CZECH MYCOLOGY 66(1): 61–69, JUNE 4, 2014 (ONLINE VERSION, ISSN 1805-1421)

First record of *Skeletocutis ochroalba* (*Polyporales*) in the Czech Republic

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Zíbarová L., Kout J. (2014): First record of *Skeletocutis ochroalba* (*Polyporales*) in the Czech Republic. – Czech Mycol. 66(1): 61–69.

The polypore species *Skeletocutis ochroalba* is newly reported from the Czech Republic and for the first time from Central Europe. Both morphology and sequencing of DNA (ITS region) confirmed the identity of the species. It is characterised by pileate basidiocarps, narrow allantoid spores and ecology. Its similarity to *S. nivea* is discussed. An in situ photo of *S. ochroalba* is included.

Key words: polypore, boreal species, taxonomy, rare species.

Zíbarová L., Kout J. (2014): První nález *Skeletocutis ochroalba* (*Polyporales*) z České republiky. – Czech Mycol. 66(1): 61–69.

Choroš *Skeletocutis ochroalba* je nově zaznamenán z České republiky a poprvé ze střední Evropy. Morfologické znaky i DNA sekvenace (ITS oblast) potvrdily správnost určení. Druh je charakterizován kloboukatými plodnicemi, úzkými alantoidními sporami a ekologií. Podobnost k bělochoroši poloklobokatému (*Skeletocutis nivea*) je diskutována. Uvedena je fotografie z místa nálezu.

INTRODUCTION

Polypores of the genus *Skeletocutis* Kotl. & Pouzar are well delimited by some microscopic features, especially by their allantoid to ellipsoid spores and remarkably encrusted hyphae in dissepiments (Ryvarden 1991, Ryvarden & Gilbertson 1994). One of them, *Skeletocutis ochroalba* Niemelä, a very rare boreal species, was newly found in the Central Europe, during a field inventory of Žofinka National Nature Reserve, Czech Republic in 2012.

The aim of this paper is to provide a description of macro- and microscopic features of this specimen as well as data on the global distribution and ecology of the species. In addition we will try to clarify its taxonomic status and compare it to related taxa, namely *Skeletocutis nivea* (Jungh.) Jean Keller using molecular methods (sequencing of ITS segment of DNA).

MATERIAL AND METHODS

The macroscopic description of the specimen was based on fresh material. Microscopic features were observed in a 3% KOH solution at 1000× magnification under an oil immersion lens using an Olympus BX41. Microscopic characterisation including measurements (with the exception of spores) was carried out using fresh material. Dimensions of spores were derived from measurements of 25 individual spores in dried material. The spores were measured directly under an optical microscope using an eyepiece micrometer. Both macro- and microscopic descriptions are solely based on the specimen from the Czech Republic.

The identification was based on the description in Ryvarden & Gilbertson (1994, p. 629–630).

The specimen was dried at room temperature, stored in a PE bag with slide fastener and was subsequently frozen. Parts of the original voucher specimen are now deposited in the Jihočeské Muzeum, České Budějovice (CB) and the private herbarium of J. Vlasák (http://mykoweb.prf.jcu.cz/polypores/index.html).

DNA extraction and PCR methods were applied as described by Vlasák & Kout (2011). Phylogram analysis was carried out according to Vampola & Vlasák (2011). The list of sequences used for phylogram construction is given in Tab. 1. There were a total of 2156 positions in the final dataset.

Tab. 1. List of sequence data used for construction of the phylogram.

