Data - Read In and Notes

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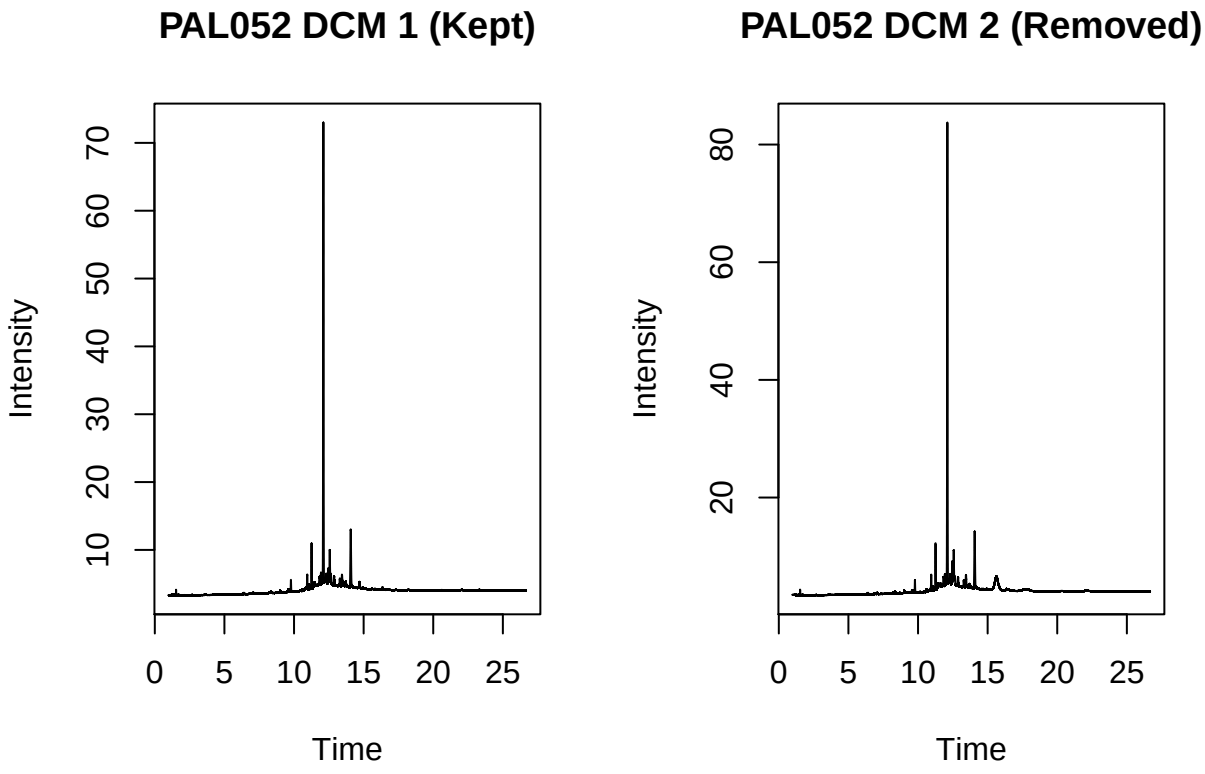
June 15, 2016

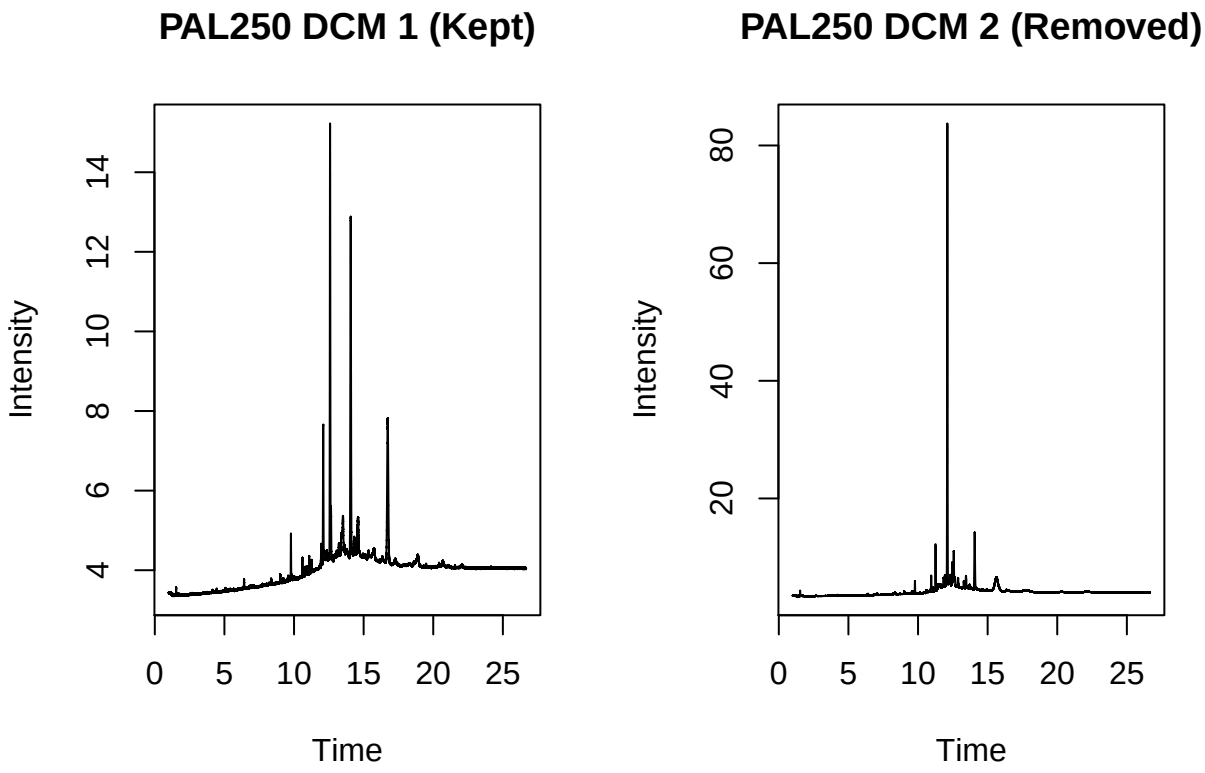
Data Entry

Data Notes:

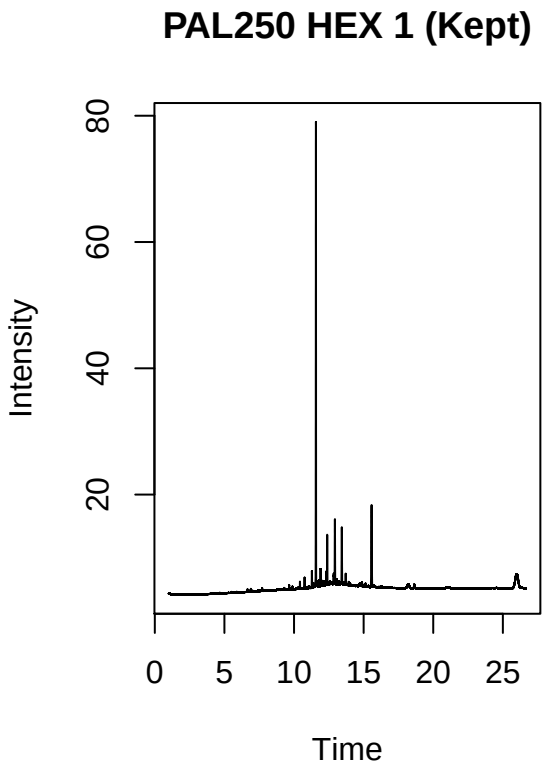
- Readings occur at delta = 0.00033333333333 seconds apart
- Several of the Trocheliophorum samples were removed due to duplication or obvious corruption. See below for details:
  - Remark that Trocheliophorum is not the main focus of this research and these samples are used mainly as a reference for the S. Glaucum clades.
- Two data files found for sample PAL052 (DCM) - discarded 2nd sample (file PAL052D2.csv)
  - Incongruent with other DCM samples
  - Apparently identical to 2nd sample found for sample PAL250 (DCM)
  - Broad peak @ 15.8 looked less like rest of data.

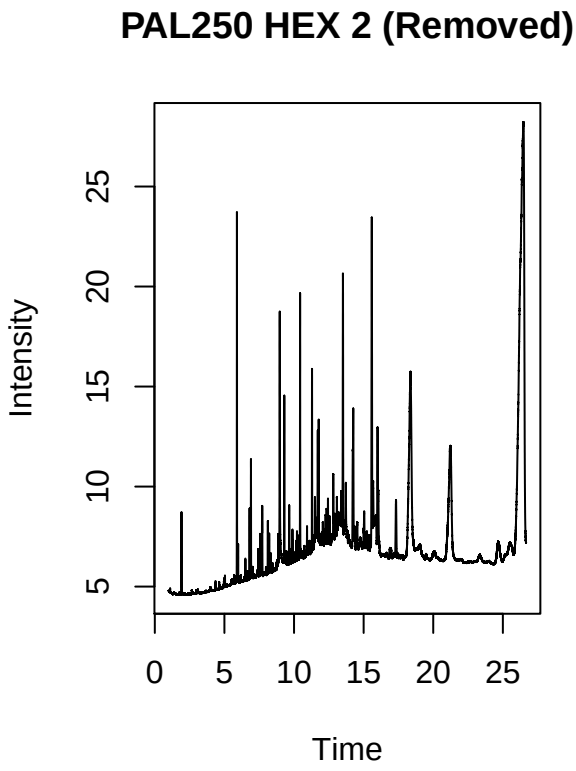


- Two data files found for sample PAL250 (DCM) - discarded 2nd sample (file PAL250D2.csv)
  - Incongruent with other DCM samples
  - Apparently identical to 2nd sample found for sample PAL052 (DCM)

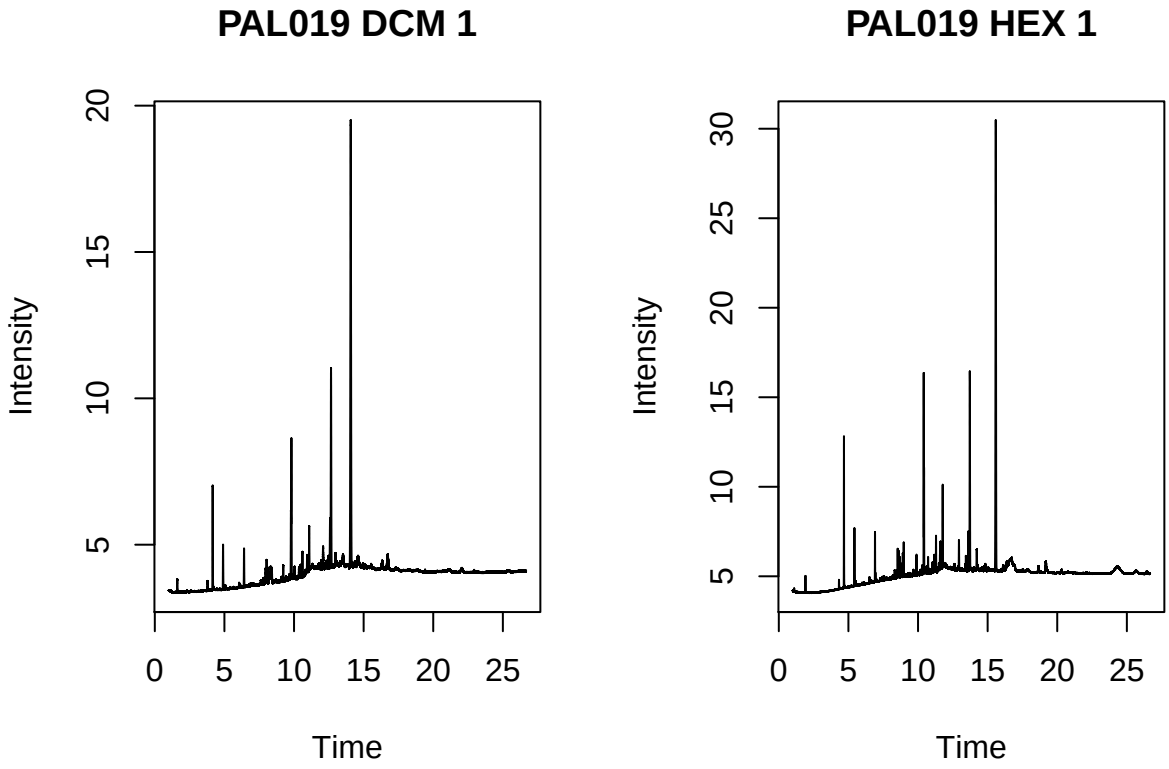


- Two data files found for sample PAL250(HEX) - discarded 2nd sample (file PAL250H2.CSV)
  - Incongruent with other HEX Trocheliophorum samples
  - Choosing sample 2 is consistent with earlier choices where 2 data files found

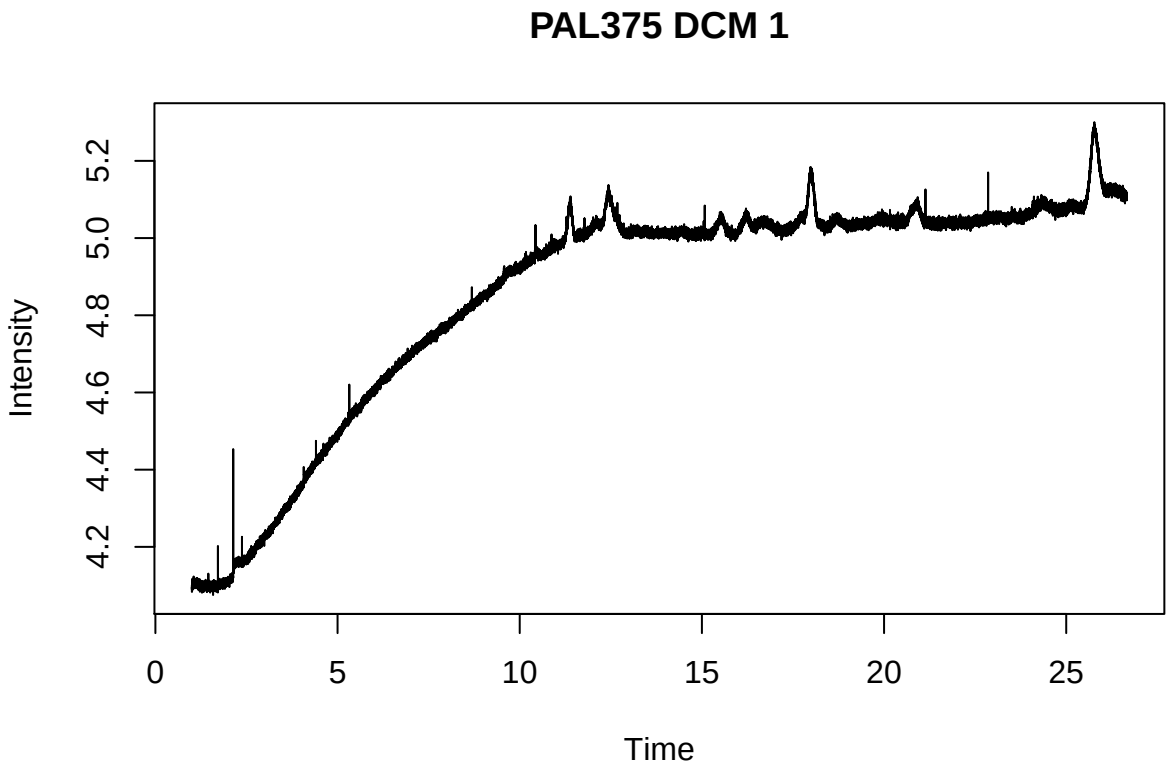




- Trocheliophorum Comparison Plot: PAL019 DCM and HEX



- Chromatogram of sample PAL375 (DCM) indicates that nothing was injected and was discarded (file PAL375D1.csv)



- Some readings originally had 77,002 time points while others had 77,001
  - We omit the last time point and align based on the initial time which begin at approximately 1 minute
    - We observe that the standard deviation is greater among the final time points than among either the initial time points or the 77001th time points, and conclude that the samples start at approximately the same time, but some extend for an extra time point.

```
sd(initialDCMTimes) #standard deviation of initial time point for each sample
```

```
## [1] 9.001046e-05
```

```
sd(DCMTime77001) #standard deviation of 77001st time point for each sample
```

```
## [1] 9.001046e-05
```

```
sd(finalDCMTimes) #standard deviation of final time point for each sample
```

```
## [1] 0.000110811
```

Number of DCM (“medium polarity”) Samples of Each Type:

Species	Count
S.glaucum Clade F	24
S.glaucum Clade D	10
Trocheliophorum	11
Total	45

Number of Hexane (“greasy”) Samples of Each Type:

Species	Count
S.glaucum Clade F	18
S.glaucum Clade D	8
Trocheliophorum	7
Total	33

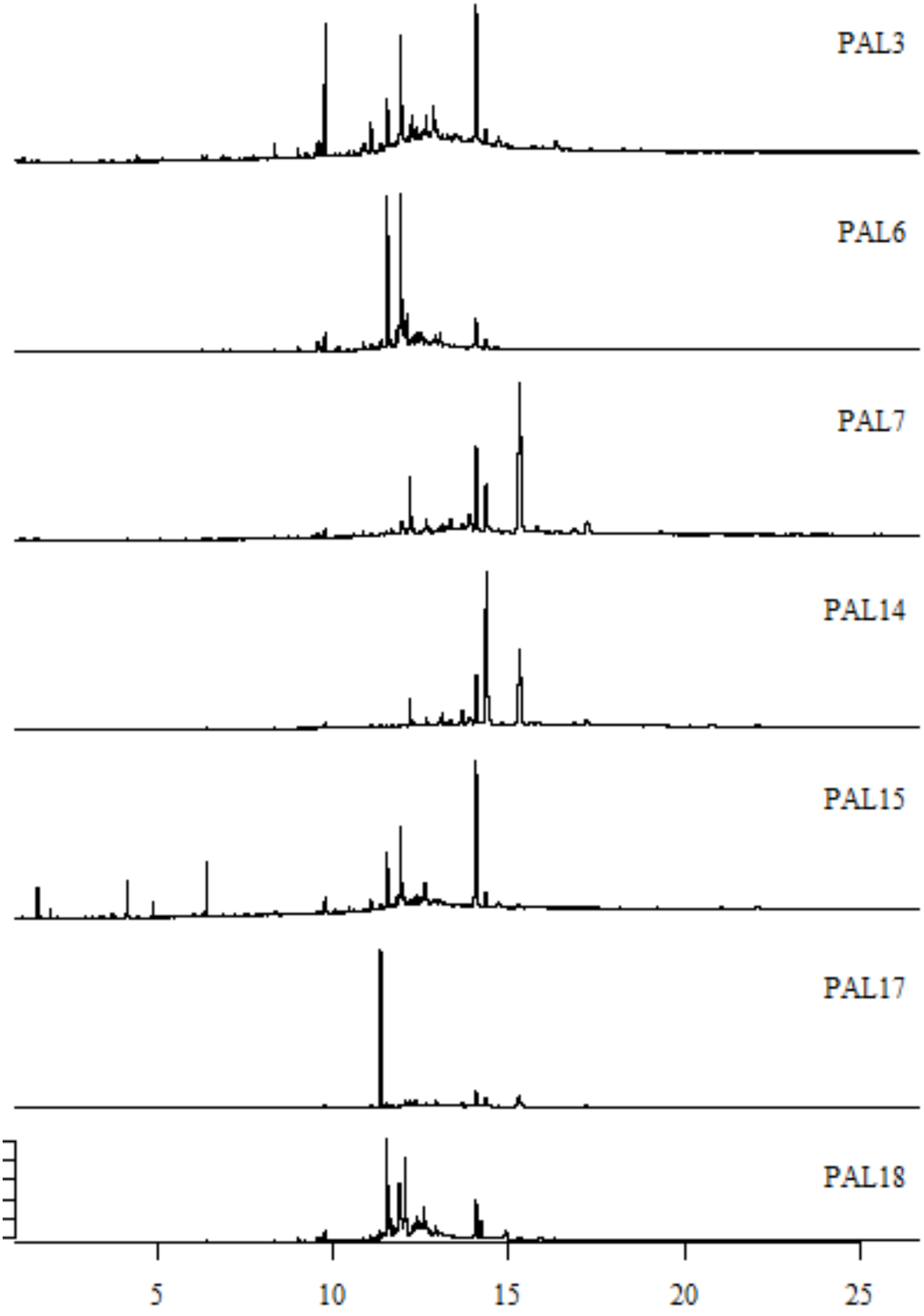
Table 3: Snapshot of All Time Points Dataframe (DCM)

