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Diversity of xylariaceous symbionts in *Xiphydria* woodwasps: role of vector and a host tree

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ABSTRACT

Siricid woodwasps live in obligatory nutritional symbiosis with fungi. Screening of symbionts from mycetangia of emerging *Xiphydria* females (*X. longicollis*, *X. prolongata*, *X. camelus*, *X. picta*) from 28 locations and four tree genera yielded 1 389 isolates. Each female carried a pure culture of a single fungus. In *X. longicollis* (*Quercus*), *Daldinia childiae* was either the only fungus or a highly dominant one in the samples from moderately dry oak-hornbeam (*Quercus–Carpinus betula*) forests. Females from the alluvial sites harboured *D. childiae* and *Daldinia decipiens* (approx. 1:1). *X. camelus* and *X. picta* (*Alnus*) shared the dominant symbiont *D. decipiens* whereas *X. camelus* from *Betula* carried *D. decipiens* and *D. petriniae* (approx. 1:1). In *X. prolongata*, *D. childiae* was the dominant species followed by an undescribed *Daldinia* sp. (0–20 % of isolates); *D. decipiens* was rare and in three females *Hypoxylon macrocarpum* was found. No symbiont occurred in a significant number among endophytes from the host trees.

Introduction

Siricid woodwasps (Hymenoptera: Siricidae) are wood borers acting as secondary and mostly minor pests in their native forests, but becoming aggressive and damaging after their introduction to new areas. Similarly to other wood borers, woodwasps live in obligatory nutritional symbiosis with fungi, which are themselves of economic importance as wood-destroyers. The fungal propagules are carried in mycangia of adult females and transferred during oviposition to sapwood. Woodwasp larvae then feed on mycelium of the symbionts that spread in their galleries (Morgan 1968). Woodwasps of the family Siricidae have basidiomycetous symbionts; *Sirex* and *Urocerus* transfer species of *Amylostereum* (Slippers *et al.* 2003). *Tremex* harbours *Cerrena unicolor* (Stillwell 1965; Palma *et al.* 2005; Pažoutová & Šrůtka 2007). In the genus *Xiphydria* (*Xiphydriidae*), xylariaceous symbionts were found (Šrůtka *et al.* 2007). Some siricids (*Xeris* spp. and *Urocerus japonicus*) do not have any symbionts but their females oviposit only in trees that have already been

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"inoculated" by siricids of other species (Fukuda 2003; Fukuda & Hijii 1997).

There are four common and three rather rare *Xiphydria* species recorded in Europe. *Xiphydria longicollis* (oak woodwasp) preferentially colonizes oak (*Quercus*) in warmer locations, but it has been found also on maple (*Acer*), alder (*Alnus*), birch (*Betula verrucosa*), cherry (*Prunus*), pear (*Pyrus*), and elm (*Ulmus carpinifolia*) (Liston 1997; Schimitschek 1974). The first records from the Czech Republic originate from South Moravia (Schimitschek 1935) but since 1990 *X. longicollis* is quite common in oak forests in the whole country (Pádr 1990; Šrůtka *et al.* 2007). Interestingly, at approximately the same time this species was recorded in Bavaria and the United Kingdom (Kraus 1997; Liston 1997).

Xiphydria camelus (alder woodwasp) prefers weakened host trees of *Alnus* and *Betula* predominantly in floodplain forests. *Xiphydria picta* occurs mainly on *Alnus* at the same locations. *Xiphydria prolongata* (willow woodwasp) colonizes various broadleaved trees, predominantly willow (*Salix*), poplar (*Populus*), and *Ulmus* (Eichhorn 1982). These three species were recorded Europe-wide, including Scandinavia.

Rare species include *Xiphydria betulae* and *Xiphydria megapolitana* (living in birch and alder) and newly described *Xiphydria irrorata* (Pesarini 1995), which were not recorded in the course of the present study.

Horizontal transfer of the fungal symbiont is ensured by mycangia of female adults and larvae. Whereas female mycangia occur in all siricids associated with fungi, larval mycangia are known only in *Sirex* and *Tremex*. These organs are located in hypopleural sacs (Stillwell 1965; Talbot 1977) from which the fungus is released on shed wax plates which serve as a source of inoculum for the emerging female (Francke-Grosmann 1957). So far, no such specific organs have been found in the larvae of *Xiphydria*, where the mechanism of transfer is unknown. In healthy *Sirex* and *Tremex* woodwasp females, the content of mycangia consists of a pure culture containing a clonal population of the original inoculum (Pažoutová & Šrůtka 2007; Thomsen & Koch 1999). In a previous study, symbionts of three *Xiphydria* species were isolated and characterized (Šrůtka *et al.* 2007). In *X. camelus* reared from alder, *Daldinia decipiens* was found, whereas *X. longicollis* (from oak) harboured either *D. decipiens* or *Daldinia childiae*. *D. childiae* was also found in a small sample of females of *X. prolongata* from willow.

