Cryptogamie, Mycologie, 2013, 34 (3): 203-209

Antrodia kmetii, a new European polypore similar to *Antrodia variiformis*

Josef VLASÁK[a*], *Josef VLASÁK Jr. & Bao-Kai CUI*[b]

[a]*Biol. Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic*

[b]*Institute of Microbiology, P.O. Box 61, Beijing Forestry University, Beijing 100083, P. R. China*

Abstract – *Antrodia variiformis*, a species originally described from the USA, has been reported from a few localities in southern Europe, including Slovak Republic, Croatia, and southern France. The identity of these collections was questioned and both morphological and molecular data showed that it represents a distinct undescribed species. It is described as *Antrodia kmetii* and compared to related species such as *A. variiformis* and *A. serialis*.

Molecular phylogeny / ITS / polypore / Brown-rot fungi / taxonomy

INTRODUCTION

A small group of phylogenetically related polypores around *Antrodia serialis* (Fr.) Donk (Cui, 2013) contains a few species characterized by annual but rather tough, resupinate to effused-reflexed basidiocarps that often produce small, nodulose pilei elongated along the substrata. Whitish colors, sometimes light brown on pileus surface, and cylindrical to slightly fusoid spores are also typical. This group includes at least four members: *A. serialis, A. variiformis* (Peck) Donk, *A. serialiformis* Kout & Vlasák (Kout & Vlasák, 2009) and *A. leucaena* Dai & Niemelä (Dai & Niemelä, 2002).

Antrodia variiformis differs from the common *A. serialis* (Fr.) Donk by much larger pores. It was described in 1889 from north-eastern America where it is widely distributed on dead wood of gymnosperms, rarely angiosperms (Overholts, 1953; Sarkar, 1959). It has also been recorded from China (Dai & Niemelä, 2002) and Africa (Ryvarden & Johansen, 1980). In Europe, Kotlaba (1985) reported several collections of *A. variiformis* from Croatia, Slovakia and Ukraine. All collections, some of them dating back to late nineteenth century (1891!), originated from relatively warm forested regions and were growing on *Abies alba* L. They were originally determined as *A. serialis* or *A. heteromorpha* (Fr.) Donk. David & Tortic (1986) provided some cultural characteristics of this fungus for a collection from the French Pyrenees. According to Ryvarden & Gilbertson (1993), *A. variiformis* is known also from Italy. Nonetheless, it is still regarded as a very rare species in Europe.

* Corresponding author: vlasak@umbr.cas.cz

doi/10.7872/crym.v34.iss2.2013.203

Kotlaba (1985) was the first to express some doubts about the identity of the European collections of *A. variiformis.* In America, this species grows often far north and has distinctly larger spores compared to European collections of *A. variiformis*. David & Tortic (1986) mentioned also smaller spores as well as larger pores for European collections. More importantly, they recorded incompatibility in nearly all crossings of American and European monokaryotic mycelia and only partial compatibility in others, which indicates a different species.

For the present paper, the authors compared European and American specimens of *A. variiformis* to explore whether or not these represent the same species from morphological and phylogenetic perspectives.

MATERIAL AND METHODS

Morphological studies. — Macroscopical and microscopical characters were studied on fresh fruitbodies and herbarium specimens deposited at the Prague Museum Herbarium, Czech Republic (PRM) and the private herbarium of J. Vlasák (JV). Microscopic characteristics were observed in Melzer's reagent and Cotton Blue with an oil immersion lens at a magnification of 1000×. A total of 20 basidiospores from each specimen were measured. In presenting the variation in the size of the basidiospores, 5% of measurements were excluded from each end of the range and given in parentheses.

Molecular procedures and phylogenetic analysis. — DNA was isolated and sequenced as described by Vlasák & Kout (2011). Some sequences obtained comprised of two haplotypes differing in several positions and both were included in phylogeny. All newly generated sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov; Fig. 1). Phylogenetic analysis followed Li & Cui (2013). Maximum parsimony analysis was conducted for the ITS dataset. All characters were equally weighted and gaps were treated as missing data. For the maximum parsimony analysis, the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford, 2002). Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein, 1985). Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI), were calculated for each Maximum Parsimonious Tree (MPT) generated. Branches that received bootstrap support for maximum parsimony (MP) greater or equal than 80% (MP) were considered as well-supported.

