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A genetic study on subtropical *Anadenanthera colubrina* (Vell.) Brenan var. *cebil* (Griseb.) Altschul tree from Northwestern Argentina

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ABSTRACT

Anadenanthera colubrina (Vell.) Brenan var. *cebil* (Griseb.) Altschul is a tree species in South America important for its cultural, economic, and medicinal uses. In addition, it represents a trace in memory of the forests that have decreased over the years and for this reason it is not only interesting to study but also important to preserve the tree species for future generations. In this paper, we have characterized the genetic diversity of four populations. We collected seeds from four different sites: San Bernardo (B), El Cebilar (C), Metán (M), and El Gallinato (G) in Salta Province, North Argentina. We then compared the intergenic transcribed sequences of ribosomal DNA, a known genetic molecular marker. Our previous results, obtained through the morphological and genetic analysis of only four individuals (one for each zone), have showed that the individuals from B and M sites were more similar to each other as well as the individuals from G and C sites. In this paper, a larger number of individuals (25) were characterized and their phylogenetic relationships were computed. The results confirmed the previously found similarities.

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deforestation; conservation;
phylogenetics

Introduction

In South America, a region that lies within the tropic, a series of humid-dry cycles occurred during the Pleistocene which altered the ecosystems. Several studies have been carried out, which have analysed the distribution of flora in relation to the climate during the last ice age, aimed at understanding the processes that produced vast areas of vegetation in South America (such as the Amazon rainforest and the grasslands of Patagonia) (Pennington et al. 2004). Glaciations and associated phenomena have had an important effect on the flora and fauna of South America, causing extinction and changes in the geographical distribution of species. They have produced the contraction of the forests during arid periods and their expansion during wet periods (Van der Hammen 1974). Climate changes during the Quaternary ages have caused fluctuations of population size (Hewitt 2000).

Anadenanthera colubrina (Vell.) Brenan var. *cebil* (Griseb.) Altschul belongs to the Fabaceae family, Mimosoideae subfamily (<http://www.kew.org/science-conservation/research-data/resources/legumes-of-the-world>), and has a wide distribution in Brazil, Paraguay, Perú, Bolivia, and Argentina (<http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB18071>). In Argentina, it is located in the northern provinces. Today, *A. colubrina* var. *cebil* is intermittently distributed in the seasonally dry tropical forests (Särkinen et al. 2011). This species was categorized as one of “least concern” with a low risk of extinction by IUCN (<http://www.iucnredlist.org>), although del Valle Perea (www.atlas.catamarca.gov.ar) considered it a vulnerable species in Catamarca, with many populations at risk

due to its continuous exploitation. A large area of this forest has destroyed, due to the improvement of agricultural soils, and so the number of *A. colubrina* var. *cebil* has decreased in recent decades representing an example of species fragmentation after deforestation (Barrandeguy et al. 2014).

The further importance of this tree species is due to its economic and medicinal applications in South America. In fact, the wood contains tannins, useful in processing animal hides; it is hard and termite resistant and for this reason it is used in furniture. In popular medicine, it is used to treat respiratory diseases (Carod-Artal & Vásquez-Cabrera 2007).

Intergenic transcribed spacers (ITS) of ribosomal DNA (rDNA) represents genetic molecular markers known to be useful in phylogenetics used in detecting both interspecific and intraspecific variability, as they contain conserved and variable regions (Simon & Weiß 2008). For example, in fungi, the use of ITS marker has allowed a better understanding of intraspecific variability (Schoch et al. 2012).

As demonstrated in a previous study (de Viana et al. 2014), the analysis of the morphological traits in the fruits and seeds of *A. colubrina* var. *cebil* showed similarities among trees from different regions of the same province of Salta (north Argentina): Metán, El Cebilar, San Bernardo, and El Gallinato. The authors analysed the ITS region of fresh seeds, from four trees of *A. colubrina* var. *cebil* (one from each site) collected in Salta Province, showing a wide variability among individuals at both morphologic and genetic levels.

The aim of this work was to deepen the previous study by including a larger number of individuals (25) collected from different locations in Salta Province (Figure 1) and conserved in the Germplasm Bank of Native Species (BGEN-INEAH) of the National University of Salta through

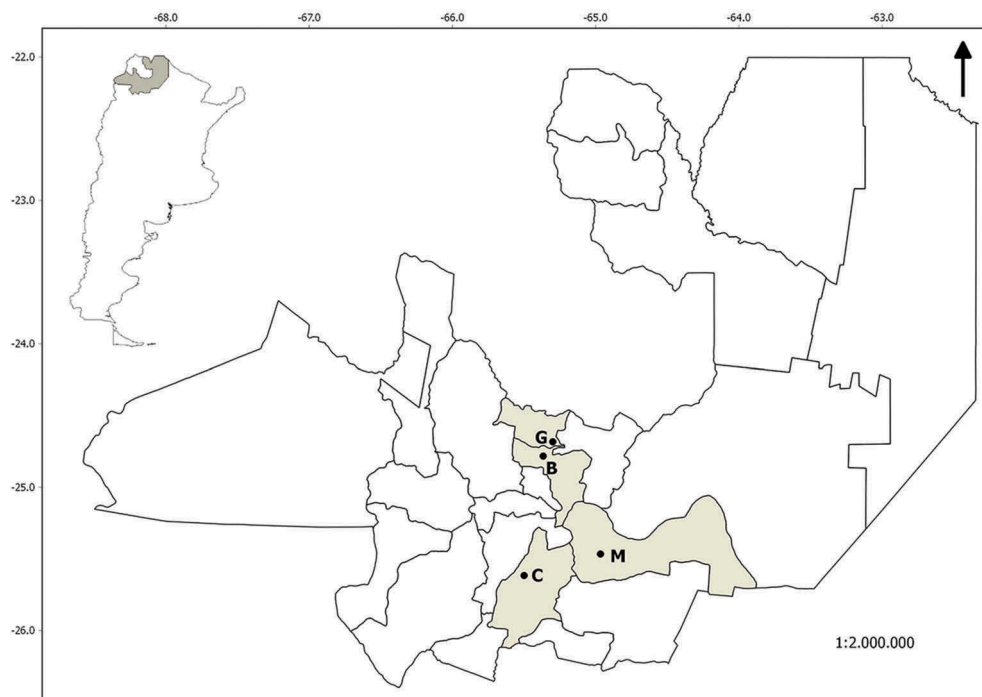


Figure 1. Map of Argentina and Salta Province. The four sites of sampling, San Bernardo (B); El Cebilar (C); El Gallinato (G); and Metán (M).

the use of the ITS-rDNA marker. Study on the genetic diversity of *A. colubrina* var. *cebil* may improve our knowledge about plant genetic characteristics and indicate a tool for propagation and conservation policies.

Materials and methods

Sampling and DNA extraction from plant seeds

Seeds of *A. colubrina* var. *cebil* were collected from four regions in Salta Province including San Bernardo (B), El Cebilar (C), Metán (M), and El Gallinato (G), which are located within about 100 km of each other, as shown in Figure 1. We collected 100 fruits per individual tree, for a total of 10 individuals per population. In the laboratory, the fruits were opened manually and most of the seeds were kept for long-term *ex situ* conservation at the germplasm bank in Salta Province. The seeds used in this study, obtained from different trees, were sterilized and shredded; then the genomic DNA was extracted as previously described (de Viana et al. 2014). Environmental and geographical data of the collecting sites are reported in Table 1.

