Genetic Partitioning Within the Metapopulation of Endangered Bakersfield Cactus (*Opuntia basilaris* var. *treleasei*): Implications for Translocation Efforts.

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ABSTRACT

This study addresses the genetic variation within and genetic relationships among fragmented populations of the endangered Bakersfield cactus, *Opuntia basilaris* var. *treleasei* (OBT) using comparative DNA sequence analysis of the chloroplast maturase K (*matK*) gene and amplified fragment length polymorphism (AFLP). The plant material used in this study consisted of 203 individuals/accessions from 32 populations. Eleven members representing two populations of *Opuntia basilaris* var. *basilaris* (OBB; Beavertail cactus), the sister variety to Bakersfield cactus, were also included as part of the 203 individuals analyzed. Neighbor-joining analysis of chloroplast *matK* gene sequences were insufficient to resolve relationships among OBT populations.

Three AFLP primer combinations produced a total of 195 fragments, with an average of 65 fragments per primer pair, of which, 168 bands (86.1%) were polymorphic. The average pairwise distance across all populations was 15.9%. AMOVA indicated that 70% of the genetic variance was due to variation among populations and that 30% of the genetic variance was due to variation within populations ($P < 0.001$).

Principle coordinates analysis, and dendrograms resulting from UPGMA and neighbor-joining analyses, indicated: (1) OBB and OBT samples are genetically distinct; (2) cactus populations that are south (Tejon; TCAC) and southeast (OC) of the mountain ranges surrounding the southern San Joaquin Valley are clustered together and are positioned

between the OBB cluster and all OBT clusters; and (3) 24 out of 30 OBT populations were recovered as distinct clusters; however, minimal geographic partitioning among the OBT populations was observed. The implications of the results with respect to future transplantation efforts are discussed.

BACKGROUND AND OBJECTIVES

Members of the genus *Opuntia* range from Mexico, across the United States and into southern Canada. There are ~50 described species of *Opuntia* in North America of which 12 are native to California (Hunt et al. 2006). *Opuntia basilaris* Engelm. & Bigelow is among the most widely spread of the North American *Opuntia* with a distribution that includes portions of Mexico, Utah, Arizona, Nevada and California (Baldwin et al. 2012).

Opuntia basilaris is a morphologically diverse species comprised of several distinct varieties. The number of recognized *O. basilaris* varieties has varied considerably over the years. Perhaps one of the more conservative estimates is that of Hunt (2006), who recognized four varieties that differ in structural characters and geographic distribution. One of the four varieties, *O. b.* var. *longiareolata* (Clover & Jotter) L. D. Benson, is found in Utah and Arizona. The other three varieties occur natively in California and two of the three occur in portions of Kern County, CA. (Baldwin et al 2012). *Opuntia b.* var. *brachyclada* (Griffiths) Munz is endemic to the San Bernardino and San Gabriel mountains outside of Kern County and is 5 – 15 cm in height and spineless. *Opuntia b,* var. *basilaris* (hereafter referred to as OBB) is found in California (including Kern

County), Nevada, Utah, Arizona and Mexico, is spineless, have joints 8 to 21 cm in length, 5 – 13 cm wide, and is flat and typically obovate. *Opuntia b.* var. *treleasei* (J.M. Coult.) Toumey (hereafter referred to as OBT; Figure 1), is endemic to parts of the southern San Joaquin Valley and the Tehachapi Mountains in Kern County, has yellowish spines (2 – 8 per areole), and joints 9 to 20 cm in length and 5 to 7.5 cm wide. One of the most prominent structural characteristics often used to distinguish OBT and OBB is the presence of spines in addition to glochids contained in OBT areoles.

Figure 1. Bakersfield cactus (*Opuntia basilaris var. treleasei*) from the Wheeler Ridge area. Photo by Robert Atwood (used with permission).

OBT is currently State and Federally listed as endangered due to historical losses and ongoing threats. Approximately one third of the historical occurrences of OBT have been lost due to agricultural, urban, and industrial development. Although factors such as fire, off-road vehicles, and competition from non-native grasses (Cypher and Fiehler 2006), have undoubtedly impacted OBT, the primary threat to the remaining populations continues to be loss of physical habitat (Cypher et al. 2011).

Small isolated populations of OBT on fragmented land caused by the destruction of habitat in the southern San Joaquin Valley likely has resulted in decreased gene flow among adjacent OBT populations. Fragmentation of habitat may significantly reduce or even prevent gene flow, which could result in the adverse biological effects often associated with inbreeding depression (Klug et al. 2005). In small isolated populations, random genetic drift may cause the attrition of genetic diversity by overwhelming the force of natural selection and resulting in the loss of evolutionary potential (Hartl 2000; Keyghobadi et al. 2005; Klug et al. 2005). Loss of genetic diversity in small, highly fragmented, populations can have deleterious effects on fitness and ultimately may increase the risk for population extinction (Charlesworth and Charlesworth 1987; Lynch 1991; Newman and Pilson 1997).

Population level genetic studies of *Opuntia* species are limited. Indeed, little is known about the population genetic structure of OBB throughout its range, or the impact of habitat loss on the genetic diversity of remaining OBT populations. Past and ongoing loss

of OBT populations has reduced the prospects for recovery of this species. Additional OBT populations potentially could be established via translocation to reverse the declines. A significant concern in translocating OBT is outbreeding depression. If the remaining OBT populations are genetically partitioned into local demes, then translocating cacti between demes could result in reduced fitness and/or the loss of unique alleles. Successful establishment of additional populations while maintaining genetic diversity of the metapopulation could contribute significantly to the conservation and ultimate recovery of OBT.

The goal of this project is to examine genetic diversity and partitioning within the OBT metapopulation. Specific objectives are to (1) assess the genetic diversity within and among populations of OBT using DNA sequence and AFLP analyses, (2) determine whether genetic demes exist within the metapopulation based on genetic clustering algorithms and principle coordinate analysis, and (3) provide translocation recommendations in light of the genetic analyses.

MATERIALS & METHODS

Tissue samples were extracted from individual pads collected from each of up to ten spatially distinct clumps. Spatially distinct clumps were sampled to reduce the likelihood of collecting from vegetative clones. A total of 203 samples were collected and analyzed from 32 populations (Appendices 1 & 2). Samples were placed in sealed plastic, or brown

paper bags, labeled with location, date and GPS coordinates and stored at – 24˚C. Voucher specimens are housed at the Department of Biology, CSUB.

DNA Sequence Analysis/Barcoding

DNA was extracted from a ~2 x 2 cm^2 portion of cactus tissue using a modified version of a CTAB DNA extraction protocol following the procedure outlined by Doyle and Doyle (1987) and Cullings (1992). We used universal primers reported in Ford et al. (2009) to amplify and sequence an ~800 bp portion of the chloroplast maturase K (*matK*) gene from all 203 individuals.

DNA barcoding (Hebert & Gregory 2005) is a tool to provide rapid and taxonomic identification using a specific DNA region. A two-marker combination of *matK* + *rbcL* was formally approved by the *Consortium for the Barcode of Life* (CBOL) to serve as the barcode for land plants. The chloroplast maturase K gene (*matK*) is one of the most variable coding genes of angiosperms and has been suggested by many authors to be among the best "barcodes" for land plants.

We carried out polymerase chain reaction (PCR) amplifications of the *matK* gene in 20 µl volume and annealing temperatures ranging between 47.8°C and 52.8°C. Successfully amplified PCR products were visualized on a 1% (w/v) agarose gel + ethidium bromide and documented using a BIO-RAD ChemiDoc™ system. Successfully amplified PCR products were purified by either using QiaQuick PCR columns or using shrimp

phosphatase and exonuclease (ExoSAPit, USB-Affymetrix). DNA sequencing reactions were performed using ABI's Big Dye Terminator following the manufacturer's instructions. DNA sequencing reactions were purified using the DyeEx 2.0 Spin Kit (Qiagen). We submitted purified sequencing products to the University of Florida's DNA Sequencing Core Facility for sequencing both forward and reverse strands on an ABI 377 DNA sequencer. DNA sequence electropherograms were read, edited, and aligned using Geneious v5.0 (Drummond *et al.* 2010). DNA sequence alignment was straightforward and did not necessitate the insertion of any gaps.

Phylogenetic analysis of the aligned OBT and OBB DNA sequences, as well as selected *Opuntia* sequences obtained from GenBank, was carried out using neighbor-joining analysis based on the p-distance in MEGA 5.0 (Tamura et al. 2011)

AFLP

The AFLP technique is based on the amplification of short restriction endonuclease digested genomic DNA fragments onto which adaptors have been ligated at both ends. Primers complementary to the adaptors and possessing 30 selective nucleotides of 1 to 4 bases are used in a selective amplification reaction. The presence or absence of these selective nucleotides in the genomic fragments being amplified provides the basis for revealing polymorphism.

Thirty-two primer combinations using MseI and EcoRI primers were tested using ABI's Regular Plant Genome kit. Of these, three primer pairs were selected based on the

number of polymorphic bands produced across samples and populations (Table 1). The AFLP Plant Mapping protocol (Applied Biosystems), a modification of the AFLP originally developed by Vos et al. (1995) was used throughout this study. DNA was digested with Eco RI and Mse I at 37ºC for 2 hrs. A small aliquot of the digested DNA was run on a 2.0% (w/v) agarose + ethidium bromide gel to check if the DNA digestion was complete. EcoRI and MseI adapters were ligated (10 ul 10X T4 DNA ligase buffer, 10 ul (micro liter) 0.5 M NaCL, 5 ul 1 mg/mL BSA, 100 units MseI, 500 units EcoRI and 100 Weiss Units T4 DNA ligase) to the digested DNA samples to generate template DNA.

We used the ABI Ligation and Preselective Amplification Module for preamplification. The reaction mixture consisted of the following: 1.0 uL 10 T4 DNA ligase buffer with ATP, 1.0 uL 0.5M NaCl, 0.5 uL 1.0 mg/ml BSA, 1.0 ul MseI adaptor, 1.0 ul EcoRI adaptor, and 1.0 ul Enzyme Master Mix. This mixture was then incubated at 37˚C for two hours. The resulting solution was then diluted 1:2 with TE buffer and the fragments amplified by PCR. The PCR parameters for AFLP pre-amplification were carried out as follows: one cycle at 72˚C for 2 min, followed by twenty-one cycles of 94˚C for 20 sec, 56˚C for 30 sec, and 72 ˚C for 2 min). A final step of 60˚C for 30 min was also added. The pre-amplification product was diluted 1:10 with TE buffer and stored at -25˚C.

The pre-amplification product was then used in the following selective amplification procedure using the AFLP Regular Plant Genome kit, which consists of eight EcoRI primers and eight MseI selective primers. For selective amplification the following were

combined: 1 ul of MseI primer, 1 ul of EcoRI primer, 3.0 ul of pre-selective amplification product and 15 ul of AFLP Core Mix in a 0.65 ul microcentrifuge tube. The PCR conditions consisted of an initial denaturation step of 94˚ C for 2 min, followed by 10 cycles of (94˚C for 20 sec, 66˚C for 30 sec and 72˚C for 2 min) with the annealing temperature decreased 1˚C each cycle from 66˚C to 56˚C. The 56˚C annealing temperature was then repeated in 23 cycles followed by a final extension step of 60˚C for 30 min. The resulting products were submitted to the University of Florida's ICBR genotyping core for fragment analysis.

DNA fragment peaks generated by the University of Florida's ICBR genotyping core were subject to selection criteria using GeneMarker v 1.75 (SoftGenetics Corporation). Fragment sizes less than 100 base pairs (bp) were excluded from the analyses to eliminate artifacts such as residual primers or degraded DNA fragments. Low quality fragment peaks (i.e., those with a score of < 6.9) were also excluded. Following the selection criteria, bands that showed clear polymorphisms were scored as present (1) or absent (0) and analyzed.

The AFLP fragment data was analyzed using GeneMarker v 1.75 (SoftGenetics Corporation), GenAlEx 6.1 (Peakall and Smouse 2006) and MEGA ver. 5.05 (Tamura et al. 2011). In MEGA 5.05, present (1) and absent (0) binary characters were transformed into alphanumeric characters and analyzed. GeneMarker 1.75 was used to create an individual sample UPGMA dendrogram. GenAlEx 5.0 was used for AMOVA, principal coordinates analysis (PCoA), and to create a pairwise genetic distance matrix. MEGA

5.05 was used to construct a neighbor-joining distance tree and perform pairwise population comparisons.

RESULTS & DISCUSSION

This study addresses the genetic relationships among fragmented populations of the endangered Bakersfield cactus, *Opuntia basilaris* var. *treleasei* (OBT), by comparative DNA sequence analysis of the chloroplast maturase K (*matK*) gene and amplified fragment length polymorphism (AFLP). Neighbor-Joining analysis of the *matK* gene amino acid sequences indicated that this gene is insufficient for addressing varietal and/or population-level relationships within *O. basilaris* as the amino acid sequence was invariant among all OB samples analyzed.

Three AFLP primer combinations produced a total of 195 fragments, with an average of 65 fragments per primer pair, of which, 168 bands (86.1%) were polymorphic. The average pairwise distance across all populations was 31 (15.9%) (Table 2a). Within-group genetic distances ranged from 0 (EO20) to 29 for IW + MJ (=OBB) across all populations. The average pairwise distance for all OBT populations (exclusive of OBB) was 6.9 (3.5%). The most variable OBT population was EO3 (11.3%) followed by EO28 (8.7%). An analysis of molecular variance (AMOVA) indicated that 70% of the genetic variance was due to variation among populations and that 30% of the genetic variance was due to variation within populations ($P < 0.001$) (Table 3.). This result is an indication that some populations are not experiencing substantial gene flow, which is not surprising

given the highly fragmented nature and geographic distances between some OBT populations. However, there is still substantial variation (>4%) within some populations indicating that, at present, alleles are not being lost to genetic drift and/or inbreeding is not having a substantial impact. Confounding this result is the fact that OBT can reproduce by vegetative cloning. Thus, populations with little or no genetic variation (e.g., EO20, n=3) may be due to the fact that the clumps, from which samples were collected, are merely clones.

Principle coordinates analysis (PCoA) provided an exploratory visualization of which populations may constitute genetic demes (Figure 2.). Based upon PCoA, and dendrograms that resulted from both unweighted pair group method based on arithmetic average (UPGMA) analysis (Figure 3.) and neighbor-joining (p-distance) analysis (Figure 4), the following is indicated: (1) OBB and OBT samples were genetically distinct. Within OBB the Mojave (MJ) and Indian Wells (IW) populations are joined together in a cluster (Figure 4.), but each may also represent independent genetic demes (Figure 5); (2) populations that are south (Tejon; TCAC) and southeast of the mountain ranges surrounding the southern San Joaquin Valley are clustered together and are positioned in between the OBB cluster and all OBT clusters (Figure 4); however, the OC and TCAC samples exhibited a closer genetic affinity to OBT samples than to the OBB samples included in the study, and (3) Within OBT many populations (24 out of 30, 71.2%) were recovered as distinct clusters; however, substantial geographic patterns within this large cluster were not observed except for those populations associated with the Wheeler Ridge area (Figure 4).