In the previous study (Šrůtka *et al.* 2007), *D. childiae* was erroneously identified as *Entonaema cinnabarinum* based on comparison of its nrDNA with sequence databases. The misidentification was obvious as additional *D. childiae* sequences were deposited in the databases (for e.g., Bitzer *et al.* 2008). Discussions with Prof. M. Stadler on the origin, collection and location metadata of the *E. cinnabarinum* herbarium specimen led to the conclusion that the culture derived from this specimen, and sequenced by Triebel *et al.* (2005), was *D. childiae*, growing inside the *Entonaema* stroma. The collection site (France, Pyrénées Atlantiques, Auterrive, Ile du Gave d'Oloron) (Stadler *et al.* 2008) of the *Entonaema* specimen became reported as heavily infested with *D. childiae*.

Daldinia species do not rely solely on insect symbioses for spread – they produce fruit bodies on weakened or fire-damaged trees that are considered their typical hosts and then propagate through ascospores (Johannesson *et al.* 2001). Some of them are known as endophytes of asymptomatic plants. An example is *D. loculata* whose fruit bodies occur typically on *Betula* (Johannesson *et al.* 2000) but rarely also on *Alnus* (Stadler *et al.* 2004) and *Fagus* (Wollweber & Stadler 2001). However, endophytes that may be conspecific with *D. loculata* were also detected in leaves of *Dryas integrifolia* (Higgins *et al.* 2007, supplementary data) and in liverworts.

The biology of the symbiosis in *Xiphydria* is, in contrast to that of economically important pests like *Sirex* or *Urocerus*, poorly known. *Sirex* and *Urocerus* have a narrow host range (trees from the family *Pinaceae* only) and predominantly monophilic association with one symbiotic partner per wasp species in contrast with the broad host range and oligophilic association in *Xiphydria*. Such fundamental differences preclude generalisation of knowledge from *Sirex* symbiosis to that of *Xiphydria*.

The aim of the present study was to enrich the knowledge about the biology of the *Xiphydria* symbiosis, particularly, the diversity of symbionts and factors shaping their communities. Fungal symbionts carried in mycetangia of emerging females were screened using an intensive sampling of four *Xiphydria* spp. in 29 locations and six host tree species yielding 1 389 fungal isolates. The mechanisms driving the structure of symbiont communities were investigated based on a sampling design involving combination of locality types, host trees and woodwasp species.

Material and methods

Sites and collections

The sampling was performed in 2005–2009 in 29 locations in the Czech and Slovak Republic (Fig 1, Table 1). The plant associations and alliances of the locations were checked on site and compared with the map of Czech habitats http://mapy.nature.cz available online at the website of the Agency for Nature Conservation and Landscape Protection of the Czech Republic. The habitat descriptions were according to Chytrý *et al.* (2001) and Kučera (2005); habitat codes of the project Natura 2000 were also included (Anonymous 2007) (Table 1). Floodplain forests and alder carrs were classified according to Douda (2008).

Cultivation of symbionts and endophytes

Rearing woodwasps, dissecting female woodwasps and microscopy of the fungal material were done as described previously (Šrůtka *et al.* 2007). Content of both mycangia was plated on a 2 % malt extract agar plate (MEA) (Difco, Detroit, MI, USA) and only fungi that grew directly from them were considered true symbionts. Isolates were maintained on slants of 2 % MEA grown in the dark at 24 °C for 4 weeks and stored at 4 °C afterwards. All isolates were first sorted in morphotype groups according to culture appearance and their identification into species was confirmed by DNA fingerprinting and nrDNA sequencing (Table 2). The purity of fungal inoculum carried in a single mycangium was tested by plating the diluted mycangial content on 2 % MEA. One female woodwasp from each species was dissected. Twenty

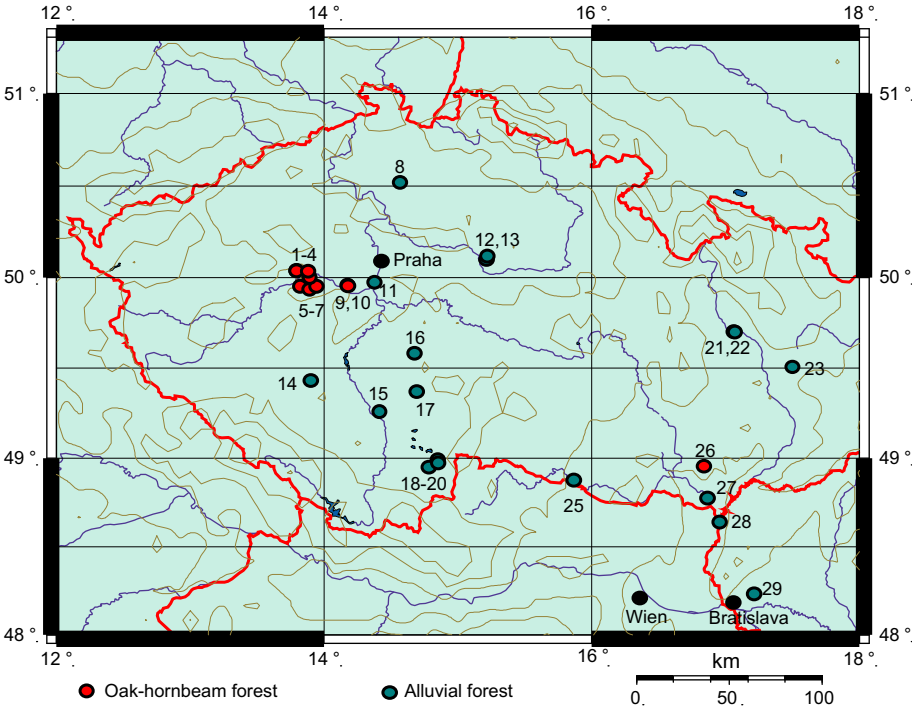

Fig 1 – Map of the Czech Republic showing the collection sites (created with the help of Online Map Creation at http://www.aquarius.ifm-geomar.de/).