In vitro amplification (PCR) of ITS regions and analysis of the nucleotide sequences

PCR was carried out as previously described (de Viana et al. 2014). The size of the specific isolated region was 450bp.

The 25 amplicons nucleotide composition was determined by the MWG sequence service (Eurofin-operon) and published in GeneBank. The relative accession numbers are included in Table S1.

Nucleotide alignment, phylogenetic and polymorphic analyses

Multiple alignments of the ITS-rDNA sequences were performed with Clustal W2 software (<http://www.expasy.org>). Shading of the multiple alignment sequences were obtained by Boxshade 3.21 program (http://www.ch.embnet.org/software/BOX_form.html).

The phylogenetic tree was achieved by MEGA6 (Tamura et al. 2013), using the neighbor-joining method (with 500 bootstrap replications). The genetic distances were computed using the Maximum Composite Likelihood Method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+noncoding. *Cojoba undulatomarginata* L.Rico (Accession number EF638187) and *Calliandra physocalyx* H.M. Hern. & M. Sousa (Accession number JX870727) were utilized as outgroups because of their belonging to the same family (Fabaceae) of *A. colubrina* but are appropriately distant.

The DnaSP (Version 5.0) (<http://www.ub.edu/dnasp>) platform was used to sort the number of variable sites.

Table 1. Geographical and climate data of the Argentina collecting sites (period 1934–1990) (http://anterior.inta.gob.ar/prorenea/info/resultados/Precip_NOA/base_precipitaciones_noa.asp).

Recording station	Collecting sites	Geographical coordinates Latitude–longitude	Altitude (MASL)	Average annual rainfall (mm)	Average annual temperature (°C)	N° individuals for each site
Salta	San Bernardo	24°47'S–65°22'W	1410	695 ± 170	16	5
Mojotoro	El Gallinato	24°41'S–65°18'W	1190	607 ± 187	16	8
Talapampa	El Cebilar	25°37'S–65°30'W	1432	376 ± 73	16	8
Metán	Metán	25°38'S–64°58'W	884	902 ± 259	18	8

MASL = meters above sea level.

Results

Phylogenetic and polymorphic analysis

The phylogenetic relationship between 25 different individuals of *A. colubrina* var. *cebil*, collected in four regions of Salta Province, San Bernardo (B), El Cebilar (C), Metán (M), and El Gallinato (G), was investigated through the ITS-rDNA region sequences. In this study, the sequences previously identified as AcC, AcG, AcM, AcB (de Viana et al. 2014) were also included. The aligned nucleotidic sequences are shown in Table S2 and the related nucleotidic differences are shaded in Table S3. The phylogenetic tree constructed by using the 29 sequences is shown in Figure 2. All of the individuals were sorted into two main groups, which branch out separately, containing 16 and 13 sequences, respectively. The two outgroup sequences of the plants *C. undulatomarginata* and *C. physocalyx* have clustered in a third group, as expected. In this phylogenetic tree, all individuals are divided into two branches: one giving rise to the AcC/AcG group and another giving rise

to the AcM/AcB group. Polymorphic analysis was sorted using DnaSP software. A total of 225 variable sites were detected out of 477 total nucleotides. Genetic distance among the four groups of populations, found by using MEGA 6 software, showed the following results: the distance between M and G populations was 0.191, between M and C was 0.203, and between M and B was 0.07; the distance between C and G populations was 0.08, and between C and B was 0.174; finally, the distance between B and G populations was 0.163 (see Table S4).

Discussion

The choice of ITS-rDNA as a marker capable of establishing the genetic variability of *A. colubrina* var. *cebil* is related to the variability of these nonfunctional sequences on which a low-selective pressure acts. Thus, the ITS-rDNA marker is widely used to study interspecific and intraspecific variations in plants such as Asteraceae and Cyperaceae (Baldwin et al. 1995; Kollipara et al. 1997). In

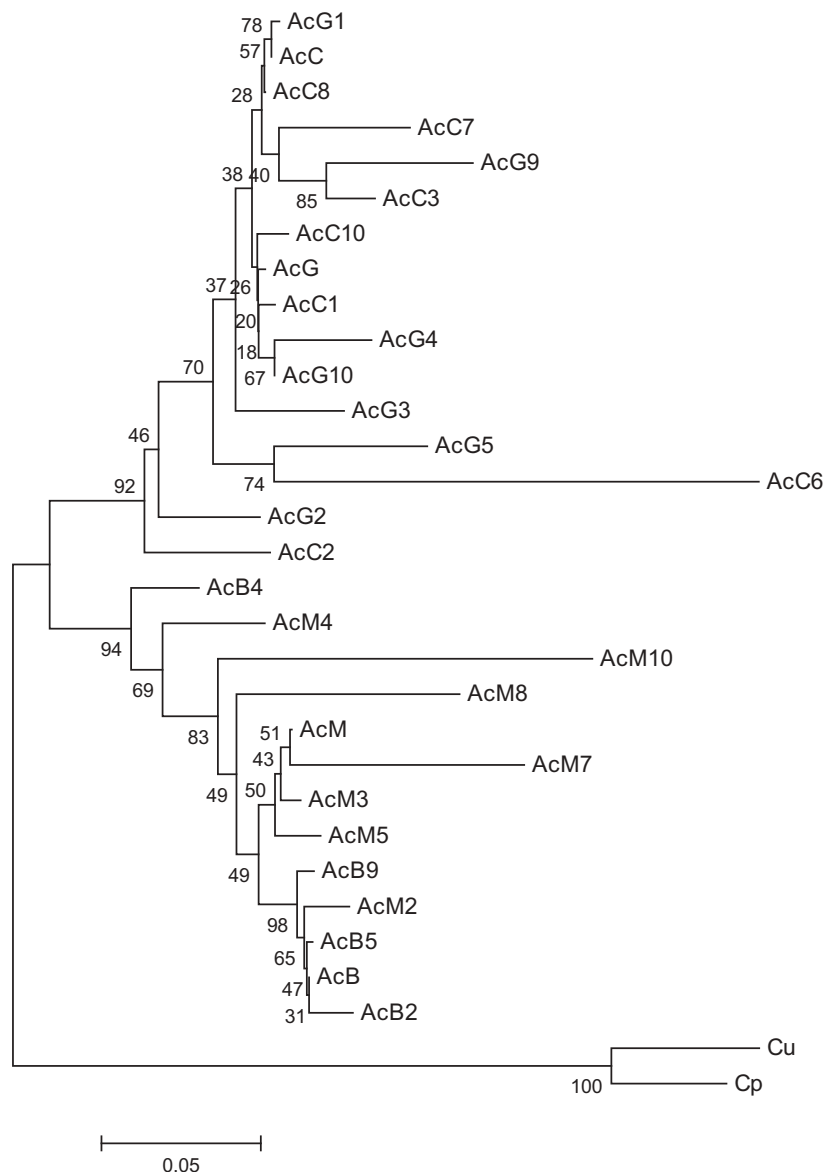


Figure 2. Phylogenetic tree of *Anadenanthera colubrina* var. *cebil* populations. The four sites of sampling were B = San Bernardo; C = El Cebilar; G = El Gallinato; M = Metán. *Cojoba undulatomarginata* (Cu) and *Calliandra physocalyx* (Cp), which are plants of the same family (Fabaceae) as *A. colubrina*, used as outgroups. The tree is drawn to scale, with branch lengths in the same units as those of the genetic distances. The genetic distances are in units of the number of base substitutions per site. The high bootstrap values indicate the accuracy of the data.

