circ}4.9\text{--}13.2\ \mu\text{m}$  wide; in  $^{\circ}\text{IKI}$  texture unstained, in  $^{\circ}\text{CRB}$  some cylindrical cells with violet blue (19A6, 19B6-19B7) flocculated globules; in  $^{\circ}\text{CB}$  walls turquoise white (24A2) to pale turquoise (24A3), cytoplasm bluish grey (23B2); in  $^{\circ}\text{MLZ}$  without dextrinoid reaction; (2) internal continuous excipular layer  $^{\circ}458\text{--}577\ \mu\text{m}$  thick, composed of hyaline *textura angularis* with  $\pm$  compact polyhedral short prismatic to subangular isodiametric cells,  $^{\circ}12.1\text{--}45.8\ \mu\text{m}$  wide; and (3) discontinuous outermost excipular part shaped as truncate pustules that are  $^{\circ}100\text{--}266\ \mu\text{m}$  high and  $^{\circ}82.5\text{--}372\ \mu\text{m}$  wide, composed of loosely arranged hyaline *textura globulosa*, cells  $^{\circ}14.5\text{--}68\ \mu\text{m}$  wide; in  $^{\circ}\text{MLZ}$  without dextrinoid reaction.

**STIPE – Ectal excipulum** consisting of discontinuous and continuous layer: (1) outermost discontinuous layer composed of densely set narrow subulate to truncate fascicles that are  $^{\circ}110\text{--}195\ \mu\text{m}$  high and  $^{\circ}76\text{--}210\ \mu\text{m}$  wide, base composed of hyaline *textura globulosa*, very loosely organized, cells  $^{\circ}15\text{--}44.5\ \mu\text{m}$  wide, apical part composed of 1-2(3) celled chains that are vertically oriented (perpendicular to the surface), cells shortly to elongated prismatic, terminal cells  $^{\circ}25.8\text{--}91.7 \times 11.2\text{--}19.3\ \mu\text{m}$ ,  $\pm$  cylindrical obtuse, walls hyaline but often with  $\pm$  pronounced hyaline plaques that finally turn to vivid yellow (3A8), highly refractive, cytoplasm highly vacuolated; (2) continuous layer  $^{\circ}322\text{--}380\ \mu\text{m}$  thick, hyaline, composed of  $\pm$  densely packed hyaline *textura angularis*, cells  $^{\circ}18.5\text{--}71.5\ \mu\text{m}$  wide. **Medullar excipulum**  $^{\circ}402\text{--}976\ \mu\text{m}$  thick, hyaline, composed of *textura intricata*, hyphae  $^{\circ}4.8\text{--}15.1\ \mu\text{m}$  wide. **Internal excipulum** composed also of continuous and discontinuous layers: (1) continuous layer  $^{\circ}439\text{--}551\ \mu\text{m}$  thick, composed of hyaline densely packed *textura angularis*, cells  $^{\circ}20.7\text{--}81\ \mu\text{m}$  wide; (2) discontinuous layer composed of large irregular truncate pustules that are  $^{\circ}170\text{--}283\ \mu\text{m}$  high and  $^{\circ}195\text{--}412\ \mu\text{m}$  wide, cells hyaline, drum-shaped or ellipsoid to oblong,  $^{\circ}32.2\text{--}59.5\ \mu\text{m}$  wide; overall in all stipe parts in  $^{\circ}\text{MLZ}$  without dextrinoid reaction.

### Specimen examined:

CROATIA, Hrvatsko Primorje region, islet of Ilovik (SE from the Lošinj island), 15 m asl, 31 Oct 2012, degraded Thermo-Mediterranean, fully evergreen, sclerophyllous maquis on thin calcareous soil, dominated by *Quercus ilex*, accompanied with *Pistacia lentiscus*, *Myrtus communis* and *Arbutus unedo*, leg. Ervin Raguzin, (CNF-2/9281).

On the same date the following fungal taxa were recorded on the islet: *Agaricus bitorquis*, *A. silvaticus*, *A. silvicola*, *A. xanthoderma*, *Boletus queletii*, *Cantharellus lilacinoprui-natus*, *Ganoderma lucidum*, *Gyroporus castaneus*, *Lactarius sanguifluus* and *Phallus impudicus*.

