

***Armillaria socialis* – morphological-anatomical and ecological characteristics, pathology, distribution in the Czech Republic and Europe and remarks on its genetic variation**

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Antonín V., Jankovský L., Lochman J. and Tomšovský M. (2006): *Armillaria socialis* – morphological-anatomical and ecological characteristics, pathology, distribution in the Czech Republic and Europe and remarks on its genetic variation. – Czech Mycol. 58(3–4): 209–224.

Armillaria socialis belongs to subgen. *Desarmillaria*. Its correct specific name (in comparison with the frequently used name *A. tabescens*) is discussed, and its detailed macro- and microscopic description and distribution in the Czech Republic and Europe are given. It is a thermophilic species occurring in Europe, USA, Japan and China. The northern distributional limit in Europe runs through Central Europe. In the Czech Republic, *A. socialis* occurs in thermophilic oak stands and also in alluvial forests. It was found on oaks, field maple and ash. A molecular study using sequence data from ribosomal DNA (ITS region) revealed a relatively high intraspecific genetic variation among sequences of *A. socialis* from various localities of the Czech Republic and France. The results resemble those published earlier from the USA.

Key words: *Armillaria*, *Desarmillaria*, description, distribution, pathology, ecology, ITS sequences, genetic variation

Antonín V., Jankovský L., Lochman J. a Tomšovský M. (2006): *Armillaria socialis* – morfologicko-anatomická a ekologická charakteristika, patologie, rozšíření v České republice a v Evropě a poznámky ke genetické variabilitě. – Czech Mycol. 58(3–4): 209–224.

Václavka bezprstenná, *Armillaria socialis*, patří do podrodu *Desarmillaria*. Je diskutováno její správné jméno oproti často používanému jménu *A. tabescens*. Je publikován její podrobný makroskopický a mikroskopický popis a rozšíření v České republice a v Evropě. Václavka bezprstenná je teplomilným druhem vyskytující se v Evropě, USA, Japonsku a Číně. Severní hranice jejího rozšíření prochází přes střední Evropu. U nás roste v teplomilných dubových porostech a v lužních lesích; sbírána byla na dubech, javoru babyce a jasanu. Molekulární studie zkoumající ITS sekvence ribozomální DNA odhalila relativně velké vnitrodruhové genetické rozdíly mezi sekvencemi *A. socialis* pocházejícími z různých lokalit z České republiky a Francie. Tyto výsledky odpovídají dříve publikovaným výsledkům z USA.

INTRODUCTION

In Europe, the genus *Armillaria* (Fr.) Staude contains seven species identified in cytogenetic studies in the late 1970s and early 1980s (e.g. Korhonen and Hintikka 1974; Korhonen 1978, 1980; Ullrich and Anderson 1978; Marxmüller 1982, 1987; Guillaumin et al. 1981; Pegler 2000; Termorshuizen and Arnolds 1987); five of them are annulate, two species are exannulate. Subgenus *Armillaria* contains the annulate species and subgen. *Desarmillaria* Herink the exannulate ones. Pegler (2000) mentioned the taxon *Desarmillaria* on a sectional level but without any formal transfer. *Armillaria socialis* (DC.) Fayod represents one of two European exannulate *Armillaria* species.

Armillaria socialis mostly occurs in the southern part of Europe (e.g. Guillaumin 1985). The northern distribution limit runs through Central Europe, including the Czech Republic and Slovakia. In the USA, the fungus is very common in the south-eastern states, west to Texas and Oklahoma, especially as a severe pathogen of oaks and fruit trees (Kuo 2004). In Japan, it was found in Kyushu and the central and southern parts of Honshu (Ota et al. 1998) and is also recorded from ornamental cherries plantations of alley trees (Hasegava 2005). It is also mentioned from China, where it is considered a pathogen of fruit trees like pears and apples.

The aim of this paper is to offer a complete characterisation of this species and a comparison of collections from several regions of Europe and different substrates.

MATERIAL AND METHODS

Microscopic features are described from material mounted in Melzer's reagent and Congo Red. For the basidiospores the following factors are used: E (quotient of length and width in any one spore) and Q (mean of E-values). Authors of fungal names are cited according to Kirk and Ansell (1992), colour abbreviations to Kornerup and Wanscher (1983), herbarium abbreviations follow Holmgren (2003).

One aim of this study was to investigate the genetic variation of *Armillaria socialis* among samples collected by the authors and DNA sequences of the species deposited under the name of *A. tabescens* in the NCBI Database online (updated 10th June 2006). Such studies based on comparison of DNA sequences are often employed to represent the genus *Armillaria* (Chillali et al. 1998a, 1998b; Kim et al. 2006; Lochman et al. 2004; Schnabel et al. 2005). In our study, DNA was isolated only from mycelium from cultures because of the low yield of DNA isolated from the herbarium specimens. Four cultures of *A. socialis* were chosen for DNA isolation. Two of them were isolated by the authors in the year 2005 and are deposited in the Laboratory of Forest Protection, Mendel University, Brno, under

the numbers 519 and 525. The source basidiocarps for the cultures were collected in the Czech Republic, in the Křivé jezero National Nature Reserve (isolate no. 519), and in the Cahnov National Nature Reserve (isolate no. 525). The dried source basidiocarp of isolate no. 525 is deposited in BRNM (no. 699839), while isolate no. 519 was taken from a basidiocarp strongly damaged by animals and furthermore damaged during culture isolation. Moreover, three specimens (BRNM 695685, 695686 and 695687) were collected during the same excursion at the locality. The remaining two isolates (T2, T4) were obtained from INRA (Clermont-Ferrand, France). These two isolates are often studied by various researchers (e.g. Bragança et al. 2004, Lochman et al. 2004).