random-picked single-colony isolates from these experiments were also analyzed by DNA fingerprinting.

An experiment was set up to ascertain if the symbiotic fungi of woodwasps occur in a significant number among xylariaceous endophytes and saprotrophs of the host trees studied; this might indicate whether endophytes are switched for symbionts in the course of larval development (Table 3). The fungi were isolated from phloem and neighbouring sapwood of alder, birch, oak, and willow; samples were chosen to represent *Xiphydria*-infested, healthy (asymptomatic), and decaying branches and trunks.

Fungi in healthy, *Xiphydria* – infested and decaying specimens of four tree species were investigated to determine the xylariaceous mycobiota related to *Daldinia*. Endophytic fungi were isolated from trunks and branches of trees felled in Feb. 2009. Pieces of inner bark (phloem) and neighbouring sapwood were sterilized using ethanol–sodium hypochlorite method (Sieber & Hugentobler 1987) and afterwards aseptically cut into slivers (ca 2 × 15 mm). From each wood sample, 100–160 slivers, half from phloem and half from sapwood, were plated on 2 % MEA. Only the fungi visibly growing out from wood were subcultured and stored as above. Fungi were sorted into morphotypes and representatives of each morphotype occurring at least on two slivers were identified by both classical taxonomic methods and by comparing their nrDNA sequences (ITS regions and D1D2 region of 28S) to public databases using BLAST (Altschul *et al.* 1990).

DNA analyses

DNA was purified from young (preferably uncolored) mycelium using UltraClean Microbial DNA Isolation Kit (Mo-Bio Laboratories, Solana Beach, California) according to the manufacturer's manual. DNA fingerprinting was performed with ISSR primers 834(C + T) (Wolfe *et al.* 1998). PCR reaction mixtures (14 μl) were loaded on 2 % agarose gel in 0.1 % TBE containing EtBr (0.2 μg ml^{-1}) and run for 3–4 hr at 165 V. The bands were visualized and photographed using GeneGenius2 Imaging System (Syngene, Frederick, Maryland).

Randomly selected representatives of each ISSR type were sequenced. The region of nuclear rDNA containing the internal transcribed spacers and D1D2 region of 28S rDNA (ITS1 and ITS2) was amplified with primers ITS5 and NL4 on Mastercycler Gradient (Eppendorf, Hamburg, Germany) as follows: 1 cycle of 3 min at 95 °C, 30 sec at 55 °C and 1 min at 72 °C, 30 cycles of 30 sec at 95 °C, 30 sec at 55 °C and 1 min at 72 °C and 1 cycle of 30 sec at 95 °C, 30 sec at 55 °C and 10 min at 72 °C. The reaction mix consisted of PCR buffer (Finnzymes, Oy), 0.2 mM deoxynucleotides, 2 pmol of each primer, 1 U of DynaZyme (Finnzymes, Oy) and 5–50 ng of DNA in 25 μl of total volume.

Sequencing was performed at Macrogen Inc. Sequencing Center (Seoul, Korea). The sequences were submitted to GenBank under accession numbers HM192904, HM192905, HM192906, HM192907, HM192908, HM192909, HM192910, HM192911, HM192912.

Phylogeny

Sequence alignment was produced using MUSCLE web interface (http://www.ebi.ac.uk/Tools/muscle/) (Edgar 2004) and manually corrected in BioEdit (Hall 1999). The optimum model, GTR + G + I with four gamma categories, was selected by jModelTest (Posada 2008) and the phylogeny was obtained using PhyML v3.0 (http://atgc.lirmm.fr/phyml) (Guindon *et al.* 2005) under the following parameters: log-likelihood –1872.84302;