The branch lengths leading to most populations within the inclusive OBT cluster are very short (Figure 4), an indication that while many populations are distinct there is not substantial genetic variation among populations. An alternative explanation is that the OBT populations are in the process of diverging, but that divergence, due to lack of gene flow and/or genetic drift, is a relatively recent phenomenon. The distinct clusters and relatively short branch lengths of some populations, when viewed in the light of the PCoA results, suggest the following genetic demes within OBT (see Figure 5.): (1) EO36 + EO37 + EO45 + WW, (2) CATR 1-4, (3) all EO10 samples, and (4) all EO51 samples. It is not know if transplantation to (or from) any of these four grouping would have a negative impact (=outbreeding depression and/or loss of unique alleles). The relatively short branch lengths among all OBT clusters suggest that transplantation to and/or from any of the OBT clusters would not result in any deleterious effects associated with outbreeding depression. However, a prudent recovery approach would be to select individuals from nearby clusters as indicated in the dendrograms and/or use the pairwise population differences (see Table 2b.) as a basis for transplant selection to existing populations.

The most genetically diverse OBT population was EO3, which also comprised the largest single sampling of individuals (n=20). EO3 was widely dispersed among the various other OBT populations (Figures 2 & 3.). Because of the genetic diversity represented in EO3 this population may represent an excellent source population from which to select individuals for transplantation to establish new populations in new geographic areas.

Several factors could account for the current level of genetic variation (16.9% total; 3.5% within OBT)) and the distribution of this variation (30% within, 70% among) within the metapopulation. Prior to the influx of non-native people, OBT were densely distributed throughout the southern San Joaquin Valley (USFWS 1998). Widespread development of the southern San Joaquin Valley occurred after the late nineteenth century, a very recent event on an evolutionary time scale. Thus, substantial variation persists within many of the highly fragmented OBT populations that exist today because not enough time has past for fragmentation and the ill effects of small population size to have a significant impact. Further light shed on this phenomenon comes by way of a report where at least one OBT plant persisted for ~48 years in a botanical garden (USFWS 1998). The potentially long-lived nature of OBT coupled with the fact that most habitat loss is a relatively recent event suggests that, at present, most OBT populations are genetically diverse and viable. However, the future viability and prevention of population extinction will depend heavily on concerted conservation efforts, which include transplantations, due to the highly fragmented nature of the remaining populations. The present study represents an invaluable tool for guiding such conservation efforts.

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TABLES

Table 1. Informative primers for selective AFLP amplification.

Primer Code	Restriction Enzyme	Selective Sequence
FB	EcoRI	ACA
1	MseI	CAA
2	MseI	CAC
6	MseI	CTC

Table 2. (a) Summary of mean group genetic distance. (b) pairwise population distances.

(a)

Population	Mean Group Distance	%
Overall	31	16.9
Within OBB	29	14.8
Within OBT	6.9	3.5

(b)

1. EO2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. EO1	20																													
3. NCHEV	23	16																												
4. EO20	24	14	19																											
5. EO18	39	28	34	27																										
6. EO16	25	16	24	22	33																									
7. EO7	23	16	22	19	35	18																								
8. PAN2	30	20	21	12	25	28	26																							
9. EO10	43	32	37	30	23	39	37	32																						
10. EO11	30	18	25	19	16	25	25	22	19																					
11. EO15	26	15	21	20	29	24	23	24	33	19																				
12. EO17	29	17	21	19	30	26	23	22	31	18	17																			
13. EO28	32	33	39	37	49	33	34	45	53	39	34	38																		
14. NCKLS	29	24	29	31	46	25	24	34	47	35	29	25	36																	
15. EO32	35	23	29	24	18	30	30	27	18	11	25	25	44	40																
16. EO51	50	42	44	38	24	48	46	33	29	27	42	39	59	56	31															
17. EO36	26	24	26	34	44	25	25	40	49	35	31	32	36	22	41	57														
18. WW	22	23	25	28	36	22	25	34	44	29	28	29	33	23	35	50	15													
19. EO45	26	28	31	38	44	27	28	43	50	36	33	34	35	24	41	57	12	14												
20. EO37	35	38	38	48	59	34	38	53	65	51	41	43	39	28	56	72	19	25	20											
21. EN37	22	17	17	20	32	23	21	25	38	22	20	20	35	24	28	45	23	21	25	31										
22. EO21	28	22	24	20	34	28	26	24	37	23	23	23	37	29	29	44	33	30	35	40	17									
23. EO38	26	17	25	20	33	22	21	24	37	22	21	25	34	28	26	45	28	26	30	42	23	25								
24. CATR	38	29	31	23	29	34	31	25	35	24	31	28	42	40	26	38	44	38	45	57	30	29	30							
25. EO23	26	21	30	25	34	24	21	31	41	25	22	28	35	32	30	48	31	29	33	42	22	25	22	32						
26. EO24	34	28	36	33	43	26	26	38	47	34	35	37	31	30	36	54	27	30	30	35	34	37	28	40	31					
27. EO25	25	16	21	22	36	26	21	30	39	25	22	21	37	27	31	48	26	27	31	36	18	22	19	34	23	34				
28. EO3	25	19	22	19	30	23	21	25	33	22	22	23	36	30	27	40	31	26	33	43	23	26	23	30	26	32	24			
29. OBB	49	48	50	48	59	44	42	51	63	53	49	53	49	47	55	67	49	53	52	51	48	47	44	48	44	42	49	50		
30. TCAC	35	28	31	28	34	32	29	30	44	33	33	33	44	42	35	44	39	37	42	51	33	35	33	27	36	39	36	33	43	
31. OC	39	30	32	28	35	32	28	30	44	34	34	36	45	44	36	46	40	39	45	52	33	36	35	29	36	38	38	35	39	6

Table 3. AMOVA table for AFLP pairwise distances. (df: degrees of freedom, SS: sum of squares, MS: mean squares).

Source	df	SS	MS	Est. Var.	%
Among Pops	31	2347.324	75.720	11.279	70%
Within Pops	171	831.917	4.865	4.865	30%
Total	202	3179.241		16.144	100%
Stat	**Value**	**P**			
Φ_{PT}	0.699	0.001			

Figure 2. Principal coordinates analysis (PCoA)/multidimensional scaling. The amount of variation from the first and second principal coordinates was 39.1% and 27.8 %, respectively.

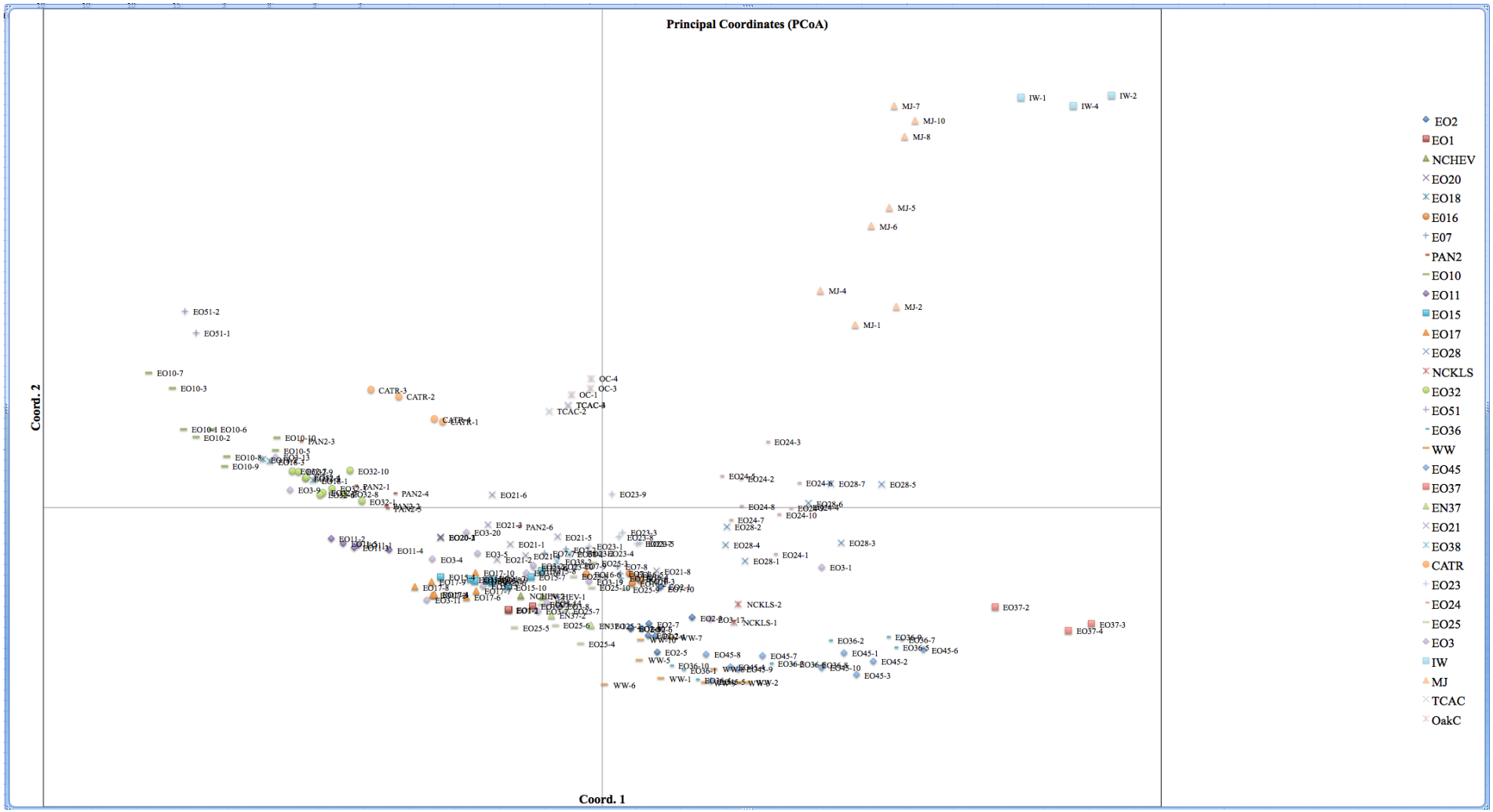
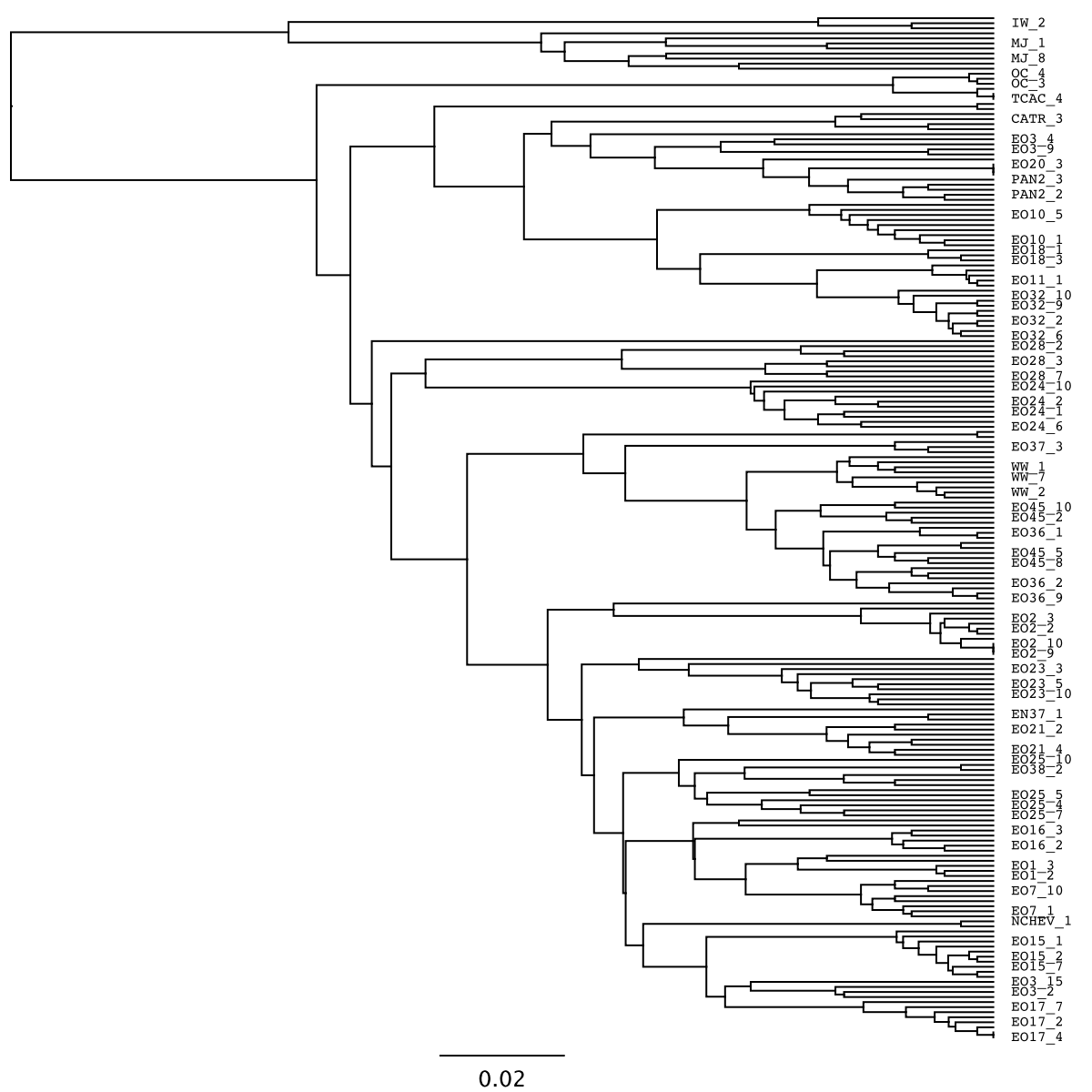

Principal Coordinates (PCoA)
Coord. 2
Coord. 1
EO2
EO1
NCHEV
EO20
EO18
E016
E07
PAN2
EO10
EO11
EO15
EO17
EO28
NCKLS
EO32
EO51
EO36
WW
EO45
EO37
EN37
EO21
EO38
CATR
EO23
EO24
EO25
EO3
IW
MJ
TCAC
OakC

Figure 3. UPGMA dendrogram derived from pairwise AFLP distances. Single tree followed by successive expanded views of all clusters.


