Eur. J. Entomol. **111**(4): 561–565, 2014
doi: 10.14411/eje.2014.070
ISSN 1210-5759 (print), 1802-8829 (online)

Fungi associated with the red-haired bark beetle, *Hylurgus ligniperda* (Coleoptera: Curculionidae) in the forest-steppe zone in eastern Ukraine

Kateryna DAVYDENKO[1], Rimvydas VASAITIS[2], Valentyna MESHKOVA[1] and Audrius MENKIS[2]

[1]Ukrainian Research Institute of Forestry & Forest Melioration, Pushkinska st. 86, 61024 Kharkiv, Ukraine;
e-mails: davidenkokv@mail.ru; valentynameshkova@gmail.com

[2]Department of Forest Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences,
P.O. Box 7026, SE-75007 Uppsala, Sweden; e-mails: rimvys.vasaitis@slu.se; audrius.menkis@slu.se

Key words. Coleoptera, Curculionidae, *Hylurgus ligniperda*, red-haired bark beetle, scots pine, pathogens, ophiostomatoid fungi, insect-fungus interaction, forest-steppe zone, eastern Ukraine

Abstract. The aim of this study was to investigate the composition of the fungal community associated with the red-haired bark beetle (*Hylurgus ligniperda* Fabricius) in two plantations of *Pinus sylvestris* L. located in the Kharkiv and Luhansk regions (ca. 250 km apart) in the forest-steppe zone in eastern Ukraine. In each plantation, 48 beetles were collected from butts of living trees and 48 beetles from stems of fallen trees, i.e., a total of 96. Half of the beetles from each site were used for culturing fungi and the other half for direct sequencing the internal transcribed spacer of fungal ribosomal RNA (ITS rRNA). Thirty distinct fungal taxa were identified by culturing and 31 by direct sequencing. When pooled, there were 40 fungal taxa among which *Ophiostoma piceae* (Münch) Sydow & P. Sydow (10.3%), *Alternaria alternata* (Fries) Keissler (9.7%), *Ogataea neopini* Nagatsuka, S. Saito & Sugiyama (8.0%), *Botryotinia fuckeliana* (de Bary) Whetzel (5.1%), *Cladosporium* sp. Link (5.1%) and *Sydowia polyspora* (Brefeld & Tavel) E. Müller (4.6%) were the most common. Species of the genus *Ophiostoma* were the most abundant and included five different taxa *O. piceae*, *O. bicolor* R.W. Davidson & D.E. Wells, *O. ips* (Rumbold) Nannfeldt, *O. canum* (Münch) Sydow & P. Sydow and *O. rectangulosporium* Ohtaka, Masuya & Yamaoka, all of which are known to be at most weak pathogens of trees. The plant pathogen *Botryotinia fuckeliana* and insect pathogens *Isaria farinose* (Holmskjold) Fries and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin were also detected. Basidiomycetes were rare, among which three wood-decaying fungi *Bjerkandera adusta* (Willdenow) P. Karsten, *Fomitopsis pinicola* (Swartz) P. Karsten and *Heterobasidion annosum* (Fries) Brefeld were detected. In conclusion, in the forest-steppe zone in eastern Ukraine *H. ligniperda* is a vector of diverse communities of fungi the majority of which, if at all, are only weak pathogens of trees.

INTRODUCTION

Bark beetles belonging to the family Curculionidae include economically important forest pests. They are known to vector ophiostomatoid fungi (Ascomycota), including species from two phylogenetically unrelated orders, Microascales and Ophiostomatales (Seifert et al., 2013), which may cause tree diseases and discoloration of wood (Kirisits, 2004). One of these is the well-known *Ips typographus* L. with associated fungus and primary invader, *Ceratocystis polonica* (Siemaszko) C. Moreau, which can be an aggressive pathogen (Persson et al., 2009). Among others are the Dutch elm disease pathogens, *Ophiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier, which over the last 100 years have destroyed billions of elm trees (*Ulmus* spp.) worldwide (Brasier, 1991).