For the comparison of DNA sequences the ITS region of nuclear ribosomal DNA (see details in White et al. 1990) was chosen. DNA was isolated from fresh mycelium grown on a Petri dish with Malt-extract agar (Himedia, India) using the NucleoSpin Plant isolation kit (Macherey-Nagel, Germany) according to the manufacturer's instructions.

The DNA was amplified by PCR, using the Mastercycler® ep thermocycler (Eppendorf, Germany). The PCR reaction was carried out in 25 µl reaction volume; the mixture for the PCR contained 50 ng of DNA, 20 pmol of each primer (using primer pair ITS1/ITS4), 0.2 mM dNTP's and 1U of DynaZyme™ polymerase with the appropriate buffer (Finnzymes, Finland). PCR amplifications were performed under the following temperatures: 94 °C / 3 min, 50 °C / 30 s, 72 °C / 1 min. (1×), 94 °C / 30 s, 50 °C / 30 s, 72 °C / 30 s (33×) and 94 °C / 30 s, 50 °C / 30 s, 72 °C / 5 min. (1×). The PCR products were purified with NucleoSpin Extract II (Macherey-Nagel, Germany) prior to sequencing.

Sequences were determined with an ABI PRISM 310 DNA sequencer (Perkin Elmer) at the Faculty of Science, Masaryk University, Brno, using the ABI PRISM BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems). All samples were sequenced with the primers used in the PCR. The sequences were deposited in the EMBL Nucleotide Sequence Database and their GenBank accession numbers are given in Tab. 1. Additional sequences of the examined species were downloaded from the GenBank (Tab. 1), while two sequences of *Armillaria ostoyae* (AY175811) and *A. gallica* (AY175808) were chosen to be the outgroup. All sequences were edited manually using BioEdit version 4.7.1. (Hall 1999). The ITS alignment with introduced gap consisted of 778 positions, all of them being included in the analyses. The total number of positions included 681 constant and 63 parsimony informative sites.

Phylogenetic trees were constructed using two different approaches, Bayesian analysis and Maximum Parsimony. Bayesian analysis was performed using the MrBayes 3.0 software (Ronquist and Huelsenbeck 2003). In MrBayes, base frequencies, rates for six different types of substitutions, number of invariant sites, and shape parameter of the gamma correction for rate heterogeneity with four discrete categories were allowed to vary; 2×10^6 generations of the Markov Chain Monte Carlo were run with four simultaneous chains, heating temperature 0.2. The chains were sampled in every 100th generation. When the likelihood scores of trees sampled approached similar values, they were considered to have converged. After this convergence point (after 250000 generations), the trees were included in computing the consensus tree, when the first 2500 trees were discarded as burn-in. The value of the cold chain was plotted against the generation number, so the number of discarded trees is not random. Maximum parsimony (MP) was performed with PAUP 4.0 (Swofford 1999). MP was carried out with 1000 repeated heuristic tree searches in which the starting tree was constructed by random taxa addition and swapped by the TBR algorithm. The support for the topology of MP was estimated using 1000 bootstrap-replicates. Equally parsimonious trees were found with MP searches with the following scores: consistency index (CI) = 0.8675, retention index (RI) = 0.9197, re-scaled consistency index (RC) = 0.8332, and homoplasy index (HI) = 0.9325. The trees resulted in a 50 % majority-rule MP consensus tree.

Tab. 1. Sequences of *Armillaria socialis* (= *A. tabescens*) encompassed in the study.

GenBank Acc. No.*	Geographic origin	Host species	Source	Reference
DQ784799	Czech Republic	<i>Quercus robur</i>	Isolate no. 519 (not preserved, see Material and methods)	This study
DQ784800	Czech Republic	<i>Acer campestre</i>	Isolate no. 525 BRNM 699839	This study
DQ784801	France, Puy de Dome	<i>Quercus</i> sp.	Isolate no. T2 (obtained from INRA, France)	This study
DQ784802	France, Gers	<i>Quercus</i> sp.	Isolate no. T4 (obtained from INRA, France)	This study
DQ109806	USA	<i>Butia capitata</i>	Isolate no. SC.PP1.05	Schnabel et al. unpublished*
AY175806	Czech Republic	<i>Quercus</i> sp.	Isolate no. AT171 Czech Rep., Cahnov	Lochman et al. 2004
AY213588	USA (South Carolina)	—	Isolate no. AT-MU-S2	Kim et al. 2006
AY213589	USA (Georgia)	—	Isolate no. 001-99	Kim et al. 2006
AY213590	USA (Georgia)	—	Isolate no. 001-210	Kim et al. 2006
AY695408	USA (South Carolina)	<i>Prunus persica</i>	Monokaryon of isolate no. SC.MF- 1.01	Schnabel et al. 2005
AY695409	USA (South Carolina)	<i>Prunus persica</i>	Monokaryon of isolate no. SC.MF- 1.01	Schnabel et al. 2005
U54821	France	<i>Eucalyptus dalrympleana</i>	—	Chillali et al. 1998
U54822	Italy	Unknown	—	Chillali et al. 1998