## DISCUSSION

There are numerous papers treating *Morchella* species focused on molecular datasets (starting with BUNYARD *et al.*, 1994; BUSCOT *et al.*, 1996; KELLNER *et al.*, 2005 etc.) with only one trying to include other, non-molecular characters (mostly macroscopical and/or ecological data) (KUO *et al.*, 2012). Chronic confusion in the species concept and ineffective species recognition took place from the earliest attempts in monographic studies (e.g. BOUDIER, 1905-1910) until recent times when it was finally elaborated by molecular revision of sequences deposited in GenBank (DU *et al.*, 2012b). Recent advances in *Morchella* taxonomy have proven the multispecies concept for the genus using molecular phylogenetic analyses (DU *et al.*, 2012a; DU *et al.*, 2012b; O'DONNELL *et al.*, 2011; TAŞKIN *et al.*, 2010), revealing the fact that only few taxa are apparently distributed pan-continentially or inter-continentially while tens of species show clear provincialism or endemism (O'DONNELL *et al.*, 2010; DU *et al.*, 2012a; TAŞKIN *et al.*, 2012). Additionally, some species that display distinctive macro- and microscopical characters with probably very narrow distribution have recently been described, such as *Morchella anatolica* Işiloğlu, Spooner, Alli & Solak (IŞILOĞLU *et al.*, 2010) and *M. tomentosa* M. Kuo (KUO, 2008). Recently, the multigene molecular approach (e.g. TAŞKIN *et al.*, 2010; O'DONNELL *et al.*, 2011) made advances in morel phylogenetics thus finally throwing much more light in the *Morchella* species concept after centuries of research. However, non-molecular differential characters for effective species recognition below the level of species aggregates in the genus are still lacking. KUO *et al.* (2012) constructed a key for identifying North American *Morchella* species previously characterized as phylogenetic species without binominals and descriptions (O'DONNELL *et al.*, 2011), providing both the binominals and descriptions for a number of them for the first time. In spite of some microscopic data that were provided, the resulting key is based almost solely on macroscopy, supplemented with ecological data and biogeography.

Morel taxonomy and the species concept was a matter for serious dispute for a very long time largely thanks to the prevalence of unreliable macroscopic features (cf. BUNYARD *et al.*, 1994; WEBER, 1995). Also, many morel macroscopic features may be often heavily influenced by the ecological factors and ontogenetic state and cannot be used as differential characters. Our experience based on the long-term monitoring of tens of forms of morels and hundreds of collections treated in detail indicates the necessity of employing as much microscopic, physiological and chemo-taxonomical information as possible in order to be able to find the maximum amount of reliable differential characters. Therefore, we here suggest that a detailed description of a *Morchella* collection should be a template for subsequent work aimed in revealing sufficient differential characters for any given *Morchella* species.

According to the detailed ecological research performed by BUSCOT (1989), MIHAIL *et al.* (2007) and SCHMIDT (1983) we can assume that the vernal occurrence of *Morchella* fruitbodies characteristic of mild and cool temperate belts of the Northern Hemisphere is controlled by a specific ecophysiology triggered by an abrupt increase of air/soil temperature. This could explain why morel fruitbodies normally occur in spring time in nature throughout the temperate and cool bioclimates in the Northern Hemisphere where a number of morel taxa and ecotypes are widely distributed. It has been shown that the ascospores of *Morchella esculenta* cannot germinate until soil temperature exceeds

10°C after snowmelt in early spring, whereas morel mycelia are capable of colonizing various substrata even at lower temperatures. Similarly, MIHAIL *et al.* (2007) recorded quite constant soil temperatures (around 10°C) at which they monitored the onset of fruiting season of *M. esculenta* over the five year period on the single studied site. Therefore, competitiveness of the morels seemingly relies on its psychrotolerant feature in post-hibernal time (SCHMIDT, 1983; our unpublished data). Quite an opposite situation has been described with autumnal morels found in Israel and the south-west Himalayas where soil temperatures were at least 15-25°C during the fruiting period (GOLDWAY *et al.*, 2000; SINGH *et al.*, 2004). Additionally, morels in tropical areas with two seasons or without climatic seasonality, where air temperature and humidity have negligible fluctuations, may occur virtually throughout the year: end of June (LE GAL, 1953), end of August and beginning of June (HEIM, 1966), during October or November (GUZMÁN *et al.*, 1985), from May to December for *M. rufobrunnea* or from January to November for *M. guatemalensis* (GUZMÁN & TAPIA, 1998) etc. (Plate 3).