In recent years, a gradual increase in diversity and abundance of different bark beetles has occurred in the forest-steppe zone in eastern Ukraine (Meshkova & Sokolova, 2007). Bark beetles from the genera *Hylastes* (*Hylastes angustatus* Herbst, *Hylastes ater* Paykull, *Hylastes opacus* Erichson), *Orthotomicus* and in particular *Hylurgus* (*Hylurgus ligniperda*) became common causing extensive damages to young plantations, stands and timber of *Pinus sylvestris* (Meshkova & Sokolova, 2007). *H. ligniperda* breeds in logging residues including stumps, roots and logs of pine trees (Reay & Walsh, 2001, 2002; Meshkova & Sokolova, 2007). In addition, larvae of *H. ligniperda* may also feed on roots and butts of healthy-looking and diseased seedlings and saplings (Dumouchel & Palisek, 2002). *H. ligniperda* is known to be associated with several *Ophiostoma* species, which in trees can cause reduced increment, crown thinning, chlorosis or even the death of the tree (Kirisits, 2004; Kim, 2010). It may also vector several *Grossmania* and *Leptographium* species, which cause root diseases (Zhou et al., 2001, 2004; Kirisits, 2004; Reay et al., 2006; Kim, 2010; Kim et al., 2011; Linnakoski, 2011; Jankowiak & Bilański, 2013). The latter may suggest that fungi vectored by *H. ligniperda* can be of particular importance for forest health, yet they have never been investigated in those regions of Ukraine at the south-eastern limit of the distribution of *P. sylvestris* in Europe.

The aim of this study was to investigate the composition of the fungal community associated with the red-haired bark beetle (*Hylurgus ligniperda*) in the forest-steppe zone in eastern Ukraine.

MATERIAL AND METHODS

Study sites and sampling

Study sites were two forest stands located in the Izum forest enterprise, Kharkiv region (N49°10´, E037°14´) and St. Luhansk forest enterprise, Luhansk region (N48°43´, E039°05´), eastern

Ukraine. The distance between these sites was ca. 250 km. Stands at both sites were ca. 50 year-old plantations of *P. sylvestris* with small admixture of *Betula pendula* Roth and *Alnus glutinosa* L. Sampling of *H. ligniperda* was carried out in the beginning of October 2010. At each site, eight adults of *H. ligniperda* were collected from butts of three randomly selected living trees attacked by the bark beetles and eight from stems of three randomly selected fallen trees, resulting in a total of 48 individuals sampled at each site or 96 altogether. Living trees were defoliated and weakened trees resulting from extensive damage caused by pine sawflies in the same season. The fallen trees were harvested trees left on site that were felled during clear-felling in mid May 2010 (ca. five month before sampling the beetles). At the time of sampling, the fallen trees were dead and their wood was extensively colonized by different insects. On living trees, the bark beetle entry holes were most common in the butts (between ca. 0 and 50 cm from the base), whereas in fallen trees they occurred higher up the stems (between ca. 50 and 100 cm from the base). Beetles were collected from both these areas of the trunks of these trees. In both standing living trees and fallen dead trees, two 50 cm-long sections of the bark with bark beetle entry holes were carefully removed from each tree and adults of *H. ligniperda* were collected. The time of sampling coincided with emergence of *H. ligniperda* beetles from their galleries and their flying period. *H. ligniperda* beetles were sampled using sterilized forceps, individually placed in sterile 1.5 ml centrifugation tubes, labelled and transported to a laboratory. No visible symptoms of blue-stain fungi were noted in the sapwood at the time of sampling. Half of the beetles from each site (Kharkiv and Luhansk) and each part of a tree sampled (butt and stem) were stored at 4°C for fungal culturing and the other half at –20°C for DNA analysis.

Fungal culturing and molecular identification of taxa

One to seven days after collection, 24 beetles from each site or 48 in total were placed separately without rinsing in Petri dishes containing ca. 30 ml of Hagem agar medium and incubated at room temperature (ca. 21°C) in the dark (Persson et al., 2009). Petri dishes were checked daily and outgrowing fungal mycelia were sub-cultured to new media. Fungal cultures were divided into groups based on their morphology and for species identification representative cultures from each group were subjected to sequencing of the internal transcribed spacer of fungal ribosomal RNA (ITS rRNA). Isolation of DNA, amplification and sequencing followed methods described by Menkis et al. (2006). Amplification by PCR was done using two primers – ITS1F (Gardes & Bruns, 1993) and ITS4 (White et al., 1990). Sequencing was carried out by Macrogen Inc., Korea. Sequences were analyzed in Seqman (version 5.07, DNASTAR, Madison, WI, USA). Databases in GenBank (Altschul et al., 1997) and the Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences were used to determine the identity of ITS rRNA sequences. The criteria used for identification were: sequence coverage > 80%; similarity to species level 99–100% and similarity to genus level 94–98%.