* Data obtained from the GenBank

RESULTS AND DISCUSSION

NOMENCLATORIAL NOTES

Two specific names concerning this species occur in recent mycological literature. Fayod (1889) used the epithet *socialis* (De Candolle 1815), and proposed a new combination, *Armillaria socialis* (DC.: Fr.) Fayod. Later, Herink (1973) also made this combination which represents a superfluous one. On the other hand, Volk and Burdsall (1995) preferred to use the specific name *tabescens* (Scopoli 1772). Unlike *Agaricus socialis*, *Agaricus tabescens* is not sanctioned by

Fries (1821). Additionally, Volk and Burdsall (1995) abandoned the name *A. socialis* because of its unclear identity. Fries (1821) mentioned it in tribe *Flammula* (a brown-spored group).

According to the first author, DeCandolle's description (DeCandolle 1815) agrees well with our fungus, as well as Fries' short characterisations (Fries 1821, 1838). Fries included this fungus in tribe *Flammula* probably based on the lamellae colour because he did not personally know it (see Fries 1838: 191).

Therefore, the correct name of this species is following:

Armillaria socialis (DC.: Fr.) Fayod

Fig. 4

Agaricus socialis DC., in: De Candolle and Lamarck, Fl. Franç., Ed. 3 (5/6): 48. 1815; *Agaricus (Flammula) socialis* DC.: Fr., Syst. Mycol. 1: 251. 1821; *Armillaria socialis* (DC.: Fr.) Fayod, Ann. Sci. Natur. 7(9): 232. 1889.

Syn.: *Armillaria socialis* (DC.: Fr.) Herink 1973 (superfluous combination). – *Agaricus tabescens* Scop. 1772; *Clitocybe tabescens* (Scop.) Bres. 1900; *Armillaria mellea* var. *exannulata* Peck 1893; *Armillaria mellea* var. *tabescens* (Scop.) Rea et Ramsb. 1917; *Armillaria tabescens* (Scop.) Emel 1921 (superfluous combination); *Armillaria tabescens* (Scop.) Dennis, Orton et Hora 1961 (superfluous combination); *Armillariella tabescens* (Scop.) Singer 1943 (for other synonyms see Volk and Burdsall 1995).

MORPHOLOGICAL-ANATOMIC CHARACTERISTICS OF BASIDIOMATA

Basidiomata caespitose. Pileus (10–)50–90 mm broad, conical-hemispherical when young, later convex-conical to conical with involute margin, then applanate with often uplifted, involute to straight margin, slightly undulate or irregular when old, hygrophanous, slightly pubescent at slightly radially striate margin when old, ochraceous yellow when young, then brown (6C6, 7D–E6) at margin and when moist, and ochraceous yellow (5–6D5, 4–5A–D5) at centre and when dried out. Covered entirely with tomentose-fibrillose scales when young, soon glabrescent at margin and with ± pyramidal scales at centre and with fibrillose tomentum among them; scale tops dark brown (8E–F7), basal parts and tomentum brown (7–8D6), sometimes entire scales paler (7–8D6). Lamellae distant, L = c. 60, l = 2–4, broadly adnate to slightly decurrent when young, then slightly emarginate and with a short to long decurrent tooth, rather thick when young, often irregular, anastomosed or furcate when young, later not anastomosed or only slightly anastomosed, orange-brown (6B5–6) when young, then brown (6–7D6–7) when old, more or less irregularly stained, with concolorous irregular smooth edge. Annulus absent. Stipe 60–160 × 7–15 mm, cylindrical or slightly laterally compressed, slightly broadened at apex, with cylindrical, slightly attenuated or broadened base, longitudinally fibrillose, then almost striate, fibrillose-flocculose at apex, whitish when young, becoming brown (8E6–7,

7–8E5) from the base, then red-brown (7E–F4), finally entirely brown-grey-black (8E–F3). Stipe velar remnants indistinct, in the form of scattered fibrillose-flocculose scales when young and single small fibrils when old. Context whitish, in stipe cortex in the stipe surface colour, soon hollow in the stipe, with fungoid smell.

