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ORIGINAL PAPER

Cadophora margaritata sp. nov. and other fungi associated with the longhorn beetles *Anoplophora glabripennis* and *Saperda carcharias* in Finland

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Abstract Symbiosis with microbes is crucial for survival and development of wood-inhabiting longhorn beetles (Coleoptera: Cerambycidae). Thus, knowledge of the endemic fungal associates of insects would facilitate risk assessment in cases where a new invasive pest occupies the same ecological niche. However, the diversity of fungi associated with insects remains poorly understood. The aim of this study was to investigate fungi associated with the native large poplar longhorn beetle (*Saperda carcharias*) and the recently introduced Asian longhorn beetle (*Anoplophora glabripennis*) infesting hardwood trees in Finland. We studied the cultivable fungal associates obtained from *Populus tremula* colonised by *S. carcharias*, and *Betula pendula* and *Salix caprea* infested by *A. glabripennis*, and compared these to the samples collected from intact wood material. This study detected a number of plant pathogenic and saprotrophic fungi, and species with known potential for enzymatic degradation of wood components. Phylogenetic analyses of the most commonly encountered fungi isolated from the longhorn beetles revealed an association with fungi residing in the *Cadophora–Mollisia* species complex. A commonly encountered fungus was *Cadophora spadicis*, a recently described fungus associated with wood-decay. In addition, a novel species of *Cadophora*, for which the name *Cadophora margaritata* sp. nov. is provided, was isolated from the colonised wood.

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Introduction

A nutrient-poor wood tissue (xylem), lacking readily available carbohydrates and proteins, is a challenging niche to be exploited by other organisms. However, cerambycid beetles (Coleoptera: Cerambycidae) are adapted to complete their long lifecycle in wood tissues. In this niche, symbiosis with microbes (including filamentous fungi and yeasts) has been considered to be crucial for their survival and development (Kukor et al. 1988; Schloss et al. 2006; Watanabe and Tokuda 2010; Scully et al. 2013). The role of the associated microbes can be considered as directly nutritional, in which insect larvae cultivate and feed on the fungi (Francke-Grosmann 1967), or when either ingested fungal enzymes (Kukor and Martin 1986; Kukor et al. 1988) or endosymbiotic gut microbes contribute to cellulose degradation (Uvarov 1929; Kukor et al. 1988; Grünwald et al. 2010; Watanabe and Tokuda 2010; Scully et al. 2013). These symbiotic fungi may also have other functional roles, such as nitrogen and vitamin acquisition (Gibson and Hunter 2009; Ayayee et al. 2014).

The association of Cerambycidae and fungi was recognised relatively long ago (Uvarov 1929; Jurzitza 1959; Riba 1977; Chararas and Pignal 1981; Nardon and Grenier 1989). In general, the modified colour and texture of the wood close to the larval tunnels indicate that symbiotic organisms are commonly involved in the diet of xylophagous insects. Many of the wood-inhabiting fungi and insect symbioses have long co-evolutionary histories (Vega and Blackwell 2005), recently supported by the observations of fungi and associated organisms successfully passing through the insect alimentary tract (Drenkhan et al. 2013, 2016). The associations with Cerambycidae have arisen independently in distantly related fungal genera, representing examples of convergent evolution (Jones et al. 1999). Cerambycidae are known to harbour a rich assemblage of microbes in terms of taxonomic and functional diversity (Grünwald et al. 2010; Watanabe and Tokuda 2010; Scully et al. 2013; Ayayee et al. 2014). However, the diversity of fungi associated with most Cerambycidae remains to be investigated.

An example of a common but poorly known Cerambycidae is the large poplar longhorn (*Saperda carcharias*) (Fig. 1). The biology of the insect is not fully understood, and there are no previous reports of its symbiotic fungi. The beetle is considered as the most harmful pest of hybrid poplars and aspen (*Populus tremula*) in Finland (Hallaksela 1999; Välimäki and Heliövaara 2007). The adult beetle feeds on aspen leaves and lays eggs in basal parts of the stems during August–September (Heliövaara et al. 2004). The eggs undergo overwintering for 10–11 months and the emerging larvae remain in the tunnels for 2–4 years. During this period, larvae can gnaw tunnels up to 1 m long in the xylem. These tunnels are often surrounded by a characteristic dark brown colour expanding vertically 1–3 m, which prevent the logs from being used in the mechanical wood industry. Part of the wood material gnawed by the larvae is transported through their alimentary canals. Due to long larval period of development in the tunnels, it is likely that the poplar longhorn has interactions with a number of fungi during its life cycle.

The Asian longhorn beetle (*Anoplophora glabripennis*) is a serious forest pest native to China (Lingafelter and Hoebeke 2002) (Fig. 2). In its native range, the beetle is not considered as a pest in natural forests. However, the beetle became a common pest in China as a result of widespread planting of susceptible poplar (*Populus* sp.) hybrids (EPPO 2016). As an invasive species, *A. glabripennis* is a highly destructive quarantine pest (EPPO 2016). The beetle is capable of infesting a wide range of deciduous trees, including also healthy trees. The Asian longhorned beetle has spread globally mainly in ineffectively treated wooden packaging materials originating from China. It was accidentally introduced first into North America (Haack et al. 1996; Hu et al. 2009), followed by several introductions into European countries during the past two decades (Tomiczek et al. 2002; Hérard et al. 2006, 2009; Haack et al. 2010), and recently into Finland.

