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Differentiating individuals of *Armillaria* species in New Zealand forests

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Abstract

Background: *Armillaria novae-zelandiae* and *A. limonea* occur naturally as wood decay fungi in native forests in New Zealand. As pathogens they are responsible for significant root disease in trees and shrubs in plantations, crops and urban parks and gardens. A thorough understanding of their population dynamics entails knowledge of the spatial arrangement of their individual mycelia or genets. In previous work the distributions of vegetative compatibility groups (VCGs) of these fungi were mapped in an area of native forest prior to and after replacement by a young *Pinus radiata* plantation. With the advent of molecular technology, it has become possible to test species identities made earlier using culture techniques and to verify whether or not their VCGs, determined by incompatibility reactions between paired cultures, represent distinct individual genets.

Methods: Stock subcultures of isolates representing each VCG were recovered from storage in order to obtain DNA. Extracted DNA was subjected to a polymerase chain reaction procedure (UP-PCR) using 11 universal primers to assess genetic variation between subcultures. Bands were scored as either present or absent for each primer-subculture combination and cluster analysis was undertaken by generating dendrogram trees to reveal genetic groupings among subcultures.

Results: DNA cluster analysis divided subcultures of isolates into two species groups, *A. novae-zelandiae* and *A. limonea*, corresponding to identities determined through culture morphology. Within species, subcultures grouped into clusters that matched VCGs determined by earlier culture pairing. There was little indication of genetic variation within VCGs, except for one of *A. limonea*, which comprised two sub-clusters.

Conclusions: The *Armillaria* species and VCGs identified by culture techniques in the laboratory were verified by independent molecular methodology. In general, the VCGs represent discrete individual genets or colonies in the field. Techniques that differentiate isolates based on differences in their DNA sequence provide a quick alternative to time-consuming laboratory culture methods for resolving population spatial structure. However, some complementary isolate pairing may be necessary when rationalising the significance of groupings in dendrogram trees.

Keywords: *Armillaria limonea*; *Armillaria novae-zelandiae*; DNA cluster analysis; fungal populations; genets; universally primed PCR; vegetative compatibility groups

Introduction

To fully comprehend the nature of fungal populations in ecological studies it is necessary to investigate the spatial configuration of their individual mycelia. One approach for filamentous species is to map the distribution and range of their vegetative compatibility groups (VCGs; also known as somatic incompatibility, SI, groups). Vegetative compatibility groups are recognised by the formation of an interaction zone between paired field isolates in laboratory culture, denoted macro- or microscopically by such features as a gap, a barrier zone or a region of hyphal dissolution between the two dissimilar mycelia.

However, while mutual incompatibility distinguishes mycelia that differ genotypically, isolates belonging to the same VCG may not be clones or genets of a single mycelial colony (Tyson et al. 2002). Work with ascomycete fungi has shown that compatibility is governed genetically by a series of *het* recognition loci (for *het*erokaryon compatibility; also known as *vic* loci, for *v*egetative *i*n*c*ompatibility), each of which contains two or more alleles (Glass et al. 2000; Moll et al. 2016). Isolates that share identical *het* loci alleles, and which are therefore mutually compatible in culture, may differ genetically at other sites. With basidiomycetes it appears that field isolates of the same VCG are more likely to be genetically identical (Malik & Vilgalys 1999), but even with these fungi there are examples of genetic variation among individuals of the same VCG (Jacobson et al. 1993; Matsumoto et al. 1996; Worrall 1997; Stenlid & Vasiliauskas 1998). Knowledge of incompatibility among fungi has been reviewed by a number of authors (Leslie 1993; Worrall 1997; Malik & Vilgalys 1999; Burnett 2003; Stenlid 2008; Krnjaja et al. 2013; also, Heinzelmann et al. 2019).

Identifying and plotting VCGs has been helpful in studying *Armillaria* communities in both natural vegetation and artificial ecosystems in different parts of the world (e.g., Shaw & Roth 1976; Korhonen 1978; Kile 1983, 1986; Hood & Morrison 1984; Rishbeth 1991; Rizzo et al. 1995; Guillaumin et al. 1996; Legrand et al. 1996; Abomo-Ndongo & Guillaumin 1997; Bruhn et al. 1997; Dettman & van der Kamp 2001; Ferguson et al. 2003; Prospero et al. 2003; Mihail & Bruhn 2005; Szewczyk et al. 2015; and others). Similar work in New Zealand has shown that *Armillaria novae-zelandiae* (G. Stevenson) Herink and *Armillaria limonea* (G. Stevenson) Boesewinkel occur in the form of numerous VCGs in native forests, plantations and orchards of kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson), supporting other research demonstrating that basidiospore colonisation plays an important ecological role, particularly for *Armillaria novae-zelandiae* (Horner 1992; Hood et al. 2002a,b, 2004; Power et al. 2008).

In one investigation, isolates representative of the *Armillaria* VCGs identified within plots in an area of native forest in the central North Island were placed in storage for possible future study (Hood & Sandberg 1987, 1989, 1993). With the subsequent development of newer techniques, it became possible to examine these stock isolates using a molecular approach (Dodd et al. 2006). A comparison between their VCG identities, as determined earlier by the culture pairing procedure, and the uniqueness of their DNA composition, would indicate how well the two procedures were in accord. It would, in addition, reveal the level of genetic variation, if any, among isolates of the same VCGs. Undertaking this work would also determine if the molecular procedure was a simpler, quicker and more precise technique than culture methodology for studying *Armillaria* populations. This paper describes how this was done and the outcomes that were obtained.

Materials

Field isolates from Plot 3 (Hood & Sandberg 1987), stored individually under oil or water in phials, were selected for the study. Most were held as two or more duplicate 'subcultures' of the original isolates (Hood & Sandberg 1993) and were between 15 and (mostly) 21 years old when cultured out from storage. Altogether 69 subcultures were successfully recovered of 26 original *Armillaria novae-zelandiae* isolates and 15 *A. limonea* isolates, as previously determined by culture procedures (Hood & Sandberg 1987). These isolates represented 16 VCGs of *A. novae-zelandiae* and 6 VCGs of *A. limonea*, established earlier using culture pairing (Table S1). For this study, cultures were labelled according to the formula 'Axxx_a_b.c', where 'Axxx' indicates *A. limonea* (Alim) or *A. novae-zelandiae* (Anz), 'a' is the VCG number (as in the earlier publications), 'b' is a unique isolate number and 'c' is one of up to four (1–4) subcultures of the original isolate (Table S1).

Methods

DNA extraction

Mycelium was harvested from 4- to 6- week-old potato dextrose broth cultures grown at 24 °C. DNA was extracted from 100 mg fresh mycelium of each isolate with the Gentra PureGene Plant DNA extraction kit (Progenz, Auckland, New Zealand) using a micro-pestle for cell disruption.

