

# Mediterranean fungus *Gymnopilus suberis* discovered in Central Europe – a consequence of global warming?

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Holec J., Kříž M., Kolařík M. & Žák M. (2016): Mediterranean fungus *Gymnopilus suberis* discovered in Central Europe – a consequence of global warming? – *Sydowia* 68: 69–85.

The Mediterranean fungus *Gymnopilus suberis* (Basidiomycota, Agaricales), which typically lives on wood of cork oak, has been found growing on decaying cherry logs on Oblík hill in the Czech Republic. Molecular methods have confirmed the morphological identification. We compared the species' fructification with long-term climate data from three neighbouring climatological stations and three Mediterranean ones. Although the two regions significantly differ in their macroclimate, the specific microclimate of Oblík hill approaches Mediterranean conditions, particularly in high summer temperatures. We found a statistically supported warming trend in the Oblík region. Temperatures peaked between 2000 and 2006, and the occurrence of the five warmest years after 1990 correlated with the start of the species' fructification. The warming macroclimate and hot local microclimate seem to be responsible for the occurrence of *G. suberis* in Central Europe. We discuss the biological context of this change in distribution range and host affinity, and comment on the identity of related *Gymnopilus* species.

Keywords: ecology, taxonomy, phylogeny, climate change, *Quercus suber*.

*Gymnopilus suberis* (Maire) Singer is a distinctive Mediterranean fungus that usually grows on dead wood of *Quercus suber* and rarely also of *Q. lusitanica* (Malençon & Bertault 1970). The species has been described from Algeria by Maire (1928) as *Pholiota suberis*. It is also known from Morocco (e.g., Malençon & Bertault 1970, Maire et al. 2009), Portugal (e.g., Barrento 2012), Spain (e.g., Maire 1937; Moreno et al. 1982, 1994), southern France (e.g., Bon & Roux 2002), and Italy (e.g., herbarium specimens in SAF, MCVE; for acronyms, see Thiers 2016). Its distribution corresponds well to the natural distribution area of *Q. suber* (Carrión et al. 2000, EUFORGEN 2009, Gil & Varela 2008, Lumaret et al. 2005, Magri et al. 2007, Petroselli 2013). So far, the species has been included in only one molecular study (Guzmán-Dávalos et al. 2003), which shows that *G. suberis* is closely related to some representatives of the *G. aeruginosus-luteofolius* clade.

Since 1993, Czech amateur mycologists E. Skála and V. Zíta have observed this remarkable species on Oblík hill (Czech Republic). Its basidiomata have repeatedly been found on fallen, decaying logs of cultivated cherry trees (*Prunus avium*) in an old or-

chard. The fungus has been identified as *Gymnopilus* sp. or *Gymnopilus igniculus* before 2014. The species had never been found in the Czech Republic or in any other European country outside the Mediterranean region. Oblík hill is a well-known and extensively studied model xerothermic locality (Slavíková et al. 1983). There are unique thermophilic habitats, for example a dry steppe grasslands dominated by *Stipa* species (Chytrý et al. 2007: 418–420), inhabited by thermophilic fungi such as *Calvatia fragilis*, *Flammulina ononidis*, *Marasmiellus carneopallidus*, *Polyporus rhizophilus* (Klán 1984; recent collections by M. Kříž in the PRM herbarium) and *Vuilleminia cystidiata* (PRM). However, the unexpected discovery of this thermophilic Mediterranean species in the heart of much colder Central Europe might indicate that the ongoing climate change (global warming) might play a role in this occurrence. The influence of global change on fungal biology has been widely discussed. Most studies focus on shifts in fruiting phenology (see review by Boddy et al. 2014), host shifts (e.g., Gange et al. 2011), and changes in fungal diversity (e.g. Newsham et al. 2015). However, case studies focused on the

correlation between occurrence/fructification data and shifts in local climate data are rare (e.g., Siebold & Tiedemann 2012, Venturella et al. 2012).

This paper focuses on the following questions: (1) Is the fungus recorded on Oblík hill really conspecific with the Mediterranean *G. suberis*? (2) What are the similarities or differences between the climate of Oblík hill and Mediterranean localities of *G. suberis*? (3) Is the unexpected occurrence of its basidiomata on Oblík hill correlated with a shift in the local climate?

### Materials and methods

#### Field work and traditional taxonomic methods

The Oblík locality has been visited by Czech amateur mycologists E. Skála and V. Zíta (sometimes together with J. Šutara and P. Špinar) in the period 1983–2007 (except 1992), mostly 2–3(5) times a year between May and November. Later on, the monitoring was carried out by M. Kříž (2009–2013) and J. Holec (2014). Altogether, the study period represents the interval of 32 years covered by 57 locality visits. All data are stored by J. Holec (not shown here to spare place) and can be provided upon request.

The description of macro- and microcharacters was compiled according to the documented records of M. Kříž (see below). We studied these collections microscopically by traditional taxonomic methods (e.g., Singer 1986, Bas et al. 1988), using Melzer's reagent, 5 % KOH solution (all characters), and pure water (pigmentation). Spore size values were estimated as 90 (prevailing "normal" values) and 10 (marginal values in brackets) percentiles of all spores measured (20 measurements per collection). Voucher specimens are kept in the PRM herbarium. For herbarium acronyms, see Thiers (2016).

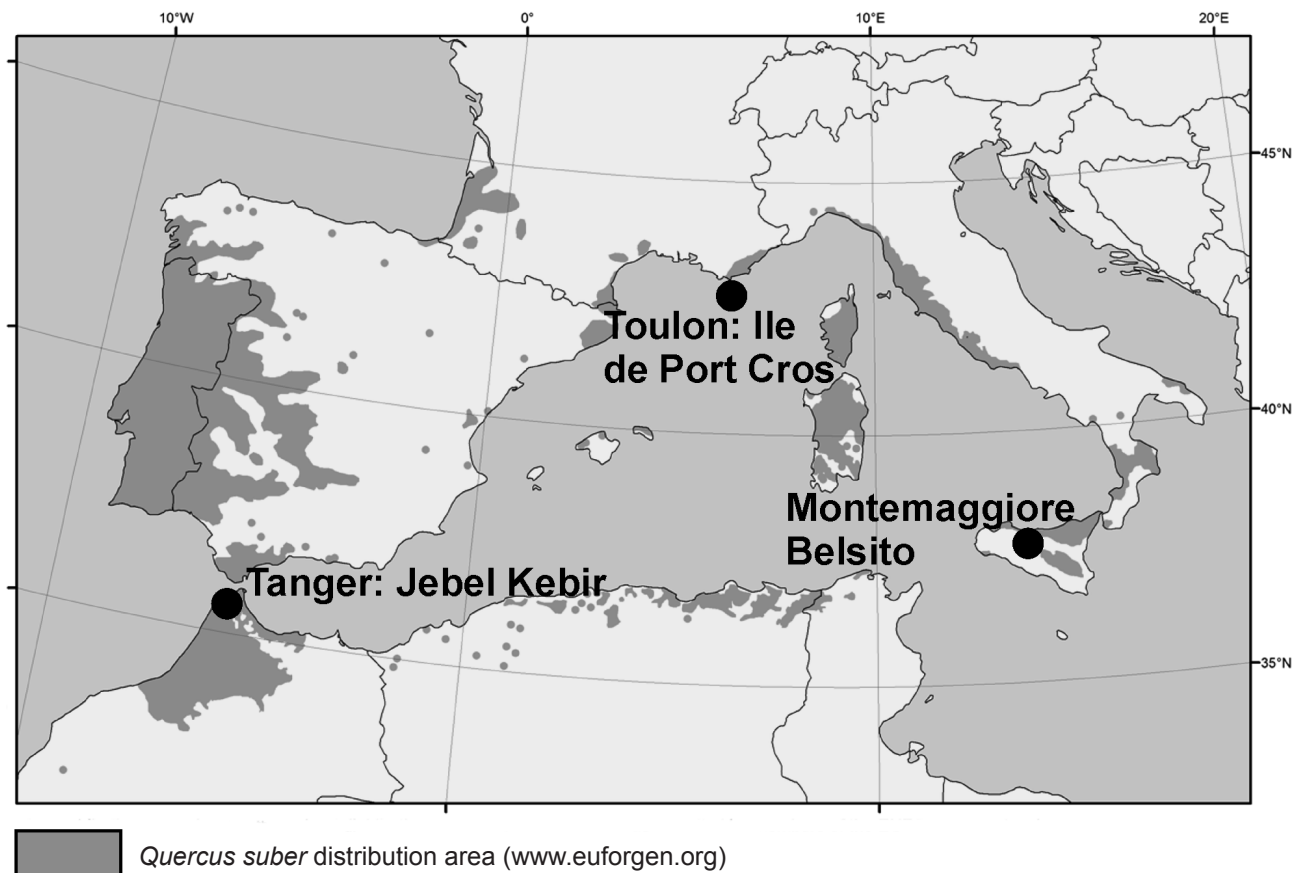
Habitat conditions of Oblík locality, collections studied

