

First records of *Cartilosoma rene-hentic* (*Polyporales*) in the Czech Republic

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The recently described polypore *Cartilosoma rene-hentic* is recorded, described and illustrated from several localities in the Czech Republic for the first time. Its identity was confirmed both based on morphology and by sequencing ITS regions. The species variability and ecology is discussed and a map of its distribution in the Czech Republic is provided.

Key words: *Antrodia*, *Fomitopsidaceae*, polypore, brown-rot fungi, lignicolous fungi.

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Nedávno popsany choroš *Cartilosoma rene-hentic* je poprvé zaznamenán, popsán a ilustrován z několika lokalit v České republice. Jeho identita byla potvrzena jak na základě morfologie, tak sekvencováním ITS úseku. Je diskutována jeho variabilita a ekologie a je uveřejněna mapa jeho rozšíření v ČR.

INTRODUCTION

The genus *Cartilosoma* Kotl. & Pouzar was erected by Kotlaba & Pouzar (1958) for polyporoid fungus *Trametes subsinuosa* Bres., now considered a synonym of *Cartilosoma ramentaceum* (Berk. & Broome) Teixeira (Spirin 2007), a species which has been known for a long time as *Antrodia ramentacea* (Berk. & Broome) Donk (e.g. Ryvarden & Gilbertson 1993, Ryvarden & Melo 2017). Characters distinguishing it from similar genera of whitish (semi-)resupinate polypores are its cartilaginous fruitbodies, white context, monomitic hyphal system in the trama and thick-walled to subsolid hyphae (Kotlaba & Pouzar 1958).

Recently, Rivoire et al. (2015) described another species of the genus, *Cartilosoma rene-hentic* B. Rivoire, Trichies & Vlasák, from western Europe.

All authors of this paper collected this recently described species in the Czech Republic independently of each other. Since there is still little data on its distribution, ecology and variability, our article aims to improve the knowledge of the species somewhat.

MATERIAL AND METHODS

The description is based on sequenced material (see below). Macroscopic characters were observed on fresh and dry material and photographs in situ. Microscopic features were examined in a Melzer's solution, Cotton Blue in lactophenol, Congo Red in 10% ammonia and 5% KOH solution at 1000× magnification under an oil immersion lens. Microscopic observations including measurements were carried out on dried material. Dimensions of spores were derived from measurements of 20 individual spores in Cotton Blue (the specimen from Plzeň-Orlík was not included in the measurements as it is almost sterile). The spores were measured directly under an optical microscope using an eyepiece micrometer.

The identification of the specimens was based on the descriptions in Rivoire et al. (2015) and Ryvarden & Melo (2017), supplemented with ITS segment sequencing (methods followed according to Vlasák & Kout 2011) for selected specimens and comparison with available data in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

The specimens were dried at room temperature, stored in a PE bag with a slide fastener and were subsequently frozen. Voucher specimens are now deposited in the herbarium of the Museum of East Bohemia in Hradec Králové (HR), the herbarium of the University of West Bohemia (CBG) and the National Museum in Prague (PRM).

Abbreviations used: avg. (range of average values in particular collections), CBG (herbarium of Department of Biology, Geosciences and Environmental Education, Faculty of Education, University of West Bohemia), det. (identified by), leg. (collected by), n_{spec} (number of collections from which the average value is calculated), Q (range of spore length to width ratio), Q_{avg} (range of average Q values in particular collections), rev. (revised by). Nomenclature of plants follows Kubát (2002). Abbreviations of public herbaria (except herb. CBG) follow Thiers (on-line).

RESULTS

Cartilosoma rene-hentic B. Rivoire, Trichies & Vlasák, Bull. mens. Soc. linn.

