## **Supporting Information**

# Adaptations of *Pseudoxylaria* towards a comb-associated lifestyle in fungus-farming termite colonies

Janis Fricke,<sup>#1</sup> Felix Schalk,<sup>#1</sup> Nina B. Kreuzenbeck,<sup>1</sup> Elena Seibel,<sup>1</sup> Judith Hoffmann,<sup>1</sup> Georg Dittmann,<sup>2</sup> Benjamin H. Conlon,<sup>3</sup> Huijuan Guo,<sup>1</sup> Z. Wilhelm de Beer,<sup>4</sup> Daniel Giddings Vassão,<sup>5</sup> Gerd Gleixner,<sup>2</sup> Michael Poulsen,<sup>3</sup> Christine Beemelmanns<sup>1,6,7\*</sup>

- Group Chemical Biology of Microbe-Host Interactions, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Beutenbergstraße 11a, 07745 Jena, Germany,
- 2. Department of Biogeochemical Processes, Max Planck Institute for Biogeochemistry, Hans-Knöll-Straße 10, 07745 Jena
- 3. Department of Biology, Section for Ecology and Evolution, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark
- 4. Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Hatfield 0028, Pretoria, South Africa
- 5. Department of Biochemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, 07745 Jena
- 6. Department Anti-infectives from Microbiota, Helmholtz Institute for Pharmaceutical Resea rch Saarland (HIPS), Helmholtz Centre for Infection Research, Saarland University Camp us, 66123 Saarbrücken, Germany
- 7. Saarland University, 66123 Saarbrücken, Germany

<sup>#</sup> contributed equally; alphabetical order

\*Corresponding author: Christine Beemelmanns,

E-mail: Christine.Beemelmanns@helmholtz-hips.de

#### **List of Figures**

igure S1. Maximum-likelihood ITS tree of Xylariales fungi calculated with 1000 bootstrap	
replicates. For tree generation, a symmetric model with unequal rates but equal base	
frequencies were used. The model of rate heterogeneity is invariant using discrete gamma	а
model with 4 categories. The tree is unrooted	13

Figure S2. Maximum likelihood tree of *Xylaria* sp. using ACT as phylogenetic marker, calculated with 1000 bootstrap replicates. Clades are assigned according to Hsieh et al 2005.Phylogeny was predicted using TIM2 model as base substitution rates with empirical base frequencies. The model of rate heterogeneity is invariant using discrete gamma model with 4 categories. The tree is unrooted.

- Figure S3. Maximum likelihood tree of *Xylaria* sp. using RPB2 as phylogenetic marker, calculated with 1000 bootstrap replicates. Clades are assigned according to Hsieh et al 2005. RPB2 gene tree was calculated using general time reversible model with unequal rates and empirical unequal base frequencies. For rate heterogeneity, a free rate model with 5 categories was used.
- Figure S4. Maximum likelihood tree of *Xylaria* sp. using TUB as phylogenetic marker, calculated with 1000 bootstrap replicates. Clades are assigned according to Hsieh et al 2005. Phylogeny was predicted using TIM2 model as base substitution rates with empirical, unequal base frequency. For rate heterogeneity, a free rate model with 16 categories was used.

- Figure S9. GNPS network of EtOAc extracts from six *Pseudoxylaria* spp. grown on PDA for two weeks. Colored nodes represent molecular ions from extracts of: Pseudoxylaria sp. OD126 (red), Pseudoxylaria sp. X802 (blue), *Pseudoxylaria* sp. X187 (green), *Pseudoxylaria* sp. Mn132 (orange), *Pseudoxylaria* sp. X3.2 (yellow) and *Pseudoxylaria* sp. X170LB (black). Identified metabolite clusters are represent as A) xylacremolides (X187/Mn132), B) pseudoxylariamides (X187/Mn132), C) pseudoxylallemcycins (X802/OD126), D) cytosporin/xylasporins (X802/OD126 and X187/Mn132) and E) cytochalasins (X802/OD126).

Figure S11. Semipreparative HPLC chromatogram of culture extracts showing fractions	of
xylasporin I (Fraction 0) and xylasporin G (Fraction 4). Gradient of ACN and $H_2O+0$	.1% FA
is displayed in light blue	33
Figure S12. Integrated <sup>1</sup> H-NMR spectrum of xylasporin G in CDCl <sub>3</sub> , 500 Mhz	36
Figure S13. Peak-picked <sup>13</sup> C-NMR spectrum of xylasporin G in CDCl <sub>3</sub> , 500 Mhz	37
Figure S14. <sup>13</sup> C-NMR spectrum of xylasporin G in MeOH-d3, 500 Mhz	38
Figure S15. Peak-picked DEPT135 spectrum of xylasporin G in CDCl <sub>3</sub> , 500 Mhz	39
Figure S16. HSQC spectrum of xylasporin G in CDCI <sub>3</sub> , 500 Mhz	40
Figure S17. HMBC spectrum of xylasporin G in CDCl <sub>3</sub> , 500 Mhz.	41
Figure S18. COSY spectrum of xylasporin G in CDCI <sub>3</sub> , 500 Mhz	42

Figure S19. <sup>1</sup> H-NMR of xylasporin I in CDCl <sub>3</sub> , 500 Mhz
Figure S20. Comparison of <sup>1</sup> H NMR spectra (CDCI <sub>3</sub> , 500 Mhz) for xylasporin G (top) and
xylasporin I (inverted, bottom). Spectra are inverted based on the ppm axis and are
displayed from 0 to 4 ppm44
Figure S21. Comparison of <sup>1</sup> H NMR spectra (CDCI <sub>3</sub> , 500 Mhz) for xylasporin G (top) and
xylasporin I (inverted, bottom). Spectra are inverted based on the ppm axis and are
displayed from 3.8 to 5.1 ppm45
Figure S22. Comparison of <sup>1</sup> H NMR spectra for xylasporin G (top side) and xylasporin I
(inverted, bottom). Spectra are inverted based on the ppm axis and are displayed from 5.5
to 10.5 ppm. CDCl <sub>3</sub> , 500 Mhz
Figure S23. ESI(+)-HRMS spectrum of xylasporin G. A strong double water loss and adduct
formation, $m/z [M+H]^+ = 307.15347$ , $C_{17}H_{23}O_5^+$ calcd1.726 ppm. RT = 6.48 min was
observed
Figure S24. ESI(+)-HRMS spectrum of xylasporin I. A strong double water loss and adduct
formation, $m/z [M+H]^+ = 309.16910$ , $C_{17}H_{25}O_5^+$ calcd1.683 ppm. RT = 5.53 min was
observed
Figure S25. ESI(+)-HRMS spectrum of hypothesized xylasporin H carbonate ( $m/z$ [M+H] <sup>+</sup> =
335.1484, C <sub>18</sub> H <sub>23</sub> O <sub>6</sub> <sup>+</sup> calcd. 1.417 ppm. RT = 6.70 min)
Figure S26. Broth dilution assay of Pseudoxylaria sp. extracts against Saccharomyces
cerevisiae BY474152