Species	Country	Herbarium specimen	GenBank	Reference
Cinereomyces lindbladii	Finland	Heikki Kotiranta 19911	FN907909.1	Miettinen & Larsson (2011)
Gloeoporus pannocinctus	USA	CFMR: DLL2011-074	KJ140594.1	
Skeletocutis amorpha	United Kingdom	K 31290	AJ006677.1	Yao et al. (1999)
Skeletocutis chrysella	Finland	Otto Miettinen 9472	FN907916.1	Miettinen & Larsson (2011)
Skeletocutis diluta	USA	JV 100861	JF692198.1	Vlasák et al. (2011)
Skeletocutis kuehneri	United Kingdom	K 39915	AJ006678.1	Yao et al. (1999)
Skeletocutis nivea	USA	CFMR: DLL2011-060	KJ140581.1	
Skeletocutis nivea	USA	CFMR: DLL2011-144	KJ140645.1	
Skeletocutis nivea	USA	CFMR: DLL2011-070	KJ140590.1	
Skeletocutis ochroalba	Czech Republic	CB 18402	KF840389	
Skeletocutis ochroalba	Finland	Markkanen 1855		
Skeletocutis odora	Czech Republic	JV 1007_7	JN592508.1	Vlasák et al. (2012)

Results

Skeletocutis ochroalba Niemelä, Naturaliste Can. 112(4): 466, 1985. Figs. 1, 2

Selected illustrations. Niemelä (2008), fig. 268, p. 255 (colour photograph of basidiomata); Niemelä (1985), p. 466 (microcharacters).

Macroscopic description. Basidiocarps annual, possibly perennial, pileate, knob-like, tough, up to 18 mm wide, up to 12 mm thick at the base, projecting up to 10 mm; upper surface convex, yellowish (at first cream-coloured) with warm ochraceous tint, matt, with darker gelatinous spots or bands; margin even, blunt, outline semicircular; pore surface concave, cream with slight orange (salmon) tint, orange tone more pronounced when bruised; pores entire, angulate, 6–9 per mm; context coriaceous, whitish, uniform; tube layer cream coloured. Smell slightly acidulous, taste none.

Microscopic description. Hyphal system trimitic in context, monomitic in trama; generative hyphae with clamps, hyaline, thin-walled but more often with thickened walls (especially in the context), sparingly branched, 2–3.5 µm in diam.; skeletal hyphae dominating, without clamps, non-septate, rarely with secondary septa, hyaline to faintly yellowish, thick-walled to solid, non-septate, unbranched,

Fig. 1. Basidiocarps of *Skeletocutis ochroalba*. Czech Republic, Žofinka Nature Reserve, 13 Oct. 2012 (CB 18402). Photo by Lucie Zíbarová.

