Electronic supplementary material

Methods – DArTseq

Genome-wide single nucleotide polymorphism (SNP) data was generated at Diversity Arrays Technology (DArT). DArTseq™ is genotype-by-sequencing technology which represents a combination of a DArT complexity reduction methods and next generation sequencing platforms. DArTseq methods are optimized for different organisms and applications by selecting the most appropriate complexity reduction method (size of the representation and the fraction of a genome selected for the assays). Four methods of complexity reduction were tested and the PstI-HpaII method was selected. Genomic DNA was processed in digestion/ligation reactions principally as per Kilian *et al*. (2012) but replacing a single PstI-compatible adaptor with two different adaptors corresponding to two different Restriction Enzyme (RE) overhangs. The PstI-compatible adapter was designed to include Illumina flowcell attachment sequence, sequencing primer sequence and "staggered", varying length barcode region, similar to the sequence reported by Elshire et al. (2011). Reverse adapter contained the flowcell attachment region and HpaII-compatible overhang sequence. Sequencing was carried out on a single lane of an Illumina Hiseq2500 and processed using proprietary DArT analytical pipelines. In the primary pipeline, the FASTQ files were first processed to filter away poor quality sequences. In the barcode region the minimum Phred pass score was 30 and the minimum pass was 75%, while in the whole read quality the minimum Phred pass score was 10 and the minimum pass quality was 50%. Approximately 2,500,000 sequences per barcode/sample were identified and used in marker calling. Identical sequences were collapsed into fastqcall files and these files were used in the secondary pipeline for DArT's proprietary SNP calling algorithms (DArTsoft14). Sequences were blasted against a *Symbiodinium* reference genome to ensure that only sequences belonging to the coral host and not the symbiont were included in the dataset; however, no symbionts were detected among the markers anyway due to the strength of DArT PL's filtering software. This software (DArTsoft14) has the capacity to filter contaminants such as viral and/or bacterial sequences in SNP marker selection through training the program to distinguish allelic sequence variants from

paralogs and "contaminating" sequences based on analysis of Mendelian behaviour of DArTseq markers in thousands of control crosses in a large diversity of organisms.

DaRTseq generates two types of data: (a) "Silico DaRT" which are presence/absence dominant markers based on a range of DNA variation types such as SNPs, indels and methylation variation; and (b) SNPs in fragments of approximately 100 bp. In this study we used only the fragment data, and SNPs were extracted from each fragment and concatenated into supermatrices using IUPAC codes for heterozygous loci.

Methods – Phylogenetic analyses

For the CR and *PaxC*, the most appropriate model of DNA substitution was determined in MEGA6 (Tamura *et al.* 2013) using the Bayesian Information Criterion (CR = HKY model, *PaxC* = K80), and these models were used in phylogenetic analyses run in PhyML 3.0 (Guindon *et al.* 2010). Support for each node was based upon 1000 bootstrap replicates. For the Bayesian analyses, MCMC chains were run for 3,000,000 generations (for each gene/SNP matrix) and sampled every 100th generation, with the first 7,500 runs discarded as burn-in. The *PaxC* alignment contained 14 indels (including several large indels up to 390 bp), and the CR contained three large indels, and many of the indels were phylogenetically informative. To take a conservative approach, each indel was coded as a single base change, based on the view that indels arise from single mutation events (Simmons & Ochoterena 2000).

ESM References

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Kilian A, Wenz lP, Huttner E, Carling J, Xia L, *et al*.. 2012 Diversity Arrays Technology (DArT) - a generic genome profiling technology on open platforms. *Methods in Molecular Biology* Edited by Francois Pompanon and Aurelie Bonin, Humana Press: 67–91

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S 2013 MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. . *Mol Biol Evol* **30**, 2725-2729.

Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O. 2010 New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59(3), 307-321**.**

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence based phylogenetic analyses. *Syst Biol* **49**, 369-381

Table S1. Locality and GPS co-ordinates of sites from which each sample was collected in this study. Species are arranged into morphological species groups as traditionally defined by Wallace (1999). Clade number and letter refers to the clades highlighted in Fig S1. Registration numbers are for the Western Australian Museum where samples are housed. Samples annotated with _A = autumn spawner and _S = spring spawner. * = sample couldn't be amplified in CR; ^ = sample couldn't be amplified in *PaxC*; # = sample not included in SNP analysis.

