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Short communication

First record of *Inocutis tamaricis* in Romania with comments on its cultural characteristics

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Abstract – *Inocutis tamaricis* is a lignicolous basidiomycete associated exclusively with *Tamarix* species. The first Romanian record of this species is reported from Constanţa city near the Black Sea coast where it was detected on *Tamarix tetrandra*. We noticed that in pure culture it forms swollen hyphae in the aerial mycelium, which have not been reported so far for *I. tamaricis*.

Keywords: *Inocutis tamaricis*, Romania, swollen hyphae, *Tamarix tetrandra*

Introduction

Inocutis tamaricis (Pat.) Fiasson & Niemelä is a thermophilous basidiomycete typical for the Mediterranean region that grows exclusively on *Tamarix* species (Ryvarden and Gilbertson 1993) and causes a white rot of the heartwood (Bondartseva and Parmasto 1986). This taxon was renamed by Fiasson and Niemelä (1984) from the widely known *Inonotus tamaricis* (Pat.) Maire to *Inocutis tamaricis*. Later, on the basis of molecular data, Wagner and Fischer (2001) confirmed this new combination.

The global distribution of *I. tamaricis* includes the southern part of the Palearctic. It is particularly widely distributed in the countries bordering the Mediterranean Sea and the Black Sea (Piątek 2001). Reviewing the distribution of this species, Klán (1978) mentions its presence in Portugal, Spain, France, Italy, Greece, Croatia, Montenegro, Bulgaria, Ukraine, Georgia, Algeria, Morocco, Syria, and Kazakhstan. Furthermore, *I. tamaricis* was reported to be present in Egypt (Hejný and Kotlaba 1984), Uzbekistan (Bondartseva and Parmasto 1986), Macedonia (Karadelev 1993), South of European Russia, Central Asia, Israel, Senegal (Ryvarden and Gilbertson 1993), China (Dai et al. 1997), Canary Islands (Beltrán-Tejera and Rodríguez-Armas 1999), Turkey (Doğan et al. 2005) and Iran (Ghobad-Nejhad and Kotiranta 2008).

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Inocutis tamaricis is sporadically present in some countries and although it is a pathogen of *Tamarix* spp., in Macedonia (Karadelev 2000) and Bulgaria (Gyosheva et al. 2006) it is included in the Red list of fungi.

In this study we present the first record of *I. tamaricis* in Romania and provide new data on its cultural characteristics.

Materials and methods

In the field, three basidiomata were collected from two trunks of living *Tamarix tetrandra* Pall. ex M. Bieb. Observations of microscopic characters were made in 5% KOH and in 1% aqueous solution of Congo red using a compound microscope. Spores and swollen hyphae measurements were based on thirty observations. Statistical data (length and width) are given as arithmetic mean ± standard deviation (SD).

Species identification was performed based on Bondartsev 1953, Bondartseva and Parmasto 1986, Jülich 1989, Ryvarden and Gilbertson 1993, and Bernicchia 2005. The analyzed material was dried and deposited in the Herbarium of the Faculty of Biology, Alexandru Ioan Cuza University, Romania (Index Herbariorum acronym: I).

In order to investigate the cultural and morphological characteristics of *I. tamaricis*, dikaryon culture isolated from one basidioma (voucher I 137309, leg. Chinan and Mânzu) was grown on 9 cm Petri dishes containing the following nutrient media:

- Sabouraud: 10 g peptone, 40 g dextrose, 15 g agar, 1000 mL distilled water;
- Potato dextrose agar (PDA): 200 g unpeeled potatoes, 20 g dextrose, 20 g agar, 1000 mL distilled water);
- Malt extract agar (MEA): 15 g malt extract, 15 g agar, 1000 mL distilled water;
- Czapek: 30 g sucrose, 20 g agar, 2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \times 7\ H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \times 7\ H_2O$, 1000 mL distilled water.

The culture media were inoculated with small blocks of actively growing mycelium at a distance of 1.5 cm from the edge of the Petri dish and incubated at 25 °C in the dark. Three replicates were used for each medium.

Species identification and the identity of the cultured mycelium were confirmed using molecular markers (ITS, internal transcribed spacer region). DNA sequences were generated from a dried basidioma (voucher I 137309) and cultured mycelium derived from it using primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990) and sequenced at a commercial facility (Alvalab, Santander, Spain). The obtained ITS sequence (GenBank accession number KJ755854) was compared with those of *Inocutis rheades* (Pers.) Fiasson & Niemelä, *I. dryophila* (Berk.) Fiasson & Niemelä and *I. tamaricis* available in GenBank. The first two species were used in the analysis because of a possible confusion in identification due to the morphological resemblance between these species and *I. tamaricis* (Ghobad-Nejhad and Kotiranta 2008). A sequence of *Inonotus tricolor* (Bres.) Y. C. Dai was used as out-group. Sequences were aligned using ClusatlW (Larkin et al. 2007) followed by minor alignment corrections by eye. Phylogenies and p-distances were estimated in MEGA 6.06 (Tamura et al. 2013). The phylogenetic analysis was run using maximum likelihood and a K2P + G model of nucleotide substitution as this was determined to be the most appropriate model with MEGA 6.06 using the Bayesian information criterion (BIC). Support for monophyly of clades was evaluated by bootstrap values (1000 replicates).