this paper, the sequence analysis of the 29 samples showed that the Argentine population of *A. colubrina* var. *cebil* possesses a genetic variability within species, as deduced by the phylogenetic tree (Figure 2). In particular, the cluster analysis displayed a high similarity between individuals collected from the regions of San Bernardo and Metán, and between individuals collected from the regions of El Cebilar and El Gallinato (Figure 1). The genetic distance, found between the four analysed populations, clearly showed the same results. In fact, individuals from G/C and B/M sites belonged to the same cluster in the phylogenetic tree. The genetic distance confirmed these findings. In fact, the distance between B and M (corresponding to a value equal to 0.07) was lower than that between B and C/G (0.203 and 0.163, respectively). Analogously, the distance between G and C was 0.08 (see Table S4).

These results are in agreement with the previous study (de Viana et al. 2014) in which the comparison between the sequences of four individuals, one per each region, revealed high identity (98.1%) between B and M, as well as between C and G (97.9%). It could be conceivable to hypothesize that all these individuals belong to a single large population of *A. colubrina* var. *cebil* with a distribution area that extends from G/B (north) and C/M (south). The environmental conditions and geographical data of the seeds-collecting sites, relating to the period 1934–1990, are reported in Table 1.

The distribution of different individuals may suggest progressive divergence, due to isolation, as in the study on molecular markers in Anacardiaceae reported by Caetano et al. (2008). Alternatively, but not exclusively, populations of *A. colubrina* var. *cebil* from Salta Province had genetic diversity in ITS-rDNA sequences, simply as a consequence of genetic drift.

Our results are in agreement with those reported by Barrandeguy et al. (2012) and Garcia et al. (2014). They found genetic variability and high levels of polymorphism in natural populations of *A. colubrina* var. *cebil* collected from northern provinces of Argentina, other than Salta. In addition, they used chloroplast microsatellite (cpSSR) plus nuclear SSRs loci (nSSRs), other than ITS marker.

As regards the high grade of intraspecific polymorphism detected in our study, it is known that ITS polymorphism within individuals is quite common (Baldwin et al. 1995; Rosselló et al. 2006).

As trees have a crucial role in the social, ecological, and economic life of the Earth, covering 30% of its surface, the application of genetics to plant diversity may determine improvements in knowledge about conservation and forests management (Rao & Hodgkin 2002).

The assessment of intraspecific genetic variability of *A. colubrina* var. *cebil* is important to design appropriate conservation policies. Further studies are needed to assess the levels of variability of a larger population which also includes neighbouring countries of subtropical South America.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table S1 Accession numbers of the *Anadathera colubrina* samples

Samples	Accession numbers
AcM	JQ910930
AcM2	LN827632
AcM3	LN827633
AcM4	LN827634
AcM5	LN827635
AcM7	LN827636
AcM8	LN827637
AcM10	LN827638
AcC	HF564642
AcC1	LN827646
AcC2	LN827647
AcC3	LN827648
AcC6	LN827649
AcC7	LN827650
AcC8	LN827651
AcC10	LN827652
AcB	HF564640
AcB2	LN827653
AcB4	LN827654
AcB5	LN827655
AcB9	LN827656
AcG	HF564641
AcG1	LN827639

AcG2	LN827640
AcG3	LN827641
AcG4	LN827642
AcG5	LN827643
AcG9	LN827644
AcG10	LN827645