Table 1 – Collection sites and their communities

Site[a]	Name	Latitude	Longitude	Community type	Association or alliance	Biotop[b]	Natura 2000 habitat code
1	Křivoklát – Malá Buková 1	50.038661	13.791736	Alders in a spring area inside an oak-hornbeam forest	*Alnenion glutinoso-incanae*	L2.2B	91E0
2	Křivoklát – Malá Buková 2	50.036049	13.793619	Oak-hornbeam forest, moderately dry	*Melampyro nemorosi-Carpinetum*	L3.1	9170
3	Křivoklát – Bušohrad	49.952006	13.821647	Oak-hornbeam forest, moderately dry	*Stellario-Tilietum*	L3.1	9170
4	Křivoklát – Sokolí	50.033964	13.879939	Oak-hornbeam forest, moderately dry	*Stellario-Tilietum*	L3.1	9170
5	Křivoklát – Kolna 1	49.931625	13.900567	Alders in a spring area inside an oak-hornbeam forest	*Alnenion glutinoso-incanae*	L2.2B	91E0
6	Křivoklát – Kolna 2	49.928054	13.851206	Birch at the margin of an oak-hornbeam forest, moderately dry	*Melampyro nemorosi-Carpinetum*	L3.1	9170
7	Křivoklát – Hudlice	49.952681	13.944536	Oak-hornbeam forest, moderately dry	*Melampyro nemorosi-Carpinetum*	L3.1	9170
8	Beškovský důl	50.51924	14.566882	Alder-ash floodplain forest	*Alnion glutinosae*	L2.2A	91E0
9	Czech karst – Čeřinka	49.959678	14.177808	Oak-hornbeam forest, moderately dry	*Stellario-Tilietum*	L3.1	9170
10	Czech karst, Mořina	49.954653	14.178856	Oak-hornbeam forest, moderately dry	*Stellario-Tilietum*	L3.1	9170
11	Krňák	49.973153	14.37635	Alder carr	*Carici acutiformis-Alnetum*	L1	
12	Libický luh – Velký Osek	50.099311	15.213547	Old acidophilous oak woods with *Quercus robur* on sandy plains	*Genisto germanicae-Quercion*	L7.2	9190
13	Libický luh – Sány	50.117536	15.220833	Old acidophilous oak woods with *Quercus robur* on sandy plains	*Genisto germanicae-Quercion*	L7.2	9190
14	Blatná, Kaneček pond	49.432222	13.899167	Secondary vegetation	*Saliceto-Alnetum*	L2.2B	91E0
15	Koloděje nad Lužnicí	49.258611	14.411944	Secondary vegetation, ruderal			
16	Miličín, V olších	49.583115	14.677844	Alder carr	*Carici elongatae-Alnetum*	L1	
17	Sezimovo Ústí	49.369444	14.694722	Alder carr	*Carici elongatae-Alnetum*	L1	
18	Ruda fishpond	48.949267	14.78455	Old acidophilous oak woods		L7.2	9190
19	Lužnice bifurcation	48.991053	14.850292	Alder carr	*Carici elongatae-Alnetum*	L1	
20	Majdalena	48.971944	14.854444	Alder carr	*Carici elongatae-Alnetum*	L1	
21	Litovelské Pomoraví 1	49.701478	17.061247	Softwood floodplain forest	*Salicetum albae*	L2.4	91E0
22	Litovelské Pomoraví 2	49.699359	17.070324	Softwood floodplain forest	*Salicetum albae*	L2.4	91E0
23	Osek nad Bečvou	49.508235	17.501609	Softwood floodplain-like secondary vegetation, degraded	*Salicetum albae*		
24	Albrechtičky	49.701387	18.071534	Softwood floodplain forest	*Salicetum albae*	L2.4	91E0
25	Podyjí (National Park)	48.875554	15.866835	Mixed ash-alder alluvial forests, degraded	*Saliceto-Alnetum*	L2.2B	91E0
26	Kuntínov hill	48.952598	16.838924	Pannonian–Carpathian oak-hornbeam forests	*Carici pilosae-Carpinetum*	L3.3A	91G0
27	Břeclav, Kančí obora	48.775199	16.865156	Mixed oak-elm-ash forests of great rivers	*Fraxino pannonicae-Ulmetum*	L2.3B	91F0
28	Morava–Dyje junction	48.640683	16.957678	Mixed oak-elm-ash forests of great rivers	*Fraxino pannonicae-Ulmetum*	L2.3A, B	91F0
29	Jurský Šúr	48.232796	17.211771	Alder carr, primeval	*Carici elongatae-Alnetum*	L1	

a Site numbers are those used in Fig 1.
b Biotop classification according to Kučera (2005).

Table 2 – The occurrence of fungal symbionts in the woodwasps reared from wood specimens