Lyon 84(1–2): 8, 2015

Figs. 1–4

≡ *Antrodia rene-hentic* (Rivoire, Trichies & Vlasák) Melo & Ryvarden

Macroscopic description. Basidiocarps annual, effused forming irregular bulges, or less often effused-reflexed with narrow pilei (up to 8 mm) and slightly undulating and bent margin, sometimes imbricate, widely attached, cartilaginous, up to 50 mm wide and up to 4 mm thick; upper surface (if present) whitish, cream-coloured to ochre, matt, smooth, azonate; margin of pilei blunt; margin of basidiocarps whitish, velutinous, lighter than pore surface; pore surface convex; pores entire or slightly lacerate, isodiametric, elongate to almost daedaleoid, (1.5)2–3 per mm (in some specimens larger near basidiocarp margin); dissepiments whitish velutinous; tubes at first white, then becoming cream-coloured, ochre to brownish in old fruitbodies, 2–4 mm high; context tough, whitish, contrasting with tube layer in older specimens, homogenous, up to 1 mm thick. Smell slightly acidulous, taste indistinct.

Exsiccates. Tubes brownish, resinous and brittle, dissepiments whitish; basidiocarp margin white to whitish, not or barely loosening from the substrate, context and trama whitish.

Microscopic description. Basal layer of basidiocarps with dimitic hyphal system, skeletal hyphae dominating, non-septate, thick-walled to sub-solid, but lumen usually discernible, often tortuous, sparingly branched, irregularly arranged, hyaline, 2.5–4 µm in diam., swelling only slightly in KOH, non-amyloid, non-congophilous. Context with monomitic or dimitic hyphal system; generative hyphae dominating, with clamps, hyaline, mostly thick-walled, sparingly branched, irregularly arranged, hyaline, (2)3–4.5 µm in diam., swelling conspicuously in KOH, congophilous, skeletal hyphae penetrating from basal layer, absent to rare, similar as above; bipyramidal crystals present on hyphae. Trama with monomitic hyphal system; generative hyphae with clamps, easy to find, hyaline, slightly to distinctly thick-walled, with walls often irregularly thickened and lumen usually discernible, sparingly branched, tortuous, irregularly arranged, 2.5–4 µm in diam. Cystidial elements absent. Cystidioles scattered, fusoid, 18–22 × 4–4.5 µm, with a basal clamp. Dissepiments sterile, terminal elements cylindrical to almost subulate, thin-walled, not incrustated, 2.5–3 µm in diam. Basidia clavate, 4-spored, 18–21 × 5.5–6.5(9) µm, with a basal clamp. Basidiospores (sub-)cylindrical, often slightly ventrally concave, rarely slightly sigmoid, hyaline, thin-walled, weakly cyanophilous and neither amyloid nor dextrinoid, 6–8(10) × 2.2–3 µm (avg. 6.88–7.49 × 2.49–2.72 µm, $n_{\text{spec}} = 3$); $Q = 2.2–3.3$ ($Q_{\text{avg}} = 2.77–3.09$, $n_{\text{spec}} = 3$).



Fig. 1. Fresh basidiocarp of *Cartilosoma rene-hentic*, reclaimed mine spoil Antonín near Sokolov, Czech Republic (HR B000001, PRM 951454). Photo L. Zíbarová.



Fig. 2. Fresh basidiocarp of *Cartilosoma rene-hentic*, Radotínské údolí Nature Reserve, Český kras (Bohemian Karst), Czech Republic (PRM 951086). Photo M. Kříž.



Fig. 3. Dried basidiocarp of *Cartilosoma rene-hentic* (above, HR B000001) and *C. ramentaceum* (below, herb. L. Zíbarová no. 6568). Photo L. Zíbarová.

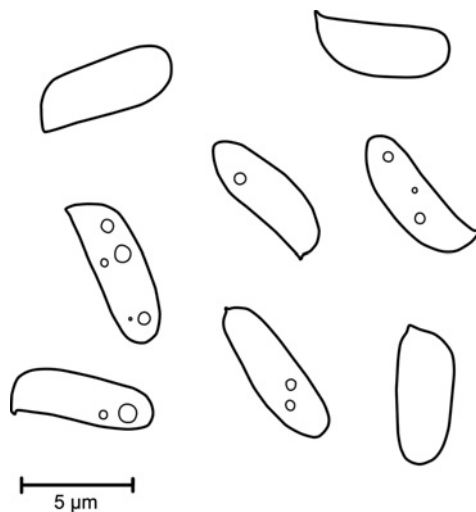


Fig. 4. Line drawing of spores (HR B000001, PRM 951454). Scale bar = 5 μ m. Del. L. Zíbarová.