The role of precipitation dynamics is also mentioned as a presumably important ecological factor for the extent of morel fruiting in nature (MIHAIL *et al.*, 2007; WURTZ *et al.*, 2005). This suggestion is in full agreement with our experience and data obtained through our long-term monitoring (unpublished data) in several Croatian sites as well, when the amount of rainfall in several weeks or a month prior to morel fructification is taken into account (Plate 4). In our experience there is a positive correlation between fruitbody productivity and amount of snowfall/rain during the preceding months (cf. also MIHAIL *et al.*, 2007). However, quantitative monitoring on abiotic factors performed in Israeli sites on a specific ecotype of *M. esculenta* s. lat. (GOLDWAY *et al.*, 2000; cf. also MASAPHY *et al.*, 2009) showed that morel fruitbody production followed long after the winter rainy season when soil humidity is significantly decreased, which may suggest a completely different strategy and also the occurrence of a species distinct from those occurring in cooler areas. It should be noted as well, that the site where this ecotype occurs is strongly influenced by river or irrigation channel inundation, which necessarily means that substrate (soil) drying takes a much longer time than in cases normally recorded in temperate/cooler northern areas. In temperate/cooler areas where *M. esculenta* s. lat. and the other members of the family *Morchellaceae* appear during spring for just a few weeks, the soil temperature just begins to reach about 10°C and soil humidity is still high (MIHAIL *et al.*, 2007; SCHMIDT, 1983). On the contrary, Israeli sites of the same species aggregate and those of south-west Himalayas (SINGH *et al.*, 2004) where the main fructification season lasts for months during autumn or late summer, the soil temperature is higher (15-20°C) and the soil humidity drops considerably (compare bioclimatic representatives nos. 1-8 vs. 9-13, Plate 3). The difference in these two modes of fruiting behaviour in *M. esculenta* s. lat. may rely on differences in its life cycle elements/phases, which could represent two different adaptive life strategies (ecotypes or species). In both cases the morel season follows sooner or later after the rainy period and in that way decreased accessibility of water uptake may lead to the production of the morel fruitbodies. This could happen, either because of the overall substrate drying or by repetitive surface soil freezing during still cold spring nights. This is supported by the experiments performed by OWER *et al.* (1986) showing the role of substrate wetting and successive drying.

In our case on the Ilovik islet, *M. esculenta* s. lat. ascomata occurred two weeks after two incidences of heavy rain during a short period: around 100 mm in September and 140 mm in October per cyclonal activity that lasted for 2-3 days. This constituted nearly 100% of the mean monthly rainfall for September and ca. 125% for October. The total rainfall in October 2012 on Ilovik islet was 217 mm, which is double the average for the

month. The preceding summer was unusually dry for months with continuous exceptionally high soil temperatures (approx. 20–33°C measured on nearby Rab island during August and September). Plate 4 gives air temperature and precipitation for Lošinj and soil temperature (10 cm depth) for Rab, islands close to Ilovik with the same climate.

Morel fruiting on Ilovik was very probably triggered firstly by the vigorous rainfall over a three-day period (Plate 4b, triggered event labelled by red cones) and/or by air and soil temperature decreased roughly by 5°C to form primordia. This was followed by rapid decrease of soil humidity during the next two weeks since no or very little precipitation occurred while soil temperature remained stable for 16 days and ranged 16–18°C, measured on the somewhat cooler Rab island (minimal and maximal monthly mean air temperatures measured on Ilovik islet ranged 15–24°C in October) when the transition from primordial to rapidly developing ascomata must have taken place (Plate 4b, event labelled by green arrow). Fully ripened fruitbodies were discovered on the fourth day after that. The exact collecting site on Ilovik islet is locally well drained due to the terrain configuration (inclination), the porous limestone bedrock and strong influence of the common cold dry bora wind. This is in agreement with the total time (21 days) that *M. esculenta* s. lat. required to develop its primordia to submature ascomata by OWER (1982) who conducted the experiment under controlled conditions (15–18°C). This also corresponds well to autumnal morel fructification in the high soil temperature regime known from Israel and south-west Himalayas (see above). Therefore, we can assume that this type of behaviour is merely the life mode of the *M. esculenta* species aggregate. Repeated subsequent site visitation by the fourth author during November recorded no further morel fructifications when both air and soil temperature rapidly decreased (8–13°C air temperature for 1–19 Nov) combined with new heavy rainfall (145 mm for 1–19 Nov) that rewetted and cooled the soil below the temperature level required for developing fruitbodies in this mode of *M. esculenta* s. lat.