UP-PCR

DNA obtained from all isolate subcultures was subjected to universally primed-polymerase chain reaction methodology (UP-PCR) with 11 primers to assess genetic variation between the isolates. In this method single primers are used in a reaction and these were 0.3-1, 3-2, AA2M2, AS4, AS15, AS15inv, L15, L15/AS19, L21, L45 and FokI (Lübeck et al.1998; Lübeck & Lübeck 2005). Each 25 µL amplification reaction contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each of dATP, dTTP, dGTP, and dCTP, 20 pmoles primer, 2.5 mM $MgCl_2$, 25 ng genomic DNA, and 0.25 U Taq DNA polymerase (Roche Diagnostics N.Z. Ltd).

The PCR reactions consisted of an initial 5 min at 94°C followed by 45 cycles of 50 s at 94°C, 1 min at the specified annealing temperature for that primer as reported in Tyson et al. (2002) or 52°C for Fok1, and 1 min at 72°C, with a final extension of 72°C for 7 min.

PCR products were separated by electrophoresis in 1.6% agarose gels (Fig. 1). Four gels were run for each UP-PCR primer, with subcultures of the same VCGs placed in adjacent lanes (Table S2; the order on each gel was the same for all primers). Selected lanes across the gel were loaded with a 1Kb ladder (Invitrogen, Auckland NZ) to use for the gel normalisation process.

Bands were visualised using SYBR Gold nucleic acid gel stain (Invitrogen, Auckland, NZ) to increase band resolution. For each primer the four gels were assessed together. Gels were normalised and bands at a single distance migratory position scored as either present or

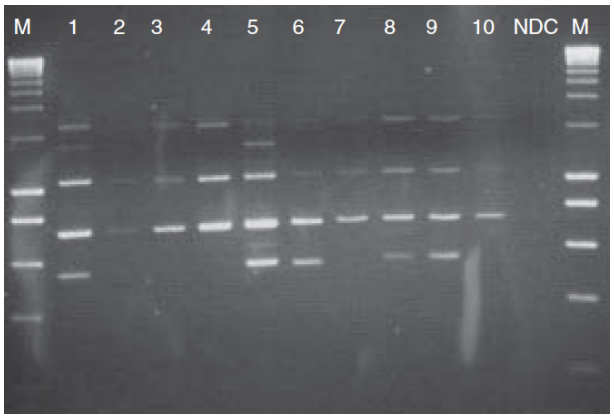

FIGURE 1: Early gel of AS15 UP-PCR products showing differences between VCG groups among isolates of *Armillaria limonea*. Lanes 2, 3, 4, 7 and 10 represent isolates of VCG 33; lanes 1 and 5, of VCG 32; lanes 6, 8 and 9, of VCG 34. M, size marker lane; NDC, no DNA control lane.

absent for each primer-isolate combination using the software BioNumerics (Applied Maths, https://www.applied-maths.com/bionumerics). Note, faint bands were excluded from the analysis as preliminary studies showed their presence/absence varied in multiple reactions run for the same DNA/primer combinations making them unreliable for pattern comparisons between isolates.

Analysis

The UP-PCR procedure yields an array of binary data suitable for cluster analysis, with bands at each unique distance migratory position from the loading combs treated as an independent character for each primer. However, not all primers produce meaningful groupings. In addition, small differences in band identification were found between some subcultures of the same isolate with different primers. In order to use only data from the most functional primers, including those with fewest discrepancies, 2 × 2 grid matrices were prepared for all primers combined (Table S3), as well as for each individual primer. Cladistic dendrograms were constructed with combined data from all primers and from just three selected primers (3-2, AS4, 0.3-1; Table S4) using Ward's minimum variance method (Ward 1963), which at each step merges the two clusters that provide the smallest increase in the combined error sum of squares. This analysis was performed using the hclust function in R (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/hclust). In addition, the R pvclust function (https://www.rdocumentation.org/packages/pvclust/versions/2.2-0/topics/pvclust; Suzuki & Shimodaira 2006) was used to assign p values to each cluster using bootstrap resampling techniques.

Results

Species identities determined earlier by culture methods in the laboratory were confirmed. For all isolates, both species grouped separately using UP-PCR and cluster analysis by means of the Ward procedure with data for the three selected primers combined (Fig. 2).

In addition, there was a clear trend for most VCGs to separate out from one another in a systematic fashion using cluster analysis of the DNA data even if these groupings were not always judged to be statistically significant (Fig. 2). Exceptions were the VCG pairs 22, 65; 16, 23; 21, 56 of *A. novae-zelandiae*, and 34, 35 of *A. limonea*, whose components were not distinguished in the cladogram (Fig. 2).

There was little evidence of genetic variation within VCGs using cluster analysis. One exception was VCG 33 of *A. limonea*, which separated out into two related subclusters (Fig. 2). Apparent variation within *A. novae-zealandiae* VCGs 15 and 18 was not considered significant (Fig. 2). Likewise, the apparent separations of two subcultures of each of two isolates were not significant (Fig. 2; ANZ_15_9.1, ANZ_15_9.2; and ANZ_18_12.1, ANZ_18_12.2).

Discussion

Armillaria species are important wood decomposer fungi in native forests in New Zealand. Four species are known in southern beech forests (*Fuscospora* and *Lophozonia*, Nothofagaceae), of which at least two (*Armillaria novae-zelandiae* and *A. limonea*) also occur in podocarp-hardwood forests (Hood et al. 2004, 2019; Dodd et al. 2010). *Armillaria novae-zelandiae* and *A. limonea* are significant root pathogens of *Pinus radiata* D.Don and at one time caused severe mortality in young trees when plantations of this host were established on sites cleared of indigenous forest. Losses were also formerly sustained in orchards of kiwifruit as a result of root and collar infection by *A. novae-zelandiae*. For these reasons, much research has been undertaken to understand and manage the disease in these crops. This work has included molecular studies to develop PCR primers facilitating the ready identification of individual *Armillaria* species (Dodd et al. 2010). Although less important than previously, *Armillaria* species still cause significant disease and mortality in trees and shrubs in plantations and orchards, as well as in urban and rural settings in New Zealand. An awareness of the nature of the populations of the disease agents therefore remains relevant. The purpose of the present investigation was to examine and compare two approaches to recognising and distinguishing individual mycelia in order to be able to map their spatial distribution in ecological studies.