Specimen code[a]	Species	Host	Location	Year	No. of isolates	*Daldinia childiae*	*Daldinia decipiens*	*Daldinia* sp. (%)	*Daldinia petriniae*	*Hypoxylon macrocarpum*
01LOQU12	*Xiphydria longicollis*	*Quercus robur*	Libický luh – Velký Osek	2005	9	33.3	66.7			
02LOQU12				2006	25	80.0	20.0			
03LOQU12				2007	20	40.0	60.0			
04LOQU12				2008	34	61.8	38.2			
05LOQU12				2009	32	40.6	59.4			
06LOQU28			Morava–Dyje junction	2009	51	56.9	43.1			
07LOQU10			Czech karst, Mořina	2007	118	87.3	12.7			
08LOQU10				2008	7	100.0				
09LOQU03			Křivoklát – Bušohrad	2007	35	100.0				
10LOQU04			Křivoklát – Sokolí	2007	4	100.0				
11LOQU09		*Quercus petraea*	Czech karst – Čeřinka	2007	1	100.0				
12LOQU26			Kuntínov hill	2008	23	95.7	4.3			
13LOQU07			Křivoklát – Hudlice	2008	3	100.0				
14CAAL27	*Xiphydria camelus*	*Alnus glutinosa*	Břeclav, Kančí obora	2006	44		100.0			
15CAAL20			Majdalena	2006	19		100.0			
16CAAL20				2008	25		100.0			
17CAAL17			Sezimovo Ústí	2006	12		100.0			
18CAAL19			Lužnice bifurcation	2006	58		100.0			
19CAAL19				2008	48		100.0			
20CAAL18			Ruda fishpond	2008	26		100.0			
21CAAL16			Miličín – V Olších	2009	14		100.0			
22CAAL01			Křivoklát – Malá Buková 1	2009	28		100.0			
23CAAL11			Krňák	2007	115	1.7	98.3			
24CAAL21			Litovelské Pomoraví 1	2007	30	10.0	90.0			
25CAAL05			Křivoklát – Kolna	2007	47		97.9		2.1	
26CAAL09			Beškovský důl	2008	38		97.4		2.6	
27CABE12	*Xiphydria camelus*	*Betula pendula*	Libický luh – Velký Osek	2008	4		75.0		25.0	
28CABE13			Libický luh – Sány	2009	5		20.0		80.0	
29CABE06			Křivoklát – Kolna 2	2009	4		50.0		50.0	
30CABE02			Křivoklát – Malá Buková 2	2009	5		80.0		20.0	
31PRSA24	*Xiphydria prolongata*	*Salix alba*	Albrechtičky	2008	59	79.7		20.3		
32PRSA24				2009	13	92.3		7.7		
33PRSA23			Osek nad Bečvou	2008	25	88.0		12.0		
34PRSA28			Morava–Dyje junction	2009	64	79.7	1.6	18.8		
35PRSA14			Blatná, Kaneček pond	2008	52	90.4	3.8	1.9		3.8
36PRSA15			Koloděje nad Lužnicí	2008	16	93.8	6.3			
37PRSA19			Lužnice bifurcation	2008	41	97.6				2.4
38PRSA22			Litovelské Pomoraví 2	2006	3	100.0				
39PRSA21			Litovelské Pomoraví 1	2007	66	100.0				
40PRSA25			Podyjí	2008	6	100.0				
41PRSA29			Jurský Šúr	2009	37	100.0				
42PRSA21		*Salix caprea*	Litovelské Pomoraví 1	2007	10	100.0				
43PIAL28	*Xiphydria picta*	*Alnus glutinosa*	Jurský Šúr	2009	103		100.0			
44PIAL29			Morava–Dyje junction	2009	14		100.0			

a Specimen codes are those used in the CANOCO analyses and Fig 4.

gamma shape parameter 0.394; P_{inv}: 0.285; GTR relative rate parameters: A – C 1.04438; A – G 3.07739; A – T 1.06310; C – G 1.45533; C – T 6.56618; G – T 1.00000. The phylogram was drawn using MEGA 4.0 (Tamura *et al.* 2007). Reference sequences of *Daldinia* spp. (EF026144, AM292044, AM749931, AF176958, AM292042, AB284189, AF176981, AF176968, AF176971, AM749939, AM749927) and of *Hypoxylon macrocarpum* (AY616705) were downloaded from GenBank.

Table 3 — Frequency of xylariaceous endophytes in the host trees

Host tree	*Alnus glutinosa*		*Quercus robur*				*Salix alba*					
Site	Morava–Dyje junction		Libický luh – Velký Osek		Morava–Dyje junction		Jurský Šúr		Albrechtičky		Morava–Dyje junction	
Specimen type	Healthy	Decaying	*Xiphydria*		Healthy	Decaying	*Xiphydria*	Healthy	*Xiphydria*	Healthy	Healthy	Decaying
Daldinia childiae	2.0[a]											
Xylariales spp. H39	0.5											
Hypoxylon howeanum												47.0
Biscogniauxia nummularia	1.0						1.8	3.0				
Obolarina dryophila							0.3					
Nemania serpens	3.0	0.5			0.4		0.3		0.7	1.0	0.3	
Nemania aff. *diffusa*	0.5	1.0										
Xylaria longipes										0.3		
Xylaria hypoxylon			0.5									
Xylaria spp. H55		1.5								0.3	0.7	
Kretzschmaria deusta									0.3			
Arthrinium phaeospermum						1.2			0.3			
Arthrinium arundinis									0.3			
Coelomycete spp. H34		0.5	0.5			0.4	5.3	10.5	5.0	1.3	7.3	1.0
Diatrypella pulvinata		4.5										

a Frequency was calculated as a percentage of colonized slivers from all plated slivers (both phloem and sapwood).

Analysis of the symbiotic fungal communities

The dataset consisting of frequencies of fungal species in each sample was subjected to multivariate analysis, implemented in CANOCO 4.5 and its add-on WinKyst 1 (ter Braak & Šmilauer 2002). Detrended correspondence analysis (DCA), calculating the length of environmental gradients, suggested that the species respond in a linear manner along hypothetical environmental gradients. Data were analyzed by non-metric multidimensional scaling (NMDS) and DCA whenever a "horseshoe effect" in the results was detected. NMDS was performed using Bray–Curtis distance without transformation of the input data to graphically evaluate any pattern in the grouping of samples. Redundancy analysis (RDA) was used to explore the specificity of relationships between the vector and host tree species and their fungal associates. Settings included downweighting rare species and biplot scaling focusing on interspecies distances. Woodwasp or host tree species were used as environmental variables and their significance was assessed using Monte Carlo permutation tests ($n = 1000$).