Basidiospores (162 spores of 9 basidiocarps of 9 collections) (6.0–)7.5–10(–11) × (4.5–)5.0–7.0 μm, E = 1.3–1.8, Q = 1.3–1.7, (broadly) ellipsoid, fusoid-ellipsoid, obovoid, sometimes almost subglobose, both thin- and slightly thick-walled (crassospores), hyaline, non-dextrinoid. Basidia 31–45 × (7.5–)8.0–12 μm, 4-spored, clavate; crassobasidia present. Basidioles 15–45 × 4.0–10 μm, clavate, cylindrical; crassobasidioles present. Cheilocystidia (12–)17–41 × 5.0–10 μm, clavate, subfusoid, subcylindrical, subutriform, ± regular, irregular to coralloid, one- or more-celled, ± thin-walled. Trama hyphae cylindrical, thin- to slightly thick-walled, non-dextrinoid, up to 12 μm wide. Pileipellis a cutis consisting of cylindrical, radially arranged, ± thin-walled, hyaline to pale-yellowish, up to 10 μm wide hyphae. Pileus scales composed of chains of ellipsoid, cylindrical, subfusoid, slightly thick-walled (up to 0.5 μm), incrusting, up to 90 × 3.0–18 μm large cells, 0–1(–2–3)-septate and yellow-brown pigmented; terminal cells 12–65(–75) × 4.0–23 μm, cylindrical, conical or (sub)subulate. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, smooth, non-dextrinoid, up to 6.0(–7.0) μm wide hyphae. Caulocystidia (apex) (11–)20–50 × 7.0–14 μm, adpressed to erect, clavate, cylindrical, fusoid, utriform, often irregular or with projection(s), one- or more-celled or in the form of chains of cells, thick-walled (up to 1.5 μm wide). Basal mycelium of cylindrical, slightly thick-walled (up to 0.5 μm wide), smooth, up to 5.0 μm wide hyphae; terminal cells 30–65 × 4.5–6.0 μm, cylindrical, obtuse. Clamp-connections present in hymenium and subhymenium, absent in other structures (Fig. 1).

ECOLOGY AND PATHOLOGY

Armillaria socialis is a typically thermophilic species, distributed especially in southern countries in Europe. It is more thermophilic than *A. mellea* (Guillaumin et al. 1993).

Guillaumin et al. (1993) recorded *A. socialis* on *Arbutus unedo*, *Betula pendula*, *B. pauciflora*, *Eucalyptus macarthurii*, *E. gunnii*, *Eucalyptus* sp., *Quercus conferta*, *Q. ilex*, *Q. robur*, *Q. suber* and *Q. cerris*. They did not find it on conifers in Europe. However, Minter and Dudka (1996) published one record of this fungus from the Crimea, Nikita Botanic Garden, made by L. I. Vasilyeva on roots of *Cedrus atlantica*. It may represent the only European collection on conifers.

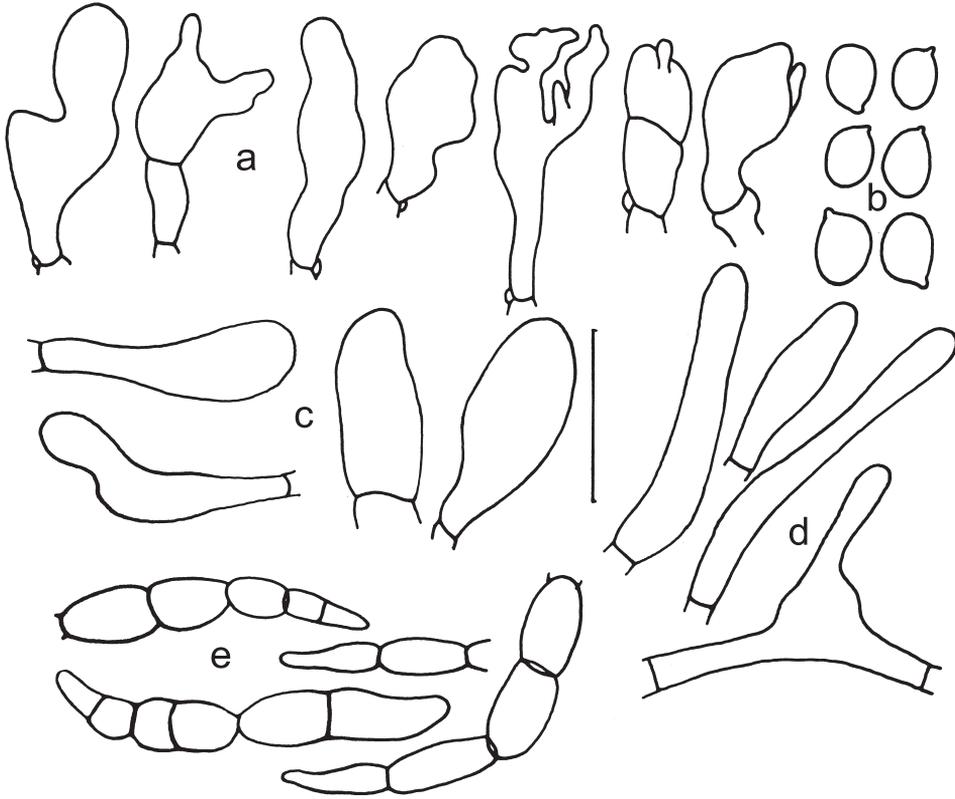


Fig. 1. Microscopic characters of *Armillaria socialis*: **a.** cheilocystidia, **b.** basidiospores, **c.** caulocystidia, **d.** terminal cells of basal mycelium, **e.** pileus scales. Scale bar = 20 µm for a – d, 50 µm for e.