The results of the DNA-based technology confirmed the findings from the earlier laboratory culture work with both *Armillaria* species. The previously determined species identities of the field isolates were verified by the molecular method, as was the distinctiveness of the individual VCGs. In addition, the substantial variation observed between the VCG genets, indicating that

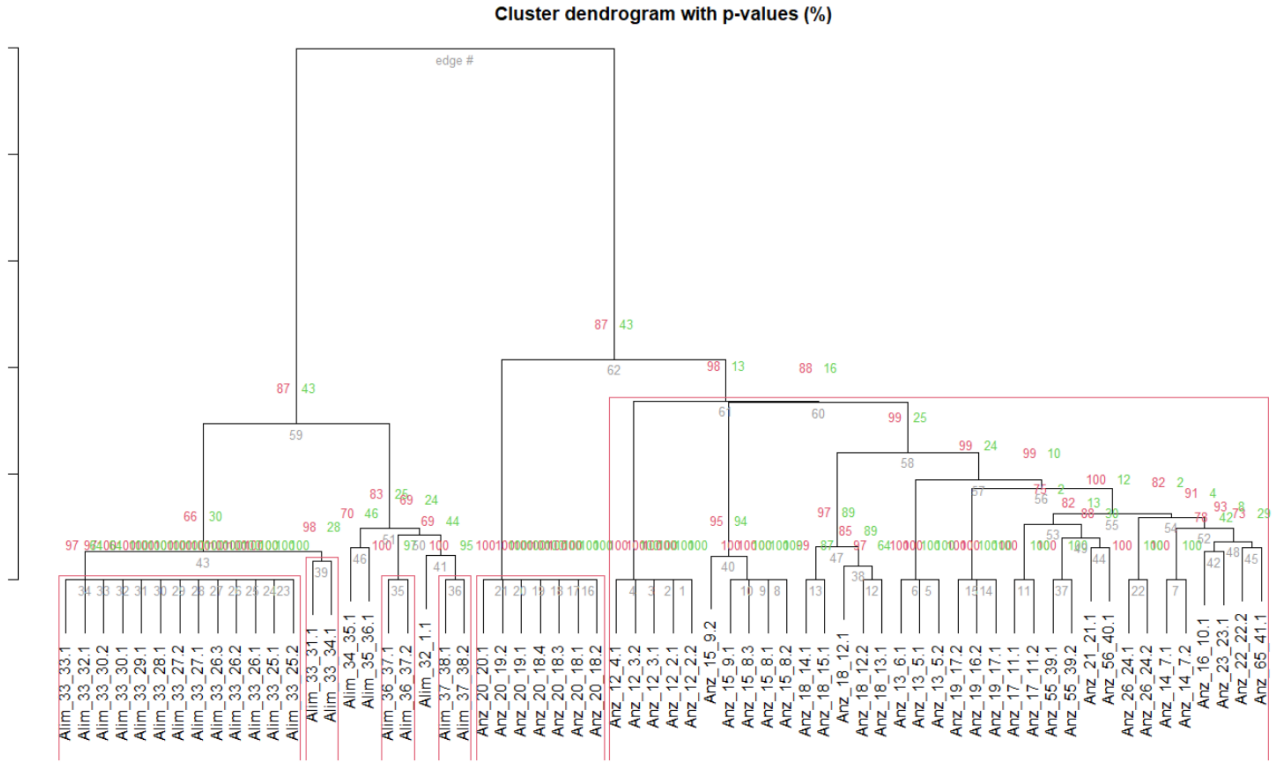

FIGURE 2: Cluster analysis of subcultures of *Armillaria* field isolates for primers 3_2, AS4, 0.3_1 combined, using Ward's minimum variance method (Ward 1963). Label code: Axxx_a_b.c, where Axxx indicates *A. novae-zelandiae* or *A. limonea* (as identified in culture), a is the VCG number (determined by incompatibility between pairs of cultures), b is the assigned isolate number and c is a laboratory subculture of that isolate (1-4). Results of bootstrap resampling using the R pvclust procedure (Suzuki and Shimodaira 2006): xx (red), approximately unbiased (AU) p-value % (generally considered the best assessment of strength of support for the cluster), yy (green), bootstrap probability % (BP), zz (grey) rank of the cluster. Red rectangles indicate clusters with AU>95.

recombination has occurred, corroborates the role of basidiospores as significant in dispersal, especially of *A. novae-zelandiae*. However, there was little evidence of genetic variation within VCGs, implying that each represented a distinct mycelial colony (diploid, as indicated by their dark, crustose, depressed culture morphology; haploid isolates tend to take on a different appearance). An earlier acceptance of variation within VCGs of both *Armillaria* species is therefore here qualified (Hood 2012; Hood et al. 2019). However, this does not mean that such within-VCG variation does not occur with *Armillaria* species (Kile 1983; Guillaumin et al. 1996; Szewczyk et al. 2015), just that it appears to be less likely among isolates taken from the same individual vegetative field colonies. In the present study a difference was apparent between subcultures of two isolates (Alim_33_31.1 and Alim_33_34.1) and those of the other isolates within VCG 33 (*A. limonea*). Both these isolates were obtained, two years apart, from a restricted zone, 4 m across, within the full area, ≥ 14 m wide, occupied by VCG 33 in Plot 3. Possible explanations include somatic mutation or perhaps the formation of adjacent sib-related colonies with a common parent derived from basidiospores from one local fruitbody cluster (Kile 1983; Rishbeth 1991; Hood & Sandberg 1993; Hood 2012). A change during storage appears less likely (Hood & Sandberg 1993). VCG 33 was represented by a comparatively large number of isolates. It may be possible that more isolates might have revealed similar variation within some other VCGs. Likewise, four pairs of VCGs that each grouped together were represented by only one isolate per VCG. Would these VCGs have been differentiated using the DNA-based approach had there also been more isolates?

In the UP-PCR technique, the selected primer binds to regions of the entire genome of the same (or nearly the same) base pair sequence. In this procedure, the primers employed are designed to target non-coding genome regions that retain mutations and are hence more variable than coding segments. Primers are longer and binding conditions more stringent than the similar RAPD technique (Bardakci 2001). Nevertheless, despite this, these highly variable regions may or may not be phylogenetically informative. Only some primers are successful in revealing meaningful groupings, depending on the genome regions being sampled. However, once obtained, useful data from appropriate primers can be combined to increase precision and reduce "noise". In this study, data from some primers resulted in only partially workable, semi-rational patterns of clustering in

which VCG clusters were, nonetheless, still recognisable. However, the three primers AS4, 0.3-1 and 3-2, provided functional data suitable for cluster analyses, giving rise, when combined, to fully coherent results that matched those from the earlier research.

The culture pairing and DNA-based procedures proved complementary, and in combination were successful in identifying discrete mycelial field colonies of *A. novae-zelandiae* and *A. limonea* previously mapped as VCGs. The methods for identifying species and VCGs in culture are laborious and time consuming, although a multiple pairing design procedure has been prescribed for reducing some of the effort (Burgess et al. 2009). The UP-PCR technique was quicker and effective, using the three primers 3-2, AS4, 0.3-1 followed by clustering analysis by means of the Ward procedure. However, in this study, resolving which sub-groups represented authentic field colonies relied on information from pairing of cultures. One way to realise this goal in a future study would be to subject DNA data to cluster analysis, "calibrating" resultant groups by pairings among a limited sample of isolates in the laboratory to support statistical estimates of group significance.