IW_2
MJ_1
MJ_8
OC_4
OC_3
TCAC_4
CATR_3
EO3_4
EO3_9
EO20_3
PAN2_3
PAN2_2
EO10_5
EO10_1
EO18_1
EO18_3
EO11_1
EO32_10
EO32_9
EO32_2
EO32_6
EO28_2
EO28_3
EO28_7
EO24_10
EO24_2
EO24_1
EO24_6
EO37_3
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WW_7
WW_2
EO45_10
EO45_2
EO36_1
EO45_5
EO45_8
EO36_2
EO36_9
EO2_3
EO2_2
EO2_10
EO2_9
EO23_3
EO23_5
EO23_10
EN37_1
EO21_2
EO21_4
EO25_10
EO38_2
EO25_5
EO25_4
EO25_7
EO16_3
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EO3_15
EO3_2
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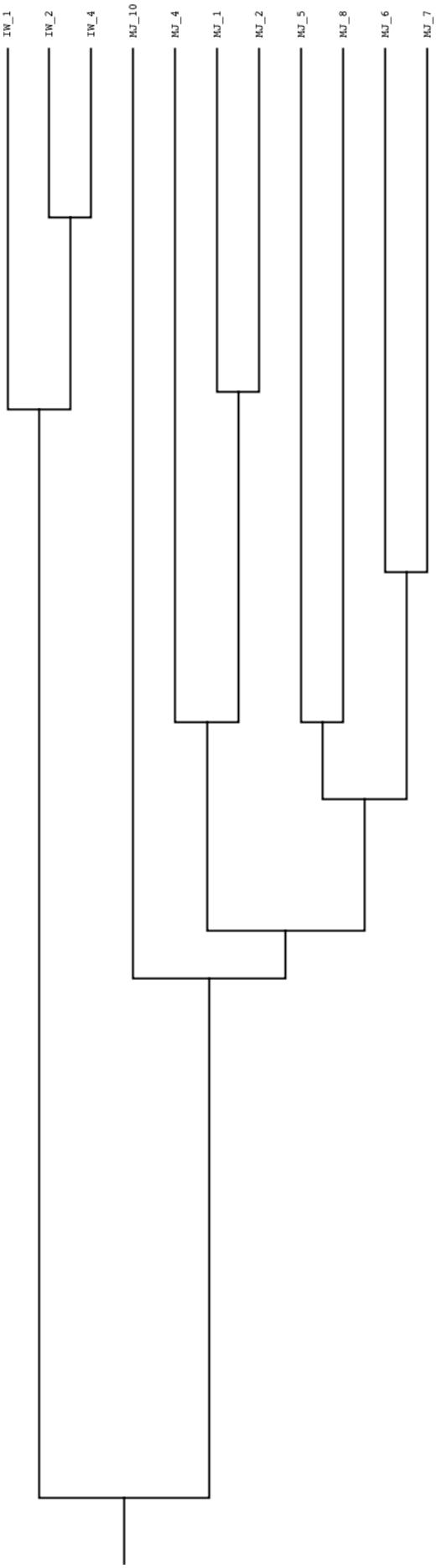
EO17_2
EO17_4
0.02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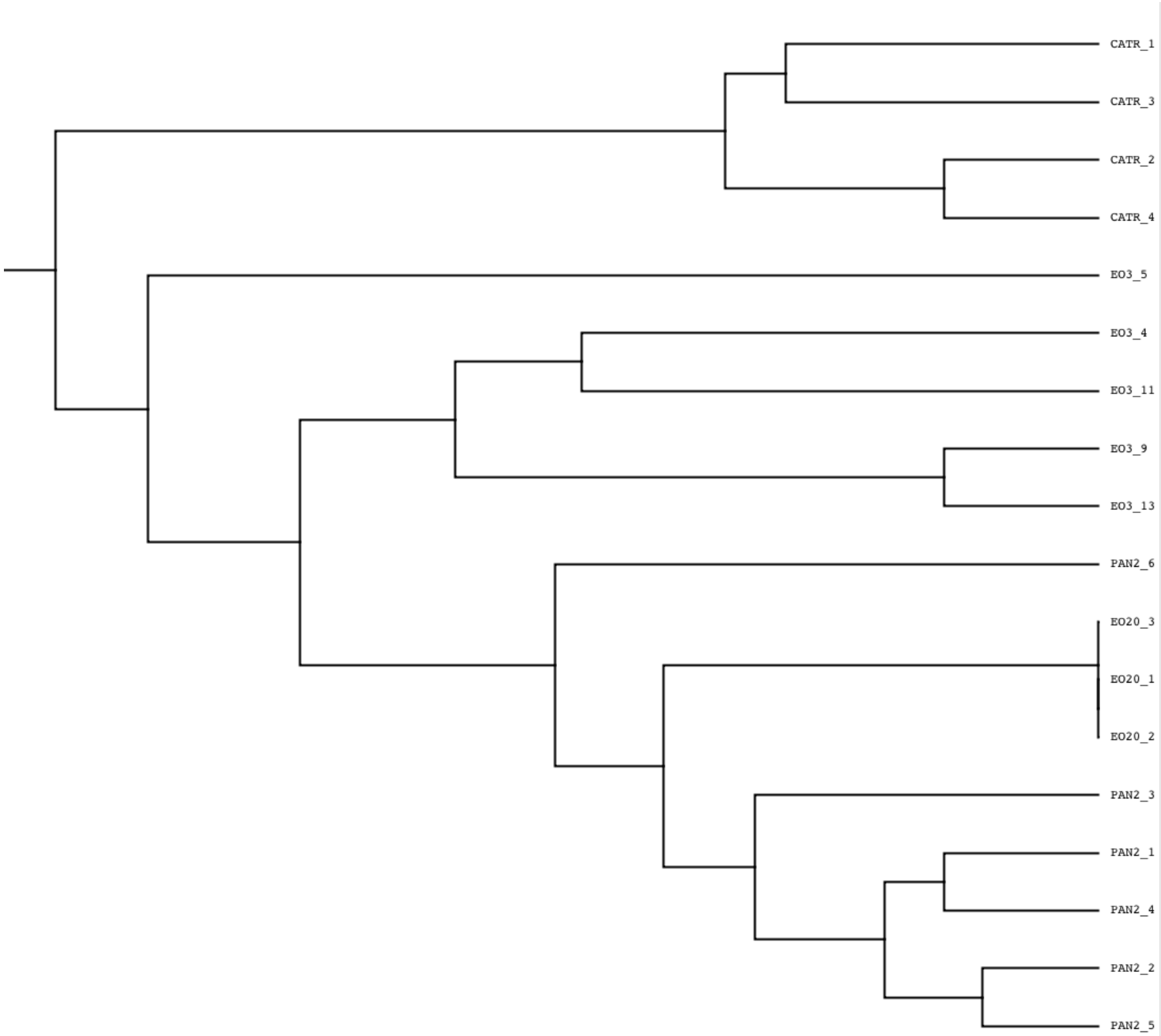
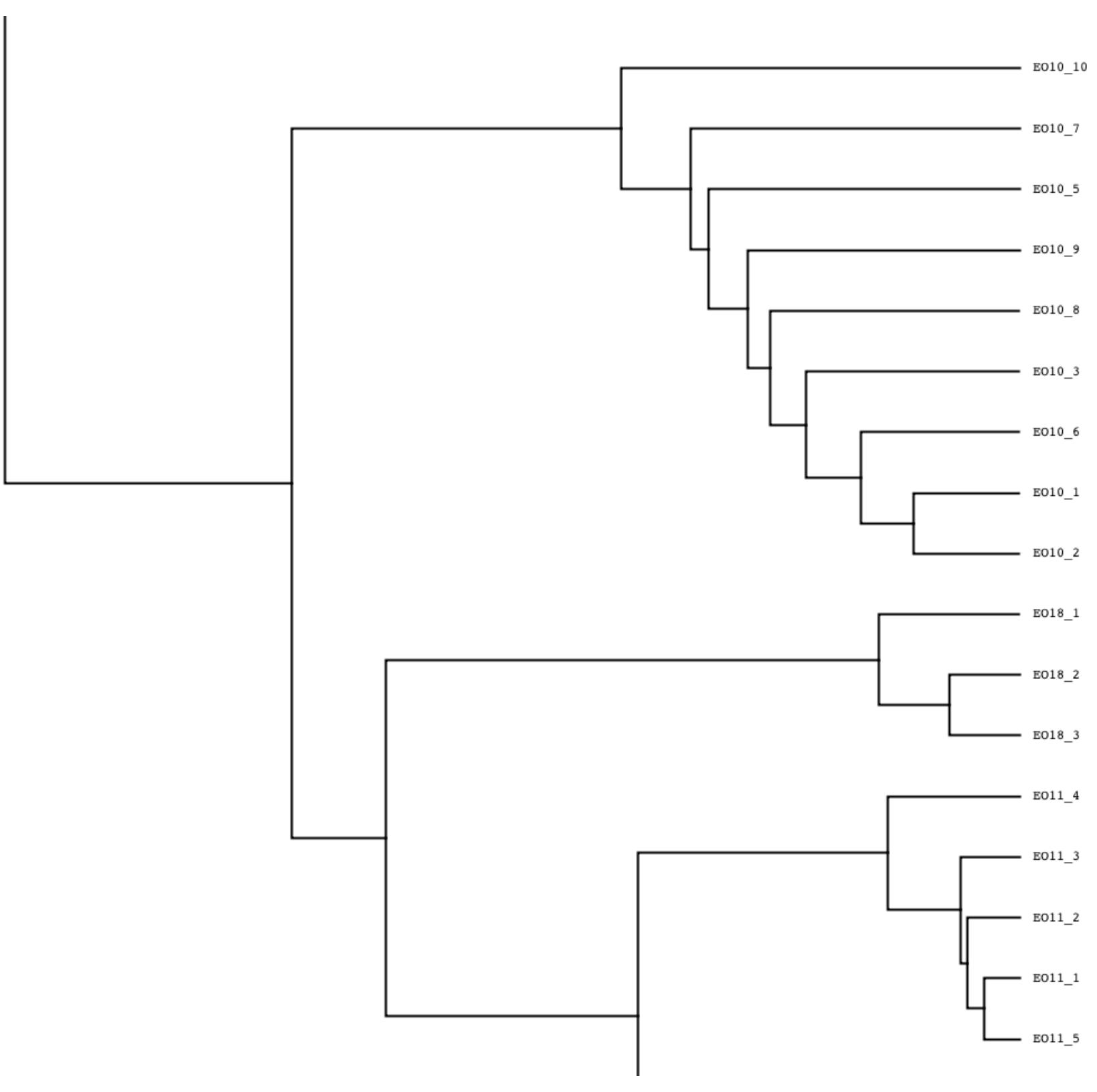
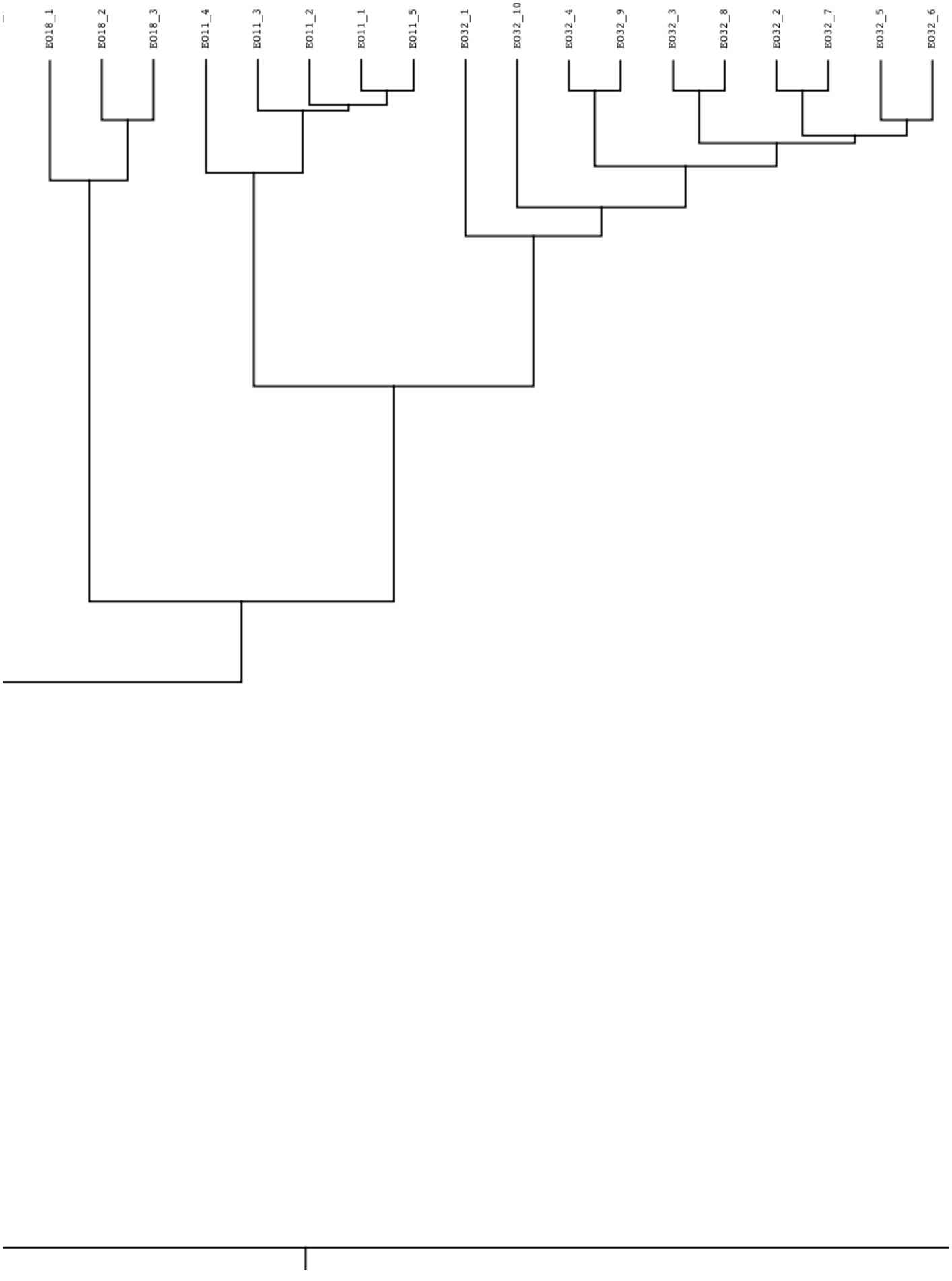
IW_4
MJ_10
MJ_4
MJ_1
MJ_2
MJ_5
MJ_8
MJ_6
MJ_7


OC_4
OC_1
OC_3
TCAC_2
TCAC_3
TCAC_4


CATR_1
CATR_3
CATR_2
CATR_4
EO3_5
EO3_4
EO3_11
EO3_9
EO3_13
PAN2_6
EO20_3
EO20_1
EO20_2
PAN2_3
PAN2_1
PAN2_4
PAN2_2
PAN2_5


EO10_10
EO10_7
EO10_5
EO10_9
EO10_8
EO10_3
EO10_6
EO10_1
EO10_2
EO18_1
EO18_2
EO18_3
EO11_4
EO11_3
EO11_2
EO11_1
EO11_5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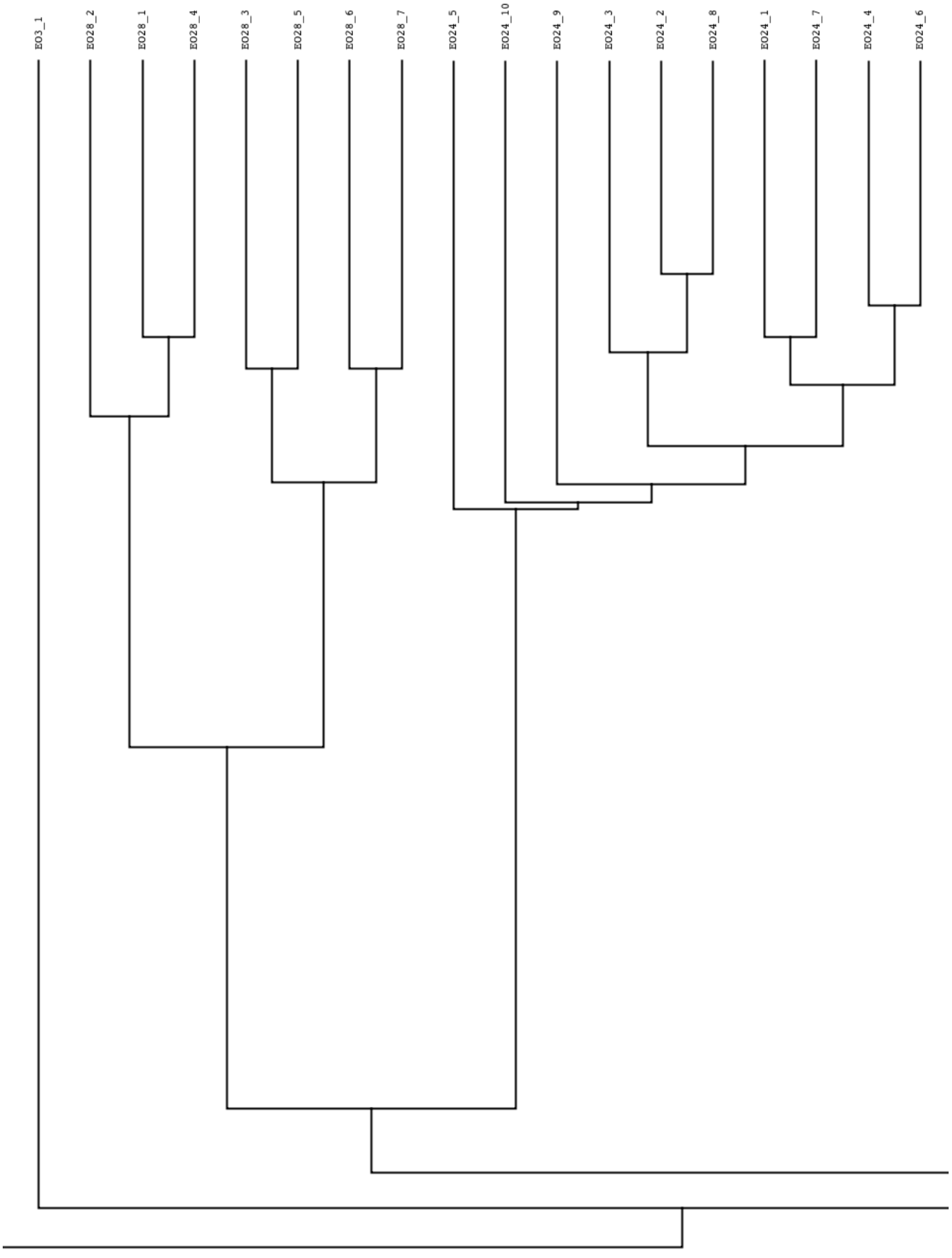
EO18_2
EO18_3
EO11_4
EO11_3
EO11_2
EO11_1
EO11_5
EO32_1
EO32_10
EO32_4
EO32_9
EO32_3
EO32_8
EO32_2
EO32_7
EO32_5
EO32_6