#### *Gymnopilus suberis*

Czech Republic, central Bohemia, České Středohoří Hills, 6 km N of the town of Louny (Fig. 1), Oblík hill (National Nature Reserve), small flat area under the steep WSW slope of the hill (Fig. 3a), 50.40939N, 13.80297E (coordinates of the locality centre), alt. 345–360 m a.s.l., contact zone of the basaltic hill and its limestone to marlstone background, old cherry orchard on clayey soil, formerly grazed by sheep, then abandoned, recently partly recultivated



**Fig. 1.** Geographic position of Oblík hill (black circle) and surrounding climatological stations (white circles).



**Fig. 2.** Distribution of *Quercus suber* (dark grey area) and positions of Mediterranean climatological stations selected for this study. Names of stations are followed by names of *Gymnopilus suberis* localities; only Montemaggiore Belsito indicates both the name of the station and the locality. Basic map kindly provided by EUFORGEN (www.euforgen.org), adapted for this paper.

and grazed again. Altogether, at least 5 microlocalities distributed in the area of about  $200 \times 200$  m have been discovered. Basidiomata of *G. suberis* were found on fallen decaying logs of a cherry tree (cultivar of *Prunus avium* = *Cerasus avium*), often 0.1–0.5 m above ground (on protruding parts or log heaps). – Observations of basidiomata: 26 June 1993, 28 June 1997, 30 September 2001, 16 August 2005 (E. Skála and V. Zíta), 19 July 2012, 4 November 2013 (M. Kříž), 11 June 2014 (J. Holec). – Documented records: 24 June 2001, leg. E. Skála and V. Zíta (LIT 3817/4459); 6 June 2004, leg. E. Skála and V. Zíta (PRM, Fig. 3b); 8 June 2009, leg. M. Kříž (PRM 923694, Fig. 3c); 29 May 2010, leg. M. Kříž (PRM 923698, Fig. 3d–e); 24 June 2011, leg. M. Kříž (PRM 923695); 21 September 2011, leg. M. Kříž (PRM 923696); 7 Jun 2013, leg. M. Kříž (PRM 923697, Fig. 3f).

Spain, Extremadura, Badajoz, Herrera del Duque, *Quercus suber*, 20 April 1981, leg. P. Domínguez, det. G. Moreno (PRM 923203, representing a duplicate of AH 1854); Cádiz, near Algeciras, Parque

Natural Los Alcornocales, alt. 560 m a.s.l., *Quercus suber*, 30 November 2013, leg. & det. M. À. Pérez-De-Gregorio (PRM 933004).

#### *Gymnopilus luteofolius*

Russia, Siberia, Sakha Republic, Yakutsk, Chochu, alt. 100 m, 61°60' N, 129°36' E, on a rotten trunk of *Betula platyphylla*, 14 August 1999, leg. & det. U. Peintner (IB 1999/0678).

#### Molecular methods

We selected representative specimens identified as *G. suberis* (Czech Republic, Spain). *Gymnopilus luteofolius* IB 1999/0678 turned out to be identical with our collections based on its ITS rDNA sequences (AF325668) and was therefore included into our study. We also included two specimens of *G. igniculus*, an ecologically and morphologically related species whose phylogenetic position is unknown. Isolation of DNA from dried specimens fol-



lowed Holec & Kolařík (2013). ITS-LSU rDNA was amplified using primers ITS1F and LR6, and the same, together with ITS4 and NL1, were used for sequencing (see Holec & Kolařík 2013 for details). ITS rDNA sequences were combined with related sequences published by Guzmán-Dávalos et al. (2003), Rees et al. (2004) and Guzmán-Dávalos et al. (2008) (Tab. 1) selected based on a BlastN similarity search and subsequent phylogenetic analyses. Outgroups (*G. spectabilis* and *G. junonius*) were selected based on the whole genus phylogeny published by Guzmán-Dávalos et al. (2003). Sequences were aligned using MAFFT 6 (<http://mafft.cbrc.jp/alignment/software>; Katoh & Toh 2008). The final dataset comprised a total of 30 sequences (of which six were obtained in this study) and 648 positions (612 bp of the ITS region, 36 bp of LSU rDNA), of which 71 were variable and 6 were singletons. Maximum likelihood (ML) analyses were performed in PhyML 3.0 (Guindon et al. 2010), using the HKY+G parameter model. Bootstrap support was obtained using 300 replicates. Evolutionary models were computed using MEGA 5.05 (Tamura et al. 2011). Bayesian phylogenetic analyses were done in MrBayes 3.0 (Ronquist & Huelsenbeck 2003) software using the same methods as described in Holec & Kolařík (2013). Sequences of the LSU rDNA gene were compared with those deposited in GenBank database, but not evaluated phylogenetically due to their low variability in this fungal group.

Additional DNA markers, reported to be variable in basidiomycetes (Balasundaram et al. 2015), were amplified and sequenced using various protocols. The high quality sequences were obtained only from the mitochondrial SSU rDNA (mtSSU) gene and partially also transcription elongation factor (*TEF1α*). *TEF1α* gene sequences were obtained only from the recently sampled material (5 collections) using the protocol of Kolařík & Jankowiak (2013). The couple of primers V6U/V6R and V9U/V9R and methods of Gonzalez & Labarère (1998) were used to amplify the mtSSU rDNA regions containing variable domains V6 and V9, respectively. DNA replication licensing factor (*mcm7*), another gene used for cryptic species discovery (Stefani et al. 2014), was amplified using the primers *Mcm7-709f* and *Mcm7-1348r* according to Schmitt et al. (2009). The haplotype network was constructed using the statistical parsimony algorithm TCS (Clement et al. 2002) implemented in the software PopArt (Leigh & Bryant 2015). The indels were recoded as new characters to be informative in TCS analysis.

Sequences were deposited in the EMBL sequence database and their accession numbers are

LN889949–LN889962 in mtSSU, *mcm7* and *TEF1α* or are listed in Tab. 1 in the case of nuclear rDNA sequences.

#### Climate data

No climatological station is located directly on Oblík hill. There are several stations in the vicinity of Oblík hill that are operated by the Czech Hydrometeorological Institute. From its network, M. Žák expertly selected three stations close to the hill (Tab. 2, Fig. 1). The stations measure temperature regularly three times a day (7, 14, and 21 o'clock) and precipitation once a day (7 o'clock a.m.).

Because the Žatec station has the longest record of temperature measurements, we used its data (period 1947–2013) to study long-term trends. Besides the continuous linear trend, we focused on the course of progressive monthly air temperature deviation sums. These deviations are computed as differences between the monthly average of a given month and the long-term monthly average. We computed average annual trends of various days (with the following arbitrary thresholds: maximum temperature above 25 and 30 °C and minimum temperature below 10 °C). Finally, we calculated the statistical significance of these trends as described in Huth & Pokorná (2004).

The selected stations provide different lengths of observation periods (Tab. 2). For this reason, we constructed technical time series for all three stations to obtain complete time series of temperature (daily mean, maximum, minimum) and precipitation data (daily sums). This is a standard climatological procedure for filling in and correcting time series using data from surrounding stations (for details, see Květoň & Žák 2004).