Ecology. All our records are from wood of broadleaves (*Corylus avellana*, *Fagus sylvatica*, *Syringa vulgaris*, *Salix* sp. and an unidentified hardwood), always from branches – either still attached or fallen. The habitats of our records are rather diverse (mine spoil, suburban forests, willow thickets, etc.), often at man-influenced localities.

Type of rot. Brown rot.

Molecular analysis. We obtained ITS region sequences from four specimens identified as *C. rene-hentic*. Subsequently, we confirmed the identity of the species based on our microscopic work in concordance with GenBank data (BLAST tool). All of them were 100% identical with the sequence of *C. rene-hentic* type LYBR 4111, GenBank KM068100 (not shown).

Material studied

Czech Republic. West Bohemia. Sokolov, reclaimed mine spoil Antonín, 428 m a.s.l., planted *Syringa* stand, attached dead branches of *Syringa vulgaris*, 18 Oct 2016 leg. L. Zíbarová & A. Lepšová, det. L. Zíbarová (HR B000001, PRM 951454), rev. J. Kout & J. Vlasák based on DNA (GenBank MK558724). – Plzeň, Bolevecké rybníky, yellow-marked hiking trail by Malý Bolevecký rybník, 315 m a.s.l., suburban forest, verge of path between ponds, on small fallen hardwood branch, 17 Nov 2016 leg. & det. J. Kout (herb. CBG, PRM 944766), rev. J. Vlasák based on DNA (GenBank MK558725). – Plzeň, approx. 300 m south of Plzeň-Orlík railway station, 345 m a.s.l., scattered scrub vegetation along railway embankment, on fallen branch of hardwood (*Salix?*), 29 Sept 2017 leg. & det. J. Kout (herb. CBG, J. Vlasák 1709/7 Kout), rev. J. Vlasák based on DNA (GenBank MK558726). – Chrást, near railway station, 345 m a.s.l., unkempt shrubs along path, attached dead branch of standing hardwood (probably *Syringa vulgaris*), 30 Sept 2017 leg. & det. J. Kout (herb. CBG). – Central Bohemia. Zadní Kopanina, Český kras (Bohemian Karst) Protected Landscape Area, Radotínské údolí Nature Reserve, Kopaninský les, 270 m a.s.l., broadleaf forest (beech, oak, hornbeam) with admixed pine, on limestone, on fallen branch of *Fagus*, 24 Nov 2017 leg. M. Kříž, det. P. Vampola (PRM 951086), rev. J. Vlasák based on DNA (GenBank MK558727). – Městec Králové, by tributary to Krčský Pond, 210 m a.s.l., broadleaf forest on edge of pond, attached dead branch of *Corylus avellana*, 29 Dec 2017 leg. et det. J. Kout (herb. CBG). – East Bohemia. Pohránov, U Pohránovského rybníka Nature Monument, 220 m a.s.l., water-logged *Salix* thicket, attached branch of *Salix*, 14 Apr 2017 leg. L. Zíbarová & T. Tejklová, det. L. Zíbarová (HR 104363).

DISCUSSION

Taxonomy and similar species

Although Ryvar den & Melo (2017) recently transferred *Cartilosoma rene-hentic* to genus *Antrodia* P. Karst., it is clear that this was done to accommodate species corresponding to the traditional concept of morphological genera used in the publication. While we acknowledge that this solution is certainly useful in identification literature, it is nevertheless obvious that the type of *Antrodia*, *A. serpens* (Fr.) P. Karst., is located in phylograms very distant from the type of *Cartilosoma*, *C. ramentaceum* (Spirin et al. 2013), and therefore its position in

Antrodia is untenable. Even though it is almost certain that generic delimitations inside the *Fomitopsidaceae* will be subject of further changes, we believe that the genus *Cartilosoma* is well-delimited both by means of morphology (see Introduction) and molecular phylogeny. Therefore we prefer to use the combinations *Cartilosoma rene-hentic* and *Cartilosoma ramentaceum* here.

