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Antrodia serialiformis from the eastern USA, a new and abundant polypore similar to *A. serialis*

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Abstract — A new and common polypore *Antrodia serialiformis* from eastern USA is described. The species is similar enough to *A. serialis* that previously both species were probably confused, but *A. serialiformis* seems exclusively confined to oaks. We present evidence that the new species differs from *A. serialis* based on much smaller basidiospores, mating incompatibility, rDNA sequence differences, and ecology.

Key words — taxonomy, brown-rot polypore, *Basidiomycota*, North America.

Introduction

Nearly all trees and herbaceous plants as well as some agaricoid fungi (Vilgalys 1991) native to the eastern USA represent species different from those described from Europe. Similarly looking "twin species" are frequent, reflecting evolution from common ancestors. In contrast, approximately 75% of temperate zone polypores represent species that have been regarded as the same on both continents (Gilbertson & Ryvarden 1986, 1987). Not surprisingly, the advent of recombinant DNA technology has uncovered new American polypore twin-species that differ from those found in Europe (Miettinen et al. 2006). In this paper, we present evidence that a polypore similar to *Antrodia serialis* (Fr.) Donk, very common on oak logs in Pennsylvania, Maryland, Virginia, North Carolina, Tennessee and probably other US southeastern states, is a related but distinct species named here *Antrodia serialiformis.*

Materials and methods

Specimens

We collected 31 specimens from the eastern USA during 2001, 2003, 2005, 2007, and 2008. Pieces of dried basidiocarps mounted in 5% KOH solution or Melzer`s

Table 1. *Antrodia serialiformis* specimens examined.

Specimen	*	Locality	Collected
JV0108/11	*s*	French Creek St. Park, Hopewell, PA, USA	2. VIII. 2001
JV0108/12a	*f*	French Creek St. Park, Hopewell, PA, USA	2. VIII. 2001
JV0108/13a,b	*f*	French Creek St. Park, Hopewell, PA, USA	2. VIII. 2001
JV0108/118a	*s*	Ralph Stover St. Park, Point Pleasant, PA, USA	21. VIII. 2001
JV0109/C3-J	*s*	Shenandoah Nat. Park, Front Royal, VA, USA	19. IX. 2001
JV0308/35	*f*	Evansburg St. Park, Norristown, PA, USA	28. VIII. 2003
JV0308/36	*s*	Evansburg St. Park, Norristown, PA, USA	28. VIII. 2003
JV0308/37	*s*	Evansburg St. Park, Norristown, PA, USA	28. VIII. 2003
JV0308/38	*s*	Evansburg St. Park, Norristown, PA, USA	28. VIII. 2003
JV0309/98	*s*	Ricketts Glen St. Park, Wilkes-Barre, PA, USA	11. IX. 2003
JV0309/113	*s*	Ralph Stover St. Park, Point Pleasant, PA, USA	16. IX. 2003
JV0309/153	*s*	Tuckahoe St. Park, Hillsboro, MD, USA	20. IX. 2003
JV0309/154	*s*	Tuckahoe St. Park, Hillsboro, MD, USA	20. IX. 2003
JV0402/5-J	*s*	Elkneck St. Park, Elktown, MD, USA	10. II. 2004
JV0404/13-J	*s*	Boogerman Trail, Great Smoky Mt., NC, USA	9. IV. 2004
JV0404/26-J	*f*	Nat. Historical Park, Harpers Ferry, MD, USA	18. IV. 2004
JV0508/27	*s*	Valley Forge, Norristown, PA, USA	31. VIII. 2005
JV0509/96	*f*	Abrams Falls, Great Smoky Mt., TN, USA	8. IX. 2005
JV0509/124	*s*	Promised Land St. Park, Pike County, PA, USA	13. IX. 2005
JV0709/15	*f*	Wissahickon Creek, Philadelphia, PA, USA	1. IX. 2007
JV0709/186	*s*	Spring Mt., Schwenksville, PA, USA	24. IX. 2007
JV0709/187	*s*	Spring Mt., Schwenksville, PA, USA	24. IX. 2007
JV0709/187A	*s*	Spring Mt., Schwenksville, PA, USA	24. IX. 2007
JV0808/47	*Wf*	Wissahickon Creek, Philadelphia, PA, USA	31. IX. 2008
JV0809/29	*Sf*	Spring Mt., Schwenksville, PA, USA	4. IX. 2008
JV0809/124	*s*	Spring Mt., Schwenksville, PA, USA	22. IX. 2008
JV0809/126	*s*	Spring Mt., Schwenksville, PA, USA	23. IX. 2008
JV0809/127	*s*	Spring Mt., Schwenksville, PA, USA	23. IX. 2008
JV0809/128	*s*	Spring Mt., Schwenksville, PA, USA	23. IX. 2008
JV0809/130	*s*	Green Lane County Park, PA, USA	23. IX. 2008
JV0809/132	*Gf*	Green Lane County Park, PA, USA	23. IX. 2008