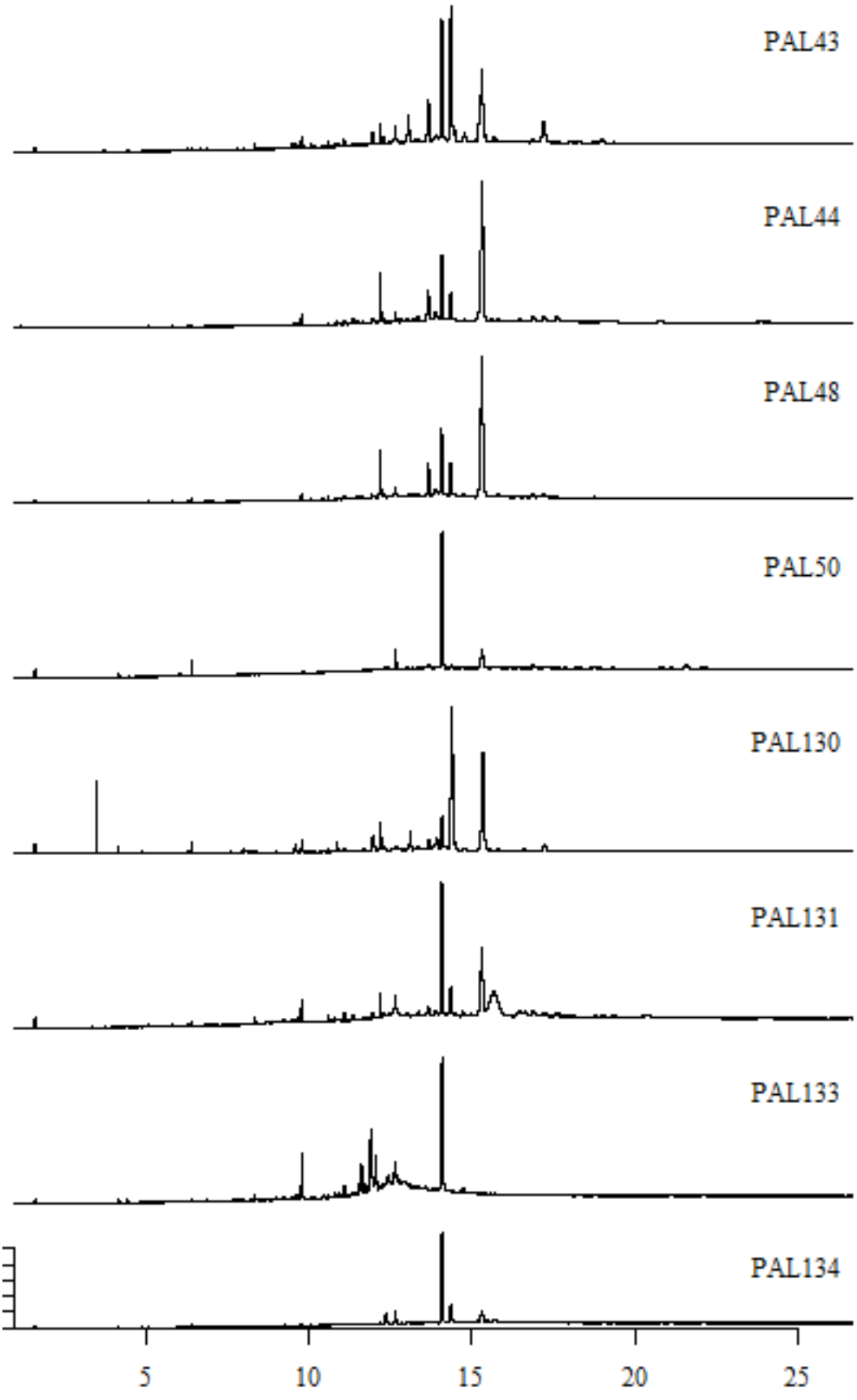
Sample	Greasiness	Clade	t1	t2
3	DCM	F	4.05859396167216	4.06250021187589
6	DCM	F	3.93515645523439	3.93190124673129
7	DCM	F	4.01484395939042	4.0098960424657
14	DCM	F	3.90481791198545	3.90065124510147
15	DCM	F	3.90716166210768	3.9088543705293

Table 4: Snapshot of All Time Points Dataframe (HEX)

Sample	Greasiness	Clade	t1	t2
1	HEX	F	4.82070337641926	4.81888045965752
3	HEX	F	4.79622420847591	4.7972658751969
7	HEX	F	4.80494816726423	4.79661483349628
14	HEX	F	4.82669296006497	4.82916691852734
15	HEX	F	4.73854191380087	4.73411483023665

Gas Chromatograms - Clade F (DCM)

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PAL133
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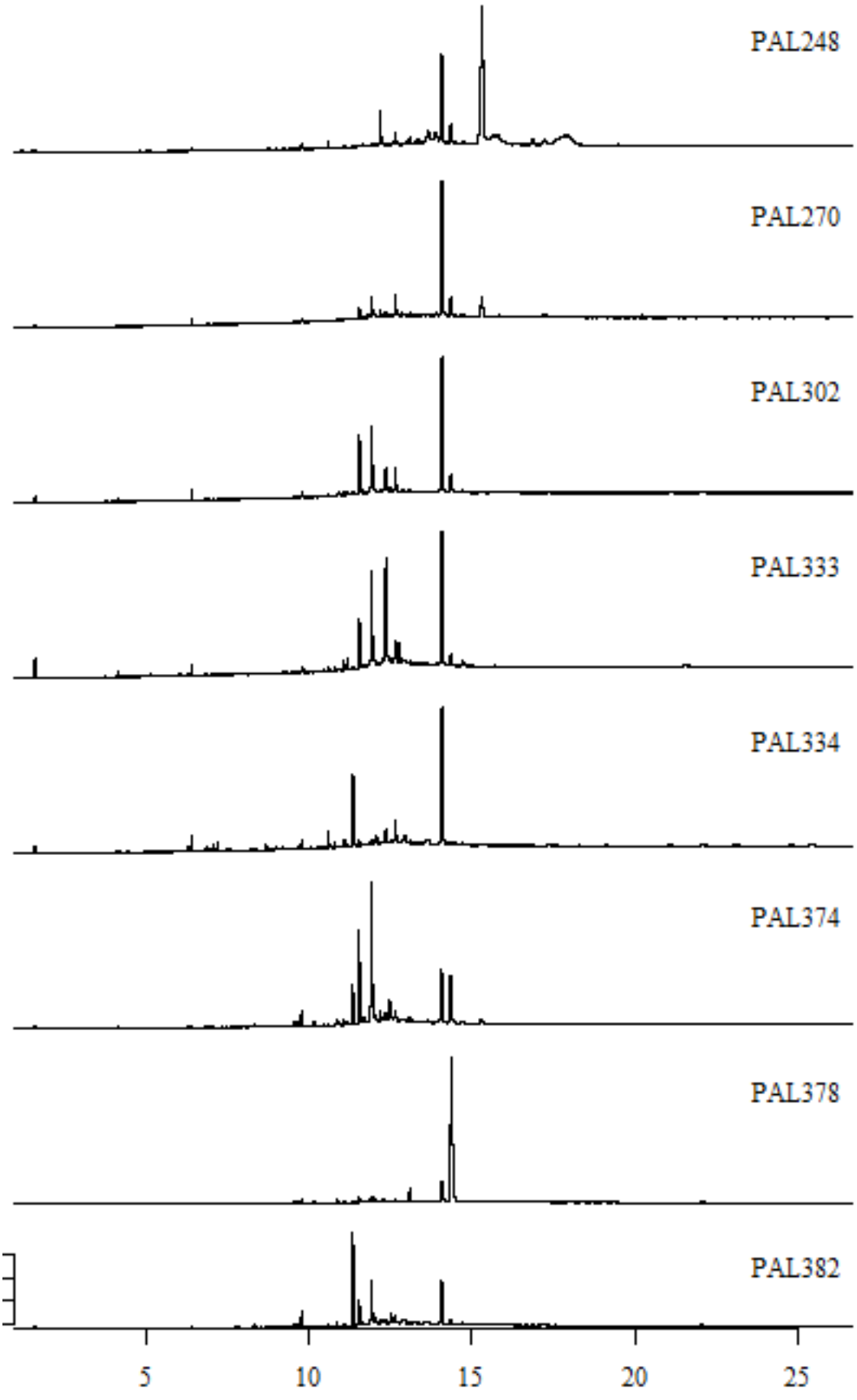
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PAL270
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PAL334
PAL374
PAL378
PAL382
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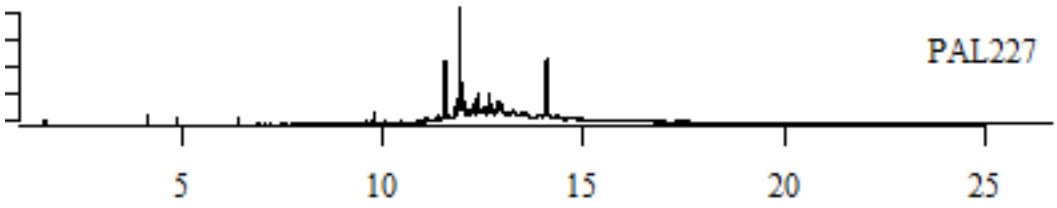
PAL227
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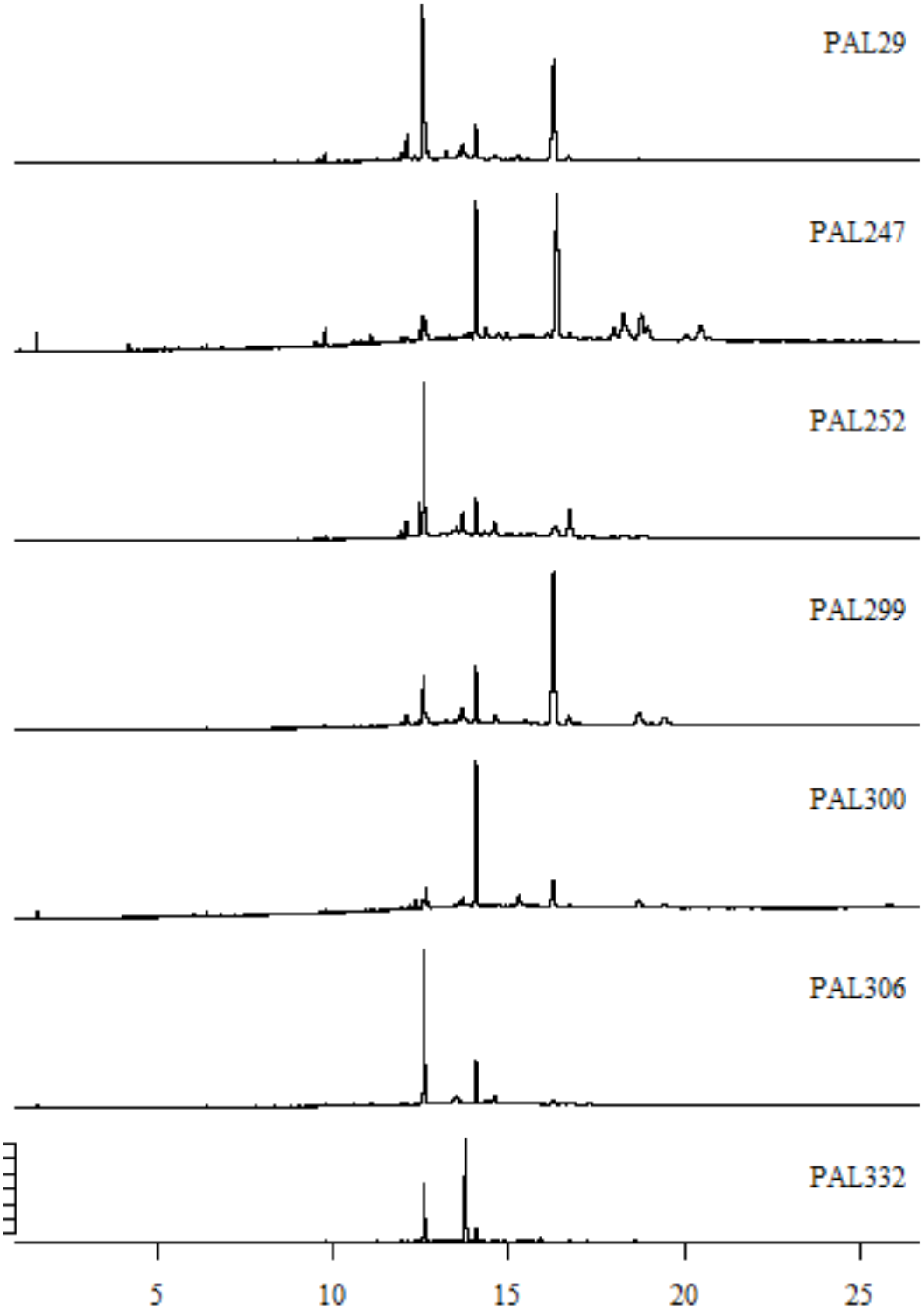
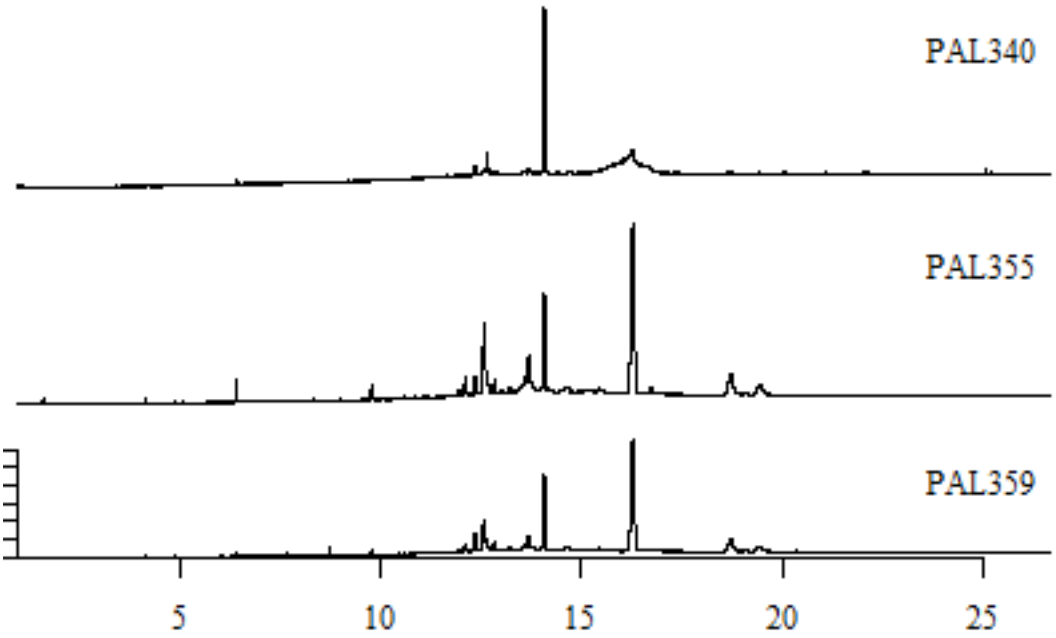
Gas Chromatograms - Clade D (DCM)




PAL340
PAL355
PAL359
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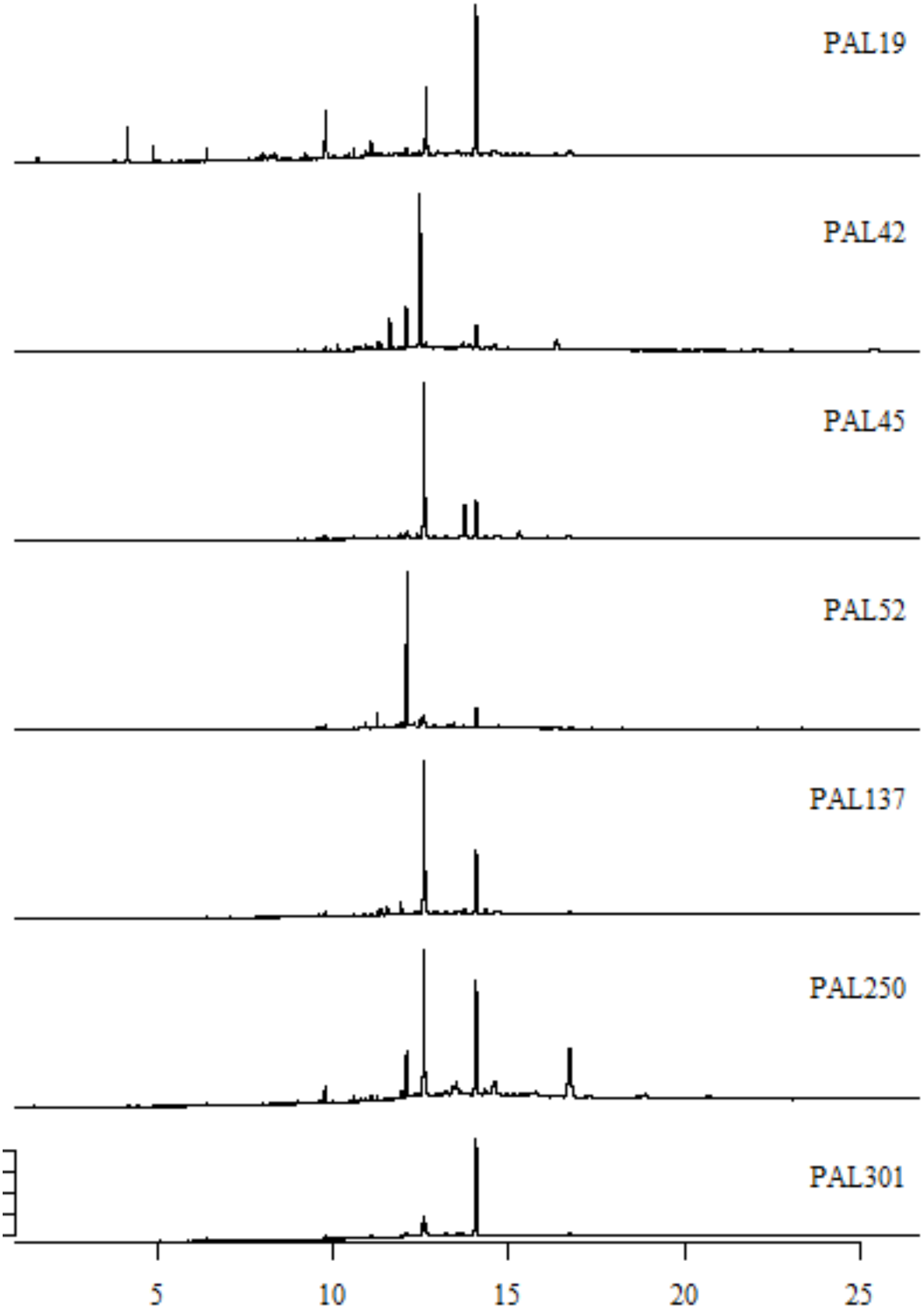
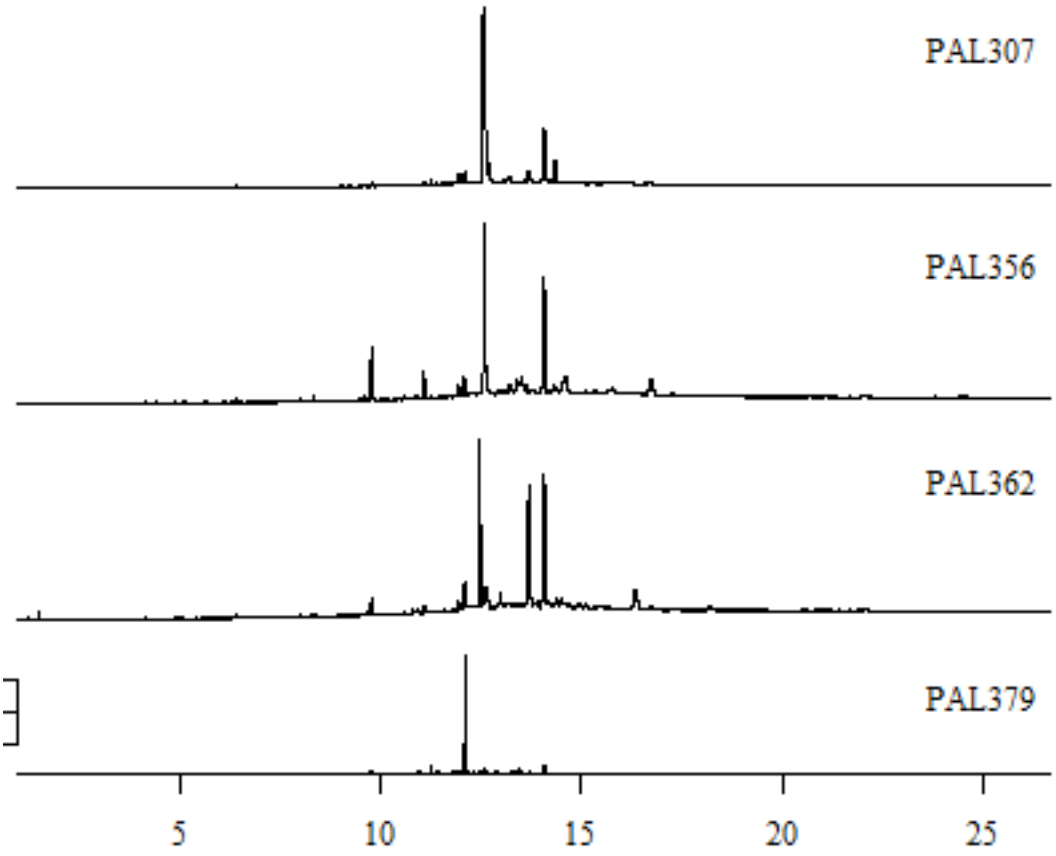
Gas Chromatograms - Trocheliophorum (DCM)

