Title: **Lack of fidelity revealed in an insect-fungal mutualism after invasion**

Short title: Symbiont fidelity breakdown during an invasion

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1. Summary

Symbiont fidelity is an important mechanism in the evolution and stability of mutualisms. Strict fidelity has been assumed for the obligate mutualism between *Sirex* woodwasps and their mutualistic *Amylostereum* fungi. This assumption has been challenged in North America where a European woodwasp, *Sirex noctilio*, and its fungal symbiont *Amylostereum areolatum*, have recently been introduced. We investigate the specificity of the mutualism between *Sirex* and *Amylostereum* species in Canada, where *S. noctilio* co-infests *Pinus* with native *S. nigricornis* and its mutualist *A. chailletii*. Using phylogenetic and culture methods, we show that extensive, reciprocal exchange of fungal species and strains is occurring, with 75.3% of *S. nigricornis* carrying *A. areolatum* and 3.5% of *S. noctilio* carrying *A. chailletii*. These findings show that the apparent specificity of the mutualism between *Sirex* spp. and their associated *Amylostereum* spp. is not the result of specific biological mechanisms that maintain symbiont fidelity. Rather, partner switching may be common when shifting geographic distributions driven by ecological or anthropogenic forces bring host and mutualist pairs into sympatry. Such novel associations have potentially profound consequences for fitness and virulence. Symbiont sharing, if it occurs commonly, may represent an important but overlooked mechanism of community change linked to biological invasions.

2. Keywords

Mutualism, insect-fungus symbiosis, symbiont fidelity, invasion, *Sirex* woodwasp

3. Introduction

A frequently cited consequence of globalisation is the growing homogenisation of biotic communities, commonly driven by biological invasions. Invasive species can have serious negative impacts on the ecosystems in which they become established. Such impacts include invasive species altering existing

mutualisms among native species, or acquiring novel symbionts that affect virulence in one partner [1-3].

In the Southern Hemisphere the invasive wood-boring wasp *Sirex noctilio* and its obligate nutritional fungal mutualist *Amylostereum areolatum*, is a highly aggressive pest complex infesting and killing healthy plantation pines [4]. In its native range in Eurasia and North Africa this complex is a secondary pest, infesting dead or dying conifers, primarily in the genus *Pinus* [5]. The complex has recently been introduced into eastern North America (ENA), where it poses a potential threat to planted and natural pine forests [6].

The introduction of *S. noctilio* into ENA provides an opportunity to study the specificity of the mutualism between *Sirex* and *Amylostereum* species. This mutualism was until recently assumed to be highly specific as a result of fungal mutualists being vertically transmitted as asexual spores [7]. This dogma has been questioned recently with the discovery of specimens of native *S. nigricornis* and *S. nitidus* carrying *A. areolatum* [8].

We investigate the specificity of the *Sirex-Amylostereum* mutualism in invasive and native populations in Canada. In this study we question whether native *S. nigricornis* and invasive *S. noctilio* are strictly associated with their known symbionts, *A. chailletii* and *A. areolatum* respectively. We further examine evidence for recent exchange of *A. areolatum* strains between *S. nigricornis* and *S. noctilio* by identifying shared clonal lineages of *Amylostereum* in native and invasive wasp populations.

4. Materials and Methods

A collection of 134 *Sirex* woodwasps and their mutualistic fungi, isolated from female mycangia [as described in 9], was obtained from collaborators in Canada (see electronic supplementary material S1 and S2 for sampling locations and storage details). Wasp and fungal samples were identified to species using sequence data from the mitochondrial cytochrome *c* oxidase subunit I (COI) and mitochondrial small subunit (mtSSU) genes respectively (electronic supplementary material S3

and S4). The internal transcribed spacers (ITS) and intergenomic spacer (IGS) regions of the rRNA locus were sequenced for representative samples of each *A. areolatum* mtSSU haplotype (electronic supplementary material S5), to compare them with isolates from previous studies which produced multilocus genotypes (MLG's). Where cloning was necessary, the Promega pGEM®-T Easy Vector System was used. PCR products were sequenced on an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems) at the sequencing facility of the University of Pretoria.

Bidirectional sequences were assembled and edited in CLC Main Workbench 6.6.2 (CLC Bio Inc. Denmark) and aligned using MEGA 5 [10] and MAFFT [11]. Sequence Evolution Models were selected using AIC in jModelTest 0.1.1 [12]. Species identification was based on group membership in neighbour-joining trees constructed in PAUP 4.0 [13]. Haplotype analysis was carried out using Splitstree4 [14]. Maximum likelihood (PhyML [15]) and Bayesian (MrBayes [16]) approaches were used for phylogenetic analysis of ITS sequence data from representative samples. Laboratory methodologies, sequence evolution models and programme parameters can be found in electronic supplementary material S6-S8.

Vegetative incompatibility assays using randomly selected representative samples from each identified *A. areolatum* mtSSU haplotype were performed using established methods to determine vegetative compatibility groups (VCG's; electronic supplementary material S9) [17]. VCG richness was then used as an additional measure of genetic diversity.

5. Results

COI sequencing identified 77 *S. nigricornis* and 57 *S. noctilio* specimens. Fungal species switching has occurred in Canadian siricid populations with 75.3% of *S. nigricornis* females carrying *A. areolatum* (n=58), and 3.5% of *S. noctilio* females carrying *A. chailletii* (n=2; electronic supplementary material S10). We detected a single mtSSU haplotype for *A. chailletii* and three mtSSU haplotypes for *A. areolatum*, which differed by a maximum of two base pairs (figure 1). Two of the *A. areolatum* haplotypes, H3 and H2, were uniquely detected in *S. noctilio* and *S. nigricornis* respectively, whereas the third (H1) was carried by both species.

Representative *A. areolatum* isolates for which we obtained MLGs grouped into two clades, (A and B), based on ITS sequence data (table 1, electronic supplementary material S12). Both MLG1 and

MLG3 corresponded with previously isolated samples [MLG3 and MLG2 in 18 respectively]. MLG2 was unique in this study, although ITS (MLG2a and MLG2b) and IGS sequences (MLG2a) have been previously isolated [8, 19].