RESULTS

Fifteen ITS sequences were generated from specimens of *A. variiformis* and similar *A. serialis* and *A. heteromorpha,* collected in the USA and Europe in nature or obtained from PRM (Prague) herbarium. Other sequences of

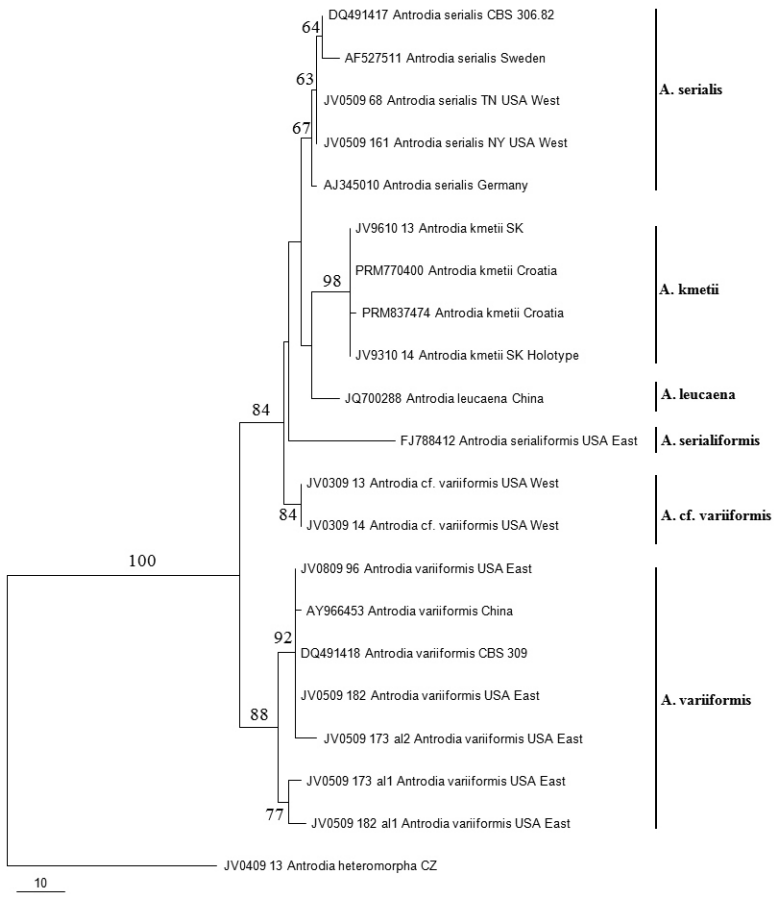

Fig. 1. Phylogenetic relationships of 21 *Antrodia* taxa inferred from ITS rRNA sequences. *A. heteromorpha* was used to root the tree. Topology from maximum parsimony (MP) analysis. Support values along branches from MP bootstrap (1000 replicates). Branch lengths are drawn proportional to genetic distances (bar indicates 10 changes per ITS1-5.8S-ITS2 region). Some of the specimens show two haplotypes. GenBank numbers indicate sequences retrieved from GenBank; other GenBank numbers in specimens examined.

A. variiformis and similar species were retrieved from GenBank (Fig. 1). The alignment of 630 characters (35 parsimony informative) demonstrated that the specimens of "*Antrodia variiformis*" from Europe differ from the American specimens in many positions, including 20 transitions/transversions and 8 one base indels. Based on ITS sequences, the European species is in fact more closely related to *A. serialis* than to American *A. variiformis.* In MP phylogeny, European collections form a strongly supported clade separated from the *A. serialis* as well as *A. variiformis* clades (Fig. 1). Identical results were obtained with Maximum Likelihood phylogeny (not shown). This, together with obvious differences in morphology (including spore size) and ecology justifies the recognition of the European "*A. variiformis*" as a distinct species. It is described and illustrated below as *Antrodia kmetii*.