E03_1
E028_2
E028_1
E028_4
E028_3
E028_5
E028_6
E028_7
E024_5
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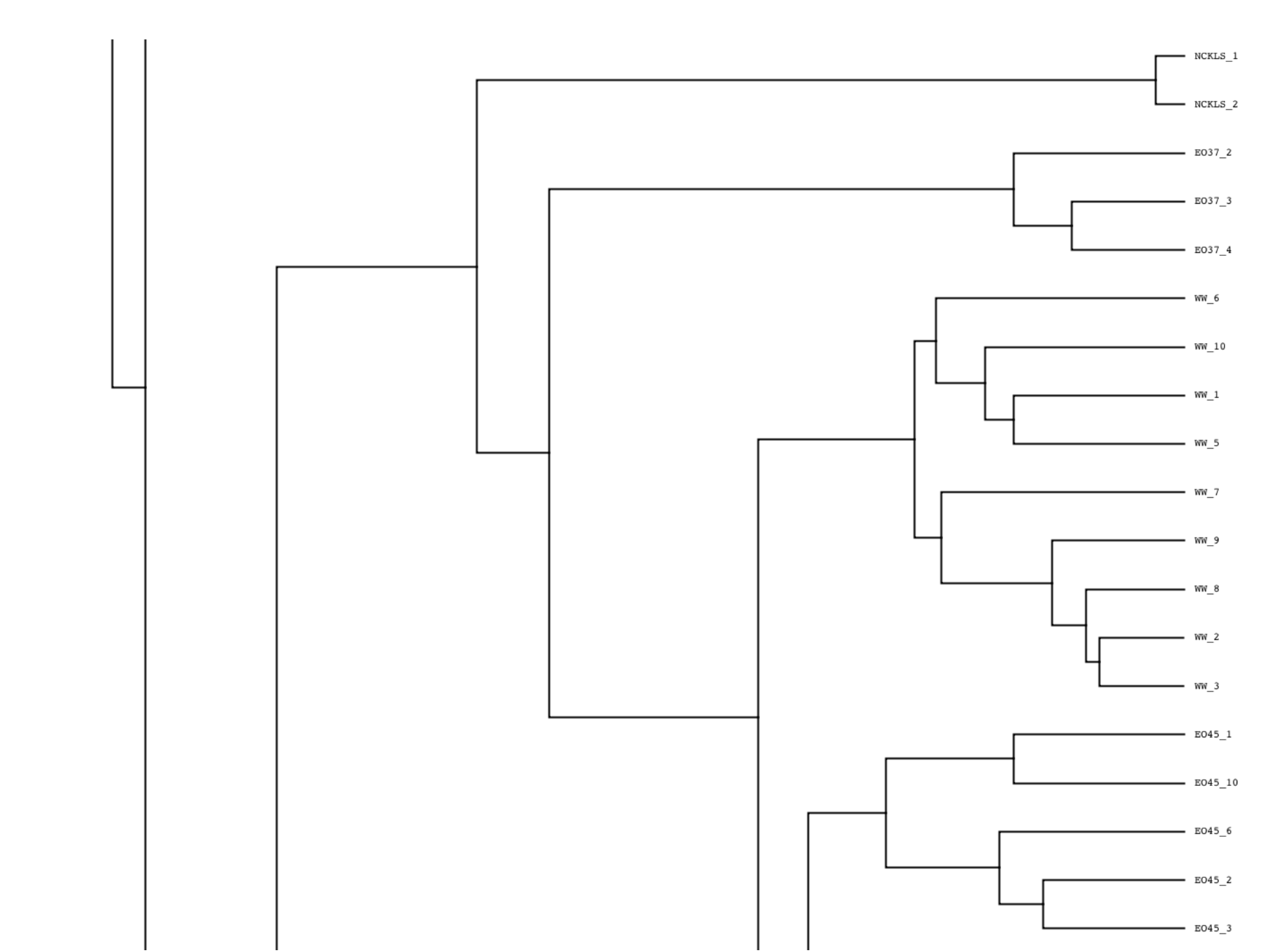
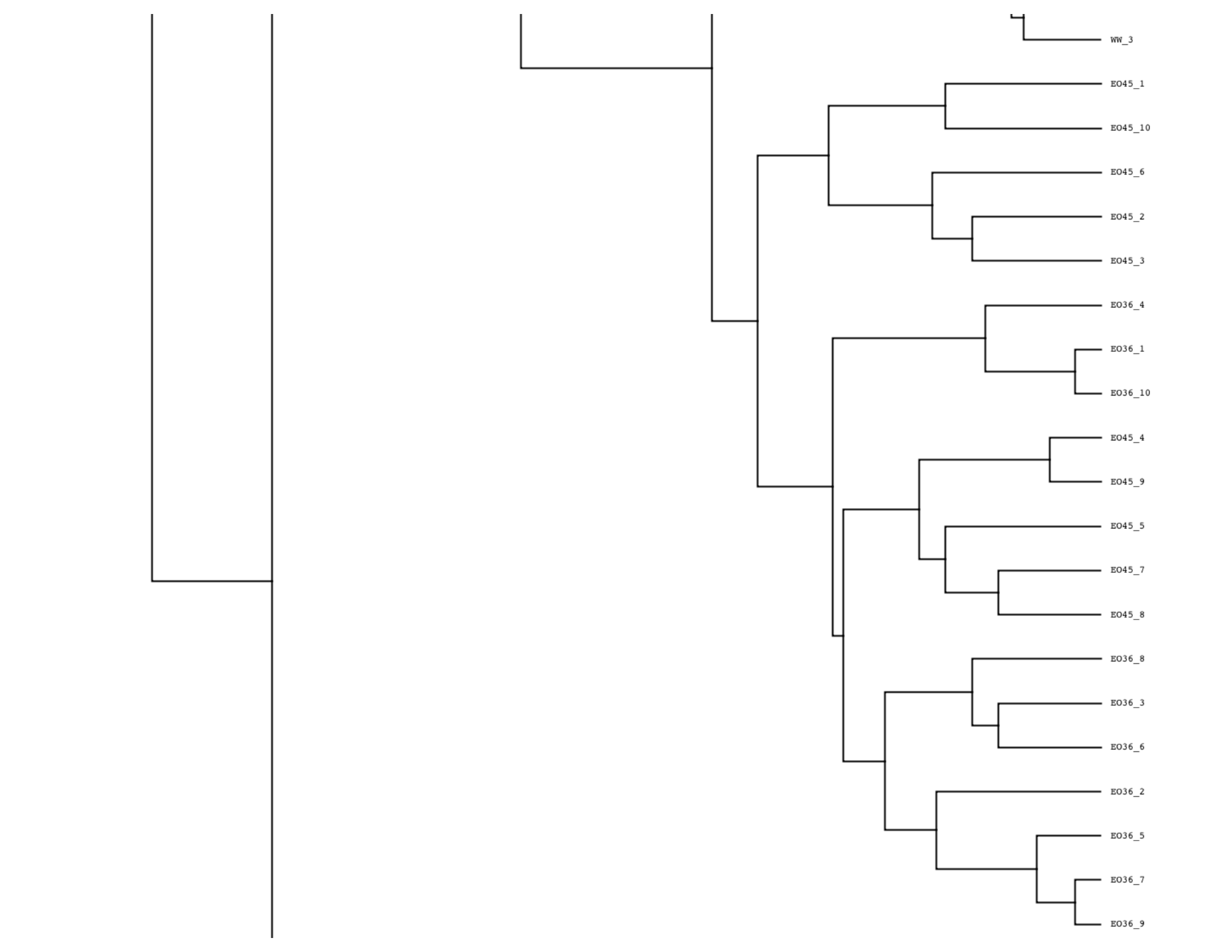
E024_9
E024_3
E024_2
E024_8
E024_1
E024_7
E024_4
E024_6