Multiple VCG's were identified within each mtSSU haplotype. VCG richness was high; 14 VCG's were identified from 27 isolates of *A. areolatum.* Ten isolates were incompatible with all others, and up to 5 distinct VCGs were isolated from wasps emerging from the same tree. One VCG was shared between *S. noctilio* and *S. nigricornis,* confirming recent lateral transfer of strains between invasive and native Canadian *Sirex* populations.

6. Discussion

The identification of a shared MLG and VCG of *A. areolatum* between newly sympatric *Sirex* species strongly supports direct lateral transfer of symbionts. This transfer is bi-directional, as ~3% of *S. noctilio* females carried *A. chailletii*, but is skewed towards *A. areolatum* transfer to *S. nigricornis*. This skewed directionality of transfer could result from temporal patterns in oviposition and emergence, or disproportionate utilization of *S. noctilio*-weakened trees by *S. nigricornis*.

The lack of host-symbiont fidelity detected in this study, also shown in an independent concurrent study [19], calls into question the mechanisms maintaining the apparent fidelity of symbiont associations in the native range of *S. noctilio*. It is possible that geographic, host, or temporal segregation among native siricids may be sufficient to maintain the low rates of transfer observed in Europe and elsewhere [5]. Alternatively, fungal and or wasp mis-identification and sparse sampling across Europe could have led to underestimates of symbiont switching [6] We propose that the process of invasion of *S. noctilio* into Canada has facilitated symbiont switching among *S. noctilio* and *S. nigricornis*. The sequence data produced in this study (GenBank; electronic supplementary

material S3-S5) will serve as an important reference for further studies examining the mechanism of horizontal symbiont transfer among siricid woodwasps.

Symbiont transfer is stable over at least one generation; 54 of 58 *S. nigricornis* females carrying *A. areolatum* emerged from trees where no *S. noctilio* emerged in the sampling season. Similarly, *S. noctilio* specimens carrying *A. chailletii* emerged from logs that were not infested with *S. nigricornis*. However, these wasps could have entered trees pre-infected via wind dispersed spores (as can occur with *A. chailletii* in Europe [20]), un-emerged woodwasp infestation, or aborted woodwasp attacks. Important questions which should be addressed by further studies are whether carrying the "wrong" symbiont influences wasp or fungal fitness, and whether novel symbiont associations are stable over longer time periods. It is evident however, that fungal switching increases opportunities for the fungi to spread by increasing the pool of potential vectors.

Previous studies have shown that diversity of *A. areolatum* in the Southern Hemisphere is low, with only two VCG's identified [17]. We identified 14 VCG's demonstrating higher than expected diversity in an invaded area, even higher than in the putative native range in Northern Europe [20]. This diversity could reflect more than one introduction of the fungus into ENA, including the possibility of introduction prior to the *S. noctilio* invasion, together with *S. juvencus* [21]. The high amount of VCG diversity could also be influenced by sexual reproduction of the fungus in ENA. However, fruiting bodies of *A. areolatum* have not been reported in North America, and are rare in the native European range [17].

The detection of an *A. areolatum* MLG unique to Canada supports the results of Nielsen *et al.* and Bergeron *et al.* [8, 18], who identified *A. areolatum* isolates unique to ENA. These findings suggest that this genotype could have been introduced from a previously unsampled *S. noctilio* source population, as identified by a recent analysis of a global *S. noctilio* collection [22], or that ENA harbours an unsampled native *A. areolatum* population. A concurrent study of woodwasp-fungal fidelity in eastern USA revealed that two native woodwasps, *S. nigricornis* and *S. nitiudus*, carried *A.*

areolatum in their mycangia [19]. The majority of these fungal isolates were shown to contain IGS type BE, which was identified in MLG2 in this study. MLG2 was associated with 55% of *A. areolatum* carrying *S. nigricornis* specimens. This IGS type, and IGS type E unique to the present study, have been identified exclusively from ENA. This lends further support to the hypothesis of a native population *A. areolatum* in ENA.

The ecological and evolutionary consequences of symbiont switching in the *Sirex-Amylostereum* mutualism are not known, but could be significant [1]. One dramatic possibility is that symbiont swapping could induce changes in wasp virulence with respect to their ability to attack and kill healthy host trees [3]. Undoubtedly, the potential threat of the *S. noctilio – A. areolatum* complex to native and commercial forest ecosystems in ENA is more complex than might previously have been anticipated. Given the specificity of interactions between native Siricids and their parasites, these relationships could also be altered by symbiont switching [6]. The discovery of symbiont switching at considerable frequency also calls into question the wisdom of importing foreign strains of the nematode *Deladenus siricidicola*, which feeds on *A. areolatum* during part of its life-cycle, as a biological control agent [6], as the nematode could easily escape into native siricid populations. The lack of specificity observed between *Sirex* and *Amylostereum* species after the invasion of *S. noctilio* into ENA highlights a need to reassess the specificity of the mutualism in Eurasia and North Africa.

7. Acknowledgements

We thank members of the Tree Protection Cooperative Programme (TPCP), the Department of Trade and Industry (DTI) THRIP programme and the National Research Foundation (NRF) of South Africa for funding. We also thank our collaborators, Mr Chuck Davis (Natural Resources Canada, Canadian Forest Service) for providing the samples used in this study, and Sarah Drabble (Ontario Ministry of Natural Resources) for monitoring emergence of Sirex specimens.

The authors declare no competing interests.

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9.Figure and table legends

Figure 1. (a) Haplotype network of *Amylostereum areolatum* (n=113). Numbers of isolates per wasp species are shown within haplotype nodes. Dark grey indicates samples isolated from *S. noctilio*, white from *S. nigricornis*. (b) Haplotype network of *A. chailletii* isolates. Connecting lines specify one substitution, cross-bars specify additional substitutions separating haplotypes. Below the nodes are countries from which the haplotypes have previously been sampled (accession numbers in electronic supplementary material S11).

