Pyrofomes juniperinus, comb. nova, the North American sibling of *P. demidoffii* (Polyporales, Basidiomycota)

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Received 18 Apr. 2017, final version received 8 Oct. 2017, accepted 10 Oct. 2017

Vlasák J., Vlasák J.Jr., Harvey P.G., Leacock P.R. & Spirin V. 2018: *Pyrofomes juniperinus*, comb. nova, the North American sibling of *P. demidoffii* (Polyporales, Basidiomycota). — *Ann. Bot. Fennici* 55: 1–6.

Polyporus juniperinus is reinstated as a good species to encompass the North American collections labelled as *P. demidoffii*, and a new combination, *Pyrofomes juniperinus* (H. Schrenk) Vlasák & Spirin, is proposed. Identity of *P. juniperinus* and *P. juniperinus* ssp. *earlei* is discussed based on morphological and DNA evidence.

Introduction

Pyrofomes demidoffii is a polypore inhabiting living juniper trees. It can be easily recognized by ungulate, perennial, dark-colored basidiocarps, peculiar host and brownish, thick-walled, truncate basidiospores. The species has been reported from Europe, Middle Asia, East Africa and North America (Gilbertson & Ryvarden 1987, Ryvarden & Gilbertson 1994). However, recent DNA-based studies (Assefa *et al.* 2015) showed that the North American specimens named *P. demidoffii* differ from the European specimens.

Murrill (1903) introduced two species from living juniper trees, *Pyropolyporus juniperinus* and, as a new species, *P. earlei*, collected from *Juniperus* sp. in New Mexico. The first species was described as *Polyporus juniperinus* a few years earlier from Tennessee growing on *Juni-*

perus virginiana (Schrenk 1900). Murrill (1903) emphasized larger pores and thicker tube walls in *P. earlei*, and a strongly rimose pileal surface in the latter species but only slightly furrowed in *P. juniperinus*. Hedgcock and Long (1912) studied the rot of both species, which they found differing in several macro- and microscopic characteristics. In addition, they published rather informative photos of both species and defined *P. juniperinus* as a rare species from the eastern USA growing on *J. virginiana*, and *P. earlei* as a common western species growing on *J. monosperma*, *J. utahensis* and *J. sabinoides*. Later, however, Murrill (1920) suggested that both species should be considered to be *Fomes demidoffii*, a European species growing on *J. excelsa*. Thereafter, all North American collections have been named *Pyrofomes demidoffii* (Gilbertson & Ryvarden 1987).

Some years ago, we realized, however, that our collections of *Pyrofomes* from the eastern and western parts of the USA showed macroscopic differences corresponding to those described by Murrill (1903) and Hedgcock and Long (1912). To solve this taxonomic problem, we conducted morphological and DNA studies of European and North American collections. Type specimens of *Polyporus juniperinus* H. Schrenk and *Pyropolyporus earlei* Murrill (both described from USA) were checked to settle the correct name for the North American species.

Material and methods

Type material and voucher specimens from herbaria BPI, F, H, O, PRM, as well as from the private herbarium of the author JV, were studied. Herbarium acronyms are given according to *Index Herbariorum* (<http://sweetgum.nybg.org/ih>). Morphological routine of this study follows Miettinen *et al.* (2012). In all cases, 20 context and tramal skeletal hyphae and 30 basidiospores per specimen were measured. Basidiospore measurements are reported as follows: the main range is for 90% of basidiospores and the values in parentheses are for 5% that are smaller and 5% that are larger than the main-range lower and upper limits, respectively. For hyphal diameter, the main range is for 60% of hyphae and the values in parentheses are for 20% that are smaller and 20% that are larger than the main range lower and upper limits, respectively. The following symbols are used below: *L* = mean spore length, *W* = mean spore width, *Q* = *L/W* ratio, *n* = number of measurements/ number of measurements specimens.