# List of Tables

Table S1. Geographic locations of termite colonies used for isolation of Pseudoxylaria strains7
Table S2. Media Composition.    7
Table S3. Growth of <i>Pseudoxylaria</i> strains on PDA medium at RT and at different time points.
Top-down view and bottom up view8
Table S4.         Sequencing, assembly and annotation statistics of genomes from for Pseudoxylaria
spp. and Xylaria spp. included in this study (I: BGIseq, IT: Illumina, PB: PacBio, ONT:
Oxford Nanopore Technologies; * genome was excluded from the study due to low quality
10
Table S5. Xylaria ITS reference sequences from NCBI fungal ITS RefSeq targeted loci database
(marked in grey) and isolated Pseudoxylaria strains (upper part). Gene accession numbers
of ITS sequences used for phylogenetic analysis as reference data was selected from Hsieh
et al 2005 to provide a framework for phylogenetic classification (lower part)11
Table S6. Gene accession numbers of partial protein-coding genes ACT, RPB2 and TUB used
for phylogenetic analysis and identified sequences from isolated Xylaria strains (lower part).
Table S7. Primers and PCR conditions for amplification of fungal phylogenetic marker
sequences13
Table S8. Mitochondrial assemblies and annotation statistics of species from the Xylariales used
in this study17
Table S9. Identified transposable elements (TEs) in Pseudoxylaria and Xylaria species using
EDTA
Table S10. Additional identified redox active enzymes in Pseudoxylaria and Xylaria species 20
Table S11. Schematic representation of co-cultivation set-ups to determine inhibition of growth:
Method A) Simultaneous co-cultivation of <i>Termitomyces</i> sp. T153 and <i>Pseudoxylaria</i> sp.
(black square) after 2 or 3 weeks (W), and Method B) <i>Pseudoxylaria</i> sp. (black circle); was
first incubated on a PDA plate and then challenged with <i>Termitomyces</i> sp. T15323
Table S12. Pictures of co-culture studies and axenic controls of Termitomyces sp. T153 and
Pseudoxylaria sp. X170LB, X802 on PDA media after 6, 12 and 35 days. Inoculation of
Pseudoxylaria (black square) was performed A) on top of a vegetative Termitomyces culture
(grey color), B) next to a vegetative Termitomyces culture or C) both fungi were inoculated
simultaneously
Table S13. Pictures of Pseudoxylaria sp. X170LB and X802 cultures on water-agarose medium
and water-agarose medium supplemented with lyophilized Termitomyces sp. T112 biomass
or 1/3 PDA
S5

Table S14. Pictures of fungal co-cultures of different Termitomyces (T112, T153, P5HKI) and	
Pseudoxylaria strains (X802, X170LB) on wood-rice medium (WRM)	28
Table S15. Results for stable carbon isotopic fractionation experiments for sugars and lipids	

 Table S16. NMR assignment of xylasporin G and I based on HMBC and key <sup>1</sup>H-<sup>1</sup>H COSY correlations (CDCl<sub>3</sub>), 500 MHz.
 34

Table S18. Disc diffusion assay against Termitoymces sp. 153 using cytochalasines and
Pseudoxylaria sp. extracts51
Table S19. Feeding studies investigating the effect of fungus consumption on the relative growth
rate (RGR) of S. littoralis caterpillars. Insects were fed with PDA (A), Termitomyces sp.
T153 (B) or <i>Pseudoxylaria</i> sp. X802 (D) growing on PDA and PDA after removing fungal

mycelium of the latter fungi, respectively (C, E)	53
Table S20. Results of additional insect feeding studies	53

Strain	Termite species	Excavation site	Isolation date
Mn132	Macrotermes natalensis	S24 40.484 E28 48.271	2016
Mn153	Macrotermes natalensis	S25 44.492 E28 15.663	2016
X167	Odontotermes sp.	S25 43.777 E28 14.423	2016
X170	Odontotermes sp.	S25 56.636 E30 35.833	2016
X3-2	Macrotermes natalensis	S26 50.163 E30 30.490	2016
X187	Macrotermes natalensis	S24 40.434 E28 48.275	2018
X802	Microtermes sp.	S25 43 55.7 E28 14 08.2	2008
OD126	Microtermes sp.	S25 43 55.7 E28 14 08.2	2008

Table S1. Geographic locations of termite colonies used for isolation of *Pseudoxylaria* strains.

Table S2. Media Composition.

Medium	Compound	Concentration	Vendor
PDA (Potato extract dextrose agar)	Potato extract	6,5 g/l	Carl Roth
	Dextrose	20 g/l	Carl Roth
	Agarose	20 g/l	
1/3 PDA (diluted PDA)	Potato extract	2,2 g/l	Carl Roth
	Dextrose	6,7 g/l	Carl Roth
	Agarose	20 g/l	
Agar-agar	dd H <sub>2</sub> O		
	Agarose	20 g/l	
Rice wood	Sawdust	50 Vol%	Perfecto Nager
	Rice	50 Vol%	Alnatura
Wood	Sawdust	100 Vol%	Perfecto Nager
Fungus comb	Fungus comb	100 Vol%	
Wood-Fungus comb	Sawdust	50 Vol%	Perfecto Nager
	Fungus comb	50 Vol%	
PDA-Termitomyces (1/3 PDA-T112)	Potato extract	2,2 g/l	Carl Roth
	Dextrose	6,7 g/l	Carl Roth
	<i>Termitomyces</i> (dry)	~ 5 g/l	
Termitomyces (T112)	dd H <sub>2</sub> O	-	
	Termitomyces (dry)	~ 5 g/l	

Strain						
X802	5 d - Top	5 d - bottom	21 d - Top	21 d- bottom		
	X802		X802			
OD126	3 d - top	3 d - bottom	21 d - top	21 d – bottom		
		OLAS PDA		<b>P0-26</b> P3 T		
X187-2	3 d – top	3 d - bottom	10 d - top	30 d - bottom		
			(i)	X (1)-2 B DF HT BUT		
X3-2	5 d - top	5 d - bottom	17 d – top	17 d – bottom		
				X322 X32		
X167	7 d - top	3 d - bottom	21 d – top	21 d - bottom		
		XHE (2) TO THE STATE				

Table S3. Growth of *Pseudoxylaria* strains on PDA medium at RT and at different time points. Top-down view and bottom up view.

X170 LB	4 d – top	4 d – bottom	17 d – top	21 d - bottom
		×14016 27.03 24741 27.03 24741 200 134 0 0 0 134 0 0 0 134 0 0 0 134 0 0 0 134		
MN132H12	3 d - top	6 d – bottom	12-d - top	14 d - bottom
	C C C C C C C C C C C C C C C C C C C	М. М. АЗ 2 НА2		WV/IS2.R/D Krop-Kors Brand Response Brand Response Respon
MN153 H3	10 d – top	6 d – bottom	15 d – top	15 d - bottom
		MJ/153H3 00000000000000000000000000000000000		ANIS HS

**Table S4.** Sequencing, assembly and annotation statistics of genomes from for *Pseudoxylaria* spp. and *Xylaria* spp. included in this study (I: BGIseq, IT: Illumina, PB: PacBio, ONT: Oxford Nanopore Technologies; \* genome was excluded from the study due to low quality