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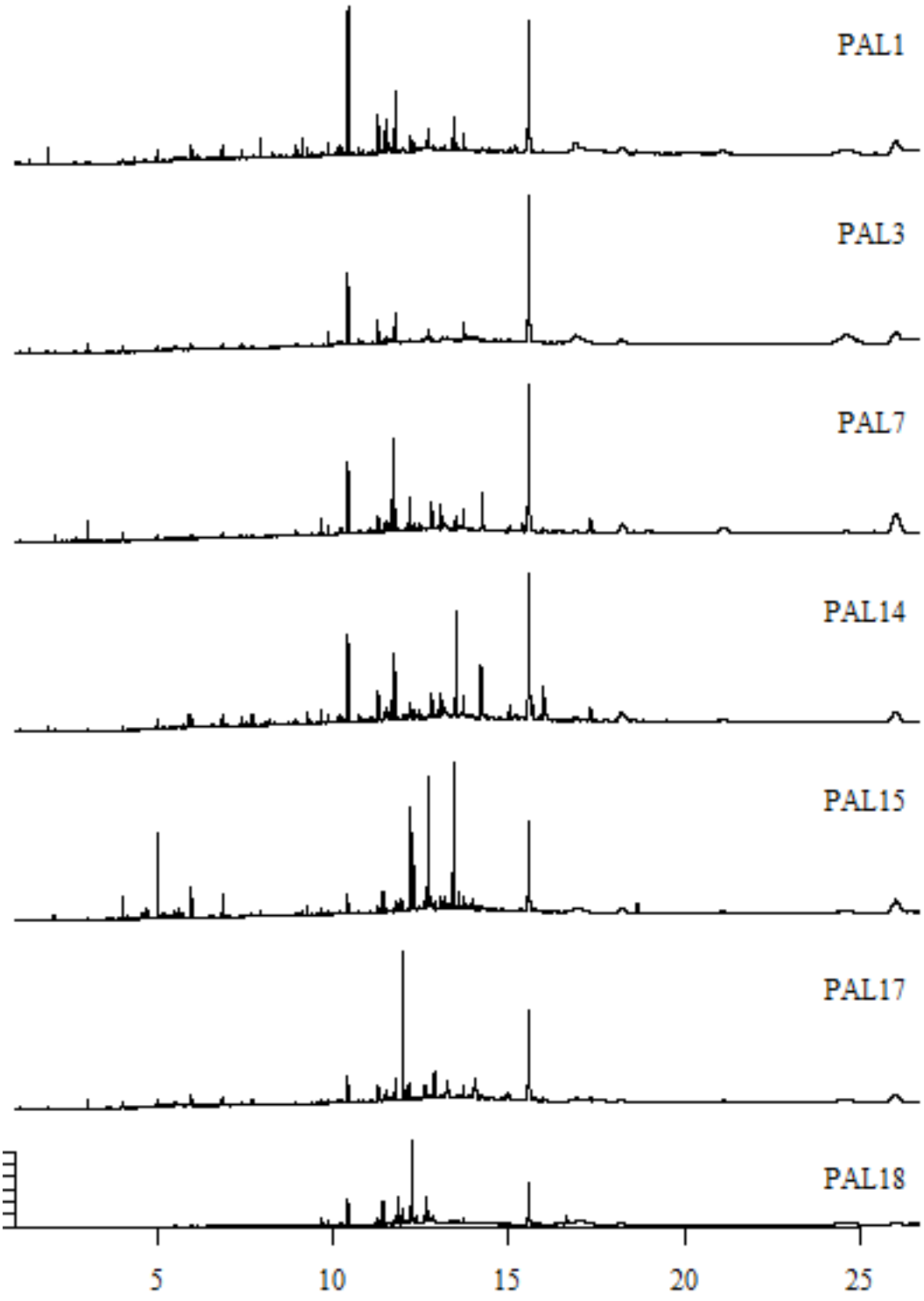
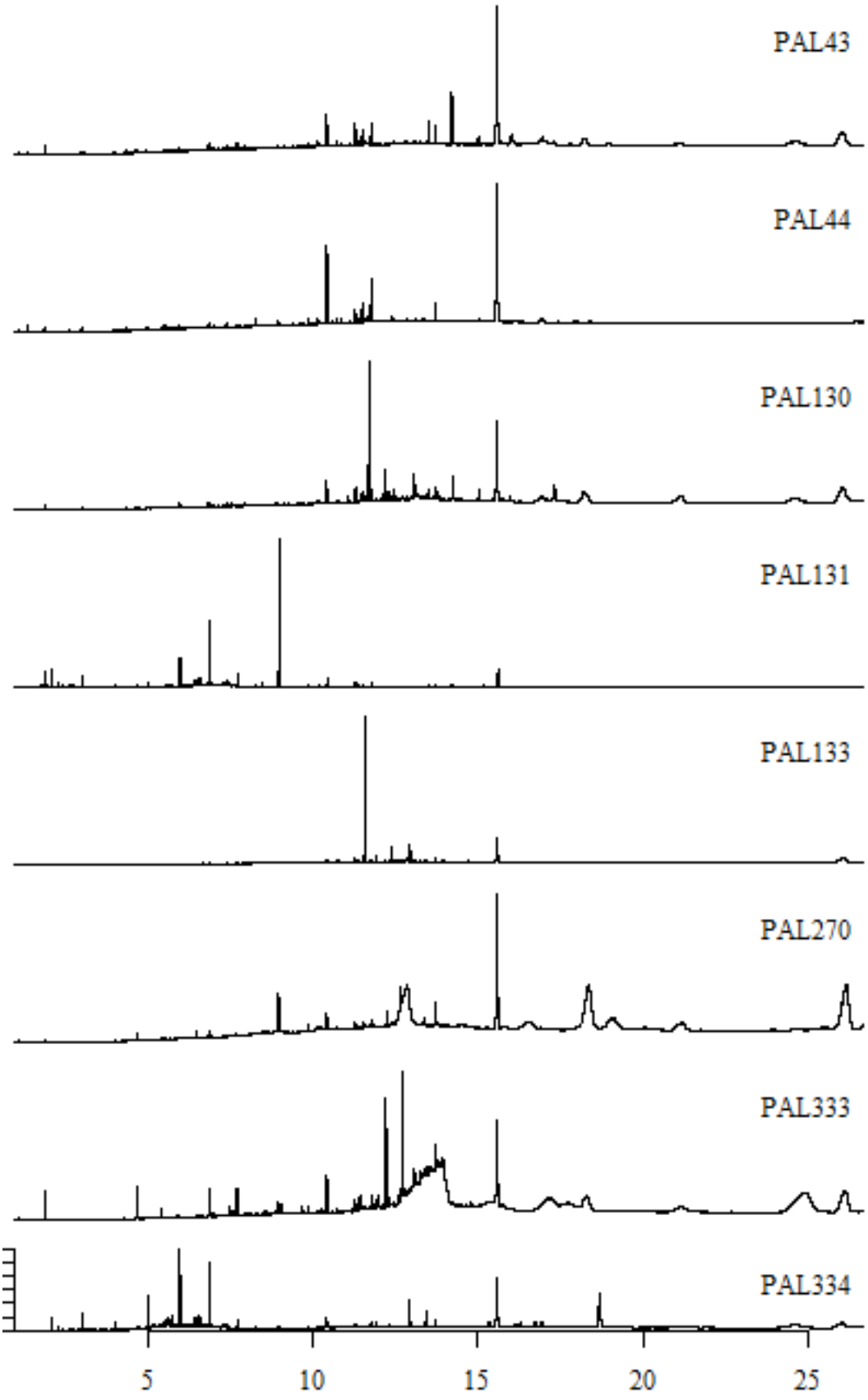
Gas Chromatograms - Clade F (HEX)

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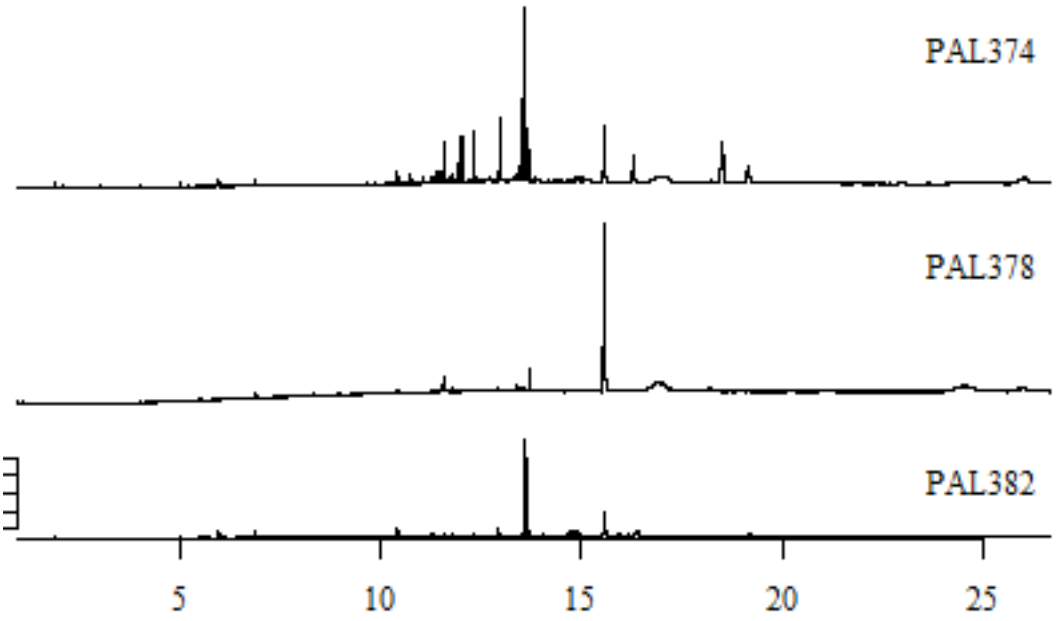
PAL374
PAL378
PAL382
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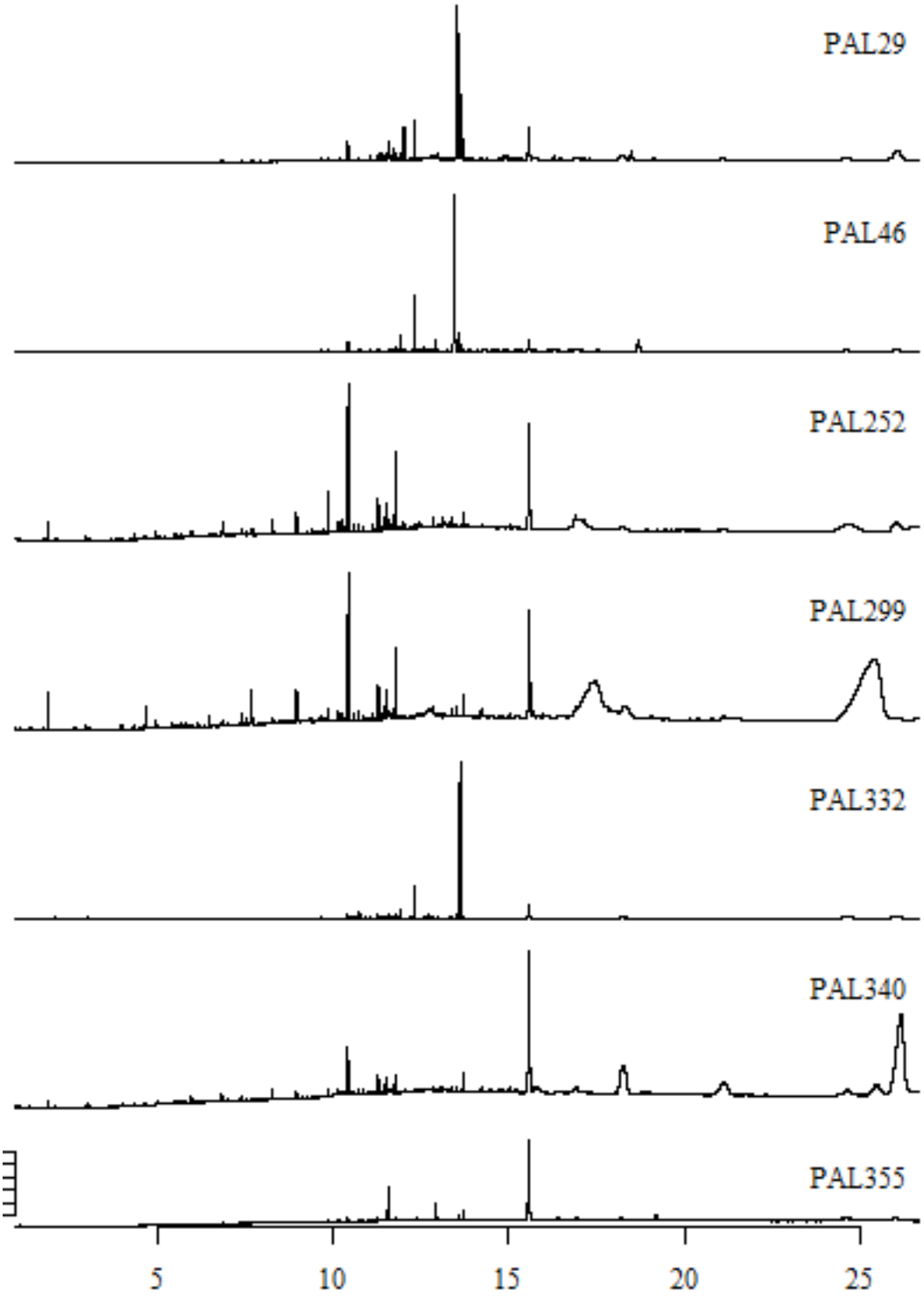
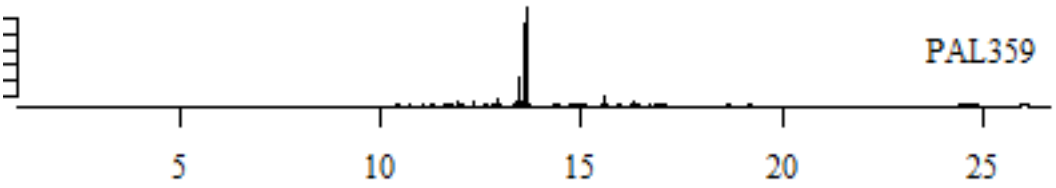
Gas Chromatograms - Clade D (HEX)




PAL359
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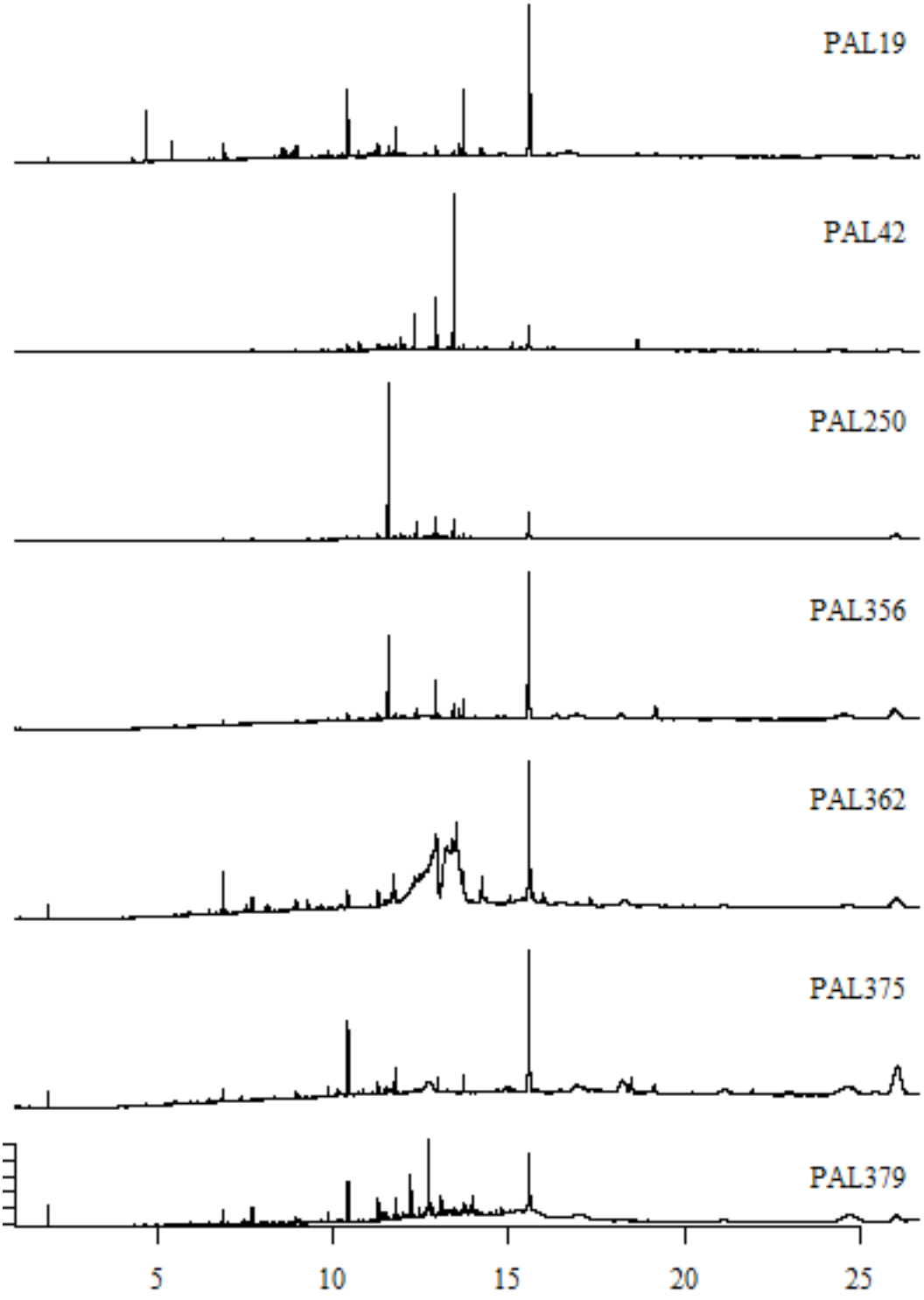
Gas Chromatograms - Trocheliophorum (HEX)



Truncated Normalized Trapezoidal Areas

Note:

- We omit the first 15000 time points (about 5 minutes) where there is little activity
- We omit the last 15002 time points (about 5 minutes) where there is little activity

```
#computes the trapezoidal area of each time interval in the given vector using the delta as the height
trap <- function(intens, delta) {
  intens1 <- rep(intens)
  intens1 = intens1[-1]
  intens = intens[1:length(intens)-1]
  trapArea = (intens + intens1)*delta/2
  return(c(as.numeric(trapArea), 0))
}

#normalizes the vector input so that the total area adds to 1
norm <- function(trapArea){
  tot = sum(trapArea)
  return(trapArea/tot)
}

#We omit the first and last 15000 time observations (5 minutes) because there is little observed activi
TRUNC_BEGIN = 15003
TRUNC_END = 62002

#Process as dataframe - CHANGE TO AS.NUMERIC
dfDCM_area = dfAllT_DCM[,TRUNC_BEGIN:TRUNC_END]
for(i in 1:nrow(dfDCM_area)){
  dfDCM_area[i, ] = norm(trap(as.numeric(dfDCM_area[i, ]), DELTA))
}
rownames(dfDCM_area) = dfAllT_DCM[,1]

dfHEX_area = dfAllT_HEX[,TRUNC_BEGIN:TRUNC_END]
for(i in 1:nrow(dfHEX_area)){
  dfHEX_area[i, ] = norm(trap(as.numeric(dfHEX_area[i, ]), DELTA))
}
rownames(dfHEX_area) = dfAllT_HEX[,1]
```

Binned Areas

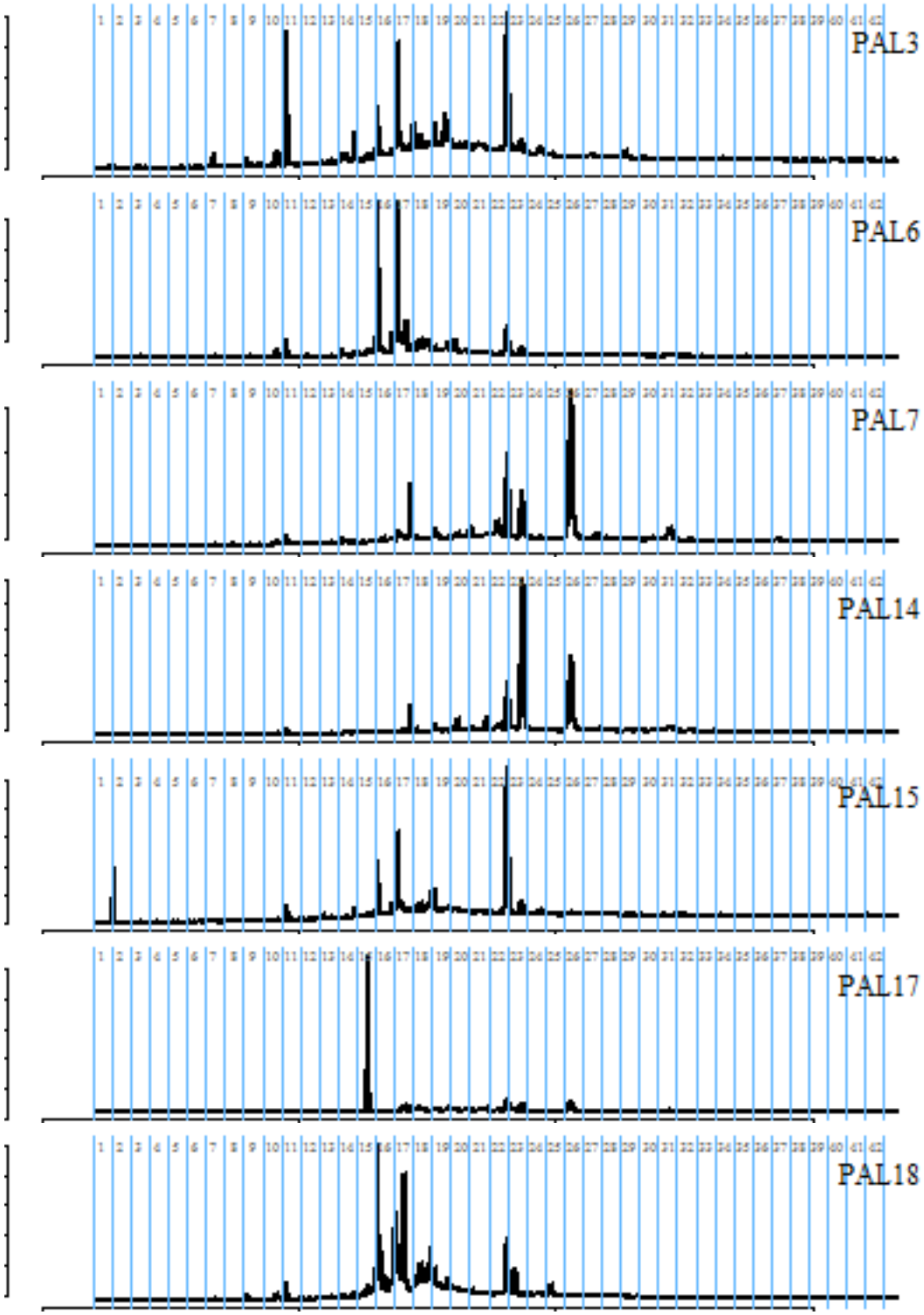
```
#returns a binned matrix whose bins are the sums of the areas df for each bin interval
#each bin has as many time intervals as specified by binWidth
#any remainder from ncol(df)/binwidth will not be included in the binned matrix
binner <- function(df, binWidth, delta) {
  rowz = c()
  for(j in 1:nrow(df)) {
    col = c()
    for(i in seq(1, ncol(df)-binWidth, binWidth)){
      end = min(ncol(df),i + binWidth - 1)
      col = c(col, sum(as.numeric(df[j,i:end])))
    }
    rowz = rbind(rowz, col)
  }
```