DNA was isolated, amplified and sequenced as in Spirin *et al.* (2013). Three data sets were compiled for the analysis: ITS data set containing newly prepared sequences of eight *Pyrofomes* specimens and five sequences retrieved from GenBank; LSU data set containing newly prepared sequences of seven *Pyrofomes* specimens plus three sequences retrieved from GenBank; and mtSSU data set containing newly prepared sequences of seven *Pyrofomes* collections.

The sequences were aligned with Clustal X and manually pruned. The maximum likeli-

hood (ML) analyses were conducted in MEGA6 (Tamura *et al.* 2013) using Kimura 3 parameter model with gamma distribution, which was tested as the best one for all data sets.

SPECIMENS EXAMINED (GenBank sequence numbers are given in parentheses): — *Pyrofomes demidoffii*. **Macedonia**. Resen: Golem Grad, *Juniperus excelsa*, 29 November 1987 Karadelev (PRM 869997) (GenBank KY940246, 940247, 940259). **Morocco**. Ifrane: Azrou, *Juniperus thurifera*, 4 October 1995 Uotila 40936 (H). **Tanzania**. Arusha: Mt. Meru, *Juniperus procera*, 26 May 1988 Niemelä 4242 (H). **Turkmenistan**. Ahal: Dushak, *Juniperus turcomanica*, 2 October 1971 Parmasto (H ex TAAM 55663). **Ukraine**. Crimea: Aiza, *J. excelsa*, 16 November 2002 Marcz (PRM 869999) (GenBank KY940248, 940249, 940260). — *Pyrofomes juniperinus*. **USA**. Maryland: Baltimore Co., Sparrow's Point, *Juniperus virginiana*, November 1908 Spaulding (BPI US 0231386, 0231387, 0231388). Missouri: Jefferson Co., Myron and Sonya Glassberg Family Conservation Area, *J. virginiana*, 6 November 2015 Harvey 221923 (F) (GenBank KY940257, 940265); Taney Co., Drury Wildlife Refuge, *J. virginiana*, 17 August 2016 Wyman (F); Wayne Co., Patterson, *J. virginiana*, 9 November 2015 Bishop 222395 (F) (GenBank KY940258, 940264). New Jersey: Ocean Co., Lanoka Harbor, *J. virginiana*, 14 September 1932 Martin (BPI US 0231317) (GenBank KY940256). Oklahoma: Payne Co., Ripley Bluffs, *J. virginiana*, 11 August 1979 Cooke 56497 (O F-912228) (GenBank JX968555). Tennessee: Rutherford Co., Murfreesboro, *J. virginiana*, September 1899 Schrenk (BPI US 0231341, lectotype of *Polyporus juniperinus*); Anderson Co., Norris, *J. virginiana*, August 1950 Zimmerman (BPI US 0231389). Virginia: Prince William Co., Woodbridge, *Juniperus* sp., 21 September 2007 Vlasák 0709/173 (JV). — *Pyrofomes juniperinus* ssp. *earlei*. **USA**. Arizona: Coconino Co., Flagstaff, *Juniperus* sp., May 2002 Vlasák 0205/10 (JV) (GenBank KY940254, 940255, 940263). Tusayan, *Juniperus* sp., 16 May 2010 Vlasák 1005/1 (PRM 915981, JV) (GenBank KY940250, 940251, 940261). Vlasák 1005/2 (JV) (GenBank KY940252, 940253, 940262); Navajo Co., Sitgreaves Nat. Forest, *Juniperus monosperma*, 18 July 1967 Gilbertson 6960 (H ex O F-12234). New Mexico, El Capitan Mts., *Juniperus* sp., 31 August 1900 Earle (BPI US 0231334, lectotype of *Pyropolyporus earlei*). Idaho: Owyhee Co., Owyhee Mts., *Juniperus occidentalis*, 2 August 1958 Gilbertson 2090 (H ex NYS).