Genotype	mtSSU-ITS profile	IGS profile	mtSSU previously sampled	ITS previously sampled	IGS previously sampled	Region
MLG1	H1-A	D	Bergeron *et. al.* (2011)	Bergeron *et. al.* (2011)	Bergeron *et. al.* (2011) and Hajek *et al.* (2013)	ENA, Southern Hemisphere
MLG2a*	H2-B	BE	**No**	Nielsen *et. al.* (2009)	Nielsen *et. al.* (2009) and Hajek *et al.* (2013)	USA
MLG2b*	H2-B	E	**No**	Nielsen *et. al.* (2009)	**No**	USA
MLG3a*	H3-A	BD	Bergeron *et. al.* (2011)	Bergeron *et. al.* (2011)	Bergeron *et. al.* (2011) and Hajek *et al.* (2013)	ENA
MLG3b*	H3-A	D	Bergeron *et. al.* (2011)	Bergeron *et. al.* (2011)	Bergeron *et. al.* (2011) and Hajek *et al.* (2013)	ENA

Table 1. Multilocus genotypes (MLGs) of representative *Amylostereum areolatum* samples in comparison to previously sampled *A. areolatum* isolates

*a and b represent IGS profiles

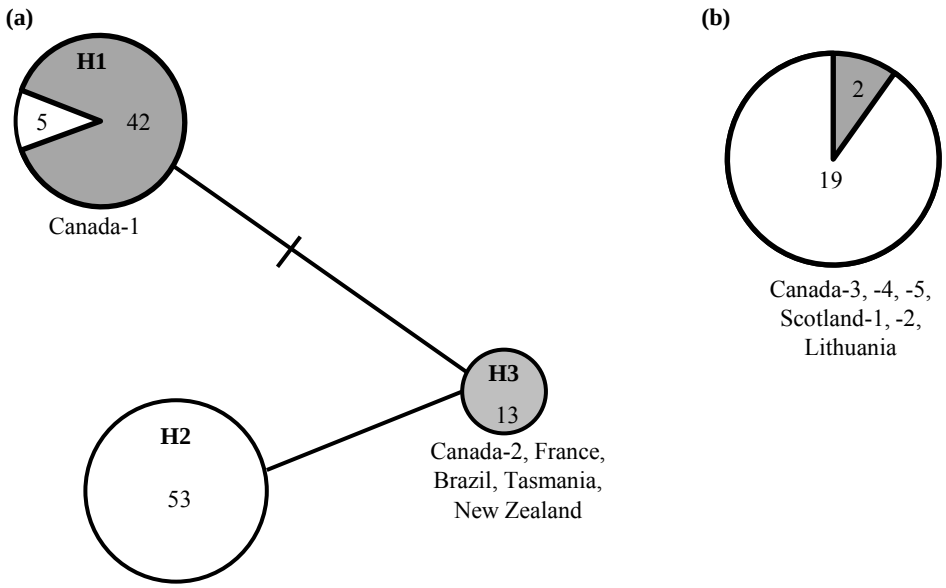
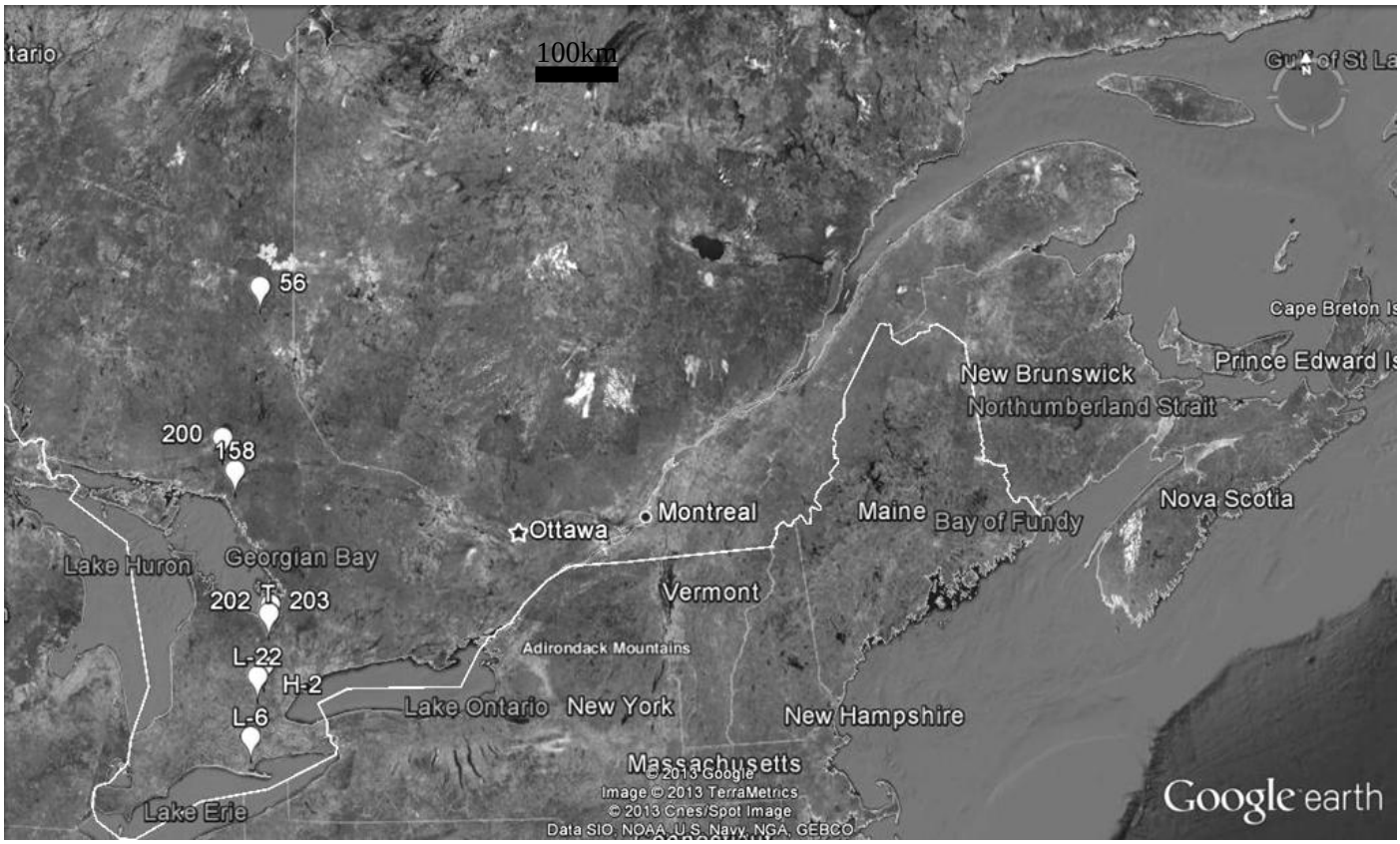