It seems that the occurrence of *M. esculenta* s. lat. on Ilovik fully corresponds with occurrences in previous years in the vicinity of Brindisi, southern Italy during autumn, i.e. from the beginning of November to the end of December (AGNELLO & LIVIERI, 2011). Both localities are characterised by pure sclerophyllous, evergreen Mediterranean vegetation dominated by evergreen *Quercus* species and represent areas where *M. esculenta* s. lat. may occasionally produce fruitbodies both in spring and autumn (Plate 3; note that autumnal fructifications period is presented in grey colour). The fourth author repeatedly searched for morels both during April in previous years and four times in autumn 2013 without positive results. At another site in southern Italy, AGNELLO & LIVIERI (2011) with their collaborators recorded autumnal *M. esculenta* fruitbodies in three years while no fruiting productivity was recorded in another three years. Regular vernal fructification period extends there from mid-February to mid-April while autumnal occurrence is quite irregular.

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## SAŽETAK

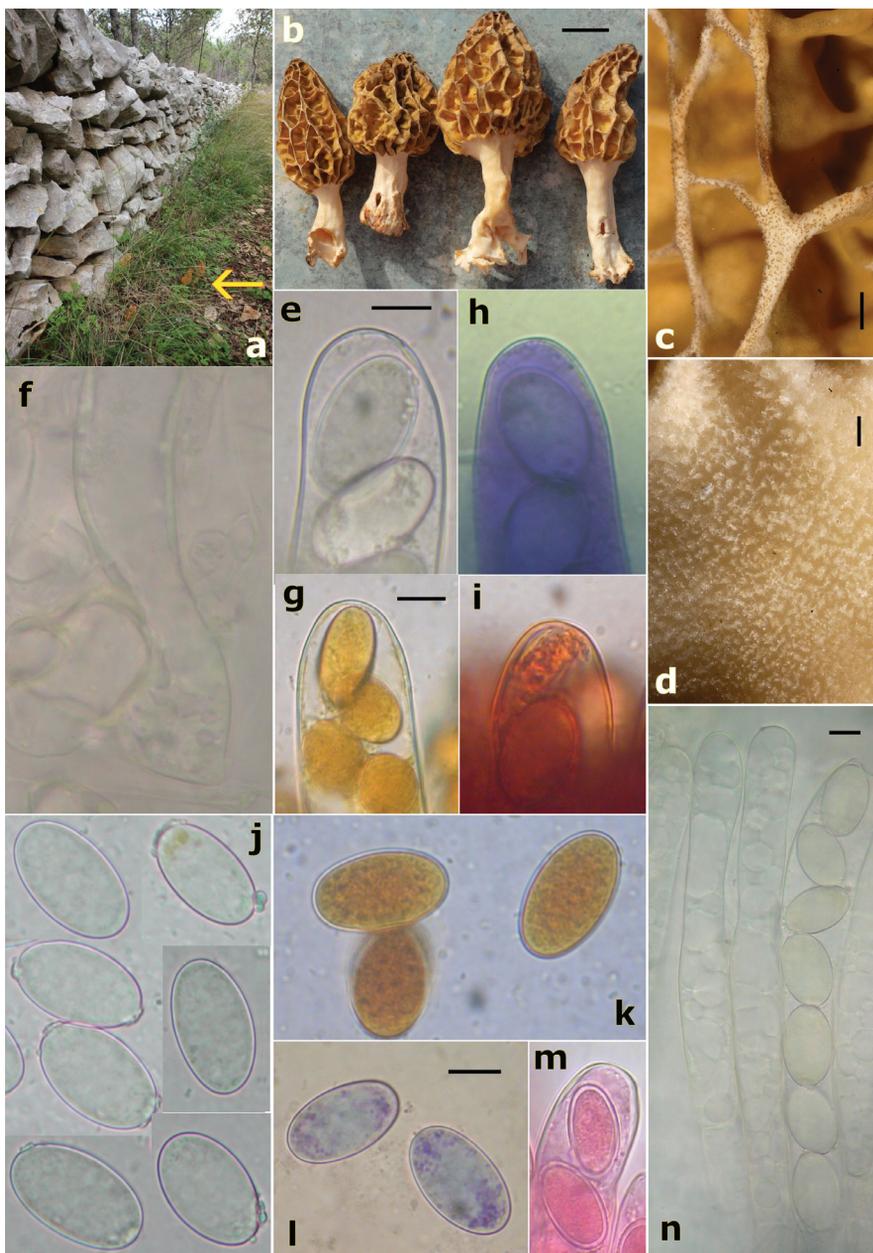
### Pojava proljetnog roda *Morchella* u jesen (*Ascomycota*, *Fungi*)