Results

Types of *P. juniperinus* and *P. earlei*, plus 14 other specimens from North America were studied under a microscope and compared with specimens of *P. demidoffii* from Eurasia. According to our observations, two extremes of basidiospore variation exist in North America. Specimens from Maryland, New Jersey and Tennessee, including

Fig. 1. Phylogenetic relationships among 12 *Pyrofomes* specimens inferred from ITS rRNA sequences. *Donkioporia expansa* was used as outgroup. Topology from maximum likelihood (ML) analysis. Support values next to the branches from ML bootstrap (500 replicates). Branch lengths are drawn proportional to the number of substitutions per site. Large dots indicate sequences generated for this study.

the type specimen of *P. juniperinus*, have basidiospores smaller than in *P. demidoffii* and distinctly smaller than in specimens from Arizona, Idaho and New Mexico. In addition to macroscopic features, these differences could offer grounds for recognizing three geographically separated species. However, three specimens from Missouri and Oklahoma blurred this scheme. Their macroscopic characters, as well as host species, *J. virginiana*, would suggest *P. juniperinus sensu* Mur-

rill but the basidiospore dimensions are almost identical to *P. demidoffii* and partly overlapping with those of the specimens from western United States, including the type specimen of *P. earlei* (Table 1).

Results of our phylogenetic study correspond with geographic and morphological data. nrLSU, ITS and mtSSU sequences clearly segregate European and North American specimens (Fig. 1). Since *P. demidoffii* was described from Crimea,

Table 1. Basidiospore dimensions in *Pyrofomes demidoffii* s. lato. L' = spore length, L = mean spore length, W' = spore width, W = mean spore width, $Q' = L'/W'$ ratio, $Q = L/W$ ratio. The measurements are given as follows: the main range is for 90% of basidiospores and the values in parentheses are for 5% that are smaller and 5% that are larger than the main-range lower and upper limits, respectively.

Species/specimen	L'	L	W'	W	Q'	Q	n
<i>P. demidoffii</i>	(5.7)5.8–7.8(8.3)	6.72	(4.8)4.9–6.3(6.5)	5.46	1.1–1.4(1.5)	1.23	120
PRM 869997	(5.9)6.0–7.3(7.7)	6.58	(4.8)4.9–6.1(6.5)	5.56	1.1–1.3	1.19	30
PRM 900899	(5.7)5.8–7.8(8.0)	6.49	(4.8)4.9–6.3(6.5)	5.40	1.1–1.3(1.4)	1.20	30
TAAM 55663	(6.2)6.3–7.8(8.3)	6.97	(5.0)5.1–6.2	5.54	1.1–1.4(1.5)	1.26	30
Uotila 40936	(5.9)6.2–7.8(8.3)	6.84	(4.8)4.9–6.1(6.3)	5.34	1.1–1.4	1.28	30
<i>P. juniperinus</i>	(5.1)5.2–7.8(8.2)	6.16	(3.7)3.9–6.2(6.6)	4.90	(1.0)1.1–1.4(1.6)	1.26	210
holotype	(5.4)5.5–6.8(7.1)	6.07	(4.0)4.2–5.2(5.3)	4.80	(1.1)1.2–1.4(1.6)	1.27	30
Bishop 2015-11-09	(5.3)5.5–7.8(8.2)	6.49	(4.1)4.2–6.2(6.6)	5.21	1.1–1.4	1.25	30
Cooke 56497	(5.6)5.7–7.2(7.3)	6.31	(4.7)4.9–5.8(6.0)	5.27	1.1–1.4	1.20	30
Harvey 2015-11-06	(5.3)5.5–7.8(8.2)	6.39	(4.1)4.2–6.2(6.6)	5.26	(1.0)1.1–1.4	1.22	30
US 0231389	(5.2)5.3–6.2(6.3)	5.62	(4.0)4.2–5.1(5.4)	4.55	(1.0)1.1–1.3(1.4)	1.24	30
US 0231386	(5.1)5.2–7.1(7.2)	5.97	(3.7)3.9–5.9(6.1)	4.60	(1.1)1.2–1.4(1.5)	1.30	30
US 0231317	(5.2)5.5–6.8(7.0)	6.25	(4.0)4.1–5.2(5.3)	4.61	(1.2)1.3–1.4(1.5)	1.36	30
<i>P. juniperinus</i> ssp. <i>earlei</i>	(6.1)6.2–8.8(9.2)	7.25	(4.4)4.5–7.2(7.3)	5.68	1.1–1.7(1.8)	1.28	90
holotype	(6.2)6.6–8.3(8.4)	7.31	(4.7)4.8–6.5(6.7)	5.50	(1.2)1.3–1.4(1.5)	1.33	30
Gilbertson 2090	(6.1)6.2–8.0(9.2)	7.02	(4.4)4.5–6.2(6.7)	5.36	(1.1)1.2–1.7(1.8)	1.32	30
Gilbertson 6960	(6.2)6.3–8.8(9.0)	7.43	(5.1)5.2–7.2(7.3)	6.19	1.1–1.3(1.4)	1.20	30