Table S2 Clustal W2 alignment

ACG-HF564641.1	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCCCGGTACCTCAGGTGC	50
ACC1	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCCCGGTACCTCAGGTGC	50
ACC10	-----TGAAAGCCT-CCCAAGCCCGGGCACAACG--CCAACCCCGGCACCTCAGGTGC	50
ACG1	-----TGAAAGCCT-CCCAAGCCCGGGTACAACA--CAAACGCCGGTACCTCAGGTGC	50
ACC-HF564642.1	-----TGAAAGCCT-CCCAAGCCCGGGTACAACA--CAAACCCCGGTACCTCAGGTGC	50
ACG9	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACGCCGGTACCTCAGGTGC	50
ACC3	-----TGAAAGCCT-CCCAAGCCCGGGTACAACA--CAAACGCCGGTACCTCAGGTGC	50
ACC7	-----TGAAAGCCT-CCCAAGCCCGGGTACGACG--CAAACGCTGGTACCTCAGGTGC	50
ACC8	-----TGAAAGCCT-CCCAAGCCCGGGTACAACA--CAAACGCCGGTACCTCAGGTGC	50
ACG4	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCCCGGTACCTCAGGTGC	50
ACC10	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCCCGGTACCTCAGGTGC	50
ACG3	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCCCGGTACCTCAGGC	50
ACG5	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CATACCCCGATACCTCAGGC	50
ACG2	-----TGAAAGCCT-CCCAAGCCCGGGTACAACA--CAAACCCCGGTACCTCAGGTGC	50
ACC2	-----TGAAAGCCT-CCCAAGCCCGGGTACAACA--CAAACGCCGGTACCTCAGGTAC	50
ACC6	-----TGAAAGCCT-CCCGAGCGCGGGCACAACG--CCTACCCCGGCACCTTAGGTGC	50
ACM-JQ910930.1	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACM3	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACM5	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACB-HF564640.1	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACB2	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACB5	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACM2	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACB9	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACM7	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
ACM8	-----TGAAAGCCT-CCCAAGCCCGGTATAACA--CAACCCCTGGTGCCTCAGGCAC	50
ACM10	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTGCCTCAGGCAC	50
AcM4	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCCCGGTCCCTCAGGTAC	50
AcB4	-----TGAAAGCCT-CCCAAGCCCGGGCACAACA--CAAACCTGGTACCTCAGGTAC	50
CU	GGGAGGGCGACGGCGTGCCTGAGGCCTCCCCGAGAAACAAACCCCGGCCTCAGGC	60
CP	----GG-----GGCGCGCCGAGGCC-CCCCGACAACCAACCCCGGCACAGGC	50
	. ** *** . ** . . * . * . : * * * . ** *** . *	
ACG-HF564641.1	CAAGGAAC TGCAACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACC1	CAAGGAAC TGCAACAGAGCGAG-AGTGCCTTACGTTCGATTGCACCCGAAATGACTCTCAA	109
ACC10	CAAGGAAC TGCAACAGATCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACG1	CAAGGAAC TGCAACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACC-HF564642.1	CAAGGAAC TGCAACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACG9	CAAGGAAC CGCAACAAAGCGAG-AACGCCTCATGCCGATTGCACCCGAAATGACTCTCAA	109
ACC3	CAAGGAAC CGCAACAAAGCGAG-CACGCCTCAGCCGATTGCACCTGAAATGACTCTCAA	109
ACC7	CAAGGAAC AGCAACAGAGCCGG-AGCGCCTAACGCCAATTGCACCCGAAATGACTCTCAA	109
ACC8	CAAGGAAC TGCAACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACG4	CAAGGAAC TGCAACAGAGCGAG-CGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACG10	CAAGGAAC TGCAACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACG3	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACG5	CAAGGAAC TCGACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA	109
ACG2	CAGGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCGAAATGACTCTCAA	109
ACC2	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCGAAACGACTCTCGG	109
ACC6	CAAGCAAC TGCAACAGAGGGAG-AGCGCCTCTTGCCGATTGCACCCAAATGACTCTTAA	109
ACM-JQ910930.1	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACM3	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACM5	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACB-HF564640.1	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACB2	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACB5	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACM2	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACB9	CAAGGAAC TGCAACAGAGCGAG-TAGGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACM7	AACGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACM8	CAAGGAAC TGCAACAGAGTGAG-GGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
ACM10	CAAGAAAC CGCAACAAAGCGAG-CGCGCCTCAGCCGATTGCACCCAAAATGACTCTCGG	109
AcM4	CAAGGAAC TGCAACAGAGCGAG-TGCGCCTCATGCCGATTGCACCCAAAACGACTCTCGG	109
AcB4	CAAGGAAC TGCAACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCAAAACGACTCTCGG	109
CU	CAAGGAAC GAAACAAAGCAGGGC ACTCGT ---GCCATTTGCATCCGAAACGACTCTCGG	117
CP	CAAGGAAC AGCAAGAAGCAGGCACGCATGATGCCATTTGCATCCGAAACGACTCTCGG	110
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ACG-HF564641.1 CAACGGATATCTCAGCTCTCGCATCGATG-AGAACGTAGTAAAATGCAATGCTTGGTGTG 168
ACC1 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGTAAAATGCAATGCTTGGTGTG 169
ACC10 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGTAAAATGCAATGCTTGGTGTG 169
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ACC3 TAACGGATATCTCAGCTCTCGCATCAATGAAGAACGTAGTAAAATGCGATGCTTGGTGTG 169
ACC7 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGGAAAATGCAATACTTGGTGTG 169
ACC8 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGTAAAATGCAATGCTTGGTGTG 169
ACG4 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGTAAAATGCAATGCTTGGTGTG 169
ACG10 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGTAAAATGCAATGCTTGGTGTG 169
ACG3 CAACGGATATCTCAGCTCTCGCATCAATGAAGAACGTAGTAAAATGCAATGCTTGGTGTG 169
ACG5 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGTAAAATGCGATGCTTGGTGTG 169
ACG2 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTAGTGTG 169
ACC2 CAACGGATATCTCAGCTCTCGCATCGATGAAGAACGTAGCGAAAATGCAATGCTTAGTGTG 169
ACC6 CAACGGATATCTCACTTCTGGCATCGATGAAGAACGTAGCGAAAATGCAATGCTTGGTGTG 169
ACM-JQ910930.1 CAACGGATATCTCGGCTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTGGTGTG 169
ACM3 CAACGGATATCTCGGCTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTGGTGTG 169
ACM5 CAACGGATATCTCGGCTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTGGTGTG 169
ACB-HF564640.1 CAACGGATATCTCGACTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTAGTGTG 169
ACB2 CAACGGATATCTCGACTCTTGGATCGATGAAGAACATAGCGAAAATGCGATGCTTAGTGTG 169
ACB5 CAACGGATATCTCGACTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTAGTGTG 169
ACM2 CAACGGATATCTCGACTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTAGTGTG 169
ACB9 CAACGGATATCTCGACTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTGGTGTG 169
ACM7 TACCGATATCTCGGCTCTCGCATCGATGAAGAACATAGCGAAAATGCGATGCTTGGTGTG 169
ACM8 CAACGGATATCTCGGCTCTCGCATCGATGAAGAACATAGCGAAAATGTGATGCTTGGTGTG 169
ACM10 CAACGTATATCTTAGCTCTCATATCTAAAAAGAACAGAGCGAAAATATGATACTTGGTGTG 169
AcM4 CAAAGGATATCTCGGCTCTCGCATCGATGAAGAACATAGCGAAAATGCAATGCTTGGTGTG 169
AcB4 CAACGGATATCTCGACTCTCGCATCGATGAAGAACATAGCGAAAATGCAATGCTTGGTGTG 169
CU CAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAATGCGATACTTGGTGTG 177
CP CAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAAATGCGATACTTGGTGTG 170
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ACG-HF564641.1 AATTGCAGAAATCCTGTGAACTATCGAGTCTTTGAATGTAAGTTGCG-CCAAAGCTATTAG 227
ACC1 AATTGCAGAAATCCTGTGAACTATCGAGTCTTTGAATGTAAGTTGCGCCCAAAGCTATTAG 229
ACC10 AATTGCAGAAATCCTGTGAACTATCGAGTCTTTGAATGTAAGTTGCGCCCAAAGCTATTAG 229
ACG1 AATTGCAGAAATCCTGTGAACTATTGAGTCTTTGAATGTAAGTTGCGCCCAAAGCTATTAG 229
ACC-HF564642.1 AATTGCAGAAATCCTGTGAACTATTGAGTCTTTGAATGTAAGTTGCG-CCAAAGCTATTAG 228
ACG9 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAATGTAAGTTACGCCCGAAGCTATTAG 229
ACC3 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAATGCAAGTTACGCCCAAAGCTATTAG 229
ACC7 AATTGCAAAATCCCGTGAACCATGGAGTCTTTGAATGCAAGTTGCGCCCAAAGCCATTAG 229
ACC8 AATTGCAGAAATCCTGTGAACTATCGAGTCTTTGAATGTAAGTTGCGCCCAAAGCTATTAG 229
ACG4 AATTGCAGAAATCCTGTGAACTATCGAGTCTTTGAATGTAAGTTGCGCCCAAAGCTATTAG 229
ACG10 AATTGCAGAAATCCTGTGAACTATCGAGTCTTTGAATGTAAGTTGCGCCCAAAGCTATTAG 229
ACG3 AATTGCAGAAATCCTGTGAACTATCGAGTCTTTGAATGTAAGTTGCGCCCAAAGCTATTAG 229
ACG5 AATTGCAGAAATCCTGTGAACTATTGAGTCTTTGAATGTAAGTTATGCCCATGCTATTAG 229
ACG2 AATTGCAGAAATCCTGTGAACTATTGAGTCTTTGAACGCAAGTTGCGCCCAAAGCTATTAG 229
ACC2 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTG-CCAAAGCCATTAG 228
ACC6 AATTGCGGAATCCTGGAACCATGGAGTCTTTGAAGGGAACTTGTGCCCAAATTGATTAG 229
ACM-JQ910930.1 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCGAAGCCATTAG 229
ACM3 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCGAAGCCATTAG 229
ACM5 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCGAAGCCATTAG 229
ACB-HF564640.1 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCAAAGCCATTAG 229
ACB2 AATTGCAGAAATCCCGTGAACCATTTAGTCTTTGAACGCAAGTTGTGCCCAAAGCCATTAG 229
ACB5 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCAAAGCCATTAG 229
ACM2 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCAAAGCCATTAG 229
ACB9 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCAAAGCCATTAG 229
ACM7 AATTGCAGAAATCCCGTGAACCATGGAGTCTTTGAACGCAAGTTGTGCCCGAAGCCATTAG 229
ACM8 ACTTGCAGAAATCCTGTGAACTATCGAGTCTTTGAACGCAAGTTGTGCCCGAAGTATTAG 229
ACM10 AAGTGGAGAAATCCCGTGAACCATGGAGTCTTTGAATGCAAGTTACGCCCGAAGCCATTAG 229
AcM4 AATTGCAGAAATCCTGTAAACCATGGAGTCTTTGAACGGAAGTTGTGCCCGAAGCCATTAG 229
AcB4 AATTGCAGAAATCCCGTGAACCATCGAGTCTTTGAACGTAAGTTGCGCCCAAAGCTATTAG 229
CU AATTGCAGAAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAG 237
CP AATTGCAGAAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAG 230
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ACG-HF564641.1	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCACGCG--GGGCAC	285
ACC1	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCACGCG--GGGCAC	287
ACC10	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCACGCG--GGGCAC	287
ACG1	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCACGCG--GGGCAC	287
ACC-HF564642.1	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCACGCG--GGGCAC	286
ACG9	GCTGAGGGCACGCCTGCCTAGGCGTCACGTAACATCGCCGCCACCCACGCG--GGGCAC	287
ACC3	GCCGAGGGCACGCCTGCCTGGGCGTCACGTAACGTCGCCGCCACCCACGCG--GGGCAC	287
ACC7	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAAGGTCGCCGCCACCCACGCG--GGGCAC	287
ACC8	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCACGCG--GGGCAC	287
ACG4	GCCGAGGGCACGCCTGCCTGGGCGACACGCAACGTCGCCGCCACCCACGCG--GAGCAC	287
ACG10	GCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCACGCG--GGGCAC	287
ACG3	GCCGAGGGCACGCCTGCCTGGGCATCACGCAACGTTGCCGCCACCCACGCG--GGGCC	287
ACG5	GCCGAGGGCACGCCTGCCTGGGCGTCAGGCAACGTCGCCGCCACCTCACGCG--GGGCGC	287
ACG2	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTTGCCGCCGCTCACGCG--GGGCAC	287
ACC2	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTTGCCGCCGCTCACGCG--AGGCGC	286
ACC6	GCCGAGGGCCCCGCTGATAGGCTGAGGCAACGCCGCCGCTCACGCG--GGGCAC	287
ACM-JQ910930.1	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTCGCCGCCACCTCACGCG--GGGCGC	287
ACM3	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTCGCCGCCGCTCACGCG--GGGCGC	287
ACM5	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTCGCCGCCGCTCACGCG--GGGCGC	287
ACB-HF564640.1	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTTGCCGCCGCTCACGCG--GGGCGC	287
ACB2	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTTGCCGCCGCTCACGCG--GGGCGC	287
ACB5	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTTGCCGCCGCTCACGCG--GGGCGC	287
ACM2	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTTGCCGCCGCTCACGCG--GGGCGC	287
ACB9	GCCGAGGGCACGCCTGCCCAGGCATCACGCAACGTTGCCGCCGCTCACGCG--GGGCGC	287
ACM7	GCCAAGGACACGCCTGCTTGGGCATCACGCATCGTCGCCCTACCTCACGCG--GGGCGC	287
ACM8	GCCGAGGGCACGCCTGCCCTTGGCTCACGTATCGTCGCCGCCCTCAAGCG--GGGCGC	287
ACM10	GCTGAGGGCACCCCTGCCTGGGCGTCACGTAACGTCGCCGCCCTACCTGACGCG--GGGCGC	287
AcM4	GCCGAGGGCACGCCTGCCCAGGCCTCACGGAACGTCGCCGCCACCCACGCG--GGGCGG	287
AcB4	GCCGAGGGCACGCCTGCTGGGATCAGGCAACGTCGCCGCCACCTCACGCG--GGGCGC	287
CU	GCCGAGGGCACGCTGCTGGTGTACGCAACGTCGCCAACGCCGCTCACCTTGGGCGT	297
CP	GCCGAGGGCACGCTGCTGGTGTACGCAACGTCGCCAACGCCGCTCACCTTGGGCGT	290

