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Low intraspecific genetic diversity indicates asexuality and vertical transmission in the fungal cultivars of ambrosia beetles

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ABSTRACT

Ambrosia beetles farm ascomycetous fungi in tunnels within wood. These ambrosia fungi are regarded asexual, although population genetic proof is missing. Here we explored the intraspecific genetic diversity of *Ambrosiella grosmanniae* and *Ambrosiella hartigii* (Ascomycota: Microascales), the mutualists of the beetles *Xylosandrus germanus* and *Anisandrus dispar*. By sequencing five markers (ITS, LSU, TEF1α, RPB2, β-tubulin) from several fungal strains, we show that *X. germanus* cultivates the same two clones of *A. grosmanniae* in the USA and in Europe, whereas *A. dispar* is associated with a single *A. hartigii* clone across Europe. This low genetic diversity is consistent with predominantly asexual vertical transmission of *Ambrosiella* cultivars between beetle generations. This clonal agriculture is a remarkable case of convergence with fungus-farming ants, given that both groups have a completely different ecology and evolutionary history.

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1. Introduction

Insect agriculture evolved once in ants, once in termites and at least twelve times in wood-boring weevils (Curculionidae: Scolytinae and Platypodinae), the so-called ambrosia beetles (Mueller et al., 2005; Jordal and Cognato, 2012). While fungus-farming ants and termites collect substrate to grow their fungal mutualists within the nests, ambrosia beetles live within wood, which serves as substrate for their cultivars. Despite these ecological differences, there are some striking similarities: new nests of ants and beetles are founded by single individuals, which transmit the fungal cultivars from their parental nest by vertical transmission (Francke-Grosmann, 1967; Korb and Aanen, 2003; Himler et al., 2009). Furthermore, in termites, where two individuals found the colony, in some species one of the two founding individuals transmits the fungus vertically (Korb and Aanen, 2003). This corresponds to clonal farming across many farmer generations, as all vertically transmitted fungi appear asexual. By contrast, the horizontally acquired symbionts of most other termite species undergo regular meiosis and sexual recombination (Mueller et al., 2005; de Fine Licht et al., 2006; Nobre et al., 2011). In theory vertical symbiont transmission is expected to strengthen the mutualism by linking the fitness between host and symbiont, whereas horizontal transmission may lead to the deterioration of the partnership (Frank, 1997).

For ambrosia beetles both vertical transmission and asexuality of fungal cultivars are based on circumstantial evidence, however. First, specialized fungal-spore-carrying organs (i.e., mycetangia; Francke-Grosmann, 1956, 1967) were regarded as evidence for sole

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vertical cultivar transmission, although beetle nests typically co-occur at high densities, which may also facilitate horizontal propagation of cultivars between neighbouring nests. Second, beetle cultivars have long been assumed asexual, because sexual states remained unknown (Harrington, 2005). The latter has been proven wrong by recent discoveries of a sexual state (Musvuugwa et al., 2015) and two mating types in the *Raffaelea* lineage (Ascomycota: Ophiostomatales) of ambrosia beetle-associated fungi (Wuest et al., 2016). However, even though sex is possible it remains unknown to what extent this affects the genetic population structure of those fungal symbionts.

Like other farming insects, ambrosia beetles are obligately dependent on fungi as their sole food source. The best studied ambrosia beetles are found in the inbreeding tribe Xyleborini, which contains about 1300 species (Farrell et al., 2001). These beetles bore tunnel systems in the wood of recently dead or dying trees and inoculate the walls of the tunnels with fungal spores. Glands in their spore-carrying mycetangia ensure specificity of the fungal inoculum during foundation of a new nest (Francke-Grosmann, 1967; Schneider and Rudinsky, 1969). Typical fungal cultivars of ambrosia beetles are species in the genera *Raffaelea* and *Ambrosiella* (Ascomycota: Ophiostomatales and Microascales). Related to plant pathogens, both genera are polyphyletic and it appears that domestication by beetles has occurred several times independently (Cassar and Blackwell, 1996; Jones and Blackwell, 1998; O'Donnell et al., 2015). While the association of ambrosia beetles with *Raffaelea* symbionts appears usually quite loose (i.e., often several different *Raffaelea* species are found within one beetle species and also in a single nest; e.g. Harrington et al., 2010), associations with *Ambrosiella* symbionts are tighter (i.e., only a single ambrosia fungus per nest and species; Mayers et al., 2015).