In Italy, *A. socialis* is generally not common and has an irregular distribution, but may be locally abundant. The fungus is widespread in the dry thermophilic evergreen Mediterranean forests (macchia) in particular on oaks (*Quercus ilex*, *Q. cerris* and *Q. robur*; Intini 1989). Siccoli et al. (1992, 1994) described *A. socialis* only from *Quercus cerris*, *Q. frainetto* and *Q. pubescens* stands at altitudes of 200–1000 m. Chillali et al. (1998a, 1998b) also mentioned *Eucalyptus dalrympleana*, *Prunus domestica* ssp. *oconomica* and *Ulmus* sp. Tsopelas (1994, 1997) mentioned *A. socialis* from an almond orchard in central Greece.

Schnabel et al. (2005) published records of it on *Ilex cornuta*, *Ilex* sp., *Juniperus squamata*, *J. virginiana*, *Liquidambar styraciflua*, *Prunus domestica*, *P. persica*, *Quercus nigra*, *Quercus* sp., *Raphiolepis indicus* and *Thuja occidentalis* from North America. Moreover, it was recorded as a pathogen of peach trees, *Prunus persica* (Rhoad 1954, Volk and Burdsall 1995), and lemon trees (Rhoad 1948).

In Central Europe, *A. socialis* grows in two different habitats – thermophilic dry oak stands and floodplain forests with *Q. robur*. Thus far, it has mostly been found at the bases of trunks, on roots and stumps of both living and dead oak trees (*Q. cerris*, *Q. frainetto*, *Q. petraea*, *Q. robur*), and also on *Acer* spp. In floodplain forests along the Dyje river and of the confluence of the Morava and Dyje rivers close to the Czech, Slovak and Austrian border, it was recorded on *Acer campestre*, *Fraxinus angustifolia*, *Quercus robur*, and *Ulmus* sp. Kautmanová collected it in a beech stand (Slovakia, Bratislava, Turecký vrch hill; BRA CR 4994), however, she did not record the substrate.

The fructification period of this species is from (July) August to October under the climatic conditions of Central Europe.

Rishbeth (1982, 1985) mentioned that the pathogenicity of *A. socialis* appears to be very low. In the UK, *A. socialis* is less common, and is restricted to mild and relatively dry regions in south-east England (Rishbeth 1982, 1985; Guillaumin et al. 1993), where it is not known to kill standing trees but, locally, is an important agent of root decay. Also in Italy, this fungus is mentioned as a mainly saprophytic species (Intini 1989, 1994). Rhoads (1942, 1948) reported this species from tropical plantations in Florida (USA) as an important root rot of fruit trees and other plants. The same author also indicated that this species was a pathogen of lemon (Rhoads 1948) and peach (Rhoads 1954) trees. The American Phytopathological Society web site (APS 2006) also lists *A. socialis* (as *A. tabescens*) as a pathogen of banana and plantain trees (*Musa* spp.). It also is considered a pathogen of mango, *Mangifera indica* (Pernezny and Simone 2000), and it was further collected on apple- (*Malus* spp.) and pear-trees (*Pyrus* spp.) by Drake (1997). Hasegawa (2003) reported *A. socialis* as a species connected with declined ornamental cherries (e. g. *Prunus jamasakura*, *Prunus* × *yedoensis*).

In Europe, *A. socialis* is mainly considered a saprophytic species with low pathological impact on native trees, while in the USA, Japan and China it represents a pathogenic species of ornamental and fruit trees (e.g. Rhoads 1942, 1948, 1954; Hasegawa 2003; Schabel et al. 2005). According to Guillaumin et al. (1993), the species is saprotrophic in most cases, but it becomes highly pathogenic in *Eucalyptus* plantations in Europe as well.

Most finds origin from broadleaved trees, including non-woody plants like bananas, and rarely conifers. Schnabel et al. (2005) reported *Thuja occidentalis*, Hasegawa and Ota (2003 ex Hasegawa 2005) reported finds on Himalayan cedars (*Cedrus deodara*), while Minter and Dudka (1996) reported *Cedrus atlantica* as a host. It was found also on *Podocarpus macrophyllus* (Sato and Suzuki 2002 ex Hasegawa 2005).

The behaviour of this species is similar to other *Armillaria* species. They infect root systems of weakened trees, predominantly exotic species growing outside their ecological optimum. While the native species are not strongly infected,

introduced trees can be seriously affected by this fungus. In the Czech Republic, *A. socialis* was observed mostly on butts and on root systems of dead or dying trees and on stumps. There are no observations of basidiomes on apparently fully healthy living trees in the Czech Republic, but colonisation of living weakened trees without apparent fructification may be assumed. Production of rhizomorphs is reduced in comparison with other *Armillaria* species. The main ecological role of this species is to destroy lignocellulose in roots and stumps. Unlike other species such as *A. cepistipes* and *A. gallica*, it is not known to grow from buried wood in soil.