Authors' contributions

SD and FS conducted the molecular and laboratory work with cultures supplied by IH. Statistical analyses were performed by MK. CS undertook some preliminary analyses. The paper was written by IH, SD and MK and the final version accepted by all co-authors.

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Additional File

Tables S1-S4

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Differentiating individuals of *Armillaria* species in New Zealand forests

Sarah Dodd, Farhat Shah, Mark Kimberley, Chanatda Somchit and Ian Hood

Supplementary material

Table S1. Details of isolates.

Isolate code[1]	Species (by culture procedure)	VCG (by culture pairing)	Source[2]	Published reference (Hood & Sandberg 1987, 1989, 1993)
Anz_12_2.1	Anz	12	00.08	1987
Anz_12_2.2	Anz	12	00.08	1987
Anz_12_3.1	Anz	12	04.16	1987
Anz_12_3.2	Anz	12	04.16	1987
Anz_12_4.1	Anz	12	06.20	1987
Anz_13_5.1	Anz	13	00.34	1987
Anz_13_5.2	Anz	13	00.34	1987
Anz_13_6.1	Anz	13	02.34	1987
Anz_14_7.1	Anz	14	06.28	1987
Anz_14_7.2	Anz	14	06.28	1987
Anz_15_8.1	Anz	15	10.22	1987
Anz_15_8.2	Anz	15	10.22	1987
Anz_15_8.3	Anz	15	10.22	1987
Anz_15_9.1	Anz	15	14.20	1989
Anz_15_9.2	Anz	15	14.20	1989
Anz_16_10.1	Anz	16	18.32	1987
Anz_17_11.1	Anz	17	18.26	1987
Anz_17_11.2	Anz	17	18.26	1987
Anz_18_12.1	Anz	18	18.34	1987
Anz_18_12.2	Anz	18	18.34	1987
Anz_18_13.1[3]	Anz	18	28.24	1987
Anz_18_14.1[3]	Anz	18	28.24	1987
Anz_18_15.1	Anz	18	34.22	1987
Anz_19_16.2	Anz	19	20.16	1987
Anz_19_17.1	Anz	19	24.16?	1987
Anz_19_17.2	Anz	19	24.16?	1987
Anz_20_18.1	Anz	20	28.10	1987
Anz_20_18.2	Anz	20	28.10	1987
Anz_20_18.3	Anz	20	28.10	1987
Anz_20_18.4	Anz	20	28.10	1987
Anz_20_19.1	Anz	20	32.10	1987
Anz_20_19.2	Anz	20	32.10	1987
Anz_20_20.1	Anz	20	34.06	1989
Anz_21_21.1	Anz	21	20.22	1987
Anz_22_22.2	Anz	22	22.32	1987

Anz_23_23.1	Anz	23	26.30	1987
Anz_26_24.1	Anz	26	F243	1987
Anz_26_24.2	Anz	26	F243	1987
Alim_32_1.1	Alim	32	04.26	1987
Alim_33_25.1	Alim	33	20.34	1987
Alim_33_25.2	Alim	33	20.34	1987
Alim_33_26.1	Alim	33	22.26	1987
Alim_33_26.2	Alim	33	22.26	1987
Alim_33_26.3	Alim	33	22.26	1987
Alim_33_27.1[4]	Alim	33	22.30	1987
Alim_33_27.2[4]	Alim	33	22.30	1987
Alim_33_28.1[4]	Alim	33	22.30	1987
Alim_33_29.1	Alim	33	26.24	1987
Alim_33_30.1	Alim	33	30.30	1987
Alim_33_30.2	Alim	33	30.30	1987
Alim_33_31.1	Alim	33	30.32	1987
Alim_33_32.1	Alim	33	32.32	1987
Alim_33_33.1	Alim	33	32.34	1987
Alim_33_34.1	Alim	33	34.32	1989
Alim_34_35.1	Alim	34	28.00	1987
Alim_35_36.1	Alim	35	12.16	1989
Alim_36_37.1	Alim	36	26.12	1987
Alim_36_37.2	Alim	36	26.12	1987
Alim_37_38.1	Alim	37	F239	1987
Alim_37_38.2	Alim	37	F239	1987
Anz_55_39.1	Anz	55	S650	1993
Anz_55_39.2	Anz	55	S650	1993
Anz_56_40.1	Anz	56	S633	1993
Anz_65_41.1	Anz	65	S780	1993

[1] Code key: Axxx_a_b.c, where Axxx is Alim (*A. limonea*) or Anz (*A. novae-zelandiae*), determined in culture; a= VCG N$^{o.}$, determined by culture pairing; b=assigned isolate N$^{o.}$; c=laboratory subculture N$^{o.}$

[2] Grid coordinates (in metres) locating sample position across Plot 3 (from rhizomorphs), except (locations also known) 'F' from fruitbody tissue and 'S' from *Pinus radiata* D.Don seedling.

[3,4] Separate isolates from rhizomorphs in the same soil cores.

Note: data from five subcultures, selected arbitrarily within their own isolate group to leave one remaining, were discarded where it was no longer clear whether they were separate isolates from the same core or subcultures from the one isolate (not shown in the above table).

Table S2. Arrangement of subcultures of isolates during electrophoresis runs.

Four gels were run for each UP-PCR primer, with subcultures of the same VCGs placed in adjacent lanes (the order on each gel was the same for all primers). Empty lanes were used for ladders. For each primer the four gels were assessed together.

Lane	Gel 1	Gel 2	Gel 3	Gel 4
1				Data omitted[1]
2	Data omitted[1]	Anz_12_2.1	Anz_20_18.1	Anz_19_16.2
3	Alim_33_25.1	Anz_12_2.2	Anz_20_18.2	Anz_19_17.1
4	Alim_33_25.2	Anz_12_3.1	Anz_20_18.3	Anz_19_17.2
5	Alim_33_27.1	Anz_13_5.1	Anz_20_18.4	Alim_33_28.1
6	Alim_33_26.1	Anz_12_4.1	Anz_20_19.1	
7	Alim_33_26.2	Anz_12_3.2	Anz_20_19.2	Alim_34_35.1
8	Alim_33_26.3	Anz_13_5.2	Anz_20_20.1	Alim_35_36.1
9	Alim_33_27.2	Anz_13_6.1	Anz_15_8.1	Alim_32_1.1
10				Alim_37_38.1
11	Alim_33_29.1	Data omitted[1]	Anz_15_8.2	Alim_36_37.1
12	Alim_33_31.1	Data omitted[1]	Anz_15_8.3	Alim_36_37.2
13	Alim_33_30.1	Anz_23_23.1	Anz_15_9.1	Alim_37_38.2
14	Alim_33_30.2	Anz_14_7.1	Anz_15_9.2	
15	Alim_33_32.1	Anz_14_7.2	Anz_18_12.1	Anz_26_24.1
16	Alim_33_33.1	Data omitted[1]	Anz_18_12.2	Anz_26_24.2
17	Alim_33_34.1	Anz_16_10.1	Anz_18_13.1	Anz_65_41.1
18	Anz_22_22.2	Anz_17_11.1	Anz_18_14.1	Anz_56_40.1
19	Anz_21_21.1	Anz_17_11.2	Anz_18_15.1	Anz_55_39.1
20				Anz_55_39.2

[1] See note, Table S1.