Direct sequencing of fungi from the beetles

Isolation of DNA (without rinsing of the beetles), amplification and sequencing of fungal ITS rRNA obtained directly from the beetles was carried out as described by Persson et al. (2009). Amplification by PCR was done in to steps: firstly using fungal specific primers NLC2 (GAGCTGATTCCCAAACAACTC) and NSA3 (AAACTCTGTCGTGCTGGGGATA) (Persson et al., 2009), and then in a second (nested) PCR using primers ITS1F and ITS4. If only one DNA band was present per sample following nested PCR, the PCR product was used for sequencing. Multiple-banded PCR products were separated on 2.0% agarose gels, individual bands were excised and re-amplified using universal primers ITS1 and ITS4 (White et al., 1990). Resulting single-banded products were sequenced in both directions using the same primers as for PCR amplification.

Statistical analyses

Richness of fungal taxa detected in beetles from different tree parts (butts and stems), different sites (Kharkiv and Luhansk) and by different methods (culturing and direct sequencing) was compared using chi-squared tests (Mead & Curnow, 1983). The relative abundance of fungal taxa was calculated from actual numbers of observations (presence/absence data) as the percentage of observations (isolates/sequences) for the total fungal community. Shannon diversity indices and quantitative Sorensen similarity indices were used to characterise the diversity and composition of fungal communities (Shannon, 1948; Magurran, 1988).

RESULTS

Of the 48 beetles of *H. ligniperda* used for fungal culturing, 43 (89.5%) yielded fungal growths and between one and six different fungal cultures per beetle or 77 cultures (1.8 on average) in total. Sequencing of representative cultures revealed the presence of 30 distinct fungal taxa of which 18 (60.0%) could be identified to taxon level, 8 (26.7%) to genus level and 4 (13.3%) remained unidentified (Table 1). Successful amplification were obtained for all the 48 beetles used for direct amplification and sequencing of fungal ITS rRNA, with from one to three amplicons, or an average of 2.0 amplicons per beetle. Separation and sequencing of individual amplicons resulted in 98 high-quality sequences representing 31 distinct fungal taxa among which 17 (54.8%) were identified to taxon level, 9 (29.1%) to genus level and 5 (16.1%) remained unidentified (Table 1). In total, 40 fungal taxa were detected using culturing and direct sequencing. Among these, 9 (22.5%) were exclusively detected by culturing, 10 (25.0%) by direct sequencing and 21 (52.5%) by both methods (Tables 1). A chi-square test revealed no significant difference in richness of fungal taxa detected by the two methods ($p > 0.05$). Sorensen index of similarity of fungal communities was high (0.69) when compared between culturing and direct sequencing. In the pooled dataset, there were 32 different taxa from Kharkiv and 36 from Luhansk (Table 1), and a chi-square test revealed no significant difference in richness of fungal taxa between these sites ($p > 0.05$). Twenty-eight (70.0%) taxa were common to both sites. As a result, Sorensen index of similarity of fungal communities was very high (0.82) between these sites. Associated with *H. ligniperda* collected on different parts of trees, there were 36 fungal taxa associated with those from butts and 14 from stems, when the data from both Kharkiv and Luhansk were analyzed together (Table 1). As a result, the chi-square test revealed that the richness of fungal taxa was significantly higher for beetles from butts than from stems ($p < 0.0001$). Ten (25%) fungal taxa were common, resulting in a moderate (0.40) value of the Sorensen index of similarity of fungal communities associated with beetles from butts and stems. In different datasets (e.g. Kharkiv culturing butts, Kharkiv culturing stems, etc.), Shannon di-

Table 1. Relative abundance of fungal taxa cultured/directly sequenced from adults of *Hylurgus ligniperda* collected from butts and stems of *Pinus sylvestris* growing in the Kharkiv and Luhansk regions in eastern Ukraine. Number of the beetles used is given in the parentheses.