Species group	Species	Sample	Clade	Region	Lat	Long	Reg. No.
aspera	*A. aspera*	asp1	IIID	Kimberley	S15.505	E123.608	Z65722
	A. aspera	asp2	IVA	Kimberley	S16.054	E123.350	Z65735
	A. aspera	asp3	IVA	Abrolhos	S28.681	E113.860	
	*A. millepora*_A	mil1	IIIA	Ningaloo	S22.168	E113.865	
	*A. millepora*_A	mil2	IIIA	Ningaloo	S22.168	E113.865	
	*A. millepora*_A	mil3#	-	Ningaloo	S22.168	E113.865	
	*A. millepora*_A	mil4#	-	Ningaloo	S22.168	E113.865	
	*A. millepora*_S	mil5	IIIA	Ashmore	S12.245	E122.986	
	*A. millepora*_S	mil6	IIIA	Ashmore	S12.245	E122.986	
	*A. millepora*_S	mil7#	-	Ashmore	S12.245	E122.986	
	*A. millepora*_S	mil8#	-	Ashmore	S12.245	E122.986	
	A. pulchra	pul1	IIIC	Kimberley	S13.956	E125.623	Z65654
	A. pulchra	pul2*^	IIIA	Kimberley	S15.551	E124.177	Z65621
	A. pulchra	pul3	IIID	Kimberley	S13.956	E125.623	Z65683
	A. spicifera	spi1	IIIB	Kimberley	S15.324	E123.076	Z65612
	A. spicifera	spi2	IIIB	Abrolhos	S 28.852	E114.0122	
	A. spicifera	spi3	IIIB	Montebello	S20.516	E115.466	
divaricata	*A. divaricata*	div1	IIIE	Kimberley	S13.956	E125.623	Z65637
	A. divaricata	div2	IIIC	Kimberley	S15.324	E123.076	Z65609
	A. divaricata	div3	IIIE	Kimberley	S13.956	E125.623	Z65673
	A. stoddarti	sto1*^	IIIA	Kimberley	S15.324	E123.076	Z65616
	A. stoddarti	sto2	IIIE	Kimberley	S13.956	E125.623	Z65677
	A. stoddarti	sto3	IIIE	Kimberley	S13.856	E125.824	Z65706
florida	*A. florida*	flo1	IVC	Kimberley	S14.061	E125.366	Z65765
	A. florida	flo2	IV	Kimberley	S12.237	E123.160	Z66287
	A. florida	flo3	IV	Kimberley	S15.038	E124.427	Z65712
humilis	*A. digitifera*	dig1	IIID	Kimberley	S15.282	E124.105	Z65717a
	A. digitifera	dig2	IIID	Kimberley	S15.282	E124.105	Z65717b
	A. digitifera	dig3	IVB	Kimberley	S13.956	E125.623	Z65671
	A. gemmifera	gem1	IVC	Kimberley	S14.117	E123.538	Z65750
	A. gemmifera	gem2	IVA	Kimberley	S14.117	E123.538	Z65756
	A. humilis	hum1	IVB	Montebello	S20.405	E115.581	