Fig. 2. Phylogenetic relationships among nine *Pyrofomes* specimens inferred from nuLSU rRNA sequences. *Donkioporia expansa* was used as outgroup. Topology from maximum likelihood (ML) analysis. Support values next to the branches from ML bootstrap (500 replicates). Branch lengths are drawn proportional to the number of substitutions per site. Large dots indicate sequences generated for this study.

Fig. 3. Basidiocarps of *Pyrofomes juniperinus* (Harvey 221923, F).

we apply the latter name to the species from Eurasia. In turn, nrLSU, ITS and mtSSU regions of North American collections demonstrate only negligible differences between eastern and western samples which, therefore, should be considered conspecific (Figs. 1 and 2). Particularly, there are approximately 25 differences in both ITS1 and ITS2 regions between Eurasian and American *Pyrofomes* specimens but only four inconstant dif-

ferences between eastern and western collections from North America (Fig. 1). Similarly, there are 23 differences in the 1100-bp-long LSU sequence between Eurasian and American collections but only a few individual differences between collections from the American East and West (Fig. 2). mtSSU sequence of Eurasian *Pyrofomes* is 570 bp long (between MS1 and MS2 primers) and is highly conserved also in American *Pyrofomes* which differs in one position only; there is, however, a very long (about 1470 bp) insert behind the mtSSU base 368 in all American specimens. This insert (of the mitochondrial intron 2 nature) contains some base changes between *P. earlei* and *P. juniperinus*, accumulated in five short (10 bp) regions (not shown), which, however, cannot support phylogenetic species separation.

Polyporus juniperinus was described three years earlier than *P. earlei*, and thus it has priority over the latter name. Here we formally transfer *P. juniperinus* to the genus *Pyrofomes* for naming the North American collections formerly synonymized with *P. demidoffii*. Moreover, macroscopic features, geographic distribution and host preferences, in addition to tiny microscopic and DNA differences, allow us to consider *P. earlei* as a subspecies of *P. juniperinus*. Both taxa are briefly described below.

***Pyrofomes juniperinus* (H. Schrenk)
Vlasák & Spirin, *comb. nova* (Fig. 3)**

Polyporus juniperinus H. Schrenk, U.S. Department of Agriculture Bulletin 21: 9. 1900.

MB 822963. — LECTOTYPE (designated by Lowe 1957: 62): USA. Tennessee, Rutherford Co., Murfreesboro, *Juniperus virginiana*, September 1899 Schrenk (BPI US 0231341!).

Basidiocarps perennial, at first hemispherical, later triquetrous, broadly attached, projecting up to 10 cm. Upper surface with annual zones, grey, indistinctly hispid, later dark grey, in oldest parts often greenish (covered by algae), not or only indistinctly cracking. Pileal edge blunt, concolorous with pore surface, often covered by incomplete pores. Pore surface yellowish brown or ochraceous brown, flat or oblique; pores roundish or slightly elongated, (2.5)3–4 per mm, with rather thin, entire dissepiments. Section: context hard corky, rusty brown, indistinctly fibrillose, up to 1 cm thick, gradually merging with tubes; tube layer ochraceous brown to rusty brown, indistinctly stratified, up to 6 cm thick. Hyphal structure dimitic; generative hyphae clamped. Context skeletal brownish, thick-walled, with distinct lumen, (2.1)2.2–3.7(4.2) μm in diam. ($n = 80/4$), mostly arranged in parallel bundles. Tramal skeletal brownish, thick-walled, with distinct lumen, (1.7)1.9–3.2(3.3) μm in diam. ($n = 80/4$), subparallel. Basidiospores broadly ellipsoid to subglobose, with flattened distal end (truncate), brownish, thick-walled, (5.1)5.2–7.8(8.2) $\times$ (3.7)3.9–6.2(6.6) μm ($n = 210/7$), $L = 6.16$, $W = 4.90$, $Q = 1.20$ –1.36, apiculus often indistinct.