Research has mainly focused on the identity of ambrosia fungi and only a single study has investigated genetic variation of a *Raffaelea* symbiont (Wuest et al., 2016). *Raffaelea lauricola*, associated with the Redbay ambrosia beetle *Xyleborus glabratus* showed very low genetic variation in its invasive range in the US, but higher diversity in its native Asian range, where also two mating types were discovered. The mating system in the independently evolved and probably more beetle-specific lineage of *Ambrosiella* symbionts remains unstudied so far, however. If the latter indeed are truly asexual and predominantly vertically transmitted between beetle generations, this would lead to low intraspecific variation. Alternatively, sexuality associated with horizontal exchange of cultivars is expected to lead to a higher degree of genetic variation (Charlesworth and Willis, 2009).

Here we tested the hypothesis that genetic variation within and between *Ambrosiella* symbiont populations is low. This was done by comparing the amount of genetic variation by sequencing five polymorphic genetic markers (ITS, LSU (Schoch et al., 2012), TEF1α (Stielow et al., 2015), RPB2, and β-tubulin), which have been used successfully to confirm high genetic variation and frequent horizontal exchange of fungal cultivars in *Macrotermes* fungus-farming termites (de Fine Licht et al., 2006; Nobre et al., 2011) and other non-mutualistic fungal species (Johannesson et al., 2001). Two species of *Ambrosiella* were collected from two beetle species from seven different populations across Europe. *Ambrosiella grosmanniae* was isolated from the ambrosia beetle *Xylosandrus germanus,* which originates from Asia, and *Ambrosiella hartigii* was isolated from the pear blight beetle *Anisandrus dispar*, a species endemic to Europe.

2. Materials and methods

2.1. Beetle collection and fungal extractions

We collected beetles and their symbionts between May and July 2014 at six different locations in six different countries (for details see Fig. 1). The fungi from an additional population in Switzerland were collected in 2012. Additional samples for *A. dispar* beetles were collected in Wageningen, the Netherlands in May 2015. Beetles were trapped using ethanol (96%) baited traps. After collection we immediately stored living beetles in 1.5 ml Eppendorf tubes with a small piece of wet tissue. *X. germanus* and *A. dispar* beetles were stored at 4 °C until they were used for fungal extraction. Prior to fungal extraction the beetles were surface sterilized by dipping them briefly in 70% ethanol and rinsing them afterwards with sterile demineralized water. We isolated fungi by first grinding individual beetles in 1 ml of sterile PBS buffer solution (1:10 dilution), vortexing of the mixture and spreading 200 μl of the pure or diluted (10 ×, 20 ×, 50 ×, 100 ×) mixtures on SMEA plates (3% malt extract, 1.5% agar and 100 ppm streptomycin added after autoclaving) with a metal hockey. Plates were incubated in the dark at 25 °C until fungal colonies appeared. When present, two to three CFUs of all suspected *Ambrosiella* morphotypes were picked and purified on MEA (3% malt extract, 1.5% agar) for molecular identification and sequencing of the different markers. In total we isolated 35 *Ambrosiella* strains from 31 collected beetles. Three additional fungal isolates from *X. germanus* from the USA were provided by T.C. Harrington. An extra ITS sequence of a fungal isolate from a Swiss *X. germanus* beetle was added to the alignment.