To compare the climate of Oblík hill with the Mediterranean, we selected three climatological stations close to *G. suberis* localities in different Mediterranean regions (Tab. 3, Fig. 2). The stations cover different landscapes (islands versus inland, low versus high altitude) and geographical gradients running from the north to the south (France – Morocco) and from the west to the east (Morocco – Italy). The source of data for the Montemaggiore Belsito station is the web page of the Meteorological service of Italy (<http://clima.meteoam.it>). Data for the Tanger and Toulon stations come from the web page of the National Climate Data Center of NOAA ([www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)). The period of observation is 1961–2000 for all three stations. For the purposes of this study, we used monthly long-term

**Tab. 1.** Collections used for DNA analysis and sequences obtained from the NCBI GenBank for comparison. Collections printed in bold were obtained during this study. (n.d. = no data, s.n. = without number)

Species	Country	Voucher	DNA sequence	Publication
<i>G. aeruginosus</i>	USA	SAR-88/431 (WTU)	AY280975	Guzmán-Dávalos et al. (2003)
<i>G. aeruginosus</i>	Canada	P. Kroeger 1336 (DAOM-210322)	AY280974	Guzmán-Dávalos et al. (2003)
<i>G. aeruginosus</i>	Canada	F.M. Brigham s.n. (DAOM-137158)	AY280976	Guzmán-Dávalos et al. (2003)
<i>G. cerasinus</i>	n.d.	Herb. E.A. Burt (S)	AY280978	Guzmán-Dávalos et al. (2003)
<i>G. cf. punctifolius</i>	USA	L. Norvell 92-04.20-1)(WTU)	AY280993	Guzmán-Dávalos et al. (2003)
<i>G. cyanopalmicola</i>	Mexico	F. Ramírez-Guillén 254a (XAL, type)	EU401711	Guzmán-Dávalos et al. (2008)
<i>G. dilepis</i>	Malaysia	R. Treu (IMI-370900)	AY280980	Guzmán-Dávalos et al. (2003)
<i>G. hispidellus</i>	Honduras	Standley 53856 (F-1112036)	AY280983	Guzmán-Dávalos et al. (2003)
<i>G. hispidus</i>	Dominica	D.N. Pegler 3254 [K(M) 75211]	AY280985	Guzmán-Dávalos et al. (2003)
<b><i>G. igniculus</i></b>	<b>Czech Republic</b>	<b>PRM 900986</b>	<b>HG969655</b>	<b>This study</b>
<b><i>G. igniculus</i></b>	<b>Belgium</b>	<b>PAM 01120901, isotype</b>	<b>HG969656</b>	<b>This study</b>
<i>G. junonius</i>	France	L. Guzmán-Dávalos 8220 (IBUG)	AY280987	Guzmán-Dávalos et al. (2003)
<i>G. lepidotus</i>	Mexico	G. Guzmán 30602 (XAL)	AY280991	Guzmán-Dávalos et al. (2003)
<i>G. luteofolius</i> s. Peintner	Russia	IB 1999/0678	AF325668	n.d.
<i>G. luteofolius</i>	USA	L.R. Hesler & H. Ford (DAOM-80626)	AY280992	Guzmán-Dávalos et al. (2003)
<i>G. medius</i>	Mexico	J. García-Franco s.n. (IBUG, isotype)	AY280994	Guzmán-Dávalos et al. (2003)
<i>G. purpuratus</i>	Switzerland	ZT02.01	AY386818	Rees et al. (2004)
<i>G. purpuratus</i>	Australia	BRWA99.14	AY386822	Rees et al. (2004)
<i>G. purpureo-squamulosus</i>	Panama	C.L. Ovrebo 3594 (IBUG)	EU401712	Guzmán-Dávalos et al. (2008)
<i>G. purpureo-squamulosus</i>	Zimbabwe	L. Ryvarden & K. Høiland G24 (O-72839, holotype)	EU401713	Guzmán-Dávalos et al. (2008)
<i>G. purpureo-squamulosus</i> (as <i>G. cf. peliolepis</i> in Guzmán-Dávalos et al. 2003)	Switzerland	O. Röllin 89-16 (IBUG)	AY280998	Guzmán-Dávalos et al. (2003, 2008)
<i>G. purpureo-squamulosus</i> (as <i>G. cf. palmicola</i> in Guzmán-Dávalos et al. 2003)	Nigeria	M.H. Zoberi 342 [K (M) 75214]	AY280979	Guzmán-Dávalos et al. (2003, 2008)
<i>G. spectabilis</i>	Argentina	E. Albertó s.n. (BAFC-32.321)	AY281009	Guzmán-Dávalos et al. (2003)
<i>G. spectabilis</i>	France	L. Guzmán-Dávalos 8221 (IBUG)	AY281008	Guzmán-Dávalos et al. (2003)
<b><i>G. suberis</i></b>	<b>Czech Rep.</b>	<b>PRM 923695</b>	<b>HG969651</b>	<b>This study</b>
<b><i>G. suberis</i></b>	<b>Czech Rep.</b>	<b>PRM 923698</b>	<b>HG969653</b>	<b>This study</b>
<b><i>G. suberis</i></b>	<b>Czech Rep.</b>	<b>PRM 923697</b>	<b>HG969652</b>	<b>This study</b>
<b><i>G. suberis</i></b>	<b>Spain</b>	PRM 923203 (duplicate from AH 1854)	HG969654	<b>This study</b>
<b><i>G. suberis</i></b>	<b>Spain</b>	PRM 933004	LN889948	<b>This study</b>
<i>G. suberis</i>	Spain	M.A. Pérez-de-Gregorio s.n. (IBUG)	AY281015	Guzmán-Dávalos et al. (2003)
<i>G. subpurpuratus</i>	Mexico	L. Guzmán-Dávalos 5303 (IBUG)	AY281016	Guzmán-Dávalos et al. (2003)





**Fig. 3.** *Gymnopilus suberis* basidiomata from Oblík hill, Czech Republic. **a:** Oblík hill with locality of *G. suberis* (red line), **b:** PRM 6 Jun 2004, **c:** PRM 923694, **d–e:** PRM 923698, **f:** PRM 923697. **a, c–f:** photo by M. Kříž, **b:** photo by V. Zita.

**Tab. 2.** Climatological stations in the vicinity of Oblík hill, Czech Republic.

Station	Altitude (m above sea level)	Latitude (°)	Longitude (°)	Data available since	Distance from Oblík hill (km)
Kopisty	240	50.54	13.62	1970	19
Smolnice	345	50.31	13.86	1978	12
Žatec	210	50.34	13.54	1947	18

**Tab. 3.** Climatological stations in the Mediterranean.

Station	Region	Nearest locality of <i>G. suberis</i> (source)	Altitude (m)	Latitude (°)	Longitude (°)
Toulon	Southern France	Ile de Port Cros (Bon and Roux 2002)	25	43.12	5.92
Montemaggiore Belsito	Italy: Sicily	Montemaggiore Belsito: 10 km E (herb. SAF)	830	37.85	13.48
Tanger	Northwest Morocco	Jebel Kebir (costal forest W of Tanger) (Malençon and Bertault 1970, as "Jbel Kbir")	29	35.77	- 5.80

averages (of mean, maximum, and minimum temperature and precipitation sums).

The climatic differences between the periods of *G. suberis* absence and presence were tested statistically using the non-parametric Mann-Whitney U test in Past software (Hammer et al. 2001). This test shows whether the medians of two independent distributions are different.

