CZECH MYCOL. 63(2): 195–201, 2011

Antrodiella niemelaei, a new polypore species related to *Antrodiella americana*

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Vampola P., Vlasák J. (2011): *Antrodiella niemelaei*, a new polypore species related to *Antrodiella americana*. – Czech Mycol. 63(2): 195–201.

A new polypore species, *Antrodiella niemelaei* Vampola et Vlasák, occurring on dead fruitbodies of *Pseudochaete tabacina*, is described. In Europe, the species has to date been identified as *Antrodiella americana*. The most important macro– and microscopic features of the two species are discussed and molecular characteristics are provided.

Key words: *Antrodiella*, polypores, taxonomy, identification, internal transcribed spacer.

Vampola P., Vlasák J. (2011): *Antrodiella niemelaei*, nový druh choroše příbuzný druhu *Antrodiella americana*. – Czech Mycol. 63(2): 195–201.

Je popsán nový druh chorošů *Antrodiella niemelaei* Vampola et Vlasák rostoucí na mrtvých plodnicích *Pseudochaete tabacina*. V Evropě byl dosud určován jako *Antrodiella americana*. Jsou diskutovány hlavní rozdílné makroskopické i mikroskopické znaky obou druhů a je připojena jejich molekulární charakteristika.

INTRODUCTION

Antrodiella americana Ryvarden et Gilb. was collected for the first time on 23 June 1932 by the prominent American mycologist L. O. Overholts in Cook Forest, Pennsylvania, on old fruitbodies of *Hymenochaete corrugata*, growing on an old log of *Fagus grandifolia*. The second specimen also comes from Pennsylvania; it was collected in Stone Valley, again on old fruitbodies of *Hymenochaete corrugata*. Ten years later, Overholts published his collections as a new species, *Poria aestivale* Overholts (1942). From the standpoint of the International Code of Botanical Nomenclature (ICBN) this name is however invalid, because no Latin diagnosis was provided. This was the reason why Ryvarden and Gilbertson (1984) formally described this fungus once again as a new species *Antrodiella americana* Ryvarden et Gilb.

In North America, *A. americana* is presently known from several states of the USA and Canada, mostly from the eastern part of the continent (Gilbertson & Ryvarden 1986, Vlasák 2011). Most of the collections come from old fruitbodies of *Hymenochaete* spp. occurring on hardwoods. *Antrodiella americana* basidiomes are thin, strictly resupinate, cream-colored to straw-yellowish. The species is distinguished from similar, but small-pored species of the *Antrodiella semisupina* complex by its large, angular pores. These are about 1–3 per mm, on oblique surfaces elongated and opened, in older basidiomes often a bit lacerated. Large pores are a very constant feature, typical of all American collections. Macroscopically, *A. americana* markedly reminds *Ceriporiopsis aneirina*.

In Europe, *Antrodiella americana* is only reported from Finland and Norway (Ryvarden & Gilbertson 1993, Johannesson et al. 2000, Miettinen et al. 2006) and recently also from Russia and Estonia (Spirin & Zmitrovich 2003, Parmasto 2004). Nonetheless, all European collections have distinctly smaller pores, 3–4 per mm. Fundamental doubts regarding the identity of American and European *A. americana* were already published by Vampola & Pouzar (1996) and recently again by Vampola (2011). Spirin & Zmitrovich (2003) also assumed that *A. americana* could be a complex of several sibling species. To solve this problem, we have performed a more detailed examination of herbarium material from North America and North Europe, supplemented with a DNA sequence analysis. We conclude that the North European fungus is really different from *A. americana* and therefore describe it as a new species.

MATERIALS AND METHODS

Macroscopic and microscopic study. Macroscopical and microscopical features were studied on herbarium specimens from PRM (National Museum Prague, Mycological Department), PACMA (Pennsylvania State University), MJ (Muzeum Vysočiny Jihlava) and also on specimens from the private herbaria of both authors. Microscopic characteristics were observed in Melzer's reagent and Cotton Blue under Olympus BX41 and Meopta D816Bi microscopes with an oil immersion lens at a magnification of 1000×. A total of 20 spores from each specimen were measured.