Fungal taxa	Genbank accession number	Kharkiv							Luhansk							Total
		Cultured			Sequenced			All	Cultured			Sequenced			All	
		Butt (12)	Stem (12)	All (24)	Butt (12)	Stem (12)	All (24)	(48)	Butt (12)	Stem (12)	All (24)	Butt (12)	Stem (12)	All (24)	(48)	(96)
Ascomycota																
Alternaria alternata (Fries) Keissler	KC768067	–	27.3	8.8	–	16.7	4.1	6.0	–	15.0	7.0	10.3	30.0	18.4	13.0	9.7
Alternaria sp. Nees	KC768068	4.3	–	2.9	–	–	–	1.2	–	–	–	–	–	–	–	0.6
Beauveria bassiana (Balsamo-Crivelli) Vuillemin	KC768069	4.3	–	2.9	–	–	–	1.2	4.3	–	2.3	–	–	–	1.1	1.1
Botryotinia fuckeliana (de Bary) Whetzel	KC768099	8.7	–	5.9	10.8	–	8.2	7.2	–	–	–	10.3	–	6.1	3.3	5.1
Candida sp. Berkhout	KC768071	–	–	–	5.4	–	4.1	2.4	–	–	–	3.4	–	2.0	1.1	1.7
Chaetomium globosum Kunze ex Fries	KC768072	–	–	–	2.7	–	2.0	1.2	4.3	–	2.3	3.4	–	2.0	2.2	1.7
Chaetomium sp. Kunze	KC768073	–	–	–	–	–	–	–	4.3	–	2.3	–	–	–	1.1	0.6
Chalara sp. (Corda) Rabenhorst	KC768074	–	–	–	2.7	–	2.0	1.2	–	–	–	3.4	–	2.0	1.1	1.1
Cladosporium sp. Link	KC768075	13.0	–	8.8	8.1	–	6.1	7.2	13.0	–	7.0	–	–	–	3.3	5.1
Eupenicillium sp. F. Ludwig	KC768077	–	–	–	–	–	–	–	–	5.0	2.3	–	5.0	2.0	2.2	1.1
Fungal sp. HD6_31	KC768105	–	–	–	–	–	–	–	4.3	–	2.3	–	–	–	1.1	0.6
Fungal sp. HH74_18	KC768101	–	–	–	2.7	–	2.0	1.2	4.3	–	2.3	–	–	–	1.1	1.1
Fungal sp. HH78_19	KC768102	–	–	–	2.7	–	2.0	1.2	4.3	–	2.3	–	–	–	1.1	1.1
Fungal sp. HK2_22	KC768104	–	–	–	2.7	–	2.0	1.2	4.3	–	2.3	–	–	–	1.1	1.1
Graphium sp. Corda	KC768079	4.3	–	2.9	–	8.3	2.0	2.4	–	–	–	–	–	–	–	1.1
Ilyonectria radicicola (Gerlach) Chaverri & C. Salgado	KC768082	4.3	–	2.9	–	–	–	1.2	–	–	–	–	–	–	–	0.6
Isaria farinosa (Holmskjold) Fries	KC768083	4.3	9.1	5.9	–	8.3	2.0	3.6	4.3	10.0	7.0	–	5.0	2.0	4.3	4.0
Mariannaea elegans (Corda) Samson	KC768084	4.3	–	2.9	5.4	–	4.1	3.6	4.3	–	2.3	3.4	–	2.0	2.2	2.9
Ogataea neopini Nagatsuka, S. Saito & Sugiyama	KC768085	–	18.2	5.9	–	16.7	4.1	4.8	–	25.0	11.6	3.4	20.0	10.2	10.9	8.0
Ophiostoma bicolor R.W. Davidson & D.E. Wells	KC768086	4.3	9.1	5.9	5.4	8.3	6.1	6.0	–	5.0	2.3	–	–	–	1.1	3.4
Ophiostoma canum (Münch) Sydow & P. Sydow	KC768087	4.3	–	2.9	2.7	8.3	4.1	3.6	4.3	5.0	4.7	–	10.0	4.1	4.3	4.0
Ophiostoma ips (Rumbold) Nannfeldt	KC768089	–	–	–	2.7	–	2.0	1.2	8.7	–	4.7	3.4	10.0	6.1	5.4	3.4
Ophiostoma piceae (Münch) Sydow & P. Sydow	KC768090	17.4	9.1	14.7	13.5	–	10.2	12.0	–	10.0	4.7	13.8	10.0	12.2	8.7	10.3
Ophiostoma rectangulosporium Ohtaka, Masuya & Yamaoka	KC768088	–	–	–	2.7	–	2.0	1.2	4.3	–	2.3	3.4	–	2.0	2.2	1.7
Penicillium sp. Link HK36_7	KC768091	–	–	–	–	16.7	4.1	2.4	–	–	–	–	10.0	4.1	2.2	2.3
Penicillium sp. HK80_14	KC768092	–	–	–	–	16.7	4.1	2.4	–	10.0	4.7	–	–	–	2.2	2.3
Penicillium sp. HK83_22	KC768093	–	18.2	5.9	–	–	–	2.4	–	10.0	4.7	–	–	–	2.2	2.3
Phoma macrostoma Montagne	KC768094	–	–	–	5.4	–	4.1	2.4	–	–	–	3.4	–	2.0	1.1	1.7
Pochonia bulbillosa (W. Gams & Malla) Zare & W. Gams	KC768095	–	–	–	–	–	–	–	4.3	–	2.3	–	–	–	1.1	0.6
Sordariomycetes sp. O.E. Eriksson & Winka	KC768100	–	–	–	2.7	–	2.0	1.2	–	–	–	–	–	–	–	0.6
Sydowia polyspora (Brefeld & Tavel) E. Müller	KC768096	17.4	9.1	14.7	5.4	–	4.1	8.4	4.3	–	2.3	–	–	–	1.1	4.6
Trichoderma asperellum Samuels, Lieckfeldt & Nirenberg	KC768097	–	–	–	–	–	–	–	–	–	–	3.4	–	2.0	1.1	0.6
Unidentified Helotiales HH79	KC768103	–	–	–	8.1	–	6.1	3.6	–	–	–	10.3	–	6.1	3.3	3.4
Unidentified Pezizales HG88	KC768106	–	–	–	2.7	–	2.0	1.2	–	–	–	3.4	–	2.0	1.1	1.1
All Ascomycota		91.3	100	94.1	94.6	100	95.9	95.2	78.3	95.0	86.0	79.3	100	87.8	87.0	90.9
Basidiomycota																
Bjerkandera adusta (Willdenow) P. Karsten	KC768070	–	–	–	–	–	–	–	4.3	5.0	4.7	–	–	–	2.2	1.1
Cryptococcus sp. Vuillemin	KC768076	–	–	–	2.7	–	2.0	1.2	–	–	–	6.9	–	4.1	2.2	1.7
Fomitopsis pinicola (Swartz) P. Karsten	KC768078	4.3	–	2.9	2.7	–	2.0	2.4	4.3	–	2.3	6.9	–	4.1	3.3	2.9
Hebeloma sp. (Fries) P. Kummer	KC768080	4.3	–	2.9	–	–	–	1.2	4.3	–	2.3	–	–	–	1.1	1.1
Heterobasidion annosum (Fries) Brefeld	KC768081	–	–	–	–	–	–	–	–	–	–	3.4	–	2.0	1.1	0.6
All Basidiomycota		8.7	–	5.9	5.4	–	4.1	4.8	13.0	5.0	9.3	17.2	–	10.2	9.8	7.4
Mucoromycotina																
Umbelopsis isabellina (Oudemans) W. Gams	KC768098	–	–	–	–	–	–	–	8.7	–	4.7	3.4	–	2.0	3.3	1.7
No. of taxa		14	7	17	21	8	27	32	19	10	26	18	8	22	36	40
Shannon diversity index		2.5	1.8	2.7	2.9	2.0	3.2	3.2	2.9	2.2	3.1	2.7	1.9	2.8	3.2	3.3