N. Matočec, I. Kušan, D. Mrvoš & E. Raguzin

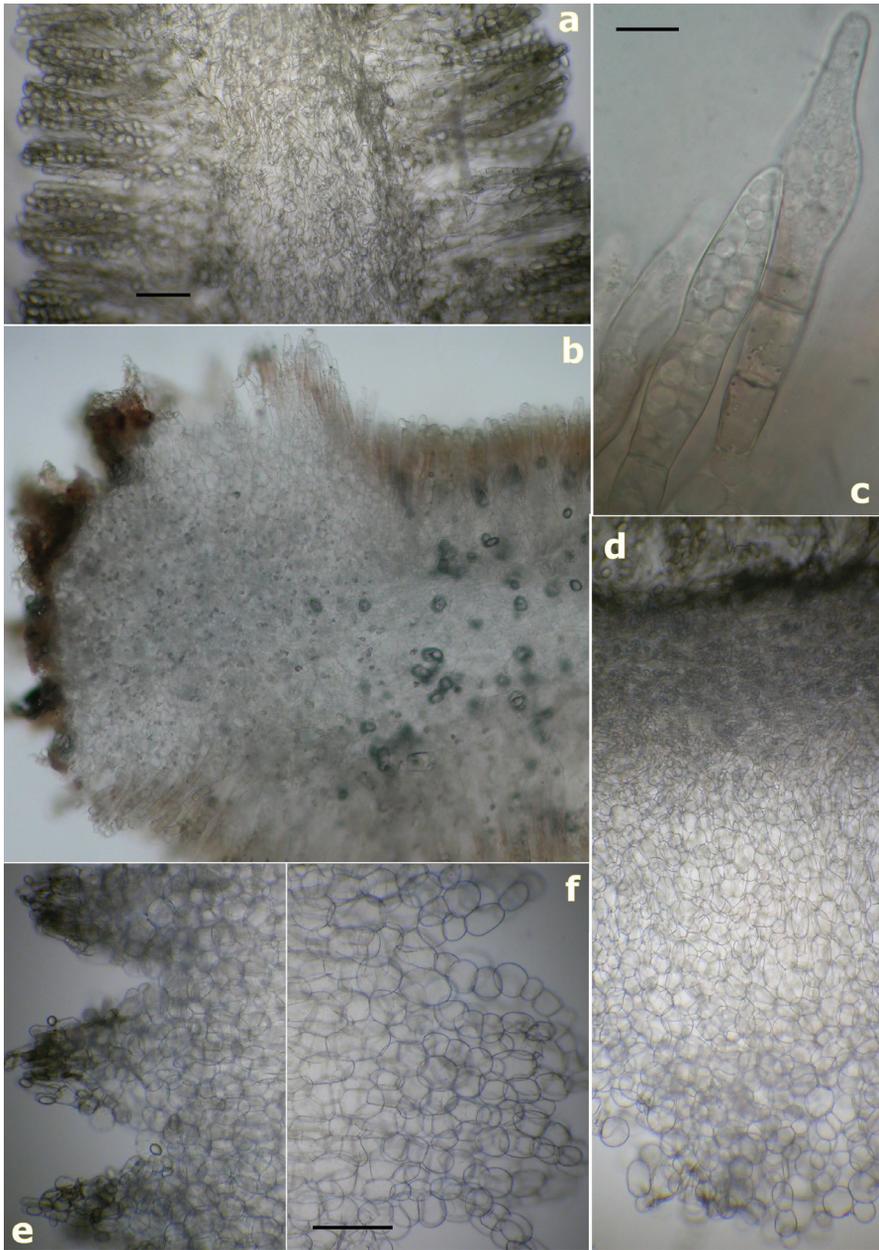
Na području sjeverne hemisfere vrste iz roda *Morchella* (smrčci) kao i drugi članovi porodice *Morchellaceae*, koji su na tom području široko rasprostranjeni, dobro su poznati kao tipične proljetne vrste gljiva čija se plodišta razvijaju od ožujka do srpnja. Prema nekim autorima sezona fruktificiranja smrčaka odvija se zimi u suhim mediteranskim područjima u kojima je to ujedno i jedino vlažno i svježije razdoblje. S druge strane, u širem tropskom pojasu, zbog neznatnih kolebanja temperature i vlage, ove se gljive plodištima mogu pojaviti bilo kada tijekom godine. U umjerenim područjima južne hemisfere smrčci razvijaju plodišta u rujnu / listopadu što je usporedivo pojavi smrčaka u ožujku / travnju na području sjeverne hemisfere. U hladnijim i umjerenim područjima sjeverne hemisfere zabilježen je veći broj svojiti iz roda *Morchella*, dok se u široj tropskoj zoni pojavljuju isključivo vrste iz agregata *Morchella esculenta* s. lat. i *M. rufobrunnea*.