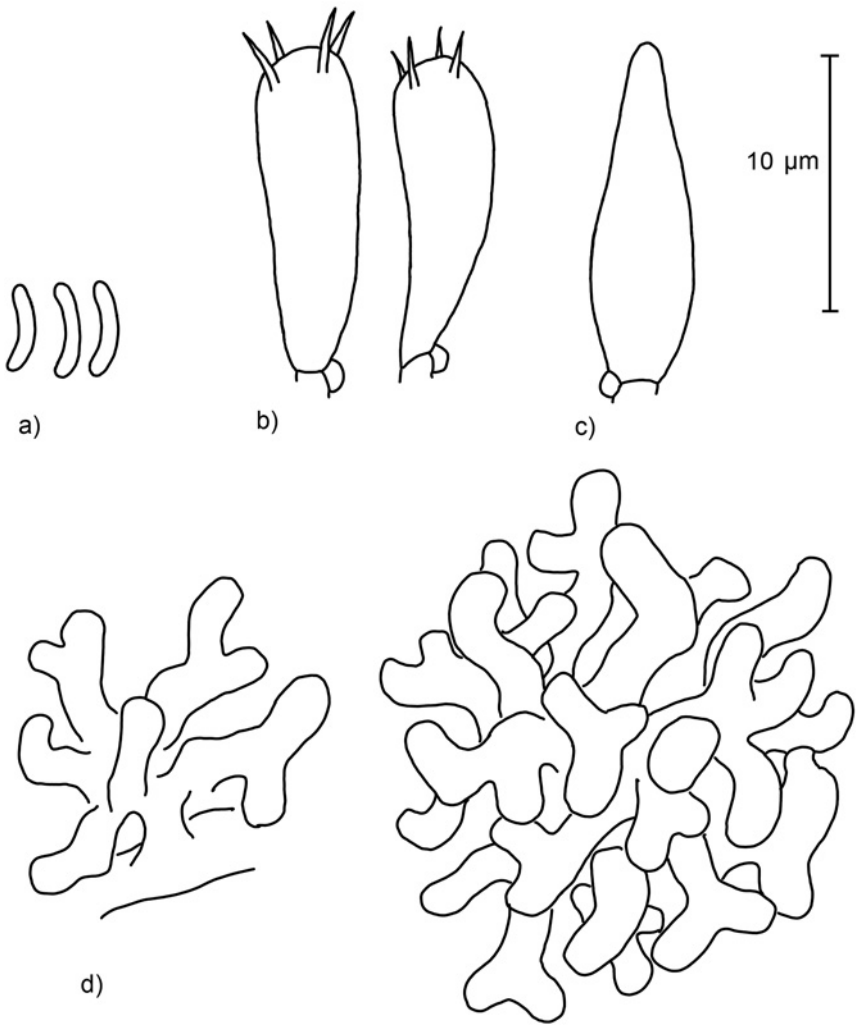

Fig. 2. Line-drawing of microscopic characters (CB 18402): **a** – basidiospores; **b** – basidia; **c** – cystidioles; **d** – clusters of binding hyphae (context). Del. Lucie Zíbarová.

3.5–5.5 µm in diam.; binding hyphae originating from generative hyphae, densely branched, coralloid, slightly thick-walled, with blunt ends, 1.5–2.5 µm in diam. Cystidial elements absent. Cystidioles scattered, fusoid, 10–15 × 3–4 µm, with a basal clamp. Basidia clavate, 4-spored, 10–14 × 3.5–4 µm, with a basal clamp. Basidiospores narrowly allantoid, hyaline, thin-walled, acyanophilous and neither amyloid nor dextrinoid, 3–4.5 (ave. 3.8) × 0.7 µm.

Type of rot. White rot.

Substrate and habitat. Three basidiocarps were found in August 2012 in Žofinka National Nature Reserve in the Třeboň Basin, South Bohemia by the first author. The nature reserve was founded in 1975 in order to protect a vast nutrient-poor raised bog with dominance of *Pinus sylvestris*, *Pinus rotundata* and its hybrids with characteristic vegetation (*Rhododendron tomentosum*, *Andromeda polifolia* etc.). The geological bedrock consists of Tertiary clay sediments overlaid by Quaternary organic peat deposits (Albrecht et al. 2003).

The fungus was not found in the bog itself, but in a strip of water-logged spruce stands which line the bog. The stand at the site itself was rather young (pole-stage) and dense, with numerous recently fallen logs. All basidiocarps stemmed from a single fallen dead log of Norway spruce (*Picea abies*), growing at most about 20 cm apart. At the time of collection, the log, fairly thin in diameter (ca. 5 cm) and touching the ground only at one end, was in the initial stage of decay, still with most bark attached (Stage 1 according to Renvall 1995). Other macromycete species present on the log were *Trichaptum abietinum* (Dicks.) Ryvarden and *Exidia pithya* Fr.

ITS sequence analysis. The sequence of our specimen of *Skeletocutis ochroalba* (GenBank access number KF840389), according to Blast, agreed completely with O. Miettinen's sequence of this species from Finland (pers. comm.). The second closest species is *S. nivea* with 97% concordance in GenBank (e.g. KJ140581.1, KJ140645.1), quite in agreement with the morphological similarity of these species. No ITS sequence of *Skeletocutis ochroalba* has been published to the date. However, available sequences of *Skeletocutis nivea* come very close, although they are sufficiently different (97%) for distinguishing two separate species.