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ACG-HF564641.1	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	345
ACC1	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	347
ACC10	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	347
ACG1	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	347
ACC-HF564642.1	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	346
ACG9	GGTGAATGATGGCCTCCCGAGGCCTCTGTCCCCAGCTGGCTGAAATGACGGCTAGCGC	347
ACC3	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	347
ACC7	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	347
ACC8	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	347
ACG4	GGCGAATGATGGCCTCCCAGAGGCTCTATCCCCTAACTGGCCGAAATAACTGCCTAGCGC	347
ACG10	GGCGAATGATGGCCTCCCAGAGGCTCTATCCCCAACTGGCCGAAATAACTGCCTAGCGC	347
ACG3	GGTGAATGATGGCCTCCCGAGGCCTCCATCCCCAGCTGGCCGAAATAACTGCCTAGCGC	347
ACG5	CGCAAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGACGAAATCACTGCCTAGCGC	347
ACG2	GGCGAATGATGGCCTCCCGAGGCCTCTATCCCCGGCTGGCCGAAATAACTGCCTAGCGC	347
ACC2	GGCGAATGATGGCCTCCCGGGGCTCCATCCCCTAGCTAGCCGAAATGACGGCTAGCGC	346
ACC6	CGCAGATGATGGCTCCCGAGGCCTCTATCCCCGGCTAGCCGAAATGACTCCCTAGCGT	347
ACM-JQ910930.1	GGTAGATGATGGCCTCCTAGGGGCTCCATCCCCTGGCTAGCTGAAATGATGGCTCGCGC	347
ACM3	GGTAGATGATGGCCTCCTAGGGGCTCCATCCCCTGGCTAGCTGAAATGATGGCTTACGC	347
ACM5	GGTAGATGATGGCCTCCTAGGGGCTCCATCCCCTGGCTAGCTGAAATGATGGCTAGCGC	347
ACB-HF564640.1	GGTAGATGATGGCCTCCCAGGGGCTCCATCCCCTGGCTAGCTGAAATGATGGCTCGCGC	347
ACB2	GGTAGATGATGGCCTCCCAGGGGCTCCATCCCCTGGCTAGGTGAAATGATGGCTCGCGC	347
ACB5	GGTAGATGATGGCCTCCCAGGGGCTCCATCCCCTGGCTAGCTGAAATGATGGCTCACGC	347
ACM2	GATAGATGATGACCTCCCAGGGGCTCGATCCCCTGGCTAGCTGAAATGATGGCTCGCGC	347
ACB9	GGTAGATGATGGCCTCCCAGGGGCTCCATCCCCTGGCTAGCTGAAATGATGGCTCGCGC	347
ACM7	GGTAGAAGATGGCTTCTTAGGGCTCCATCCCCTGGCTACTTGAATTGATGGCTCCCC	347
ACM8	GGTAGATGATGGCCTCTTGGGGCTCCATCCTCCGGCTAGCCGAAATGATGGCTTAGTGC	347
ACM10	GGTTGATGATGGCCTCCTAGGGGCTCCATCCCCTGGCTAGCTGAAATGTTGGCTCGCGC	347
AcM4	GGTAGATGATGGCCTCCCAGGGGCTCCATCCCCTGGCTGAAATGATGGCTCGCGC	347
AcB4	GGTAGATGATGGCCTCCCAGGGGCTCCATCCCCTGGCTGAAATAATGGCTAGCGC	347
CU	GGCGGATGATGGCCTCCCGGAGCCTCGCTCCCCTGGCTGGCCGAAATAAGGGGCCGACGT	357
CP	GGCGGATGATGGCCTCCCGGAGCCTCGCTCCCCTGGCTGGCCGAAATAAGGGGCCAACGT	350