Jesenski nalaz vrste iz agregata *M. esculenta* uzorkovan u Hrvatskoj 31. 10. 2012. na otoku Iloviku, detaljno je analiziran i obrađen metodologijom koja se temelji na radu sa živim materijalom. Detaljan opis makro- i mikroskopskih karaktera te primjena spektra mikroke-mijskih reakcija i bojila predstavljaju predložak za obradu svih nalaza vrsta roda *Morchella*.

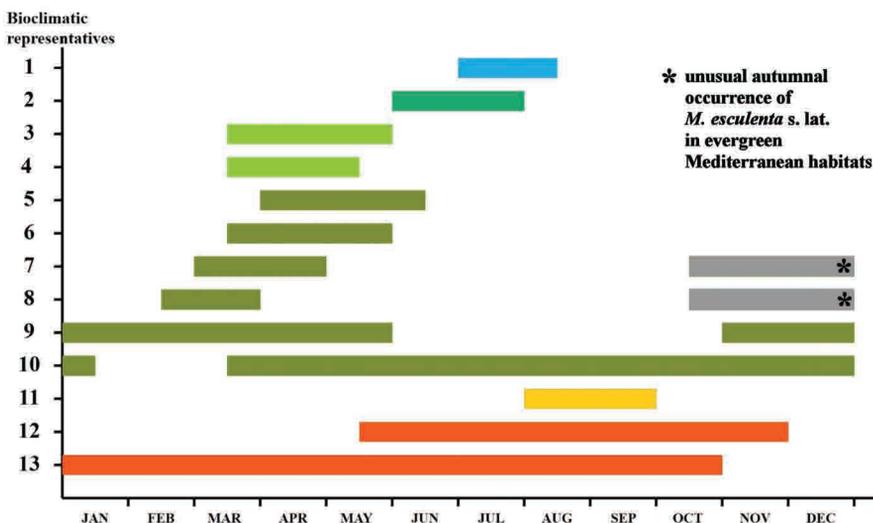
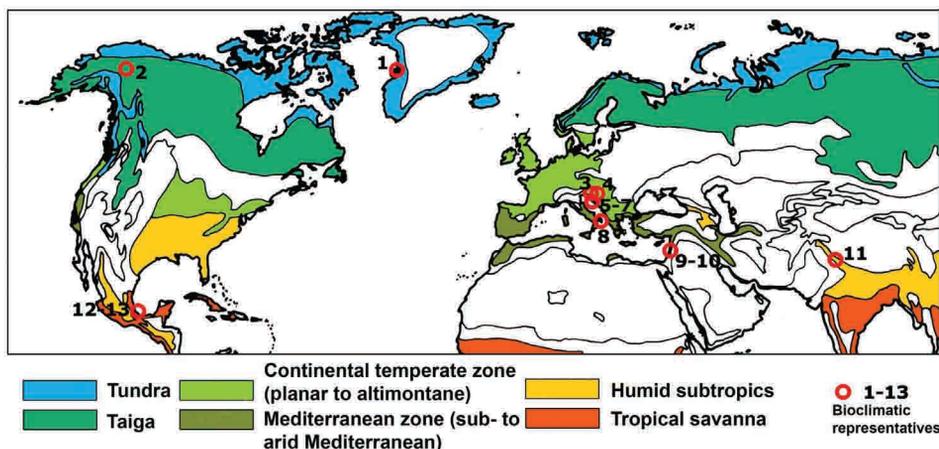
Ekološka istraživanja roda *Morchella* pokazuju da su količina oborina, temperatura te vlažnost tla (dobavljivost vode) bitni ekološki čimbenici za razvoj plodišta. Zajednička obilježja pojave plodišta smrčaka jesu uvjeti vlažnosti tla gdje se plodišta razvijaju prilikom sušenja supstrata, bilo nakon otapanja snijega i/ili obilnih kiša u proljeće u umjerenj zoni ili obilnih kiša u jesen na Mediteranu. Uvjeti pod kojima je nastupilo jesensko fruktificiranje smrčaka na Iloviku uspoređeni su sa sličnom situacijom na jugu Italije te s detaljno analiziranim ekotipom smrčaka s jesenskim modusom fruktificiranja u uvjetima visoke temperature tla u Izraelu kao i s laboratorijskim uvjetima pod kojima je uspjelo dobivanje plodišta u kulturi.