In several instances, baseline information on fungal associates of insects in their endemic environment is lacking. This hinders risk assessment measures in cases where a novel forest pest and/or pathogen is introduced. Novel insect–fungal interactions represent

Fig. 1 **a** Aspen (*P. tremula*) stand in Urjala, Finland, **b**, **c** the large poplar longhorn beetle (*S. carcharias*) adult and larvae, and **d** brown decay surrounding the beetle gallery on *P. tremula*

an increased risk to forest health (Humble and Allen 2006; Hulcr and Dunn 2011; Wingfield et al. 2016; Stenlid and Oliva 2016). Examples of devastating tree disease problems that have arisen from these associations are increasing in number (Lu et al. 2011; Akbulut and Stamps 2012; Ploetz et al. 2013; Santini and Faccoli 2014; Wingfield et al. 2016). Recent introductions of plant pathogens (Wingfield et al. 2016) suggest that latent microbial pathogens commonly remain undetected in quarantine inspections.

The aim of the study was to provide baseline information on fungal diversity associated with *S. carcharias* in its native host tree and environment, and with the introduced *A. glabripennis* infestation in Finland. Our hypotheses were that the longhorn beetles are associated with specific fungi, which may improve nutritional quality of the larvae facilitating larval development inside the wood. Furthermore, we hypothesised that differences in fungal species compositions between intact wood and wood colonised by beetles would reflect the specific symbiotic fungi associated with each beetle. In addition, we hypothesise that the introduced insect pest would establish interactions with native fungal associates of

Fig. 2 The Asian longhorned beetle (*A. glabripennis*) adult and larvae, and brown decay surrounding the beetle gallery on *B. pendula*

ecologically and taxonomically closely related insect species.

Materials and methods

Study areas and collection of samples

Populus tremula trees damaged by *S. carcharias* were collected from a 15-year-old aspen stand in Urjala, southwestern Finland (66°00′N, 23°29′E) in May 2013 (Fig. 1). The aspen clonal stand 2120/01 was established on 25 May 1998 as a part of the aspen clonal research programme of the Finnish Forest Research Institute. The average diameter at breast height of trees was 12 cm and their average height was ca. 9.0 m. The stand consisted of both aspen and hybrid aspen, which were planted at intervals of 3 m in 3.0 × 3.0 m blocks. All the five sampled trees were of the same clone, collected from different blocks. The logs including the entire beetle tunnels were cut with a chain saw and immediately closed in plastic bags and stored at + 4 °C.

The logs (60–90 cm of length) were sawn with table mounted circular saw longitudinally into two halves in a laboratory for fungal isolations. Two samples 5 mm × 10 mm (separated by 5 cm) from three points (separated by at least 15 cm) were cut from the brown-coloured wood adjacent to larval tunnels with surface disinfected carpenter's knife and surgical knife. Control isolations were made from the non-coloured, undamaged distal ends of the logs as described above. All samples were surface disinfected in sterile conditions with 70% ethanol (10 s) and 10% sodium hypochlorite (NaClO) (5 s) and finally washed four times with fresh sterile water. The samples were placed onto both water agar (WA: 2% AMRESCO® agar, bacteriological grade) and malt extract agar (MEA: 2% Bacto™ malt extract agar and 1% AMRESCO® agar, bacteriological grade) in 90 mm Petri dishes. A subset of unsterilised wood samples was incubated at 25 °C on sterilised filter paper placed in clean Petri dishes (90 mm).

Anoplophora glabripennis-infested trees were detected in Vantaa, Finland (60.303′N, 25.119′E) in October–November 2015. During the inspections and eradication measures, a total of 20 *Betula pendula* and 13 *Salix caprea* trees showing signs of *A. glabripennis* infestation were detected (Fig. 2). The trees were removed by the Finnish Food Safety Authority (Evira), and transported to a quarantine facility for careful examination and destruction. At the same time, fungal isolations were made from decayed wood surrounding the galleries of *A. glabripennis* on five *B. pendula* and two *S. caprea* trees, using a similar sampling scheme as described earlier. Isolations were also made from the surfaces of five living larvae found in galleries on *S. caprea*. Small pieces of wood and the

larvae were placed onto the surface of WA and MEA. The larvae were removed from the plates after approximately 15 min. Control isolations were made from the non-coloured, undamaged distal ends of the logs.

The cultures obtained from *S. carcharias* and *A. glabripennis* were grown for 1 week in the dark at + 25 °C and inspected daily. Emerging mycelia was sub-cultured to obtain pure cultures. Purified fungal isolates were grown on MEA and grouped based on their colony and morphological characteristics. Representative isolates from each group were chosen for identification based on DNA sequences. Isolates were deposited in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, and the ex-type isolates also in the CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (Table 1). Herbarium specimens were deposited in the Herbarium of the University of Turku (TUR), Finland.

DNA extraction, PCR and sequencing

Pure cultures for DNA extractions were cultivated on MEA plates. DNA was extracted from at least one isolate of each morphological group using the methods described previously (Terhonen et al. 2011; Linnakoski et al. 2016). Gene regions sequenced in the study were the internal transcribed spacer (ITS) regions (ITS1 and ITS2) including the 5.8S gene (all samples), as well as the partial beta-tubulin gene.