* One-capital letter names ***W, S, G*** indicate specimens used for mating experiments.
Specimens labeled ***f*** are fertile, specimens ***s*** sterile or with very rare spores.

reagent were examined microscopically and 30 basidiospores from each specimen were measured. Single-basidiospore isolate (SBI) cultures were obtained from three dried two-month-old specimens from different Pennsylvania localities (labeled G, S, W, see Table 1). All specimens are deposited in the private herbarium of the senior author (http://mykoweb.prf.jcu.cz/polypores), with two split collections housed in PRM. The mycelial cultures are maintained at the Biology Centre of the Academy of Sciences of the Czech Republic.

Cultivation of monosporic mycelia and isolation of SBI

Sporocarp blocks ~5 mm thick containing the upper part of tubes were cut with a sterile scalpel, extracted with 500 µl of sterile water for five minutes at room

temperature, and diluted 100× with sterile H_2O. 20 μl of diluted spore suspension was plated on 3 10-cm plates with 3% malt extract FLUKA (Cat. No. 70167) supplemented with 1.5% agar. Plates were incubated at room temperature in low-light conditions. The spores germinated in 6 days. Approximately 6 germinating spores from each specimen were picked out singly and transferred to 6-cm plates with the same medium. After 7 days, the isolates were checked for typical microscopic features and absence of clamp connections. Oxidase tests were performed according to Kaarik (1965). Selected isolates were then transferred into test tubes with malt agar medium.

Compatibility tests

Petri dishes (60 mm diam) with the same malt agar medium as above were used for compatibility tests. Pairings were made by placing 2 × 4 mm mycelial mat pieces of each SBI pair 0.6 cm apart on the culture surface and mycelia were allowed to grow for 10 days. The character of the demarcation zone was evaluated and pieces of the agar with mycelial mat 5 mm on both sides of the demarcation line were transferred to 1.5 mL Eppendorf tubes containing 0.1 mL of Quiagen QX1 buffer to dissolve the agar. Small pieces of mycelium were added to water drops to check for clamp connections.

ITS amplification and sequencing

Sterile homokaryotic single-spore cultures G6 and S8 were isolated from plates after dissolving the agar in Quiagen QX1 buffer and washing with sterile water. Mycelia were frozen and disintegrated 60 s in mixer mill MM301 RETSCH under liquid nitrogen. DNA was isolated using CTAB/NaCl extraction buffer as described by Murray & Thompson (1980), followed by repeated extraction with chloroform and isopropanol precipitation. 18S rDNA (part), ITS1, 5.8S rDNA, ITS2 and 28S rDNA (part) were amplified according to White et al. (1990), using 55 °C annealing temperature with primers ITS1 and ITS4. Amplified DNA was purified using Wizard Clean Up kit PROMEGA and sequenced in the Genomics laboratory of Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on ABI 3730xl DNA analyzer, using BigDye Terminator 3.1 kit. The sequence of the G6 isolate was deposited in GenBank (FJ788412).

Results

Antrodia serialiformis Kout & Vlasák, **sp. nov.** Figure 1

MycoBank MB512890; photos in (http://mykoweb.prf.jcu.cz/polypores)

Basidiocarpi perennes, effuso-reflexi vel resupinati, suberosi, 8–20 cm in diam., 0.5–1 cm crassi. Pilei rugosi, 0.5–2 cm lati, sed in serie confluenti, brunnei, margine acuto, albo. Pori albi, rotundati, 3–4 per mm. Systema hypharum dimiticum, hyphae generaticae tenuiter tunicatae, fibulatae, hyphae skeletales hyalinae, subsolidae, rectae, 3–5 μm in diam. Basidia clavata, 4–sterigmatica, 12–18 × 4–6 μm, basidiosporae hyalinae, laeves, IKI–, ellipsoideae vel subfusoideae, 4.5–5.5(6) × 2.0–2.3(2.5) μm. Cariem brunneam in ligno quercino producet.