NCKLS_1
NCKLS_2
EO37_2
EO37_3
EO37_4
WW_6
WW_10
WW_1
WW_5
WW_7
WW_9
WW_8
WW_2
WW_3
EO45_1
EO45_10
EO45_6
EO45_2
EO45_3


WW_3
EO45_1
EO45_10
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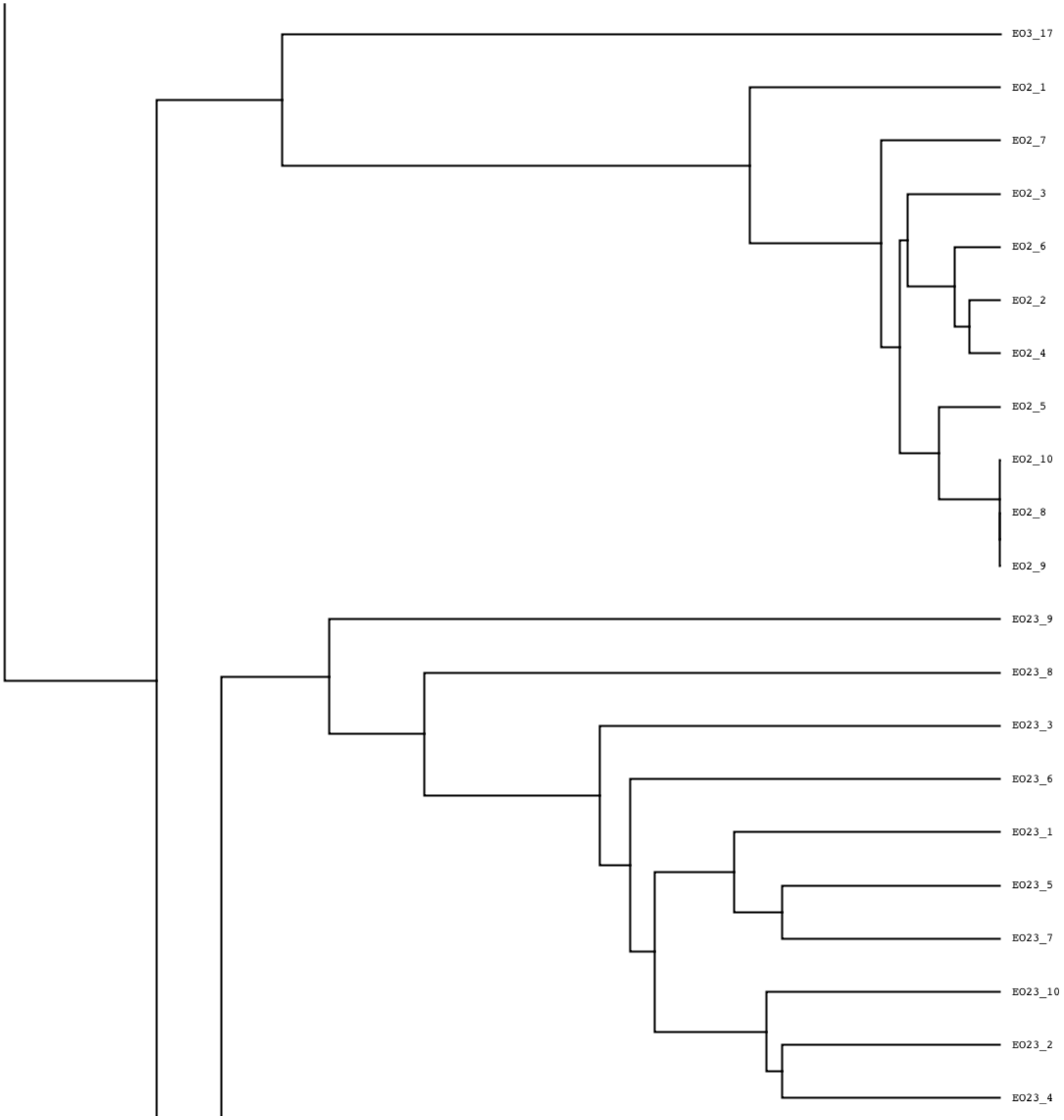
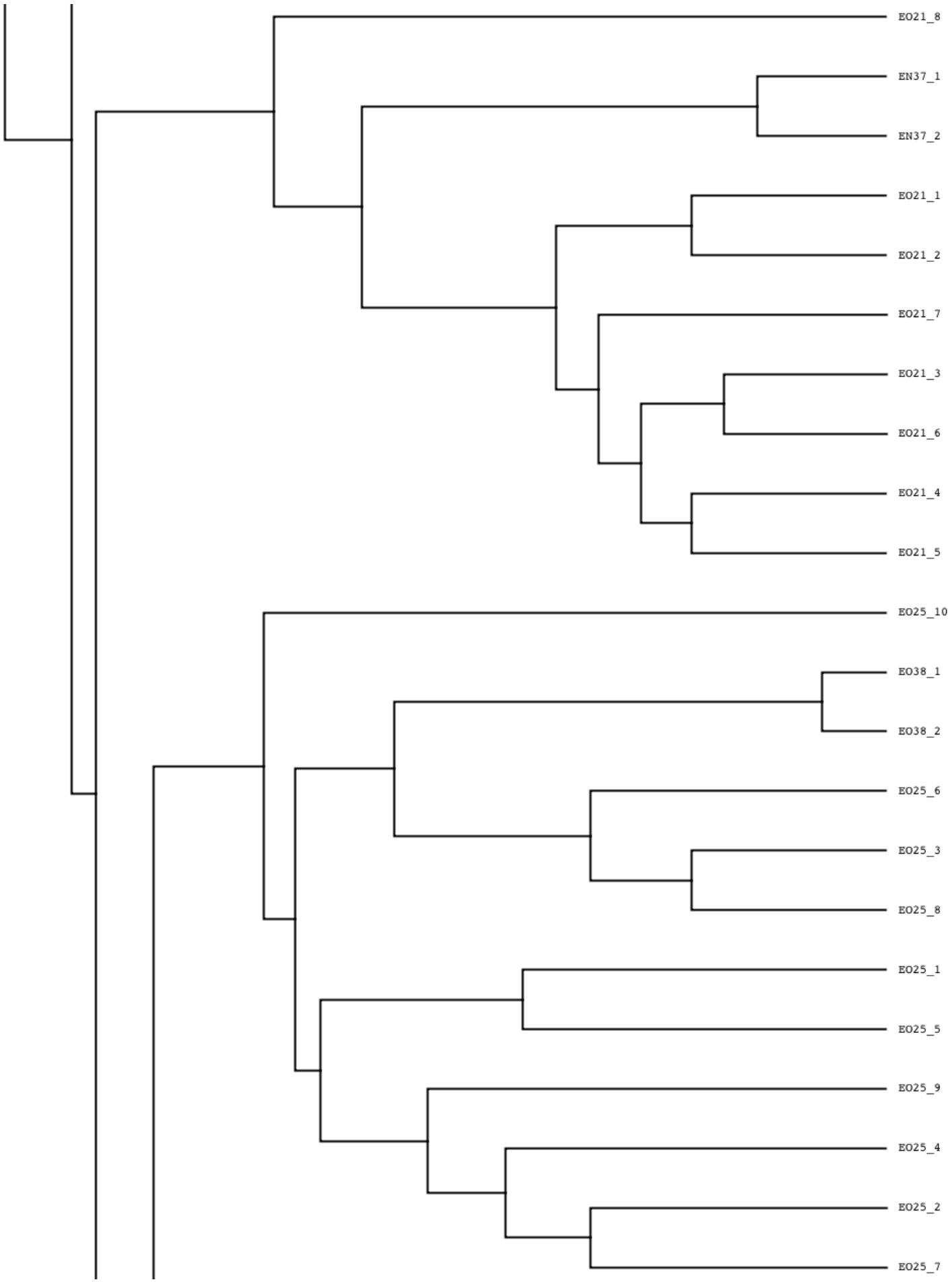
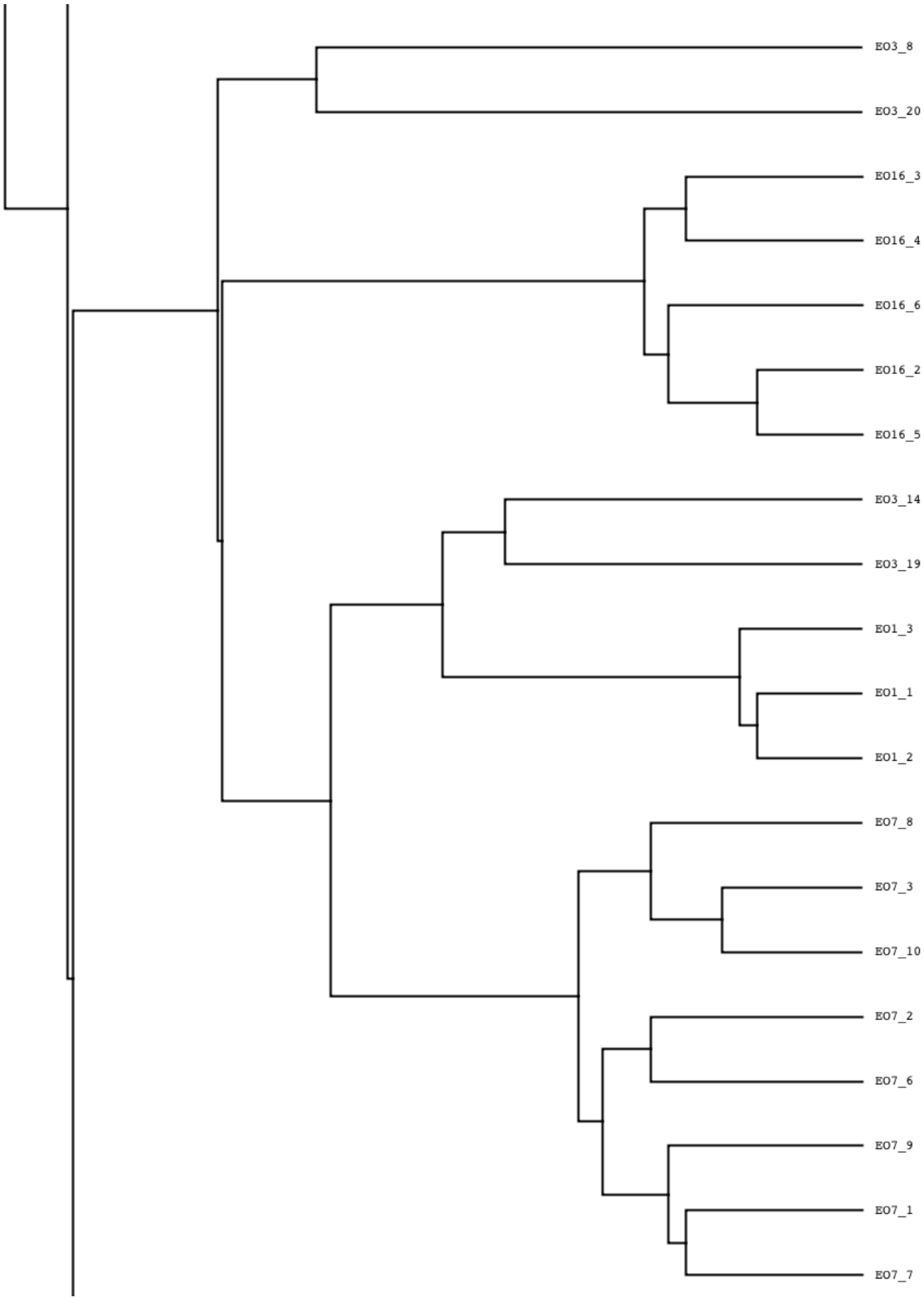
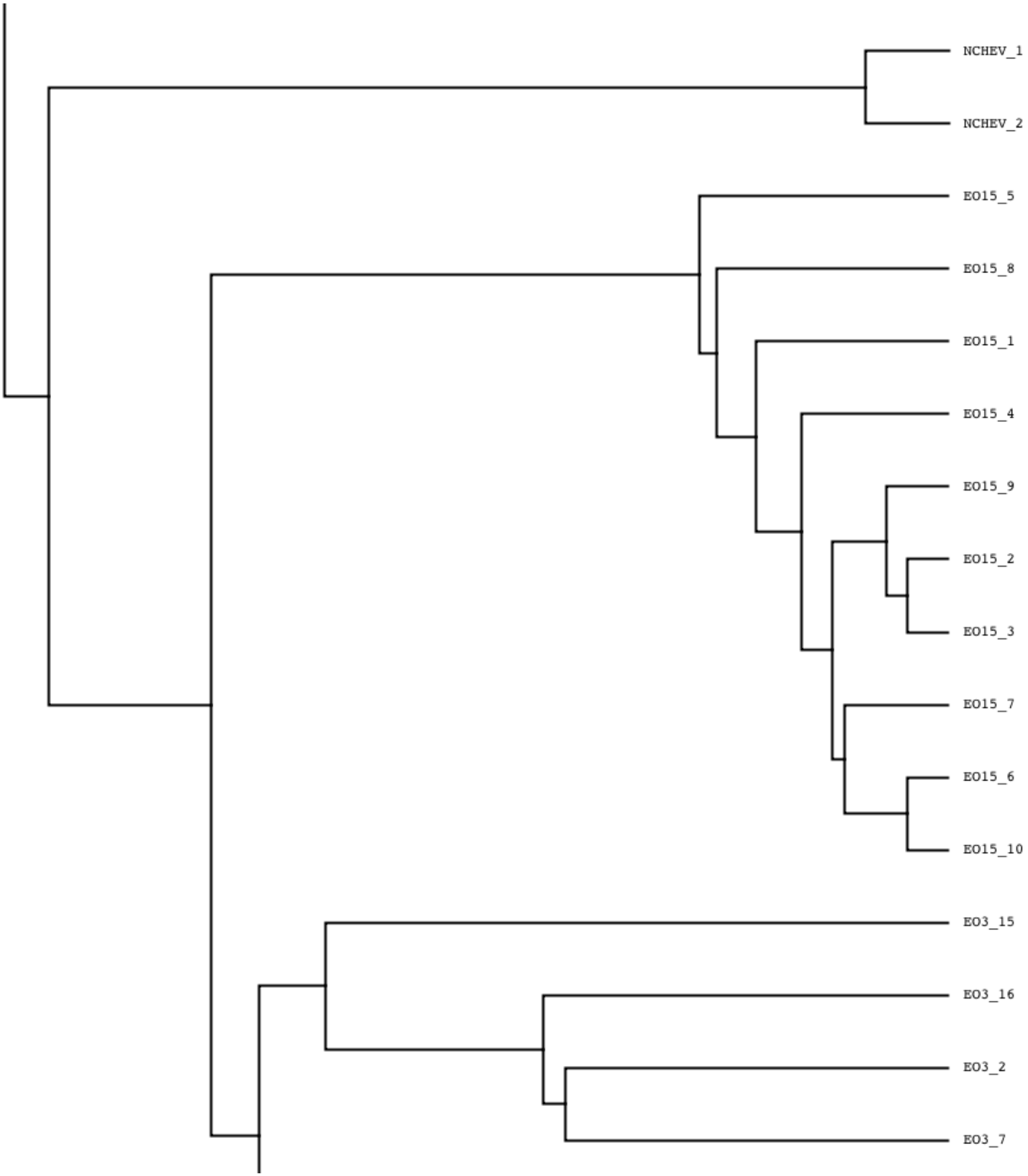
EO45_2
EO45_3
EO36_4
EO36_1
EO36_10
EO45_4
EO45_9
EO45_5
EO45_7
EO45_8
EO36_8
EO36_3
EO36_6
EO36_2
EO36_5
EO36_7
EO36_9


EO3_17
EO2_1
EO2_7
EO2_3
EO2_6
EO2_2
EO2_4
EO2_5
EO2_10
EO2_8
EO2_9
EO23_9
EO23_8
EO23_3
EO23_6
EO23_1
EO23_5
EO23_7
EO23_10
EO23_2
EO23_4


EO21_8
EN37_1
EN37_2
EO21_1
EO21_2
EO21_7
EO21_3
EO21_6
EO21_4
EO21_5
EO25_10
EO38_1
EO38_2
EO25_6
EO25_3
EO25_8
EO25_1
EO25_5
EO25_9
EO25_4
EO25_2
EO25_7


EO3_8
EO3_20
EO16_3
EO16_4
EO16_6
EO16_2
EO16_5
EO3_14
EO3_19
EO1_3
EO1_1
EO1_2
EO7_8
EO7_3
EO7_10
EO7_2
EO7_6
EO7_9
EO7_1
EO7_7