### Results

#### Identity of *Gymnopilus suberis* from Oblík hill

##### Macrocharacters (Fig. 3)

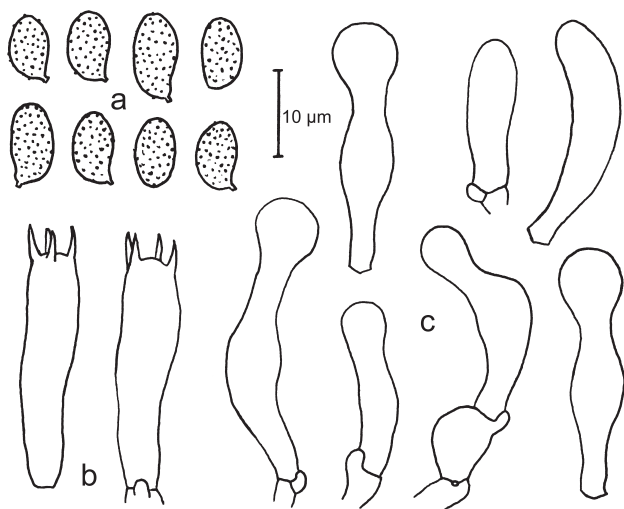
**B**asidiomata variable in size and habit, either small and slender or medium-sized and robust. – **P**artial veil only on youngest basidiomata, quickly disappearing, pale yellow to ochre, tomentose, seen as disrupted floccose-tomentose scales on the pileus surface and cover layer between the pileus margin and stipe apex, leaving pale yellow scales on the margin. – **P**ileus 10–20 mm when small, 40–70(85) mm when medium-sized, at first subglobose to broadly obtusely conical with an inflexed to almost involute margin, then subglobose to convex with a deflexed to reflexed margin, sometimes with a low broad umbo. – **P**ileus cuticle dry, at first fibrillose-scaly (more scaly towards the centre) and brown-violet to rhubarb reddish with a violet tinge, which quickly disappears, background colour becoming rusty yellow to golden yellow-brown, sometimes almost yellow, at other times (later) deeply rusty, either with an almost glabrous surface (the fibrillose-scaly covering can be almost washed away, and the pileus becomes brown radially striped, especially towards the margin) or with contrasting, brownish to rusty-brown, finally dark brown scales, which are mostly innate, rarely with upturned ends, more distinct towards the centre, usually soft, sometimes (especially in dry weather) becoming cracked into frames with broad gaps, es-

pecially in the centre. The cuticle is separable to the half of the pileus radius. – **L**amellae sparse, 30–50 reaching the stipe, with three levels of lamellae, emarginate, adnate, to adnate with a decurrent tooth, at first yellow-ochre to pale yellow, then rusty yellow to vividly rusty, edge concolorous, changing brown when bruised. – **S**tipe 20–45 × 2–11 mm, cylindrical, sometimes with an indistinct basal bulb when slender, pale yellowish or with a pale violet tinge, sometimes with a contrast of yellowish lamellae and purplish stipe apex, covered with a fibrillose annulus-like zone, which is rusty from spore deposit when mature, later darkening to rusty brown, with pale greyish-brownish tinges when mature, ± innately fibrillose, then slightly glossy, base changing brown when bruised. – **C**ontext yellow in stipe, paler in pileus, dirty yellowish, later darkening to brown in stipe. – **T**aste unpleasantly bitter. – **S**mell indistinct, alternatively rather fungoid-fruity (like *Gymnopilus penetrans*). – Some parts of the basidiomata yellow in UV light (lamellae, context under the pileus surface, pileus margin when young), but not very distinctly.

##### Microcharacters (Fig. 4)

**B**asidiospores 7.2–8.8(9.6) × (4.0)4.4–4.8 µm, ellipsoid to oblong in face view, ellipsoid to ovoid-amygdaliform with slight suprahilar depression, finely to moderately verrucose, ochre-rusty to rusty brown in 5 % KOH. – **B**asidia 24–27 × 6.5 µm, 4-spored, cylindrical to narrowly clavate, with slight median constriction, sterigmata 3–4 µm long. – **B**asidiolae 15–20 × 5.0–5.5 µm, cylindrical to narrowly clavate. – **L**amellae edge sterile, composed of cheilocystidia. – **C**heilocystidia 16–32 × 5.5–7 µm, some of them cylindrical to narrowly clavate, most of them irregularly narrowly lageniform with a short thick neck and (sub)capitate apex having a diameter of 5–7 µm, hyaline or completely filled with a homogeneous yellow-rusty pigment. –





**Fig. 4.** *Gymnopilus suberis*, microcharacters (PRM 923698). a: basidiospores, b: basidia, c: cheilocystidia.

Pleurocystidia not observed. – Lamellar trama regular, of 2–12  $\mu\text{m}$  broad hyphae. – Pileipellis a cutis of densely arranged parallel hyphae 3–8(10)  $\mu\text{m}$  broad, with yellow membranous and encrusting pigment, uppermost layer (epicutis) of more pigmented hyphae 2.5–3  $\mu\text{m}$  broad, forming a net of hyphal cords (best seen on scalps from pileus surface). Narrowly clavate elements (“pileocystidia”) up to 17  $\mu\text{m}$  broad, rarely observed. – Scales formed by densely arranged cords or conglomerates of interwoven and branched hyphae different from those of the epicutis, 3–10.5  $\mu\text{m}$  broad, filled with homogeneous rusty brown pigment in 5% KOH. – Pileocystidia resembling cheilocystidia not observed. – Veil (annulus) composed of densely arranged parallel hyphae 2.5–5.5  $\mu\text{m}$  broad, mostly hyaline but some of the narrowest hyphae filled with homogeneous ochre-rusty pigment, other hyphae with yellow wall and dense rusty yellow incrustations. – Stipitipellis a cutis of densely arranged parallel hyphae 2.5–5.5  $\mu\text{m}$  broad, with yellow membranous pigment and rusty yellow incrustations.

#### Phylogeny and species identity (Figs. 5, 6)

Czech and Spanish specimens of *G. suberis* had identical LSU rDNA sequences (800 bp). The most closely related sequences from the NCBI GenBank were 99 % similar (3–4 differences in 800 bp) and belonged to *G. aeruginosus* (AF261650), *G. purpuratus* (AY219604), *G. luteofolius* (AY219599), and *G. megasporus* (AY219601).

Phylogenetic relationships of more variable ITS rDNA was studied using Maximum likelihood and