	A. humilis	hum2*^	IVB	Kimberley	S14.117	E123.538	Z65748
	A. humilis	hum3	IVB	Montebello	S20.907	E115.462	
	*A. samoensis*_S	sam1	IVB	Barrow	S20.786	E115.506	
	*A. samoensis*_S	sam2	IVB	Barrow	S20.786	E115.506	
	*A. samoensis*_S	sam3	IVB	Barrow	S20.786	E115.506	Z84440
	*A. samoensis*_S	sam4	IVB	Barrow	S20.786	E115.506	Z84441
	*A. samoensis*_S	sam5	IVB	Barrow	S20.786	E115.506	Z84442
	*A. samoensis*_S	sam6	IVB	Barrow	S20.786	E115.506	Z84443
	*A. samoensis*_S	sam7	IVB	Barrow	S20.786	E115.506	Z84446
	*A. samoensis*_S	sam8	IVB	Barrow	S20.786	E115.506	Z84447
	*A. samoensis*_A	sam9	IVC	Barrow	S20.786	E115.506	Z84451
	*A. samoensis*_A	sam10	IVC	Barrow	S20.786	E115.506	
	*A. samoensis*_A	sam11	IVC	Barrow	S20.786	E115.506	Z84461
	*A. samoensis*_A	sam12	IVC	Barrow	S20.786	E115.506	Z84452
	*A. samoensis*_A	sam13	IVC	Barrow	S20.786	E115.506	Z84463
	*A. samoensis*_A	sam14	IVC	Barrow	S20.786	E115.506	Z84453
	*A. samoensis*_A	sam15	IVC	Barrow	S20.786	E115.506	Z84454
	*A. samoensis*_A	sam16	IVC	Barrow	S20.786	E115.506	Z84455
hyacinthus	*A. cytherea*	cyt1	IIIB	Kimberley	S13.956	E125.623	Z65641
	A. cytherea	cyt2	IIIB	Kimberley	S14.117	E123.538	Z65792
	A. cytherea	cyt3	IIIB	Montebello	S20.786	E115.506	
latistella	*A. subulata*	sub1	IIIB	Kimberley	S13.956	E125.623	Z65682
	A. subulata	sub2	IIIA	Kimberley	S16.054	E123.350	Z65738
	A. subulata	sub3	IIIA	Kimberley	S13.856	E125.824	Z65700
muricata	*A. muricata*	mur1	IIIC	Kimberley	S13.956	E125.623	Z65648
	A. muricata	mur2	IIIE	Kimberley	S14.254	E125.159	Z65775
	A. muricata	mur3	IIID	Kimberley	S13.956	E125.623	Z65684
nasuta	*A. lutkeni*	lut1	IIID	Kimberley	S13.956	E125.623	Z65647
	A. lutkeni	lut2	IVA	Kimberley	S13.956	E125.623	Z65649
	A. lutkeni	lut3*^	IVA	Kimberley	S15.525	E124.177	Z65624
selago	*A. donei*	don1	IIIB	Kimberley	S13.956	E125.623	Z65636
	A. donei	don2	IE	Kimberley	S13.856	E125.824	Z65697
	A. donei	don3	IIIB	Kimberley	S14.117	E123.538	Z65759
	A. loisetteae	los1*	IIIA	Abrolhos	S 28.852	E114.012	
	A. loisetteae	los2	IIIA	Abrolhos	S28.681	E113.860	
	A. loisetteae	los3	IIIA	Abrolhos	S28.681	E113.860	
	*A. tenuis*_A	ten1	IC	Montebello	S20.516	E115.466	
	*A. tenuis*_A	ten2	IA	Montebello	S20.516	E115.466	

	*A. tenuis*_A	ten3	IA	Montebello	S20.516	E115.466	
	*A. tenuis*_A	ten4	IC	Montebello	S20.516	E115.466	
	*A. tenuis*_A	ten5	IB	Kimberley	S13.956	E125.623	Z65663
	*A. tenuis*_A	ten6	IB	Kimberley	S13.856	E125.824	Z65695
	*A. tenuis*_S	ten7	IE	Kimberley	S13.956	E125.623	Z65667
	*A. tenuis*_S	ten8	IE	Kimberley	S13.956	E125.623	Z65655
	*A. tenuis*_A	ten9	IC	Montebello	S20.516	E115.466	
	*A. tenuis*_S	ten10	IA	Ashmore	S12.240	E122.980	
	*A. tenuis*_S	ten11	IA	Ashmore	S12.240	E122.980	
	*A. tenuis*_S	ten12	IC	Ashmore	S12.240	E122.980	
	*A. tenuis*_S	ten13	IC	Ashmore	S12.240	E122.980	
	*A. tenuis*_A	ten14	IC	Ashmore	S12.184	E123.109	
	*A. tenuis*_S	ten15	IC	Ashmore	S12.240	E122.980	
	*A. tenuis*_S	ten16	IC	Ashmore	S12.240	E122.980	
	A. selago	sel1	ID	Kimberley	S13.956	E125.623	Z65665
	A. selago	sel2	ID	Kimberley	S13.856	E125.824	Z65698
	A. selago	sel3	IC	Montebello	S21.044	E115.470	
robusta	*A. intermedia*	int1	IVA	Kimberley	S13.956	E125.623	Z65689
	A. intermedia	int2*^	IVA	Abrolhos	S 28.852	E114.012	
	A. intermedia	int3	IVA	Abrolhos	S 28.852	E114.012	

Table S2. Descriptive statistics of DArT single nucleotide polymorphism dataset for each *Acropora* species sampled (± SE).