NCHEV_1
NCHEV_2
EO15_5
EO15_8
EO15_1
EO15_4
EO15_9
EO15_2
EO15_3
EO15_7
EO15_6
EO15_10
EO3_15
EO3_16
EO3_2
EO3_7

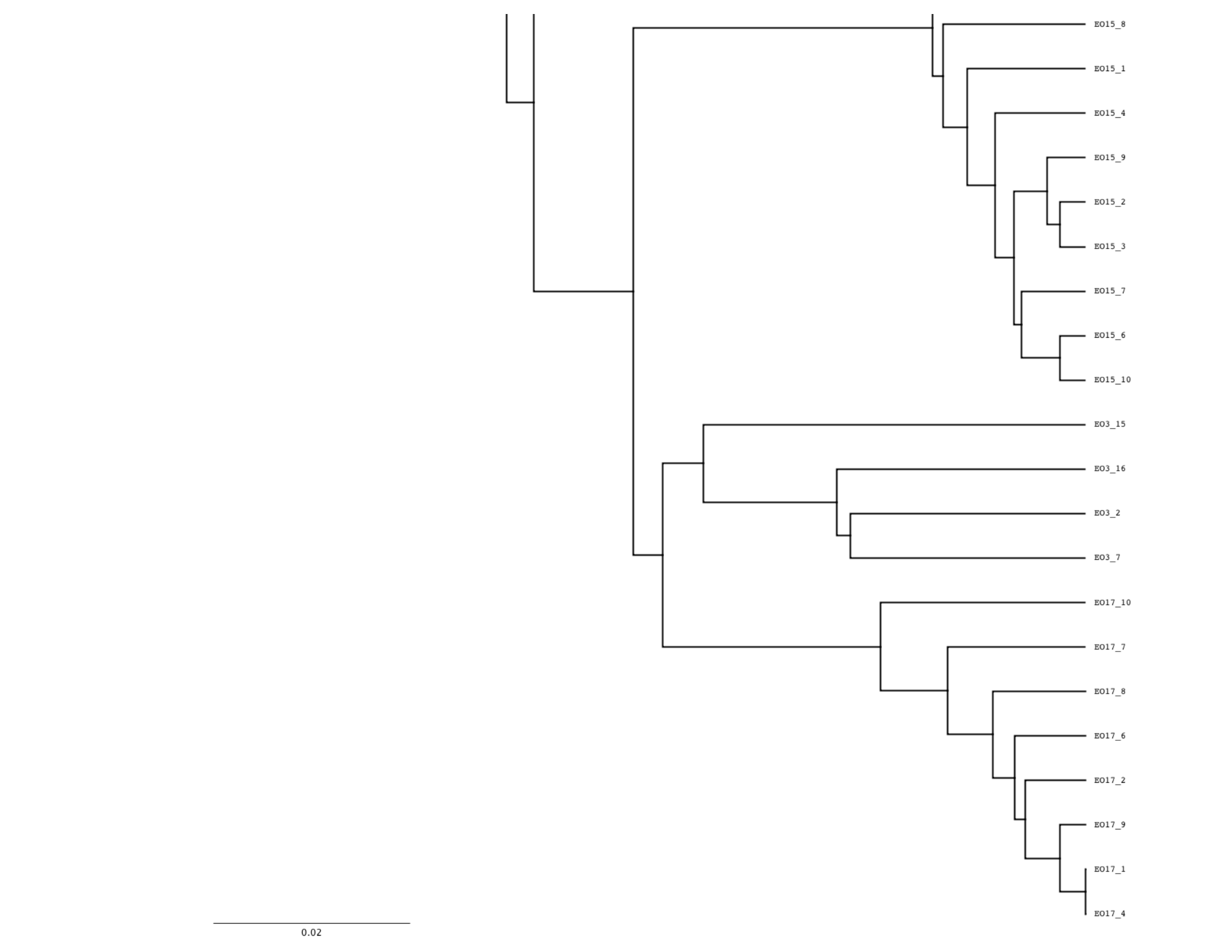
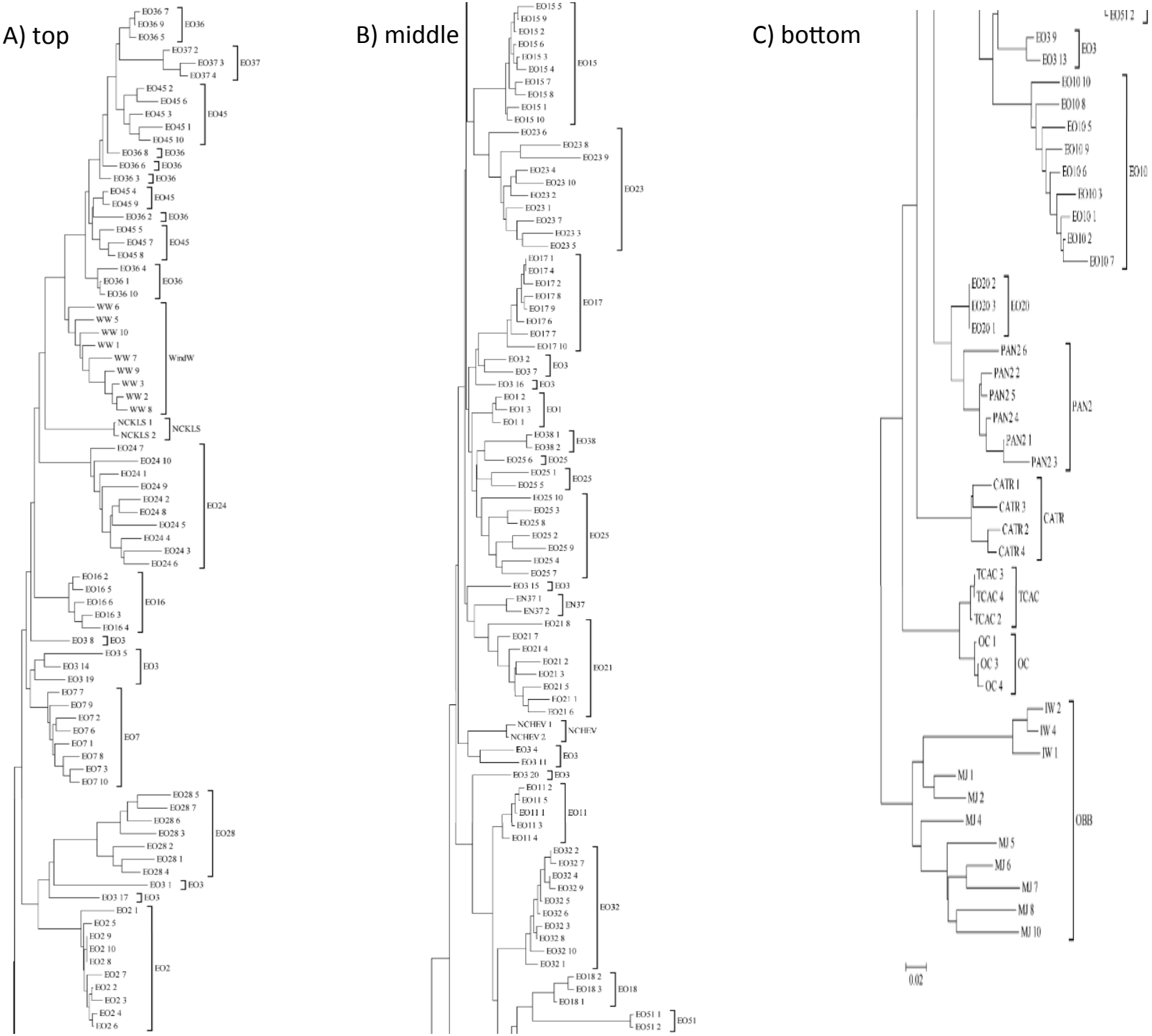
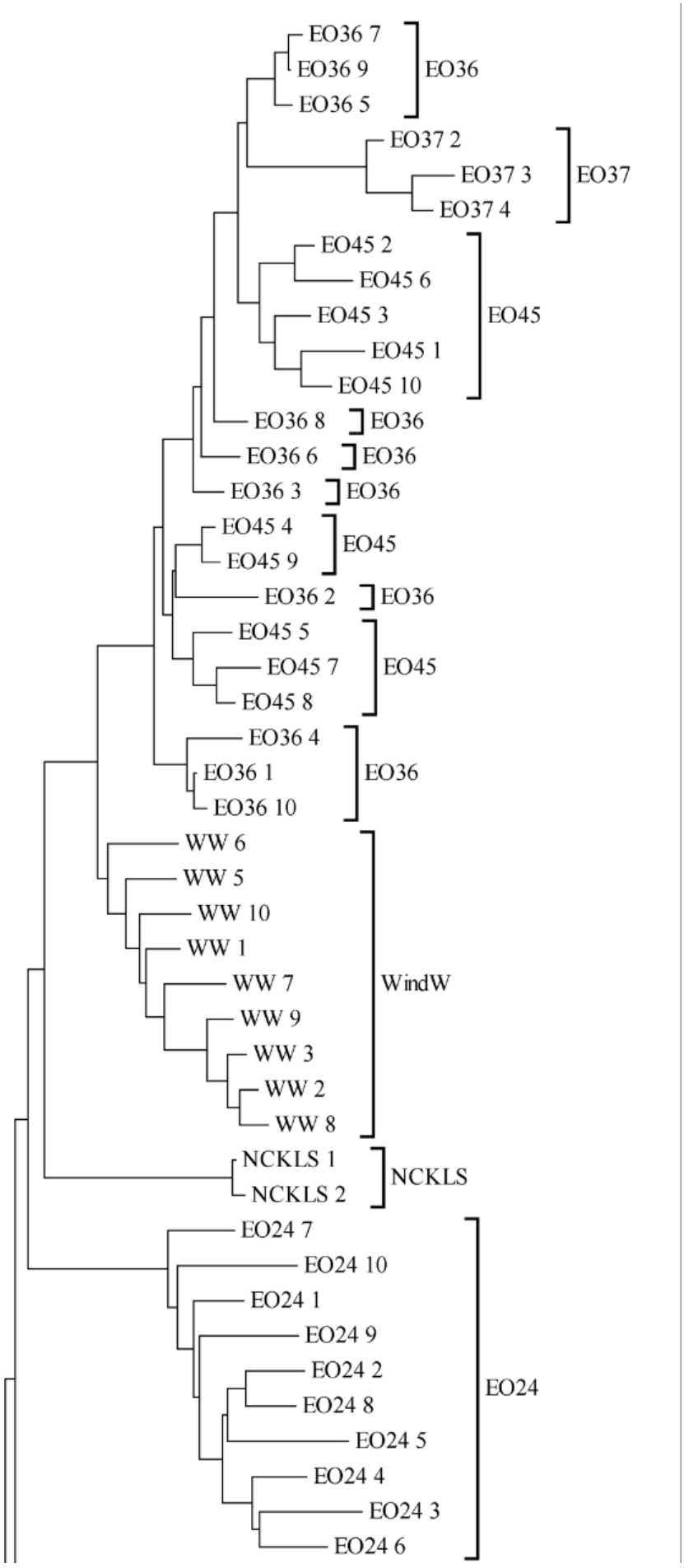
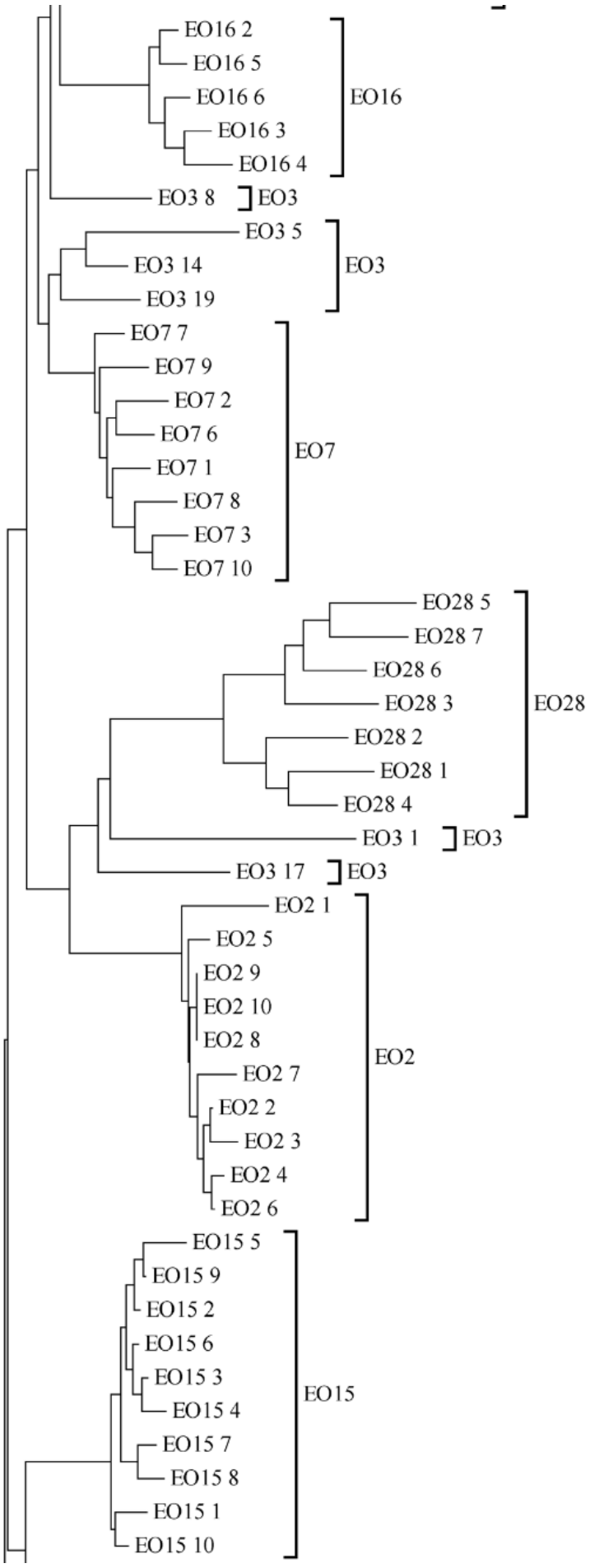

EO15_8
EO15_1
EO15_4
EO15_9
EO15_2
EO15_3
EO15_7
EO15_6
EO15_10
EO3_15
EO3_16
EO3_2
EO3_7
EO17_10
EO17_7
EO17_8
EO17_6
EO17_2
EO17_9
EO17_1
EO17_4
0.02

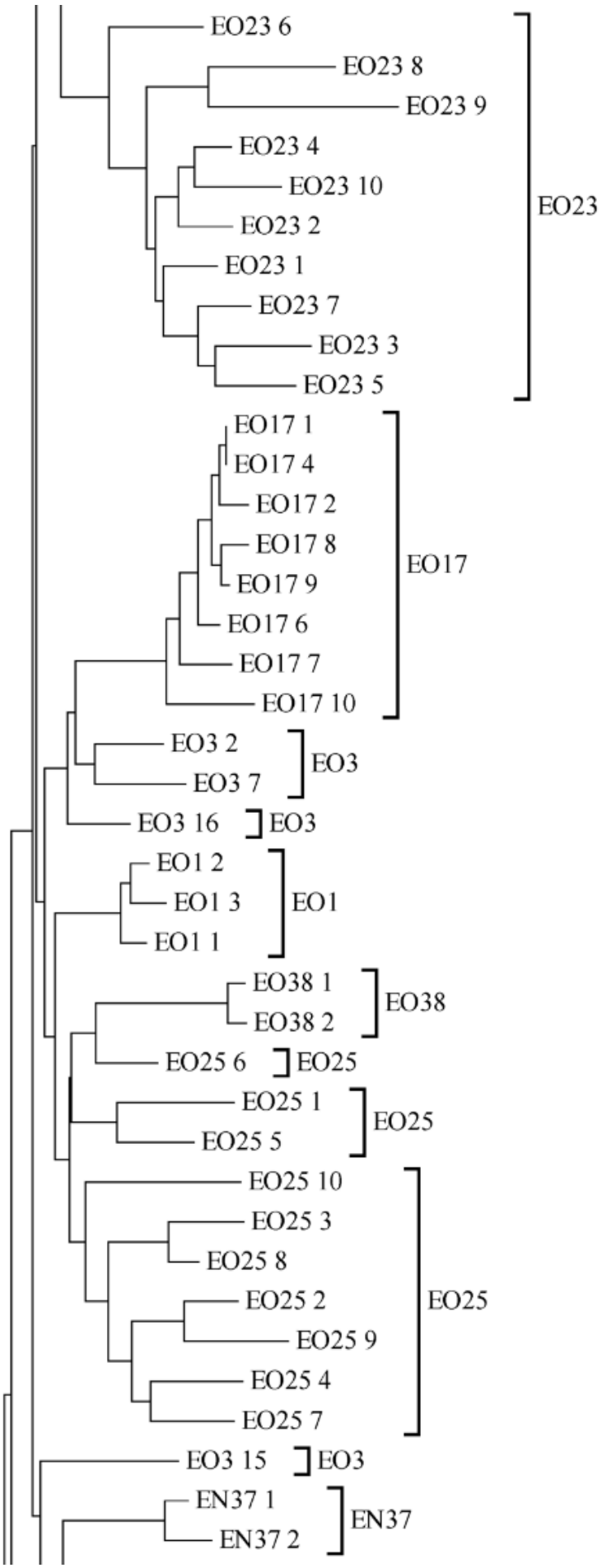
Figure 4. Neighbor-joining dendrogram derived from pairwise AFLP distances. Single tree (a) top portion, (b) middle portion, (c) bottom portion, followed by successive expanded views of all clusters.

Fig 4. A) top B) middle C) bottom




EO36 7
EO36 9
EO36 5
EO36
EO37 2
EO37 3
EO37 4
EO37
EO45 2
EO45 6
EO45 3
EO45 1
EO45 10
EO45
EO36 8
EO36
EO36 6
EO36
EO36 3
EO36
EO45 4
EO45 9
EO45
EO36 2
EO36
EO45 5
EO45 7
EO45 8
EO45
EO36 4
EO36 1
EO36 10
EO36
WW 6
WW 5
WW 10
WW 1
WW 7
WW 9
WW 3
WW 2
WW 8
WindW
NCKLS 1
NCKLS 2
NCKLS
EO24 7
EO24 10
EO24 1
EO24 9
EO24 2
EO24 8
EO24 5
EO24 4
EO24 3
EO24 6
EO24


EO16 2
EO16 5
EO16 6
EO16 3
EO16 4
EO16
EO3 8
EO3
EO3 5
EO3 14
EO3 19
EO3
EO7 7
EO7 9
EO7 2
EO7 6
EO7 1
EO7 8
EO7 3
EO7 10
EO7
EO28 5
EO28 7
EO28 6
EO28 3
EO28 2
EO28 1
EO28 4
EO28
EO3 1
EO3
EO3 17
EO3
EO2 1
EO2 5
EO2 9
EO2 10
EO2 8
EO2 7
EO2 2
EO2 3
EO2 4
EO2 6
EO2
EO15 5
EO15 9
EO15 2
EO15 6
EO15 3
EO15 4
EO15 7
EO15 8
EO15 1
EO15 10
EO15