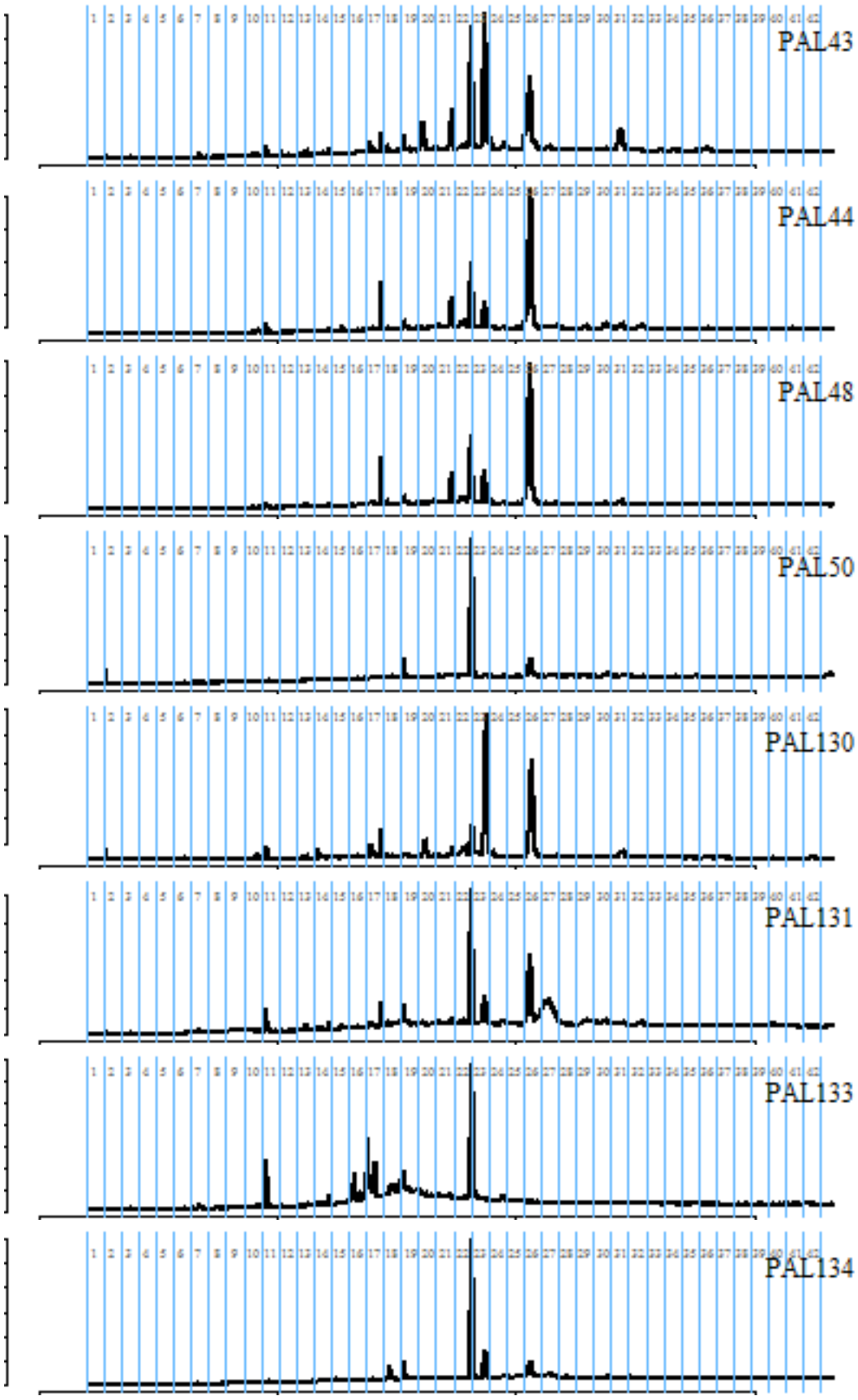
```
  return(rowz)
}

binned_matrix_DCM = binner(dfDCM_area, 1100, DELTA)
bins = 1:ncol(binned_matrix_DCM)
for (i in 1:ncol(binned_matrix_DCM)){
  bins[i] = paste("Bin", as.character(i))
}
dimnames(binned_matrix_DCM) = list(dfAllT_DCM[,1], bins)

binned_matrix_HEX = binner(dfHEX_area, 1100, DELTA)
dimnames(binned_matrix_HEX) = list(dfAllT_HEX[,1], bins)
```

	Bin 1	Bin 2	Bin 3	Bin 4	Bin 5
3	0.0196983	0.0197666	0.0200023	0.0198696	0.0201119
6	0.0178640	0.0179466	0.0188559	0.0181555	0.0182886
7	0.0194413	0.0195503	0.0196366	0.0196392	0.0197458
14	0.0190575	0.0191769	0.0192920	0.0192632	0.0194491
15	0.0204703	0.0218515	0.0206287	0.0207084	0.0209207

	Bin 1	Bin 2	Bin 3	Bin 4	Bin 5
1	0.0204115	0.0207935	0.0211689	0.0210199	0.0209994
3	0.0204800	0.0207290	0.0209415	0.0209799	0.0211188
7	0.0202705	0.0204378	0.0207545	0.0207985	0.0209747
14	0.0199825	0.0202935	0.0207790	0.0206028	0.0209530
15	0.0196398	0.0197934	0.0205295	0.0200836	0.0204511

Gas Chromatograms (Truncated and Showing Bins) - Clade F (DCM)

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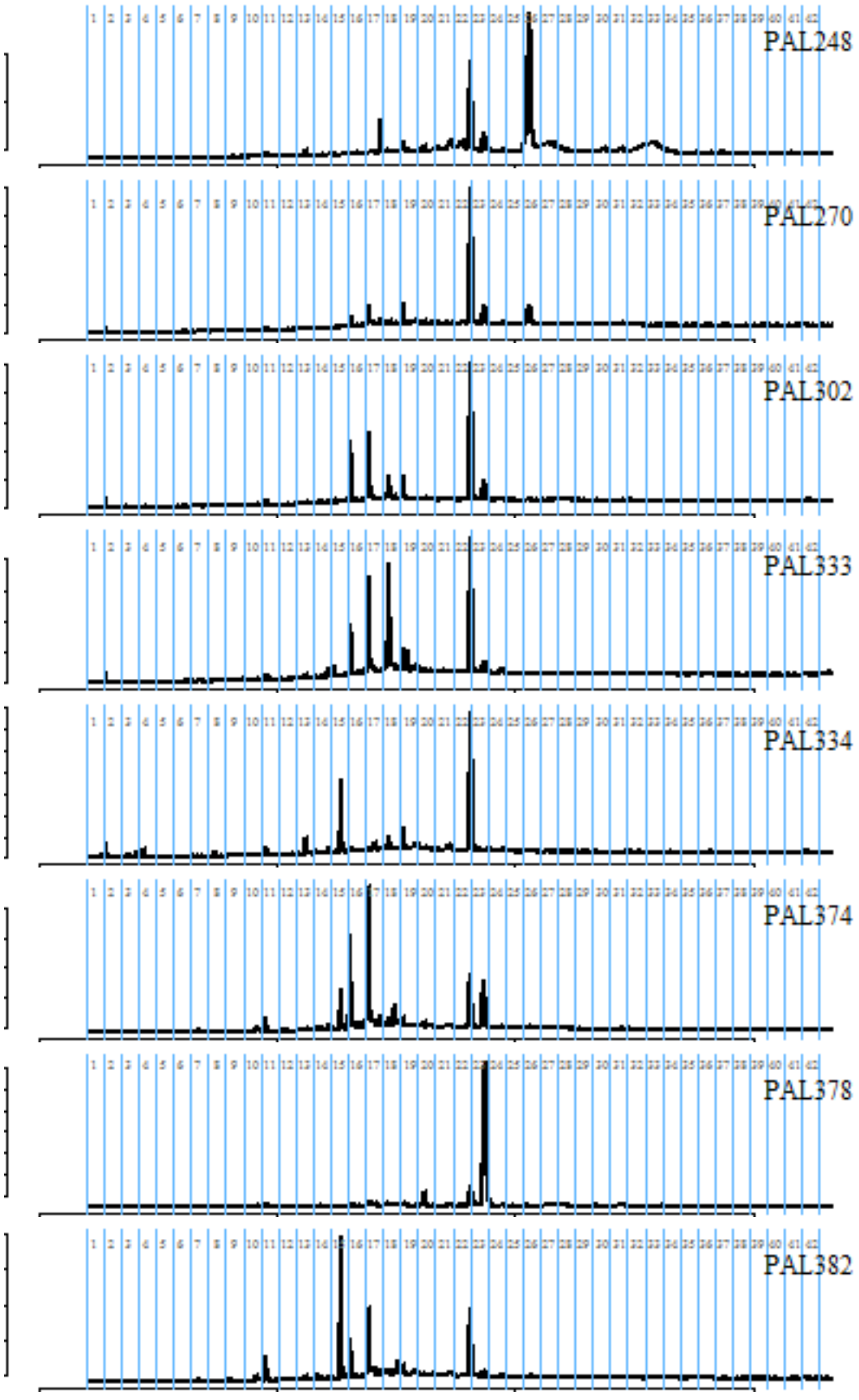
PAL131
PAL133
PAL134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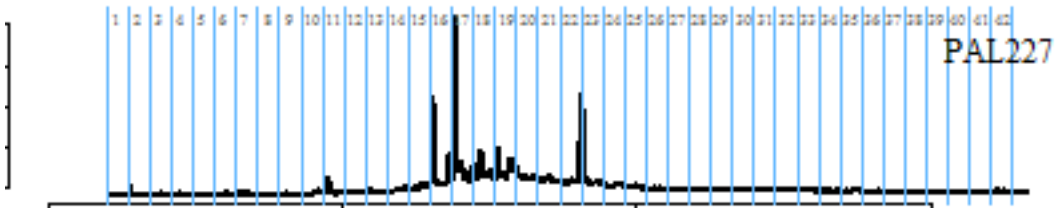
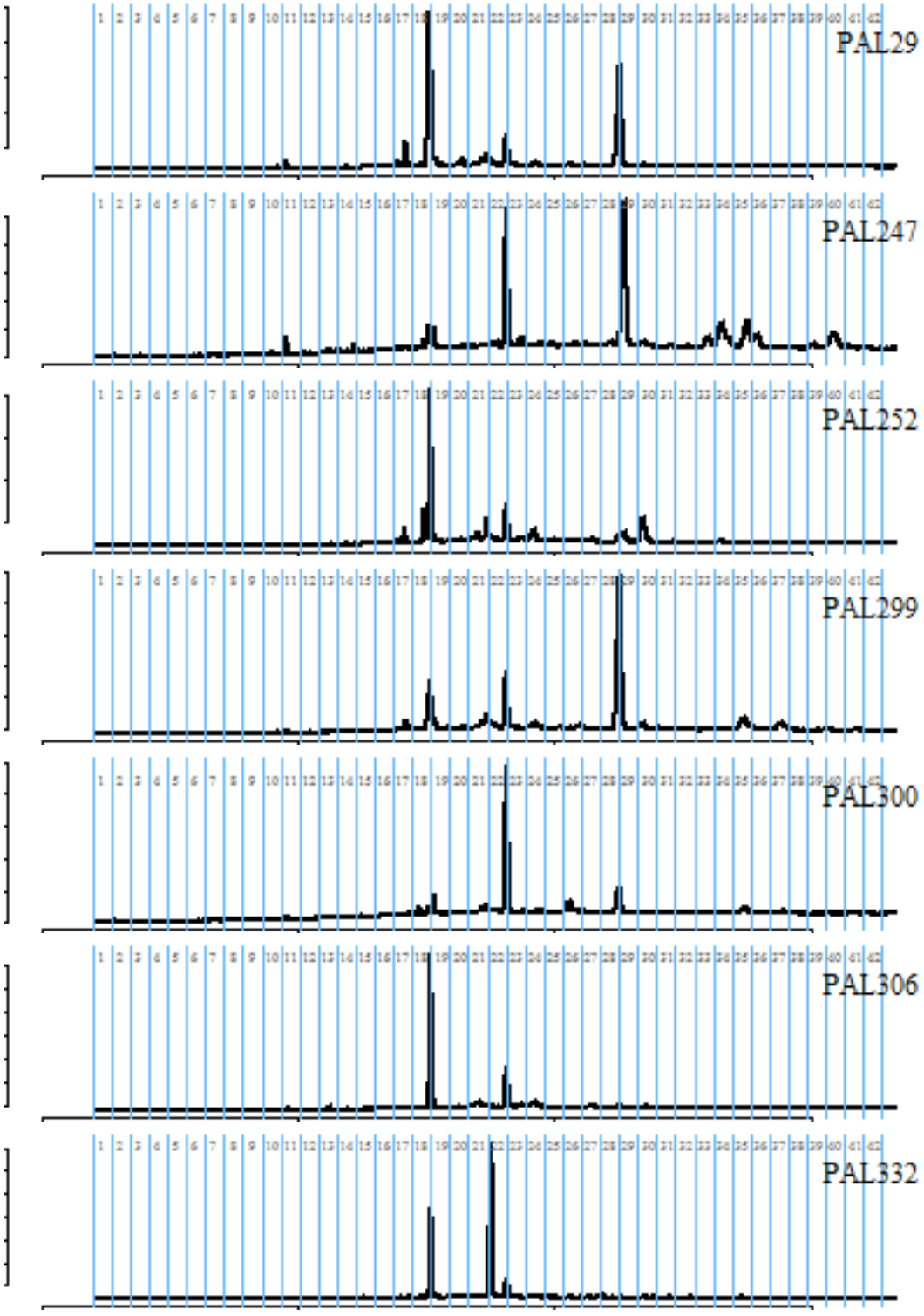
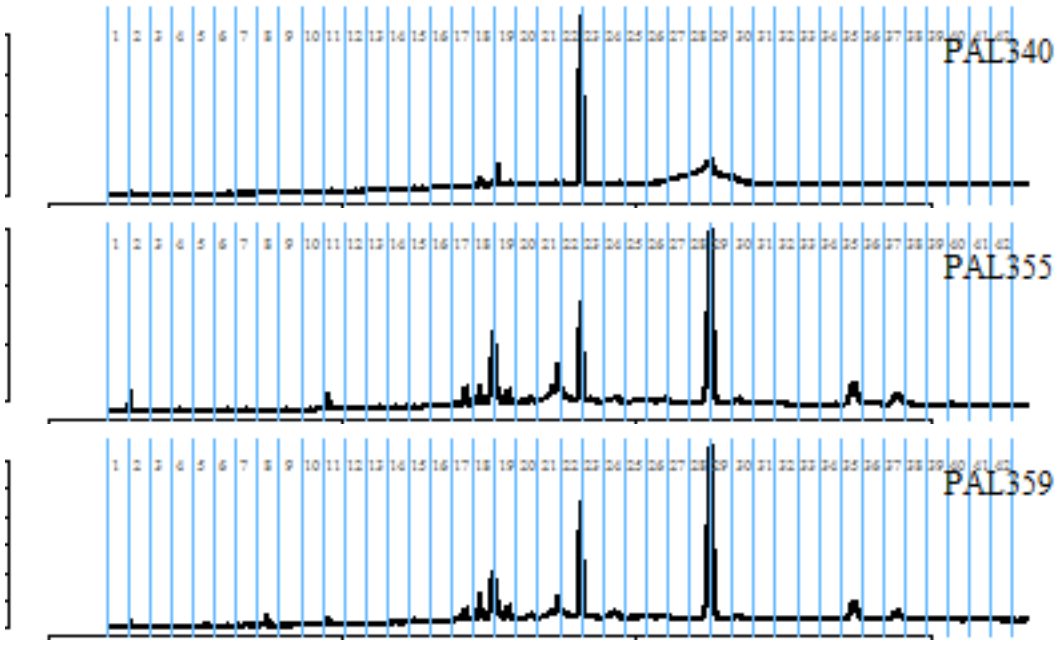
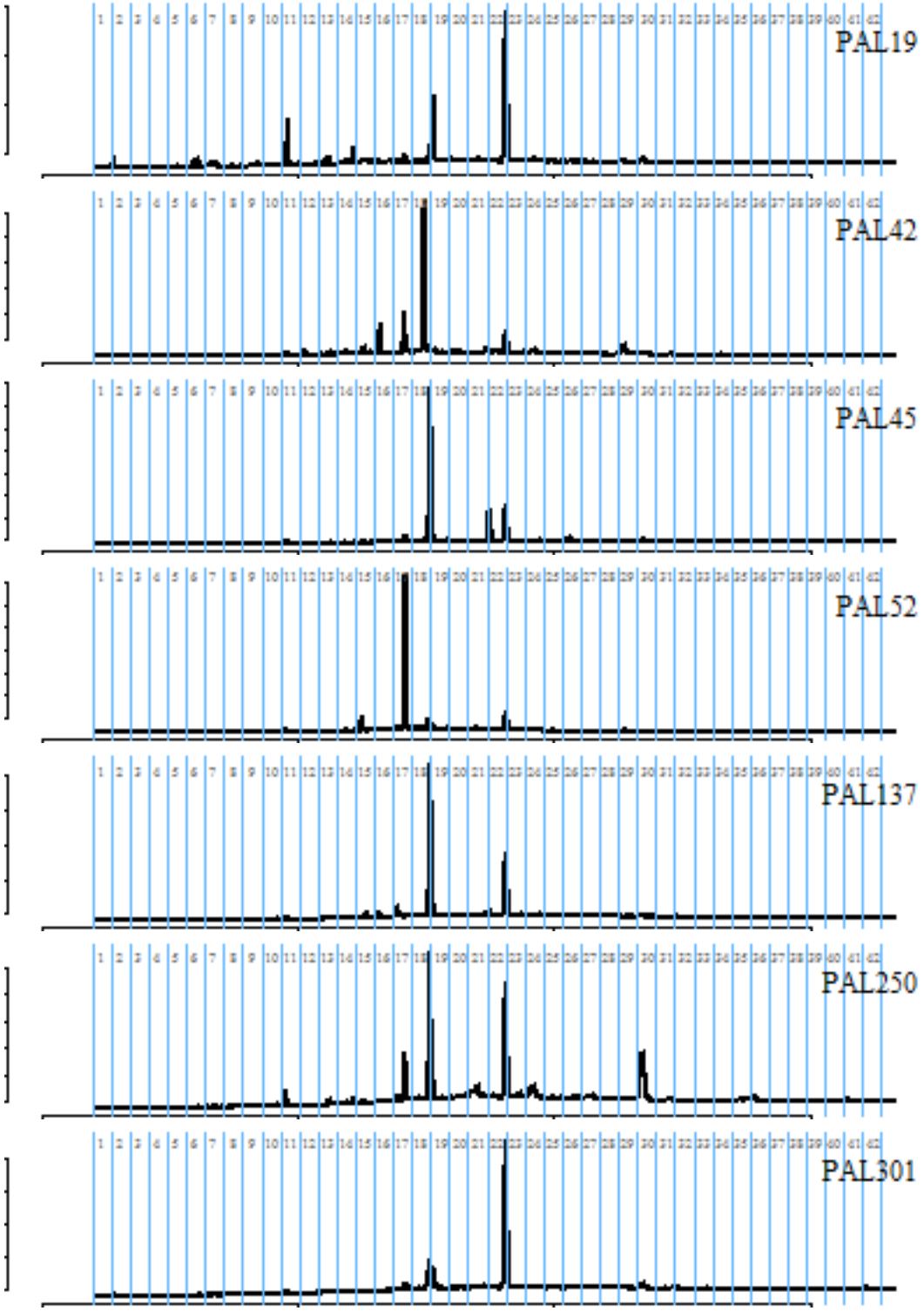