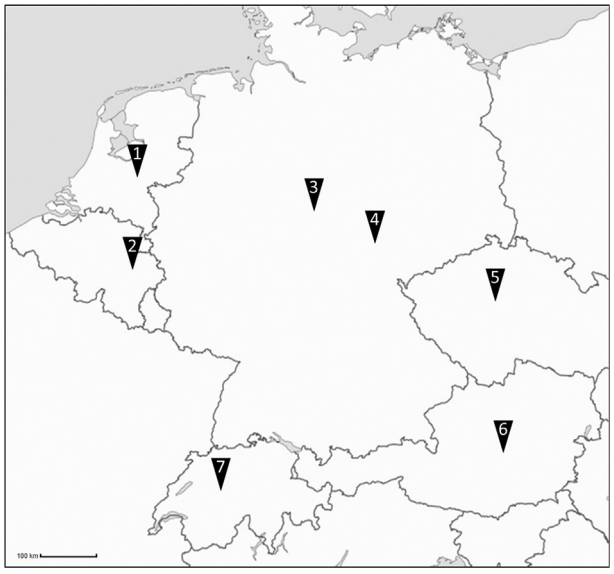

Fig. 1. Origin of the fungal isolates from Europe. Pin number (1) Wageningen, NL, (51°58′44.1″N 5°42′31.0″E) (2) Comblain-au-Pont, BE, (50°28′31.91″N 5°35′26.47″E) (3) Reinhausen (51°27′36.4″N 9°59′54.1″E) and Göttingen (51°34′13.3″N 9°58′24.5″E), DE, (4) Jena (50°59′18.0″N 11°44′44.3″E), DE, (5) Prague (50°01′30.7″N 14°28′07.5″E), CZ, (6) Gesäuse (47°36′28.4″N 14°37′13.4″E), AT, (7) Bern (46°54′37.5″N 7°20′34.1″E), CH. Modified map of NordNordWest, Wikimedia Commons, licensed by CreativeCommons (CC BY-SA 3.0), URL: https://creativecommons.org/licenses/by-sa/3.0/deed.de.

2.2. DNA extraction, sequencing and phylogenetic analyses

Pure cultures of all *Ambrosiella* morphotypes were kept for 7–10 d on MEA plates with cellophane so that the mycelium could be easily harvested. We extracted DNA by placing around 1 g of mycelium in a 1.5 ml Eppendorf tube with glass beads. After freezing in liquid nitrogen the tubes were shaken for 1 min in a Beadbeater machine; this step was repeated once. After grinding, 100 μl of 5% Chelex100 and 10 μl of proteinase K (20 mg/ml) were added. Dilutions were vortexed and incubated for 30 min at 56 °C and for

10 min at 95 °C to inactive proteinase K. Because of problems with the purity of the DNA, we repeated some extractions using a Qiagen DNeasy Blood & Tissue kit (Qiagen, Valencia, CA, USA).

To identify the fungal species and to determine intraspecific variation, we first amplified the nuclear ribosomal region containing internal transcribed spacer 1 (ITS1), 5.8S and internal transcribed spacer 2 (ITS2) using a regular polymerase chain reaction (PCR) with the primers ITS1-F and ITS4 (Paulin-Mahady et al., 2002). To support our initial results with more evidence we amplified four additional markers using regular PCRs and a touchdown PCR (Supplementary document 1): a partial sequence of the gene that encodes for the second largest nuclear RNA Polymerase II subunit (RPB2) using the primers RPB2-6F and RPB2-7.1R (de Fine Licht et al., 2006), a partial sequence of the D1 and D2 domains of the nuclear ribosomal large subunit (LSU, 28S) using the primers LROR and LR5 (Harrington et al., 2014), the sequence encoding for translation elongation factor 1-alpha (TEF1α) using the primers EF595F and EF1160R (Maphosa et al., 2006) and a partial sequence of the gene encoding β-tubulin using the primers Bt2a and Bt2b (Dreaden et al., 2014). For the additional four markers we sequenced only a subset of samples from different geographical regions. All amplified products were sequenced by Eurofins Genomics, Ebersberg (Germany) using the forward primer for the amplification reaction. Fungi were identified using NCBI BLAST.

ITS sequences were obtained for 38 isolates. For the other markers, 13 sequences were obtained for LSU, ten for TEF1α, ten for RPB2 and 12 for β-tubulin.