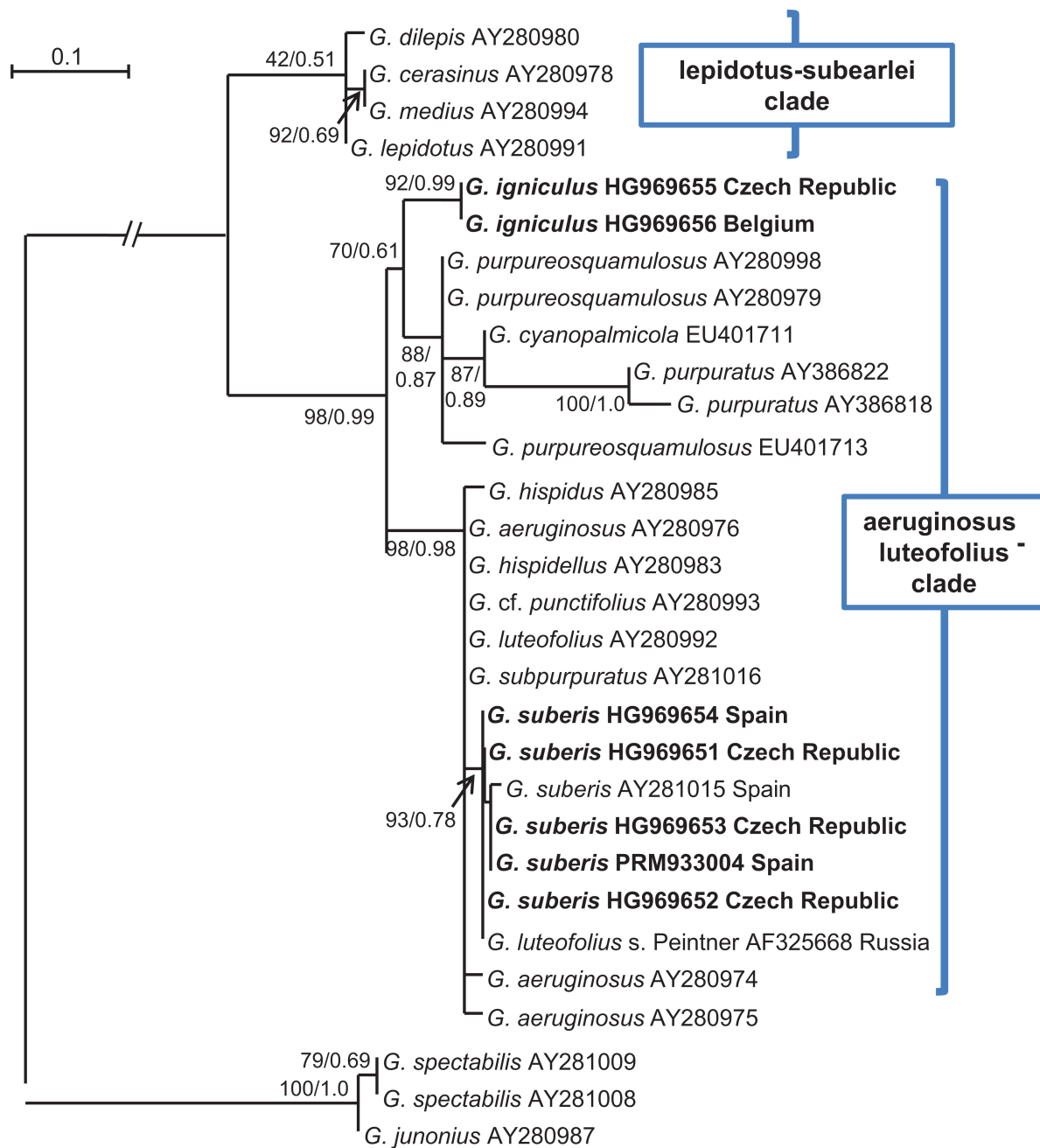
Bayesian analyses. Both analyses resulted in trees with the same topology and recognized a strongly supported *suberis-aeruginosus* clade comprising *G. aeruginosus*, *G. luteofolius*, *G. hispidus*, *G. hispidellus*, *G. cf. punctifolius*, *G. suberis*, and *G. subpurpuratus* with no or very small (1–4 bp) interspecies variability (Fig. 5). Within this group, it is possible to distinguish a statistically supported sub-clade comprising *G. suberis* from the Czech Republic and Spain together with *G. luteofolius* s. Peintner from Russia. The sequences of *G. suberis* and those of the species listed above differed by 2–4 bp. The alignment of the *G. suberis* sub-clade consisted of seven sequences with three variable positions. Most of the sequences were heterozygous in these positions (i.e., contained two bases at the same position). Some Czech and Spanish collections (HG969652, HG969654) were identical with *G. luteofolius* s. Peintner from Russia (AF325668). This whole set of species formed a strongly supported clade with *G. purpureosquamulosus*, *G. purpuratus*, *G. cyanopalmicola* and *G. igniculus*, which is a sister clade to species such as *G. dilepis*, *G. lepidotus*, etc. belonging to the *lepidotus-subearlei* clade recognized by Guzmán-Dávalos et al. (2003). In the ITS region, there were two variable positions from 663 bp among five collections of *G. suberis* sequenced by us. Haplotypes with heterozygous positions were recoded and more homozygous haplotypes from each collection were obtained. Three haplotypes were found in total, two of them containing both the Czech and Spanish collections (Fig. 6). Mitochondrial SSU sequences (V6 and V9 domain, 753 pb) contained two variable positions (both indels) and separated specimens to three haplotypes, from which two contained both the Czech and Spanish collections. Finally, the *mcm7* gene (667 bp) showed two haplotypes and both Spanish collections differed from the Czech ones in a single substitution. There were no shared haplotypes between collections when the concatenated dataset (2082 bp) was analysed. The collections differed in one mutation from each other and there was no apparent clustering according to their geographic origin (Fig. 6).

As regards *G. igniculus*, the Czech and Belgium specimens have identical ITS sequences (four positions are heterozygous).

#### Macroclimate of Oblík and Mediterranean localities of *Gymnopilus suberis*

Based on data from three climatological stations close to Oblík hill (Tab. 2, Figs. 1, 7), the annual average temperature is around 8.8 °C with July being



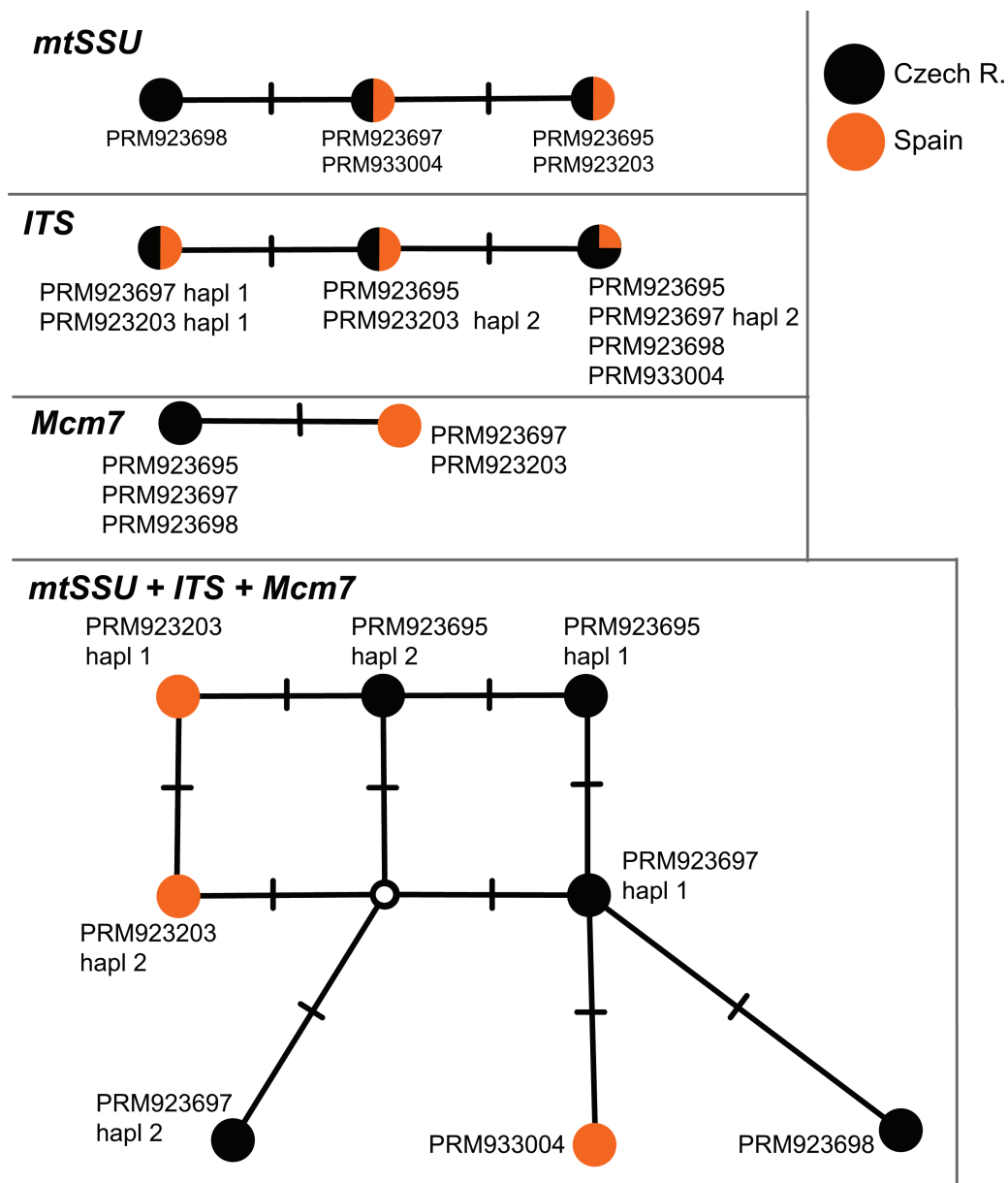


**Fig. 5.** Phylogenetic placement of *Gymnopilus suberis* inferred from ITS rDNA data using PhyML. Bayesian posterior probabilities are given after Maximum likelihood bootstraps. The tree is rooted with *G. spectabilis* and *G. junonius*. Sequences printed in bold were obtained in this study. The branch leading to the outgroup was shortened by half for illustrative purposes.

the warmest month (18.2 to 19 °C) and January the coldest (−1.5 to −0.9 °C). The average daily maximum is around 13 °C and minimum 4.5 °C, the difference among the stations being only a few tenths of a degree Celsius. Variation in annual precipitation is straightforward, with its main maximum in July and main minimum in February (Fig. 7). The

average annual precipitation total is 462–506 mm. Again, the differences among the stations remain relatively small, around 5 mm in winter and over 10 mm in summer months.