Figure 1. (a) Haplotype network of Amylostereum areolatum (n=113). Numbers of isolates per wasp species are shown within haplotype nodes. Dark grey indicates samples isolated from S. noctilio, white from S. nigricornis. (b) Haplotype network of A. chailletii isolates. Connecting lines specify one substitution, cross-bars specify additional substitutions separating haplotypes. Below the nodes are countries from which the haplotypes have previously been sampled (accession numbers in electronic supplementary material S11).

S1: Supplementary figure 1: Sampling locations of trees felled for collection of *Sirex* wasps are indicated by white balloons. The black scale bar indicates a distance of 100km.



S2: Supplementary table 1: Storage collection information for wasp (EntoStock Collection*) and fungal (CMW* samples used in this study

Sample Number	EntoStock number	CMW number of fungal isolate	Sampling site	Tree
92	296	36936	202	1
95	297	36937	202	1
98	298	36938	202	1
105	299	37078	L6	2
107	300	37032	202	2
121	301	36989	L6	3
123.1	302	37053	202	1
123.2	303	36939	202	1
123.3	304	37033	202	1
124	305	37054	202	1
125	306	36940	202	1
126	307	36941	203	2
127	308	37055	203	2
128	309	37056	203	1
141	310	36942	L6	2
142	311	36990	L6	3
144	312	37057	T	3
146	313	36943	202	1
150	314	37058	L6	3
151	315	39644	L6	2
152	316	36991	L6	2

153	317	36945	L6	2
154	318	36992	L6	2
161	319	36993	203	2
163	321	37035	202	1
166	323	36946	L6	2
167	324	36947	L6	2
168	325	37206	L6	1
169	326	36994	L22	1
170	327	36995	T	2
192	328	36996	L22	1
193	329	36997	L6	2
200	331	36998	202	2
208	333	36999	203	2
209	334	37000	202	2
213	335	37079	202	2
214	336	37060	L6	2
215	337	37061	H2	1
220	338	37062	202	2
222	339	37001	L6	2
228	340	36949	L6	3
229	341	37002	L6	2
230	342	37003	L6	2
231	343	36950	202	1
232	344	36951	202	2
250	345	37063	56	1
251	346	37064	202	2
254	347	37005	201	2
260	348	37006	202	2
265	349	37036	201	1
266	350	37080	201	1
267	351	36952	203	2
277	353	37065	56	1
278	354	36954	203	2
283	355	36955	56	1
284	356	36956	203	2
285	357	36957	200	1
286	358	36958	200	1
287	359	36959	200	1
288	360	36960	200	1
289	361	37007	200	1
290	362	37037	200	1
291	363	37081	200	1
292	364	36961	200	1
293	365	37066	200	1
294	366	37067	200	1
295	367	36962	200	2
296	368	37038	200	2
309	369	37008	200	1
310	370	36963	200	1
311	371	36964	200	1
312	372	36965	200	1

313	373	37009	200	2
314	374	37010	203	2
315	375	37011	56	1
324	376	36966	200	1
325	377	37207	201	1
332	378	37012	200	1
334	379	37039	200	1
335	380	36967	200	1
337	381	36968	158	1
338	382	37068	200	2
348	383	37013	56	1
357	384	36969	200	1
358	385	36970	200	1
359	386	36971	200	1
360	387	36972	200	1
361	388	37040	200	2
362	389	37041	203	2
375	390	37014	158	2
376	391	36973	158	1
378	392	36974	T	2
380	393	37015	158	1
382	394	36975	200	1
384	395	36976	200	1
385	396	37208	200	1
386	397	36977/37016	200	1
387	398	37017	158	2
388	399	37083	158	2
389	400	37069	158	1
390	401	36978	158	1
393	402	36979	L22	1
407	403	36980	200	1
408	404	37084	158	2
412	405	37085	158	1
414	407	37043	158	1
421	408	37070	158	2
422	409	37071	158	2
423	410	37072	158	2
424	411	37018	158	2
425	412	37073	158	2
426	413	37209	158	2
427	414	36981	158	2
428	415	37074	158	1
429	416	37086	158	1
430	417	37019	158	1
431	418	37075	158	1
432	419	37087	158	1
433	420	37020	158	1
434	421	37044	158	1
437	422	37021	158	1
440	423	37076	201	1
441	424	36982	201	1

442	425	36983	201	1
443	426	37077	L22	1
444	427	36984	T	2
459	428	37022	158	2
460	429	36985	158	2
462	430	36986	158	1
464	431	36987	158	1
476	432	37045	L22	1
477	433	37023	L22	1
514	434	37024	158	1
517	435	36988	L22	1