Species	N	Freq. heterozygotes	Freq. homozygote ref.	Freq. missing data	Freq. polymorphic loci
A. aspera	3	0.028	0.736	0.160	0.182
A. cytherea	3	0.040	0.719	0.172	0.138
A. digitifera	3	0.037	0.731	0.118	0.163
A. divaricata	3	0.048	0.702	0.206	0.169
A. donei	3	0.036	0.721	0.171	0.155
A. florida	3	0.077	0.702	0.060	0.203
A. gemmifera	2	0.052	0.729	0.127	0.078
A. humilis	3	0.075	0.709	0.041	0.150
A. intermedia	3	0.047	0.719	0.094	0.134
A. loisetteae	3	0.026	0.735	0.151	0.078
A. lutkeni	3	0.023	0.732	0.166	0.166
A. millepora	4	0.044	0.725	0.104	0.115
A. muricata	3	0.020	0.727	0.211	0.180
A. pulchra	3	0.025	0.734	0.187	0.226
A. samoensis	16	0.049	0.716	0.088	0.226
A. selago	3	0.024	0.711	0.378	0.226
A. spicifera	3	0.029	0.723	0.150	0.226
A. stoddarti	3	0.100	0.681	0.071	0.226
A. subulata	3	0.042	0.720	0.128	0.226
A. tenuis	16	0.045	0.694	0.387	0.226
Min		0.020	0.681	0.041	0.078
Max		0.100	0.736	0.387	0.226
Mean		0.046 (± 0.006)	0.716 (± 0.004)	0.160 (± 0.019)	0.179 (± 0.010)

Table S3. Comparison of trees showing the placement of species within major clades (clades I, III or IV) and the placement of individuals within species (tight or split; MS = major split between clades; ms = minor split within a clade), and where discrepancies in topologies occurred. The criteria for a discrepancy is where there is support for different topologies on the two trees, but does not apply where one tree is more highly resolved than another.

Species	***PaxC***	**CR**	**SNPs**	**Discrepancy in topology**
A. aspera	III+IV: split = MS+ms	III+IV: split = MS+ms	III+IV: split = MS+ms	-
A. cytherea	III: tight	IV: tight	III: tight	CR ≠ SNP; *PaxC* ≠ CR
A. digitifera	III+IV: split = MS	III+IV: split = MS	III+IV: split = MS	-
A. divaricata	III: split = ms	III: split = MS	III: split = ms	CR ≠ SNP; *PaxC* ≠ CR
A. donei	I+III: split = MS	I+II+IV: split = MS	I+III: split = MS	CR ≠ SNP; *PaxC* ≠ CR
A. florida	IV: split = ms	IV: tight	IV: split = ms	-
A. gemmifera	IV: split = ms	IV: tight	IV: split = ms	-
A. humilis	IV: tight	IV: tight	IV: tight	-
A. intermedia	IV: tight	IV: tight	IV: tight	-
A. loisetteae	III: tight	III: tight	III: tight	-
A. lutkeni	III+IV: split = MS	III+IV: split = MS	III+IV: split = MS	-
A. millepora	III: split = ms	III: tight	III: tight	*PaxC* ≠ SNP
A. muricata	III: split = ms	III+IV: split = MS	III: split = ms	CR ≠ SNP; *PaxC* ≠ CR
A. pulchra	III: tight	III+IV: split = MS	III: tight	CR ≠ SNP; *PaxC* ≠ CR
A. samoensis	IV: split = ms	IV: tight	IV: split = ms	-
A. selago	I: split = ms	I: tight	I: split = ms	*PaxC* ≠ SNP
A. spicifera	III: tight	III+IV: split = MS	III: split = ms	CR ≠ SNP; *PaxC* ≠ CR
A. stoddarti	III: tight	III: tight	III: split = ms	-
A. subulata	III: split = ms	III+IV: split = MS	III: split = ms	CR ≠ SNP; *PaxC* ≠ CR
A. tenuis	I: split = ms	I: tight	I: split = ms	*PaxC* ≠ SNP