Table S3. Distance matrix, combined primers (numbers of band differences between pairs of subcultures).

	Alim_32_1.1	Anz_12_2.1	Anz_12_2.2	Anz_12_3.1	Anz_12_3.2	Anz_12_4.1	Anz_13_5.1	Anz_13_5.2	Anz_13_6.1	Anz_14_7.1	Anz_14_7.2	Anz_15_8.1	Anz_15_8.2	Anz_15_8.3	Anz_15_9.1	Anz_15_9.2	Anz_16_10.1	Anz_17_11.1	Anz_17_11.2	Anz_18_12.1	Anz_18_12.2	Anz_18_13.1	Anz_18_14.1	Anz_18_15.1	Anz_19_16.2
Alim_32_1.1	0	49	49	50	50	59	43	42	47	50	62	65	62	62	62	52	54	50	59	57	55	55	55	60	62
Anz_12_2.1	49	0	2	3	1	10	26	27	34	39	49	52	53	53	53	41	39	33	42	56	54	54	50	55	51
Anz_12_2.2	49	2	0	3	1	10	24	25	32	37	47	50	51	51	51	39	37	31	40	54	52	52	48	53	49
Anz_12_3.1	50	3	3	0	2	9	27	28	35	40	48	53	52	52	52	40	38	32	41	55	53	53	49	54	52
Anz_12_3.2	50	1	1	2	0	9	25	26	33	38	48	51	52	52	52	40	38	32	41	55	53	53	49	54	50
Anz_12_4.1	59	10	10	9	9	0	18	19	30	35	43	48	49	49	49	43	39	29	42	56	54	54	50	59	55
Anz_13_5.1	43	26	24	27	25	18	0	1	12	27	37	42	43	43	43	37	37	25	40	50	48	48	44	53	45
Anz_13_5.2	42	27	25	28	26	19	1	0	11	26	36	41	42	42	42	36	36	24	39	49	47	47	43	52	46
Anz_13_6.1	47	34	32	35	33	30	12	11	0	17	45	48	51	51	51	35	35	31	46	54	52	52	48	51	47
Anz_14_7.1	50	39	37	40	38	35	27	26	17	0	28	51	54	54	54	40	26	28	43	55	53	53	51	54	50
Anz_14_7.2	62	49	47	48	48	43	37	36	45	28	0	27	28	28	28	58	42	36	25	41	39	39	37	56	52
Anz_15_8.1	65	52	50	53	51	48	42	41	48	51	27	0	5	5	5	39	61	45	36	38	40	40	36	55	55
Anz_15_8.2	62	53	51	52	52	49	43	42	51	54	28	5	0	0	0	34	62	42	35	33	35	35	35	54	52
Anz_15_8.3	62	53	51	52	52	49	43	42	51	54	28	5	0	0	0	34	62	42	35	33	35	35	35	54	52
Anz_15_9.1	62	53	51	52	52	49	43	42	51	54	28	5	0	0	0	34	62	42	35	33	35	35	35	54	52
Anz_15_9.2	52	41	39	40	40	43	37	36	35	40	58	39	34	34	34	0	30	38	51	53	53	53	53	58	54
Anz_16_10.1	54	39	37	38	38	39	37	36	35	26	42	61	62	62	62	30	0	34	43	55	53	53	53	58	52
Anz_17_11.1	50	33	31	32	32	29	25	24	31	28	36	45	42	42	42	38	34	0	31	53	51	51	49	54	54
Anz_17_11.2	59	42	40	41	41	42	40	39	46	43	25	36	35	35	35	51	43	31	0	28	26	26	24	53	53
Anz_18_12.1	57	56	54	55	55	56	50	49	54	55	41	38	33	33	33	53	55	53	28	0	2	2	8	37	51
Anz_18_12.2	55	54	52	53	53	54	48	47	52	53	39	40	35	35	35	53	53	51	26	2	0	0	6	35	49
Anz_18_13.1	55	54	52	53	53	54	48	47	52	53	39	40	35	35	35	53	53	51	26	2	0	0	6	35	49
Anz_18_14.1	55	50	48	49	49	50	44	43	48	51	37	36	35	35	35	53	53	49	24	8	6	6	0	29	51

Anz_18_15.1	60	55	53	54	54	59	53	52	51	54	56	55	54	54	54	58	58	54	53	37	35	35	29	0	24
Anz_19_16.2	62	51	49	52	50	55	45	46	47	50	52	55	52	52	52	54	52	54	53	51	49	49	51	24	0
Anz_19_17.1	64	49	47	50	48	53	43	44	45	48	50	53	54	54	54	56	50	54	53	53	51	51	49	22	2
Anz_19_17.2	62	47	45	48	46	51	41	42	47	50	32	35	36	36	36	52	46	54	37	39	37	37	35	50	30
Anz_20_18.1	63	46	46	47	47	52	52	53	58	51	33	38	37	37	37	53	53	53	38	36	34	34	34	49	53
Anz_20_18.2	65	48	48	49	49	54	54	55	60	53	35	40	39	39	39	55	55	55	40	38	36	36	36	47	53
Anz_20_18.3	64	47	47	48	48	53	53	54	59	52	34	37	38	38	38	54	54	54	39	37	35	35	35	50	54
Anz_20_18.4	64	47	47	48	48	53	53	54	59	52	34	37	38	38	38	54	54	54	39	37	35	35	35	50	54
Anz_20_19.1	63	48	48	49	49	54	54	55	58	51	35	36	39	39	39	55	55	55	40	38	36	36	36	51	55
Anz_20_19.2	63	48	48	49	49	54	54	55	58	51	35	36	39	39	39	55	55	55	40	38	36	36	36	51	55
Anz_20_20.1	65	50	50	51	51	54	54	55	54	47	61	62	65	65	65	57	57	51	64	60	58	58	58	55	59
Anz_21_21.1	45	48	46	47	47	50	38	37	36	39	53	64	59	59	59	43	43	47	56	52	50	50	52	49	49
Anz_22_22.2	40	35	33	36	34	41	33	32	33	32	46	57	54	54	54	36	28	36	47	45	43	43	45	50	42
Anz_23_23.1	52	23	21	22	22	27	29	30	31	32	44	55	56	56	56	36	24	30	39	55	53	53	49	54	50
Anz_26_24.1	66	53	53	52	54	57	57	58	57	46	48	57	56	56	56	54	50	54	47	55	53	53	53	38	34
Anz_26_24.2	71	58	58	57	59	62	62	63	62	51	49	58	57	57	57	49	45	57	50	58	56	56	56	53	49
Alim_33_25.1	49	68	68	69	69	70	56	55	56	67	69	78	79	79	79	71	65	63	68	72	70	70	68	69	71
Alim_33_25.2	47	66	66	67	67	68	54	53	54	65	67	76	77	77	77	73	67	63	66	70	68	68	66	67	69
Alim_33_26.1	47	66	66	67	67	68	54	53	54	65	67	76	77	77	77	73	67	63	66	70	68	68	66	67	69
Alim_33_26.2	46	67	67	68	68	69	53	52	53	64	66	75	76	76	76	72	66	62	65	69	67	67	65	66	68
Alim_33_26.3	49	66	66	67	67	68	52	51	52	63	65	74	75	75	75	71	65	61	64	68	66	66	64	65	67
Alim_33_27.1	47	66	66	67	67	68	54	53	54	65	67	76	77	77	77	73	67	63	66	70	68	68	66	67	69
Alim_33_27.2	47	68	68	69	69	70	54	53	54	65	67	76	77	77	77	73	67	63	66	70	68	68	66	67	69
Alim_33_28.1	44	69	69	70	70	71	55	54	55	66	68	75	76	76	76	72	68	64	67	71	69	69	67	68	68
Alim_33_29.1	49	66	66	67	67	68	52	51	52	63	65	74	77	77	77	73	65	63	66	72	70	70	66	67	69
Alim_33_30.1	48	65	65	66	66	67	51	50	51	62	64	73	76	76	76	72	64	62	65	71	69	69	65	66	68
Alim_33_30.2	46	67	67	68	68	69	53	52	53	64	66	75	76	76	76	72	66	62	65	69	67	67	65	66	68
Alim_33_31.1	47	68	68	67	69	70	54	53	54	65	67	74	75	75	75	71	67	63	68	70	68	68	64	65	71
Alim_33_32.1	47	66	66	67	67	68	52	51	52	63	65	74	75	75	75	71	65	61	64	68	66	66	64	65	67
Alim_33_33.1	46	67	67	68	68	69	53	52	53	64	66	75	76	76	76	72	66	62	65	69	67	67	65	66	68