Organism	Acc. No.	Raw Data	Assembly length (Mbp)	# Seq.	N50 (Mbp)	L50	BUSCO (C %)	Location	Lifestyle	Reference
Pseudoxylaria sp. Mn132	JAJFDL000000000	1	35.0	342	0.26	46	96.6	South Africa	Termite-associated stowaway (Macrotermes natalensis)	This study
Pseudoxylaria sp. Mn153	JAJFDM000000000	I	34.5	355	0.23	49	93.3	South Africa	Termite-associated stowaway (Macrotermes natalensis)	This study
Pseudoxylaria sp. X167	JAJFDK000000000	I	33.2	454	0.22	48	95.2	South Africa	Termite-associated stowaway (Odontotermes sp.)	This study
Pseudoxylaria sp. X170	JAJFDJ000000000	I	33.7	339	0.22	49	96.6	South Africa	Termite-associated stowaway (Odontotermes sp.)	This study
Pseudoxylaria sp. X187	JAJIZN000000000	PB, ONT	39.7	34	2.94	6	96.4	South Africa	Termite-associated stowaway (Macrotermes natalensis)	This study
Pseudoxylaria sp. X3-2	JAJFD1000000000	I, ONT	36.9	742	0.267	41	96.2	South Africa	Termite-associated stowaway (Macrotermes natalensis)	This study
Pseudoxylaria sp. X802	JAJFDH000000000	I, ONT	40.4	106	0.63	20	96.4	South Africa	Termite-associated stowaway ( <i>Microtermes</i> sp.)	This study
<i>Xylaria</i> sp. BCC 1067	GCA_005188305.1	РВ	54.1	43	5.57	5	-	Thailand: Phetchabun	Saprotroph; Isolated from petiole of <i>Nenga</i> pumila	Sutheeworapong et al. 2019 <sup>1</sup>
<i>Xylaria</i> sp. JS573	GCA_000966885.1	I	40.0	100	0.94	14	-	South Korea: Muiu	Parasite; Isolated from <i>Phragmites australis</i>	-
<i>Xylaria</i> sp. MSU_SB201401	GCA_002288965.1	IT	56.8	5,995	0.06	245	94.7	USA: Louisiana	Parasite: Isolated on soy bean roots	Sharma et al. 2018 <sup>2</sup>
Xylaria flabelliformis G536	GCA_007182795.1	I	41.2	155	0.49	28	93.6	USA: North Carolina	Saprotroph and Endophyte; Isolated on Asimina triloba	Mead et al. 2019 <sup>3</sup>
Xylaria grammica EL000614	GCA_004353285.1	PB, I	55.6	44	3.88	6	99.8	South Korea	Endolichenic	Park et al. 2021 <sup>4</sup>
Xylaria grammica IHI A82	007_004014013.1	IT	47.0	1,053	0.08	172	-	Kakamega Forest	Endophyte; Isolated on deadwood	-
Xylaria hypoxylon CBS 122620	GCA_902806585.1	I, ONT	54.3	88	3.89	6	-	-	Saprotroph	Wibberg et al. 2020 <sup>5</sup>
Xylaria hypoxylon DSM 108379	GCA_004768795.1	IT	42.8	635	0.12	102	96.0	Germany: Bad Lobenstein	Saprotroph; Isolated on deadwood	Büttner et al. 2019b <sup>6</sup>
Xylaria longipes IHI A66	GCA_003426265.1	IT	43.2	1,006	0.07	185	90.6	Germany: Bavarian Forrest	Saprotroph; Isolated from Acer pseudoplatanus	Büttner et al. 2019a <sup>7</sup>
Xylaria multiplex DSM 110363	GCA_011057905.1	IT	45.6	389	0.24	57	96.4	USA: Puerto Rico	Saprotroph; Isolated from deadwood	Büttner et al. 2020 <sup>8</sup>
Xylaria polymorpha DSM 105756	GCA_003426235.1	IT	43.5	947	0.07	173	-	Germany: Zittau, Westpark	Saprotroph; isolated from beech	-
Xylaria striata RK1-1*	GCA_002749545.1	IT	59,8	79,106	0.001	15,261	-	China: Yunnan	Parasite: Isolated on rice roots	-

**Table S5.** *Xylaria* ITS reference sequences from NCBI fungal ITS RefSeq targeted loci database (marked in grey) and isolated *Pseudoxylaria* strains (upper part). Gene accession numbers of ITS sequences used for phylogenetic analysis as reference data was selected from Hsieh et al 2005 to provide a framework for phylogenetic classification (lower part).

Family	Genus	Species	Strain	Accession ID
	Amphirosellinia	fushanensis		NR_153514
	Amphirosellinia	nigrospora		NR_153513
	Biscogniauxia	arima		NR_167683
	Podosordaria	muli		NR_158883
	Poronia	pileiformis		NR_158882
	Xylaria	acuminatilongissima		NR_147516
	Xylaria	bambusicola		NR_153200
	Xylaria	brunneovinosa		NR_153201
	Xylaria	ellisii		NR_172972
	Xylaria	eucalypti		NR_166326
	Xylaria	fabacearum		NR_171104
	Xylaria	fabaceicola		NR_171103
	Xylaria	hongkongensis		NR_154905
	Xylaria	insolita		NR_171861
	Xylaria	longissima		NR_147567
	Xylaria	ripicola		NR_153251
	Xylaria	subescharoidea	Vooo	NR_171862
	Pseudoxylaria	sp.	X802	KX097055.1
	Pseudoxylaria	sp.	Mn132	OM443072
	Pseudoxylaria	sp.	IVIN153	MT012094.1
	Pseudoxylaria	sp.	X167	MT012092.1
	Pseudoxylaria	sp.	X170ID	MT012093.1
	Pseudoxylaria	sp.	X187	MT012001
Amphisphoracoao	Pseudoxylaria	sp. foliicola	X3.Z	NP 172090
Amphispheraceae	Iodosphaeria	nhvllonhila		NR_173900 NP 173081
	Noopostalationsis	macadamiaa		NR_173901
Aniosporação	Arthrinium	neosubalohosum		NP 15/737
Diatrypaceae	Diatrype	lijangonsis		NR 165220
Hypoglacogo	Hypoxylon	hellicolor		NP 160071
Пуроаласеае	Hypoxylon	ourasiaticum		NIC_103371
	Hypoxylon	fuscum		NP 172330
	Hypoxylon	nseudofuscum		NR_172213
	Hypoxylon	sporistriatatunicum		NP 160072
	Furfurolla	nigroscons		NP 164061
Leptosnaceae	Furfurelle	stromatica		NR 164062
	l ontosilia	acorina		NP 164063
	Leptosilia	macrospora		NP 164064
	Lopiosilia Lentosilia	muelleri		NR 164065
	Lopiosilia Lentosilia	slantonensis		NR 16/066
	Lopiosilia Lentosilia	wienkamnii		NR 164067
Pseudomassariaceao	Psoudomassariello	vovata		NR 164007
i seudomassanatede	Tristratingridium	microsporum		NR 16/238
Xylariaceae	Calceomyces	lacunosus		NR 167686
Aylallabeae	CalceUniyces	acunosus		1111_107000

**Table S6.** Gene accession numbers of partial protein-coding genes ACT, RPB2 and TUB used for phylogenetic analysis and identified sequences from isolated *Xylaria* strains (lower part).

Clade	Genus	Species	Strain	TUB	ACT	RPB2
outgroup	Annulohypoxylon	cohaerens		AY951655.1	AY951766	GQ844766
	Biscogniauxia	arima		AY951672.1	AY951784	GQ304736.1
	Biscogniauxia	mediterranea		AY951684.1	AY951796.1	GQ844765
	Podosordaria	mexicana		AY951684.1	GQ455451.1	GQ853039
	Podosordaria	muli		GQ844839.1	GQ455450	GQ853038
	Poronia	pileiformis		GQ502720.1	GQ455449	GQ853037
HY	Kretzschmaria	clavus		EF025611.1	EF025596.1	GQ844789
	Kretzschmaria	megalospora		EF025609.1	EF025594.1	GQ844791
	Kretzschmaria	sandvicensis		GQ478211.1	GQ398234.1	GQ844786
	Xylaria	adscendens		GQ487709.1	GQ438746.1	GQ844818
	Xvlaria	arbuscula		GQ478226.1	GQ421286.1	GQ844805.1
	Xvlaria	bambusicola		GQ478223.1	GQ408910.1	GQ844801
	Xvlaria	coccophora		GQ487701.1	GQ421289.1	GQ844809.1
	Xvlaria	hvpoxvlon		GQ260187.1	GQ427196.1	GQ844812
	Xvlaria	liquidambaris		GQ487702.1	GQ421290.1	GQ844810
	Xvlaria	multiplex		GQ487705.1	GQ427198.1	GQ844814
	Xvlaria	oligotoma		GQ487700.1	GQ421288.1	GQ844808
	Xvlaria	striata		GQ478224 1	GQ421284 1	GQ844803
	Xvlaria	venustula		GQ487699 1	GQ421287 1	GQ844807
NR	Nemania	beaumontii		GQ470222 1	GQ389694 1	GQ844772
	Nemania	binanillata		GQ470221 1	GQ389693 1	G0844771
	Nemania	maritima		GQ470225.1	GQ389697 1	G0844775 1
	Rosellinia	huxi		GQ470228 1	GQ398228 1	G0844780 1
	Rosellinia	lamprostoma		EE025604 1	EE025589 1	G0844778
	Rosellinia	merrillii		GO470229 1	GO398229 1	GO844781
PO	Xvlaria	frustulosa		GQ495943 1	GQ449237 1	G0844837
10	Xylaria	sp 8		GO495931 1	GQ438752 1	G0844824
	Discovularia	myrmeconhila		GO487710 1	GQ438747 1	GO844819 1
	Stilbohypoxylon	elaeicola		GQ495933 1	GQ438754 1	G0844827
	Xvlaria	berteri		GO502698 1	GO455442 1	GO848363
	Xylaria	oxvacanthae		GO495927 1	GO438748 1	GQ040000 GO844820
	Xylaria	cubensis		GO502700 1	GO455444 1	GO848365
	Xylaria	curta		GO495936 1	GO438757 1	GO844830
	Xylaria	feeieensis		GO495945 1	GO449241	GO848334
	Xylaria	laevis		GQ502695 1	GQ455439 1	G0848359 1
	Xylaria	polymorpha		GO495954 1	GO452364 1	GO848343
	Xylaria	sn 7		GO495928 1	GO438749 1	GO844821
	Xylaria	anoda		GO495930 1	GQ438751 1	G0844823 1
	Xylaria	alohosa		GQ502684 1	GQ452369 1	G0848348 1
	Xylaria	scruposa		GO495952 1	GO452362 1	GO848341 1
TF	Xylaria	acuminatilongissima		GQ502711	G0853046	G0853028 1
	Xylaria	atrodivaricata		GO502713	GO853048	GO853030
	Xylaria	brunneovinosa		GO502706 1	GO853041	GO853023 1
	Xvlaria	cirrata		GQ502707	GQ853042 1	GQ853024
	Xvlaria	escharoidea		GQ502709	GQ853044	GQ853026 1
	Xylaria	fimbriata		GQ502705	G0853040 1	G0853022.1
	Xylaria	ariseoseniacea		GQ502714	G0853049 1	G0853031
	Xylaria	intraflava		GQ502718	G0853053	G0853035 1
	Xylaria	niarines		GO502710	GO853045	GO853027
	Xylaria	ochraceostroma		GQ502717	GO853052	GO853034 1
	Xylaria	sn 1		GO5027191	GO853054	GQ000004.1
	Yylaria	sp. 1		GQ502713.1	GQ000004 GQ8530/3	GQ000000.1
	Xylaria	sp. 2		GQ502700 GQ502712	GO853047	GQ000020 GQ853029 1
	Xylaria	sp. 6		GQ502712 GQ502715	GO853050	GQ000020.1
	Xylaria	sp. 4		GQ502716	GQ853051	GQ000002.1
	Pseudovularia	sp. 0	X802	OM562/30	OM562432	OM562446
	Pseudoxylaria	sp.	Mn132	OM562440	OM562433	OM562447
	Pseudoxylaria	sp.	Mn153	OM562441	OM562434	OM562448
	Pseudoxylaria	sp.	X167	OM562442	OM562435	OM562449
	Pseudoxylaria	sp.	X170lb	OM562443	OM562436	OM562450
	Pseudoxylaria	sp.	X187	OM562444	OM562437	OM562451
	Pseudoxylaria	SD.	X3.2	OM562445	OM562438	OM562452
		-1				_ · · · • • • • • • • • • • • • • • • •