versity indices ranged between 1.8 and 3.3 (Table 1). Overall fungal community was composed of 90.9% Ascomycota, 7.4% Basidiomycota and 1.7% Mucoromycotina. The most commonly detected fungi were *Ophiostoma piceae* (10.3%), *Alternaria alternata* (9.7%), *Ogataea neopini* (8.0%), *Botryotinia fuckeliana* (5.1%), *Cladosporium* sp. (5.1%) and *Sydowia polyspora* (4.6%) (Table 1).

DISCUSSION

The results of the present study indicate that in the forest-steppe zone in eastern Ukraine there is a species-rich community of fungi associated with *H. ligniperda* (Table 1). From the methodological point of view, the use of fungal culturing and direct sequencing to a slight extent complemented each other as they both detected similar fungal

communities as indicated by Sorensen index of similarity, suggesting that both methods can be successfully used to study fungi associated with *H. ligniperda*. Furthermore, fungal communities detected at Kharkiv and Luhansk were also very similar, indicating low site specificity. This resemblance may probably be attributed to similar climatic, edaphic and forest stand conditions present at both sites e.g. high temperatures and low humidity during the growing season, nutrient poor sandy soils, similar age and composition of forest stands and low overall forest coverage. In contrast, there was notable within-tree habitat specificity since richness of *H. ligniperda*-associated fungi differed significantly for beetles collected on butts vs. stems (Table 1). The observed difference may probably be explained by differences in substrate quality, i.e. dead dry wood of fallen trees vs. moist living tissues of living trees. The latter should be taken into account when sampling *H. ligniperda* in similar studies in the future.