We aligned all our sequences from the different markers using the online version of the MAFFT Multiple Sequence Alignment software (version 7) using the default settings (Katoh and Standley, 2013). Twenty-seven additional ITS sequences of related *Ambrosiella* and *Ceratocystis* species were added to this alignment (Table 1). *Thielaviopsis paradoxa* (KF697686) was used as an outgroup (de Beer et al., 2014). The alignment was trimmed down to a total length of 551 bp before phylogenetic tree reconstruction. To find a fitting nucleotide substitution model for the dataset, jModelTest was used (Guindon and Gascuel, 2003; Darriba et al., 2012). We reconstructed a Bayesian tree of the aligned ITS sequences and calculated the posterior probability (Bayesian) estimates with Mr. Bayes 3.2.6 (Ronquist et al., 2012) using a general time-reversible model with gamma distribution (GTR + G), 10.000.000 generations and a sampling frequency of 5.000. The first 25% of the samples were discarded (burninfrac = 0.25). The 50% majority rule consensus tree was constructed using the post burn-in samples from the posterior distribution of trees. For LSU an additional 26 sequences were added from GenBank and the alignment was trimmed down to a length of 573 characters. For TEF1α 17 sequences were added from GenBank and the alignment was trimmed down to a length of 424 characters. Phylogenetic trees were reconstructed for both these markers with Mr. Bayes 3.2.6. using the same settings. The RPB2 and β-tubulin markers were not used for phylogenetic reconstruction because of the lack of closely matching sequences in GenBank.

3. Results

Our identifications revealed a single *Ambrosiella* species per beetle species. By sequencing and reconstructing a phylogeny of the ITS marker two major clades, each with nearly identical sequences were found (Fig. 2). The first clade contained eleven fungal isolates from *A. dispar,* with sequences that were nearly identical (2 bp or a 0.36% difference) to the GenBank sequence of *A. hartigii* (KF669873). The second clade consisted of 23 fungi isolated from *X. germanus* and was most similar (2 bp or a 0.36% difference) to a *Ceratocystis* sp. isolate from Korea (HQ538467) and to an *A. grosmanniae* isolate from the United States of America (KR611324, 2 bp or a 0.36% difference). The isolate from Korea was recently classified as *A. grosmanniae* (Mayers et al., 2015). All 23 isolates were identical except for three sequences (B9, C3383, and C3149) with a single base-pair difference (0.18% difference). Sequencing of the additional four markers confirmed the absence of intraspecific variation within each species. One LSU sequence (B9) also had a single G→A substitution at position 437, which was in agreement with the results from the ITS marker (see Supplementary Fig. 1 for the phylogenetic tree). All isolates sequenced for TEF1α, RPB2 and β-tubulin had identical sequences for each fungal species.

4. Discussion

Our analyses revealed that the ambrosia beetles *X. germanus* and *A. dispar* are both associated with a single *Ambrosiella* species all over Europe. These are *A. grosmanniae* and *A. hartigii*, respectively, which is in accordance with a recent study by Mayers et al. (2015). Remarkably, between populations genetic variance was extremely low for both fungal species, even though forest habitats differed strongly between populations and locations were up to 900 km apart. In total, the dataset contained 39 *Ambrosiella* sequences of which 27 were *A. grosmanniae* and twelve were *A. hartigii*.

The *A. hartigii* sequences were all identical, while three *A. grosmanniae* sequences grouped separately because of one base-pair substitution in the ITS and for one of them in the LSU sequence. Interestingly, this point mutation in the ITS sequence was not restricted to geographical location since it occurred both in the European and the USA samples: the European strain (B9) was identical to two USA strains (C3383, C3149) and two Korean samples from GenBank, but all five differed from the rest of the European samples and one USA strain (C3385). This suggests that two different *A. grosmanniae* strains are present in both Europe and the USA. This could mean that there have been at least two independent introductions of this Asian beetle to both regions. For Germany (and Europe) the introduction of *X. germanus* is relatively well documented and most likely occurred with wood of different oak species from Japan during the years 1907–1914 and 1919–1929 (Wichmann, 1957). In the USA, this species was first reported in 1932 from Long Island, NY, originating probably from Korea or Japan (Felt, 1932). This scenario seems likely because both the dominant European and American *A. grosmanniae* strain are found in Japan (Ito and Kajimura, 2017) (Supplementary Fig. 1). Overall, both the exotic *X. germanus* and native *A. dispar* were associated with *Ambrosiella* spp. with a similarly low genetic variability throughout Europe. Therefore, the low genetic variation cannot solely be attributed to the rapid invasion of Europe by *X. germanus* from a small founding population. Instead, it is likely that good dispersal capabilities of ambrosia beetles and/or occasional events of horizontal transmission of cultivars enable sweeps of highly successful symbiont clones through beetle populations.