able S7. Primers and PCR	conditions for	amplification	of fungal	phylogenetic	marker sequences
--------------------------	----------------	---------------	-----------	--------------	------------------

Gene	Sequence	t <sub>R</sub>	Product length (bp)
ACT-512F	ATGTGCAAGGCCGGTTTCGC	<b>66 %</b>	200
ACT-783R	TACGAGTCCTTCTGGCCCAT	60 °C	300
fRPB2-5F	GACGACAGAGATCATTTTGG		1000
fRPB2-7cR	CCCATAGCTTGTTTACCCAT	60 °C	1300
ITS 1	TCCGTAGGTGAACCTGCGG		
ITS 4	TCCTCCGCTTATTGATATGC	55 °C	700



0.05

**Figure S1.** Maximum-likelihood ITS tree of *Xylariales* fungi calculated with 1000 bootstrap replicates. For tree generation, a symmetric model with unequal rates but equal base frequencies were used.<sup>9</sup> The model of rate heterogeneity is invariant using discrete gamma model with 4 categories.<sup>10</sup> The tree is unrooted.





**Figure S2.** Maximum likelihood tree of *Xylaria* sp. using ACT as phylogenetic marker, calculated with 1000 bootstrap replicates. Clades are assigned according to Hsieh et al 2005. Phylogeny was predicted using TIM2 model as base substitution rates with empirical base frequencies. The model of rate heterogeneity is invariant using discrete gamma model with 4 categories. The tree is unrooted.



0.05

**Figure S3.** Maximum likelihood tree of *Xylaria* sp. using RPB2 as phylogenetic marker, calculated with 1000 bootstrap replicates. Clades are assigned according to Hsieh et al 2005. RPB2 gene tree was calculated using general time reversible model with unequal rates and empirical unequal base frequencies. For rate heterogeneity, a free rate model with 5 categories was used.



0.05

**Figure S4.** Maximum likelihood tree of *Xylaria* sp. using TUB as phylogenetic marker, calculated with 1000 bootstrap replicates. Clades are assigned according to Hsieh et al 2005. Phylogeny was predicted using TIM2 model as base substitution rates with empirical, unequal base frequency. For rate heterogeneity, a free rate model with 16 categories was used.



**Figure S5.** Mauve alignment of the *Pseudoxylaria* mitochondrial genome assemblies. For the assembly the software Mauve was used.<sup>11</sup> Locally collinear blocks are highlighted in the same color.

Organism	Acc. No.	Genome length (kbp)	Circular assembly	Annotated genes	Annotated tRNAs	Reference
Pseudoxylaria						
Mn132	OL598078	40.7	Yes	4	13	This study
Mn153	OL598079	18.6	Yes	4	13	This study
X167	OL598081	33.8	Yes	7	14	This study
X170	OL598082	38.9	Yes	12	13	This study
X187	OL598083	36.4	Yes	5	12	This study
X3-2	OL598080	27.4	Yes	8	13	This study
X802	OL598084	63.8	Yes	13	22	This study
Xylariales						-
Annulohypoxylon stygium	MH620794.1	143.3	Yes	60	26	Deng et al. 2018 <sup>12</sup>
Annulohypoxylon stygium	KF545917.1	133.8	Yes	58	26	n/a (direct submission)
Arthrinium arundinis	KY775582.1	49.0	Yes	45	23	n/a
Nemania diffusa	MN780510.1	258.9	Yes	41	25	Tang et al. 202013
Pestalotiopsis fici	KX870077.1	69.5	Yes	78	32	Zang et al. 2017 <sup>14</sup>
Xylaria hypoxylon	NC_046734.1	129.3	Yes	48	27	Zhou et al. 2019 <sup>15</sup>

Table S8. Mitochondrial assemblies and annotation statistics of species from the Xylariales used in this study.

Table S9. Identified transposable elements (TEs) in Pseudoxylaria and Xylaria spec	vies using EDTA.
--	------------------

Organiam		LTR				TIR			nonTIR		% of
Organism	Copia	Gypsy	unknown	CACTA	Mutator	PIF_Harbinger	Tc1_Mariner	hAT	Helitron	total	genome
Pseudoxylaria spp.											
Mn132	0	58	45	163	204	59	52	149	382	1112	2.30
Mn153	81	28	27	221	310	26	8	27	239	967	2.16
X167	104	52	79	34	119	55	4	56	86	589	2.17
X170	101	31	2	26	33	6	1	10	19	230	1.53
X187	90	281	344	255	616	50	18	99	451	2204	5.16
X3-2	254	956	106	43	227	32	7	23	53	1703	5.53
X802	357	842	667	451	800	132	8	253	395	3905	9.93
mean	141	321	181	170	330	51	14	88	232	1530	4.11
<i>Xylaria</i> spp.											
X. flabelliformis G536	0	10	0	31	35	0	10	3	33	122	1.30
X. grammica EL372	0	112	52	206	326	363	219	4	904	2186	2.45
X. grammica IHI A82	0	8	72	562	79	105	9	68	1163	2066	2.41
X. hypoxylon CBS 122620	1087	0	1711	283	306	75	35	28	612	4137	7.48
X. hypoxylon DSM 108379											
X. longipes IHI A66	16	10	50	754	791	8	72	513	1459	3673	2.94
X. multiplex DSM 110363	70	61	937	705	347	131	7	173	1667	4098	3.07
X. polymorpha DSM 105756	0	7	298	974	182	76	5	155	1523	3230	2.65
Xylaria sp. BCC 1067	0	0	506	180	610	12	15	452	1836	3611	2.79
Xylaria sp. JS573	0	111	59	76	133	3	147	46	323	898	1.66
Xylaria sp. MSU_SB201401	2853	0	722	511	1507	212	19	250	555	6629	8.10
mean	490	32	429	533	595	74	44	265	1227	3690	3.54

(LTR) long terminal repeats retrotransposons; (TIR) Two inverted tandem repeats DNA transposons



Figure S6. Comparative CAZY analysis of free-living Xylaria, termite-associated Pseudoxylaria-clade and Termitomyces.