Armillaria socialis is mentioned as an important symbiont of the achlorophyllous orchid *Galeola septentrionalis* (Terashita and Schuman 1987, 1989) as well. This species was found in a third of isolates from roots of this symbiotic host.

DISTRIBUTION IN THE CZECH REPUBLIC

Armillaria socialis is known from 15 localities in the Czech Republic; all of them are situated in Moravia (see Fig. 2). Except for three finds in central Moravia from 1930, one in the vicinity of the city of Brno in 1994 (BRNM 603064) and near Žarošice in the Ždánický les Hills (Vacek 1937), this fungus was collected in southern Moravia. Except for the locality in the vicinity of Brno and near Žarošice, it has been collected in alluvial and floodplain forests in the Czech Republic.

DISTRIBUTION IN EUROPE

In Europe, *Armillaria socialis* has been found in Austria (Aron et al. 2005), Bulgaria (see list of revised specimens), Croatia (Tkalčec and Mešić 2002), the Czech Republic (Jankovský 2003, and see list of revised specimens), Estonia (Urbonas et al. 1986), Finland (Lochman et al. 2004), France (Guillaumin et al. 1993, Bragança et al. 2004, Chillali et al. 1998a, Lochman et al. 2004, Sierra et al. 1999, and see list of revised specimens), Germany (Guillaumin et al. 1993, Benkert et al. 1992, Kreisel 1987), Great Britain (Rishbeth 1982, England and Wales: Legon and Henrici 2005), Greece (Tsopelas 1994, Tsopelas and Tjamos 1997), Hungary (Anonymus 1996–2004), Italy (Intini 1989, 1994; Sierra et al. 1999; Zuccherelli 1993; Sicily: Chillali et al. 1998a), Latvia (Urbonas et al. 1986), Lithuania (Urbonas et al. 1986), Poland (Wojewoda 2003), Portugal (Azevedo 1976, Bragança et al. 2004, Santos et al. 2005), Romania (Bontea 1986), Serbia and Montenegro (Lazić 2005, Keča et al. 2006), Slovakia (Lizoň and Bacigálová 1998, Škubla 2003, and see list of revised specimens), Slovenia (Poler 1998), Spain (Anonymus 2004,

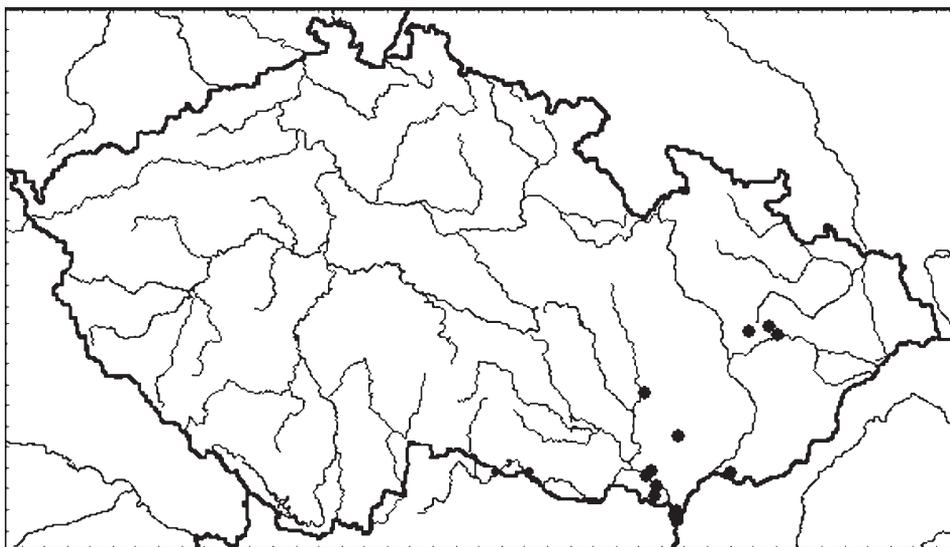


Fig. 2. Distribution map of *Armillaria socialis* in the Czech Republic (generated from the Fungi 3 programme by J. Slaviček).

Bragança et al. 2004), Sweden (Anonymus 2006), Switzerland (Guillaumin 1994, Bendel 2006) and Ukraine (Minter and Dudka 1996). It is also mentioned from Tunisia from north Africa (Delatour 1969 ex Guillaumin 1993).

Armillaria socialis is also included in three red lists in Europe. It belongs to the vulnerable species (VU) in Germany (Benkert et al. 1992, Schnittler and Ludwig 1996) and endangered ones (E) in Poland (Wojewoda and Lawrynowicz 2004); it is also included in a preliminary red list of macromycetes of Steiermark, Austria (Aron et al. 2005).

MATERIAL REVISED

Bulgaria: Banja near Nesebar, between Obzor and Slančev Briag, 30 Aug. 1983 leg. et det. F. Kotlaba (PRM 831855, as *A. tabescens*). – Stara Planina, under Lovno chanče, 2 Aug. 1979 leg. B. Bill and F. Kotlaba, det. F. Kotlaba (PRM 821423, as *A. tabescens*). – Primorsko near Burgas, in the direction of Mičurin, 21 Sept. 1984 leg. S. Hejný, det. F. Kotlaba (PRM 837720).