Our genetic data are consistent with the hypothesis that ambrosia beetles propagate their cultivars as clonal monocultures within their nests and probably across many beetle generations, which is a remarkable convergence to fungus-growing leafcutter ants in the tribe Attini (Mueller et al., 2005). The very low genetic diversity in fungal symbionts within nests and populations is consistent with clonality (Charlesworth and Willis, 2009), which decreases the effective population size relative to sexual reproduction, and also with uniparental, predominantly vertical transmission of the symbionts to the next generation, as this transmission mode is accompanied with severe bottlenecks of the symbiont population and thus decreases its effective population size (Korb and Aanen, 2003). Only a few ambrosia fungus asexual spores are taken up in the females' mycetangia from the natal nest

Table 1
Origin of the different sequences used and, if available, information on beetle host and location.

Species	Culture #	Beetle host species	Location	GenBank accession #				
				ITS	LSU (28s)	TEF1α	β-tubulin	RPB2
Ambrosiella batrae	C3130, CBS 139735	*Anisandrus sayi*	Michigan, USA	KR611322	KY744584			
A. beaveri	C2749, CBS 121750	*Cnestus mutilatus*	Mississippi, USA	KF669875	KF646765			
A. beaveri	1030LHC8	*Xylosandrus mancus*	Lianhuachi, Taiwan		LC175290			
A. beaveri	0414XX13	*C. mutilatus*	Xinxian, Taiwan		LC175287			
A. hartigii	C12	*A. dispar*	Nationalpark Gesäuse, Austria					
A. hartigii	A16	*A. dispar*	Nationalpark Gesäuse, Austria					
A. hartigii	A4	*A. dispar*	Reinhausen, Germany					
A. hartigii	D10	*A. dispar*	Reinhausen, Germany					
A. hartigii	D13	*A. dispar*	Reinhausen, Germany					
A. hartigii	A3	*A. dispar*	Göttingen, Germany					
A. hartigii	A2	*A. dispar*	Prague, Czech Republic					
A. hartigii	A5	*A. dispar*	Prague, Czech Republic					
A. hartigii	D17	*A. dispar*	Prague, Czech Republic		MG050697	MG230535	MG230540	MG230537
A. hartigii	D8	*A. dispar*	Prague, Czech Republic					
A. hartigii	C003	*A. dispar*	Wageningen, The Netherlands					
A. hartigii	C006	*A. dispar*	Wageningen, The Netherlands	MG031180				
A. hartigii	C1573, CBS 404.82, CMW25525	*A. dispar*	Germany	KF669873	KM495317			
A. hartigii	XgF28S02	*X. germanus*	Japan		LC140890			
A. hartigii	XgF28S03	*X. germanus*	Hiroshima, Japan		LC140891			
A. hartigii	XgF28S04	*X. germanus*	Hokkaido, Japan		LC140892			
A. hartigii	XgF28S05	*X. germanus*	Hokkaido, Japan		LC140893			
A. grosmanniae	1002HHS1	*X. germanus*	Hehuanshan, Taiwan	LC175288	LC175288			
A. grosmanniae	1002HHS2	*X. germanus*	Hehuanshan, Taiwan	LC175289				
A. grosmanniae	C3151, CBS 137359	*X. germanus*	Iowa, USA	KR611324	KY744587			
A. grosmanniae	D14	*X. germanus*	Nationalpark Gesäuse, Austria					
A. grosmanniae	D19	*X. germanus*	Nationalpark Gesäuse, Austria					
A. grosmanniae	D20	*X. germanus*	Nationalpark Gesäuse, Austria					
A. grosmanniae	B10	*X. germanus*	Comblain-au-Pont, Belgium					
A. grosmanniae	C7	*X. germanus*	Comblain-au-Pont, Belgium					
A. grosmanniae	B9	*X. germanus*	Comblain-au-Pont, Belgium	MG031178	MG050695		MG230538	
A. grosmanniae	A1	*X. germanus*	Reinhausen, Germany					
A. grosmanniae	A10	*X. germanus*	Reinhausen, Germany					
A. grosmanniae	A13	*X. germanus*	Reinhausen, Germany					
A. grosmanniae	A7	*X. germanus*	Göttingen, Germany					
A. grosmanniae	D1	*X. germanus*	Göttingen, Germany					
A. grosmanniae	D2	*X. germanus*	Göttingen, Germany					
A. grosmanniae	D3	*X. germanus*	Göttingen, Germany					
A. grosmanniae	D4	*X. germanus*	Göttingen, Germany					
A. grosmanniae	C14	*X. germanus*	Jena, Germany					
A. grosmanniae	D9	*X. germanus*	Jena, Germany					
A. grosmanniae	B18	*X. germanus*	Jena, Germany					
A. grosmanniae	D11	*X. germanus*	Jena, Germany					
A. grosmanniae	D15	*X. germanus*	Jena, Germany					
A. grosmanniae	D7	*X. germanus*	Jena, Germany					
A. grosmanniae	D16	*X. germanus*	Wageningen, The Netherlands	MG031179	MG050696	MG230534	MG230539	MG230536
A. grosmanniae	D6	*X. germanus*	Wageningen, The Netherlands					
A. grosmanniae	B2	*X. germanus*	Wageningen, The Netherlands					
A. grosmanniae	C3149	*X. germanus*	Michigan, USA					
A. grosmanniae	C3, C3385	*X. germanus*	Missouri, USA					
A. grosmanniae	C3383	*X. germanus*	Iowa, USA					
A. grosmanniae	Xgk74	*X. germanus*	Bern, Switzerland					
A. nakashimae	0414XX4	*X. amputatus*	Xinxian, Taiwan	LC175284				
A. nakashimae	0414XX7	*X. amputatus*	Xinxian, Taiwan	LC175285	LC175285			