Results and discussion

During a field trip to the Black Sea coast in South East Romania, *I. tamaricis* was detected in June 2012 on two cultivated *T. tetrandra* bushes, in the park of the Natural Sciences Museum Complex from Constanța City (44°12′19.19″N, 28°38′20.05″E). This is the first record of this fungus in Romania.

Macroscopic and microscopic description

The basidiomata are fan-shaped, semicircular, 7–22 cm across, 4–9 cm wide, 3–4 cm thick, surface rusty-brown, zonate, hispid to villose (Fig. 1a). Hymenial surface is pale ochraceous at first, later becoming brown, pores angular, 1–3 per mm. Tube layer is rusty-brown 5–30 mm long. Context is rusty-brown, fibrous, zonate, with granular, marbled core at base. Hyphae of the fibrous context with simple septa, yellow to rusty brown, thin to thick-walled, 4–10 µm wide. Hyphae of mycelial core are brown, encrusted, branched, thick-walled, dark brown mixed with lobed and branched sclerids. Basidia are clavate, 4-sterigmate, 14–18 × 7–9 µm. Basidiospores are rusty brown, thick-walled, ellipsoid, 6.5–8.5 µm long (mean 7.6 ± 0.6), and 5–6 µm wide (mean 5.3 ± 0.4).

Inocutis tamaricis is easy to identify in the field due to its habitat on the wood of various *Tamarix* species and the presence of a marbled core at the base of the basidioma. The macroscopic and microscopic characteristics of the specimens from Romania are in accordance with the published descriptions of this species (Bondartseva and Parmasto 1986, Ryvarden and Gilbertson 1993, Jülich 1989, Bernicchia 2005).

Cultural characteristics

On Sabouraud agar, the colony is initially white, then yellowish to ochraceous (Fig. 1b). The aerial mycelium is cottony with concentric zones. The reverse in the old part of the colony is brownish-ochraceous, but in the recently covered zone it is unchanged. On other nutrition media, such as Czapek, MEA, and PDA the colony is whitish with yellowish tints, and the reverse is unchanged to cream-yellowish. This strain of *I. tamaricis* preferred PDA, on which we recorded the highest growth rate (with a mean of 23 mm per week), followed by MEA (18 mm per week), Sabouraud (17 mm per week), and Czapek (15 mm per week).

Regarding the microstructural characteristics we observed that on all tested media the aerial mycelium presents numerous swollen hyphae (Fig. 1c). These are spherical to fusiform, 7–25 µm long (mean 13.1 ± 6) and 7–20 µm wide (mean 10.4 ± 4.3), hyaline to yellowish, single or in chains (monilioid). The swellings on Czapek, MEA, and Sabouraud media appeared after about three weeks and on PDA after two weeks.

The cultural and structural characteristics of this species were previously investigated by Stalpers (1978) and Țura et al. (2009) on different nutrient media (cherry decoction agar, MEA, PDA, beer wort, corn-meal agar, and Czapek medium). Although the morphological characteristics of the colonies examined by us are similar to the previous descriptions, our observations showed that this strain forms numerous inflated hyphae in the aerial mycelium. These were not mentioned so far for *I. tamaricis*.

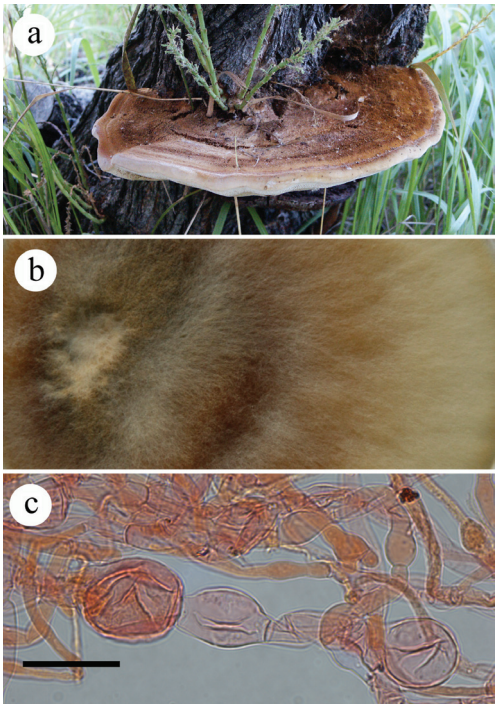

Fig. 1. *Inocutis tamaricis*: a – basidioma on *Tamarix tetrandra*; b – colony on Sabouraud agar; c – aerial mycelium with swollen hyphae (in Congo red solution). Scale bar = 20 µm (Photo by V. C. Chinan).