**Plate 1. A collection of autumnal *Morchella esculenta* s. lat. from Ilovik islet. Situation at collection site (1a), macroscopic features (1b-d) and microscopic features (1e-n). 1a – collection site, the arrow points the exact position of the fruitbodies; 1b – collected mature fruitbodies, bar = 20 mm; photos by E. Raguzin; 1c – primary rib detail, bar = 2 mm; 1d – stipe surface detail, bar = 0.5 mm; photos by N. Matočec; 1e – mature ascus apex ( $H_2O$ ), bar = 10  $\mu m$  (also valid for 1f, 1h, 1i-k); 1f – mature ascus base ( $H_2O$ ); 1g – mature ascus apex in 'IKI, bar = 10  $\mu m$  (also valid for 1m); 1h – mature ascus apex in 'CRB; 1i – mature ascus apex in 'CR; 1j – freshly discharged mature ascospores ( $H_2O$ ); 1k – freshly discharged mature ascospores in 'IKI; 1l – freshly discharged mature ascospores in 'CRB (slightly alcalinized by KOH), bar = 10  $\mu m$ ; 1m – mature ascus apical part with ascospores in 'AC, bar = 10  $\mu m$ ; 1n – paraphyses apices ( $H_2O$ ), bar = 10  $\mu m$ ; photos by N. Matočec & I. Kušan.**



**Plate 2.** A collection of autumnal *Morchella esculenta* s. lat. from Ilovik islet. Microscopic features (2a-f, all in H<sub>2</sub>O). 2a – transverse perpendicular section through median area of ascumatal primary rib, bar = 100  $\mu$ m (also valid for 2b); 2b – transverse perpendicular section through the edge of ascumatal primary rib; 2c – terminal cells on the edge of ascumatal primary rib, bar = 20  $\mu$ m; 2d – pileal median perpendicular section passing through the bottom of alveole (bar valid from 2f); 2e – outermost excipular area of the stipal longitudinal perpendicular section (bar valid from 2f); 2f – innermost excipular area of the stipal longitudinal perpendicular section, bar = 100  $\mu$ m; photos by N. Matočec & I. Kušan.



	BIOCLIMATE	LOCALITY	REFERENCE	TAXON
1	Tundra	Oqertarsuaq surrounding, Greenland, Denmark	Dissing (1982)	<i>Morchella</i> spp.
2	Taiga	Fairbanks surrounding, Alaska, USA	Wurtz et al. (2005)	<i>Morchella</i> spp.
3	Altimontane continental temperate zone	Gorski kotar region, Croatia	this research	<i>M. elata</i> s. str.
4	Plain continental temperate zone	Sava river in Zagreb area, Croatia	this research	<i>M. esculenta</i> s. lat.
5	Submediterranean zone	Coastal area of Kvarner region, Croatia	this research	<i>M. esculenta</i> s. lat.
6	Mesomediterranean zone	Adriatic islets, Croatia	this research	<i>M. esculenta</i> s. lat.
7	Thermomediterranean zone	Adriatic islets, Croatia	this research	<i>M. esculenta</i> s. lat.
8	Thermomediterranean zone	Brindisi surrounding, Apulia region, Italy	Agnello & Livieri (2011)	<i>Morchella</i> spp.
9	Arid Mediterranean zone	Dan reserve, Israel	Masaphy et al. (2009)	<i>M. rufobrunnea</i>
10	Riparian habitat in arid Mediterranean zone	Dan reserve, Israel	Goldway et al. (2000)	<i>M. esculenta</i> s. lat.
11	Humid subtropics	Shivalik hills, Himachal Pradesh, India	Singh et al. (2004)	<i>M. esculenta</i> s. lat.
12	Tropical savanna	Xalapa region (Veracruz), Mexico	Guzmán & Tapia (1998)	<i>M. rufobrunnea</i>
13	Tropical savanna	Xalapa region (Veracruz), Mexico	Guzmán & Tapia (1998)	<i>M. guatemalensis</i>

Plate 3. *Morchella* spp. populations as representatives of different geographical and bioclimatic areas in Northern Hemisphere. Map (upper diagram) displays the geographical range of different bioclimatic areas studied with 13 localities of selected morel taxa (as bioclimatic representatives) superposed; Graph (median diagram) displays normal fructification periods of 13 selected morel populations (as bioclimatic representatives) designated in corresponding colour of given bioclimatic area. Grey bar designates unusual autumnal morel occurrence; Table (lower diagram) gives further details on morel populations studied (names of localities with morel populations monitored as selected bioclimatic representatives, corresponding reference and taxa studied).