Both specimens of *S. ochroalba* form a well-supported clade in the maximum likelihood phylogram (Fig. 3) separated from specimens of *S. nivea*. Other narrow-spored *Skeletocutis* taxa are rather distant.

Specimens examined

Czech Republic. Bohemia. Třeboň Basin, Třeboňsko Protected Landscape Area, Žofinka National Nature Reserve, alt. 476 m a.s.l., 48°49'15.06" N 14°52'31.62" E, thin fallen log of *Picea abies*, young water-logged spruce stand, 13 Aug. 2012, leg. et det. L. Zíbarová (CB 18402, JV 1208/8-Zib).

DISCUSSION

Skeletocutis ochroalba was originally described from North America (Niemelä 1985) and is a polypore known from the boreal region of the entire Northern Hemisphere – Quebec, Canada (type locality), China (Dai 2000, Dai et al. 2004) and Northern Europe, namely Estonia, Finland, Norway, and Sweden (Niemelä et al. 2001). It is also known from the island of Corsica, France (Norstedt et al. 2001),

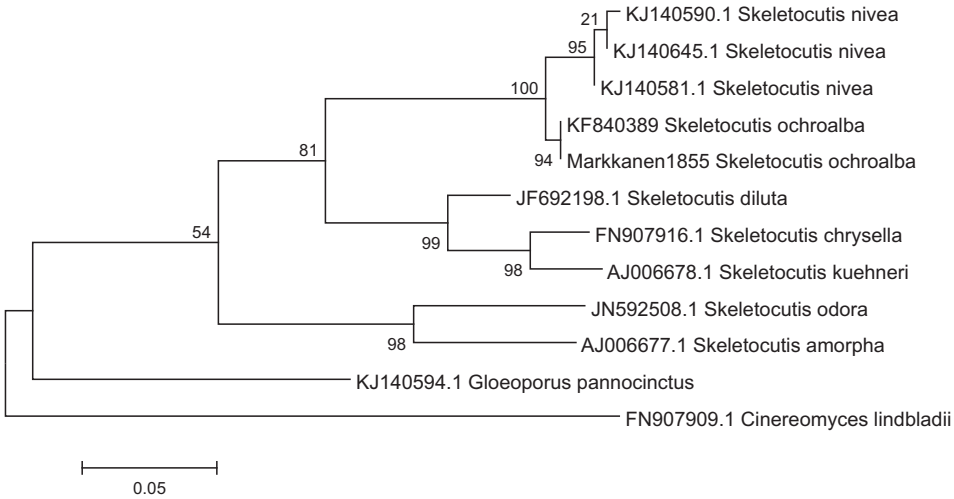

Fig. 3. Maximum-likelihood phylogram of evolutionary relationships of *Skeletocutis ochroalba*. The tree with the highest log likelihood (–6058.8012) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths corresponding to the number of base substitutions per site.

where it occurs in a montane pine forest with several other boreal polypore taxa (e.g. *Antrodia primaeva* Renvall & Niemelä, *Antrodia sordida* Ryvarden & Gilb.). Recently, two specimens were published from Mexico (Valenzuela et al. 2006). Nuñez et Ryvarden (2001) also mention it from Taiwan, but no further data are provided. Despite its wide geographical range, the species appears to be generally rare in its distribution area and is included in the Red lists of Sweden (VU) (Dahlberg et al. 2010) and Norway (DD) (Kålås et al. 2010). It has been collected from dead wood of various spruce species – *Picea abies* in Europe, *P. glauca* in N America and *P. jezoensis* in China (Dai 2000, Niemelä et al. 2001, Ryvarden & Gilbertson 1994), but also from *Pinus nigra* ssp. *laricio* (Norstedt et al. 2001). Niemelä et al. (2001) note that trunks on which *S. ochroalba* was found have a fairly small diameter. Also its position just outside a virgin forest is strikingly similar to our specimen. On the other hand, the original description (Niemelä 1985) is based on man-cut corticated logs, lying deep in moss, so its ecological amplitude could be much wider.