* Permanent collections of the Forestry and Agricultural Biotechnology Institute

S3: Supplementary table 2: Representative COI samples submitted to GenBank

Sample number	Wasp species	COI accession number	MS haplotype	Location
EB296	*Sirex noctilio*	KC310477	1	202
EB335	*Sirex noctilio*	KC310480	3	202
EB336	*Sirex noctilio*	KC310481	3	L6
EB319	*Sirex noctilio*	KC310478	1	203
EB328	*Sirex noctilio*	KC310479	3	L22
EB337	*Sirex noctilio*	KC310482	1	H2
EB342	*Sirex noctilio*	KC310483	1	L6
EB345	*Sirex nigricornis*	KC310484	1	56
EB347	*Sirex noctilio*	KC310485	1	201
EB362	*Sirex nigricornis*	KC310486	2	200
EB365	*Sirex nigricornis*	KC310487	1	200
EB375	*Sirex nigricornis*	KC310488	2	56
EB392	*Sirex noctilio*	KC310489	1	T
EB408	*Sirex nigricornis*	KC310490	2	158
EB424	*Sirex nigricornis*	KC310491	2	201
EB432	*Sirex nigricornis*	KC310492	1	L22

S4: Supplementary table 3: Representative mtSSU samples submitted to GenBank

Sample number	mtSSU accession number	Fungal species	Wasp species isolated from	mt SSU haplotype	Location
36936	KC296918	*A. areolatum*	*S. noctilio*	1	202
36993	KC296895	*A. areolatum*	*S. noctilio*	1	203
36994	KC296896	*A. areolatum*	*S. noctilio*	1	L22
37061	KC296899	*A. areolatum*	*S. noctilio*	1	H2
37003	KC296900	*A. areolatum*	*S. noctilio*	1	L6
37063	KC296901	*A. areolatum*	*S. nigricornis*	1	56
37005	KC296902	*A. areolatum*	*S. noctilio*	1	201
37066	KC296904	*A. areolatum*	*S. nigricornis*	1	200
36974	KC296908	*A. areolatum*	*S. nigricornis*	1	T
36975	KC296910	*A. areolatum*	*S. noctilio*	1	T
37077	KC296916	*A. areolatum*	*S. nigricornis*	1	L22
37037	KC296903	*A. areolatum*	*S. nigricornis*	2	200
37013	KC296907	*A. areolatum*	*S. nigricornis*	2	59

37015	KC296909	*A. areolatum*	*S. nigricornis*	2	158
36982	KC296914	*A. areolatum*	*S. nigricornis*	2	201
37019	KC296913	*A. areolatum*	*S. nigricornis*	2	158
36940	KC296893	*A. areolatum*	*S. noctilio*	3	202
36945	KC296894	*A. areolatum*	*S. noctilio*	3	L6
36996	KC296897	*A. areolatum*	*S. noctilio*	3	L22
37060	KC296898	*A. areolatum*	*S. noctilio*	3	L6
37010	KC296905	*A. chailletii*	*S. noctilio*	-	203
36968	KC296906	*A. chailletii*	*S. nigricornis*	-	158
36979	KC296911	*A. chailletii*	*S. nigricornis*	-	222
36980	KC296912	*A. chailletii*	*S. nigricornis*	-	200
36983	KC296915	*A. chailletii*	*S. nigricornis*	-	201
36984	KC296917	*A. chailletii*	*S. noctilio*	-	T

S5: Supplementary table 4: Representative samples used to assess diversity

Sample number	ITS accession number(s)*	IGS Accession number(s)*	IGS Type	mtSSU Haplotype	Location**
CMW36936	KC329719 / KC329720 / KC329721	KC310492	D	1	202-1
CMW36940	KC329722	KC296876 / KC296877	BD	3	202-1
CMW36945	KC329723	KC296878 / KC296879	D	3	L6-2
CMW36993	KC329724 / KC329725 / KC329726 / KC329727	KC296880	D	1	203-2
CMW37060	KC329748 / KC329749	KC296891 / KC296892	BD	3	L6
CMW36996	KC329728 / KC329729 / KC329730 / KC329731	KC296881	D	3	L22-1
CMW36974	KC329751 / KC329750	KC296890	D	1	T-2
CMW37006	KC329732 / KC329733 / KC329734	KC296882	D	1	202-2
CMW37037	KC329745 / KC329746 / KC329747	KC296887	E	2	200-1
CMW37009	KC329736 / KC329735	KC296883 / KC296884	BE	2	200-2
CMW37015	KC329739 / KC329740	KC296885 / KC296886	BE	2	158-1
CMW37019	KC329741 / KC329742 / KC329743 / KC329744	KC296888 / KC296889	BE	2	158-1

***Multiple accession numbers are given where multiple alleles were found**
****-1 and -2 indicate which tree wasps emerged from at each site**

S6: PCR and DNA extraction conditions

Wasp DNA was extracted from thorax tissue using the *prep*GEM™ Insect DNA extraction kit from ZyGEM Corporation Ltd, as per manufacturers' instructions. Fungal DNA was extracted using a modified phenol:chloroform extraction method [1]

All PCRs were carried out in either a BIO RAD iCycler or Veriti (Life Technologies Corporation) thermocycler.

PCR of the COI gene had a total volume of 25µl, 5µl of 5x MyTaq™ Reaction Buffer (Bioline), 0.1µM of both LCO1490 and HCO 2198 [2], 0.5µl MyTaq™ DNA polymerase (Bioline), 30-100ng of template DNA was used. Cycling conditions were 95°C for 5 minutes, followed by 41 cycles of 95°C for 30 seconds, 46.5°C for 60 seconds and 72°C for 60 seconds, with a final extension step of 72°C for 30 minutes.

PCR of the mtSSU gene had a total volume of 25µl, 2.5µl 10x FastStart Taq DNA Polymerase PCR Buffer (Roche Ltd), 50nM magnesium chloride (Roche Ltd), 400ng of each dNTP, 0.1µM of both MS1 and MS3 [3] and 2.5U FastStart Taq DNA Polymerase (Roche Ltd), 30-100ng of template DNA was used. Cycling conditions were 95^{o}C for 3 minutes, followed by 35 cycles of 95^{o}C for 45 seconds, 58^{o}C for 30 seconds and 72^{o}C for 60 seconds, with a final extension step of 72^{o}C for 10 minutes.