PAL227

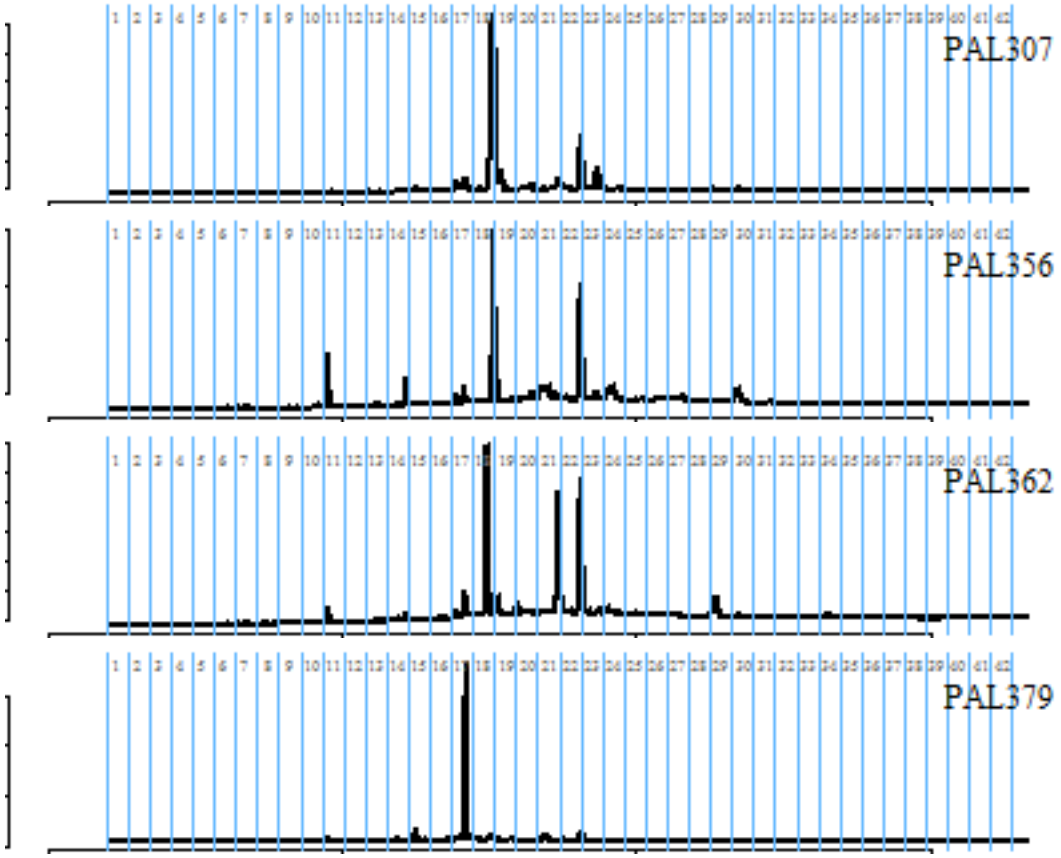
Gas Chromatograms (Truncated and Showing Bins) - Clade D (DCM)



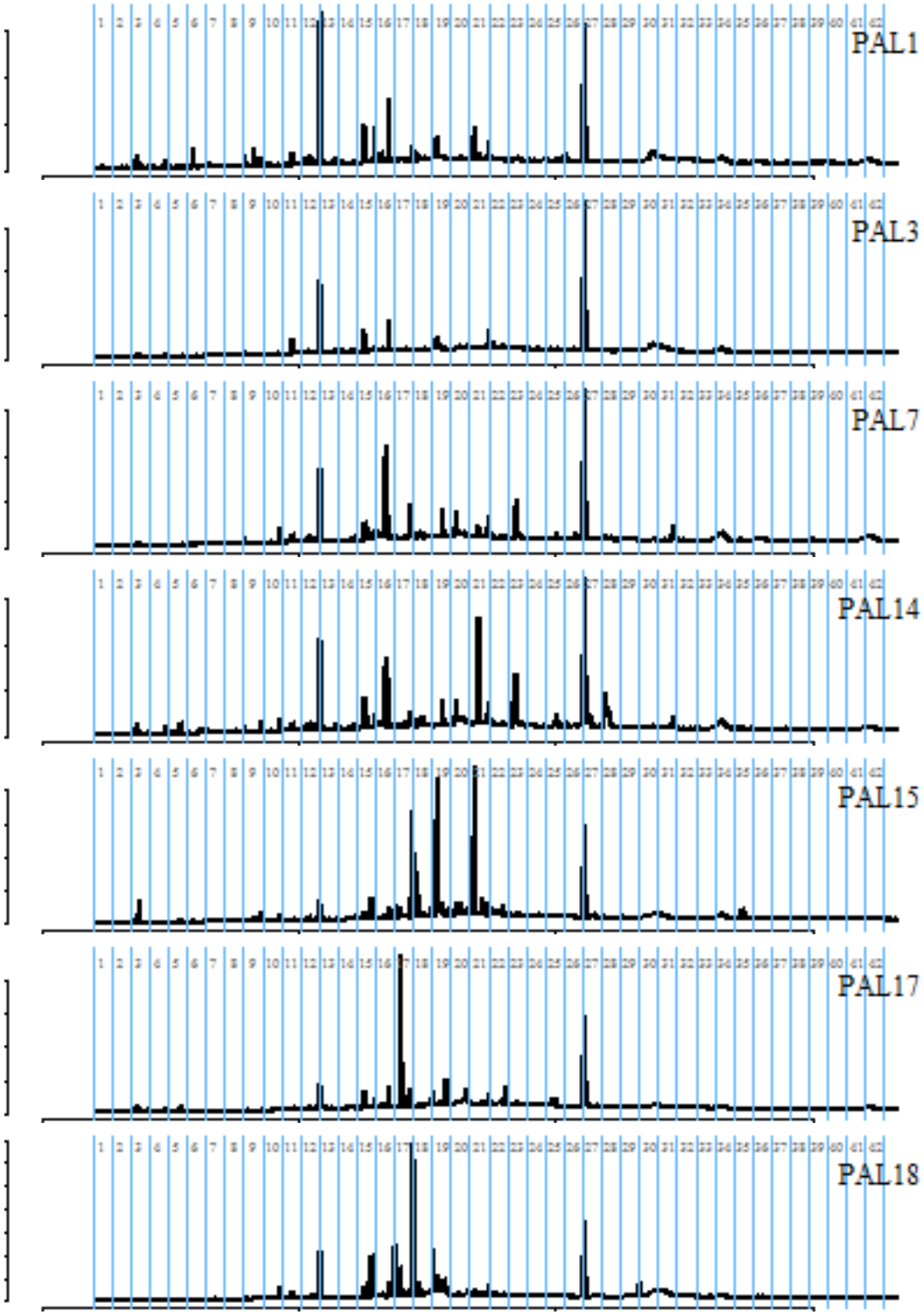

PAL340
PAL355
PAL359

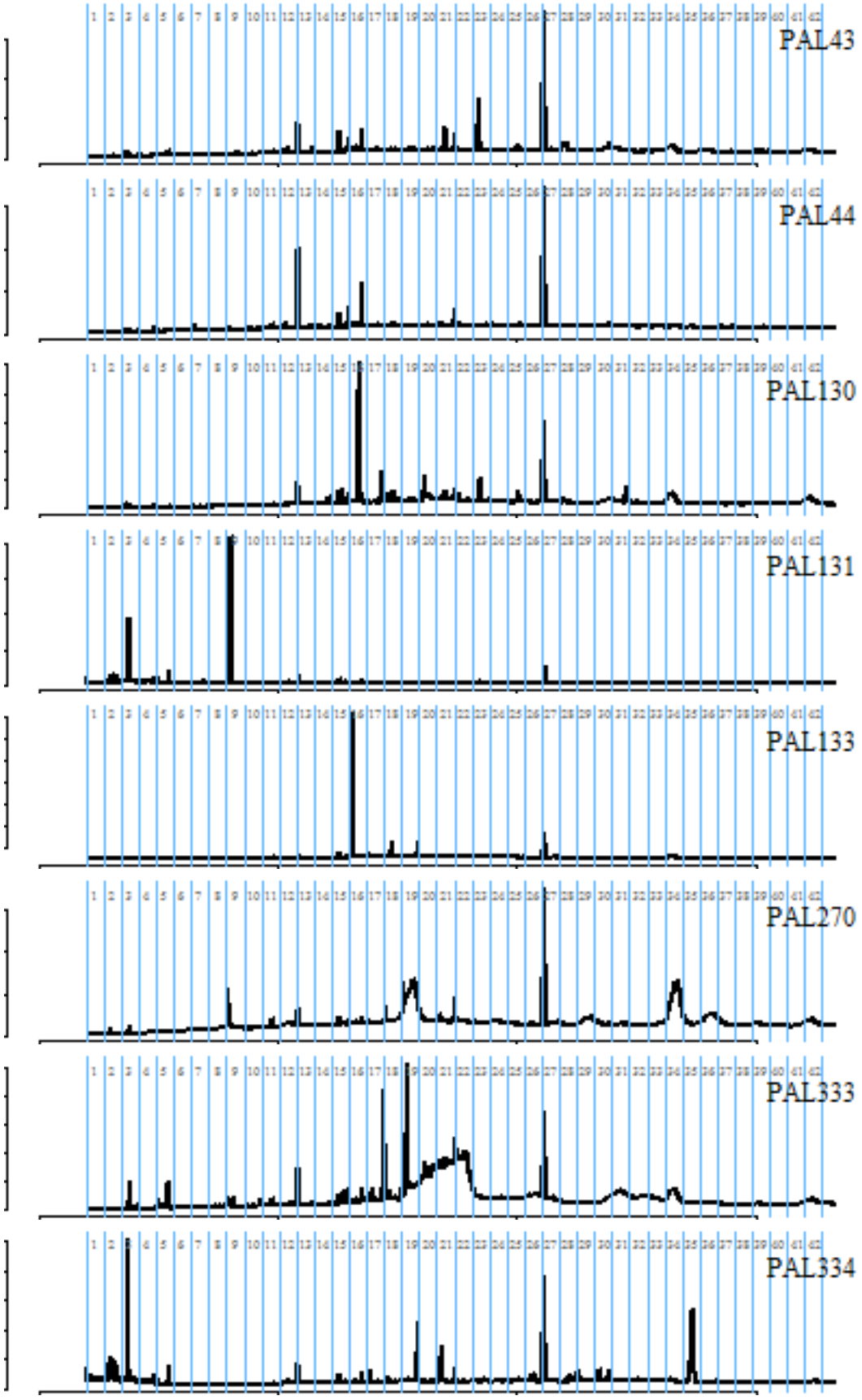
Gas Chromatograms (Truncated and Showing Bins) - Trocheliophorum (DCM)




PAL307
PAL356
PAL362
PAL379

Gas Chromatograms (Truncated and Showing Bins) - Clade F (HEX)




PAL43
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PAL131
PAL133
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PAL333
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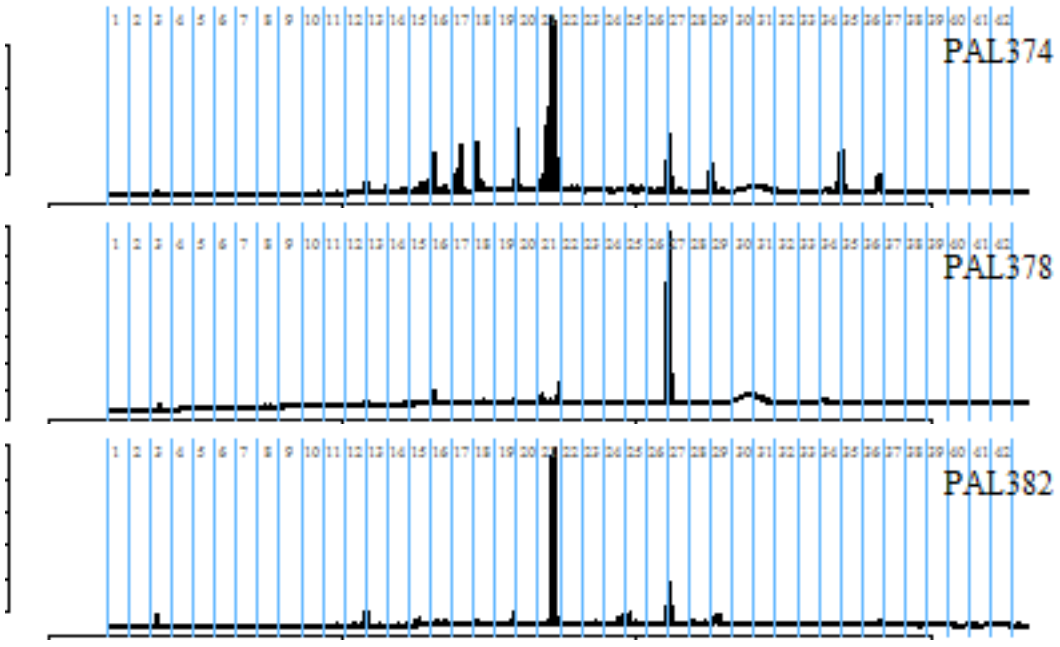
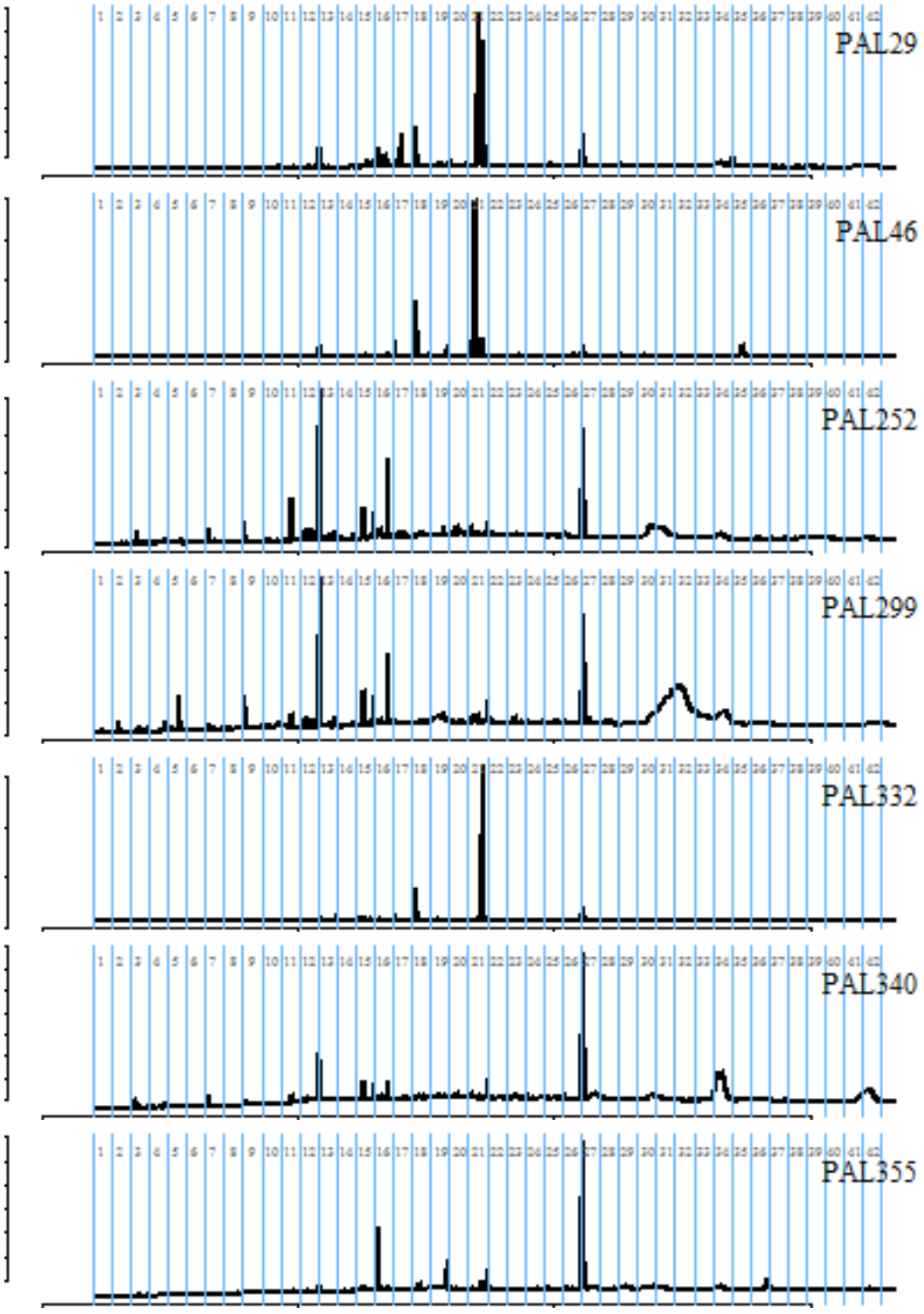
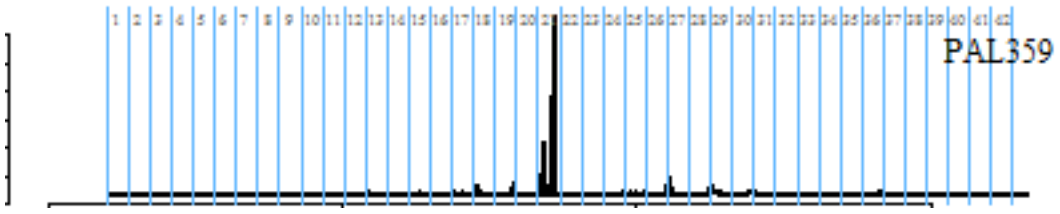
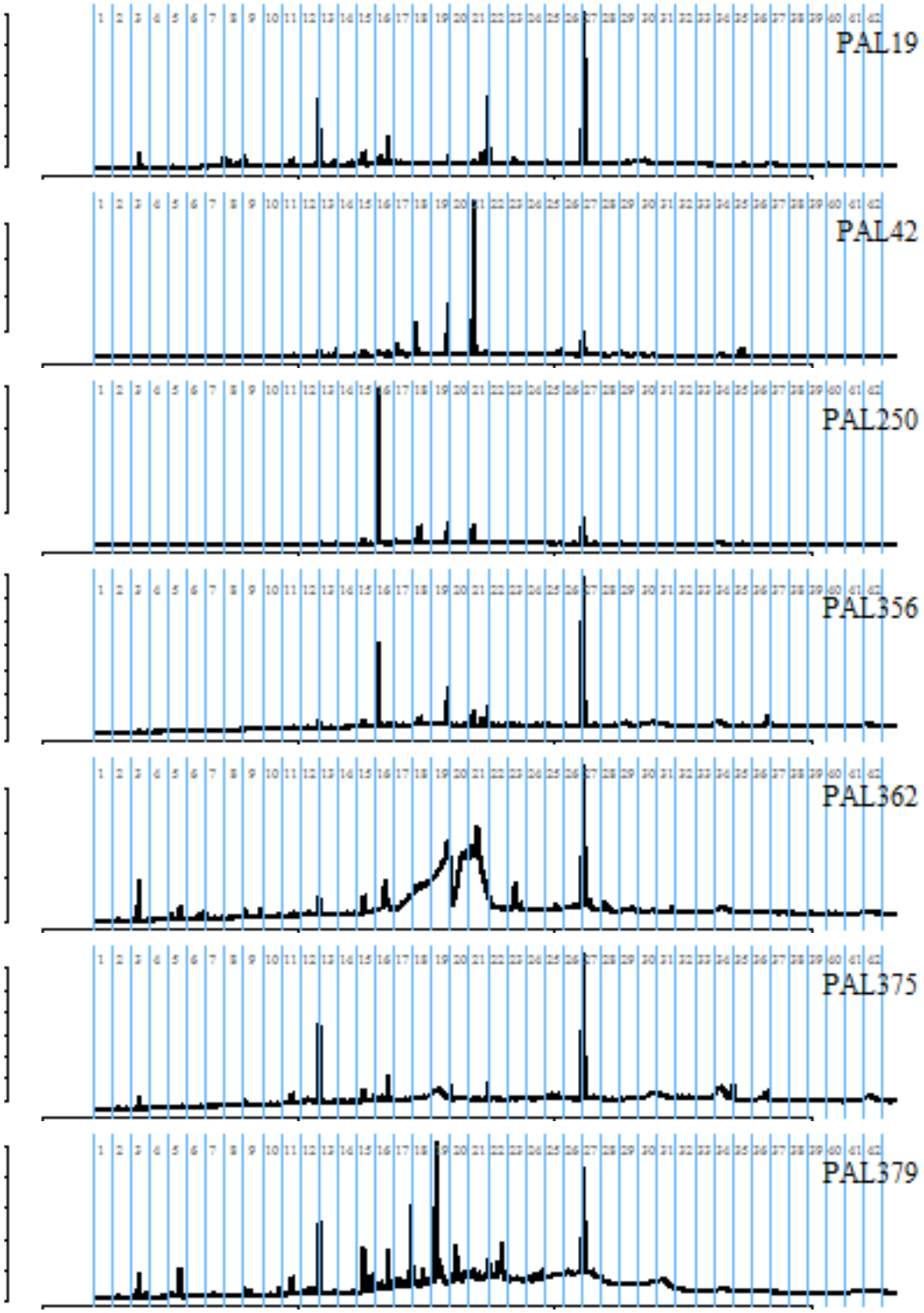

PAL374
PAL378
PAL382

Gas Chromatograms (Truncated and Showing Bins) - Clade D (HEX)