Results

Identification of symbionts

Only one fungal species per one adult was found. ISSR patterns of isolates obtained from a single mycangium were identical. ISSR fingerprinting of 1389 independent isolates revealed four distinct groups (Fig 2). *D. childiae*, *D. decipiens* and *D. petriniae* were linked with the reference sequences from GenBank. Comparison of nrDNA representing the fourth group to that of other *Daldinia* species from the public databases and database of unpublished species (D. Peršoh, personal communication) has shown that this symbiont did not belong to any *Daldinia* species sequenced so far (Fig 3). Colonies of *Daldinia* sp. resembled those of *D. decipiens*, but with grey–brown pigmentation instead of yellow–green shades. All three isolates of the fifth symbiont were identified as *H. macrocarpum* based on colony morphology and nrDNA sequence comparison.

Communities of symbiotic fungi

Whereas a single woodwasp female hosted only one symbiotic species, more diverse assemblages (1–4 species, Table 2) were associated with conspecific females reared from the same wood sample. Diversity of the symbionts varied from one to four per one *Xiphydria* species (Table 2). Three first ordination axes of NMDS explained 94 % of the variation in the data (1. axis 93.7 %, 2. axis 0.3 %, Fig 4). The environmental variable correlating best with the first ordination axis (x) was the "*Xiphydria* species", where the majority of the *X. prolongata* samples (*Salix*) appeared on the left, followed by samples of *X. longicollis* (*Quercus*) and *X. picta* (*Alnus*) together with *X. camelus* (*Alnus*, *Betula*) samples plotted on the right.

The second axis (y) separated samples originating from the same vector species. In the case of *X. camelus*, where the second axis showed an isolated position of the population

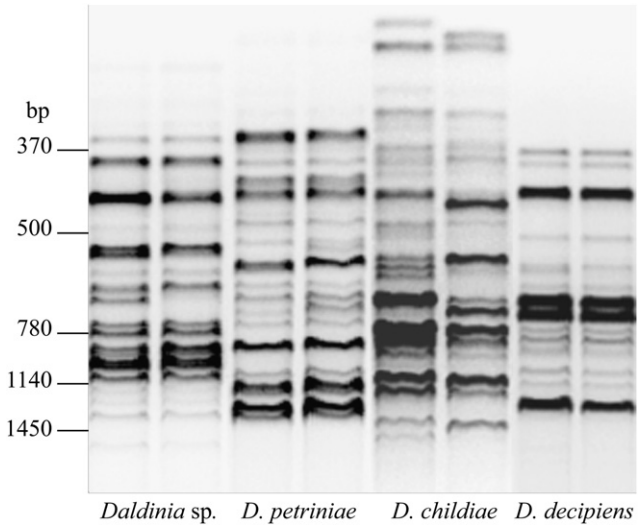

Fig 2 – ISSR patterns of the four symbiotic *Daldinia* species obtained with primers 834C + T.

from *Betula*, it was evident that the correlating environmental variable was the host tree. Analyses also separated *X. longicollis* samples into two groups, differing in the abundance of *D. decipiens*, where the only correlating environmental variable found was the vegetation type of the original locality (see below). The RDA demonstrated that the environmental variable "host tree" was correlated with our data with high significance ($P < 0.002$, Monte Carlo test), and was responsible for the 84.6 % of the variation observed. The environmental variable "*Xiphydria* species" was also significant and explained 77.1 % of the total variation in the system.

In *X. longicollis*, *D. childiae* was either the only symbiont detected or a highly dominant one in the wood samples from moderately dry oak-hornbeam (*Quercus–Carpinus betula*) forests. The females from the alluvial sites harboured *D. childiae* and *D. decipiens* in ratios around 1:1. The site at Libický luh was sampled to various extents for five subsequent years and a high proportion of *D. decipiens* was always found, sometimes even exceeding that of *D. childiae*. Only two symbiont species were recorded among the total of 362 isolates.

In *X. prolongata*, four symbiotic fungal species were found among 392 isolates, with *D. childiae* being the dominant species (80–100 % of isolates per wood sample), followed by *Daldinia* sp. (0–20 % of isolates). *D. decipiens* was encountered rarely. Two females of Blatná and one female of Lužnice junction possessed *H. macrocarpum*, instead of any *Daldinia* symbiont.

D. decipiens was a predominant symbiont of female *X. camelus* reared from alder. From 504 isolates only five were identified as *D. childiae* and two as *D. petriniae*. In the birch samples, however, the situation was different. Although the total number of females that emerged from birch was only 14, six of the isolates were to *D. petriniae*. Altogether three symbiotic species were found.

In *X. picta* from alder trees at two locations, 117 isolates of symbionts were obtained, all of them were *D. decipiens*.