The description above is based on specimens collected from *Juniperus virginiana* in the eastern and central parts of the USA. *Pyrofomes juniperinus* has been found in Kentucky, Tennessee (Schrenk 1900), Maryland (Hedgcock & Long 1912), and it is reported here from Missouri, New Jersey, Oklahoma and Virginia. *Pyrofomes juniperinus* ssp. *earlei*, distributed in the western part of the USA, has caps with strongly rimose upper surface and slightly bigger, 2–3 per mm, pores; its basidiospores are on average longer and broader than in *P. juniperinus* s. *stricto*. Basidiospores of *Pyrofomes demidoffii* are only slightly larger than in *P. juniperinus* s. *stricto* or almost identical to the latter one. However, *P. demidoffii* produces basidiocarps with distinctly cracking pileal crust and pores 2–3 per mm, and therefore it is macroscopically more similar to *P. juniperinus* ssp. *earlei*.

Fig. 4. Basidiocarps of *Pyrofomes juniperinus* ssp. *earlei* (specimen Vlasák 1005/1, JV).

Pyrofomes juniperinus* ssp. *earlei (Murrill) Vlasák & Spirin, comb. nova (Fig. 4)

Pyropolyporus earlei Murrill, Bulletin Torrey Bot. Club 30: 116. 1903.

MB 822964. — LECTOTYPE (designated by Lowe 1957: 62). USA. New Mexico, Capitan Mts., *Juniperus* sp., 31 August 1900 Earle (BPI US 0231334!; NY).

Basidiocarps perennial, at first hemispherical, later triquetrous, broadly attached, projecting up to 8 cm. Upper surface with annual zones, first grey and indistinctly hispid, later blackening and distinctly cracking in random directions, in oldest portions jagged. Pileal edge somewhat sharpened to blunt, concolorous with pore surface, sterile. Pore surface yellowish brown or ochraceous brown, flat or rarely oblique; pores roundish, 2–3 per mm, with rather thick, entire dissepiments. Section: context hard corky, rusty brown, compact, up to 1 cm thick, more or less distinctly separated from tubes; tube layer rusty brown, indistinctly stratified, up to 7 cm thick. Hyphal

structure dimitic; generative hyphae clamped. Context skeletal brownish, thick-walled, with distinct lumen, (3.1)3.2–5.0(5.3) μm in diam. ($n = 40/2$), mostly arranged in parallel bundles. Tramal skeletal brownish, thick-walled, with distinct lumen, (2.0)2.2–3.1(3.2) μm in diam. ($n = 40/2$), subparallel. Basidiospores broadly ellipsoid to subglobose, with flattened distal end (truncate), brownish, thick-walled, (6.1)6.2–8.8(9.2) $\times$ (4.4)4.5–7.2(7.3) μm ($n = 90/3$), $L = 7.25$, $W = 5.68$, $Q = 1.20$ –1.33, apiculus often indistinct.

Pyrofomes juniperinus var. *earlei* is distributed in the western part of the USA. It has been found on *Juniperus deppeana*, *J. monosperma* and *J. osteosperma* in Arizona, Colorado, Idaho, Montana, New Mexico, Oregon and Utah (Murrill 1903, Hedgcock & Long 1912, Lowe & Gilbertson 1961, Gilbertson et al. 1974). Records on *Juniperus ashei* (= *J. sabinoidea*) from Texas (Hedgcock & Long 1912) should be re-checked.

Acknowledgements

Curators of herbaria BPI, F, O and PRM kindly provided us with valuable specimens on loan.

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