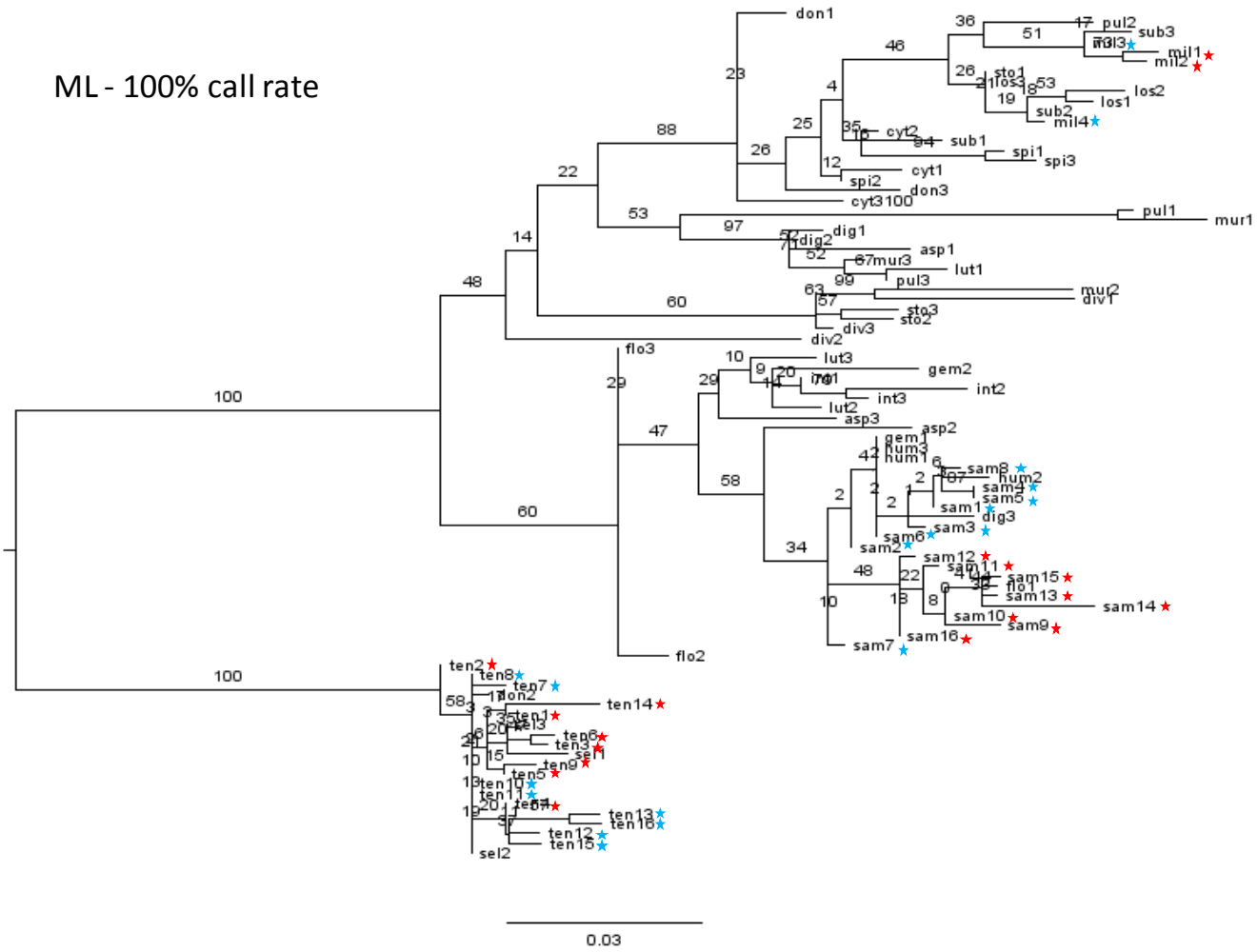

Figure S1(a). Maximum Likelihood trees generated in RAxML from SNP matrices with genotype call rate of (a) 100% (b) 90% and (c) 70% and a minimum coverage of 8X. Blue stars represent spring spawners and red stars represent autumn spawners.