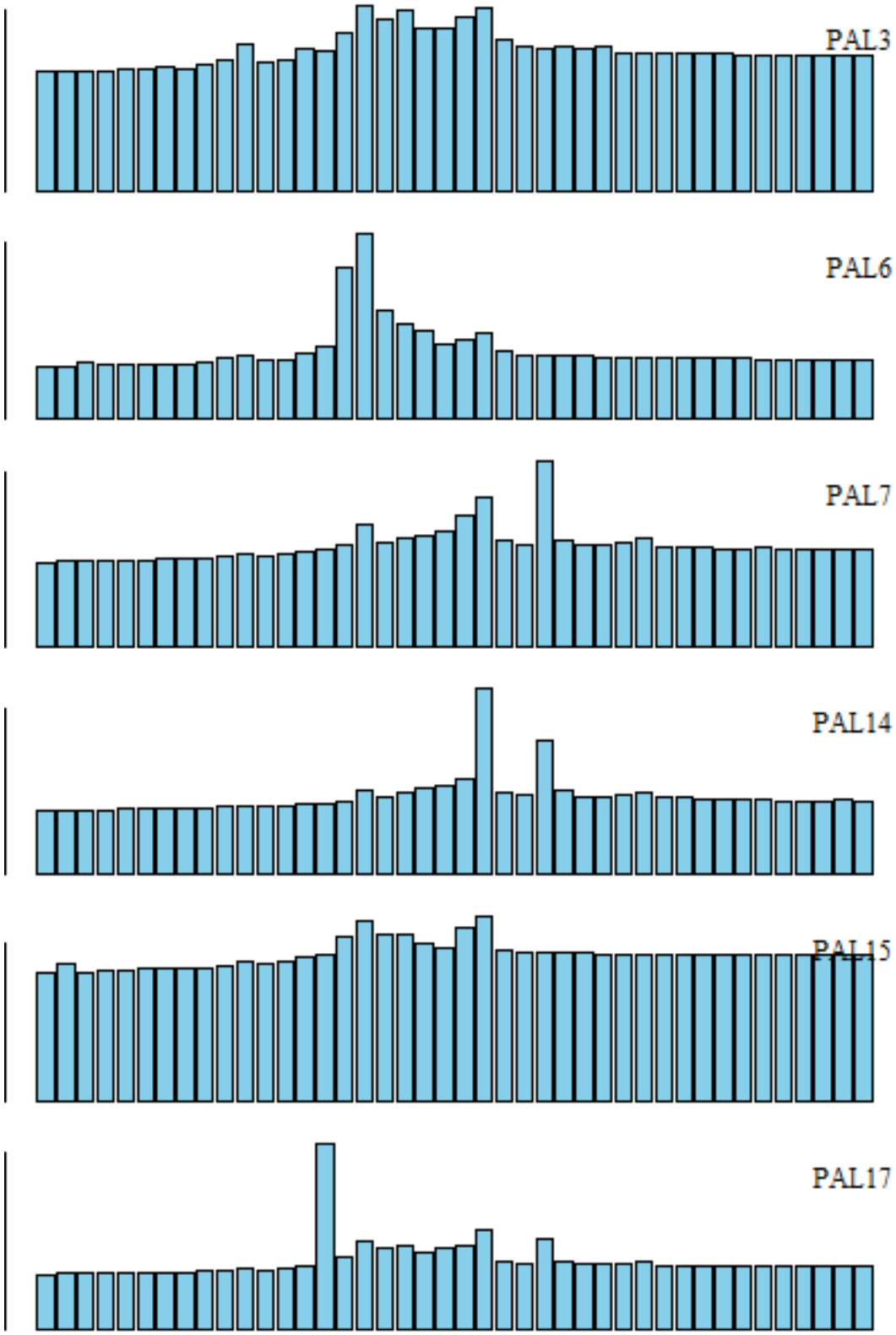


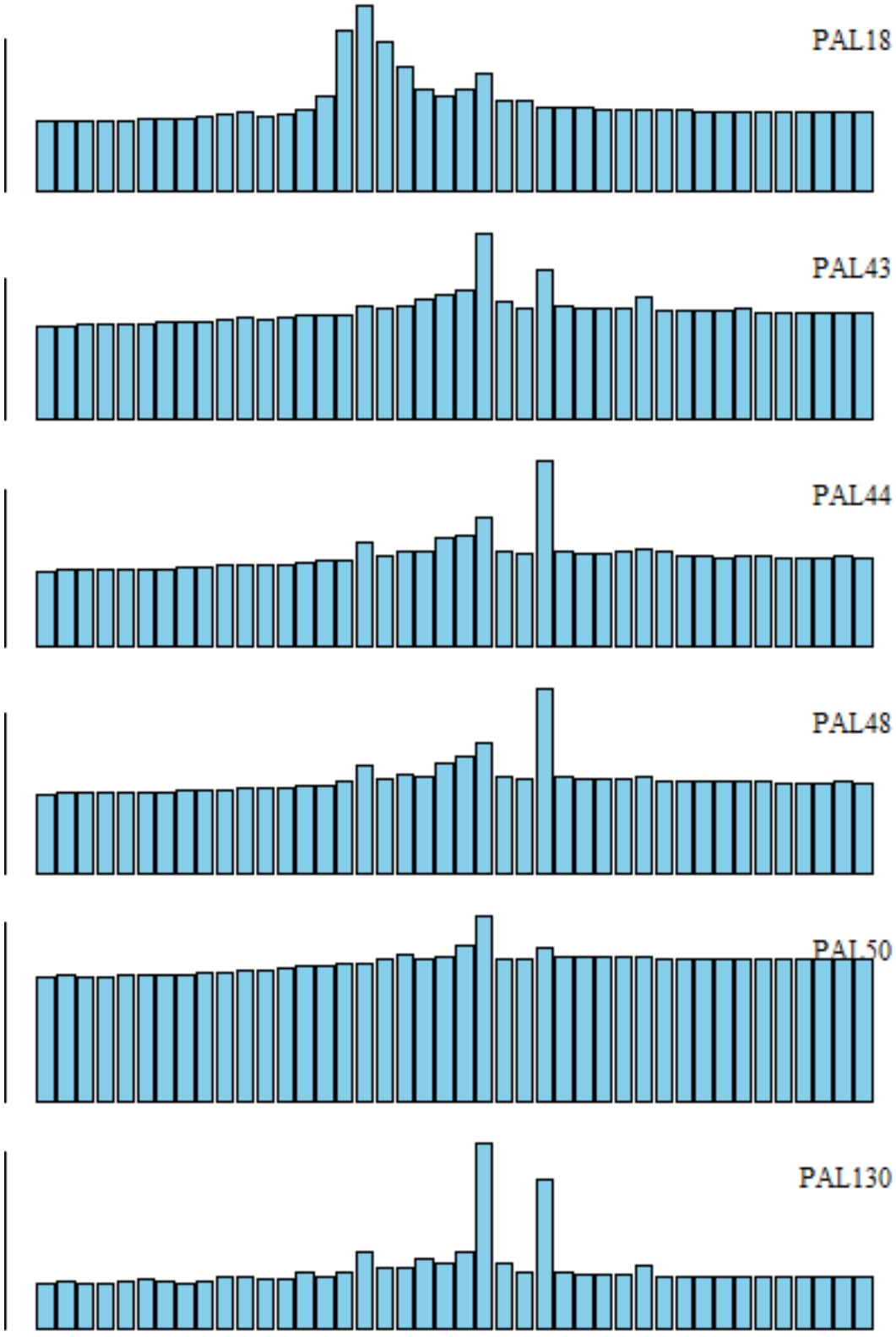

PAL359

Gas Chromatograms (Truncated and Showing Bins) - Trocheliophorum (HEX)



Gas Binned Barplots - Clade F (DCM)




PAL18
PAL43
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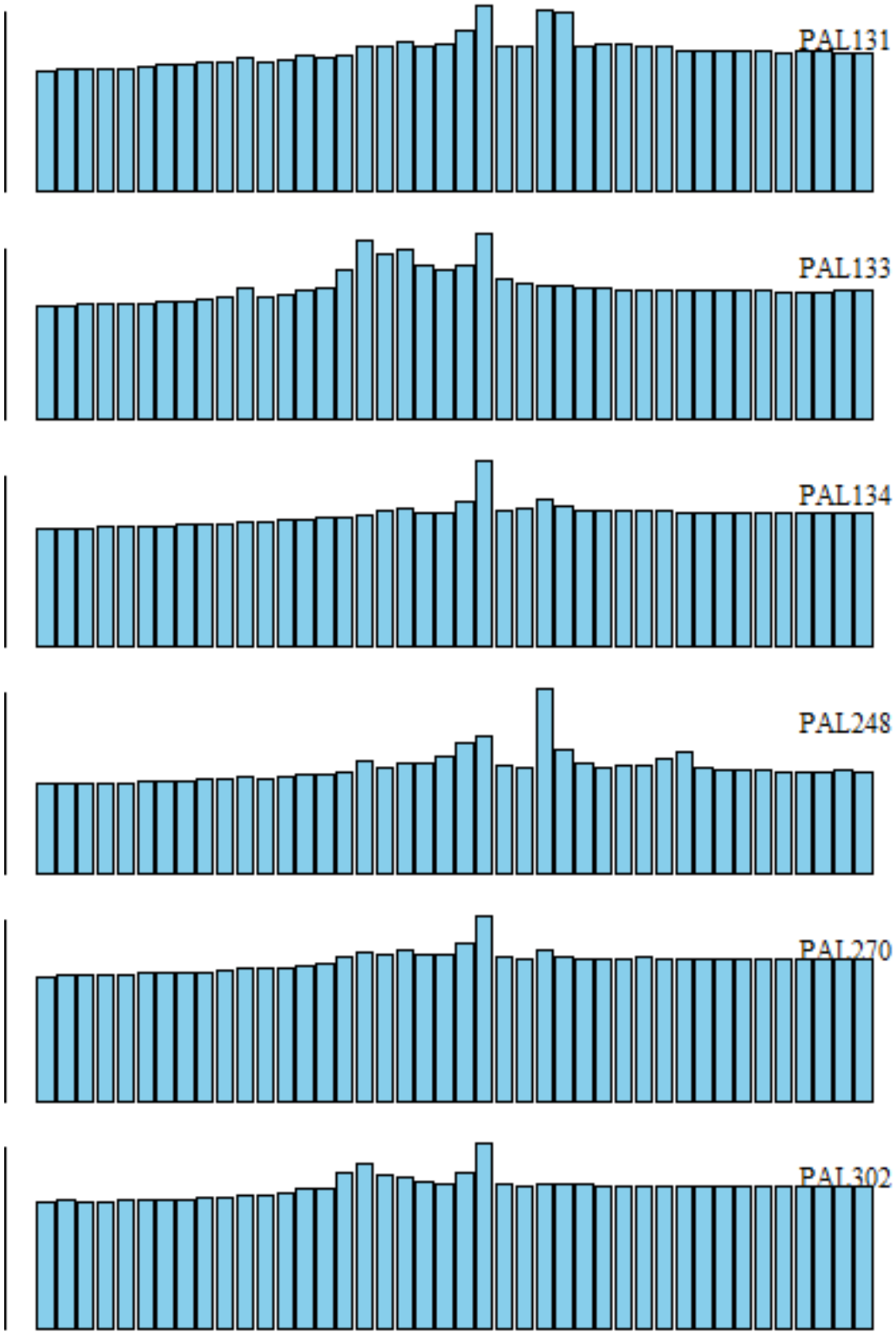
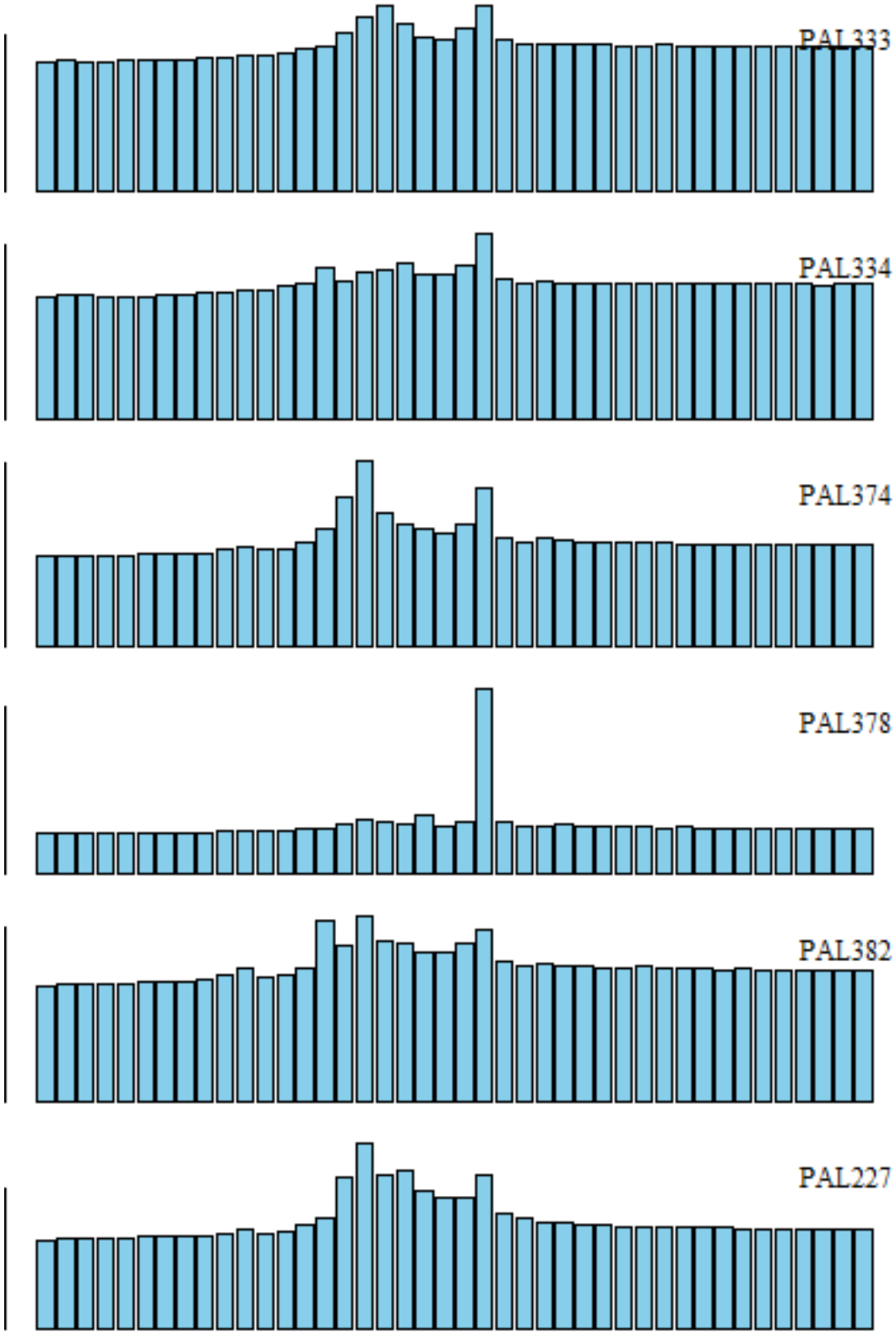
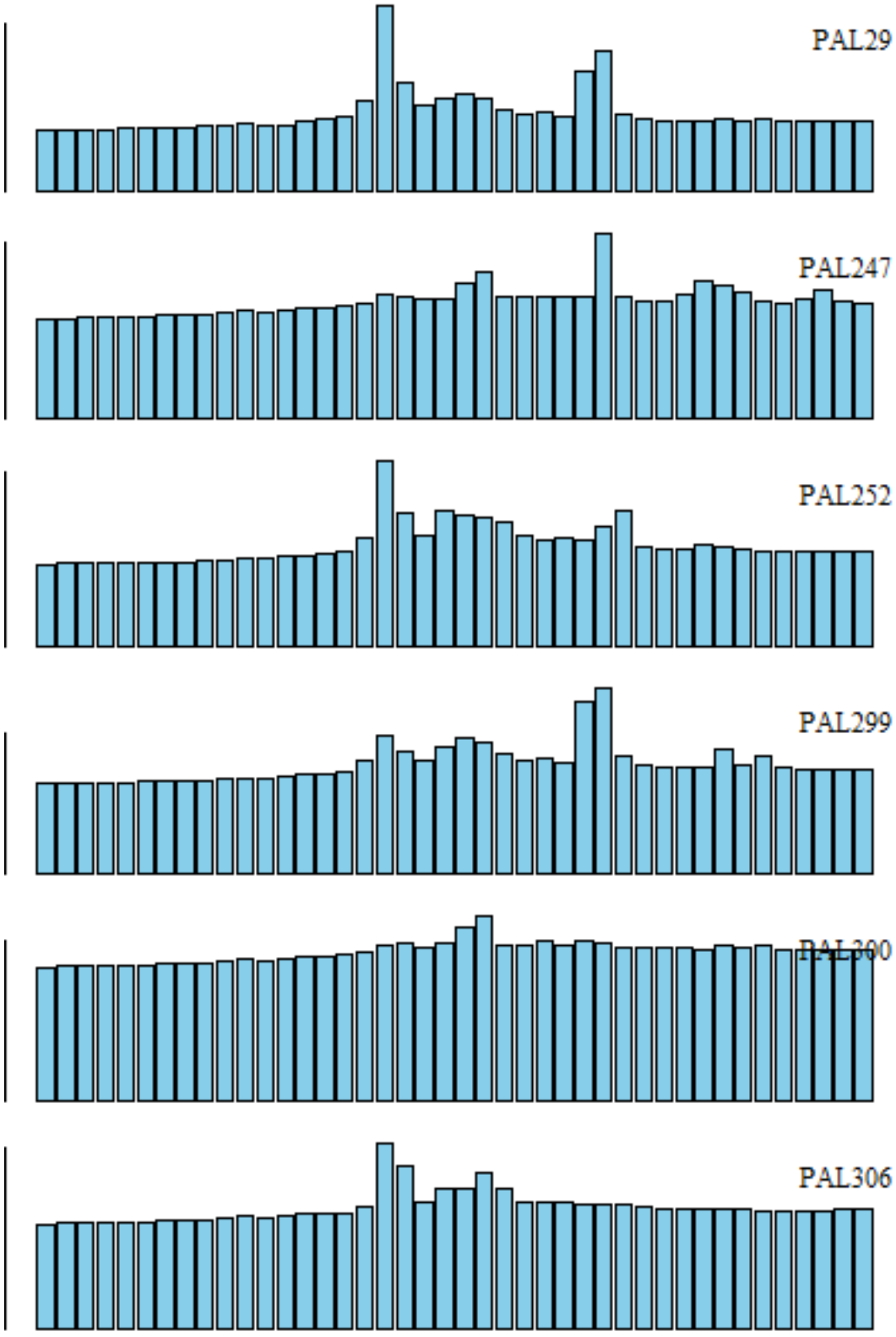
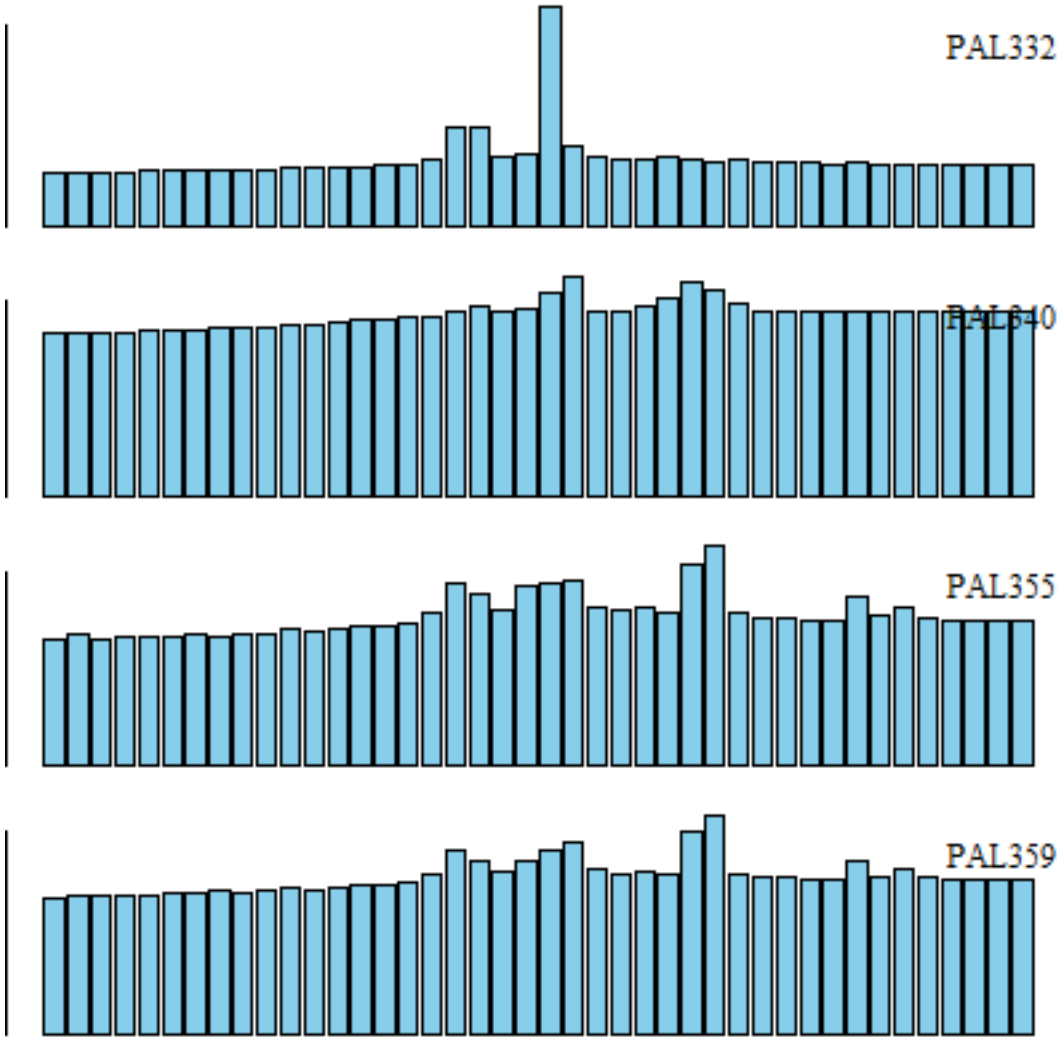
PAL50
PAL130