The DNA samples from mycelial cultures obtained from *S. carcharias* were amplified using a PCR protocol for DyNAzyme DNA® polymerase (Finnzymes, Espoo, Finland). A PCR reaction (total volume 50 µl) contained 30.5 µl of sterile water, 5 µl of DyNAzyme 10 × buffer, 1 µl of 10 mM dNTP's, 1 µl of 25 mM ITS1-F primer (Gardes and Bruns 1993), 1 µl of 25 mM ITS4 primer (White et al. 1990), 1 µl of 50 mM $MgCl_2$, 0.5 µl of DyNAzyme DNA® polymerase and 10 µl/l of DNA template. The partial beta-tubulin gene region was amplified using the primer pair T10 (O'Donnel and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995). The PCR reactions were run with BIOER XP Cycler (BIOER Technology, Hangzhou, China). The PCR reaction was started with 4 min of initial denaturation followed by 30 cycles of 45 s of denaturation (95 °C), 45 s of annealing (52 °C), and 1 min extension (72 °C). A final extension step was 10 min (72 °C). Products were visualised by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and purified using GenElute™ PCR clean-up kit according to the manufacturer's instructions (Sigma-Aldrich, Missouri, USA).

Amplification of the ITS region and purification of the PCR products of fungal samples from *A. glabripennis* were performed following the same protocols described previously (Linnakoski et al. 2016). The studied gene region was amplified using the primer pair ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The reaction mixture contained 0.15 µl of MyTaq™ DNA Polymerase (5 U/µl) (Bioline, Massachusetts, USA), 5 µl of MyTaq™ Reaction Buffer (5 ×) containing dNTPs, $MgCl_2$ and enhancers, 0.5 µl of each primer (10 mM stock concentration) (Whitehead Scientific Ltd, Cape Town, South Africa), and 1 µl fungal genomic DNA. Sterile Sabax water was added to adjust the reaction volume to 25 µl. PCR amplifications were performed using the following conditions: denaturation at 95 °C for 2 min, followed by 32 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C, and a final extension at 72 °C for 7 min. An aliquot of 5 µl of each PCR product was stained with GelRed™ nucleic acid gel stain (Biotium, Hayward, CA, USA), run on 2% agarose gel at 60 V along with a 100 bp molecular marker (Fermentas O′ Gene Ruler™) and visualised with a Gel Doc EZ Imager (Bio-Rad Laboratories Inc.). Amplified PCR products were cleaned using the Exonuclease I—Shrimp Alkaline Phosphatase Clean Up (Exo-SAP) protocol.

The sequencing reactions contained 0.5 µl of BigDye® Terminator v3.1 Ready Reaction mixture (Perkin-Elmer Applied Biosystems, Warrington, UK), 2.1 µl of sequencing buffer, 1 µl of either the forward or reverse primer (10 mM stock concentration) and 1 µl of purified PCR product. Sterile Sabax water was added to adjust the reaction volume to 12 µl. The thermal cycling conditions were: 25 cycles of 10 s at 96 °C, 5 s at 55 °C and 4 min at 60 °C. Sequencing products were then cleaned using ethanol/salt precipitation. Sequencing of the isolates from *S. carcharias* was conducted on the Applied Biosystems (ABI) with an ABI 3130xl sequencer (Thermo Fisher Scientific, Casrbad, USA) at the Haartman Institute Sequencing Unit, University of Helsinki. Sequencing of the isolates from *A. glabripennis* was conducted with an

Table 1 Fungal isolates obtained from longhorn beetles *A. glabripennis* and *S. carcharias* colonized wood and intact wood in Finland

Species	Culture collection no.[a]	Herbarium no.b	Beetle	Host tree	Substrate	Date of isolation	GenBank acc. no.		
							LSU	ITS	β-Tubulin
Alternaria sp.	N/A		*Saperda carcharias*	*Populus tremula*	Colonized wood	01 Aug 2013		KJ702019	
Arthrinium sp.	N/A		*S. carcharias*	*P. tremula*	Colonized wood	20 Sept 2013		KJ702039	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	20 Sept 2013		KJ702044	
Ascocoryne cylichnium	N/A		*S. carcharias*	*P. tremula*	Colonized wood	13 Aug 2013		KJ702030	
Cadophora spadicis	CMW49956		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188968	
	CMW49957		*A. glabripennis*	*B. pendula*	Colonized wood	09 Dec 2015		MF188969	
	CMW49958		*A. glabripennis*	*B. pendula*	Colonized wood	09 Dec 2015		MF188970	
	CMW49959		*A. glabripennis*	*B. pendula*	Colonized wood	09 Dec 2015		MF188971	
	CMW49960		*A. glabripennis*	*Salix caprea*	Larvae	11 Dec 2015		MF188972	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	13 Sept 2013		KJ702035	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	13 Sept 2013		KJ702037	
***C. margaritata* sp. nov.**	CMW51780, CBS144083[c]	TUR207199[e]	*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013	MH267288	KJ702027	MH327786
	CMW51781, CBS144084[d]	TUR207200[d]	*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		MH203866	
Cladosporium sp.	CMW49961		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188973	
	CMW49962		*A. glabripennis*	*S. caprea*	Larvae	11 Dec 2015		MF188974	MH327787
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		KJ702022	
Coniochaeta sp.	N/A		*Anoplophora glabripennis*	*Betula pendula*	Colonized wood	23 Nov 2015		MH203865	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	13 Aug 2013		KJ702032	
Coniothyrium carteri	49963		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188975	
Cosmospora sp.	N/A		*S. carcharias*	*P. tremula*	Colonized wood	02 Aug 2013		KJ702026	
Epicoccum sp.	CMW49964		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188976	MH327788
	CMW49965		*A. glabripennis*	*S. caprea*	Larvae	11 Dec 2015		MF188977	MH327789
	N/A		*S. carcharias*	*P. tremula*	Intact wood	18 Nov 2013		KJ702040	
Fusarium sp.	CMW49966		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188978	
	CMW49967		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188979	MH327790
	CMW49968		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188980	
	CMW49969		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188981	