Czech Republic: Moravia: Mährisch-Weißkirchen [Hranice na Moravě], Podhorn [Podhoří], Sept. 1930 leg. F. Petrak, in: Reliquiae Petrakianae, no. 508 (PRM 861928, as *Armillariella tabescens*). – Mährisch-Weißkirchen [Hranice na Moravě], Svrčov, Aug. 1930 leg. F. Petrak, in: Reliquiae Petrakianae, no. 1699 (PRM 874963, as *Armillariella tabescens*). – Mährisch-Weißkirchen [Hranice na Moravě], Ribař [Rybáře], Sept. 1937 leg. F. Petrak, in: Reliquiae Petrakianae, no. 1882 (PRM 876147, as *Armillariella tabescens*). – Between Brno-Líšeň and Ochoz near Brno, 18 Sept. 1994 leg. P. Fukal, det.

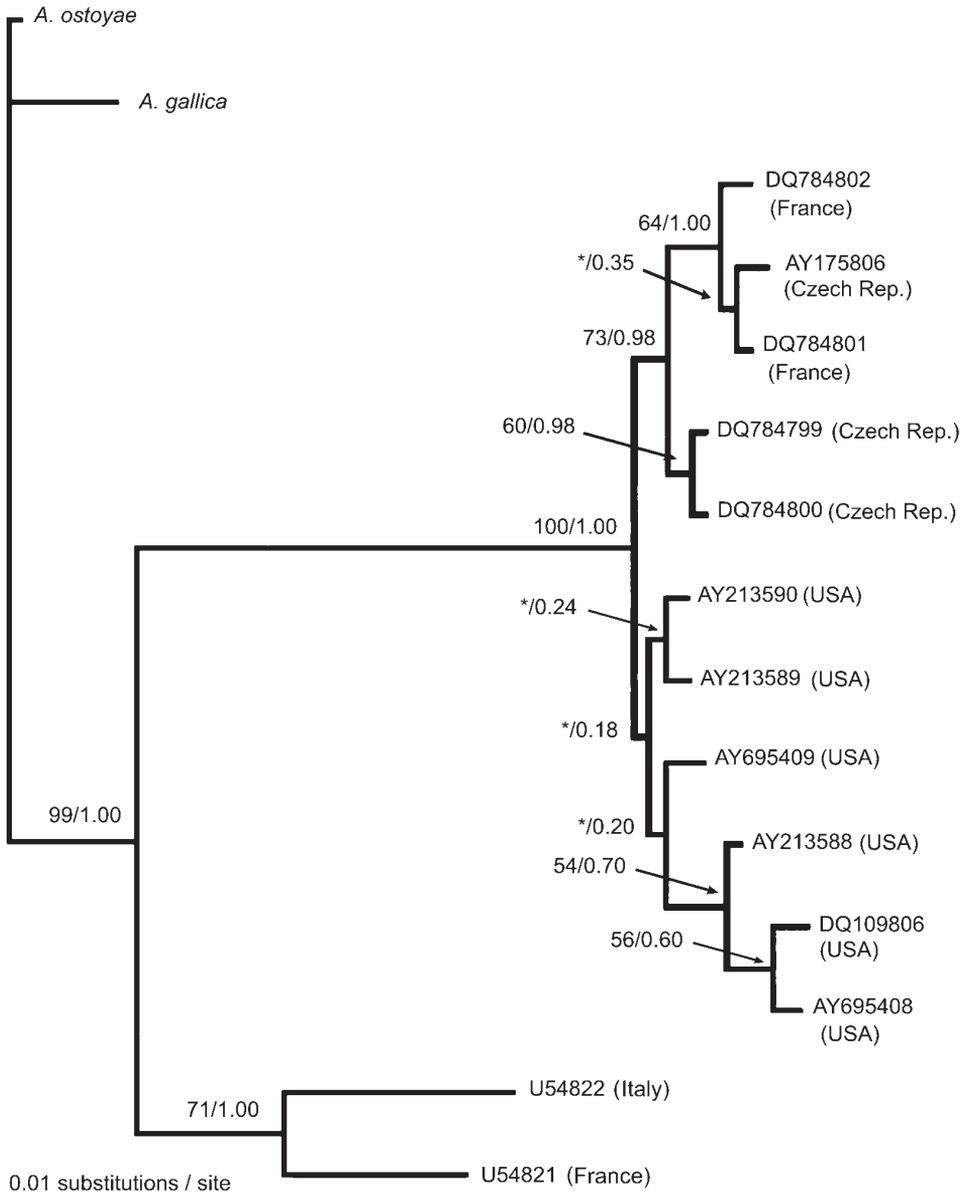


Fig. 3. Phylogenetic tree of *Armillaria socialis* based on sequences of the ITS region. The unrooted tree was constructed by the Bayesian analysis. Bootstrap values from Maximum Parsimony and Bayesian posterior probabilities, respectively, are shown at the nodes. Asterisks indicate nodes with bootstrap values below 50 %.