The three Mediterranean stations (Tab. 3, Figs. 2, 7) indicate much warmer winters compared to the Czech ones (by about 10 °C), but the difference in



**Fig. 6.** TCS haplotype networks for single gene and concatenated data set obtained from five specimens of *Gymnopilus suberis*. The network was generated using PopArt. Inferred mutational steps (hashlines) separate recovered (full circles) and hypothetical (empty circle) haplotypes.

summer months is somewhat smaller (by about 4–6 °C). The annual course of precipitation is the opposite of that at the Czech stations, which have a pronounced summer minimum and receive about 20–30 % higher annual totals.

When taking into account the most used climate classification of Köppen–Geiger (M. Kottek et al. 2006) the Mediterranean stations are within Csa climate type (warm temperate climate with dry and hot summer), while Oblík has Cfb type of climate

(warm temperate climate, humid, with warm summer; Tolasz et al. 2007).

Generally, there are significant macroclimatic differences between localities of *G. suberis* in the Mediterranean and on Oblík hill, as can be clearly seen from the climatological data and the above mentioned classification. Of course, there are also large differences among Mediterranean localities, attributable to their different location and geomorphology (Tab. 3, Fig. 2).

*Gymnopilus suberis* on Oblík hill – a consequence of climate change?

#### Records of *G. suberis* on Oblík hill

As shown in detail in Collections studied, basidiomata of *G. suberis* were first observed in 1993 and then in 1997, 2001, 2004, and 2005. Since 2009, basidiomata have been observed every year. The fruiting period lasts from late May to late September (exceptionally to early November). The highest number of records originates from June. Unfortunately, the first documented record was made only in 2001. The species has not been observed in the period 1983–1991 during 24 visits (May – November).

#### Trends in local climate

The selected climatological stations (Tab. 2, Fig. 1) provide the best possible picture of climate conditions in Oblík region. Žatec station, which provides the longest series of temperature measurements (Fig. 8), shows quite a large fluctuation in annual average temperature with the coldest year being 1956 (7.1 °C) and the warmest 2000 (10.7 °C). All the top five warmest years occurred after 1990 (1992, 1994, 2000, 2002, and 2007), and the 5-year moving average attained the highest values around 2002. The linear trend is 0.14 °C per decade, which is statistically significant. As regards linear trends of monthly averages, only those for April and August are statistically significant whereas the other months show large interannual variations leading to statistically non-significant linear trends (Tab. 4).

The graph of progressive monthly air temperature deviation sums (Fig. 9) shows an interesting

picture. From the course of these sums, we can clearly infer a change in the direction of their trend in the seventies of the 20<sup>th</sup> century with a really strong upward trend during the period of 1997–2002, followed by a slower increase in the subsequent period.

As regards extreme temperatures, we focused on trends in their occurrence frequency. For these reasons, we selected three thresholds: 25 and 30 °C for the maximum daily temperature, and –10 °C for the minimum daily temperature. We computed trends for the period of 1961–2012 (see Tab. 5 presenting average trends per decade). Extremely cold days seem to have occurred less frequently in the last 30 years, but this trend is not statistically significant which could partly be caused by the winter of 1996/1997, one of the three coldest winters since 1961. On the other hand, there is a statistically significant increase in the number of days with high and extremely high temperatures in the last 50 years, but especially in the last 30 years.

#### Correlation of local warming and beginning of *G. suberis* fructification

As shown in previous chapter, a clear warming trend since the 80s of the 20<sup>th</sup> century is apparent at the Žatec station (Fig. 9) with a temperature peak in the years 2000–2006. The beginning of *G. suberis* fructification on Oblík hill (1993) starts after the first third of the warming period (Fig. 9). Not only the visual evaluation of box graph (Fig. 10) clearly demonstrates this fact but also the Mann-Whitney U test shows statistically significant difference ( $p < 0.0001$ ) between progressive monthly air temperature sums before the year 1993 (period 1983–1992, when *G. su-*

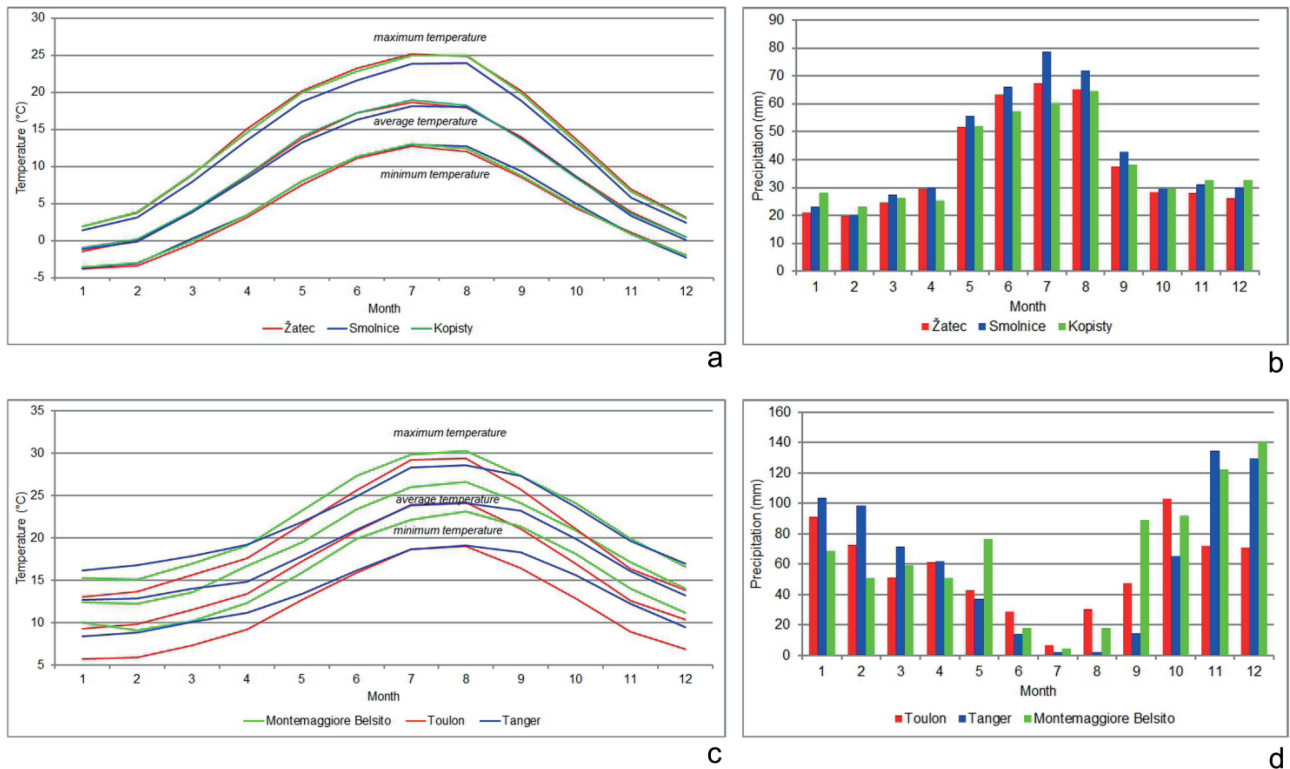
**Tab. 4.** Linear monthly and annual trends (°C/10 years) for the Žatec station (period 1947–2013). Statistically significant values are in bold.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Trend	0.32	0.35	0.24	<b>0.27</b>	0.19	0.08	0.13	<b>0.23</b>	–0.01	0.09	0.06	0.05	<b>0.15</b>

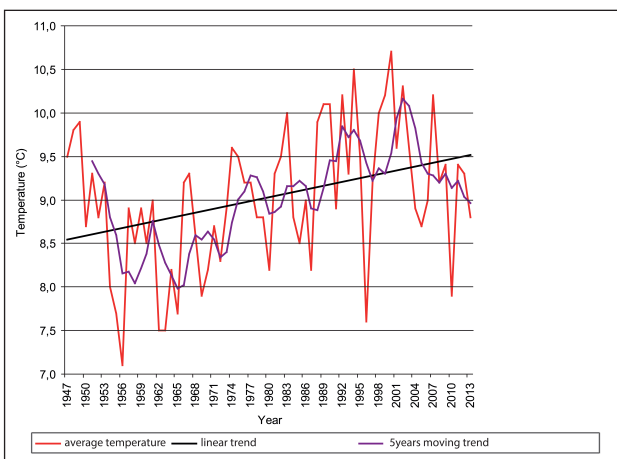
**Tab. 5.** Average linear trend of the number of days (per 10 years) with maximum temperatures above 25 and 30 °C, respectively, and with minimum temperatures below –10 °C in the period 1961–2012. Statistically significant values are in bold.