Table 1 continued

Species	Culture collection no.[a]	Herbarium no.b	Beetle	Host tree	Substrate	Date of isolation	GenBank acc. no.		
							LSU	ITS	β-Tubulin
	CMW49970		*A. glabripennis*	*B. pendula*	Colonized wood	09 Dec 2015		MF188982	MH327791
	CMW49971		*A. glabripennis*	*B. pendula*	Colonized wood	09 Dec 2015		MF188983	
	CMW49972		*A. glabripennis*	*S. caprea*	Colonized wood	11 Dec 2015		MF188984	MH327792
	N/A		*A. glabripennis*	*S. caprea*	Intact wood	11 Dec 2015		MH203867	
	N/A		*A. glabripennis*	*S. caprea*	Colonized wood	11 Dec 2015		MF188985	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	02 Aug 2013		KJ702020	
	N/A		*S. carcharias*	*P. tremula*	Intact wood	18 Nov 2013		KJ702041	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	18 Nov 2013		KJ702045	
Mollisia dextrinospora	CMW49973		*A. glabripennis*	*B. pendula*	Colonized wood	23 Nov 2015		MF188986	
Mortierella gamsii	N/A		*S. carcharias*	*P. tremula*	Colonized wood	02 Aug 2013		KJ702018	
M. cf. *hyalina*	N/A		*S. carcharias*	*P. tremula*	Intact wood	18 Nov 2013		KJ702042	
Mucor sp.	N/A		*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		KJ702017	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		KJ702024	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		KJ702025	
Phialocephala lagerbergii	N/A		*S. carcharias*	*P. tremula*	Colonized wood	13 Sept 2013		KJ702036	
Phlebia radiata	CMW49974		*A. glabripennis*	*S. caprea*	Colonized wood	11 Dec 2015		MF188987	
Pholiota sp.	N/A		*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		KJ702028	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	13 Aug 2013		KJ702029	
Phoma sp.	N/A		*S. carcharias*	*P. tremula*	Colonized wood	20 Sept 2013		KJ702031	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	20 Sept 2013		KJ702034	
Physalospora scirpi	N/A		*S. carcharias*	*P. tremula*	Colonized wood	13 Aug 2013		KJ702033	
Pseudeurotium bakeri	N/A		*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		KJ702021	
	N/A		*S. carcharias*	*P. tremula*	Colonized wood	01 Aug 2013		KJ702023	
Sarocladium strictum	N/A		*S. carcharias*	*P. tremula*	Intact wood	18 Nov 2013		KJ702043	

Table 1 continued

Species	Culture collection no.[a]	Herbarium no.b	Beetle	Host tree	Substrate	Date of isolation	GenBank acc. no.		
							LSU	ITS	β-Tubulin
Tolypocladium sp.	N/A		*A. glabripemnis*	*S. caprea*	Colonized wood	11 Dec 2015		MH203868	
Trichoderma sp.	N/A		*S. carcharias*	*P. tremula*	Colonized wood	20 Sept 2013		KJ702038	

Bold *Cadophora–Mollisia* species complex

[a]CMW, Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CBS, the CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands

[b]TUR Herbarium, Centre for Biodiversity, University of Turku, Finland

[c]Ex-type

[d]Ex-paratype

[e]Holotype

ABI 3500xl sequencer (Thermo Fisher Scientific, Carlsbad, USA) at the DNA Sequencing Facility of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

Sequence analyses and fungal identification

Consensus sequences were assembled with the Geneious R6.0.6 (Biomatters Ltd, Auckland, New Zealand), after which initial identification of the isolates was obtained using a BLAST search in GenBank nucleotide database (http://www.ncbi.nlm.nih.gov) applying a megablast algorithm and (a) excluding uncultured/environmental sample sequences and (b) limiting the search to sequences from type material (Supplementary Table 1). Identification to the closest genus and species level was made as far as possible, with caution given to the fact that the ITS sequence variability in certain fungal groups is low, and acknowledging possibly misidentified sequences in GenBank. We considered reliable identification to consist of the BLAST matches that had $\geq$ 98% sequence similarity to ex-type sequences or peer-reviewed published studies. For species in the *Cadophora–Mollisia* complex, BLAST searches were also used to retrieve similar sequences, as well as from type material to compile data sets for phylogenetic analysis. Data sets were edited with MEGA v.7 (Kumar et al. 2016). GenBank accession numbers of sequences are presented in the corresponding phylogenetic trees (Figs. 3, 4).