PAL131
PAL133
PAL134
PAL248
PAL270
PAL302

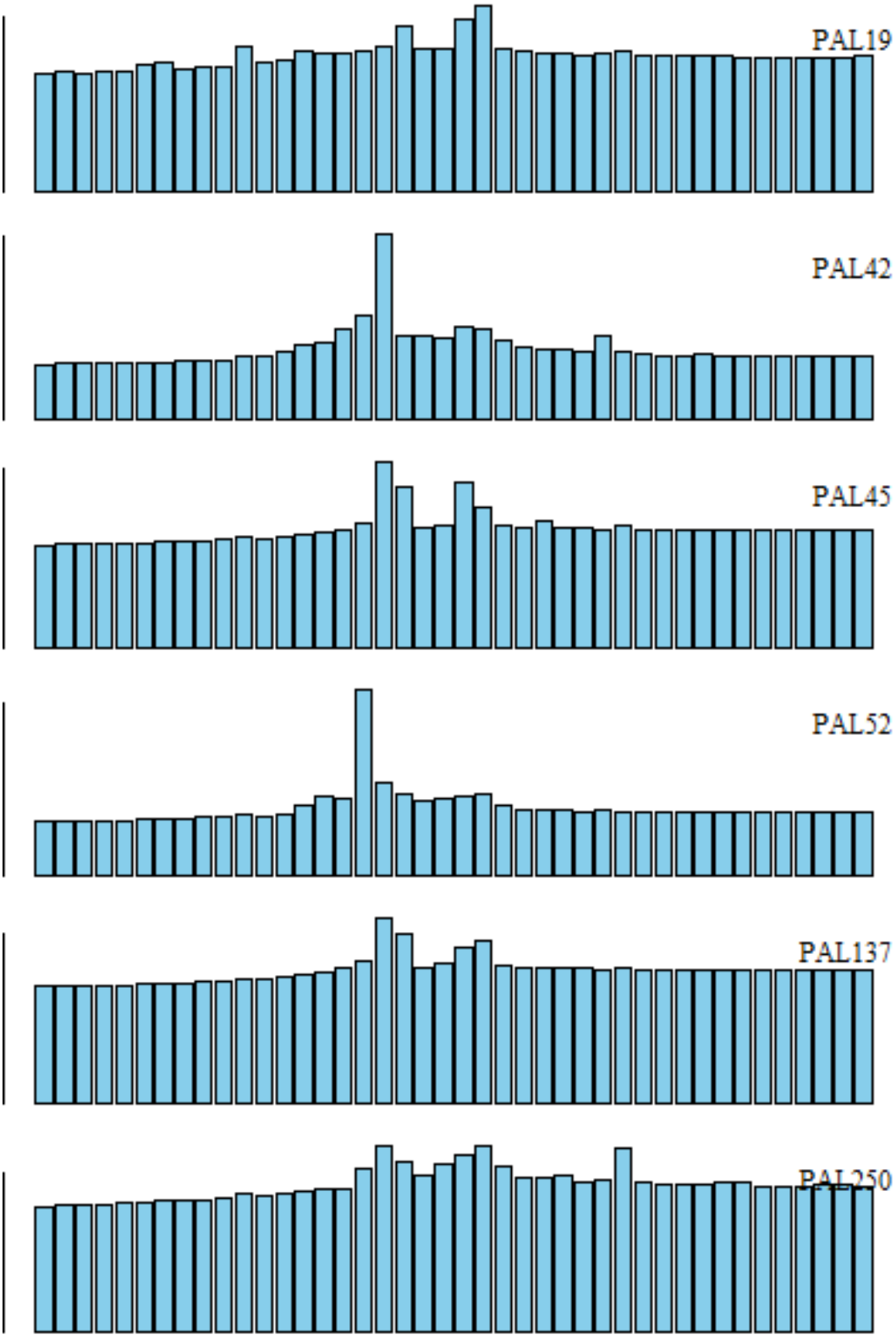

PAL333
PAL334
PAL374
PAL378
PAL382
PAL227

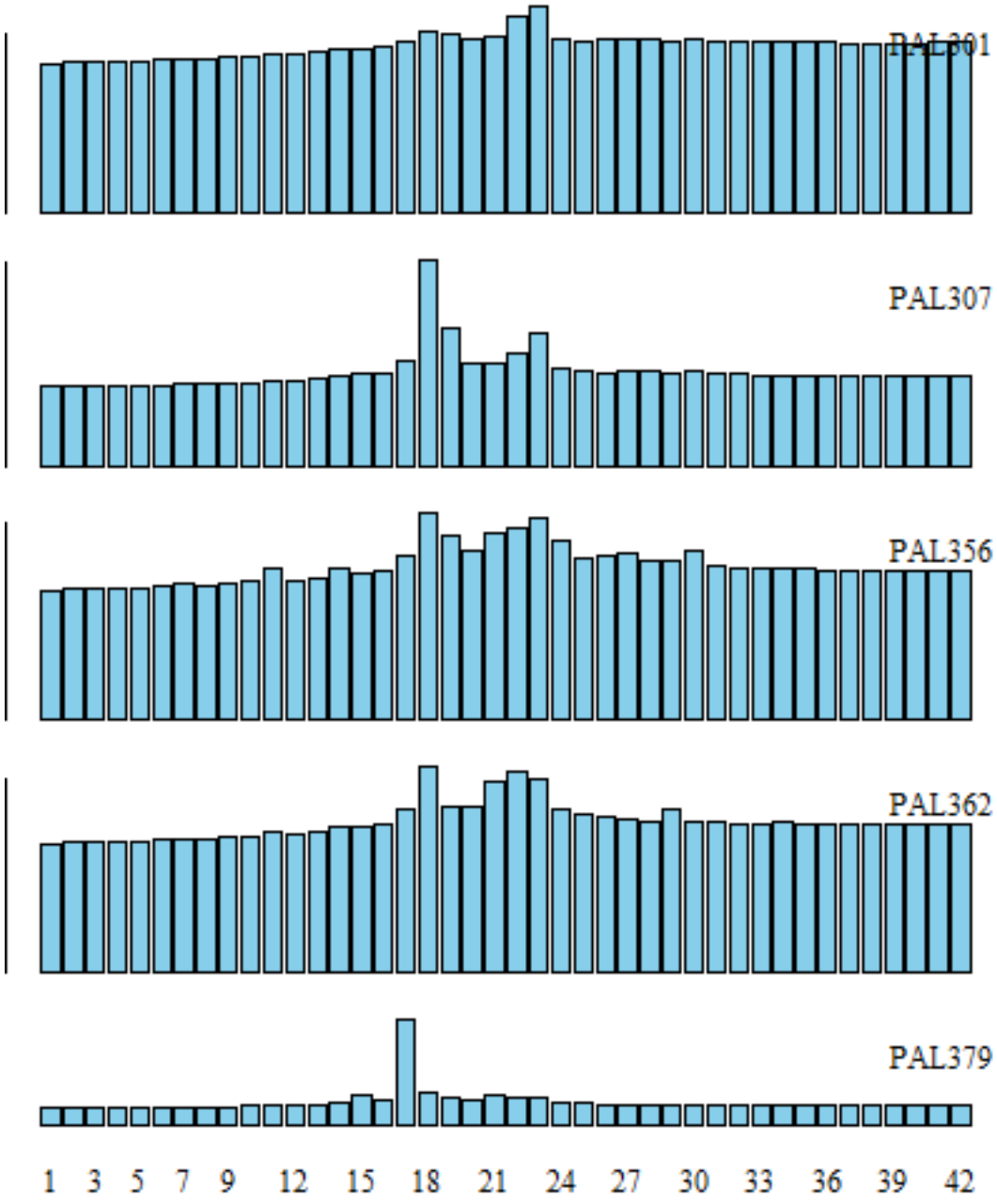
Gas Binned Barplots - Clade D (DCM)



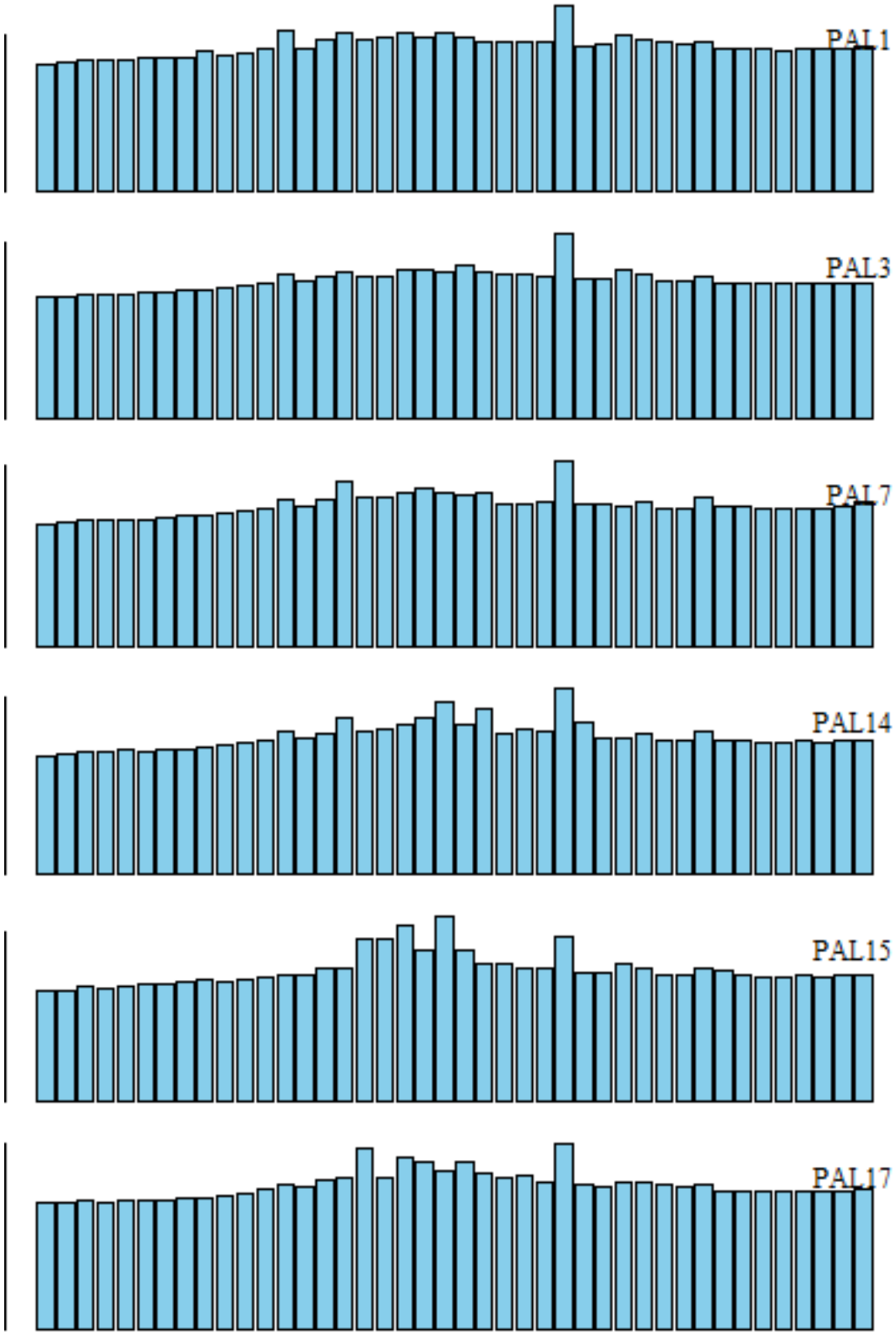

PAL332
PAL340
PAL355
PAL359

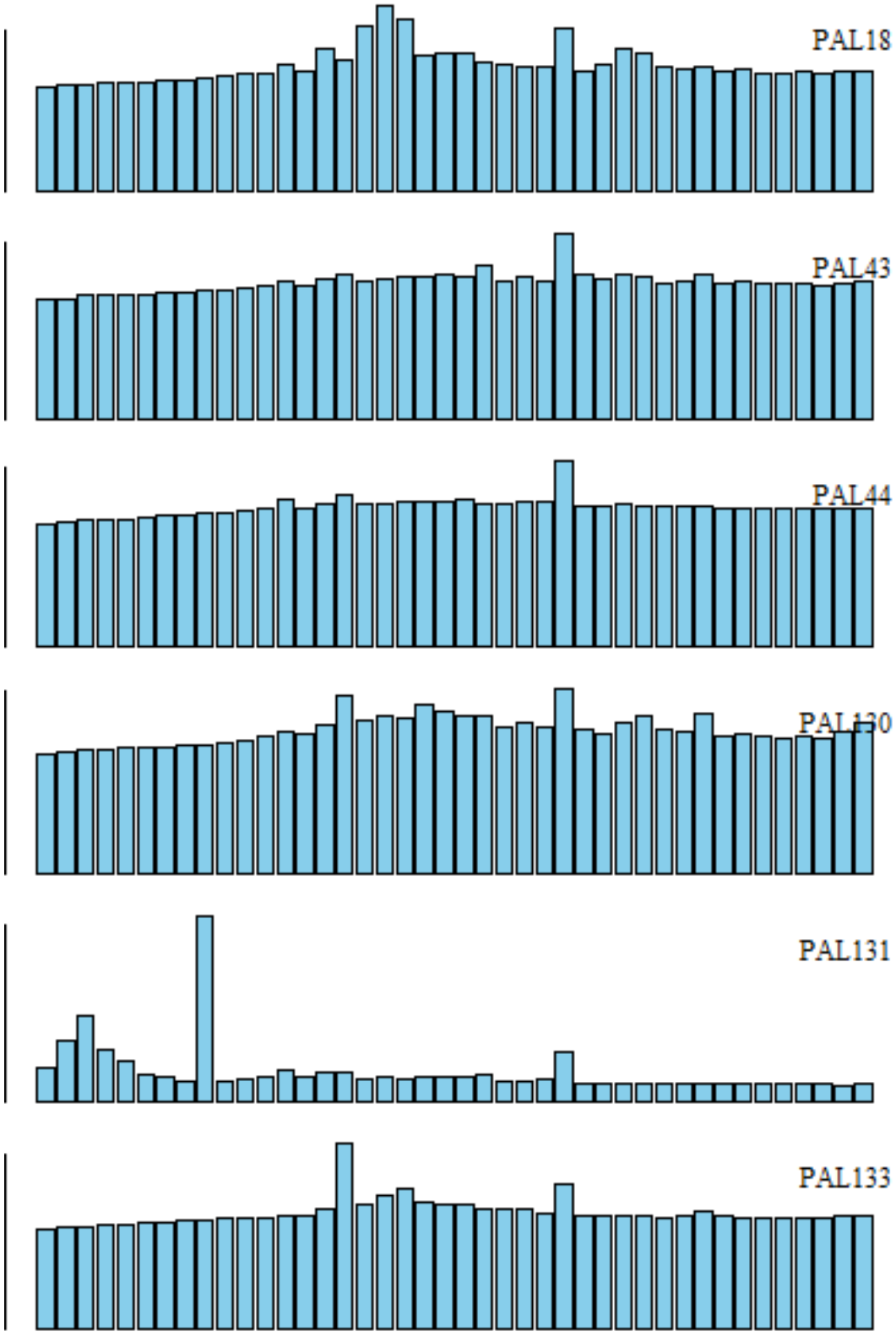
Gas Binned Barplots - Trocheliophorum (DCM)

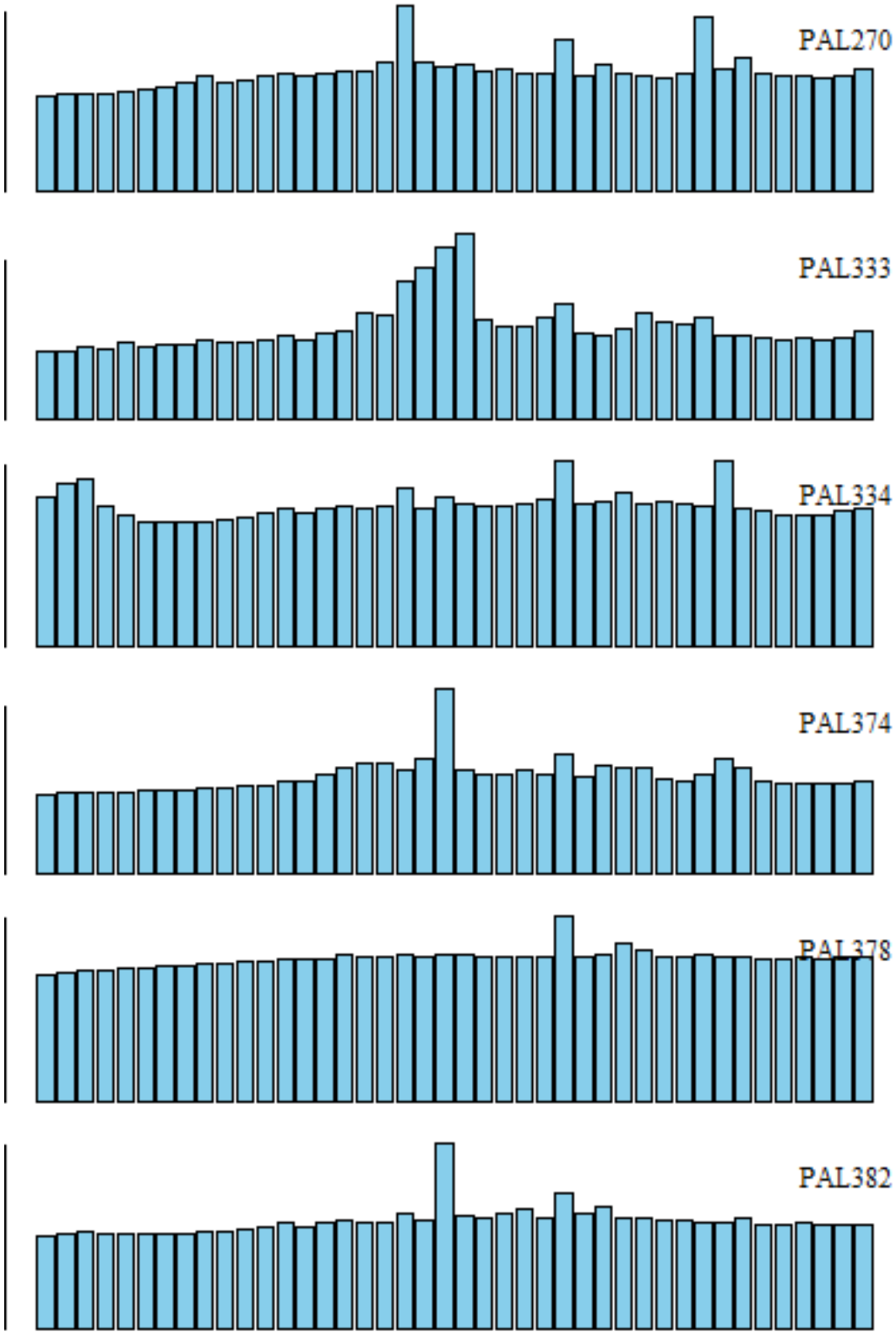



PAL301
PAL307
PAL356
PAL362
PAL379
1 3 5 7 9 12 15 18 21 24 27 30 33 36 39 42

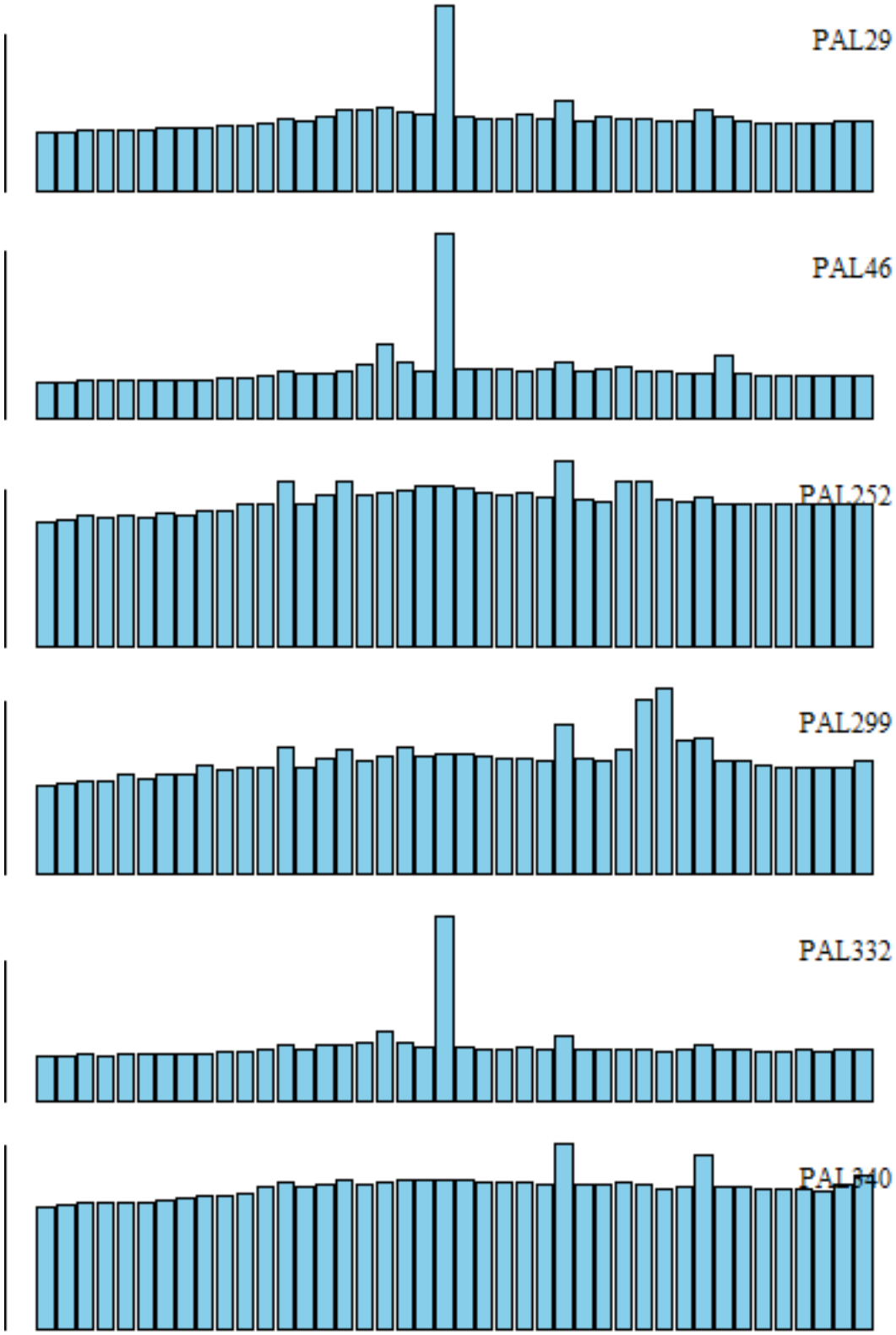
Gas Binned Barplots - Clade F (HEX)