Alim_33_34.1	43	64	64	67	65	68	50	49	50	59	63	70	73	73	73	73	65	59	62	66	64	64	60	61	65
Alim_34_35.1	62	83	83	84	84	85	73	72	71	76	70	79	80	80	80	86	80	76	71	81	79	79	79	72	72
Alim_35_36.1	46	75	75	78	76	81	71	70	69	72	70	75	74	74	74	74	70	74	61	65	63	63	65	66	66
Alim_36_37.1	46	79	79	78	80	79	63	62	61	68	60	63	62	62	62	68	68	68	63	65	63	63	61	60	64
Alim_36_37.2	45	82	82	81	83	82	66	65	64	71	63	66	63	63	63	69	71	69	64	64	62	62	62	61	65
Alim_37_38.1	48	81	81	80	82	81	69	68	67	70	64	65	62	62	62	64	68	72	69	65	63	63	63	60	62
Alim_37_38.2	53	84	84	83	85	82	70	69	66	69	67	68	65	65	65	67	69	71	72	66	64	64	66	59	57
Anz_55_39.1	62	53	53	54	54	49	43	44	47	44	50	57	56	56	56	52	48	36	47	55	53	53	53	58	58
Anz_55_39.2	61	52	52	53	53	48	44	45	50	47	51	58	55	55	55	53	51	35	46	54	52	52	54	61	59
Anz_56_40.1	63	58	58	57	59	58	50	49	50	59	57	58	59	59	59	57	53	57	48	54	52	52	48	47	45
Anz_65_41.1	52	51	49	50	50	49	41	40	43	38	40	43	42	42	42	44	40	42	39	43	41	41	37	42	36

	Anz_19_17.1	Anz_19_17.2	Anz_20_18.1	Anz_20_18.2	Anz_20_18.3	Anz_20_18.4	Anz_20_19.1	Anz_20_19.2	Anz_20_20.1	Anz_21_21.1	Anz_22_22.2	Anz_23_23.1	Anz_26_24.1	Anz_26_24.2	Alim_33_25.1	Alim_33_25.2	Alim_33_26.1	Alim_33_26.2	Alim_33_26.3	Alim_33_27.1	Alim_33_27.2
Alim_32_1.1	64	62	63	65	64	64	63	63	65	45	40	52	66	71	49	47	47	46	49	47	47
Anz_12_2.1	49	47	46	48	47	47	48	48	50	48	35	23	53	58	68	66	66	67	66	66	68
Anz_12_2.2	47	45	46	48	47	47	48	48	50	46	33	21	53	58	68	66	66	67	66	66	68
Anz_12_3.1	50	48	47	49	48	48	49	49	51	47	36	22	52	57	69	67	67	68	67	67	69
Anz_12_3.2	48	46	47	49	48	48	49	49	51	47	34	22	54	59	69	67	67	68	67	67	69
Anz_12_4.1	53	51	52	54	53	53	54	54	54	50	41	27	57	62	70	68	68	69	68	68	70
Anz_13_5.1	43	41	52	54	53	53	54	54	54	38	33	29	57	62	56	54	54	53	52	54	54
Anz_13_5.2	44	42	53	55	54	54	55	55	55	37	32	30	58	63	55	53	53	52	51	53	53
Anz_13_6.1	45	47	58	60	59	59	58	58	54	36	33	31	57	62	56	54	54	53	52	54	54
Anz_14_7.1	48	50	51	53	52	52	51	51	47	39	32	32	46	51	67	65	65	64	63	65	65
Anz_14_7.2	50	32	33	35	34	34	35	35	61	53	46	44	48	49	69	67	67	66	65	67	67
Anz_15_8.1	53	35	38	40	37	37	36	36	62	64	57	55	57	58	78	76	76	75	74	76	76
Anz_15_8.2	54	36	37	39	38	38	39	39	65	59	54	56	56	57	79	77	77	76	75	77	77
Anz_15_8.3	54	36	37	39	38	38	39	39	65	59	54	56	56	57	79	77	77	76	75	77	77
Anz_15_9.1	54	36	37	39	38	38	39	39	65	59	54	56	56	57	79	77	77	76	75	77	77
Anz_15_9.2	56	52	53	55	54	54	55	55	57	43	36	36	54	49	71	73	73	72	71	73	73
Anz_16_10.1	50	46	53	55	54	54	55	55	57	43	28	24	50	45	65	67	67	66	65	67	67
Anz_17_11.1	54	54	53	55	54	54	55	55	51	47	36	30	54	57	63	63	63	62	61	63	63
Anz_17_11.2	53	37	38	40	39	39	40	40	64	56	47	39	47	50	68	66	66	65	64	66	66
Anz_18_12.1	53	39	36	38	37	37	38	38	60	52	45	55	55	58	72	70	70	69	68	70	70
Anz_18_12.2	51	37	34	36	35	35	36	36	58	50	43	53	53	56	70	68	68	67	66	68	68
Anz_18_13.1	51	37	34	36	35	35	36	36	58	50	43	53	53	56	70	68	68	67	66	68	68
Anz_18_14.1	49	35	34	36	35	35	36	36	58	52	45	49	53	56	68	66	66	65	64	66	66
Anz_18_15.1	22	50	49	47	50	50	51	51	55	49	50	54	38	53	69	67	67	66	65	67	67
Anz_19_16.2	2	30	53	53	54	54	55	55	59	49	42	50	34	49	71	69	69	68	67	69	69