DNA isolation and sequencing. 0.1 g of the fungal tissue was disintegrated for 60 s with a MM301 RETSCH steel ball mixer mill at room temperature. DNA was isolated using the CTAB/NaCl extraction buffer as described by Murray and Thompson (1980), followed by repeated extraction with chloroform and isopropanol precipitation. Crude DNA was dissolved in 100 µl of sterile water and further purified using the PROMEGA Wizard Clean Up kit. The resulting DNA solution (50 µl) was diluted ten times and 1 µl was used as a template for amplifica-

tion with ITS5 and ITS4 primers (White et al. 1990) in 25 µl reaction mixture using an annealing temperature of 55 °C. Amplified DNA was sequenced in the Genomics laboratory of Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on an ABI 3730xl DNA analyser, using the BigDye Terminator 3.1 kit.

Phylogenetic analysis. The sequences of several *Antrodiella* species from GenBank and our sequence of recently collected typical *A. americana* specimen were aligned with Clustal X and manually pruned. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). Initial tree(s) for the heuristic search were obtained using the BIONJ method with an MCL distance matrix. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 545 positions in the final dataset. Phylogenetic analyses were conducted with the MEGA5 software package (Tamura et al. 2011).

RESULTS AND DISCUSSION

Antrodiella niemelaei Vampola et Vlasák **sp. nov.**

(MycoBank MB563473)

Diagnosis latina. Carposomata annua, resupinata, cremea; pori rotundati, parvi, cca 4 per 1 mm; systema hypharum dimiticum, cum hyphis generativis hyalinis, tenuiter tunicatis, fibulatis; hyphis skeleticis crasse tunicatis; hymenium e basidiis et cystidiis constat; basidiosporae 2,8–3,8 × 1,8–2,2 µm, ellipsoideae, hyalinae. Ad carposomata *Pseudochaete tabacina* (= *Hymenochaete tabacina*). *Antrodiellae americanae* Ryvarden et Gilb. affinis, sed cum poris minoribus.

Holotypus. Finland, Uusimaa, Vantaa, Mustavuori, *Prunus padus* in a thicket, on dead *Hymenochaete tabacina*, 29. VI. 1985 leg. T. Niemelä et R. Saarenoksa no. 3223 (holotypus in herbario PRM 879827 asservatur, isotypus in herbario H).

Etymology. Named in honour of the original collector, Finnish mycologist Tuomo Niemelä.

Description. Basidiomes annual, small, strictly resupinate and only 0.5 mm thick, cream-coloured, with whitish sterile margin. Pores circular, small, about 4 per mm. Hyphal system dimitic, generative hyphae thin-walled, hyaline, with clamps, 2–4 µm wide. Skeletal hyphae mostly thick-walled, 2–4 µm wide, their ends in the dissepiment edges reminding cystidia. Basidia 4-sterigmate, clavate, 10–14 × 4–5 µm, with basal clamps. Cystidia cylindrical, clavate or fusiform, some of them filled with refractive material (gloeocystidia), about 20–50 × 3–7 µm. Spores ellipsoid, hyaline, thin-walled, 2.8–4 × 1.8–2.2 µm (Fig. 1).

E c o l o g y. On trunks and branches of dead hardwoods, growing on or around old basidiomes of *Pseudochaete tabacina* (= *Hymenochaete tabacina*). Causes a white rot.

C u r r e n t l y k n o w n d i s t r i b u t i o n. Finland – see holotype. The fungus was reported also from Norway, Russia and Estonia (see Introduction) but we have not revised this material yet. It will be the subject of a separate taxonomic study of the *Antrodiella americana* complex.