In the present study, 27 genera of fungi were detected among which the genus *Ophiostoma* was the most abundant, with five different taxa (Table 1). Despite this richness of ophiostomatoid fungi it is lower than that recorded in similar studies in which at least ten *Ophiostoma* spp. are reported but the composition of fungal taxa is largely the same (Zhou et al., 2001, 2004; Reay et al., 2006; Kim et al., 2011; Jankowiak & Bilański, 2013). Among the ophiostomatoid fungi detected, *O. piceae* is reported to be moderately or weakly pathogenic (Kirisits, 2004). However, Krokene & Solheim (1998) report that their inoculation experiments indicate that *O. piceae* is not pathogenic. Similarly, little- or no-pathogenic behaviour is reported for *O. bicolor*, *O. ips*, *O. canum* and *O. rectangulosporium* (Linnakoski et al., 2012) though *O. ips* causes lesions on *Pinus* spp. in South Africa (Zhou et al., 2002) and *O. bicolor* small dark brown areas in the wood of Norway spruce (Solheim, 1988). In addition, *O. bicolor* is rarely found in association with pine-infesting bark beetles (Linnakoski et al., 2012). Thus, in the forest-steppe zone in eastern Ukraine *H. ligniperda* is commonly associated with many taxa of ophiostomatoid fungi all of which appear to be little or non-pathogenic. Interestingly, pathogenic fungi belonging to genus *Leptographium* were not detected although commonly found associated with *H. ligniperda* in Canada and South Africa (Zhou et al., 2001; Dumouchel & Palisek, 2002). It is possible that in the regions of Ukraine studied the extreme temperatures (up to 50°C) throughout the growing season in 2010 and low levels of precipitation (Davydenko et al., 2013) negatively affected the abundance of *Leptographium* fungi. For example, the saprotrophic taxon *Hymenoscyphus albidus* (Roberge ex Desm.) W. Phillips, which was common and widespread in the region, currently appears to be rare (Davydenko et al., 2013). Instead, *H. ligniperda* is a vector of the plant pathogen *Botryotinia fuckeliana* (anamorph *Botrytis cinerea*), which cause grey mould disease in a wide range of plants (James et al., 1995) including seedlings of *Pinus* spp., *Picea* spp. and *Abies* spp. (Capieau, 2004). It is unknown whether *B. fuckeliana* uses *H. ligniperda* as a vector but it was recently reported for the first time associated with the European spruce bark beetle (*Ips typographus*) in Italy (Giordano et al., 2013) and lesser pine shoot beetle (*Tomicus minor* Hart.) in Poland (Jankowiak, 2008).

The fungi of the genera *Alternaria, Chaetomium, Cladosporium, Penicillium, Phoma, Ilyonectria* and *Trichoderma* are mainly known as generalist saprotrophs and/or facultative parasites (Domsch et al., 2007). *Isaria farinose* and *Beauveria bassiana* are reported to be pathogenic to insects and therefore potential biocontrol agents (Linnakoski, 2011). *Umbelopsis isabellina* (Oudemans) W. Gams (Mucoromycotina) is commonly associated with *Ips typographus* in Sweden (Persson et al., 2009), and *Tomicus piniperda* L. in Poland (Jankowiak, 2006; Jankowiak & Bilański, 2007) and Finland (Linnakoski, 2011). This study also revealed that *H. ligniperda* was more commonly associated with *Sydowia polyspora* than moulds and *Trichoderma* spp., which in the past were more often associated with different pine-infesting bark beetles (Zhou et al., 2001, 2004; Reay et al., 2006, Jankowiak & Bilański, 2007, Kim et al., 2011). Basidiomycetous taxa were also occasionally detected, including the three wood-decaying fungi: *Bjerkandera adusta*, *Fomitopsis pinicola* and *Heterobasidion annosum*, yeast *Cryptococcus* sp. and the mycorrhizal fungus *Hebeloma* sp. Occasional occurrence of basidiomycetous fungi associated with bark beetles is reported and as in the present study mainly included early wound and wood colonizers (Kirsits, 2004; Jankowiak, 2006; Persson et al., 2011).