EO23 6
EO23 8
EO23 9
EO23 4
EO23 10
EO23 2
EO23 1
EO23 7
EO23 3
EO23 5
EO23
EO17 1
EO17 4
EO17 2
EO17 8
EO17 9
EO17 6
EO17 7
EO17 10
EO17
EO3 2
EO3 7
EO3
EO3 16
EO3
EO1 2
EO1 3
EO1 1
EO1
EO38 1
EO38 2
EO38
EO25 6
EO25
EO25 1
EO25 5
EO25
EO25 10
EO25 3
EO25 8
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EO25 9
EO25 4
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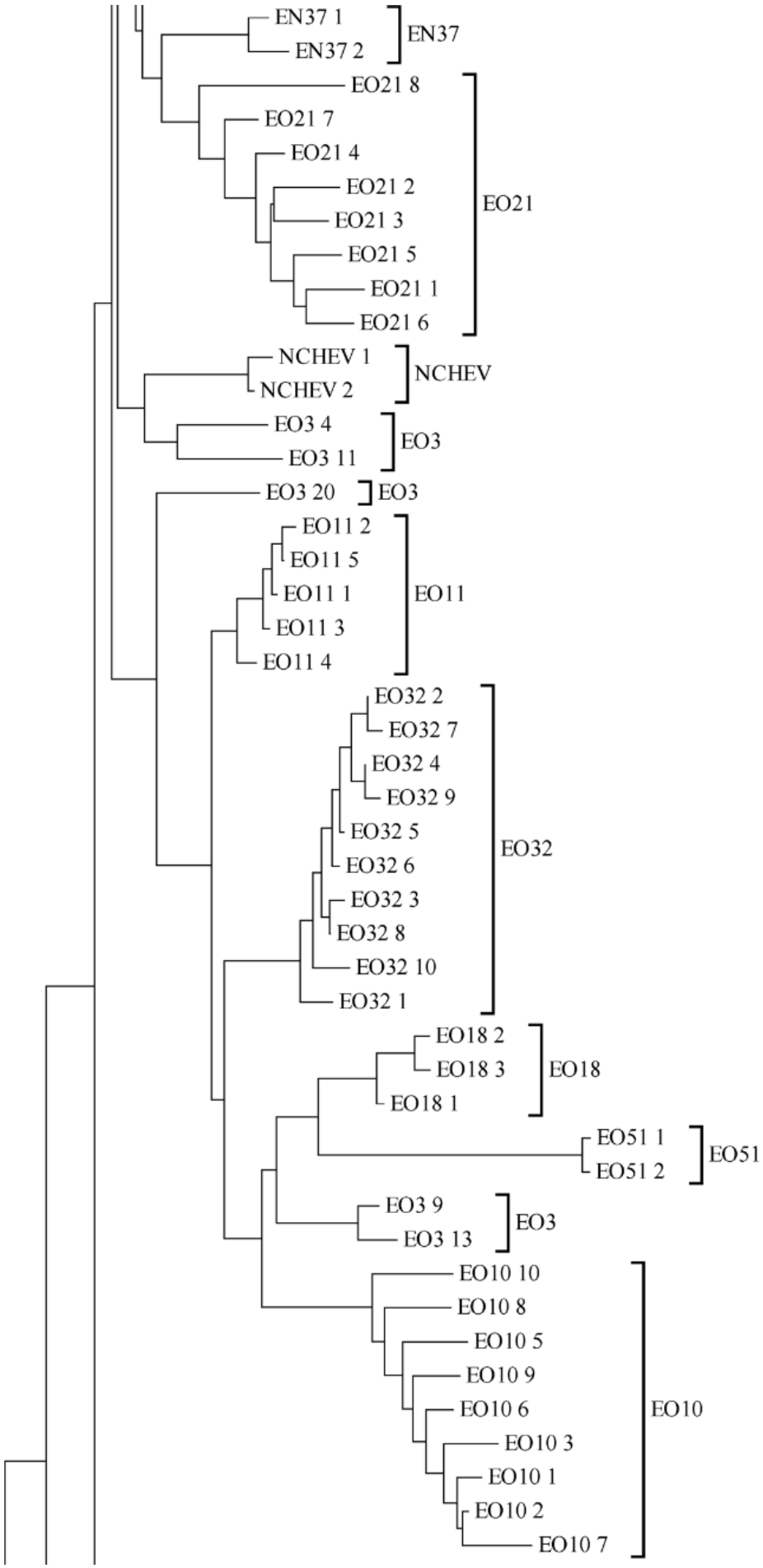
EO3 15
EO3
EN37 1
EN37 2
EN37


EN37 1
EN37 2
EN37
EO21 8
EO21 7
EO21 4
EO21 2
EO21 3
EO21 5
EO21 1
EO21 6
EO21
NCHEV 1
NCHEV 2
NCHEV
EO3 4
EO3 11
EO3
EO3 20
EO3
EO11 2
EO11 5
EO11 1
EO11 3
EO11 4
EO11
EO32 2
EO32 7
EO32 4
EO32 9
EO32 5
EO32 6
EO32 3
EO32 8
EO32 10
EO32 1
EO32
EO18 2
EO18 3
EO18 1
EO18
EO51 1
EO51 2
EO51
EO3 9
EO3 13
EO3
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EO10 3
EO10 1
EO10 2
EO10 7
EO10

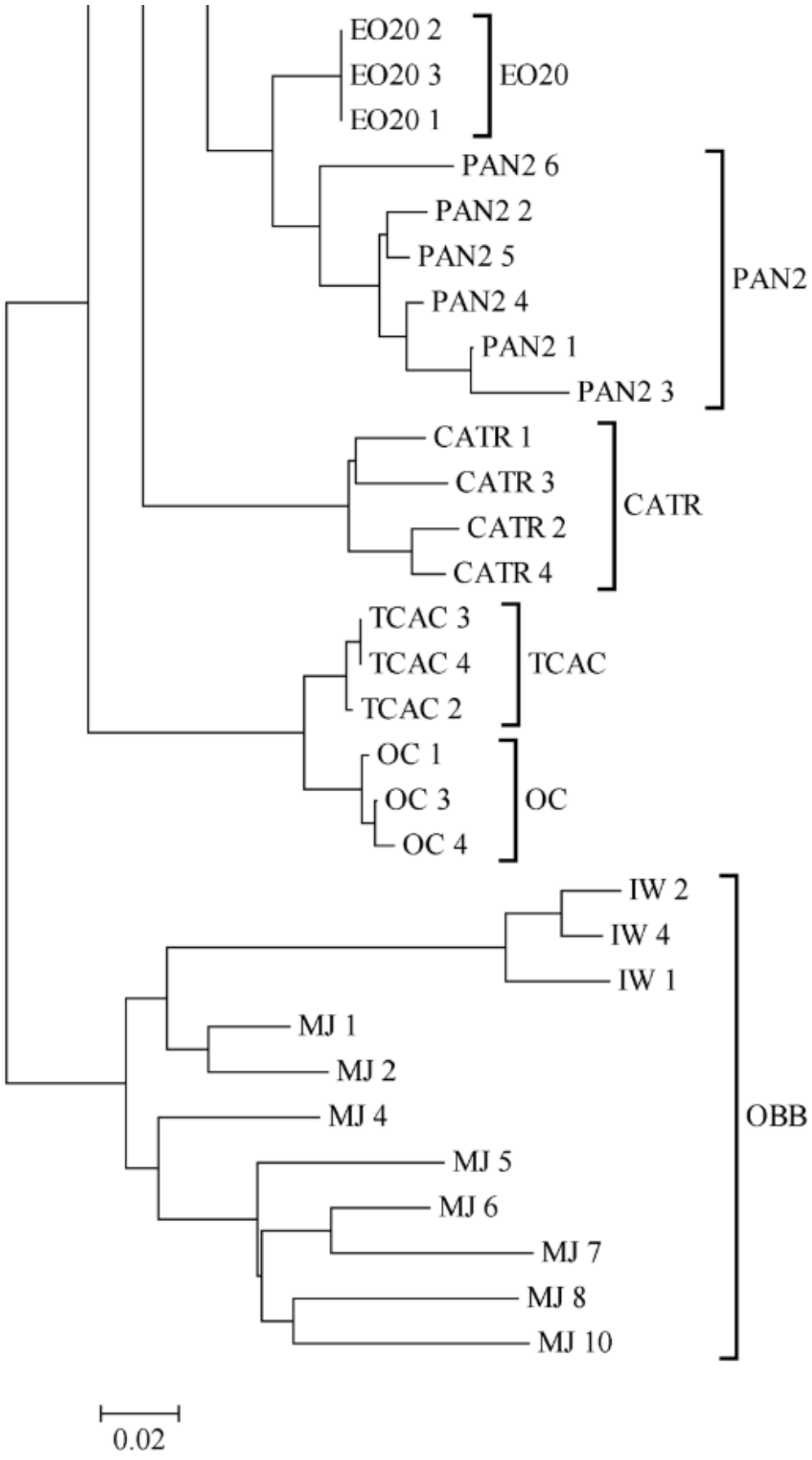
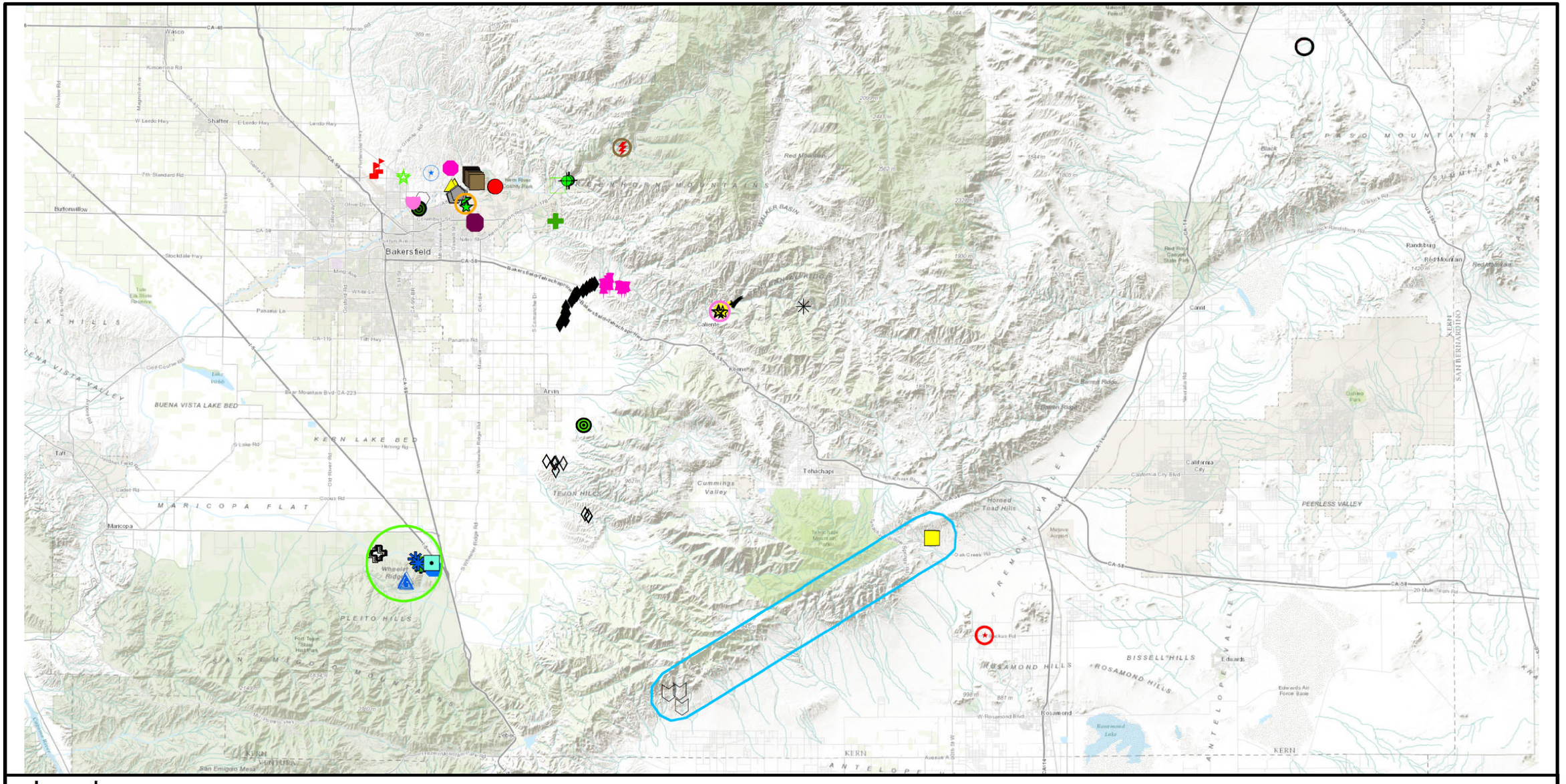

EO20 2
EO20 3
EO20 1
EO20
PAN2 6
PAN2 2
PAN2 5
PAN2 4
PAN2 1
PAN2 3
PAN2
CATR 1
CATR 3
CATR 2
CATR 4
CATR
TCAC 3
TCAC 4
TCAC 2
TCAC
OC 1
OC 3
OC 4
OC
IW 2
IW 4
IW 1
MJ 1
MJ 2
MJ 4
MJ 5
MJ 6
MJ 7
MJ 8
MJ 10
OBB
0.02

Figure 5. GPS map of sampled populations indicating genetic demes (=color-coded circles of grouped populations). See Appendix 1 for GPS points.



Legend

- BACA01
- CATR-1 -- CATR-4
- E03-5 -- E03-19
- EN37-1, EN37-2
- EO1-1 -- EO1-3
- EO10-1 -- EO10-10
- EO11-1 -- EO11-5
- EO15-1 -- EO15-10
- EO16-1 -- EO16-6
- EO17-1 -- EO17-10
- EO18-1 -- EO18-3
- EO2-1 -- EO2-10
- EO20-1 -- EO20-3
- EO21-1 -- EO21-8
- EO23-1 -- EO23-10
- EO24-1 -- EO24-10
- EO25-1 -- EO28-11
- EO28-1 -- EO28-7
- EO32-1 -- EO32-10
- EO36-1 -- EO36-10
- EO37-1 -- EO37-4
- EO38-1, EO38-2
- EO45-1 -- EO45-10
- EO51-1, EO51-2
- EO7-1 -- EO7-10
- IWXX02, IWXX04
- MJ-1 -- MJ-10
- NCHEV1, NCHEV2
- NCKLS1, NCKLS2
- NPAN1
- PAN2-1 -- PAN2-6
- TCAC-2 -- TCAC-4
- WW-1 -- WW-10
- OC-1

Grouped Populations

- EO36, EO37, EO45, WW
- TCAC and OC
- CATR
- EO10
- MJ
- IWXX
- EO51

0 50,000 100,000

SCALE IN FEET

N

Appendix 1. Collection information for sampled cactus pads.