PCR of the IGS rDNA gene had a total volume of 25µl, 5µl of 5x MyTaq^{TM} Reaction Buffer (Bioline), 0.1µM of both P-1 and O-1 [4], 0.5µl MyTaq^{TM} DNA polymerase (Bioline), 30-100ng of template DNA was used. Cycling conditions were 94^{o}C for 1 minute, followed by 35 cycles of 95^{o}C for 30 seconds, 60^{o}C for 20 seconds and 72^{o}C for 30 seconds, with a final extension step of 72^{o}C for 7 minutes.

PCR of the ITS rDNA gene had a total volume of 25µl; 5µl of 5x MyTaq^{TM} Reaction Buffer (Bioline), 0.1µM of both ITS1 and ITS4 [3], 0.5µl MyTaq^{TM} DNA polymerase (Bioline), 30-100ng of template DNA was used. Cycling conditions were 95^{o}C for 5 minutes, followed by 13 cycles of 95^{o}C for 35 seconds, 55^{o}C for 55 seconds and 72^{o}C for 45 seconds, 13 cycles of 95^{o}C for 35 seconds, 55^{o}C for 55 seconds and 72^{o}C for 2 minutes, 13 cycles of 95^{o}C for 35 seconds, 55^{o}C for 55 seconds and 72^{o}C for 3 minutes, with a final extension step of 72^{o}C for 10 minutes.

PCR of cloning products had a total volume of 50µl; 5µl 10x FastStart Taq DNA Polymerase PCR Buffer with magnesium chloride (Roche Ltd), 400ng of each dNTP, 0.1µM of both SP6 and T7 and 2.5U FastStart Taq DNA Polymerase (Roche Ltd). Cycling conditions were 95^{o}C for 3 minutes, followed by 25 cycles of 95^{o}C for 30 seconds, 51^{o}C for 30 seconds and 72^{o}C for 30 seconds, with a final extension step of 72^{o}C for 10 minutes.

PCR and sequencing success rate was 100% for all reactions for all samples.

References

1. Slippers, B., Crous, P. W., Denman, S., Coutinho, T. A., Wingfield, B. D. & Wingfield, M. J. 2004 Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia.* **96,** 83-101.
2. Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology.* **3,** 294-299.
3. White, T. J., Lee, S. & Taylor, J. 1990 *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In PCR protocols: a guide to methods and applications (eds. D. H. G. Innis, I. J. Sninsky& T. J. White) 315-322. San Diego Academic Press
4. Coetzee, M. P., Wingfield, B. D., Harrington, T. C., Steimel, J., Coutinho, T. A. & Wingfield, M. J. 2001 The root rot fungus Armillaria mellea introduced into South Africa by early Dutch settlers. *Molecular Ecology.* **10,** 387-396.

S7: Modified Promega pGEM®-T Easy Vector System Protocol

1. **Ligation reaction:** Ligation reactions contained 2.5µl 2X Ligation Buffer, 0.5µl T4 DNA Ligase, 0.5µl pGEM®-T Easy vector and 1.5µl clean PCR product.
2. **Colony Screening:** Colony screening was performed using a colony PCR (conditions described in S2 above). DNA was obtained by using a pipette tip to 'pick' a colony, this was touched onto a replica plate and then placed in the PCR reaction mix. The tip was then removed and the reaction placed in a thermocycler.

S8: Evolutionary models and analysis parameters for phylogenetic analyses

Models of nucleotide substitution used for COI and mtSSU analyses are; (i) a three-parameter model with unequal base frequencies and gamma-distributed rate variation and (ii) a Felsenstein 1981 model with no invariable sites respectively. Node support for neighbour joining analyses was estimated using non-parametric bootstrapping.

The model of nucleotide substitution used for ITS analyses was a Tamura-Nei nucleotide substitution model with no invariable sites. Node support for maximum likelihood analyses was estimated using non-parametric and nearest neighbour interchange – subtree pruning and regrafting bootstrapping. In the Bayesian analysis the Markov chain Monte Carlo was run for 10 million generations, sampled every 100 steps with the first 25% of samples discarded as burnin.

S9: Supplementary table 5: Representative *Amylostereum areolatum* isolates and their VCG groupings

Sample number	mtSSU Haplotype	VCG*	Sampling Location**	Wasp species
37041	1	1.1	203-2	*S. noctilio*
37023	1	1.1	L22-1	*S. nigricornis*
36937	1	1.1	202-1	*S. noctilio*
36993	1	1.1	203-2	*S. noctilio*
37054	1	1.1	202-1	*S. noctilio*
37055	1	1.1	203-2	*S. noctilio*
37063	1	1.2	56-1	*S. nigricornis*
37020	1	1.2	158-1	*S. nigricornis*
37066	1	1.3	200-1	*S. nigricornis*
36965	1	1.4	200-1	*S. nigricornis*
37003	1	1.5	L6-2	*S. noctilio*
37006	1	1.6	202-2	*S. noctilio*
37037	2	2.1	200-1	*S. nigricornis*
37068	2	2.1	200-2	*S. nigricornis*
37018	2	2.1	158-2	*S. nigricornis*
36961	2	2.2	200-1	*S. nigricornis*
37017	2	2.2	158-2	*S. nigricornis*
37009	2	2.3	200-2	*S. nigricornis*
36955	2	2.4	56-1	*S. nigricornis*
37019	2	2.5	158-1	*S. nigricornis*
39644	3	3.1	L6-2	*S. noctilio*
36991	3	3.1	L6-2	*S. noctilio*
36947	3	3.1	L6-2	*S. noctilio*
36997	3	3.1	L6-2	*S. noctilio*
37060	3	3.1	L6-2	*S. noctilio*
37079	3	3.2	202-2	*S. noctilio*
37001	3	3.3	L6-2	*S. noctilio*

*** VCGs are named first according to mtSSU haplotype and second VCG number identified within that haplotype. These numbers are separated by a '.'**
****-1 and -2 indicate which tree wasps emerged from at each site**