TAXONOMY

***Antrodia kmetii* Vlasák, sp. nov.** **Figs 2-4**

MycoBank: MB 804714

Basidiocarps similar to *Antrodia variiformis* (Peck) Donk or *Antrodia serialis* (Fr.) Donk, tough, whitish, effused-reflexed, usually with small, brownish pilei along the upper part, with very large, angular pores, 8–10 (–12) per cm, becoming daedaleoid on sloping substrates. Hyphal system dimitic; generative hyphae with clamp connections. Basidiospores smaller than in similar species, cylindrical, hyaline, IKI–, CB–, 5–6 × 1.9–2.2 μm. Known only from dead logs of *Abies alba* L., associated with brown rot.

Holotypus: **SLOVAKIA,** Badín, Badín NPR, log of *Abies alba*, 12 Oct 1993 J. Vlasák, JV 9310/14 (PRM 861180; isotype in JV, BJFC).

Etymology: kmetii (Lat.): In honor of A. Kmet, Slovak priest and naturalist, who collected the first specimen of this fungus already in 1891, according to Kotlaba (1985).

Basidiomes annual to biannual, resupinate to effused-reflexed, up to 20 cm or more in length, with pilei less than 1 cm wide but often elongated along the substrata, tough and flexible. **Pileal surface** velutinate, cinnamon brown, zonate, margin white, narrow, sharp. Pore surface white, sordid brown with age; *pores* angular in horizontal parts of the basidiocarp, very large, 8–10 (–12) per cm, up to 14 per cm on the very margin, on sloping substrates irregular, sinuous to daedaleoid; tubes concolorous, up to 5 mm long; context white, up to 2 mm thick, becoming brown next to substrate or at the base of the pilei.

Hyphal system dimitic, generative hyphae thin-walled, with clamp connections, 2–3 μm wide, skeletal hyphae dominating, hyaline, subsolid to thick-walled, straight, 2–5 μm wide. **Cystidia** absent, cystidioles inconspicuous. **Basidia**

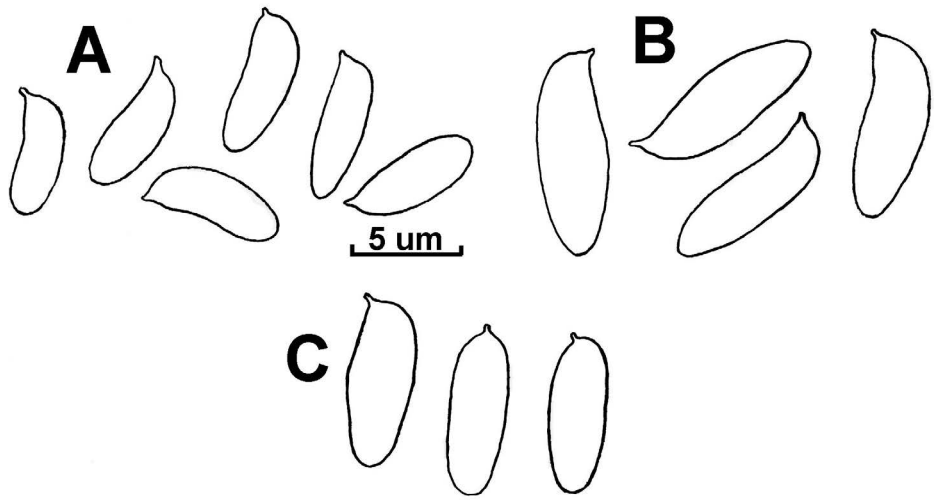

Fig. 2. Basidiospores of *Antrodia kmetii* drawn from PRM861180, holotype (**A**), *Antrodia variiformis* (JV 0809/96, **B**) and *Antrodia serialis* (JV 0509/161, **C**).

clavate, 4-sterigmatic, 20–30 × 4–6 µm with a basal clamp. **Basidiospores** cylindrical, slightly arcuate to the apiculus, hyaline, thin-walled, negative in Melzer`s reagent, (4.5–) 5–6 (–6.5) × 1.9–2.2 (–2.4) µm.