Cadophora finlandica and *Meliniomyces variabilis* were used as outgroups for the ITS data set, and *Cadophora luteo-olivacea* and *Cadophora malorum* for the beta-tubulin. The data sets were aligned using the online version of MAFFT v. 7 (Katoh and Standley 2013). Three different phylogenetic methods were applied: maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI). ML was performed in the online version of PhyML 3.0 (Guindon et al. 2010), using automatic model selection by SMS (Lefort et al. 2017) and Akaike information criterion (AIC) (Sugiura 1978). Confidence levels were estimated with 1000 bootstrap replicates. MP analyses were conducted using PAUP v. 4.0b10 (Swofford 2002). Gaps and missing data were excluded in the MP analyses. For BI analyses, the best-fitting evolutionary model using MrModeltest 2.3 (Nylander 2004) based on the Akaike Information

Fig. 3 Phylogenetic tree of species in the *Cadophora–Mollisia* species complex obtained from ML analyses of the ITS data set. Sequences obtained in this study are printed in bold type. Bootstrap support values above 70% for ML/MP are presented at the nodes. Posterior probabilities (above 70%) obtained from BI are indicated by bold lines at the relevant branching points. *bootstrap values < 70%. T, ex-type isolates. Scale bar = total nucleotide difference between taxa

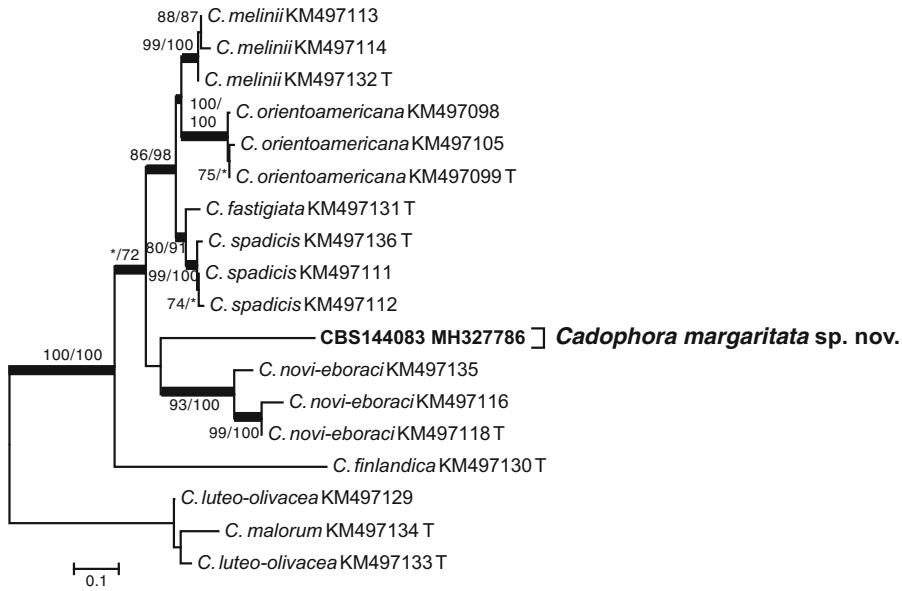

Fig. 4 Phylogenetic tree of species in the *Cadophora–Mollisia* species complex obtained from ML analyses of the beta-tubulin data set. Sequences obtained in this study are printed in bold type. Bootstrap support values above 70% for ML/MP are presented at the nodes. Posterior probabilities (above 70%) obtained from BI are indicated by bold lines at the relevant branching points. *bootstrap values < 70%. T, ex-type isolates. Scale bar = total nucleotide difference between taxa

Criterion (AIC) were determined. BI analyses based on a Markov Chain Monte Carlo (MCMC) simulation were carried out with MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003). The MCMC chains were run for five million generations using a sample frequency of 100 (resulting in 50,000 trees). Burn-in values were determined for the respective data sets, and all sampled trees having lower than the burn-in values were discarded. The resulting majority rule consensus trees were viewed with MEGA v.7 (Kumar et al. 2016), and post-processed with Adobe Illustrator CC 2018 (Adobe, San Jose, USA).

Results

Isolation and identification of fungi

Fungi were isolated from all of the collected samples associated with *S. carcharias* and *A. glabripennis* infestations in Finland. The study resulted in a total of 114 fungal isolates. The trimmed consensus sequences for the ITS gene regions ranged between 493 and 574 bp. The sequences obtained in this study were deposited in GenBank and their accession numbers are presented in Table 1.

The fungal species richness was higher in the colonised parts than in the intact parts of the wood (Table 2). The most common fungi isolated from both *A. glabripennis* and *S. carcharias*-colonised wood were members of the *Cadophora–Mollisia* species complex. These fungi were not found in the intact parts of the wood. Only a small number of fungi were detected in both the colonised and intact wood samples, including *Epicoccum* sp. and *Fusarium* sp. *Fusarium* sp. was the most commonly isolated species in the intact part of *P. tremula* sample logs. Some species were detected only from the intact wood including *Mortierella hyalina* and *Sarocladium strictum*.

In total, 83 isolates were obtained from *S. carcharias* in Finland. These represented at least 18 species of Ascomycota and one species of Basidiomycota (Table 1). The most common species isolated from *S. carcharias* colonised wood was *Cadophora spadicis*, a putatively novel *Cadophora* species and *Pseudeurotium bakeri*. The other fungal species were isolated only occasionally.