Station	Days with Tmax above 25 °C	Days with Tmax above 30 °C	Days with Tmin below –10 °C
Žatec	<b>5.1</b>	<b>1.6</b>	1.4
Kopisty	<b>7.1</b>	<b>3.3</b>	1.1
Smolnice	<b>7.6</b>	<b>2.6</b>	1.9

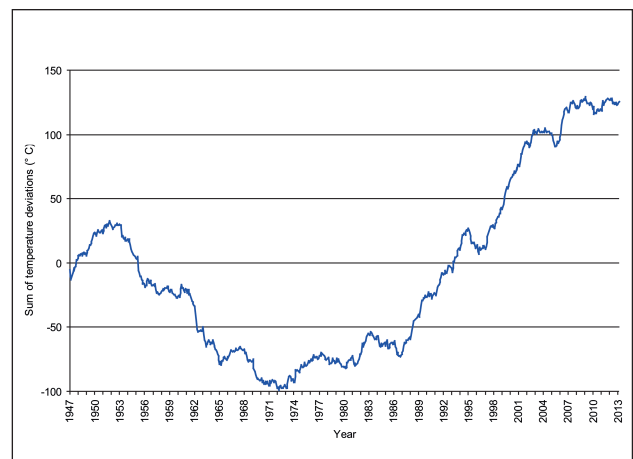




**Fig. 7.** Basic data from climatological stations in Oblík region (Žatec, Smolnice, Kopisty; period 1961–2012) and selected Mediterranean stations (Montemaggiore Belsito, Toulon, Tanger; period 1961–2012). **a, c:** annual course of air temperatures – monthly averages of daily averages, minimum, and maximum temperatures; **b, d:** annual course of monthly precipitation totals.



**Fig. 8.** Long-term course of average annual temperatures at the Žatec station for the period 1947–2013 (red line) with a linear trend (black straight line) and 5-year moving averages (blue line).



**Fig. 9.** Graph of progressive monthly air temperature deviation sums for the Žatec station in the period 1947–2013. These deviations are computed as differences between the monthly average of each given month and the long-term monthly average for the period 1947–2013.

*beris* was not observed) and after this year. Moreover, the fungus started to fructify in 1993, the year following the first of the five warmest years since 1947. The April temperature increase mentioned above probably stimulates the fructification of *G. suberis*, as the records of basidiomata start from late May.

## Discussion

### Identity of *Gymnopilus suberis* from Oblík hill

Detailed descriptions and illustrations of *G. suberis* have been published, for example, by Maire (1928), Malençon & Bertault (1970), Robich (1989), Bon & Roux (2002), and Pérez-De-Gregorio (2003). The collections from Oblík hill fit them well in all respects. The most important common morphological characters are red- to violet-tinged young pilei (tinge quickly disappearing in most cases); more or less distinct, mostly innate rusty-brown scales on a yellow, yellow-orange to yellow-brown pileus surface; pileus diameter ca. 20–100 mm; indistinct fibrillose annulus to annulus-like zone; yellow context; basidiospores  $7.0\text{--}9.5 \times 4.0\text{--}5.0(5.5)$   $\mu\text{m}$ , ellipsoid to ovoid-amygdaliform, finely to moderately verrucose; cheilocystidia mostly lageniform with a distinctly (sub)capitate apex.

High phenotype similarity is reflected by our study of variable DNA regions. ITS sequences (Fig. 5), although identical in the Czech and Spanish collections, seem to have limited taxonomic value in this group (see below) and there is a risk that the Czech collections belong to a cryptic species, differing from the Mediterranean *G. suberis* in its biology and biogeography. Thus, additional markers were studied. Two of the nine variable domains of mtSSU (V6 and V9) are reported to be species-specific and used as an efficient taxonomic marker in several genera of basidiomycetes (Gonzalez & Labarère 1998, 2000; Zhao et al. 2015; Miettinen et al. 2012). Analysis of this marker shows that the Czech collections are identical with those from Spain (Fig. 6). *Mcm7* gene is also used for species delimitation in species groups where ITS often fails to recognise biological species, e.g. in asexual moulds (e.g. *Penicillium* or *Aspergillus*) or some groups of *Cortinari* or *Hebeloma* (Eberhardt et al. 2014, Stefani et al. 2014). Single substitution in *mcm7* distinguished the Czech specimens from the Spanish ones. Such a small divergence, if found in other groups of basidiomycetes, is interpreted as a part of the intraspecific variation (Eberhardt et al. 2014, Stefani et al. 2014). We thus conclude that the Czech and Spanish collections belong to the single

species which is eventually present as two geographically delimited populations in our study.

Morphologically, *Gymnopilus suberis* is most similar to *G. luteofolius*, but the latter differs by smaller basidiospores, the presence of pleurocystidia, and a reddish context. However, Malençon & Bertault (1970: 567) report a purplish context in young, purplish-coloured basidiomata of *G. suberis*. Concerning *G. luteofolius* s. Peintner (Russia, Siberia), which has an identical ITS rDNA sequence with some *G. suberis* sequences (Fig. 5), we cannot draw a final conclusion regarding its potential conspecificity because we lack data both on its macrocharacters and other genes studied by us. Microscopically, the collection is very similar, but it has less distinctly capitate cheilocystidia (most of them are clavate or lageniform with an only slightly broadened apex), as we observed in *G. suberis*.

Our DNA study (Fig. 5) confirms that *G. suberis* is very close to other species from the *suberis-aeruginosus* clade representing a sub-clade of the previously recognized *aeruginosus-luteofolius* clade (Guzmán-Dávalos et al. 2003, 2008). The species are characterized by squamulose pilei with a purplish tinge (at least when young) and a fugacious arachnoid annulus. They share the same biogeographical pattern characterized by distribution in warmer regions (i.e., tropics, subtropics, semideserts) and disjunctive occurrences in warmer microhabitats of the temperate zone (e.g., greenhouses, compost heaps, burning coal mine dumps) (Guzmán-Dávalos et al. 2008, Holec 2005, Holec et al. 2003, Rees et al. 2004). Except for *G. suberis*, the species of *suberis-aeruginosus* clade were described from North and Central America (Hesler 1969, Guzman-Davalos et Guzman 1991). *Gymnopilus hispidus* and *G. hispidellus* are little known whereas *G. aeruginosus* and *G. luteofolius* are known from numerous records (Hesler 1969). Their morphological characters are variable with a varying degree of overlap. *Gymnopilus aeruginosus* seems to clearly differ from *G. suberis* by its blue-green tinge (Hesler 1969). However, the very close *G. subpurpuratus* (Guzman-Davalos et Guzman 1991) has red- to purple- coloured young pilei (like *G. suberis*) and a green discoloration of mature pilei (like *G. aeruginosus*), which suggests that these characters form a continuum. It, however, differs from *G. aeruginosus* (and *G. suberis*) in other characters (Guzman-Davalos et Guzman 1991), for example, shorter basidiospores.

As shown above, the taxonomic value of some morphological characters in *aeruginosus-luteofolius* clade is unclear. The same fact concerns the applicability of the ITS region for delimiting species

in this group. *Gymnopilus purpureosquamulosus*, *G. purpuratus*, and *G. igniculus* seem to be well supported by differences in this marker (Fig. 5). For distinguishing among other species, the ITS regions has been found to be less useful (see also Guzmán-Dávalos et al. 2008). This especially applies to the pair of *G. cyanopalmiticola* and *G. purpureosquamulosus*, and the species of *suberis-aeruginosus* clade (Fig. 5) having identical or very similar ITS sequences.