Xylariaceous mycobiota *of the host trees*

D. childiae was found on four slivers from healthy wood of *A. glutinosa*, but no other species of symbionts, including

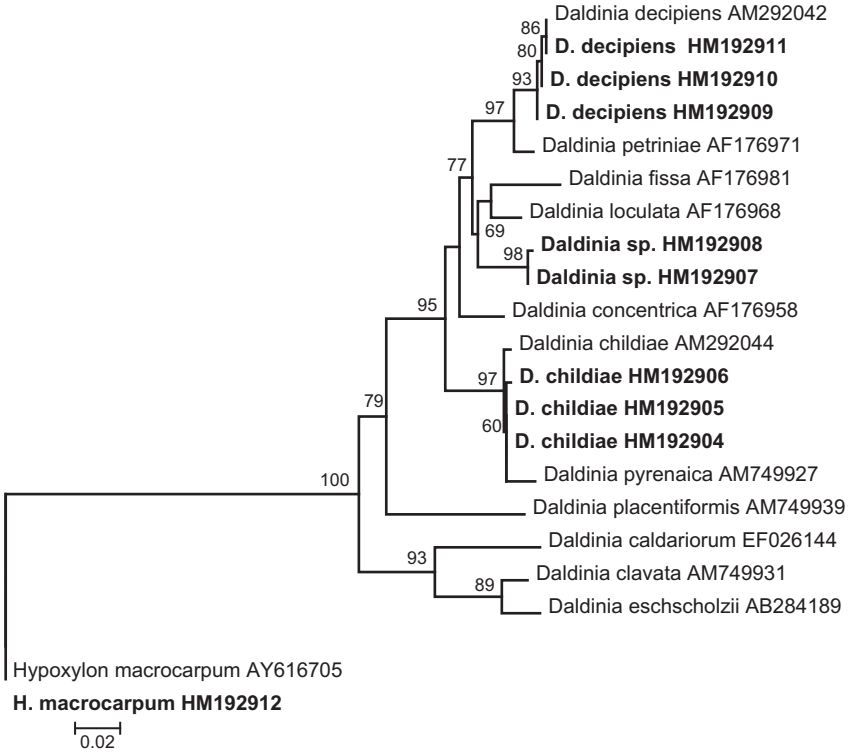

Fig 3 – Phylogenetic relationships among the isolates of *Xiphydria* symbionts (in bold) and representatives of *Daldinia* species. The unrooted tree was generated using PhyML maximum likelihood analysis of the ITS-nrDNA sequences. Bootstrap values > 50 % are given on the branches. Bar indicates the nucleotide substitutions per site.

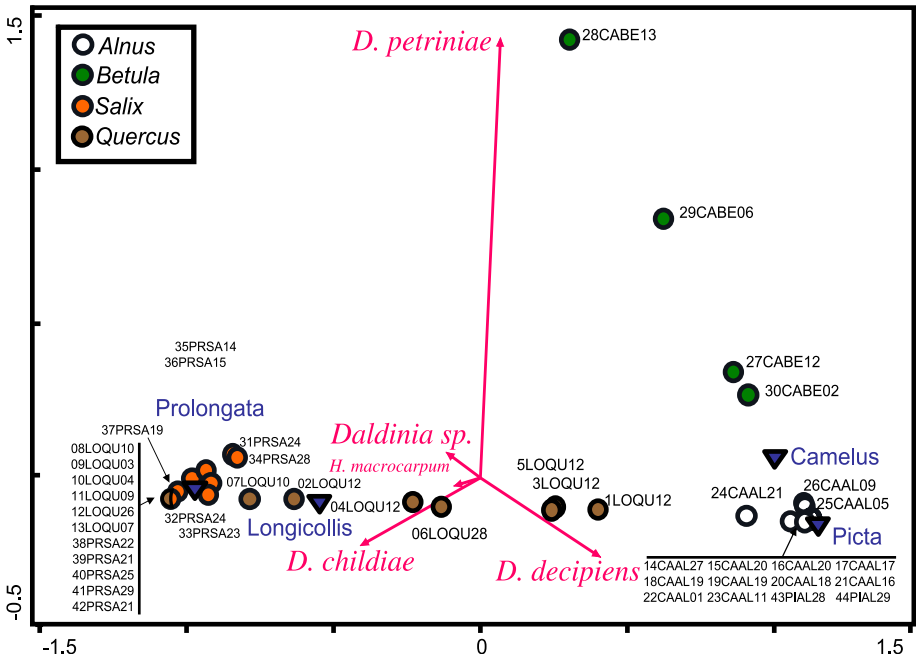

Fig 4 – NMDS ordination triplot of 44 communities based on the frequency of fungal symbionts (Table 2). Samples are indicated as dots and fungal species as arrows. *Xiphydria* spp. were included as a supplementary variable and their position in the ordination graph is marked by triangles. Abbreviations for vectors: Prolongata – *X. prolongata*, Longicollis – *X. longicollis*, Camelus – *X. camelus*, Picta – *X. picta*. Sample codes are as in Table 2.