The hyphal system of *Cartilosoma rene-hentic* is a rather complex matter. While trama and much of the context are monomitic, consisting of thick-walled generative hyphae, which swell conspicuously in KOH and are Congo Red positive, the basal layer of the basidiocarp attached to the substrate (“l’assise substratale” in Rivoire et al. 2015) is clearly dimitic, mostly made up of skeletal hyphae which do not swell markedly in KOH and are Congo Red negative. There is no distinct line between these parts of the basidiocarp, as the skeletal hyphae often penetrate into the adjoining tissue of the context. We did not see any distinct skeleto-ligative hyphae validating the description of the hyphal system as trimitic (see Rivoire et al. 2015). However, we observed scarce branching in some of the skeletal hyphae.

The more familiar *Cartilosoma ramentaceum* is morphologically and phylogenetically close to the treated species (Rivoire et al. 2015) and was considered rare for a long time in the Czech Republic (e.g. Kotlaba et al. 2006). In our experience, however, it is currently not uncommon (e.g. Kout & Vlasák 2011, Zíbarová & Kríž 2017). Such apparent increase in abundance has in Central Europe also been observed in polypores like *Pycnoporellus fulgens* (Fr.) Donk (Holec 2004) and *Antrodiella citrinella* Niemelä & Ryvarden (Holec et al. 2018). However, *C. ramentaceum* is restricted to conifers, growing almost exclusively on *Pinus* spp. (Ryvarden & Melo 2017), but also reported from other conifers such as *Abies* (Bernicchia et al. 2007a) and *Thuja* (Dueñas 2000) or even broadleaves: *Quercus ilex* (Bernicchia et al. 2007b), *Quercus cerris* (Cantini et al. 2014), *Salix* (Ainsworth 2001, Ordynets et al. 2013). The record on *Cistus albidus* from Spain (Torrejón 2007) is clearly a misidentification as we (JK) revised part of the specimen and found that it has significantly longer spores (up to 12 µm). However, the aforementioned records of *C. ramentaceum* from broadleaf wood may refer to *C. rene-hentic*. Moreover Rajchenberg et al. (2011) list *Araucaria* and *Austrocedrus* as hosts of *C. ramentaceum* in Argentina, but these records probably refer to another – yet undescribed – species (see below).

Concerning macromorphological characters, the contrast between the white context and the brownish, slightly resinous tubes – somewhat reminding of *Ceriporiopsis resinascens* (Romell) Domański – in dried specimens of *Cartilosoma rene-hentic* seems to be striking in comparison to our records of *C. ramentaceum*, in which the trama and tubes are more or less concolorous. However, Ainsworth (2001) mentions the presence of more resinous tubes in records of *C. ramentaceum* on conifers in contrast to his records on *Salix*. In *C. rene-hentic*

we neither observed curling or detachment of the margin of fruitbodies typical of *C. ramentaceum*.

Rajchenberg et al. (2011) published an ITS sequence (GenBank JF713082) of *C. ramentaceum* from Argentina. However in the phylogram presented by Rivoire et al. (2015), this sequence is different from European collections of both *C. ramentaceum* and *C. rene-hentic*, suggesting the existence of another, undescribed species in the genus. There is a sequence (GenBank KT334658.1) from an environmental sample from Poland with a 100% BLAST matching to the above-mentioned Argentinian one, so this putative species is also present in Europe. In addition, Rivoire et al. (2015) mention that according to V. Spirin there is yet another, northern *Cartilosoma* species with narrower spores. Although our specimens have spores generally narrower than indicated in the original description (2.7–3.2 µm on average), the ITS sequences of our specimens on which the measurements are based match the type material. The narrower spores of our specimens could be explained as an effect of the usage of a different medium for measuring (Cotton Blue in our work vs. ammoniacal Congo Red in the original description). We also found longer spores (8–10 × 2.2–3 µm) in the specimen from Plzeň-Orlík, whose sequence also matches *C. rene-hentic*, but this material is almost sterile, so that we were unable to find a sufficient number of spores for analysis. All in all, the spore dimensions of *C. rene-hentic* seem to be rather variable. Morphologically similar European *Antrodia* s.l. species from broadleaves, such as *A. macra* (Sommerf.) Niemelä (HR 104363 was in fact originally identified as *A. macra*) and *A. minuta* Spirin, are easily recognisable by the presence of skeletal hyphae in the tube trama.