Table S10. Additional identified redox active enzymes in *Pseudoxylaria* and *Xylaria* species.

Organism	Benzoquinone Reductase	Catalase	Gluthathione Peroxidase	HAO	Laccase	MnP	Peroxiredoxin	Superoxide Dismutase	DyP	UPO	total
Pseudoxylaria spp.											
Mn132	1	5	1	4	7	1	1	2	1	0	23
Mn153	1	4	1	4	6	1	1	2	1	0	21
X167	1	4	1	4	11	1	1	2	1	0	26
X170	1	5	1	4	10	1	1	2	1	0	26
X187	2	5	2	4	7	1	1	3	1	0	26
X3-2	1	5	1	4	12	1	1	4	1	0	30
X802	1	2	1	4	8	1	1	2	1	0	21
mean	1.1	4.3	1.1	4	8.7	1	1	2.4	1	0	24.7
<i>Xylaria</i> spp.											
X. flabelliformis G536	1	3	1	4	12	2	1	2	1	2	29
X. grammica EL372	1	4	1	4	10	2	1	2	1	2	28
X. grammica IHI A82	1	5	1	6	10	2	1	2	1	2	31
X. hypoxylon CBS 122620	1	4	1	4	9	2	1	2	1	2	27
X. hypoxylon DSM 108379	1	6	1	3	8	2	1	2	1	2	27
X. longipes IHI A66	1	5	1	6	7	2	1	2	2	2	29
X. multiplex DSM 110363	1	4	1	5	8	2	1	2	1	2	27
X. polymorpha DSM 105756	1	5	1	4	9	2	1	2	1	1	27
<i>Xylaria</i> sp. BCC 1067	1	4	1	4	10	2	1	2	1	1	27
<i>Xylaria</i> sp. JS573	1	3	1	3	6	2	2	2	0	0	20
Xylaria sp. MSU_SB201401	1	1	1	6	13	2	1	2	1	2	30
mean	1	4	1	4.5	9.3	2	1.1	2	1	1.6	27.5

(MnP) manganese-dependent peroxidase; (DyP) dye-decolorization peroxidase; (HAO) hydroxyl acid oxidase; (UPO) unspecific peroxidase



**Figure S7.** A) Numbers of BGCs classes identified in the genomes of *Pseudoxylaria* and free-living *Xylaria* species. **B**) Biosynthetic gene cluster analysis of *Pseudoxylaria* isolates. **A**) Heatmap depicting the numbers of identified BGCs classes annotated as polyketide synthases (PKS), non-ribosomal peptide synthases (NRPS), polyketide non-ribosomal peptide hybrid synthases (PKS-NRPS), NRPS-like synthases (with a domain architecture of A-T-TE and A-T-R), terpene synthases, ribosomally-synthesized and post-translationally modified peptides (RiPP), and halogenases. Shown on the bottom is the ratio of mean BGC numbers per group from not-significant (green) to significant (\*\*\*red).



**Figure S8.** BGCs encoding for the productions of cytochalasan, xylasporin/cytosporin, and xylacremolide (polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS), PKS-NRPS hybrids, short-chain reductases (SDR), cupin-fold oxidoreduxtases (cupin), monooxygenases (MO), Diels-Alderases, SnoaL-like cyclases (cyclase), aromatic ABBA-type prenyltransferases (PT),  $\alpha$ - $\beta$ -hydrolases (hydrolase), acyl transferases (transferase), transcription factors (TF), and major facilitator type transporters (MFS). Red circled numbers indicate the different strains: (1) *Pseudoxylaria* spp. Mn132, (2) Mn153, (5) X187, (7) X802, (8) *Xylaria* spp. BCC 1067, (10) MSU\_SB201401, (11) *X. flabeliformis* G536, (12) *X. grammica* EL000614, and (15) *X. hypoxylon* DSM 108379.Identified homologous biosynthetic genetic loci of the cytosporin/xylasporin (*px*) gene cluster. B) Heatmap showing the abundance and identity in % (white to dark blue) of co-localized homologous *px* genes in other ascomycete genomes deposited in the NCBI database was generated using cblaster v1.3.11.<sup>16</sup>

**Table S11.** Schematic representation of co-cultivation set-ups to determine inhibition of growth: Method **A)** Simultaneous co-cultivation of *Termitomyces* sp. T153 and *Pseudoxylaria* sp. (black square) after 2 or 3 weeks (W), and Method B) *Pseudoxylaria* sp. (black circle); was first incubated on a PDA plate and then challenged with *Termitomyces* sp. T153.

Method			Meti	Method B	
Strain	Pseudoxylaria sp. X802 (2 W)	Termitomyces sp. T153 (3W)	X802 (2W) - T153 (2W)	X802 (3w) - T153 (3W)	X802 (3W)-T153 (2W)
X802	NJ NOR	P) PAINS 29 FS Hart an	NZ POA 32		North States
MN154			S SRI MOSK	Contraction of the second seco	A CONTRACT OF A
MN164		A CONTRACT OF CONTRACTO OF CONTRACTO OF CONTRACTO OF CONTRACT OF CONTRACT OF CONTRACTO OF CONTRACT OF		22 Column the column	ANTER WIFE

	Pseudoxylaria sp. X802	Termitomyces sp. T153 (3W)	X802 (2W)- T153 (2W)	X802 (3w)-T153 (3W)	X802 (3W)-T153 (2W)
MN165	State		A THE REAL PROPERTY OF THE REA		
MN166		Section of the sectio	A RELIAND	and the Cost to Ba	COLUMN AND AND AND AND AND AND AND AND AND AN
MN171		AM S MAR	Contraction of the second seco		No the second se

**Table S12.** Pictures of co-culture studies and axenic controls of *Termitomyces* sp. T153 and *Pseudoxylaria* sp. X170LB, X802 on PDA media after 6, 12 and 35 days. Inoculation of *Pseudoxylaria* (black square) was performed A) **on top of** a vegetative *Termitomyces* culture (grey color), B) **next to** a vegetative *Termitomyces* culture or C) both fungi were inoculated **simultaneously**.

	6 days	12 days	35 days
Axenic control Pseudoxylaria sp. X170LB			
Pseudoxylaria sp. X170LB on top of Termitomyces sp. T153			
Pseudoxylaria sp. X170LB next to Termitomyces sp. T153		A STUS CITY OF	
Pseudoxylaria sp. X170LB inoculated simultaneously with Termitomyces sp. T153			

Axenic control <i>Pseudoxylaria</i> sp. X802	the set as many	A CONTRACT OF CONTRACT	
Pseudoxylaria sp. X802 on top of Termitomyces sp. T153			
Pseudoxylaria sp. X802 next to Termitomyces sp. T153	Sale and a solid contract of the solid contr		the source of th
Pseudoxylaria sp. X802 co-inoculated simultaneously on top of <i>Termitomyces</i> sp. T153			

Days	4	8	14
Axenic control <i>Pseudoxylaria</i> sp. X170LB on water- agarose medium		the second second	the state of the state
Pseudoxylaria sp. X170LB on water/agarose containing <i>Termitomyces</i> sp. T112 biomass	BOAT MAZY MADO LB TO S SZL OM O		
Pseudoxylaria sp. X802 on water- agarose medium containing 1/3PDA	A REAL PROPERTY OF THE REAL PR	And KS CON SOL	1
Pseudoxylaria sp. X802 on water/agarose containing <i>Termitomyces</i> sp. T112 biomass and 1/3 PDA	How when a set of the		

**Table S13.** Pictures of *Pseudoxylaria* sp. X170LB and X802 cultures on water-agarose medium and water-agarosemedium supplemented with lyophilized *Termitomyces* sp. T112 biomass or 1/3 PDA

Day	7	17	31
Axenic control <i>Termitomyces</i> sp. T112 on wood-rice medium			
Axenic control <i>Termitomyces</i> sp. T153 on wood-rice medium			
Axenic control <i>Termitomyces</i> sp. P5HKI on wood-rice medium			

**Table S14.** Pictures of fungal co-cultures of different *Termitomyces* (T112, T153, P5HKI) and *Pseudoxylaria* strains (X802, X170LB) on wood-rice medium (WRM).