H. macrocarpum, appeared in any of the other samples (Table 3). *Biscogniauxia nummularia*, *Nemania serpens* and an undescribed species of a coelomycete were the most often encountered xylariaceous fungi. Willow and alder had higher diversity of xylariaceous endophytes than oak, whereas no xylariaceous endophytes were found in any of the birch wood samples.

Discussion

The results show that while the fungus "culture" carried by a woodwasp female is always pure, a species has potential to host several fungal species from two genera of Xylariales. This is in contrast with *Sirex* symbioses which were considered to be limited to one symbiont per woodwasp species, although in the light of recent results (Nielsen *et al.* 2009) it is possible that the alternative symbionts may have been overlooked due to the small numbers of dissected *Sirex* females (Gaut 1970). The physiological mechanism ensuring selection of the particular symbiont might be less specific (as the "adoption" of *H. macrocarpum* has shown), but it still can support only a few suitable symbionts between the number of xylariacous fungi occurring in the ecosystem. The ability to select and maintain the pure fungus of the particular species in mycangia is enigmatic and it is surely the primary force in the evolution of this symbiosis leading to the formation of species specific assemblages of the fungal symbionts.

The clustering of the samples along the first axis confirms the woodwasp species as the most important factor determining the observed variation in fungal assemblages. The case of alder infesting *X. camelus* and *X. picta* sharing both their dominant symbiont and host tree also suggests the possibility of a combined effect of both the vector and its ecology (host tree spectrum) on the composition of associated fungal assemblages. The contact between woodwasp species may lead to unification of their symbionts; on the other hand, it may be possible that *D. decipiens* spreads better in the alder wood than other *Daldinia* species and therefore brings an advantage to larvae of its vector.

Similar interplay of factors could be responsible for the marked difference between *X. longicollis* from alluvial forest sites, where high proportions of *D. decipiens* symbionts were repeatedly found, and those from oak-hornbeam forests that rarely carried this fungus. It is possible, that some *X. longicollis* females occasionally oviposit on an alternative host tree in the vicinity (possibly alder or birch which were typical for alluvial associations) and obtain *D. decipiens* typical for *Alnus* infesting *X. camelus*.

The next important variable, the host tree itself could further shape symbiont communities. The effect of the host tree is difficult to study, because of the narrow spectrum of the main host tree in *Xiphydria* and rarity of feeding on alternative hosts. The data from *X. camelus* reared from alder and birch, sampled at the same location and time, suggested the higher proportion of *D. petriniae* symbionts associated with the population from birch might be either explained by oviposition preferences of the females towards the same tree species, from which they emerged, or that *D. petriniae* symbiont may be disadvantageous for larvae evolving in alder. The other possible way, acquisition of *D. petriniae* from the pool of endophytic fungi in birch, was not confirmed in this study – no xylariaceous fungi were found there.

Our data suggest symbiont switches between adults of the single species as well as probable switches between different

species sharing the same host tree. The same phenomenon was recently found in *Sirex*, where females of the introduced *S. noctilio* and of the native *S. edwardsii* emerging from the same wood carried the same *Amylostereum areolatum* strain, although *S. edwardsii* usually carries *Amylostereum chailletii* (Nielsen *et al.* 2009). This suggests that fungi compete with each other or may become disassociated from their insect vector for a period while growing within a host tree, which may facilitate contact with symbionts of co-occurring wood borers or other saprotrophic or endophytic fungi. Thus, the real host spectrum of *Daldinia* spp. even in its saprotrophic, symbiotic or endophytic phase is important for understanding of *Xiphydria* symbiosis biology.

We have observed symbiotic *Daldinia* spp. spreading along the larval galleries of its particular vector, but this occurrence does not necessarily correlate with the most common host upon which fruit bodies are found. *D. decipiens* is a common associate of *Alnus*-infecting woodwasps, but typically forms stromata on *Betula* with only one record of stroma from *Alnus* (Stadler *et al.* 2004). *D. petriniae* was isolated from the woodwasps reared from *Betula* and to a lesser extent from *Alnus*. However, in Central Europe, *D. petriniae* fruits predominantly on *Alnus*, and rarely on *Carpinus* (Wollweber & Stadler 2001). In Sweden, *D. petriniae* stromata were found also on *Betula*, *Corylus* and *Salix* (Johannesson *et al.* 2000). *D. childiae* consistently grows along *Xiphydria* galleries on *Quercus* (Šrůtka *et al.* 2007) and *Salix*, and its stromata occur worldwide on a variety of host trees like e.g., *Acer*, *Carpinus*, *Fagus*, *Fraxinus* and *Quercus* (Wollweber & Stadler 2001). We have shown that it also occurs endophytically on *Alnus* (Table 3). Apparently, the same *Daldinia* spp. can exist independently from woodwasps on other hosts, and propagate through the ascospores, or they can live as endophytes.

Xiphydria spp. form species specific assemblages of symbiotic fungi which are relatively stable through geographically distant locations. However, together with the results presented in this paper it is now confirmed that the symbiotic relationship between siricid woodwasp and fungus is not strictly specific and its physiological mechanism allows for symbiont switching or even acquiring other less related fungi.

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