Generally, there are two possibilities: Collections identified as *G. aeruginosus*, *G. luteofolius*, *G. suberis*, etc. (Fig. 5), either represent a single widely distributed variable species or several separate species. For the time being, whenever clearly visible morphological and ecological differences exist between the species, it is better to treat them as separate taxa until new data are accumulated and evaluated. A worldwide-based polyphasic and multi-gene study is needed. Mediterranean records reported as *G. peliolepis* or *G. luteofolius* should be revised as they could in fact represent *G. suberis*.

In addition, we have verified the conspecificity of Czech and Belgium specimens of *G. igniculus*, previously based on morphological characters only (Holec et al. 2003).

#### Mesoclimate and microclimate of Oblík localities of *Gymnopilus suberis*

Most of the Czech Republic is classified as the Cfb climate type according to Köppen's classification (Tolasz 2007), i.e., temperate with significant precipitation in all seasons and temperatures in the warmest month between 10 and 22 °C. Climate conditions directly at the localities of *G. suberis* slightly differ from those recorded by climatological stations in the vicinity of Oblík hill. The most important regional factor is the rain shadow of the Krušné hory Mts. and the Doupovské hory Mts. (Tolasz 2007). Consequently, the mesoclimate of Oblík hill exhibits some continental features. Based on data by Slavíková et al. (1983), the mesoclimate is characterized by a reduction of precipitation and humidity, higher temperatures especially in summer and stronger winter frosts. Precipitation totals are lower by 20–40 % compared to the Smolnice station, which is the nearest. During summer, the average temperature (Slavíková et al. 1983) is higher both in the course of the day and in maximum values when compared with our reference stations (Tab. 2). The same is true for night temperatures (average differences are 0.5 to 2 °C). The situation is reversed in winter: Temperatures on Oblík (Slaví-

ková et al. 1983) are significantly lower than those of the reference stations, both in their daily course and maxima (2 to 3.5 °C), and night minima (1 to 2 °C). Interestingly, the daily temperature amplitude on Oblík is smaller than at the reference stations, which is caused by higher night temperatures. This feature (i.e., contradicting characteristics of a continental climate) is evidently caused by the high heat accumulation capacity of the basalt bedrock covered by shallow skeletal soils (Slavíková et al. 1983). It plays a significant role in influencing night temperatures through heat emission.

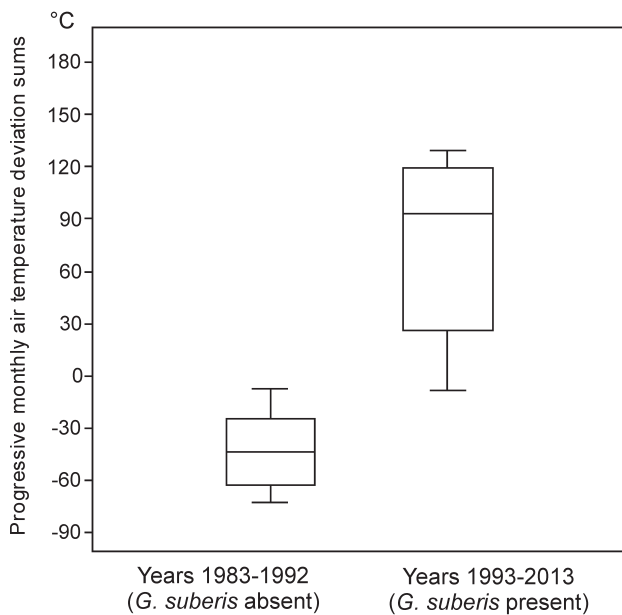
Microclimate of various sites on Oblík hill is strongly influenced by their slope and orientation. The most extreme conditions occur on sunny southwest slopes. The sites where *G. suberis* occurs are located directly in this “hot” area. Based on data collected by Slavíková et al. (1983) and our estimate, we can conclude that local day and night temperatures are higher by 1.5–4 °C in the summer half of the year compared to the reference stations and lower by 1–2.5 °C in winter. Thus, the maximum temperatures during the summer half of the year increase to 24–28 °C, and minimum temperatures increase to 12.5–15 °C. This means that the values approach temperatures in the Mediterranean (Fig. 7). In winter, the differences are higher because temperatures on Oblík hill are lower.

The importance of local microclimate conditions for fungal distribution has been stressed, for example, by Venturella et al. (2012). They observed an opposite case: an occurrence of a northern-European species of *Entoloma* in Sicily supposedly caused by exceptional cooling and extreme rainfalls during autumn.

#### Areal shift of *Gymnopilus suberis* – a consequence of climate change?

We are aware of the fact that field data on the presence/absence of *G. suberis* basidiomata on Oblík hill are far from perfect (no visits of the locality in the years 1992 and 2008; uneven number of visits in individual years). Nevertheless, they represent a long-time and reliable set of historical data, lacking for most localities/species of fungi (but see Heilmann-Clausen & Læssøe 2012). They well document absence of *G. suberis* basidiomata in the period 1983–1991, sporadic presence in 1993–2005, and annual occurrence since 2009. As *G. suberis* has striking and rather long-lasting basidiomata (they can be seen for 2–4 weeks), they certainly would not escape attention of well-trained field mycologists in the period 1983–1991.





**Fig. 10.** Box plot chart showing the difference between progressive monthly air temperature deviation sums (Žatec station, for full data range see Fig. 9) in the period 1983–1992 (no records of *G. suberis* basidiomata) and 1993–2013 (basidiomata of *G. suberis* present).

As shown in previous paragraphs, the microclimate conditions at the Oblík locality of *G. suberis* are extremely warm in the context of the Czech Republic, approaching the Mediterranean especially in summer temperatures. Moreover, local warming (observed also in other regions in the Czech Republic; Kvétoň 2001) is well-documented in previous chapters. In our opinion, these two factors account for the unexpected occurrence of this Mediterranean species in the heart of Central Europe, dated since 1993. What is the biological background, however? There are at least two possible scenarios:

1. Areal shift. The species had not been present on Oblík hill before. The records of its basidiomata document an establishing of a new population under altered climatic conditions, probably by long-distance spore dispersal.

2. Change of *G. suberis* biology. The species had been present on Oblík before (in the form of spores from long-distance dispersal or as a non-fructifying mycelium hidden in wood), and the warming enabled its fructification. Such a change in fungal activity is a complex process that involves factors such as fungal physiology, host physiology, competition, etc. (Boddy et al. 2014). Some other global factors caused by humanity, e.g. the nitrogen deposition or changes in air and water chemistry (pollut-

ants), could also play a role, however, they are not object of our study.

With our current state of knowledge, we can only speculate which of the possibilities is the correct one. In both cases, the occurrence of *G. suberis* on Oblík hill can be considered as extrazonal (Ellenberg 2009). The observed host shift from Mediterranean oaks to a cultivar of cherry is not surprising, because *Gymnopilus* species are generally not known to be very substrate-specific (Hesler 1969, Holec 2005, Rees et al. 2004). Future studies focused on DNA from spores and mycelia in the air, soil, and wood, combined with analysis of other global factors, could help us understand the complex processes governing the occurrence, spreading, and fructification strategy of *G. suberis*.

### Acknowledgements

We thank E. Skála, V. Zíta (Czech Republic), M.À. Pérez-De-Gregorio, and G. Moreno (Spain) for providing data on their records, T. Kučera and A. Kučerová (Czech Republic) for helping us research the literature, R. Kühnert and U. Peintner (Austria) for arranging a loan from the IB herbarium, C. Quesada (Spain, herb. GDA), G. Venturella (Italy, herb. SAF) and G. Robich (Italy, herb. MCVE) for sending us data on herbarium specimens, and Michele Bozzano (Biodiversity International, Rome, Italy) for providing a EUFORGEN distribution map of *Quercus suber*. The first author thanks his wife Katarína for help with Past statistics software. The work was financially supported by the Ministry of Culture of the Czech Republic (DKRVO 2015/08, National Museum, 00023272).

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(Manuscript accepted 2 February 2016; Corresponding Editor: I. Krisai-Greilhuber)