Except for the single Corsican specimen (see above), records of *S. ochroalba* (which include data on ecology) originate from boreal coniferous forests. It may be assumed that the species has survived on the Czech locality since the last glaciation, just as this is presumed for some relict plant species (*Andromeda polifolia*,

Rhododendron tomentosum, *Vaccinium uliginosum*) and invertebrates (Albrecht et al. 2003). However, as the population ecology of polypores is still poorly understood, long-distance transport of spores can neither be excluded. It should be noted that the Czech locality is rather interesting from the perspective of uncommon wood-inhabiting species. The water-logged spruce stand in the vicinity hosts some red-listed (Holec & Beran 2006) species such as *Fomitopsis rosea* (Alb. & Schwein.) P. Karst., *Phlebia centrifuga* P. Karst., *Camarops tubulina* (Alb. & Schwein.) Shear, *Pycnoporellus fulgens* (Fr.) Donk, and *Pholiota subochracea* (A.H. Sm.) A.H. Sm. & Hesler, while in the raised bog one can encounter *Auriporia aurulenta* A. David, Tortić & Jelić on several fallen pine logs, and *Diplomitoporus flavescens* (Bres.) Domański, frequently occurring on dead standing pine wood (see Zíbarová 2013 for a complete list). Additional specimens can be found in similar relict and well-preserved localities, namely in the nearby Šumava (Bohemian forest) mountain range.

The results of the ITS analysis are in agreement with both macro- and microscopic characters, as *Skeletocutis nivea* is mentioned as the closest relative to the discussed species and microscopically virtually indistinguishable from *S. ochroalba* (Niemelä et al. 2001). Bernicchia (2005) presents in her key to *Skeletocutis* a slightly larger range of spore length with higher values in *Skeletocutis nivea*, but we cannot confirm this observation. Both species are well delimited against the other European *Skeletocutis* taxa by their combination of very narrow (< 1 µm) spores and hyphal system, which is trimitic in the context but monomitic in the trama (Bernicchia 2005, Ryvarden & Gilbertson 1994). Despite the lack of significant microscopic differences it seems that both taxa can be reliably distinguished according to their ecology and basidiocarp morphology (Niemelä et al. 2001). Basidiocarps of *S. nivea* are mostly effused-reflexed, sometimes completely resupinate, pilei develop relatively late in the season and are often several centimetres in length and confluent. In contrast, basidiocarps of *S. ochroalba* are typically well-delimited, compact, relatively small (mostly about 1.5 cm), knob-like with well-developed pilei. There are also marked differences in colour. *S. nivea* completely lacks any orange or yellow tints in the pores which are typical of *S. ochroalba*. Niemelä et al. (2001) note that older and dried specimens of *S. ochroalba* may however obtain a greenish tint in the pore surface analogous to *S. nivea*, but we were unable to confirm such discolouration in our specimen. In addition, *S. nivea* shows a rather southern distribution in Europe and moreover colonises hardwoods, whereas *S. ochroalba* exhibits a boreal (Ryvarden et Gilbertson 1994) or montane (Norstedt et al. 2001) distribution and seems to be restricted to conifers.

Other members of the genus growing on conifers form resupinate basidiocarps with a dimitic trama [*S. kuehneri* A. David, *S. stellae* (Pilát) Jean Keller] or have significantly wider spores [*S. subincarnata* (Peck) Jean Keller] (Bernicchia 2005).

Niemelä et al. (2001) note that basidiocarps of *S. ochroalba* may superficially resemble very young specimens of *Fomitopsis pinicola* (Sw.) P. Karst. Any closer inspection will easily distinguish these two species, but it could be one of the reasons why *Skeletocutis ochroalba* seems to be overlooked in the field.

ACKNOWLEDGEMENTS

We thank Dr. J. Vlasák (Biology Centre ASCR, České Budějovice) for the molecular analysis and Dr. O. Miettinen (University of Helsinki) for providing access to his sequence.

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