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ACG-HF564641.1   GATGGCGGCAATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 404
ACC1             GATGGCGGCCATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACG10           GATGGCGGCCATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACG1            GATGGCGGCCATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACC-HF564642.1   GATGGCGGCCATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 405
ACG9            GACGGTGGCCACGATCCACGAGGACGCTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACC3            GATGGCGGCCATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACC7            AATGGCGGCCATGATCCACGGAGAAGTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACC8            GATGGCGGCCATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACG4            GATGGCGGCCATGATCCACGTGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCAGT 406
ACG10           GATGGCGGCCATGATCCACGTGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 406
ACG3            GATGGCGGCAATGATCCATGGGGATGTTTCGAGACCGAACGTGAACG-CGCAATCCCATGG 406
ACG5            GATGGCGGCCATGATCCACGGGGACGTTTCGAGACCGAACGTGATCG-CATAATCCCATG 406
ACG2            GATGGCGGCCACGATCTACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCACGG 406
ACC2            GATGGCGGCCACGATCTACGGGGACGTTTCGAGACCGAACGTGATCG-CACAATCCCATGG 405
ACC6            GATGGAGGCCATGATCCAGAGGGACGTTTTCGAGACCGAACGTGATCG-CATTGTCCCATGG 406
ACM-JQ910930.1  GATGGCGGCCACGATCCATAGGGATGTTTCAAGAATAGACGTGCGCG-CGTTGTCCCACGG 406
ACM3            GATGGCGGCCACGATCCATAGGGATGTTTCAAGAATAGACGTGCGCG-CGTTGTCCCACGG 406
ACM5            GATGGCGGCCACGATCCATAGGGATGTTTCAAGAATAGACGTGCGCG-CGTTGTCCCACGG 406
ACB-HF564640.1  GATGGCGGCCACGATCTATAGGGATGTTTCAAGAATAGACGTGCACG-CGTTGTCCCACGG 406
ACB2            GATGGCGGCCACGATCTATAGGGATGTTTCAAGAATAGACGTGCACG-CGTTGTCCCACGG 406
ACB5            GATGGCGGCCACGATCTATAGGGATGTTTCAAGAATAGACGTGCACG-CGTTGTCCCACGG 406
ACM2            GATGGCGGCAACGATCTATAGGGATGTTTCAAGATTAGACGTGCACG-CGTTGTCCCACGG 406
ACB9            GATGGCGGCCACGATCTATAGGGATGTTTCAAGAATAGACGTGCACG-CGTTGTCCCACGG 406
ACM7            GATGCCGGCAACAATCCATAGGGATGTTTCAAGAATGGACGTGCCCG-CGTTGACCCACGG 406
ACM8            GATGGCGGCCACGATCCATGGGGATGTTTCAAGAATAGACGTGCGCG-CGTTGTCCCACGG 406
ACM10           GATGGCGGCCACGATGCCTCGGGTGTTCATGAAGAAACGTGCGCG-CGTTGACCCACGG 406
AcM4            GATGGCGGCCACGATCCATAGGGACGTTTCAAGAATAGACGTGCGCG-CGTTATCCCATGG 406
AcB4            GATGGCGGCCACGATCCATAGGGATGTTTCAAGAATAGACGTGCACG-CGTTGTCCCATGG 406
CU              GACGACTGCCACGATCCACGTTGGT-TGGGCGAACATTTCGCTCGAGGGCCAGACGTGCG- 415
CP              GACGGCCGCCACGATCCACGTTGGA-TGAGTGATCACTCGCTCGAG-CCAGTTCGCGCG- 407
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ACG-HF564641.1   GGAGGGTTCGGACCCCGAATCCCACGGCGACCCAG-TCAA-----GCGG- 447
ACC1             GGAGGGTTCGGACCCCGAATCCCACGGCGACCCAGGTCAG-----GCGG- 450
ACG10           GGAGGGTTCGGACCCCGAATCCCACGGCGACCCAGGTCAG-----GCGG- 450
ACG1            GGAGGGTTCGGACCCCGAATCCCACGGCGACCCTAGGTCAG-----GCGG- 450
ACC-HF564642.1   GGAGGGTTCGGACCCCGAATCCCACGGCGACCCTAGGTCAG-----GCGG- 449
ACG9            GGAGGGTTCGAACCCCGAATCCCACGGCGACCCTAGGTCAG-----GCGG- 450
ACC3            GGAGGGTTCGGACCCCGAATCCCACGGCGACCCTAGGTCAG-----GCGG- 450
ACC7            GGAGGGTTCGGACCCCGAATCCCTCCGCGACCCTAGGTCAG-----GCGG- 450
ACC8            GGAGGGTTCGGACCCCGAATCCCACGGCGACCCAGGTCAG-----GCGG- 450
ACG4            GGAGGGTTCGTACCGCGAATCCGACGGTGACCCAGGTCAG-----GCGG- 450
ACG10           GGAGGGTTCGGACCCCGAATCCCACGGCGACCCAGGTCAG-----GCGG- 450
ACG3            GGAGGGCCGGACCCCGAATCCCACGGCAACCCAGGTCAG-----GCGG- 450
ACG5            GGAGGGCCGGACCCCGAATCCCACGGCGACCCAGGTCAG-----GCGG- 450
ACG2            GGAGGGTTCGAACCCCGAATCCCACGGCGACCCTAGGTCAG-----GCGG- 450
ACC2            GGAGGGTTCGGACCCCGAATCCCACGGCGACCCTAGGTCAG-----GCGG- 449
ACC6            GGAGGGCTGGACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
ACM-JQ910930.1  GGAGGGCTGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
ACM3            GGAGGGCTGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
ACM5            GGAGGGCCGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
ACB-HF564640.1  GGAGGGCCGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
ACB2            GGAGGGCCGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
ACB5            GGAGGGCCGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
ACM2            GAAGGGCCGAACCCCGAATCCCACAGCAACCCGGGTCAG-----GCGG- 450
ACB9            GGAGGGCCGAACCCCGAATCCCACAGCCACCCAGGTCAG-----GCGG- 450
ACM7            GGAGGGTTGAACCCCGAATCCAACAGTAACCCCTGGTCAG-----GCGG- 450
ACM8            GGAAGGCCGAACCCCGAATCCCACAGCAACGCCAGGTCAG-----GCGG- 450
ACM10           GGAGGGCTGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
AcM4            GGAGGGCTGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
AcB4            GGAGGGCCGAACCCCGAATCCCACAGCAACCCAGGTCAG-----GCGG- 450
CU              --CGGGTTCGTGCCACG-----GCAGGG-----GCC-- 438
CP              --TGCGCCGTGCCACGGTGATGGCCCGGCAGCAAGGGCGCTCCGACGCTT 456
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Table S3 Boxshade of Clustal W2 alignment with evidenced nucleotide differences