Days after co- inoculation	3	10	21
Axenic control <i>Pseudoxylaria</i> sp. X170LB on wood-rice medium			
Axenic control <i>Pseudoxylaria</i> sp. X802 on wood-rice medium			
<b>Co-culture</b> <i>Termitomyces</i> sp. P5HKI and <i>PseudoxyIria</i> sp. X170LB on wood-rice medium			

<b>Co-culture</b> <i>Termitomyces</i> sp. P5HKI and <i>PseudoxyIria</i> sp. X802 on wood-rice medium			
Days after co-	3	10	21
Inoculation			
<i>Termitomyces</i> sp. T112 and <i>PseudoxyIria</i> sp. X170LB on wood-rice medium			
<b>Co-culture</b> <i>Termitomyces</i> sp. T112 and <i>Pseudoxylria</i> sp. X802 on wood-rice medium			
<b>Co-culture</b> <i>Termitomyces</i> sp. 153 and <i>Pseudoxylria</i> sp. X170LB on wood-rice medium			



**Table S15.** Results for stable carbon isotopic fractionation experiments for sugars and lipids performed with *Termitomyces* sp. T112, *Pseudoxylaria* sp. X170LB in axenic cultures and cocultures.

Media	Sample	Replicates ( <i>n</i> )	∆ <sup>13</sup> C/ <sup>12</sup> C Lipids	∆ <sup>13</sup> C/ <sup>12</sup> C Sugars	Relative change lipids	Relative change sugars
PDA	T112	3	-29.99 ± 0.12	$-24.94 \pm 0.07$	-7.15 ± 0.49	-2.10 ± 0.48
PDA	X170LB	3	-26.76 ± 0.87	-24.11 ± 0.17	-3.92 ± 0.78	-1.27 ± 0.56
PDA	X170LB on vegetative T112 biomass	3	-26.95 ± 0.63	-23.87 ± 0.30	-4.11 ± 0.16	-1.03 ± 0.28
PDA	X170Lb on lyophilized T112 biomass	3	-25.05 ± 1.33	-23.57 ± 0.25	-2.21 ± 0.28	-0.73 ± 0.31
<sup>13</sup> C-PDA	T112	3	-27.86 ± 0.09	$-22.33 \pm 0.25$	-6.74 ± 0.99	$-1.22 \pm 0.50$
<sup>13</sup> C-PDA	X170LB	3	$-24.23 \pm 0.90$	-22.43 ± 0.31	-3.11 ± 1.41	-1.31 ± 0.53
<sup>13</sup> C-PDA	X170LB on vegetative T112 biomass	3	$-24.69 \pm 0.24$	-20.50± 0.28	-3.58 ± 0.91	0.62 ± 0.32
<sup>13</sup> C-PDA	X170Lb on lyophilized T112 biomass	3	-23.07 ± 0.46	-22.97 ± 0.27	-1.95 ± 0.48	-1.86 ± 0.31
PDA	Media average: -22.84 ± 0.47					
<sup>13</sup> C PDA	Media average: -21.11 ± 0.13					



Figure S9. GNPS network of EtOAc extracts from six *Pseudoxylaria* spp. grown on PDA for two weeks. Colored nodes represent molecular ions from extracts of: Pseudoxylaria sp. OD126 (red), Pseudoxylaria sp. X802 (blue), *Pseudoxylaria* sp. X187 (green), *Pseudoxylaria* sp. Mn132 (orange), *Pseudoxylaria* sp. X3.2 (yellow) and *Pseudoxylaria* sp. X170LB (black). Identified metabolite clusters are represent as A) xylacremolides (X187/Mn132), B) pseudoxylariamides (X187/Mn132), C) pseudoxylallemcycins (X802/OD126), D) cytosporin/xylasporins (X802/OD126 and X187/Mn132) and E) cytochalasins (X802/OD126).



**Figure S10.** LC-HRMS chromatogram of *Pseudoxylaria* sp. X187 raw extract (EtOAc) with chromatogram traces **A**) TIC and XICs corresponding to novel compounds: **B**) xylasporin G, *m/z* 309.1691; **C**) xylasporin I, *m/z* 307.1535 and **D**) xylasporin H, *m/z* 335.1484.



**Figure S11.** Semipreparative HPLC chromatogram of culture extracts showing fractions of xylasporin I (Fraction 0) and xylasporin G (Fraction 4). Gradient of ACN and  $H_2O+ 0.1\%$  FA is displayed in light blue.

Table S16. NMR assignment of xylasporin G and I based on HMBC and key  $^{1}H-^{1}H$  COSY correlations (CDCl<sub>3</sub>), 500 MHz.



xylasporin G					xylasporin l		
C#	δ <sup>13</sup> C	CH <sub>x</sub>	$\delta$ <sup>1</sup> H, multiplicity	НМВС	<sup>1</sup> H- <sup>1</sup> H COSY	СН <sub>х</sub>	$\delta$ <sup>1</sup> H, multiplicity
1	18.8	CH <sub>3</sub>	1.87, dd	137.6; 131.6	6.06; 6.24	CH₃	1.81, d
2	137.6	СН	6.06, m	18.8		СН	5.88, m
3	131.6	СН	6.24, m		6.83	СН	6.15, m
4	140.7	СН	6.83, m	131.6		СН	6.32, m
5	122.0	СН	6.86, m	131.6; 140.7		СН	6.62, m
6	149.3	Cq				Cq	
7	69.3	СН	4.87, s	149.3; 58.9; 62.7; 127.5		СН	4.7, s
8	58.9	СН	3.50, s	149.3; 64.9		СН	3.50, s
9	62.7	Cq				Cq	
10	29.7	CH <sub>2</sub>	2.58, dd J=12.3, 9.8 Hz 1.95, dd J=12.4, 6.7 Hz	58.9; 62.7; 72.3; 83.2	4.03	CH <sub>2</sub>	2.55, m; 1.97, m
11	83.2	СН	4.03, dd J=9.8, 6.7 Hz			СН	4.08, m
12	72.3	Cq				Cq	
13	26.3	$CH_3$	1.25, s	83.2; 72.3		CH₃	1.26, s
14	23.9	$CH_3$	1.19, s	83.2; 72.3		CH <sub>3</sub>	1.19, s
15	64.9	СН	4.82, s	149.3; 58.9; 62.7; 127.5		СН	4.6, s
16	127.5	Cq				C <sub>q</sub>	
17	191.3	СН	10.19, s	127.5		CH <sub>2</sub>	4.28, 4.50
11-OH		ОН	nd			ОН	nd
7-OH		ОН	nd			ОН	nd
						17-OH	nd