Ecology and possible spread

We found *Cartilosoma rene-hentic* both on native (*Salix* sp., *Corylus avellana*, *Fagus sylvatica*) and introduced (*Syringa vulgaris*) hosts. Kotlaba (1984) did not mention *C. ramentaceum* from broadleaf hosts in the Czech Republic, neither did we find any such specimen of *C. ramentaceum* in the major Czech herbaria (BRNM, CB, HR, PRM). Regarding phytogeography (Skalický 1988), the species was found in both the Thermophyticum (3 records) and the Mesophyticum (3 records) regions.

Our records (Fig. 5) are from completely man-made (mine spoil, vegetation along railways) or predominantly man-influenced (edges of ponds, forests with long management history) habitats, often among urban landscape. One of these is the Antonín brown coal mine spoil near the town of Sokolov, where the species was found for the first time. The locality was reforested during the late 20th century with very diverse woody plants including many alien species. Basidiocarps of *C. rene-hentic* were found in a pure stand of *Syringa vulgaris*, which is not

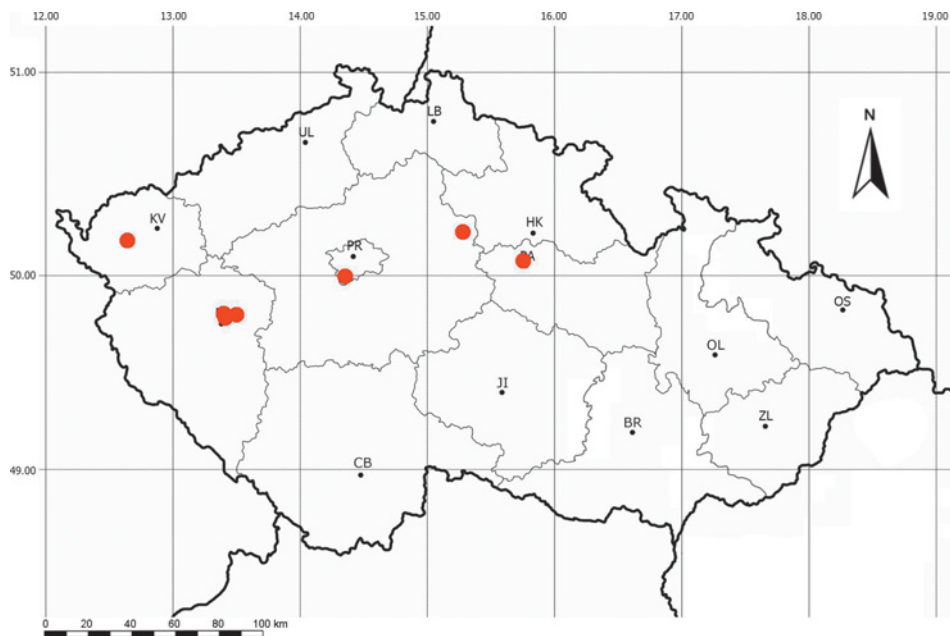


Fig. 5. Distribution map of *Cartilosoma rene-hentic* in the Czech Republic.

native to the Czech Republic, and the species was very abundant there on dead, but still attached branches or thin standing trunks of several *Syringa* individuals. The only other lignicolous fungi present on this substrate at the time were *Peniophora lycii* (Pers.) Höhn. & Litsch. and *Crepidotus cesatii* (Rabenh.) Sacc. Lignicolous fungi from the Czech Red list (Holec & Beran 2006) recorded on other alien plants on the Antonín mine spoil included *Ceriporiopsis aneirina* (Sommerf.) Domański and *Pleurotus calyptratus* (Lindblad ex Fr.) Sacc. on *Populus balsamifera*.

Even the records from protected areas (U Pohránovského rybníka Nature Monument, Radotínské údolí Nature Reserve) are from habitats with a long human management history. In contrast, there are no records of *C. rene-hentic* from virgin or natural forests in the Czech Republic. In addition, we collected *C. rene-hentic* inside or close to urban areas, often close to railroads. Although we cannot discount the possibility that a more thorough revision of herbarium material would uncover some misidentified specimens of *C. rene-hentic* from the past, the fact that none of our collections are older than 2016 and were recorded at man-influenced localities suggests the interesting hypothesis that the species might be recently introduced in the Czech Republic.

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