Anz_19_17.1	0	28	51	51	52	52	53	53	57	51	44	48	32	47	71	69	69	68	67	69	69
Anz_19_17.2	28	0	23	25	24	24	25	25	63	57	42	46	48	53	75	73	73	72	71	73	73
Anz_20_18.1	51	23	0	2	1	1	2	2	40	64	53	53	43	48	80	78	78	77	76	78	78
Anz_20_18.2	51	25	2	0	3	3	4	4	42	66	55	55	43	48	82	80	80	79	78	80	80
Anz_20_18.3	52	24	1	3	0	0	1	1	39	65	54	54	44	49	81	79	79	78	77	79	79
Anz_20_18.4	52	24	1	3	0	0	1	1	39	65	54	54	44	49	81	79	79	78	77	79	79
Anz_20_19.1	53	25	2	4	1	1	0	0	38	66	55	55	45	50	80	78	78	77	76	78	78
Anz_20_19.2	53	25	2	4	1	1	0	0	38	66	55	55	45	50	80	78	78	77	76	78	78
Anz_20_20.1	57	63	40	42	39	39	38	38	0	52	55	55	49	58	70	68	68	67	66	68	68
Anz_21_21.1	51	57	64	66	65	65	66	66	52	0	37	39	55	58	54	52	52	51	50	52	52
Anz_22_22.2	44	42	53	55	54	54	55	55	55	37	0	22	56	53	53	55	55	54	53	55	55
Anz_23_23.1	48	46	53	55	54	54	55	55	55	39	22	0	54	51	61	63	63	62	61	63	63
Anz_26_24.1	32	48	43	43	44	44	45	45	49	55	56	54	0	23	77	75	75	74	73	75	75
Anz_26_24.2	47	53	48	48	49	49	50	50	58	58	53	51	23	0	72	74	74	73	72	74	74
Alim_33_25.1	71	75	80	82	81	81	80	80	70	54	53	61	77	72	0	2	2	3	6	2	4
Alim_33_25.2	69	73	78	80	79	79	78	78	68	52	55	63	75	74	2	0	0	1	4	0	2
Alim_33_26.1	69	73	78	80	79	79	78	78	68	52	55	63	75	74	2	0	0	1	4	0	2
Alim_33_26.2	68	72	77	79	78	78	77	77	67	51	54	62	74	73	3	1	1	0	3	1	1
Alim_33_26.3	67	71	76	78	77	77	76	76	66	50	53	61	73	72	6	4	4	3	0	4	4
Alim_33_27.1	69	73	78	80	79	79	78	78	68	52	55	63	75	74	2	0	0	1	4	0	2
Alim_33_27.2	69	73	78	80	79	79	78	78	68	52	55	63	75	74	4	2	2	1	4	2	0
Alim_33_28.1	68	72	77	79	78	78	77	77	67	53	56	64	74	73	7	5	5	4	7	5	3
Alim_33_29.1	67	71	78	80	79	79	78	78	68	52	55	61	73	72	6	4	4	3	6	4	2
Alim_33_30.1	66	70	77	79	78	78	77	77	67	51	54	60	72	71	5	3	3	2	5	3	3
Alim_33_30.2	68	72	77	79	78	78	77	77	67	51	54	62	74	73	3	1	1	0	3	1	1
Alim_33_31.1	69	73	76	78	77	77	76	76	66	50	57	63	71	70	8	6	6	5	8	6	4
Alim_33_32.1	67	71	76	78	77	77	76	76	66	50	53	61	73	72	4	2	2	1	2	2	2
Alim_33_33.1	68	72	77	79	78	78	77	77	67	51	54	62	74	73	3	1	1	0	3	1	1
Alim_33_34.1	63	67	74	76	75	75	74	74	64	52	51	61	71	72	10	8	8	7	10	8	8
Alim_34_35.1	72	76	79	79	80	80	79	79	91	77	74	82	66	59	57	55	55	54	55	55	55

Alim_35_36.1	68	68	71	71	72	72	71	71	83	65	60	68	64	55	57	55	55	54	57	55	55
Alim_36_37.1	64	66	71	71	72	72	71	71	83	61	62	70	58	49	49	49	49	48	49	49	47
Alim_36_37.2	67	69	72	72	73	73	72	72	84	62	63	73	61	52	48	48	48	47	48	48	46
Alim_37_38.1	64	68	71	71	72	72	71	71	77	59	62	74	56	43	55	55	55	54	55	55	55
Alim_37_38.2	59	69	76	76	77	77	76	76	74	62	61	73	55	48	58	58	58	57	58	58	58
Anz_55_39.1	58	62	55	57	54	54	55	55	51	51	52	48	48	49	71	71	71	70	67	71	69
Anz_55_39.2	61	63	56	58	55	55	56	56	52	52	51	49	51	52	72	72	72	71	68	72	70
Anz_56_40.1	43	49	54	54	53	53	54	54	60	60	61	59	43	52	60	58	58	57	58	58	58
Anz_65_41.1	34	38	43	45	44	44	45	45	45	45	40	48	32	41	71	69	69	68	67	69	69