S10: Supplementary Figure 2: (a) Rooted neighbour-joining trees of mitochondrial small subunit gene for fungal species identification. **(b)** Rooted neighbour-joining cytochrome oxidase *c* subunit 1 gene for wasp species identification. **(c)** Table of extent of fungal symbiont switching based on wasp and fungal species identification

Figure 2(c)	Number of wasps	Number of wasps carrying incorrect* fungal symbiont	Percentage of symbiont switching
Sirex nigricornis	77	58	75.3
Sirex noctilio	57	2	3.5

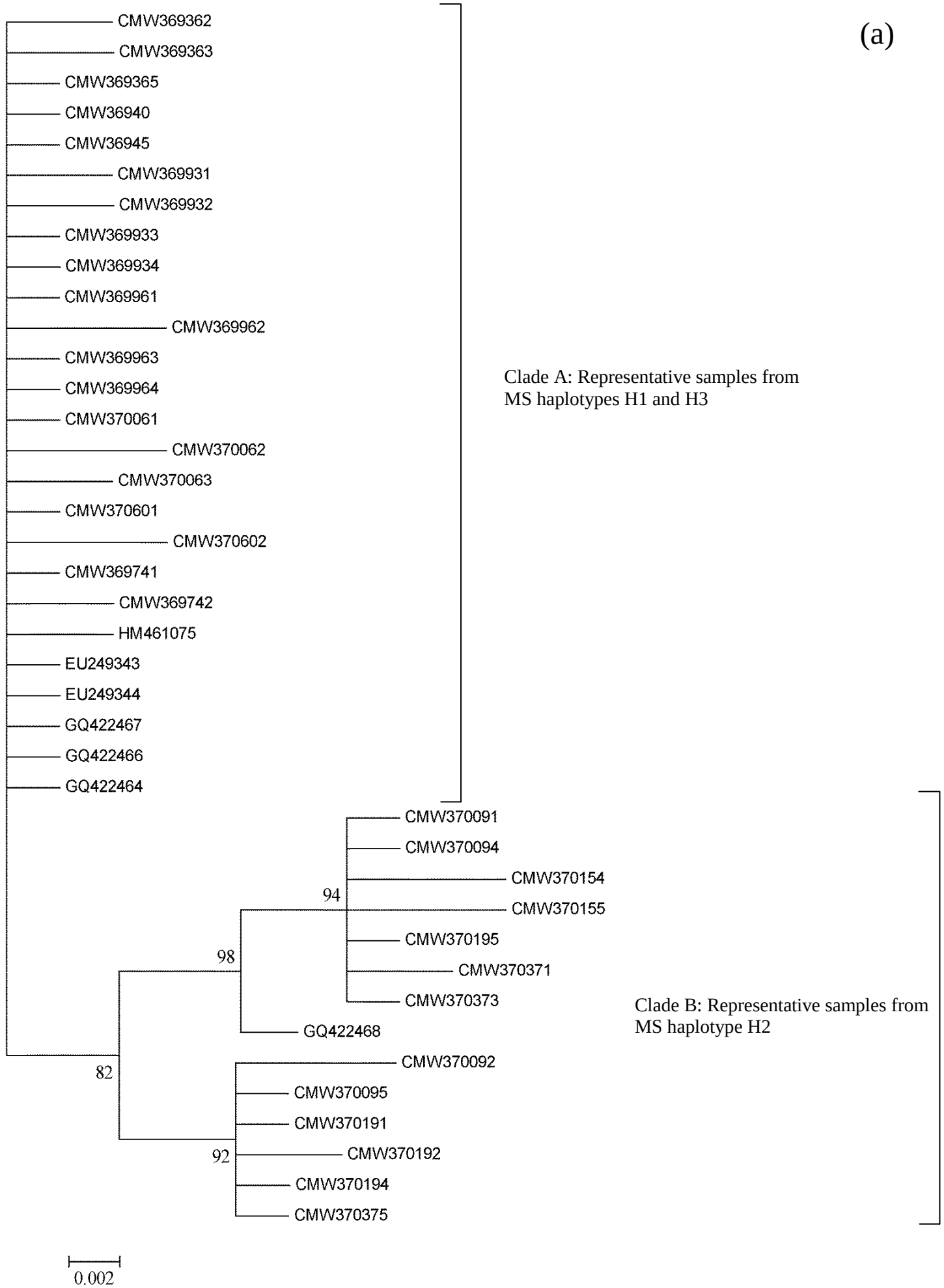
**Sirex nigricornis* is expected to carry *Amylostereum chailletii*, and *S. noctilio* is expected to carry *A. areolatum*

S11: Supplementary table 6. Accession numbers of previously isolated *Amylostereum areolatum* mtSSU used for identification of mtSSU haplotypes and comparison to previously identified *A. areolatum* multilocus genotypes

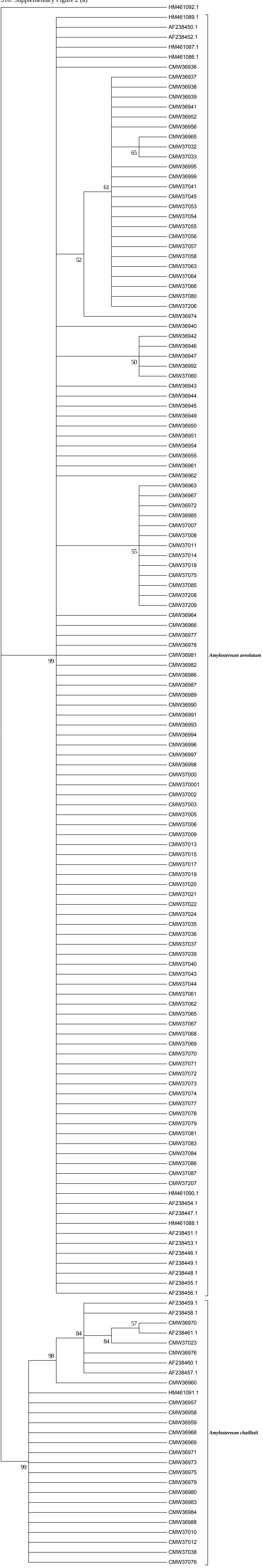
Accession number	Species	Location	Figure 1 Reference
HM461091.1	*A. chailletii*	Canada	Canada-5
HM461087.1	*A. areolatum*	Canada	Canada-1
HM461086.1	*A. areolatum*	Canada	Canada-2