N o t e s. As stated already in the introduction, *Antrodiella niemelaei* is closely related to *A. americana*, but differs in distinctly smaller pores. The detailed microscopical study revealed, however, some differences also in the hymenial layer of both species. There are three different types of cystidial elements in the hymenium of *A. americana*, often collectively called gloeocystidia. The first type represents large, cylindric, thick-walled elements up to 50 µm long, markedly protruding from the hymenium, well depicted by Lowe (1966) in Fig. 106. They may form, sometimes, also small bundles of several aggregated elements. The nature of these far protruding, strange cystidia is intriguing. They consist, probably, of deformed and thickened ends of generative hyphae, because some of them show distinct septa with clamps. The second type of cystidioid elements is depicted by Gilbertson & Ryvarden (1986) in Fig. 56. These are cylindrical to narrowly clavate ends of skeletal hyphae occurring in dissepiment edges. Only the third type can be regarded as typical gloeocystidia. They are developed as thin-walled, clavate to fusiform cystidia up to 50 µm long, mostly filled with refractive material. The European *A. niemelaei* has the same gloeocystidia and cystidioid ends of skeletals in dissepiment edges, but bundles of far protruding, thickened hyphae (first type of "cystidia") were never observed.

In the field, *A. niemelaei* can be confused with young, resupinate fruitbodies of *A. faginea* Vampola et Pouzar, which also grows quite often on basidiomes of *Hymenochaetaceae* species, including those of *Pseudochaete tabacina*. *Antrodiella faginea* also has gloeocystidia in the hymenium and, therefore, even microscopical inspection may not reveal the difference. However, gloeocystidia of *A. faginea* do not exceed 25 µm, whereas those of *A. niemelaei* may be up to 30 to 50 µm long. Moreover, basidiomes of *A. niemelaei* are always thin and resupinate, but *A. faginea* often creates thicker and effused-reflexed basidiomes with distinct pilei. Pores of *A. faginea* are slightly narrower, mostly 4–7 per mm.

A. romellii (Donk) Niemelä and *A. ichnusana* Bernicchia, Renvall et Arras are also very similar to *A. niemelaei*. Nevertheless, gloeocystidia are absent from both species and the spore size and shape are different as well. *A. romellii* has ellipsoid but larger spores 3.5–5 × 2–3 µm in size, *A. ichnusana* has very different, cylindrical spores 4–5 × 1.8–2.1 µm large.

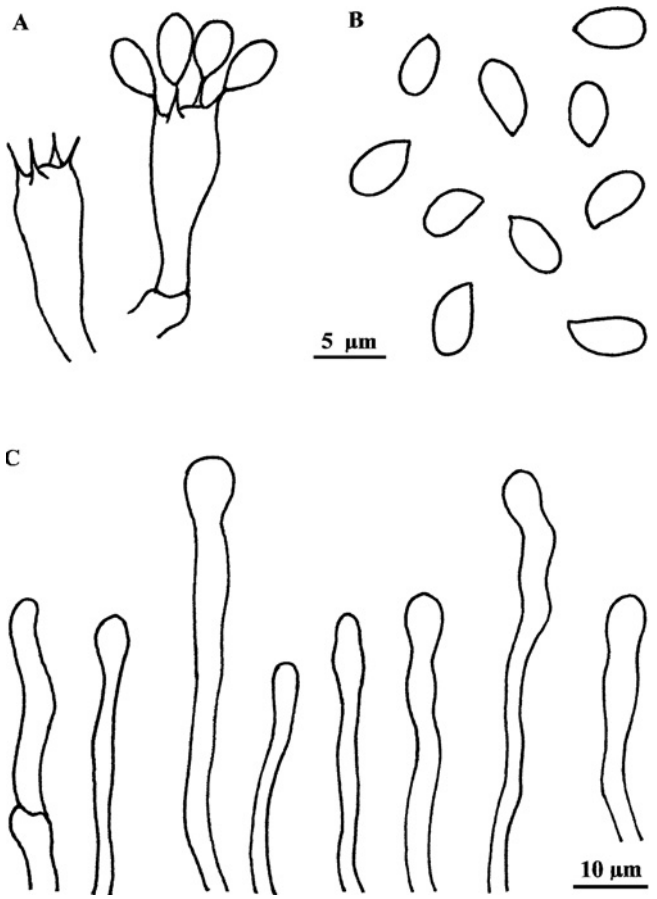

Fig 1. *Antrodiella niemelaei* (PRM 879827, holotype). **A** – basidia, **B** – spores, **C** – cystidia. Del. P. Vampola.