Holotypus: Wissahickon Creek Park, Philadelphia, PA, USA, on oak log, 31. 8. 2008, leg. Josef Vlasák, herb. J. Vlasák JV0808/47. Isotypus in PRM 915459. Paratypus: French Creek St. Park, Hopewell, PA, USA, on oak log, 2. 8. 2001, leg. Josef Vlasák, JV0108/12b, in PRM 848583.

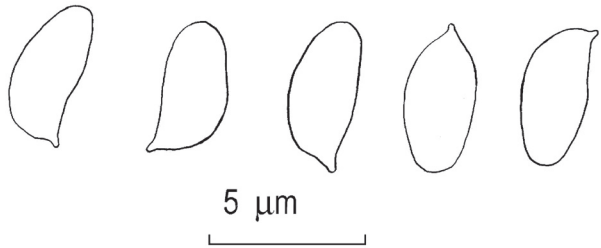

Fig. 1. *Antrodia serialiformis* (JV0809/47, holotype). Basidiospores.

Description

Basidiomes perennial, effused-reflexed, with small pilei in the upper part, often elongated along the substrata, up to 20 cm or more in length, very tough. Individual pilei up to 10 × 20 × 7 mm, with upper surface more or less horizontal, velutinate, uniformly brown, azonate or faintly zonate, margin white, narrow, sharp. Pore surface white, with age sordid brown, pores round, 3–4 per mm, tubes concolorous, up to 5 mm deep, context white, 1 mm thick.

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 clavate, 4-sterigmatic, 12–18 × 4–6 µm with a basal clamp connection. Basidiospores ellipsoid to subfusiform, hyaline, negative in Melzer`s reagent, 4.5–5.5(6) × 2–2.3(2.5) µm, thin-walled.

Remarks. *Antrodia serialiformis* is similar to *A. serialis,* but the latter has larger basidiospores (6.3–8 × 2.2–3.3 µm, Dai & Niemelä 2002). In addition, *A. serialiformis* grows on old decorticated trunk of *Quercus*, while *A. serialis* is usually on coniferous wood.

Mycelial cultures

Homokaryotic mycelia were isolated from three fertile *A. serialiformis* specimens collected from three localities in Pennsylvania, USA (Table 1). For comparison, homokaryotic mycelia were also prepared from two European *A. serialis* specimens collected near Boky, Hluboká n/Vlt., Czech Republic, on *Picea abies* logs (6.X.2007, collections JV0710/1&JV0710/2). Growth characteristics of mycelia from both species corresponded to descriptions of *A. serialis* mycelia published by Nobles (1943, 1965). Monosporic mycelia from *A. serialis* are perhaps more appressed to agar and thin ("sub-felty"), whereas those of *A. serialiformis* seemed more cottony. Variously shaped thick-walled chlamydospores were always noted for *A. serialiformis.* Mycelia from both species showed negative oxidase reactions with tetramethyl benzidine, pyrogallol, α-naphthol, pyrocatechol, tannic acid, *p*-cresol and tyrosine. Code

Symbols of *A. serialiformis* mycelia according to Nobles (1965) are 1.3.8.9.34.3 6.38.45.46.48.54.59.

Compatibility tests

Six to eight SBI from the 3 studied collections were checked for absence of clamps and then pairings were made in all possible combinations. In mating experiments, two types of contact zone morphology could be scored after 7–10 days of incubation. Compatible SBI developed a barrage zone in the young mycelia that was later more or less overgrown. Incompatible mycelia developed a sharply defined, congested contact zone. As expected, all 3 collections were determined as heterothallic with a monofactorial incompatibility system (bipolar). Still, in *A. serialiformis,* about 30% of monosporic mycelia in repeated pairing experiments produced few clamp connections in compatible pairings (scored based on contact zone morphology and abundant clamps in the pairing partner). Such mycelia were discarded.

Two compatible mating types were identified from each specimen and deposited in the culture collection. Six SBI of *A. serialiformis* from three localities were then paired in all combinations, with clamp connections formed in every pairing (results not shown) indicating that they contained different mating alleles (Raper et al. 1958). The same was true for pairings among *A. serialis* from the two European localities.