A. nakashimae	W209g3	*X. amputatus*	Lianhuachi, Taiwan	LC175304	
A. nakashimae	C3445, CBS 139739	*X. amputatus*	Georgia, USA	KR611323	KY744586
A. roeperi	C2451	*X. crassiusculus*	Georgia, USA	KF669872	
A. roeperi	C2448	*X. crassiusculus*	Georgia, USA	KF669871	KF646767
A. xylebori	C1650, CMW 2553, CBS 110.61, AFTOL-ID 1285	*X. compactus*	Ivory Coast	KF669874	KM495318
A. xylebori	Hulcr5114	*X. compactus*			KU961669
Ambrosiella sp.	W186g	*A. hirtus*	Meifong, Taiwan	LC175301	LC175301
Ambrosiella sp.	C3843	*A. maiche*	Ohio, USA		KY744585
Ambrosiella sp.	XbF28S01	*X. brevis*	Aichi, Japan		LC140894
Ambrosiella sp.	SmF28S01	*Scolytoplatypus mikado*	Wakayama, Japan		LC140895
Ambrosiella sp.	SmF28S02	*S. mikado*	Aichi, Japan		LC140896
C. adiposa	CBS 138.34			DQ318195	
C. adiposa	CMW1622		Japan	AF043606	
C. adiposa	UAMH 6973			KC305147	
C. adiposa	UAMH 6974			KC305148	
C. adiposa	xsd08011			EU918711	
C. adiposa	CMW2573				KM495320
C. adiposa	CCFC212707				AY283562
C. fagacearum	CMW2039			KC305154	
C. fagacearum	C1305				AF222483
C. major	CMW3189				KM495350
C. norvegica	WIN(M)87			DQ318194	
C. norvegica	C3124, UAMH9778				KY744591
Ceratocystis sp.	CspXger3	*X. germanus*	Korea	HQ538467	
Ceratocystis sp.	CspXger8	*X. germanus*	Korea	HQ670423	
Ceratocystis sp.	CspXapi1		Korea	HQ670422	
Meredithiella norrisii	C3152, CBS139737	*Corthylus punctatissimus*	Iowa, USA		KY744589
Meredithiella sp.	C4171	*Co. papulans*	Florida, USA		KY744590
Meredithiella sp.	M545	*Co. crassus*	French Guiana		KY744223
Phialophoropsis ferruginea	CBS 408.68	*Trypodendron retusum*	Wisconsin, USA	KC305145	
P. ferruginea	C2230, CBS 460.82	*T. domesticum*	Munden, Germany	KC305146	KF646766.2
P. ferruginea	M243	*T. lineatum*	Colorado, USA		KY744224
Thielaviopsis paradoxa	BR2		China	KF697686	
T. paradoxa	C1001, CBS 601.70				AF275498