Fig. 3. A basidiocarp of *Antrodia kmetii* (PRM 861180, holotype).

Fig. 4. Pores of *Antrodia kmetii* (JV 9610/13) and *Antrodia variiformis* (JV 0809/96).

Type of rot. Brown rot.

Remarks: Antrodia variiformis differs by somewhat smaller pores, 12-18 per cm, up to 22 per cm close to the margin, and larger spores, 8–12 × 3-4.5 µm by Gilbertson & Ryvarden (1986), but only 7–8 (–9) × 2.5–3 µm according to David & Tortic (1986), and our measurement. *Antrodia serialis* has much smaller pores, 30–40 per cm and larger basidiospores (6.3–8 × 2.2–3.3 µm, Dai & Niemelä, 2002). *Antrodia heteromorpha* (Fr.) Donk has similar small pilei and large, sinuous pores but it is white consistently in all parts of basidiocarp, and its spores are much larger. The sequence is only distantly related. On the other hand, *A. serialiformis* and *A. leucaena* show rather similar sequence. These species have, however, much smaller pores and grow on hardwood substrates (Kout & Vlasák, 2009; Dai & Niemelä, 2002).

Additional specimens examined:

***Antrodia kmetii* — SLOVAKIA**. Badín, Badín NPR, log of *Abies alba*, 22 Sep 1988 J. Vlasák, JV 8809/35; 18 Sep 1989, JV 8909/20A; 8 Oct 1996, JV 9610/13, GenBank KC886708; Dobroc, Dobroc NPR, log of *Abies alba*, 18 Sep 1989, JV 8909/40. **CROATIA**. Nat. Park Sutjeska, Perucica, log of *Abies alba*, 17 Jul 1969 M. Tortic, PRM 770400, GenBank KC88670811; 23 Jun 1972, PRM 771271; Nat. Park. Plitvice, Corkova uvala, *Abies alba,* 4 Nov 1973 M. Tortic, PRM 837474, GenBank KC886710.

***Antrodia heteromorpha* — CZECH REPUBLIC.** Strážný, *Picea abies*, Sep 2004 J. Vlasák JV 0409/13, GenBank KC886717.

***Antrodia serialis* — USA**. **TENNESSEE**: Great Smoky Mt., Road Prong Trail, *Picea* sp., 6 Sep 2005 J. Vlasák, JV 0509/68, GenBank KC886703. **NEW YORK**: Adirondack Park, Capperos Pond, *Picea* sp., 19 Sep 2005 J. Vlasák, JV 0509/161, GenBank KC886705. **CZECH REPUBLIC.** Strážný, *Picea abies*, Sep 2004 J. Vlasák JV 0409/13, GenBank KC886717.

***Antrodia variiformis* — USA**. **NEW YORK**: Adirondack Park, Capperos Pond, *Picea* sp., 19 Sep 2005 J. Vlasák, JV 0509/157; Marcy Dam, *Picea* sp., 20 Sep 2005 J. Vlasák, JV 0509/173, GenBank KC886713, KC886714; Moose Pond, *Picea* sp., 21 Sep 2005 J. Vlasák, JV 0509/182, GenBank KC886715, KC886716; Johns Brook Trail, *Picea* sp., 23 Sep 2005 J. Vlasák, JV 0509/202; **NEW HAMPSHIRE**: White Mountains, Wonalancet, The Bowl, *Picea* sp., 16 Sep 2008 J. Vlasák, JV 0809/81; Sawyer Pond, *Picea* sp., 18 Sep 2008 J. Vlasák, JV 0809/96, GenBank GenBank KC886711.

***Antrodia cf. variiformis* — USA**. **WASHINGTON**: Queets River, Queets, *Picea sitchensis*, 1 Sep 2003 J. Vlasák, JV 0309/13, GenBank KC886705; JV 0309/14, GenBank KC886706.