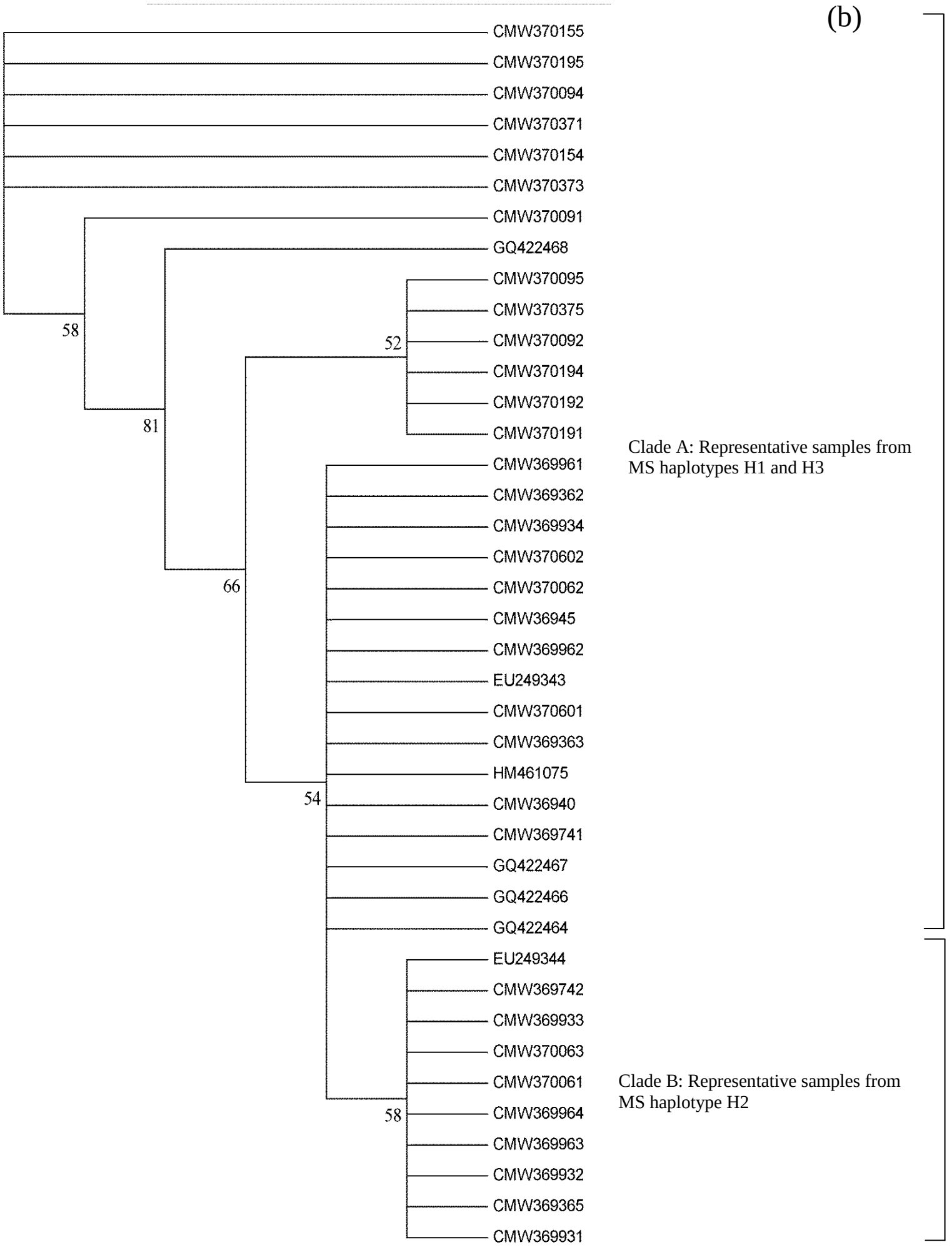
AF238459.1	*A. chailletii*	Canada	Cananda-3
AF238457.1	*A. chailletii*	Scotland	Scotland-1
AF238447.1	*A. areolatum*	France	France-1
AF238460.1	*A. chailletii*	Lithuania	Lithuania
AF238458.1	*A. chailletii*	Canada	Cananda-4
AF238456.1	*A. areolatum*	Tasmania	Tasmania
AF238452.1	*A. areolatum*	Brazil	Brazil-1
AF238446.1	*A. areolatum*	New Zealand	New Zealand
AF238461.1	*A. chailletii*	Scotland	Scotland-2

S12: Supplementary figure 3: (a) Unrooted Bayesian inference tree of ITS sequences. **(b)** Unrooted,Maximum Likelihood tee of ITS sequences. These are condensed trees where nodes with bootstrap values of less than 50% have been collapsed into polytomies.



S10: Supplementary Figure 2 (a)




(b)
CMW370155
CMW370195
CMW370094
CMW370371
CMW370154
CMW370373
CMW370091
GQ422468
CMW370095
CMW370375
CMW370092
CMW370194
CMW370192
CMW370191
CMW369961
CMW369362
CMW369934
CMW370602
CMW370062
CMW36945
CMW369962
EU249343
CMW370601
CMW369363
HM461075
CMW36940
CMW369741
GQ422467
GQ422466
GQ422464
EU249344
CMW369742
CMW369933
CMW370063
CMW370061
CMW369964
CMW369963
CMW369932
CMW369365
CMW369931
58
52
81
66
54
58
Clade A: Representative samples from MS haplotypes H1 and H3
Clade B: Representative samples from MS haplotype H2

S10: Supplementary Figure 2 (b)