ACG-HF564641.1	1	CAAGGAACTGCAACAGAGCGAG-AGCGCCTTACGCCGATTGCACCCGAAATGACTCTCAA
ACC1	1T.....T.....
ACC10	1T.....
ACG1	1T.....
ACC-HF564642.1	1
ACG9	1C.....A.....-A.....C.T.....
ACC3	1C.....A.....-CA.....C.....T.....
ACC7	1A.....G.....A.....A.....
ACC8	1
ACG4	1-C.....
ACG10	1
ACG3	1-T.....
ACG5	1G.....-.....G.....
ACG2	1	..G.....-T.....C.T.....A..C.....
ACC2	1-T.....C.T.....C.....GG
ACC6	1	...C.....G.....-.....CTT.....A..TGC.....T..
ACM-JQ910930.1	1-T.....C.T.....A..C.....GG
ACM3	1-T.....C.T.....A..C.....GG
ACM5	1-T.....C.T.....A..C.....GG
ACB-HF564640.1	1-T.....C.T.....A..C.....GG
ACB2	1-T.....C.T.....A..C.....GG
ACB5	1-T.....C.T.....A..C.....GG
ACM2	1-T.....C.T.....A..C.....GG
ACB9	1-TAG.....C.T.....A..C.....GG
ACM7	1	A.C.....-T.....C.T.....A..C.....GG
ACM8	1T.....-G.....C.T.....G..A..C.....GG
ACM10	1	...A..C.....A.....-C.....C.....A..A.....GG
AcM4	1-T.....C.T.....A..C.....GG
AcB4	1-.....A..C.....GG
CU	1AC.A...A...AG.GCA.T.G.--..AT.....T.....C.....GG
CP	1A...AGA...A..GCA...A.G.T..AT.....T.....C.....GG
ACG-HF564641.1	60	CAACGGATATCTCAGCTCTCGCATCGATG-AGAACGTAGTGAAATGCAATGCTTGG
ACC1	60A.....
ACC10	60A.....
ACG1	60A.....
ACC-HF564642.1	60A.....
ACG9	60A..A.....C.....A.....
ACC3	60	T.....A..A.....G.....A.....
ACC7	60A.....G.....A.....
ACC8	60A.....
ACG4	60A.....
ACG10	60A.....
ACG3	60A..A.....
ACG5	60A.....G.....G.....
ACG2	60A...A..C.....G.....A.....
ACC2	60A.....C.....A.....
ACC6	60CT..G.....A.....C.C.....
ACM-JQ910930.1	60G.....A...A..C.....G.....
ACM3	60G.....A...A..C.....G.....
ACM5	60G.....A...A..C.....G.....
ACB-HF564640.1	60GA.....A...A..C.....G.....A.....
ACB2	60GA...T.G.....A...A..C.....G.....A.....
ACB5	60GA.....A...A..C.....G.....A.....
ACM2	60GA.....A...A..C.....G.....A.....
ACB9	60GA.....A...A..C.....G.....
ACM7	60	T.C.....G.....A...A..C.....G.....
ACM8	60G.....A...A..C.....TG.....
ACM10	60	...T.....T.....AT...T.AAA...AG..C.....ATG..A.....
AcM4	60	...A.....G.....A...A..C.....
AcB4	60GA.....A...A..C.....
CU	58G.....A...C.....G..A.....
CP	61G.....A...C.....G..A.....
ACG-HF564641.1	115	TGTGAATTGCAGAATCCTGTGAACTATCGAGTCTTTGAATGTAAAGTTGCG-CCAAA
ACC1	116C.....
ACC10	116C.....
ACG1	116T.....C.....
ACC-HF564642.1	116T.....
ACG9	116C.....C..G.....A..C..G..
ACC3	116C.....C..T.....C.....A..C.....

ACC7	116A.....C.....C.....C.....
ACC8	116C.....
ACG4	116C.....
ACG10	116C.....
ACG3	116C.....
ACG5	116T.....AT.C..C.T
ACG2	116T.....C.C.....
ACC2	116C.....C.T...C...C.C.....T-.....
ACC6	116G.....A.....T.....G.G..C...T.C.....
ACM-JQ910930.1	116C.....C.T...C...C.C.....T.C..G..
ACM3	116C.....C.T...C...C.C.....T.C..G..
ACM5	116T.C.G...C.T...C...C.C.....T.C..G..
ACB-HF564640.1	116C.....C.T...C...C.C.....T.C.....
ACB2	116C.....C.TT...C...C.C.....T.C.....
ACB5	116C.....C.T...C...C.C.....T.C.....
ACM2	116C.....C.T...C...C.C.....T.C.....
ACB9	116C.....C.T...C...C.C.....T.C.....
ACM7	116C.....C.T...C...C.C.....T.C..G..
ACM8	116C.....C...C...C.C.....T.C..G..
ACM10	116G..G.....C.....C.G.....C...A..C..G..
AcM4	116T..C..T...C...C.G.....T.C..G..
AcB4	116C.....C.....C.....C.....
CU	114C.....C.....C.C.....C..G..
CP	117C.....C.....C.C.....C..G..

ACG-HF564641.1	170	GCTATTAGGCCGAGGGCACGCCTGCCTGGGCGTCACGCAACGTCGCCGCCACCCCA
ACC1	172
ACC10	172
ACG1	172
ACC-HF564642.1	171
ACG9	172T.....A.....T..A.....
ACC3	172T.....
ACC7	172C.....T.....G.....
ACC8	172
ACG4	172AT...A.....
ACG10	172
ACG3	172A.....T.....
ACG5	172G.....T..
ACG2	172C...A.....T.....G..T..
ACC2	171	..C.....C..A.....T.....G..T..
ACC6	172	TTG.....G.....C.....A.A...C.G.G.....C.....GC..T..
ACM-JQ910930.1	172	..C.....C...A.....T..T..
ACM3	172	..C.....C...A.....G..T..
ACM5	172	..C.....G...C...A.....G..T..
ACB-HF564640.1	172	..C.....C...A.....T.....G..T..
ACB2	172	..C.....T...C...A.....T.....G..T..
ACB5	172	..C.....C...A.....T.....G..T..
ACM2	172	..C.....C...A.....T.....G..T..
ACB9	172	..C.....C...A.....T.....G..T..
ACM7	172	..C.....A..A.....T...A.....T.....GC.T..T..
ACM8	172	..TC.....CTT.....T.T.....GC..T..
ACM10	172	..C.....T.....C.....T.....G..T..TG.
AcM4	172	..C.....C...C...G.....
AcB4	172A.....T..
CU	170	..CT.....T.....T.....AA.G..G.
CP	173	..CT.....T.....T.....G.....A..G..G.

ACG-HF564641.1	226	CGCG--GGGCACGGCGAATGATGGCCTCCCGAGGCCTCTATCCCCAGCTGGCCGA
ACC1	228--.....
ACC10	228--.....
ACG1	228--.....
ACC-HF564642.1	227--.....
ACG9	228--.....T.....G.....T..
ACC3	228--.....
ACC7	228--.....
ACC8	228--.....
ACG4	228--A.....AGA.G.....T.A.....
ACG10	228--.....A.....A.....
ACG3	228--.....C..T.....C.....
ACG5	228--.....G.C..A.....CGA.G.....A...
ACG2	228--.....G.....
ACC2	227--A...G.....G.....C.....T...A.....
ACC6	228--.....G.C..AG.....G.....G.....G..A..G..
ACM-JQ910930.1	228--.....G..TAG.....TAG..G..C.....TG...A..T..
ACM3	228--.....G..TAG.....TAG..G..C.....TG...A..T..