DISCUSSION

Several species were undoubtedly hidden for years behind the extensive "concept" variability of the very common *Antrodia serialis.* The recently described *A. serialiformis,* common in eastern USA, used to be determined as *A. serialis* from oaks (Kout & Vlasák, 2009). In China, *A. leucaena* growing on poplars is another example (Dai & Niemelä, 2002; Dai *et al.*, 2004). Based on molecular, morphological and ecological evidence, we describe in this paper *A. kmetii* from Europe, growing on *Abies alba* in temperate forests, specimens of which were

known in Europe for more than hundred years but used to be determined as *A. serialis* or *A. variiformis* (Kotlaba, 1985). Specimens of *A. kmetii* found in Slovakia and Croatia were sequenced in this study but also the collections from France belong to this species because their morphological characteristics are identical (David & Tortic, 1986).

In our opinion, other species will in all probability emerge within this group, also in the USA. For instance, two specimens from a single locality in north-western USA (JV 0309/13 and JV 0309/14) and tentatively determined as *A. variiformis,* shared with *A. kmetii* the very large pores and the small basidiospores but they lacked any pilei. They also have very similar sequences. More materials are needed to solve the identity of this fungus. Other species of similar appearance may occur because *A. serialis* is listed over the whole USA as a very common fungus (Gilbertson & Ryvarden, 1986) but we could find in 12 years of field research over the USA western states none specimen of corresponding morphology and sequence.

Acknowledgments. The authors thank Jan Holec (National Museum Prague, Mycological Department, Czech Republic) for the loan of *Antrodia kmetii* specimens. We also express our gratitude to Prof. Yu-Cheng Dai (BJFC, China) for improving the manuscript. Bao-Kai Cui gratefully acknowledges the financial support from the National Natural Science Foundation of China (No. 31170018) and the Fundamental Research Funds for the Central Universities (Project No. JC2013-1). The research of J. Vlasák was funded by institutional support RVO: 60077344.

REFERENCES

CUI B.K., 2013 — *Antrodia tropica* sp. nov. from southern China inferred from morphological characters and molecular data. *Mycological Progress* in press Doi: 10.1007/s11557-012-0829-7.

DAI Y.C. & NIEMELÄ T., 2002 — Changbai wood-rotting fungi 13. *Antrodia* sensu lato. *Annales Botanici Fennici* 39: 257-265.

DAI Y.C., WEI Y.L. & WANG Z., 2004 — Wood-inhabiting fungi in southern China 2. Polypores from Sichuan Province. *Annales Botanici Fennici* 41: 319-329.

DAVID A. & TORTIC M., 1986 — Contribution à l'étude de quatre polypores européennes peu connus. *Cryptogamie Mycologie* 7: 1-13.

FELSENSTEIN J., 1985 — Confidence intervals in phylogenetics: an approach using bootstrap. *Evolution* 39: 783-791.

GILBERTSON R.L. & RYVARDEN L., 1986 — North American polypores, Vol. 1. Fungiflora. Oslo. 433 p.

KOTLABA F., 1985 — *Antrodia variiformis* in Czechoslovakia (in Czech). *Mykologické listy* 18: 5-8.

KOUT J. & VLASÁK J., 2009 — *Antrodia serialiformis* from the eastern USA, a new and abundant polypore similar to *Antrodia serialis. Mycotaxon* 108: 329-335.

LI H.J. & CUI B.K., 2013 — Taxonomy and phylogeny of the genus *Megasporoporia* and its related genera. *Mycologia* 105: 368-383.

OVERHOLTS L.O., 1953 — The Polyporaceae of the United States, Alaska and Canada. 466 p., Ann Arbor et London.

RYVARDEN L. & GILBERTSON R.L., 1993 — European polypores, Vol. 1. Fungiflora. Oslo. 1-387 p.

RYVARDEN L. & JOHANSEN I., 1980 — A preliminary polypore flora of East Africa. Fungiflora. Oslo. 636 p.

SARKAR A., 1959 — Studies in wood-inhabiting hymenomycetes IV. The genus *Coriolellus* Murr. *Canadian Journal of Botany* 37: 1251-1270.

SWOFFORD D.L., 2002 — PAUP*: phylogenetic analysis using parsimony (*and other methods). version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

VLASÁK J. & KOUT J., 2009 — Tropical *Trametes lactinea* is widely distributed in the eastern USA. *Mycotaxon* 115: 271-279.