In conclusion, in the forest-steppe zone in eastern Ukraine *H. ligniperda* vectors diverse communities of fungi, the majority of which are either a little or non-pathogenic to trees. The community of ophiostomatoid fungi associated with *H. ligniperda* on *P. sylvestris* in eastern Ukraine appeared to be dissimilar to that previously reported for Europe (Jankowiak & Bilański, 2013) and other continents (Zhou et al., 2001, 2004; Reay et al., 2006; Kim et al. 2011).

ACKNOWLEDGEMENTS. The financial support from the Swedish Institute and European Regional Development Fund according to Commission Regulation (EC) No. 1828/2006 article 8 part 4, project no. VP1-3.1-ŠMM-07-K-02-001 is acknowledged.

REFERENCES

ALTSCHUL S.F., MADDEN T.L., SCHAFFER A.A., ZHANG J., ZHANG Z., MILLER W. & LIPMAN D.J. 1997: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. — *Nucl. Acids Res.* **25**: 3389–3402.

BRASIER C.M. 1991: *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. — *Mycopathologia* **115**: 151–161.

CAPIEAU K. 2004: *Biological Control of Grey Mould in Swedish Forest Nurseries.* Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala, 37 pp.

DAVYDENKO K., VASAITIS R., STENLID J. & MENKIS A. 2013: Fungi in foliage and shoots of *Fraxinus excelsior* in eastern Ukraine: first report on *Hymenoscyphus pseudoalbidus*. — *For. Pathol.* **43**: 462–467.

DUMOUCHEL L. & PALISEK S. 2002: *Red-haired Pine Bark Beetle, Hylurgus ligniperda (F.). Pest Risk Assessment.* Plant Health

Risk Assessment Unit, Science Division, Canadian Food Inspection Agency, 39 pp.

Domsch K.H., Gams W. & Anderson T.H. 2007: *Compendium of Soil Fungi. 2nd ed.* IHW, Eching, 322 pp.

Gardes M. & Bruns T. 1993: ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. — *Mol. Ecol*. **2**: 113–118.

Giordano L., Garbelotto M., Nicolotti G. & Gonthier P. 2013: Characterization of fungal communities associated with the bark beetle *Ips typographus* varies depending on detection method, location, and beetle population levels. — *Mycol. Prog.* **12**: 127–140.

James R.L., Dumroese R.K. & Wenny D.L. 1995: *Botrytis cinerea* carried by adult fungus gnats (Diptera: Sciaridae) in container nurseries. — *Tree Planter's Notes* **46**: 48–53.

Jankowiak R. 2006: Fungi associated with *Tomicus piniperda* in Poland and assessing their virulence using Scots pine seedlings. — *Ann. For. Sci.* **63**: 801–808.

Jankowiak R. 2008: Fungi associated with *Tomicus minor* on *Pinus sylvetris* in Poland and their succession into the sapwood of beetle-infested windblown trees. — *Can. J. For. Res.* **38**: 2579–2588.

Jankowiak R. & Bilański P. 2007: Fungal flora associated with *Tomicus piniperda* L. in an area close to a timber yard in southern Poland. — *J. Appl. Entomol.* **131**: 579–584.

Jankowiak R. & Bilański P. 2013: Ophiostomatoid fungi associated with root-feeding bark beetles in Poland. — *For. Pathol.* **43**: 422–428.

Kim S. 2010: *Ophiostomatales Isolated from two European Bark Beetles,* Hylurgus ligniperda *and* Orthotomicus erosus*, in California.* Thesis, Iowa State University, Ames, 98 pp.

Kim S., Harrington T.C., Lee J.C. & Seybold S.J. 2011: *Leptographium tereforme* sp. nov. and other Ophiostomatales isolated from the rootfeeding bark beetle *Hylurgus ligniperda* in California. — *Mycologia* **103**: 152–163.

Kirisits T. 2004: Fungal associates of European bark beetles with the special emphasis on the ophiostomatoid fungi. In Leitutier F., Dau K., Battisti A., Gregoire J.-C. & Evans H. (eds): *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer, Dordrecht, pp. 181–235.

Krokene P. & Solheim H. 1998: Phytopathogenicity of four blue-stain fungi associated with aggressive and non-aggressive bark beetles. — *Phytopathology* **88**: 39–44.