ACM5	228--....G...TAG.....TAG..G...C.....TG...A..T..
ACB-HF564640.1	228--....G...TAG.....AG..G...C.....TG...A..T..
ACB2	228--....G...TAG.....AG..G...C.....TG...A..GT..
ACB5	228--....G...TAG.....AG..G...C.....TG...A..T..
ACM2	228--....G..ATAG.....A.....AG..G...G.....TG...A..T..
ACB9	228--....G...TAG.....AG..G...C.....TG...A..T..
ACM7	228--....G...TAG.A.....T..TTAG.....C.....TG...ACTT..
ACM8	228	A....--....G...TAG.....TTTG..G...C...T..G...A.....
ACM10	228--....G...TTG.....TAG..G...C.....TG...A..T..
AcM4	228--....GG..T.G.....AG..G...C.....G.....T..
AcB4	228--....G...TAG.....AG..G...C.....AG.....T..
CU	226	TC.TCT...GT...G.....G.AG.CTCGC.T...G.....
CP	229	TCATGC...TT...G.....G.AG.GTCGC.T...G.....

ACG-HF564641.1	280	AATAACTGCCTAGCGCGATGGCGGCAATGATCCACGGGGACGTTTCGAGACCGAACG
ACC1	282C.....
ACC10	282C.....
ACG1	282C.....
ACC-HF564642.1	281C.....
ACG9	282	..G..G.....C..T..C..C.....A.....C..T.....
ACC3	282C.....
ACC7	282A.....C.....A..A.....
ACC8	282C.....
ACG4	282C.....T.....
ACG10	282C.....
ACG3	282C.....T.....T.....
ACG5	282	..C.....C.....GC.....
ACG2	282C.....C..C...T.....AG...
ACC2	281	..G..G.....C..C...T.....
ACC6	282	..G...C.....T...A...C.....GA...T...AG...
ACM-JQ910930.1	282	..G.TG...C.....C..C...TA...T...A...ATAG...
ACM3	282	..G.TG...TA.....C..C...TA...T...A...ATAG...
ACM5	282	..G.TG...C.....C..C...TA...T...A...ATAG...
ACB-HF564640.1	282	..G.TG...C.....C..C...T.TA...T...A...ATAG...
ACB2	282	..G.TG...C.....C..C...T.TA...T...A...ATAG...
ACB5	282	..G.TG...CA.....C..C...T.TA...T...A...ATAG...
ACM2	282	..G.TG...C.....C...T.TA...T...A...TTAG...
ACB9	282	..G.TG...C.....C..C...T.TA...T...A...ATAG...
ACM7	282	..T.G.TGC...CC.C...C.....CA...T...A...AT.G...
ACM8	282	..G.TG...T.....C..C...T...T...A...ATAG...
ACM10	282	..GTTG...C.....C..C..G.CTC...TT...AT..AGA...
AcM4	282	..G.TG...C.....C..C...TA...T...A...ATAG...
AcB4	282	..G.TG...C.....C..C...TA...T...A...ATAG...
CU	282	..A.GGG...CGA..T..C.A.T..C..C.....T.GT-.GG.C..A.ATT..
CP	285GGG...C.A..T..C...C..C.....T.GA-.GA.T..T.ACT..

ACG-HF564641.1	336	TGATCG-CACAATCCCATGGGGAGGGTCGGACCCCGAATCCCACGGCGACCCAG-
ACC1	338-.....G
ACC10	338-.....G
ACG1	338-.....T..G
ACC-HF564642.1	337-.....T..G
ACG9	338-.....A.....T..G
ACC3	338-.....T..G
ACC7	338-.....T.C.....T..G
ACC8	338-.....A.....G
ACG4	338-.....GT.....T..G.....G...T.....G
ACG10	338-.....G
ACG3	338	..A.-.G.....C.....A.....G
ACG5	338-..T.....AT.....CG.....G
ACG2	338-.....C.....A.....T..G
ACC2	337-.....T..G
ACC6	338-..TTG.....CT.....A..A.....G
ACM-JQ910930.1	338	..CG.-.GTTG...C.....CT.A.....A..A.....G
ACM3	338	..CG.-.GTTG...C.....CT.A.....A..A.....G
ACM5	338	..ACG.-.GTTG...C.....C..A.....A..A.....G
ACB-HF564640.1	338	..CA.-.GTTG...C.....C..A.....A..A.....G
ACB2	338	..CA.-.GTTG...C.....C..A.....A..A.....G
ACB5	338	..CA.-.GTTG...C.....C..A.....A..A.....G
ACM2	338	..CA.-.GTTG...C...A...C..A.....A..A.....G.G
ACB9	338	..CA.-.GTTG...C.....C..A.....A..C.....G
ACM7	338	..CC.-.GTTGA...C.....T.A.....A..A.TA...T..G
ACM8	338	..CG.-.GTTG...C.....A..C..A.....A..A..G...G
ACM10	338	..CG.-.GTTGA...C.....CT.A.....A..A.....G
AcM4	338	..CG.-.GTT...C.....CT.A.....A..A.....G

AcB4	338	..CA..-.GTTG.....C..A.....A..A.....G
CU	337	CTCGA.G.CA.GA.GTGC.---C.....TG..A..-----G.AG.G
CP	340	CTCGA..-CAGG..G.GC.---T.C.C..TG..A..GTGATGG.CCG.CAG.A..G
ACG-HF564641.1	390	TCAA-----GCGG- 447
ACC1	393	...G----- 450
ACC10	393	...G----- 450
ACG1	393	...G----- 450
ACC-HF564642.1	392	...G----- 449
ACG9	393	...G----- 450
ACC3	393	...G----- 450
ACC7	393	...G----- 450
ACC8	393	...G----- 450
ACG4	393	...G----- 450
ACG10	393	...G----- 450
ACG3	393	...G----- 450
ACG5	393	...G----- 450
ACG2	393	...G----- 450
ACC2	392	...G----- 449
ACC6	393	...G----- 450
ACM-JQ910930.1	393	...G----- 450
ACM3	393	...G----- 450
ACM5	393	...G----- 450
ACB-HF564640.1	393	...G----- 450
ACB2	393	...G----- 450
ACB5	393	...G----- 450
ACM2	393	...G----- 450
ACB9	393	...G----- 450
ACM7	393	...G----- 450
ACM8	393	...G----- 450
ACM10	393	...G----- 450
AcM4	393	...G----- 450
AcB4	393	...G----- 450
CU	376	-----C-- 438
CP	392	G.GCTCCGAC..CTT 456

Table S4 Estimates of Genetic Distance over Sequence Pairs between Groups

The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Analyses were conducted using the Maximum Composite Likelihood model in MEGA6. The analysis involved 29 nucleotide sequences.

M, ,
G,0.191, ,
C,0.203,0.08, ,
B,0.07,0.163,0.174, ,