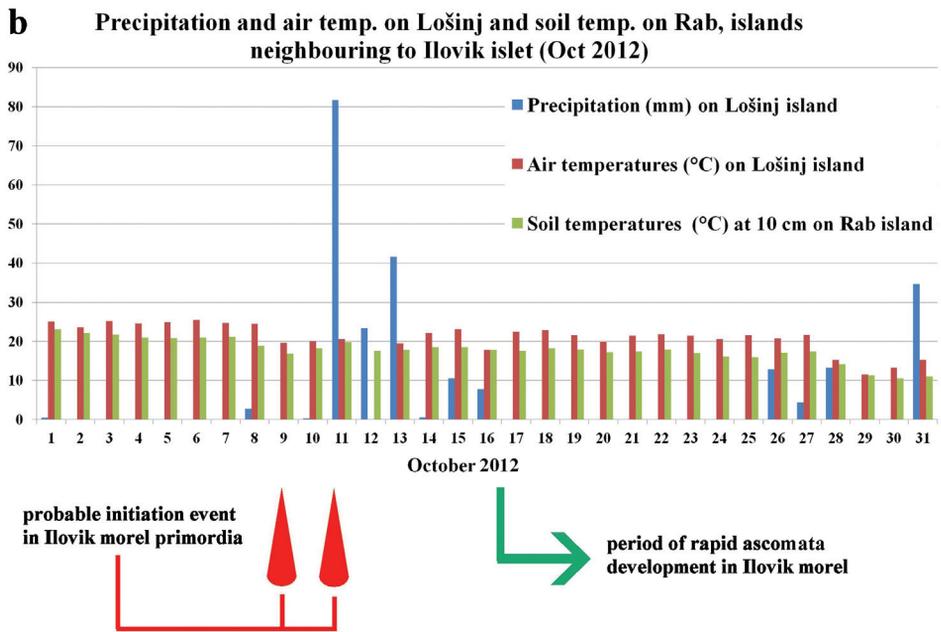
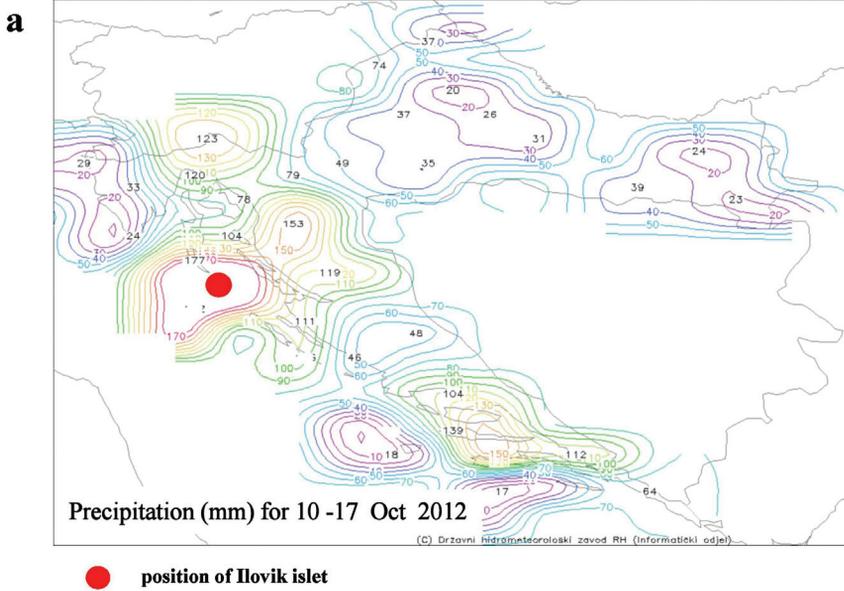


Plate 4. Meteorological events covering the Ilovik islet area under which *Morchella esculenta* s. lat. occurred in autumn. 4a – The situation in Croatia with heaviest rainfall in October that encompasses the Ilovik islet area (the position of Ilovik islet is marked by a red dot); 4b – climatologic elements (daily rainfall, soil and air temperature for October) on Lošinj and Rab, islands near Ilovik islet that are subjected to the same weather events and climatic regime. Probable triggering of the morel primordia is marked with red cones, while the period of rapid fruit body development is marked with the green arrow.