Pairings of *Antrodia serialiformis* with *Antrodia serialis*

Two compatible SBI from each of the *A. serialiformis* localities were paired in all combinations with two SBI from both European *A. serialis* specimens. Clamp connections were never formed and the contact zone morphology was consistent with incompatible pairing, indicating that *A. serialiformis* and *A. serialis* are different species.

ITS sequence analysis

Amplified sequence of 666 bp showed that only one base substitution in ITS1 discriminates between collections **G** and **S** (see Table 1) of *A. serialiformis*. The sequence blasted most significantly with five *A. serialis* ITS sequences deposited in the GenBank. In ITS1 (195b) there are 13–15 single bp substitutions or insertions/deletions (about 7% variable sites) between *A. serialiformis* and each of *A. serialis* and the same is true for ITS2 (196b). The sequence variability between each of *A. serialis* clones is only about 1.5% in ITS1 and 0.5% (one variable site) in ITS2. The second most similar ITS sequence of *A. variiformis* differs from *A. serialis* less than *A. serialiformis* (5–7 variable sites in ITS1 and about 12 in ITS2). This sequence comparison indicates that *A. serialiformis* is a distinct species related to *A. serialis*.

Discussion

During our first visit to Pennsylvania in 2001, we were surprised to find a widespread polypore very similar to *A. serialis* growing on old oak logs (Vlasák 2004). *Antrodia serialis* is very common in the Czech Republic, but there it rarely occurs on hardwoods and never on oaks (Kotlaba 1984). The same is true for the whole Europe (Ryvarden & Gilbertson 1993), perhaps with the exception of aspen as substrate in the northernmost Europe (Dai & Niemelä 2002). Moreover, we could find no *A. serialis* on conifers in lowland parts of Pennsylvania, Virginia, or Maryland, even in areas dominated by hemlock and pines woods or with occasional spruce stands. In the course of following visits in 2003, 2005, 2007 and 2008 we could only confirm the original observation and here we present experimental evidence demonstrating that the oak-growing polypore is a separate species, named here *A. serialiformis*.

Antrodia serialiformis shows some subtle macroscopic differences from *A. serialis*: it is more often pileate with more brownish and acute pilei. It never changes color to red as a result of hyphomycete infection. On the other hand, it is frequently eaten by microlepidoptera yielding powdery pore-surface, also characteristic for *A. serialis*. Despite the striking difference in the spore size (much smaller in *A. serialiformis*), unfortunately, many collections are completely sterile. We have inspected well developed, but sterile basidiocarps from three localities collected every 2–3 months from the same site over the whole year 2007, yet spores never appeared even when basidia with rudimentary sterigmata were detected in all collections from one locality.

Antrodia serialiformis, one of the most common polypore species in the eastern USA, grows abundantly in localities with lying oak logs. Sometimes it grows together with the grayish and strictly resupinate *A. oleracea* (R.W. Davidson & Lombard) Ryvarden, a species that is nonetheless far less frequently encountered.

Antrodia serialiformis undoubtedly has been previously misidentified as *A. serialis*. In the PACMA herbarium in Pennsylvania there are 19 collections of *Antrodia (Trametes) serialis* with identified substrates, 8 of which were collected on oak! We were unable to obtain these collections for direct comparison; however, given the sterility common in the species, it is probable that the collections lack spores needed for determination.

Taking into account the similarity of *Antrodia serialis* and *A. serialiformis,* the distribution in the eastern USA of true *A. serialis* is unclear. Gilbertson & Ryvarden (1986) cite *A. serialis* as present in all eastern states, including southern states, such as Florida, Louisiana, etc. Our experience suggests that *A. serialis* occurs south of New York only in the highest mountains of the Appalachians down to the Great Smoky Mountains, where it appears common on the highest peaks associated with fir and spruce. We suspect that lowland

'*A. serialis*' collections from the South are likely *A. serialiformis.* North of New York we did not find *A. serialiformis,* although *A. serialis* was locally abundant on spruce logs, especially in the White Mountains (NH) or Adirondack Park (NY). According to our experience, *A. serialiformis* does not occur on the western US coast where the *A. serialis* distribution is also unclear. Although Gilbertson & Ryvarden (1986) cite it as common in California, Oregon, and Washington, we were unable to find any specimen during extended visits in Sequoia, Kings Canyon, Yosemite, Redwoods, Olympic, and Mt. Rainier National Parks (Vlasák 2008).

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