Fig. 4. Basidiocarps of *Armillaria socialis* (Czech Republic, Nové Mlýny, Křivé jezero National Nature Reserve, 8 Sept. 2005, BRNM 695686). Photo: V. Antonín.

V. Antonín (BRNM 603064, as *A. tabescens*). – Radějov, 10 Aug. 1994 leg. ???, det. V. Antonín (BRNM 603065, as *A. tabescens*). – Pavlov, Pavlovský les forest, 25 Sept. 1975 leg. et det. K. Kříž (BRNM 289620, as *A. tabescens*). – Lednice, 20 Sept. 1972 leg. et det. J. Lazebníček (BRNL, as *A. tabescens*). – Valtice, Rendezvous National Nature Reserve, 7 Sept. 1985 leg. J. Macek, det. Z. Pouzar (PRM 866615). – Nové Mlýny, Křivé jezero National Nature Reserve, 11 Sept. 1994 leg. et det. V. Antonín 94.95 (BRNM 599047, as *A. tabescens*). – Ibid., 8 Sept. 2005 leg. et det. V. Antonín 05.123-125 (BRNM 695685, 695686 and

695687, as *A. tabescens*). – Lanžhot, Soutok National Nature Reserve, 14 July 1998 leg. et det. V. Řehořek (BRNM 652722, as *A. tabescens*). – Lanžhot, Ranšpurk National Nature Reserve, 25 Aug. 1966 leg. J. Lazebníček et A. Vágner, det. J. Lazebníček (BRNM 266006 and 826063, as *A. tabescens*). – Ibid., 25 Aug. 1966 leg. et det. J. Lazebníček (BRNM 265945, as *A. tabescens*). – Lanžhot, Cahnov National Nature Reserve, 10 Sept. 1965 leg. et det. F. Kotlaba et J. Lazebníček (PRM 613787, as *A. tabescens*). – Ibid., 14 Sept. 2005 leg. et det. L. Jankovský (BRNM 699839, as *A. tabescens*). – Lanžhot, site called Polinský vršek, 1 Oct. 1998 leg. et det. A. Vágner (BRNM 648747, as *A. tabescens*). – Lanžhot, site called Myslivecký palouk, 1 Oct. 1998 leg. et det. V. Antonín 98.153 (BRNM 642442, as *A. tabescens*).

France: Bourgogne, Aiserey, forêt d'Izeure, 12 Oct. 1992 leg. et det. J.-C. Verpeau (CB M-6803, as *A. tabescens*).

Slovakia: Budmerice near Trnava, Lindavský les forest, 15 Oct. 1985 leg. et det. F. Kotlaba and Z. Pouzar (PRM 838065, as *A. tabescens*). – Between Bojnice and Šutovce, Predné Štefankové, 16 Aug. 1970 leg. et det. J. Kuthan (PRM 795404, as *Armillariella tabescens*). – Malé Karpaty Mts., Bratislava, Turecký vrch hill, 25 Sept. 1994 leg. et det. I. Kautmanová (BRA CR 4994). – Strážovské vrchy Mts., Nitrianské Rudno, Rudnianska valley, 14 July 1984 leg. et det. J. Kuthan (BRA CR4993). – Krupinská planina, Čabradský Vrbovok, 23 Sept. 1987 leg. et det. J. Kuthan (BRA CR4992).

MOLECULAR STUDY

Bayesian and MP analyses showed congruence for the ITS region. The results are shown in Fig. 3 representing the Bayesian consensus tree. Almost all sequences of *A. socialis* (= *A. tabescens*) form one, well supported monophyletic clade. The only exceptions are two sequences published by Chillali et al. (1998b) that do not seem to be closely related to *A. socialis*. This fact is very surprising, but similarity matrices derived from the sequence data (according to Chillali et al. 1998a) confirmed the visual estimation, when both sequences revealed only 84.6–85.6 % similarity with other sequences of *A. socialis* included in the study. Other similarity matrices between all genuine *A. socialis* sequences exceeded the value of 97.4 %. This discrepancy is explainable only by a misidentification of fungal material or by an error during amplification or sequence determination of the DNA.

Inside this main resulted clade two newly sequenced Czech specimens (DQ784799, DQ784780) form one cluster, while French specimens form another one with the Czech specimen from the GenBank (AY175806). These five specimens clustered in a European subclade, while the second subclade is formed by specimens from North America. The data support the idea of possible allopatric speciation, even if branching is supported by only 73 % of bootstrap proportions (MP tree). Definitely, the intraspecific nucleotide variation of the ITS region is relatively high. For example sequences AY175806 and DQ784800 originating from one locality differ in 10 (mostly heterozygotic) sites. Also data previously published by Schnabel et al. (2005) revealed significant differences between two single spore isolates derived from one basidiocarp (sequences nos. AY695408, AY695409, see Fig. 3).

ACKNOWLEDGEMENTS

The authors wish to thank Zdeněk Pouzar (National Museum in Prague, Czech Republic) for valuable nomenclatural notes. The studies were supported by the Grant Agency of the Czech Republic, project no. 526/05/0086, and by the research concept of Ministry of Education, Youth and Sports of the Czech Republic no. 6215648902.

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