**Structure elucidation of xylasporin G:** The sum formula of xylasporin G was determined as  $C_{17}H_{22}O_5$  by ESI-(+)-HRMS (calcd. for [M+H]<sup>+</sup> C<sub>17</sub>H<sub>23</sub>O<sub>5</sub><sup>+</sup> = 307.1540, found 307.15347, -1.726 ppm) with 7 degrees of unsaturation. An additional 18<sup>th</sup> signal [ $\delta_c$  162.7] in the <sup>13</sup>CNMR spectrum (likely formic acid) could be removed by re-measuring of xylasporin G in MeOH-d3. The <sup>1</sup>H-NMR spectrum of xylasporin G revealed the presence of an aldehyde group [H-17,  $\delta_{\rm H}$  10.19,  $\delta_{\rm C}$  191.3] and three methyl groups based on chemical shift and integration [H-1 H-13 H-14, δ<sub>H</sub> 1.87 1.25 1.19, δ<sub>C</sub> 18.8 26.3 23.9] as well as four protons located on double bonds [H-2 H-3 H-4 H-5, δ<sub>H</sub> 6.06 6.24 6.83 6.86, δ<sub>C</sub> 137.6 131.6 140.7 122.0]. The DEPT135 spectra revealed the presence of only a single CH<sub>2</sub> unit in the molecule [H-10,  $\delta_{H}$  1.96 2.56,  $\delta_{C}$  29.7]. Combination of <sup>13</sup>C-NMR and DEPT135 revealed the presence of another double bond built from two quaternary carbons [C-6 C-16, δ<sub>C</sub> 149.3 127.5]. The structure of the sidechain typical for xylasporins was deduced by <sup>1</sup>H-<sup>1</sup>H COSY correlations between H-1/H-2 [бн 1.87 and 6.06], H-1/H-3 [бн 1.87 and 6.24] and H-3/H-4 [бн 6.83 and 6.86]. This connection is also supported by respective HMBC correlations between H-1/C-2 [ $\delta_H$  1.87,  $\delta_C$  137.6], H-1/C-3 [ $\delta_H$  1.87,  $\delta_C$  131.6], H-5/C-4 [δ<sub>H</sub> 6.86, δ<sub>C</sub> 140.7], H-5/C-3 [δ<sub>H</sub> 6.86, δ<sub>C</sub> 131.6] and H-4/C-3 [δ<sub>H</sub> 6.83, δ<sub>C</sub> 131.6]. The aldehyde group was located by HMBC correlations next to quaternary carbon C-16 [δ<sub>H</sub> 10.19, δ<sub>C</sub> 127.5]. The remaining two methyl groups C-13 and C-14 are connected to the same quaternary carbon C-12 by HMBC correlations [ $\delta_{H}$ 1.25  $\delta_{\rm H}$  1.19,  $\delta_{\rm C}$  72.3] which also carries an oxygen, based on its chemical shift. C-12 was located next to C-11 by additional weaker HMBC correlations of H-13/C-11 and H-14/C-11 [5H 1.25 5H 1.19, 5C 83.2]. C-11 carries oxygen based on chemical shift and was placed next to the only CH<sub>2</sub> group (C-10). Both protons H-10<sub>1</sub> H-10<sub>2</sub> give HMBC signals back to C-11 and C-12 [δ<sub>H</sub> 2.58 δ<sub>H</sub> 1.95, δ<sub>C</sub> 83.2 δ<sub>C</sub> 72.3] as well as towards quaternary carbon C-9 [δ<sub>H</sub> 2.58 δ<sub>H</sub> 1.95, δ<sub>C</sub> 62.7]. Complex mirrored HMBC 2-3 bond correlations between the four oxygenated carbons C-7, C-8, C-9 and C-15 and the related protons support the assumption of a ring structure which fits well into the predicted core structure of xylasporin (GNPS, BigScape). (H-15/C-9 [ $\delta_{H}$  4.82,  $\delta_{C}$  62.7], H-15/C-8 [δ<sub>H</sub> 4.82, δ<sub>C</sub> 58.9], H-15/C-16 [δ<sub>H</sub> 4.82, δ<sub>C</sub> 127.5], H-15/C-6 [δ<sub>H</sub> 4.82, δ<sub>C</sub> 149.3] and H-7/C-6 [δ<sub>H</sub> 4.87, δ<sub>c</sub> 149.3], H-7/C-8 [δ<sub>H</sub> 4.87, δ<sub>c</sub> 58.9], H-7/C-9 [δ<sub>H</sub> 4.87, δ<sub>c</sub> 62.7] as well as H-8/C-6 [δ<sub>H</sub> 3.50, δ<sub>c</sub> 149.3], H-8/C-15 [δ<sub>H</sub> 3.50, δ<sub>C</sub> 64.9]).

**Structure elucidation of xylasporin I:** The sum formula of xylasporin I was determined as  $C_{17}H_{24}O_5$  by ESI-(+)-HRMS (calcd. for [M+H]<sup>+</sup>  $C_{17}H_{25}O_5^+$  = 309.1697, found 309.1691, -1.68 ppm) with six degrees of unsaturation. Due to the chemical instability of xylasporin I structural analysis by NMR was limited. Based on the recorded HRMS2-pattern and characteristic <sup>1</sup>H signals the planar structure could be deduced. In addition to very similar 1H pattern, the absence of an aldehyde proton signal [307:  $\delta_H$  10.19] was characteristic, which was supported by the absence of deep field shifted aldehyde carbon [307:  $\delta_C$  191.3]. Instead the <sup>1</sup>H NMR showed additional proton signals caused by the new H-17 located at the -CH<sub>2</sub>OH group within the area between 4 and 5 ppm.



Figure S12. Integrated <sup>1</sup>H-NMR spectrum of xylasporin G in CDCl<sub>3</sub>, 500 Mhz.



Figure S13. Peak-picked <sup>13</sup>C-NMR spectrum of xylasporin G in CDCl<sub>3</sub>, 500 Mhz.



Figure S14. <sup>13</sup>C-NMR spectrum of xylasporin G in MeOH-d3, 500 Mhz.



Figure S15. Peak-picked DEPT135 spectrum of xylasporin G in CDCl<sub>3</sub>, 500 Mhz.



Figure S16. HSQC spectrum of xylasporin G in CDCl<sub>3</sub>, 500 Mhz.



Figure S17. HMBC spectrum of xylasporin G in CDCl<sub>3</sub>, 500 Mhz.



Figure S18. COSY spectrum of xylasporin G in CDCl<sub>3</sub>, 500 Mhz.



Figure S19. <sup>1</sup>H-NMR of xylasporin I in CDCI<sub>3</sub>, 500 Mhz.



Figure S20. Comparison of <sup>1</sup>H NMR spectra (CDCI<sub>3</sub>, 500 Mhz) for xylasporin G (top) and xylasporin I (inverted, bottom). Spectra are inverted based on the ppm axis and are displayed from 0 to 4 ppm.



Figure S21. Comparison of <sup>1</sup>H NMR spectra (CDCl<sub>3</sub>, 500 Mhz) for xylasporin G (top) and xylasporin I (inverted, bottom). Spectra are inverted based on the ppm axis and are displayed from 3.8 to 5.1 ppm.



Figure S22. Comparison of <sup>1</sup>H NMR spectra for xylasporin G (top side) and xylasporin I (inverted, bottom). Spectra are inverted based on the ppm axis and are displayed from 5.5 to 10.5 ppm. CDCl<sub>3</sub>, 500 Mhz.



**Figure S23.** ESI(+)-HRMS spectrum of xylasporin G. A strong double water loss and adduct formation, m/z [M+H]<sup>+</sup> = 307.15347, C<sub>17</sub>H<sub>23</sub>O<sub>5</sub><sup>+</sup> calcd. -1.726 ppm. RT = 6.48 min was observed.



Figure S24. ESI(+)-HRMS spectrum of xylasporin I. A strong double water loss and adduct formation, m/z [M+H]<sup>+</sup> = 309.16910, C<sub>17</sub>H<sub>25</sub>O<sub>5</sub><sup>+</sup> calcd. -1.683 ppm. RT = 5.53 min was observed.



Figure S25. ESI(+)-HRMS spectrum of hypothesized xylasporin H carbonate (m/z [M+H]<sup>+</sup> = 335.1484, C<sub>18</sub>H<sub>23</sub>O<sub>6</sub><sup>+</sup> calcd. 1.417 ppm. RT = 6.70 min).