Nuclear ribosomal ITS sequence analysis

There were four sequences named *Antrodiella americana* in the GenBank when we started this study. Two identical sequences supposedly derived from American cultures (H.H. Burdsall Jr. collection?) cluster with our sequence of "typical" specimen of *A. americana* JV0109_37 collected in Pennsylvania close to the original locality, even though they differ in 1 base of ITS1 and 8 bases of ITS2. They may or may not represent still another species, which we cannot decide now

having no information about the origin of the cultures (a study of them has not yet been published).

Two other identical sequences from Finland are more different featuring 7 small insertions/deletions and some additional mutations. They cluster separately with high bootstrap support, which justifies the separate position of a new species, *Antrodiella niemelaei*. Other *Antrodiella* species, either morphologically similar (*A. faginea*, *A. romellii*) or dissimilar (*A. citrinella*, *A. parasitica*, *A. pallasii*) are only distantly related (Fig. 2).

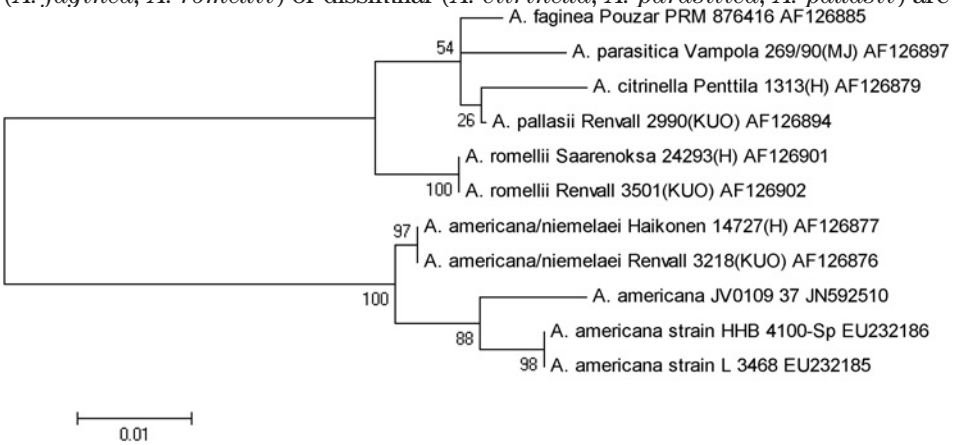

Fig 2. Evolutionary relationships of *Antrodiella* specimens from 7 allied taxa. The tree with the highest log likelihood (–1126.6656) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths corresponding to the number of base substitutions per site.

Additional specimens examined

Antrodiella americana – U S A: Pennsylvania, Cook Forest, on *Fagus grandifolia*, 23. VI. 1932 leg. L. O. Overholts et W. L. White, no. 14364, Type! (PACMA 000992, now deposited in BPI); Pennsylvania, Norristown, Evansburg State Park, hardwood, 3. VIII. 2001, leg. J. Vlasák (private herb. JV0109/37; GenBank JN592510).

Antrodiella faginea – Czech Republic: Stříbrná Skalice, Studený vrch hill, *Fagus sylvatica*, on fallen trunk, 21. IX. 1991 leg. Z. Pouzar (PRM 876416); Slovak Republic: Vysoké Tatry Mountains, Tatranská Štrba, "Brezina" forest, alt. 940 m, *Salix* sp., on dead basidiocarp of *Hymenochaete tabacina*, 14. IX. 1995 leg. P. Vampola (MJ 199/95).

ACKNOWLEDGEMENTS

The authors thank Jan Holec (National Museum Prague, Mycological Department, Czech Republic) for the kind loan of the material of *Antrodiella americana*, Zdeněk Pouzar (Prague, Czech Republic) for help with the Latin diagnosis and to anonymous referees for their valuable comments.

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