Molecular analysis

The identical ITS sequences obtained from the basidioma and cultured mycelium confirmed that observations were made on *I. tamaricis* mycelium and not on a contaminant species. The ITS sequence of *I. tamaricis* from Romania clustered with very high bootstrap support with all other *I. tamaricis* sequences available in GenBank (Fig. 2), confirming thus the identification based on microscopic characters and host preference. The p-distances between *I. tamaricis* from Romania and other conspecific sequences, varying from 0.004 to 0.036, are well below or comparable with the mean intraspecific sequence divergence of 0.025 reported for fungi (Schoch et al. 2012).

Ecology

In Romania, *I. tamaricis* was found only on living plants. Although this species has been reported from both living and dead *Tamarix* trunks (Ryvarden and Gilbertson 1993, Beltrán-Tejera and Rodríguez-Armas 1999, Ghobad-Nejhad and Kotiranta 2008), the vast majority of authors report that this species was only detected on living *Tamarix* species (Pegler 1964, Intini 1977, Klán 1978, Hejný and Kotlaba 1984, Bondartseva and Parmasto 1986, Jülich 1989, Llimona et al. 1995, Tentori 1997, Bernicchia 2005, Dai et al. 2007, Dai et al. 2009, Assyov et al. 2010, Țura et al. 2010, Zhou et al. 2011, Alexov et al. 2012, Dai 2012). Our results show that *I. tamaricis* occurs in Romania as a parasite.

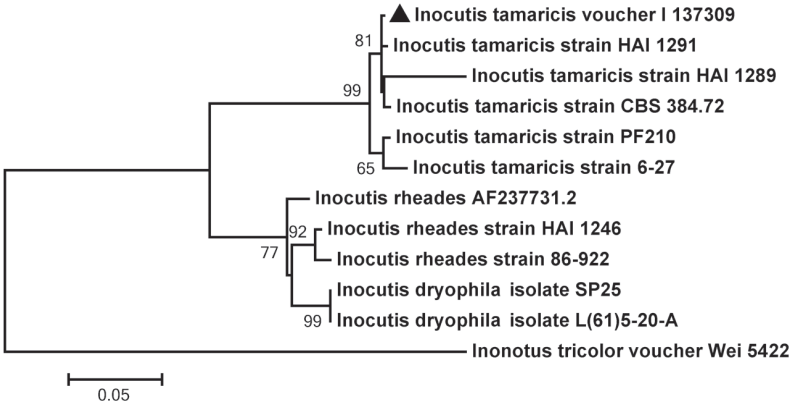

Fig. 2. Maximum likelihood tree based on ITS sequences. Bootstrap values (support > 65%) based on 1,000 replications are shown near nodes. The sequence of *Inocutis tamaricis* from Romania is indicated by a black triangle.

The host plant, *T. tetrandra*, is not a native species in Romania, but is present in urban areas and parks as an ornamental shrub. To the best of our knowledge, *I. tamaricis* has not yet been recorded living on this host plant. Interestingly, this *Inocutis* species has not been found yet in Romania on *T. ramosissima* Ledeb., which is a widespread native species, including in the Danube Delta (Ciocârlan 2011) where the ecological conditions could be favorable for this fungus. In the published data, not all of the authors mention the species of *Tamarix* on which it grows. The most frequent host mentioned in the literature is *T. gallica* L. (Intini 1977, López-Prada and Castro Cerceda 1996, Tentori 1997, Zervakis et al. 1998, Bernicchia 2005, and Ţura et al. 2009, 2010). Other species of *Tamarix* reported as hosts for this fungus are: *T. arborea* (Sieber ex Ehrenb.) Bunge (Piątek 2001), *T. canariensis* Willd. (Llimona et al. 1995, Beltrán Tejera and Rodríguez-Armas 1999), *T. chinensis* Lour. (Dai 2012), *T. nilotica* (Ehrenb.) Bunge (Hejný and Kotlaba 1984), *T. parviflora* DC. (Karadelev 1993), and *T. ramosissima* (Sinadskii and Bondartseva 1960).

As the species was already signaled in Bulgaria and Ukraine, our record of *I. tamaricis* from Romania contributes to the knowledge on its distribution in the western Black Sea region.

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