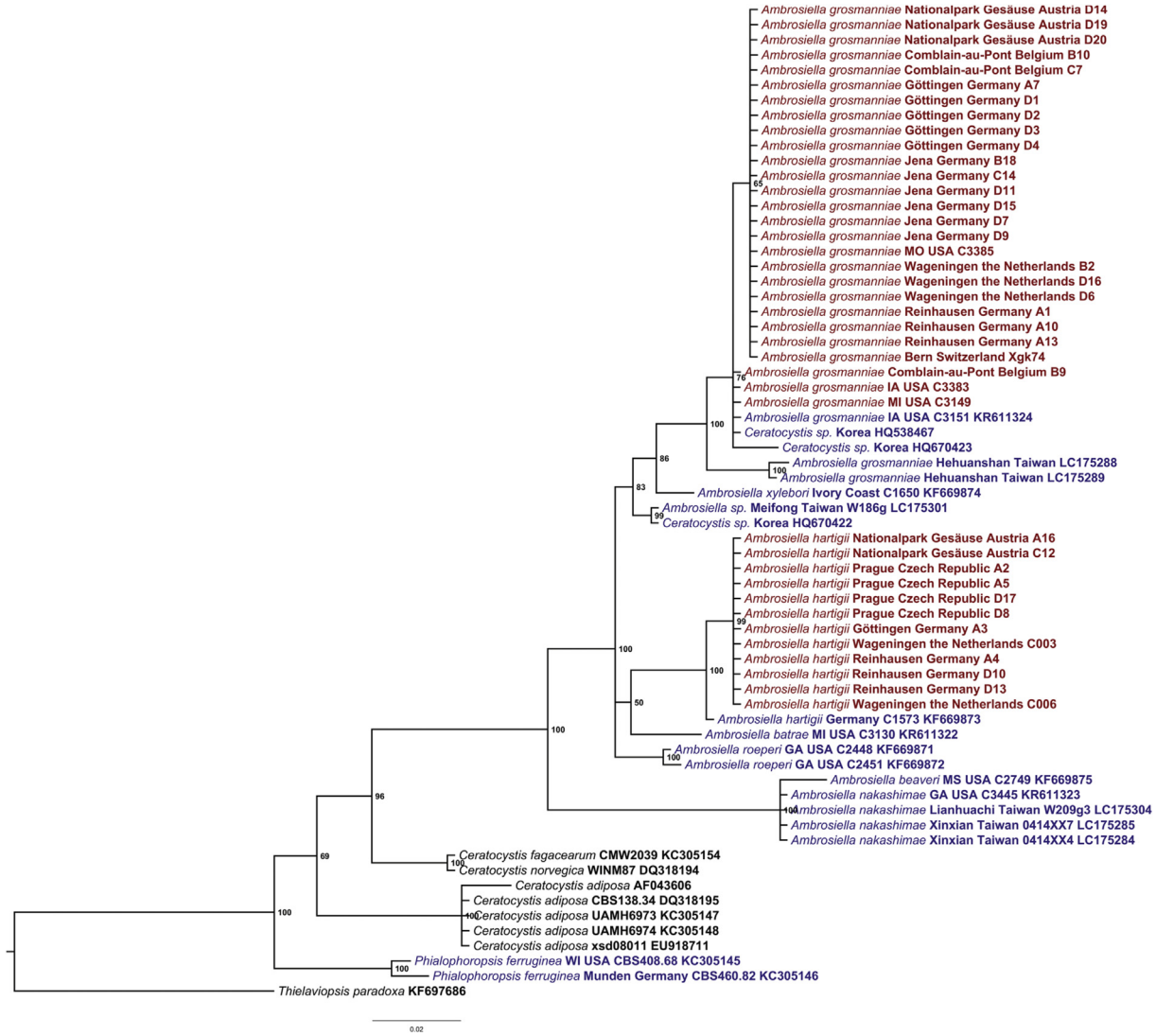

Fig. 2. Phylogenetic tree of the ITS region of our samples and samples from GenBank. Numbers at the nodes represent posterior probabilities. Our samples are highlighted in red, other ambrosia fungi in blue and additional closely related fungi in black.