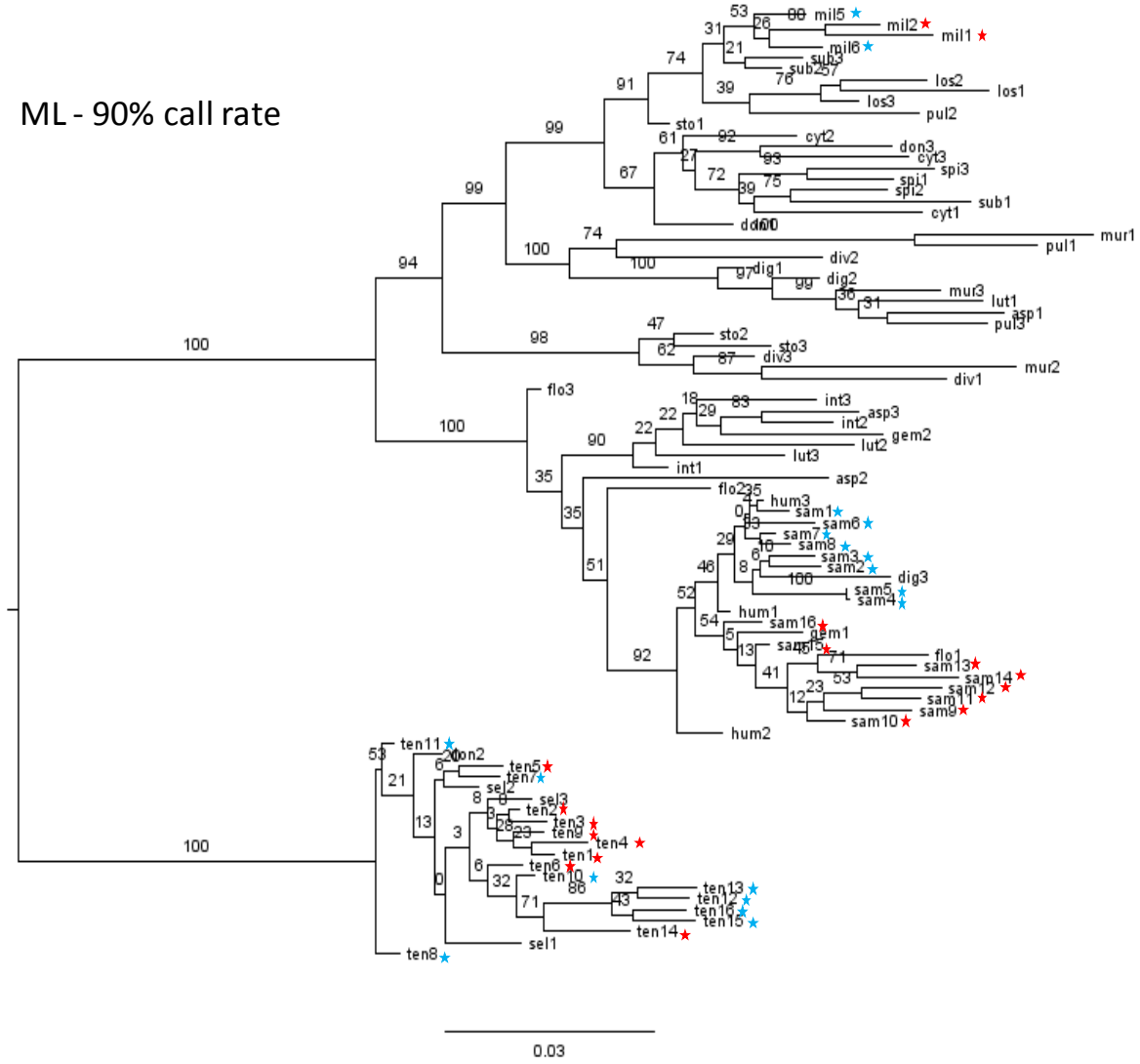

Figure S1(b). continued

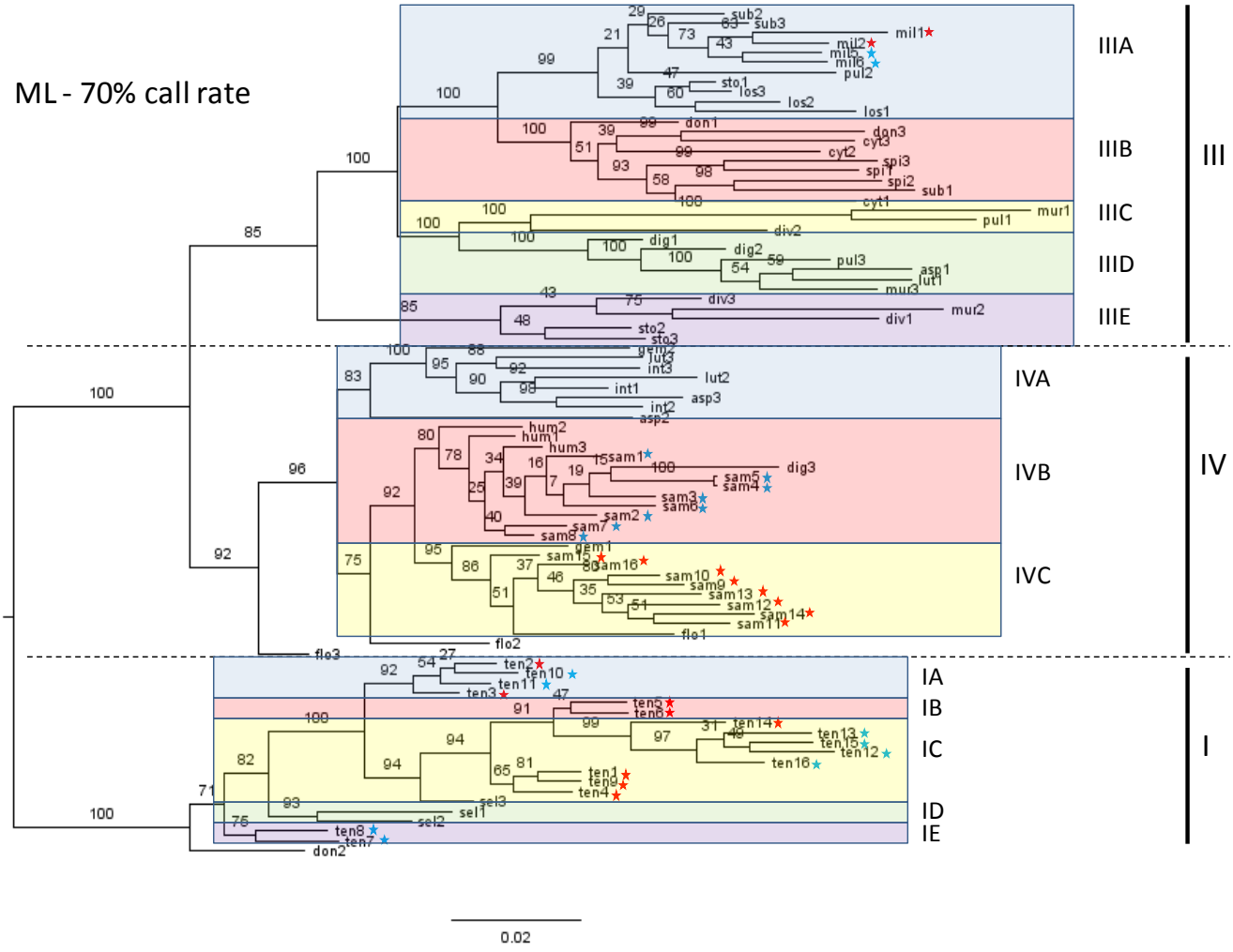

Figure S1(c). continued

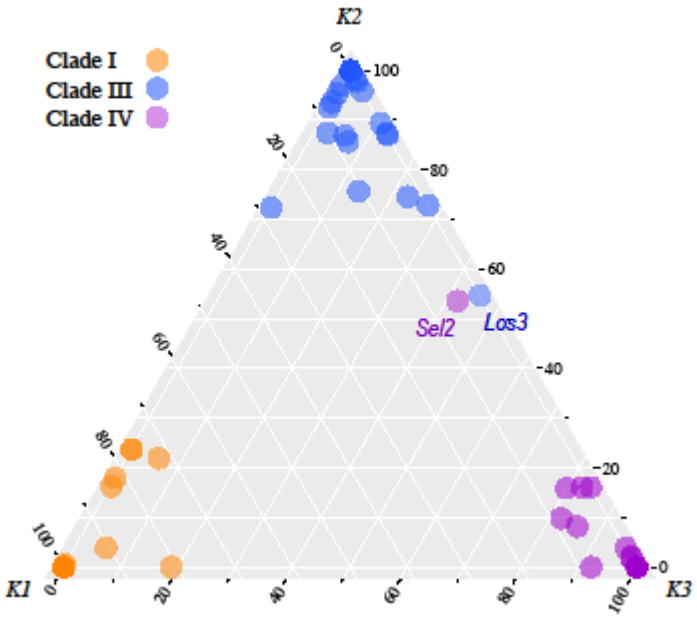

Figure S2. Admixture plot from analyses run in ADMIXTURE showing three genetic clusters that correspond to the three phylogenetic clades. The two individuals with >25% admixture were *selago*2 (purple) and *loisettae*3 (blue).