Table 2 Number of fungal isolates obtained from *A. glabripennis* and *S. carcharias* in Finland

Species	*Anoplophora glabripennis*		*Saperda carcharias*		Total
	Colonized wood	Intact wood	Colonized wood	Intact wood	
Alternaria sp.	0	0	4	0	4
Arthrinium sp.	0	0	2	2	4
Ascocoryne cylichnium	0	0	2	0	2
Cadophora spadicis	7	0	7	0	14
Cadophora margaritata sp. nov.	0	0	10	0	10
Cladosporium sp.	3	0	4	0	7
Coniochaeta sp.	1	0	7	0	8
Coniothyrium carteri	1	0	0	0	1
Cosmospora sp.	0	0	7	0	7
Epicoccum sp.	2	0	1	1	4
Fusarium sp.	12	1	1	4	18
Mollisia dextrinospora	1	0	0	0	1
Mortierella gamsii	0	0	1	0	1
Mortierella cf. *hyalina*	0	0	0	2	2
Mucor sp.	0	0	6	0	6
Phialocephala lagerbergii	0	0	2	0	2
Phlebia radiata	1	0	0	0	1
Pholiota sp.	0	0	5	0	5
Phoma sp.	0	0	2	0	2
Physalospora scirpi	0	0	1	0	1
Pseudeurotium bakeri	0	0	10	0	10
Sarocladium strictum	0	0	0	2	2
Tolypocladium sp.	0	1	0	0	1
Trichoderma sp.	0	0	1	0	1
Total	28	2	73	11	114

Isolates from *A. glabripennis* in Finland represented at least seven species of Ascomycota and one species of Basidiomycota (Table 1). In addition to the *Cadophora–Mollisia* isolates, other commonly detected fungi from *A. glabripennis*-infested wood and larvae included a *Fusarium* sp. closely related to an extensively studied gut symbiont isolate previously derived from an *A. glabripennis* larvae (Geib et al. 2012; Herr et al. 2016). The other fungi were isolated only occasionally.

DNA sequence analysis

The identities of isolates representing the most commonly encountered genera, *Cadophora–Mollisia*, were further confirmed by phylogenetic analysis. Data originating from the previous studies by Harrington and McNew (2003) and Travadon et al. (2015) served as a backbone for the phylogenetic tree construction for the *Cadophora* and allied species.

The aligned ITS data set included 44 taxa and 575 characters (with gaps). The best-fitting substitution model estimated for the *Cadophora* ITS data set was GTR + I + G. The ML, MP and BI analyses produced trees with similar topologies (Fig. 3).

The ITS data were sufficient to distinguish between most species included in the data set. However, it did not distinguish between *Cadophora melinii* and *Cadophora fastigiata*, and species in the *Phialocephala lagerbergii* clade. Phylogenetic analyses for *Cadophora–Mollisia* confirmed the identities of four species isolated in this study. These were *C. spadicis*, *P. lagerbergii*, *Mollisia dextrinospora*, and a putatively novel species (Fig. 3). The identity of the unknown species was further confirmed by