Isolate	B. subtilis	S. aureus	E. coli	P. aeruginosa	M. vaccae	S. salmonica 549	C. albicancs	P. notatum
X802 crude	10	0	0	0/A	0	0	0	0
X802 (100%)	12	11	0	0	24p	0	0	0
X802 (50%)	10	0	0	0	0	0	0	0
OD126 crude	10	0	0	0/A	0	0	0	0
OD126 (100%)	11	10	0	0	19p	0	0	0
OD126 (50%)	0	0	0	0	0	0	0	0
X187 crude	0	0	0	0	0	0	0	0
X187 (100%)	12	12	0	0	27p	0	0	0
X187-2 (50%)	0	0	0	0	0	0	0	0
X3-2 crude	10	0	0	0/A	21p	0	0	0
X3-2 (50%)	0	0	0	0	0	0	0	0
X170 LB	0	0	0	0/A	12p	0	0	0
X170 LB (50%)	0	0	0	0	0	0	0	0
X167 crude	10	0	0	0/A	24p	0	0	0
X167 (50%)	0	0	0	0	0	0	0	0
MN153 crude	25.5p	18.5	0	20p	36	0	0	0
Cip	29	18	28.5	33.5	21p	-	-	-
MeOH	0	0	0	10	0	10	0	11
Amphotericin B	-	-	-	-	-	19p	21	18p

**Table S17.** Antimicrobial activity of *Pseudoxylaria* sp. extracts was determined by measuring the inhibition zone (ZOI) in mm ((p) colonies inside inhibition zone, (P) many colonies inside inhibition zone, (A) visible hint indicating potential inhibition)

Test substance	N = 1	N = 2
cytochalasines		the second
Extracts of X802	A CONTRACTOR	
Extracts of X187		VAR3
Extracts of MN132	CC - S - S - S - S - S - S - S - S - S -	AT BE
Extracts of X170LB	1/76	100
Extracts of X3.2	A A A A A A A A A A A A A A A A A A A	

 Table S18. Disc diffusion assay against Termitoymces sp. 153 using cytochalasines and Pseudoxylaria sp. extracts.



Figure S26. Broth dilution assay of Pseudoxylaria sp. extracts against Saccharomyces cerevisiae BY4741.

**Table S19**. Feeding studies investigating the effect of fungus consumption on the relative growth rate (RGR) of *S. littoralis* caterpillars. Insects were fed with PDA (A), *Termitomyces* sp. T153 (B) or *Pseudoxylaria* sp. X802 (D) growing on PDA and PDA after removing fungal mycelium of the latter fungi, respectively (C, E). All experiments were performed with 25 replicates per treatment, a duration of 10 days, and larval weights and survival rates were recorded every day.

Treatment	Sample size (N) at the end of the	RGR	p-value (ANOVA)
A	25	0.084 ± 0.002	
В	25	0.185 ± 0.0004	
С	20	0.116 ± 0.004	< 0.001
D	6	0.065 ± 0.009	
E	0	N/A	
Pairwise Multiple Comparison Procedure	(Dunn's Method):		
B vs. D	p < 0.05		
B vs. C	p < 0.05		
C vs. D	p < 0.05		
A vs. B	p < 0.05		
A vs. C	p < 0.05		
A vs. D	p > 0.05		

Table S20. Results of additional insect feeding studies. All experiments were performed with 25 replicates per treatment, a duration of 10 days, and larval weights and survival rates were recorded every day.

Technical replicates (Date	Treatment	Final sample size at the end	RGR	p-value
	А	25	0.093 ± 0.003	< 0.001
T153_R1 (181107)	В	25	0.173 ± 0.001	
	С	22	$0.073 \pm 0.003$	
	А	22	$0.094 \pm 0.004$	< 0.001
T153_R2 (181205)	В	22	0.169 ± 0.001	
	С	15	$0.065 \pm 0.005$	
	А	24	0.095 ± 0.002	0.010
X802_R1 (180904)	В	3	0.131 ± 0.013	
	С	0	N/A	
	А	22	0.109 ± 0.001	N/A
X802_R2 (181005)	В	0	N/A	
	С	0	N/A	

## References

- <sup>1</sup> Sutheeworapong S, Suteerapongpan N, Paenkaew P, et al (2019) Draft Genome Sequence of the Wood-Decaying Fungus *Xylaria* sp. BCC 1067. Microbiol Resour Announc 8:1–2. https://doi.org/10.1128/MRA.00512-19
- <sup>2</sup> Sharma S, Zaccaron AZ, Ridenour JB, et al (2018) Draft genome sequence of *Xylaria* sp., the causal agent of taproot decline of soybean in the southern United States. Data Br 17:129–133. https://doi.org/10.1016/j.dib.2017.12.060
- <sup>3</sup> Mead ME, Raja HA, Steenwyk JL, et al (2019) Draft Genome Sequence of the Griseofulvin-Producing Fungus *Xylaria flabelliformis* Strain G536. Microbiol Resour Announc 8:14–16. https://doi.org/10.1128/MRA.00890-19
- <sup>4</sup> Park SY, Jeon J, Kim JA, et al (2021) Draft Genome Sequence of Xylaria grammica EL000614, a Strain Producing Grammicin, a Potent Nematicidal Compound. Mycobiology 49:294–296. https://doi.org/10.1080/12298093.2021.1914360
- <sup>5</sup> Wibberg D, Stadler M, Lambert C, et al (2020) High quality genome sequences of thirteen Hypoxylaceae (Ascomycota) strengthen the phylogenetic family backbone and enable the discovery of new taxa. Fungal Divers. https://doi.org/10.1007/s13225-020-00447-5
- <sup>6</sup> Büttner E, Liers C, Hofrichter M, et al (2019b) Draft Genome Sequence of Xylaria hypoxylon DSM 108379, a Ubiquitous Fungus on Hardwood. Microbiol Resour Announc 8:9–11. https://doi.org/10.1128/mra.00845-19
- <sup>7</sup> Büttner E, Gebauer AM, Hofrichter M, et al (2019a) Draft Genome Sequence of Xylaria longipes DSM 107183, a Saprotrophic Ascomycete Colonizing Hardwood. Microbiol Resour Announc 8:11–12. https://doi.org/10.1128/MRA.00157-19
- <sup>8</sup> Büttner E, Liers C, Richter A, et al (2020) Draft Genome Sequence of the Ascomycete Xylaria multiplex DSM 110363. Microbiol Resour Announc 9:9–11. https://doi.org/10.1128/mra.00262-20
- <sup>9</sup> Zharkikh, A., Estimation of evolutionary distances between nucleotide sequences. Journal of Molecular Evolution 1994, 39 (3), 315-329.
- <sup>10</sup> Yang, Z., Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. J. Mol. Evol. 1994, 39 (3), 306-314.
- <sup>11</sup> Darling, A. C. E., Mau, B., Blattner, F. R., & Perna, N. T. Mauve: Multiple alignment of conserved genomic sequence with rearrangements. Genome Research 2004, 14:1394–1403. https://doi.org/10.1101/gr.2289704
- <sup>12</sup> Deng Y, Hsiang T, Li S, Lin L, Wang Q, Chen Q, Xie B, Ming R. Comparison of the Mitochondrial Genome Sequences of Six *Annulohypoxylon stygium* Isolates Suggests Short Fragment Insertions as a Potential Factor Leading to Larger Genomic Size. Front Microbiol. 2018; 9:2079. doi: 10.3389/fmicb.2018.02079.
- <sup>13</sup> Tang D, Zhang G, Wang Y, Zhang M, Wang Y, Yu H. Characterization of complete mitochondrial genome of *Nemania diffusa* (Xylariaceae, Xylariales) and its phylogenetic analysis. Mitochondrial DNA B Resour. 2020; 5:459-460. doi: 10.1080/23802359.2019.1704665.
- <sup>14</sup> Zhang S, Wang XN, Zhang XL, Liu XZ, Zhang YJ. Complete mitochondrial genome of the endophytic fungus Pestalotiopsis fici: features and evolution. Appl Microbiol Biotechnol. 2017; 101:1593-1604. doi: 10.1007/s00253-017-8112-0.
- <sup>15</sup> Zhou H, Abuduaini A, Xie H, Kang R, Suo F, Huang L. The complete mitochondrial genome of wood-rotting fungus Xylaria hypoxylon. Mitochondrial DNA B Resour. 2019, 4. 3848-3849. doi: 10.1080/23802359.2019.1687025
- <sup>16</sup> Gilchrist CLM, Booth TJ, van Wersch B, et al. Cblaster: a Remote Search Tool for Rapid Identification and Visualization of Homologous Gene Clusters. Bioinforma Adv 2021; 1:1–19. https://doi.org/10.1093/bioadv/vbab016