Linnakoski R. 2011: *Bark Beetle-associated Fungi in Fennoscandia with Special Emphasis on Species of* Ophiostoma *and* Grosmannia*.* Doctoral Thesis, University of Eastern Finland, Joensuu, 74 pp.

Linnakoski R., De Beer Z.W., Niemelä P. & Wingfield M.J. 2012: Associations of conifer-infesting bark beetles and fungi in Fennoscandia. — *Insects* **3**: 200–227.

Magurran A.E. 1988: *Ecological Diversity and its Measurement.* Princeton University Press, Princeton, NJ, 192 pp.

Mead R. & Curnow R.N. 1983: *Statistical Methods in Agriculture and Experimental Biology.* Chapman & Hall, London, 335 pp.

Menkis A., Vasiliauskas R., Taylor A.F.S., Stenström E., Stenlid J. & Finlay R. 2006: Fungi in decayed roots of conifer seedlings from forest nurseries, afforested clearcuts and abandoned farmland. — *Plant Pathol.* **55**: 117–129.

Meshkova V. & Sokolova I. 2007: Spread of root bark beetles in pine plantations, created in the clear-cuts. — *Bull. Kharkiv Nat. Agr. Univ. (Phytopathol. & Entomol.)* **7**: 115–120 [in Ukrainian].

Persson Y., Vasaitis R., Langstrom B., Ohrn P., Ihrmark K. & Stenlid J. 2009: Fungi vectored by the bark beetle *Ips typographus* following hibernation under the bark of standing trees and in the forest litter. — *Microb. Ecol.* **58**: 651–659.

Persson Y., Ihrmark K. & Stenlid J. 2011: Do bark beetles facilitate the establishment of rot fungi in Norway spruce? — *Fungal Ecol*. **4**: 262–269.

Reay S.D. & Walsh P.J. 2001: Observations of the flight activity of *Hylastes ater* and *Hylurgus ligniperda* (Curculionidae: Scolytinae) in *Pinus radiata* forests in the central North Island, New Zealand. — *N. Z. Entomol.* **24**: 79–85.

Reay S.D. & Walsh P.J. 2002: Relative attractiveness of some volatiles to the introduced pine bark beetles, *Hylastes ater* and *Hylurgus ligniperda* (Curculionidae: Scolytinae). — *N. Z. Entomol*. **25**: 51–56.

Reay S.D., Thwaites J.M. & Farrel R.L. 2006: Survey of Ophiostomataceae associated with *Hylurgus ligniperda* (Curculionidae: Scolytinae) in New Zealand. — *N. Z. Entomol*. **29**: 21–26.

Seifert K.A., De Beer Z.W. & Wingfield M.J. 2013: *The Ophiostomatoid Fungi: Expanding Frontiers*. CBS Biodiversity Series 12. CBS-KNAW Biodiversity Centre, Utrecht, 337 pp.

Shannon C.E. 1948: A mathematical theory of communication. — *Bell Syst. Tech. J.* **27**: 379–423.

Solheim H. 1988: Pathogenicity of *Ips typographus*-associated blue-stain fungi to Norway spruce. — *Medd. Nor. Inst. Skogforsk*. **40**: 1–11.

Wingfield M., Seifert K. & Webber J. 1993: Ceratocystis *and* Ophiostoma: *Taxonomy, Ecology, and Pathogenicity*. APS Press, St. Paul, 293 pp.

White T.J., Bruns T.D., Lee S. & Taylor J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis M.A., Gelfand D.H., Sninsky J.J. & White T.J. (eds): *PCR Protocols: A Guide to Methods and Applications*. Academic Press, New York, pp. 315–322.

Zhou X.D., De Beer Z.W., Wingfield B.D. & Wingfield M.J. 2001: Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa. — *Sydowia* **53**: 290–300.

Zhou X.D., de Beer Z.W., Wingfield B.D. & Wingfield M.J. 2002: Infection sequence and pathogenicity of *Ophiostoma ips*, *Leptographium serpens* and *L. lundbergii* to pines in South Africa. — *Fungal Divers.* **10**: 229–240.

Zhou X.D., De Beer Z.W., Ahumada R., Wingfield B.D. & Wingfield M.J. 2004: *Ophiostoma* and *Ceratocystiopsis* spp. associated with two pine-infesting bark beetles in Chile. — *Fungal Divers*. **15**: 261–274.

Received January 23, 2014; revised and accepted June 19, 2014
Prepublished online August 19, 2014