before emergence, are kept alive by gland secretions during beetle dispersal and are then expelled at the new nest site to form the next generation of fungal cultivars (Schneider, 1975).

The ancestors of the cultivars of ambrosia beetles and attine ants are sexually reproducing. So why have both farming beetles and ants (and some species of fungus-growing termites as well) convergently evolved clonal fungiculture? Theory suggests that in host-symbiont association it is advantageous for the host to reduce genetic variation among their symbionts as this selects against virulent effects due to between-symbiont competition (Frank, 1996). The reason is that as relatedness between symbionts declines, a symbiont's fitness depends more on its ability to outcompete other symbionts than on the overall success of the group (Hamilton, 1972). How this affects their hosts has been nicely demonstrated in both farming ants and termites, whose productivity decreases if relatedness within their fungal cultivars is experimentally reduced (Poulsen and Boomsma, 2005; Aanen et al., 2009). It will be interesting to test if this is also the case in ambrosia beetles, whose fungi compete fiercely with other species (Klepzig and Wilkens, 1997; Klepzig, 1998) and likely also intraspecifically. Selection for vertical clonal transmission must have been very strong at the origin of beetle fungiculture as indicated by the fact that all six lineages of fungi (Cassar and Blackwell, 1996; Blackwell and Jones, 1997; O'Donnell et al., 2015) switched from sexual to primarily asexual reproduction in the course of domestication. However, not all the other fungus lineages show such a low genetic diversity as we describe here for the two Xyleborini-*Ambrosiella* mutualisms. In a Xyleborini-*Raffaelea* mutualism, *R. lauricola,* cultivated by *X. glabratus,* has high genetic variation – at least in its native Asian range – which suggests that at least occasional horizontal transmission and sexuality occur in this species (Wuest et al., 2016; Ito and Kajimura, 2017). Current data suggests that Xyleborini-*Raffaelea* mutualisms are less specific and open for several fungal symbionts. *R. lauricola*, for example, is commonly exchanged also with other *Raffaelea*-associated ambrosia beetles co-occurring in the same trees with *X. glabratus* (Ploetz et al., 2017).

It is well known that low genetic variance within a population presents special problems for disease control (Frankham, 2005; Denison, 2012). Ambrosia beetles, and generally fungus-farming insects (e.g. Mueller et al., 2005), have apparently evolved complex strategies to suppress and manage crop diseases in their clonal crops: (a) ambrosia beetles sequester their fungi from the external environment in tunnels within mostly sterile wood; (b) fungiculture co-evolved with social behaviour in ambrosia beetles, so multiple

immature and adult individuals monitor gardens intensively and can suppress the spread of pathogens (Biedermann and Taborsky, 2011; Kirkendall et al., 2015; Nuotcla, 2015); (c) this pathogen control may be partly by other symbionts, like bacteria or yeasts, which are common in ambrosia beetle nests and can provide disease-suppressing antibiotics (Cardoza et al., 2006; Scott et al., 2008; Grubbs et al., submitted); and (d) there are indications that next to their primary cultivars *Raffaelea*-associated ambrosia beetles have one or more secondary fungal symbionts (Harrington and Fraedrich, 2010; Biedermann et al., 2013) that may serve as a reservoir and ensure food security under disease outbreaks. It seems that all insect farmers embed their clonal cultivars in a holobiome of other microbes. They have maintained their monocultures against the treat of pathogens for 30–60 million years (Mueller et al., 2005). Thus, it may be fruitful to investigate how they cope with co-evolving diseases and antibiotic resistance, which are currently a big challenge for human agriculture and medicine.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funeco.2017.11.010.

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