	Alim_33_28.1	Alim_33_29.1	Alim_33_30.1	Alim_33_30.2	Alim_33_31.1	Alim_33_32.1	Alim_33_33.1	Alim_33_34.1	Alim_34_35.1	Alim_35_36.1	Alim_36_37.1	Alim_36_37.2	Alim_37_38.1	Alim_37_38.2	Anz_55_39.1	Anz_55_39.2	Anz_56_40.1	Anz_65_41.1
Alim_32_1.1	44	49	48	46	47	47	46	43	62	46	46	45	48	53	62	61	63	52
Anz_12_2.1	69	66	65	67	68	66	67	64	83	75	79	82	81	84	53	52	58	51
Anz_12_2.2	69	66	65	67	68	66	67	64	83	75	79	82	81	84	53	52	58	49
Anz_12_3.1	70	67	66	68	67	67	68	67	84	78	78	81	80	83	54	53	57	50
Anz_12_3.2	70	67	66	68	69	67	68	65	84	76	80	83	82	85	54	53	59	50
Anz_12_4.1	71	68	67	69	70	68	69	68	85	81	79	82	81	82	49	48	58	49
Anz_13_5.1	55	52	51	53	54	52	53	50	73	71	63	66	69	70	43	44	50	41
Anz_13_5.2	54	51	50	52	53	51	52	49	72	70	62	65	68	69	44	45	49	40
Anz_13_6.1	55	52	51	53	54	52	53	50	71	69	61	64	67	66	47	50	50	43
Anz_14_7.1	66	63	62	64	65	63	64	59	76	72	68	71	70	69	44	47	59	38
Anz_14_7.2	68	65	64	66	67	65	66	63	70	70	60	63	64	67	50	51	57	40
Anz_15_8.1	75	74	73	75	74	74	75	70	79	75	63	66	65	68	57	58	58	43
Anz_15_8.2	76	77	76	76	75	75	76	73	80	74	62	63	62	65	56	55	59	42
Anz_15_8.3	76	77	76	76	75	75	76	73	80	74	62	63	62	65	56	55	59	42
Anz_15_9.1	76	77	76	76	75	75	76	73	80	74	62	63	62	65	56	55	59	42
Anz_15_9.2	72	73	72	72	71	71	72	73	86	74	68	69	64	67	52	53	57	44
Anz_16_10.1	68	65	64	66	67	65	66	65	80	70	68	71	68	69	48	51	53	40
Anz_17_11.1	64	63	62	62	63	61	62	59	76	74	68	69	72	71	36	35	57	42
Anz_17_11.2	67	66	65	65	68	64	65	62	71	61	63	64	69	72	47	46	48	39
Anz_18_12.1	71	72	71	69	70	68	69	66	81	65	65	64	65	66	55	54	54	43
Anz_18_12.2	69	70	69	67	68	66	67	64	79	63	63	62	63	64	53	52	52	41
Anz_18_13.1	69	70	69	67	68	66	67	64	79	63	63	62	63	64	53	52	52	41
Anz_18_14.1	67	66	65	65	64	64	65	60	79	65	61	62	63	66	53	54	48	37
Anz_18_15.1	68	67	66	66	65	65	66	61	72	66	60	61	60	59	58	61	47	42
Anz_19_16.2	68	69	68	68	71	67	68	65	72	66	64	65	62	57	58	59	45	36

Anz_19_17.1	68	67	66	68	69	67	68	63	72	68	64	67	64	59	58	61	43	34
Anz_19_17.2	72	71	70	72	73	71	72	67	76	68	66	69	68	69	62	63	49	38
Anz_20_18.1	77	78	77	77	76	76	77	74	79	71	71	72	71	76	55	56	54	43
Anz_20_18.2	79	80	79	79	78	78	79	76	79	71	71	72	71	76	57	58	54	45
Anz_20_18.3	78	79	78	78	77	77	78	75	80	72	72	73	72	77	54	55	53	44
Anz_20_18.4	78	79	78	78	77	77	78	75	80	72	72	73	72	77	54	55	53	44
Anz_20_19.1	77	78	77	77	76	76	77	74	79	71	71	72	71	76	55	56	54	45
Anz_20_19.2	77	78	77	77	76	76	77	74	79	71	71	72	71	76	55	56	54	45
Anz_20_20.1	67	68	67	67	66	66	67	64	91	83	83	84	77	74	51	52	60	45
Anz_21_21.1	53	52	51	51	50	50	51	52	77	65	61	62	59	62	51	52	60	45
Anz_22_22.2	56	55	54	54	57	53	54	51	74	60	62	63	62	61	52	51	61	40
Anz_23_23.1	64	61	60	62	63	61	62	61	82	68	70	73	74	73	48	49	59	48
Anz_26_24.1	74	73	72	74	71	73	74	71	66	64	58	61	56	55	48	51	43	32
Anz_26_24.2	73	72	71	73	70	72	73	72	59	55	49	52	43	48	49	52	52	41
Alim_33_25.1	7	6	5	3	8	4	3	10	57	57	49	48	55	58	71	72	60	71
Alim_33_25.2	5	4	3	1	6	2	1	8	55	55	49	48	55	58	71	72	58	69
Alim_33_26.1	5	4	3	1	6	2	1	8	55	55	49	48	55	58	71	72	58	69
Alim_33_26.2	4	3	2	0	5	1	0	7	54	54	48	47	54	57	70	71	57	68
Alim_33_26.3	7	6	5	3	8	2	3	10	55	57	49	48	55	58	67	68	58	67
Alim_33_27.1	5	4	3	1	6	2	1	8	55	55	49	48	55	58	71	72	58	69
Alim_33_27.2	3	2	3	1	4	2	1	8	55	55	47	46	55	58	69	70	58	69
Alim_33_28.1	0	5	6	4	7	5	4	11	56	58	46	45	54	57	70	71	59	68
Alim_33_29.1	5	0	1	3	2	4	3	6	53	57	45	48	57	58	69	70	58	67
Alim_33_30.1	6	1	0	2	3	3	2	5	52	56	46	49	56	57	70	71	57	66
Alim_33_30.2	4	3	2	0	5	1	0	7	54	54	48	47	54	57	70	71	57	68
Alim_33_31.1	7	2	3	5	0	6	5	8	55	59	43	46	55	56	67	68	56	65
Alim_33_32.1	5	4	3	1	6	0	1	8	55	55	47	46	55	58	69	70	58	67
Alim_33_33.1	4	3	2	0	5	1	0	7	54	54	48	47	54	57	70	71	57	68
Alim_33_34.1	11	6	5	7	8	8	7	0	53	57	43	46	55	56	67	68	58	63
Alim_34_35.1	56	53	52	54	55	55	54	53	0	42	40	43	50	51	76	73	69	70

Alim_35_36.1	58	57	56	54	59	55	54	57	42	0	42	39	38	45	76	71	63	62
Alim_36_37.1	46	45	46	48	43	47	48	43	40	42	0	3	22	31	62	61	61	52
Alim_36_37.2	45	48	49	47	46	46	47	46	43	39	3	0	19	30	63	60	62	55
Alim_37_38.1	54	57	56	54	55	55	54	55	50	38	22	19	0	13	64	63	57	54
Alim_37_38.2	57	58	57	57	56	58	57	56	51	45	31	30	13	0	67	66	62	55
Anz_55_39.1	70	69	70	70	67	69	70	67	76	76	62	63	64	67	0	5	39	42
Anz_55_39.2	71	70	71	71	68	70	71	68	73	71	61	60	63	66	5	0	44	43
Anz_56_40.1	59	58	57	57	56	58	57	58	69	63	61	62	57	62	39	44	0	39
Anz_65_41.1	68	67	66	68	65	67	68	63	70	62	52	55	54	55	42	43	39	0

Table S4. Selection of primers for phylogenetic analysis.

Primer	No. of subcultures that differ
3-2	1
AS4	1
0.3-1	1
AS15	3
L15	5
L45	5
L21	5.5[1]
FokI	6
L15/AS19	8.5[1]
AA2M2	15

[1] Decimals because occasionally halving triplets as well as pairs or quadruplet of subcultures.