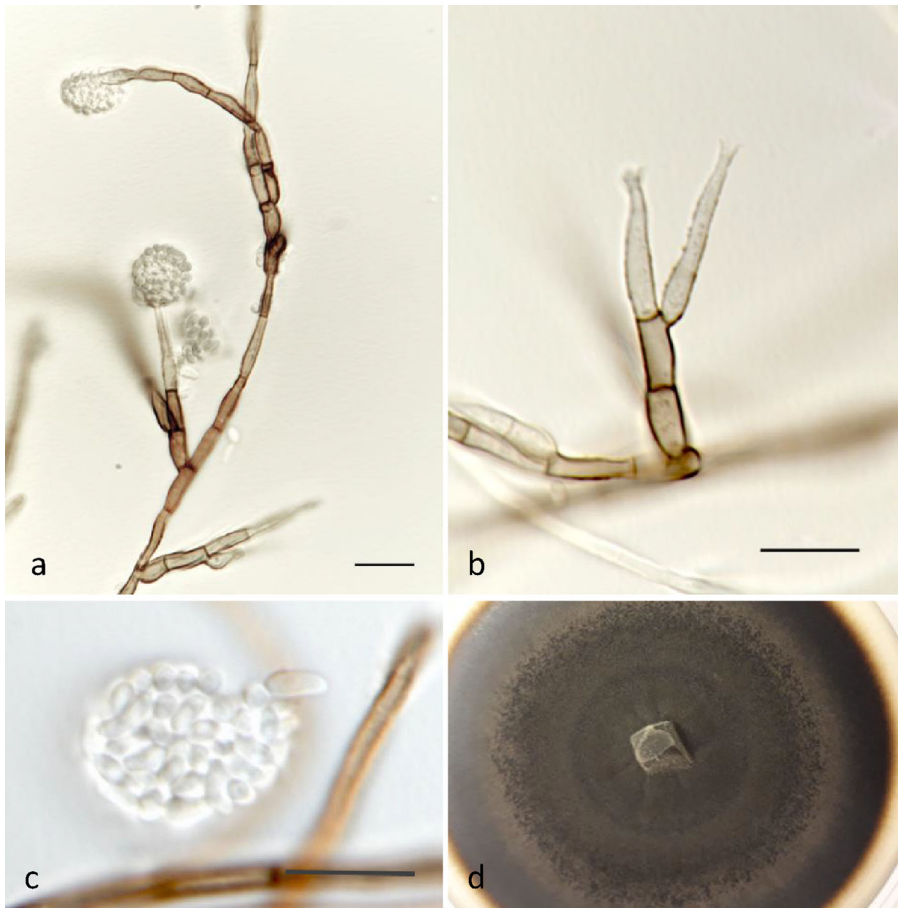

Fig. 5 Morphological characteristics of the *C. margaritata* (CMW 51780 = CBS 144083). **a** Conidiophores, **b** conidiogenous cells, **c** conidia, **d** culture on MEA. Scale bars **a**–**c** 10 μm

phylogenetic analysis of sequences for the partial beta-tubulin gene region.

The aligned beta-tubulin data set for the *Cadophora* included 18 taxa and 523 characters (with gaps). The best-fitting substitution model estimated for the data set was GTR + I + G. The novel species was clearly distinct from the other currently known species of *Cadophora* (Fig. 4). The phylogenetic analysis also supported the recent phylogenetic placement of *Cadophora* in four separate clades (Travadon et al. 2015).

Taxonomy

Based on the phylogenetic analyses for ITS region and partial beta-tubulin gene, as well as the morphological characteristics and ecology, the isolates of one of the *Cadophora* sp. isolated formed a distinct lineage that represents a novel taxon. This is described as follows with measurements presented as (min–)(mean − SD)–(mean + SD)(–max)'.

Cadophora margaritata R. Linnakoski, I. Lasarov & A.O. Oghenekaro **sp. nov.** (Fig. 5). MycoBank: MB 825227.

Etymology: The name is derived from the Latin word 'margaritata" for 'beaded" or "pearls" and refers to beaded appearance of conidial mass.

Sexual state: Not observed.

Asexual state: Aerial mycelium on MEA consisted of pale brown, tuberculate, septate hyphae up to (1.2–)1.5–2.3(–3.2) μm diam. Conidiophores arising from aerial hyphae mostly branched (2–12 branches), pale brown, septate, (93–)114.8–243(–295) μm long excluding conidia, (1.4–)2.1–3(–3.5) μm wide

(Fig. 5a, b). Conidiogenous cells terminal or lateral, mono- or poly-phialidic, hyaline (or paler brown), (8.9–)12.3–22(–29.2) µm long, slightly swollen at base, (1.6–)1.9–2.9(–3.6) µm at the widest part, tapering significantly towards collarette; collarettes cup-shaped, (1.3–)2–3(–3.7) µm wide and (0.8–)1–1.9(–2.4) µm long (Fig. 5b). Conidia aseptate, hyaline, ovoid, truncate at base (1.9–)2.5–3.7(–4.4) long × (1.3–)1.4–2.2(–3.2) µm wide, aggregating into globose ('beaded appearance') of conidial mass. *Culture characteristics*: Colonies on 2% MEA olivaceous black (Fig. 5d), mycelium superficial on agar, aerial mycelium sparse. Culture radial growth rate 3.1 mm/d (± 0.3) at 22 °C.

Specimens examined: FINLAND, Western Finland Province: Urjala, isolated from *Saperda carcharias* infested *Populus tremula*, collector Tiia Marttinen, 01 August 2013. Holotype dried specimen TUR 207199 (http://mus.utu.fi/TFU.207199), ex-holotype living culture CMW 51780 = CBS 144083; Paratype dried specimen TUR 207200 (http://mus.utu.fi/TFU.207200), ex-paratype living culture CMW 51781 = CBS 144084.
Host tree: *Populus tremula.*
Insect vector: *Saperda carcharias.*

Discussion

This study contributes to limited investigations on mycobiota associated with cerambycid beetles, and is the first to report fungi associated with the large poplar longhorn (*S. carcharias*). We isolated a number of plant pathogens, saprobes and species with known potential to cause wood degradation in the galleries of the *S. carcharias* on *P. tremula*. In total 21 fungal species residing in two phyla were identified, the most common detected in the beetle-colonised wood were species of *Cadophora* and allied species. This study also identified fungi associated with material collected from *A. glabripennis* material retained in quarantine in Finland. At least eight fungal species were detected, the most common of these were species of *Cadophora* and *Fusarium*. While *A. glabripennis* is known to harbour endosymbiotic fungi (Scully et al. 2013, 2014), these results are amongst the first to provide evidence of wood-inhabiting fungi associated with *A. glabripennis* in its introduced range. It was also noteworthy that the introduced pest shares a number of fungal associates with the native species (Table 2).