ID	Location	Latitude	Longitude
EO10-1	North west of Fairfax	35.41989950	-118.93552735
EO10-2	Rd. about 1 mile	35.41991718	-118.93551797
EO10-3	S.E. of intersection	35.42003176	-118.93554454
EO10-4	with Alfred Harrel	35.42002707	-118.93562031
EO10-5	Highway, Bakersfield	35.41997996	-118.93563875
EO10-6		35.41988583	-118.93561729
EO10-7		35.41988801	-118.93555610
EO10-8		35.41920522	-118.93548058
EO10-9		35.41940278	-118.93572877
EO10-10		35.41167785	-118.93335300
EO11-1	Both sides of Alfred	35.43341104	-118.89593015
EO11-2	Harrel Highway,	35.43345898	-118.89545280
EO11-3	south east of Hart	35.43336159	-118.89534711
EO11-4	Mem. Unit of Kern	35.43336536	-118.89523488
EO11-5	River Park	35.43332186	-118.89526673
EO15-1	South and east of	35.44645588	-118.92840282
EO15-2	Alfred Harrel	35.44545156	-118.92727763
EO15-3	Highway	35.44535458	-118.92666215
EO15-4	Hart Park Unit -	35.44479241	-118.92650616
EO15-5	large wash area	35.44392153	-118.92565448
EO15-6		35.44328207	-118.92515207
EO15-7		35.44276323	-118.92496331
EO15-8		35.44168205	-118.92321912
EO15-9		35.44062585	-118.92106631
EO15-10		35.43814053	-118.91960719
EO16-1	North side of Round	35.43471367	-118.94993543
EO16-2	Mt. 0.8 miles north	35.43484032	-118.94983510
EO16-3	East of Oil City -	35.43508373	-118.94903328
EO16-4	east of junction with	35.43505850	-118.94916538
EO16-5	China Grade loop	35.43489086	-118.94947417
EO16-6		35.43441100	-118.95361341
WW-1	Wind Wolves	35.0082	119.0094
WW-2	Preserve,	35.0068	119.0094
WW-3	Wheeler Ridge	35.0061	119.0094
WW-5		35.0054	119.0097
WW-6		35.0054	119.0094
WW-7		35.0054	119.0089
WW-8		35.0043	119.0075

WW-9		35.0052	119.0072
WW-10		35.0099	119.0075
BACA01	New area, Caliente Cr. past EO22	35.30607000	-118.48802000
EO17-1	Kern bluff on north &	35.42259721	-118.95006954
EO17-2	south sides of Alfred	35.42384159	-118.95004841
EO17-3	Harrel Highway - 1.5	35.42326491	-118.94903571
EO17-4	miles north east of	35.42582366	-118.94449725
EO17-5	Mount Vernon Ave.	35.42610898	-118.94421897
EO17-6	Bakersfield -	35.42542770	-118.94370910
EO17-7	where Fairfax Road	35.42567370	-118.94207513
EO17-8	dead ends	35.42638130	-118.94157389
EO17-9		35.42745469	-118.94535824
EO17-10		35.42633638	-118.94446121
EO18-1	Oildale - by Beardsley	35.41992749	-118.99102000
EO18-2	canal on Chevron/	35.41994710	-118.99102938
EO18-3	Panorama Preserve	35.41996454	-118.99100415
EO1-1	Chevron, Oildale	35.44482350	-119.01674548
EO1-2		35.44266743	-119.01678463
EO1-3		35.44234062	-119.01679896
EO20-1	Oildale - Chevron	35.45266603	-118.95450960
EO20-2	east side of their	35.45295211	-118.95478813
EO20-3	property	35.45275195	-118.95425663
EO21-1	Between	35.13567008	-118.81319239
EO21-2	Caliente and	35.13546062	-118.81502727
EO21-3	Grapevine -	35.13706156	-118.82499017
EO21-4	Commanche Point	35.07874061	-118.76975460
EO21-5	Tejon	35.07846359	-118.76939870
EO21-6		35.08062487	-118.77366995
EO21-7		35.12727678	-118.81306532
EO21-8		35.13482577	-118.80289145
EO23-1	Caliente Creek 0.5	35.31080609	-118.57642374
EO23-2	miles east of	35.31138034	-118.57677553
EO23-3	fig orchard and	35.31119652	-118.57657931
EO23-4	Oiler Canyon -	35.31080869	-118.57673731
EO23-5	Parker Ranch	35.31096652	-118.57660839

EO23-6		35.30940363	-118.57770944
EO23-7		35.30945711	-118.57768471
EO23-8		35.30922459	-118.57589275
EO23-9		35.30896761	-118.57590029
EO23-10		35.30944965	-118.57567884
EO24-1	Caliente Creek, 1 mile	35.29925675	-118.59122140
EO24-2	east of Caliente on	35.30028504	-118.59153061
EO24-3	sides of Caliente	35.30180611	-118.59157763
EO24-4	Creek - Bodfish Road	35.30280121	-118.58798750
EO24-5		35.30204273	-118.59098721
EO24-6		35.30203845	-118.59125937
EO24-7		35.30135000	-118.59969000
EO24-8		35.30035000	-118.60147000
EO24-9		35.30081000	-118.59882000
EO24-10		35.30099000	-118.59914000
CATR-1	Catani Ranch	35.30046215	-118.59961789
CATR-2	by EO24	35.30036258	-118.60087861
CATR-3		35.30030181	-118.60026481
CATR-4		35.29997701	-118.59466871
EO25-1	North of Bena Road	35.33297597	-118.74312999
EO25-2	adjacent to Walker	35.33061597	-118.74582980
EO25-3	Basin Creek	35.32256414	-118.72389788
EO25-4		35.32377533	-118.72220616
EO25-5		35.32453573	-118.73107614
EO25-6		35.32085231	-118.72777308
EO25-7		35.32704419	-118.75208798
EO25-8		35.32080243	-118.75169369
EO25-9		35.32480127	-118.74345328
EO25-10		35.32623441	-118.74595100
EO25-11		35.32732104	-118.74979301
EO28-1	Nickel Ranch, both	35.44087152	-118.79784194
EO28-2	sides of Hwy 178	35.44072945	-118.79831451
EO28-3	by beginning of deep	35.44069475	-118.79867393
EO28-4	V gorge of Kern River	35.44083657	-118.79884810
EO28-5		35.44095123	-118.79922068
EO28-6		35.44020692	-118.80145764
EO28-7		35.44035000	-118.80184790
EO2-1	BLM site 1.5 miles	35.45035112	-119.05364279

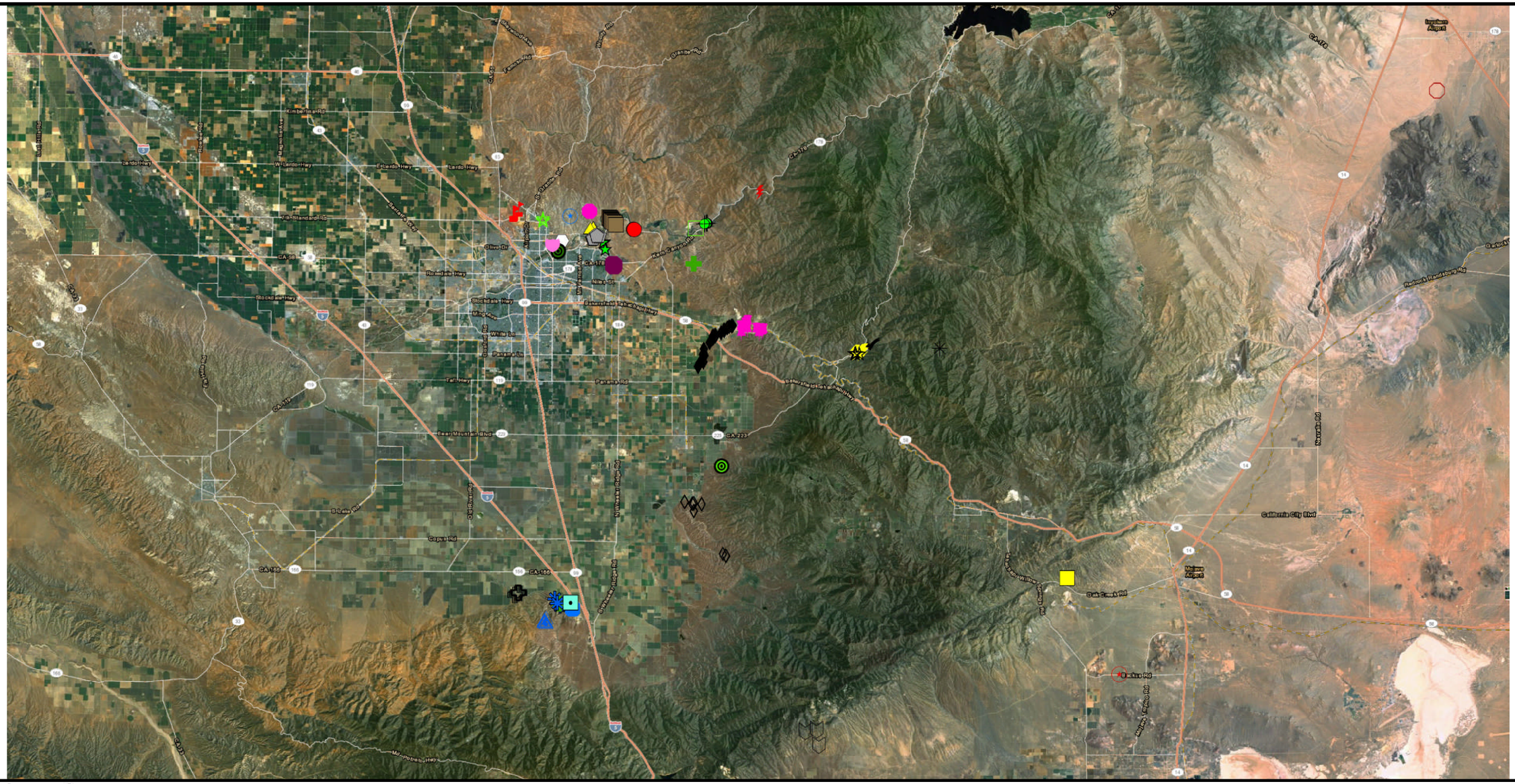
EO2-2	north of Bakersfield	35.45465766	-119.04780647
EO2-3	Airport	35.44990989	-119.05394680
EO2-4		35.44926432	-119.05413774
EO2-5		35.44877448	-119.05473637
EO2-6		35.44912174	-119.05531238
EO2-7		35.44950429	-119.05533048
EO2-8		35.44986119	-119.05520895
EO2-9		35.45025866	-119.05532839
EO2-10		35.44984770	-119.05417722
EO32-1	Junction of	35.39656721	-118.81616436
EO32-2	Breckenridge Rd. &	35.39681951	-118.81610250
EO32-3	Cottonwood Creek,	35.39680165	-118.81576655
EO32-4	south west of Rio	35.39674650	-118.81543413
EO32-5	Bravo Ranch	35.39672697	-118.81557637
EO32-6		35.39662496	-118.81570880
EO32-7		35.39641533	-118.81591885
EO32-8		35.39653125	-118.81597912
EO32-9		35.39661465	-118.81583386
EO32-10		35.39647769	-118.81611390
EO36-1	North west of	35.02270097	-118.98877373
EO36-2	Windgap pumping	35.03044426	-118.99570263
EO36-3	plant, north side of	35.02607309	-118.97893824
EO36-4	Wheeler Ridge	35.02590646	-118.98026786
EO36-5		35.02569783	-118.98329909
EO36-6		35.02489032	-118.98550018
EO36-7		35.02422430	-118.98713146
EO36-8		35.02408373	-118.98853065
EO32-9		35.02495009	-118.98974888
EO36-10		35.02595969	-118.99225105
EO37-1	0.5 miles north east	35.02013250	-118.97208922
EO37-2	of Windgap pumping	35.02024323	-118.97190867
EO37-3	plant, California	35.02051757	-118.97221511
EO37-4	Aqueduct, Wheeler Ridge	35.01931150	-118.97188973
EN37-1	New site by EO37,	35.02643201	-118.97454050
EN37-2	Wheeler Ridge	35.02644349	-118.97460010
EO38-1	Between Caliente and	35.17612911	-118.77640472
EO38-2	Grapevine - Tejon	35.17629272	-118.77631805

	Ranch		
EO3-1	Sandridge Preserve	35.32930369	-118.76324102
EO3-2	5 miles east south	35.32904847	-118.76456846
EO3-3	east of Edison.	35.32827666	-118.76745092
EO3-4	Western edge of	35.32702340	-118.76989625
E03-5	Caliente Creek	35.32472055	-118.77405762
EO3-6		35.32374817	-118.77419449
EO3-7		35.32159293	-118.77844722
EO3-8		35.31913728	-118.78611767
EO3-9		35.31755034	-118.78642176
EO3-10		35.31809717	-118.78919533
EO3-11		35.28936460	-118.80175151
EO3-12		35.28799650	-118.80708961
EO3-13		35.28459605	-118.80899540
EO3-14		35.31485648	-118.78933238
EO3-15		35.31064876	-118.79365627
EO3-16		35.29689993	-118.80017454
EO3-17		35.30389672	-118.80127206
EO3-18		35.29948985	-118.80172050
EO3-19		35.29762463	-118.80256422
EO3-20		35.29298927	-118.80276807
EO45-1	DWR California	35.03601068	-119.04273248
EO45-2	Aqueduct, Wheeler	35.03651728	-119.04345257
EO45-3	Ridge area	35.03672222	-119.04397929
EO45-4		35.03671392	-119.04411835
EO45-5		35.03677410	-119.04432320
EO45-6		35.03693679	-119.04454306
EO45-7		35.03695917	-119.04478789
EO45-8		35.03700167	-119.04492334
EO45-9		35.03406163	-119.04862823
EO45-10		35.03553492	-119.04639403
EO51-1	Hwy 178 across from	35.47471764	-118.72796603
EO51-2	Lower Richbar day use area USFS Kern River	35.47472334	-118.72793050
EO7-1	1 mile east of	35.39576992	-118.92259995
EO7-2	Bakersfield Country	35.39565643	-118.92282726
EO7-3	Club - south east	35.39581317	-118.92300328
EO7-4	junction of Hwy 178	35.39407158	-118.92017330

EO7-5	and Fairfax Road	35.39378978	-118.92321669
EO7-6		35.39391174	-118.92235796
EO7-7		35.39352148	-118.92117569
EO7-8		35.39305729	-118.92111375
EO7-9		35.39301823	-118.92161951
EO7-10		35.39228548	-118.92141500
NCHEV1	New Chevron area	35.44743966	-118.98026434
NCHEV2		35.44741427	-118.98015403
NCKLS1	Nickels Ranch	35.43525799	-118.81343848
NCKLS2	by Kern River	35.43529504	-118.81327570
PAN2-1	Panorama Preserve	35.40860396	-118.99570347
PAN2-2	By Kern River	35.40870546	-118.99571395
PAN2-3		35.40900193	-118.99547221
PAN2-4		35.40903579	-118.99568612
PAN2-5		35.40852064	-118.99589860
PAN2-6		35.40871217	-118.99588133
NPAN1	Panorama Preserve	35.41500572	-119.00344776
TCAC-2	Techachipi Mountains above Mojave Desert (Tejon Ranch)	34.88812	-118.66273000
TCAC-3		34.87309	-118.64499000
TCAC-4		34.88986	-118.64813000
IW1	Indian Wells ER by Inyokern	35.58628	-117.82648000
IW2		35.58743	-117.82648000
IW4		35.58739	-117.82682000
MJ-1	South of city of Mojave	34.951805	-118.24812400
MJ-2		34.951805	-118.24812400
MJ-4		34.951805	-118.24812400
MJ-5		34.951805	-118.24812400
MJ-6		34.951805	-118.24812400
MJ-7		34.951805	-118.24812400
MJ-8		34.951805	-118.24812400
MJ-10		34.951805	-118.24812400

OC-1	Oak Creek	35.054	-118.31067
OC-3		35.04992	-118.35235
OC-4		35.01647	-118.32893

Appendix 2. GPS map of sampled cactus pads (refer to Appendix 1 for specific information regarding GPS coordinates).


Legend
BACA01
CATR-1 -- CATR-4
E03-5 -- E03-19
EN37-1, EN37-2
EO1-1 -- EO1-3
EO10-1 -- EO10-10
EO11-1 -- EO11-5
EO15-1 -- EO15-10
EO16-1 -- EO16-6
EO17-1 -- EO17-10
EO18-1 -- EO18-3
EO2-1 -- EO2-10
EO20-1 -- EO20-3
EO21-1 -- EO21-8
EO23-1 -- EO23-10
EO24-1 -- EO24-10
EO25-1 -- EO28-11
EO28-1 -- EO28-7
EO32-1 -- EO32-10
EO36-1 -- EO36-10
EO37-1 -- EO37-4
EO38-1, EO38-2
EO45-1 -- EO45-10
EO51-1, EO51-2
EO7-1 -- EO7-10
IWXX02, IWXX04
MJ-1 -- MJ-10
NCHEV1, NCHEV2
NCKLS1, NCKLS2
NPAN1
PAN2-1 -- PAN2-6
TCAC-2 -- TCAC-4
WW-1 -- WW-10
OC-1
0
50,000
100,000
SCALE IN FEET
N