One of the intriguing findings in this study was the strong presence of a number of *Cadophora* and allied species existing in association with the longhorn beetles. This study is the first to reveal an association of these fungi with insects. Four *Cadophora* and allied species were identified, including the novel species *C. margaritata*. *Cadophora* species were originally described as fungi causing blue stain of lumber (Lagerberg et al. 1927). These fungi are now known to reside in two distinct genera *Phialophora* (Chaetothyriales, Eurotiomycetes) and *Cadophora* (Helotiales, Leotiomycetes) (Gams 2000; Harrington and McNew 2003). *Cadophora* has received little attention in the past but recent reports implicating species as potential grapevine pathogens in various countries have changed this view (Halleen et al. 2007; Casieri et al. 2009; Gramaje et al. 2011; Navarrete et al. 2011; Úrbez-Torres et al. 2014). *Cadophora spadicis* detected in the present study is one of the recently described species reported from wood-decay of grapevine in North America (Travadon et al. 2015). A previous study has also reported that *Cadophora* species are able to cause dark staining and extensive soft rot in *Betula* and *Populus* wood in vitro (Blanchette et al. 2004). Based on past findings, it is reasonable to assume that *Cadophora* species reported here play some role in wood degradation close to the larval tunnels of Cerambycidae in general.

The novel species described in this study, is characterised by production of phialides with hyaline collarettes, morphological structures typical for *Cadophora* and allied phialide-producing fungi. An example is *M. dextrinospora*, which has been reported to have a cadophora-like asexual stage (Greenleaf and Korf 1980). As shown in the present study and also in previous phylogenetic analyses (Crous et al. 2003; Harrington and McNew 2003; Day et al. 2012; Pärtel 2016), several species of *Cadophora* and *Mollisia* are closely related. Correct identification of these species is hindered by the occurrence of numerous misidentified entries in nucleotide sequence databases (Pärtel 2016). Consequently, *Mollisia* appears to be a heterogeneous assemblage of fungi that requires taxonomic treatment. The *Mollisia* isolate found in this study grouped with the ex-type strain of *M. dextrinospora* and its identity seems clear. The species was originally described from decayed *Acacia* wood in Madeira

(Greenleaf and Korf 1980), and it could have a similar role in wood decay in its association with the longhorn beetles. The identity of the fourth species belonging in the *Cadophora–Mollisia* species complex found in this study, a fungus closely related to *Phialocephala lagerbergii* could not be resolved due to lack of available type sequence data.

Fusarium is a highly diverse cosmopolitan genus that includes plant and human pathogens, saprobes and endophytes. Therefore, it is not surprising that these fungi have also been found as symbionts of cerambycid and ambrosia beetles (Geib et al. 2012; Morales-Ramos et al. 2000; Kasson et al. 2013). The presence of these fungi with *A. glabripennis* in the present study is perhaps not surprising but their consistent occurrence suggests that they may be relevant to *A. glabripennis*. This is supported by the fact that they have previously been found consistently associated with the pest in other areas and in different host trees (Geib et al. 2012). In this association, species of *Fusarium* have been considered as internal gut symbionts supporting cellulose degradation and larval fitness of *A. glabripennis* (Scully et al. 2013, 2014). Species in this group are also symbionts of ambrosia beetles (Morales-Ramos et al. 2000; Kasson et al. 2013). However, in this study *Fusarium* spp. were also detected as the most common isolates in healthy parts of *P. tremula* wood, indicating their role as endophytes on aspen.

An *Aureobasidium* sp. was commonly isolated in this study from *P. tremula*. *Aureobasidium* species appear to be a common associate of *P. tremula* (Santamaría and Diez 2005), with probable fitness effects. Albrectsen et al. (2010) reported that aspen clonal stands, where *Aureobasidium* sp. was present, were more resistant to browsing by herbivores. Another commonly detected fungus was *P. bakeri* in the *S. carcharias* colonised wood. The fungus has an ability to utilize lignin residues left from brown rot and humus (Ran et al. 2016). *Pseudeurotium bakeri* has also been found as a root-endophyte of the common grass, *Dactylis glomerata* (Sánchez Márquez et al. 2007). The other fungi were detected less commonly. These included species of *Alternaria*, *Cladosporium*, *Epicoccum*, and *Sarocladium*. These genera include well-know saprobes and pathogens of taxonomically diverse plant species. Therefore, finding them as part of the mycobiota colonizing hardwood trees was not surprising.

Unlike for example, scolytine beetles (Curculionidae: Scolytinae), very little is known regarding the relationships between cerambycid beetles and fungi. Adults of these insects never enter the wood, and any organisms such as fungi carried on their bodies would need to be associated with oviposition. While these insects are well-known to transmit nematodes such as the devastating pine pathogen *Bursaphelenchus xylophilus* (pine wood nematode) during oviposition (Wingfield 1987; Akbulut and Stamps 2012), a fixed association between them and fungi is not known. In this regard, Wingfield et al. (2016) suggested that mites carried on the bodies of Cerambycidae could be implicated in the transmission of fungi found in their galleries. The same situation could be true also for *S. carcharias* and *A. glabripennis*, but further investigations would be needed to confirm it.

Species diversity of wood colonised by the insects was higher than intact wood highlighting the importance of larval tunnel for fungal diversity. We detected a number of pathogenic fungi capable of killing living cells, although many of these are considered as weak and opportunistic pathogens or endophytes. We also found several species with known potential of enzymatic degradation of wood components. The availability of such fungi closely associated with the insect suggests that fungal enzymes ingested by the larvae play a key role in improved nutritional quality of the longhorn beetles. The study shows that cerambycid beetles are associated with a diverse microbial assemblage, which can participate in wood degradation by larvae either killing or breaking down host tissue.

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Conflict of interest The authors declare that have no conflict of interest.

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