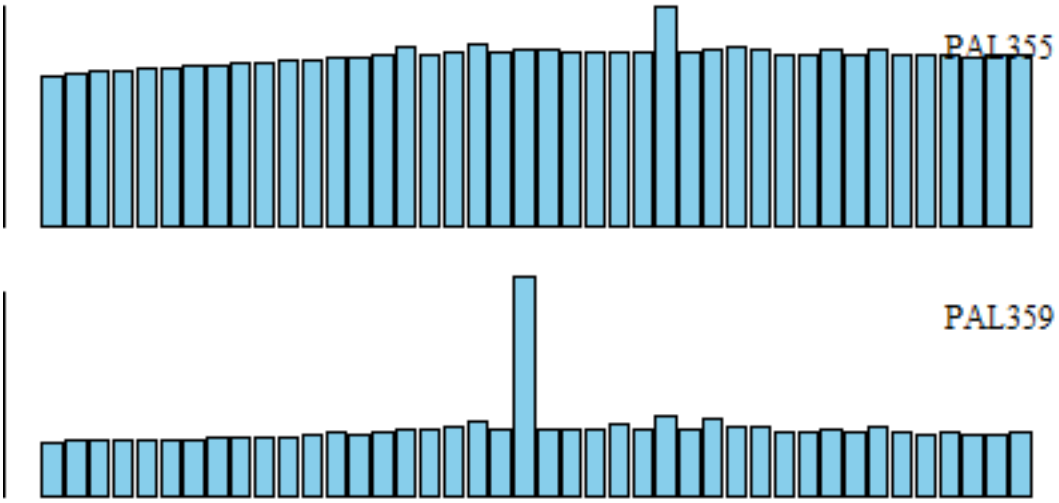



PAL18
PAL43
PAL44
PAL130
PAL131
PAL133

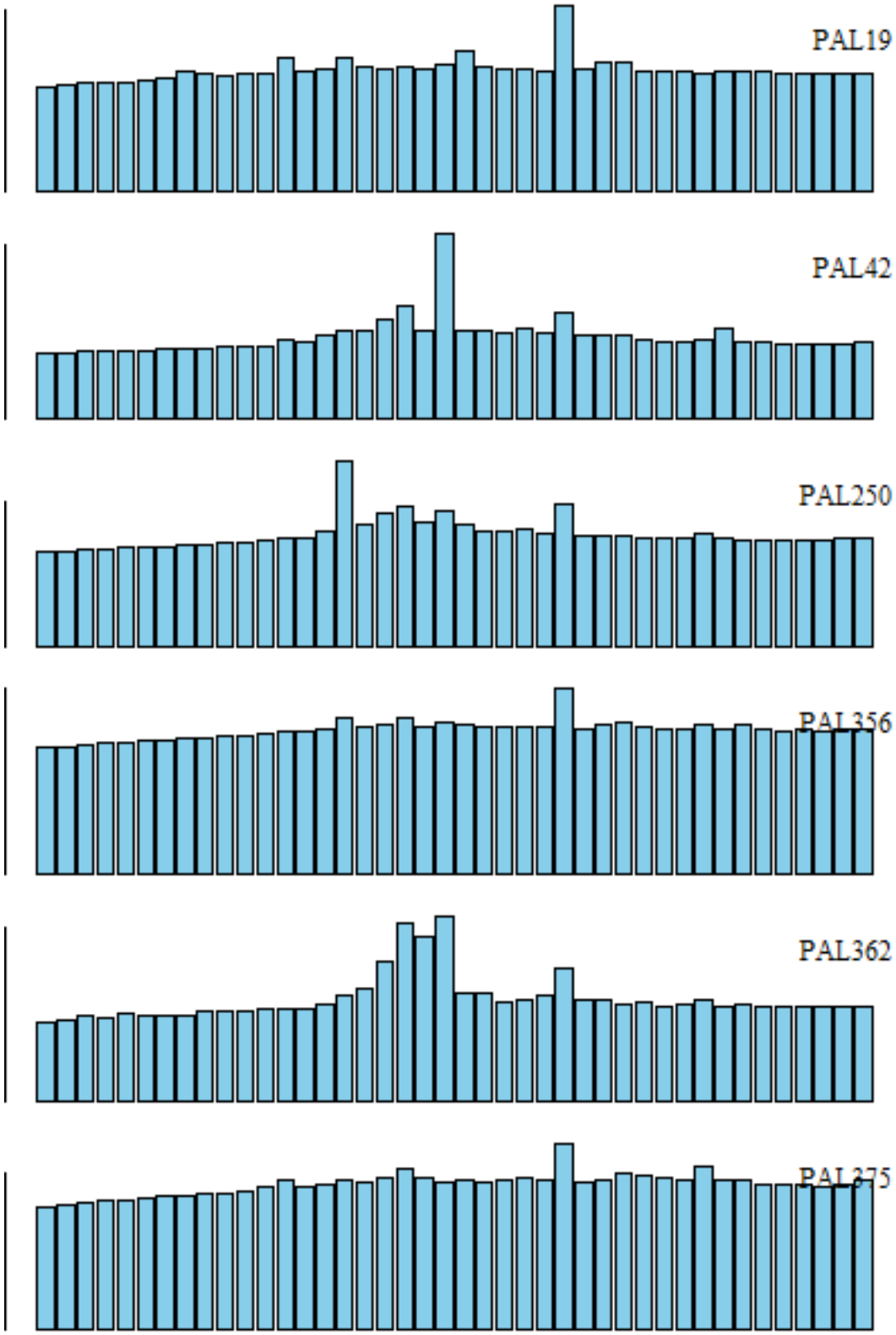

PAL270
PAL333
PAL334
PAL374
PAL378
PAL382

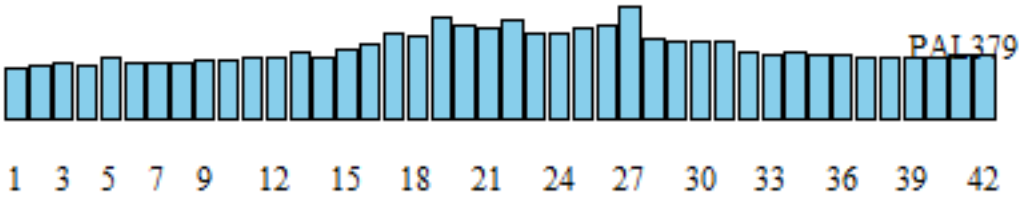
Gas Binned Barplots - Clade D (HEX)




PAL355
PAL359

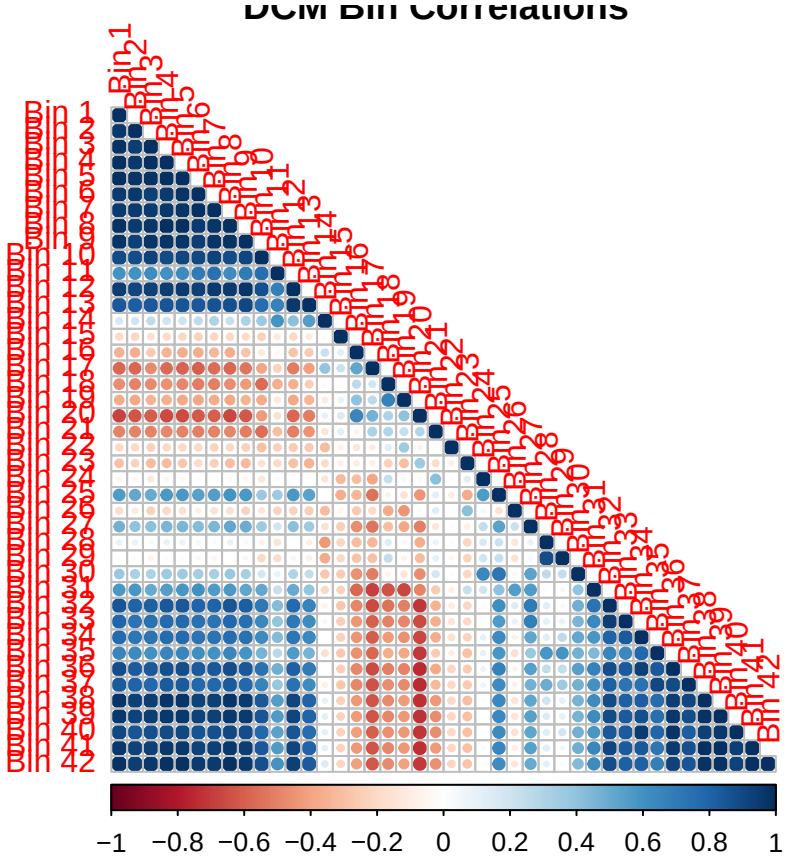
Gas Binned Barplots - Trocheliophorum (HEX)

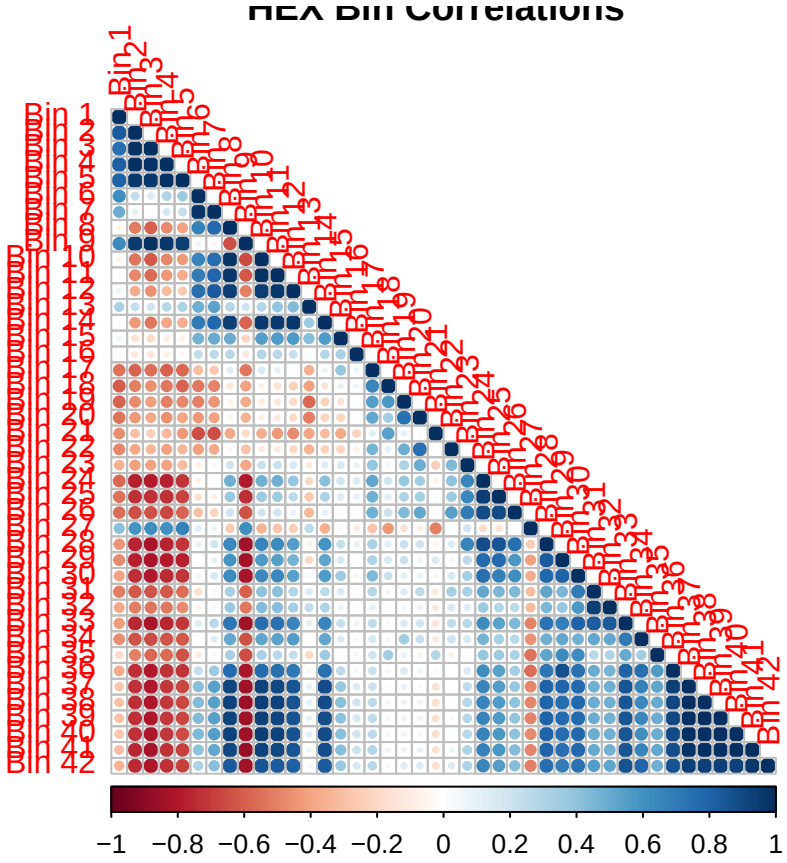



PAL379
1 3 5 7 9 12 15 18 21 24 27 30 33 36 39 42

Principal Components Analysis (PCA)

As a descriptive measure, we examine the correlation between the bins we use as our explanatory variables.

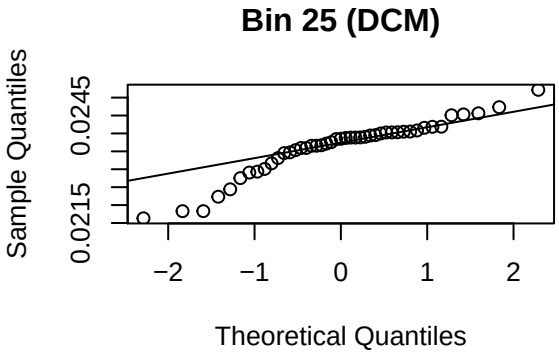


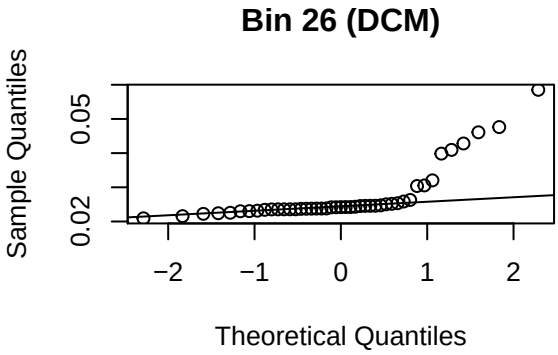


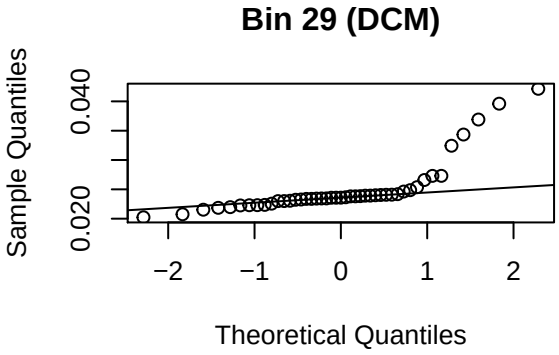
Because we see that there are fairly high correlations between bins in several regions of both correlation plots, we have reason to believe that PCA might be appropriate.

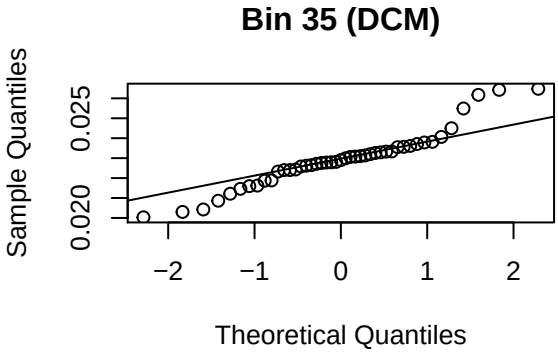
We proceed by validating the assumptions of PCA (as outlined in the Princeton Tutorial on Principal Component Analysis listed below).

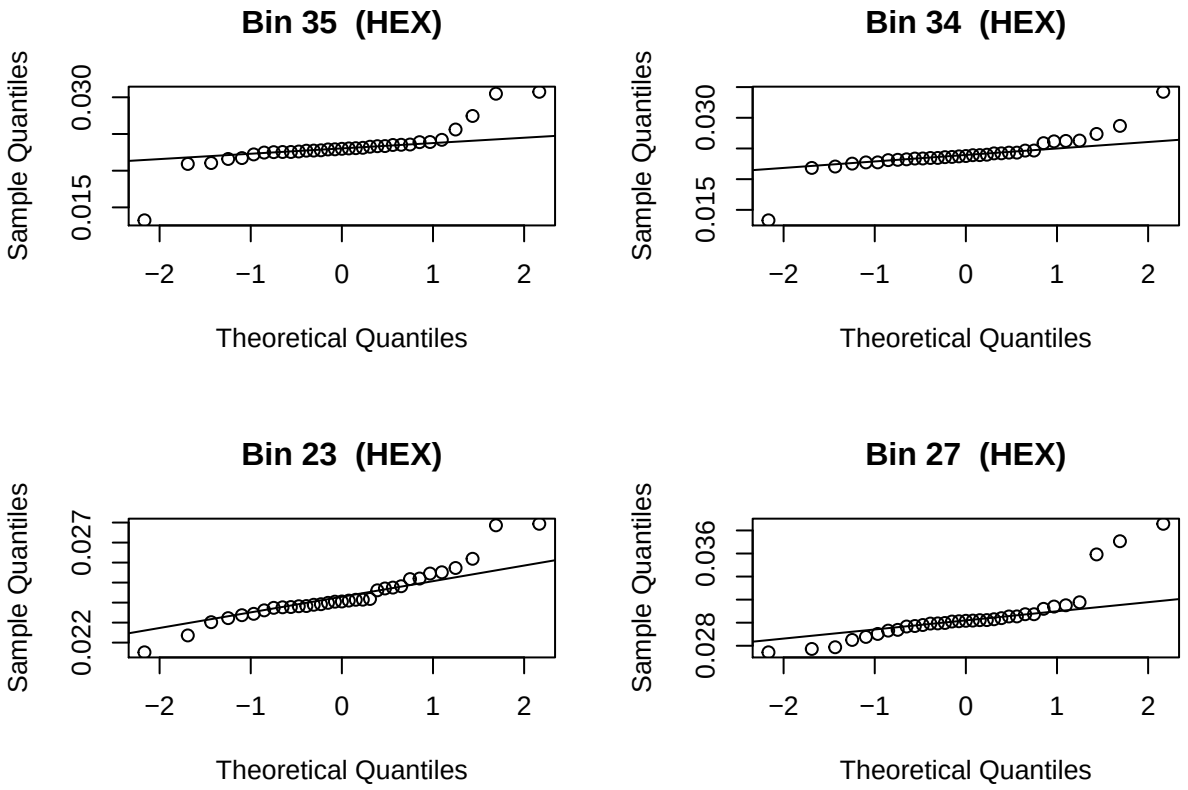
Normal Distribution of Explanatory Variables / QQ plots indicate that the explanatory variables are not normally distributed, which violates one PCA assumption. We show some of these non-normal QQ plots below as examples. PCA tends to be fairly robust to such assumptions, so we proceed cautiously with the analysis.


Bin 25 (DCM)
Sample Quantiles
0.0215
0.0245
−2
−1
0
1
2
Theoretical Quantiles


Bin 26 (DCM)
Sample Quantiles
0.02
0.05
−2
−1
0
1
2
Theoretical Quantiles


Bin 29 (DCM)
Sample Quantiles
0.020
0.040
−2
−1
0
1
2
Theoretical Quantiles


Bin 35 (DCM)
Sample Quantiles
0.020
0.025
−2
−1
0
1
2
Theoretical Quantiles



LINEARITY ASSUMPTION?

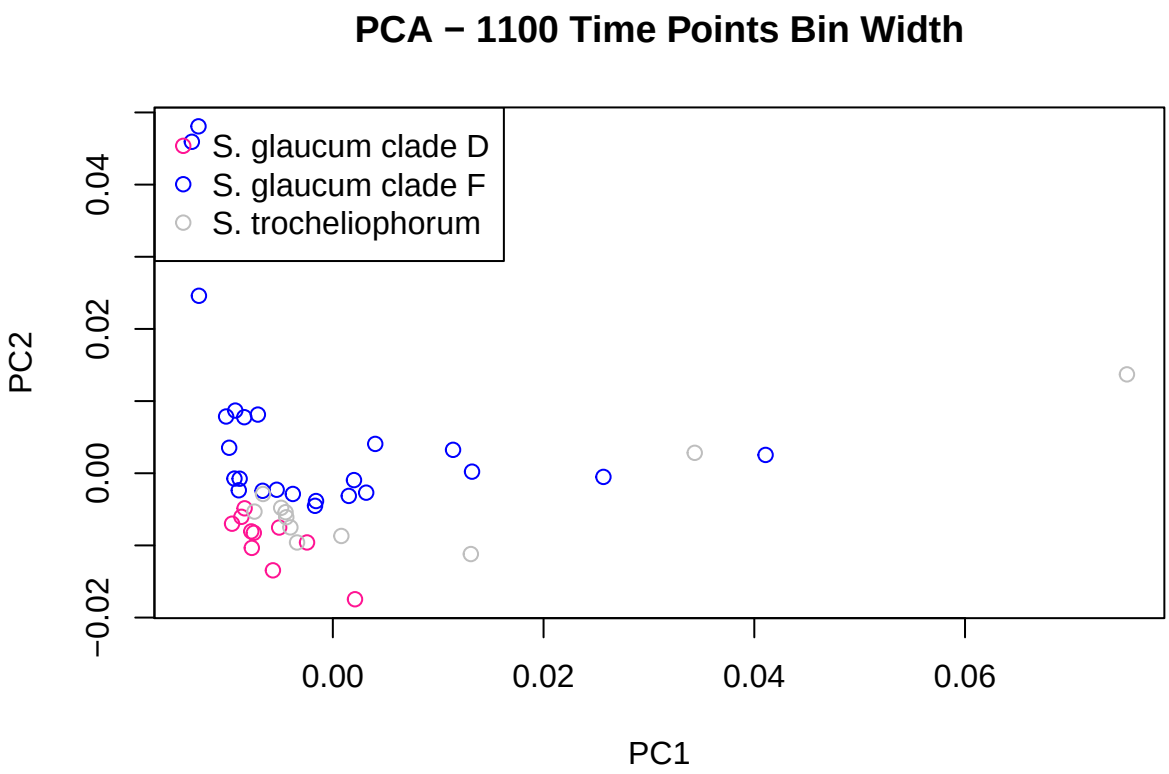
LARGE VARIANCES HAVE IMPORTANT DYNAMICS (SNR)?

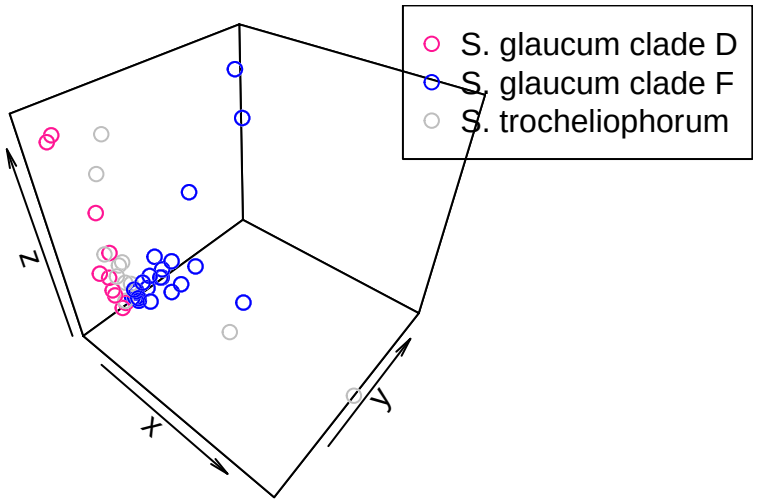
ORTHOGONALITY OF PRINCIPAL COMPONENTS?

We center the explanatory variables, but do not scale them, since all explanatory variables are measured in the same units.

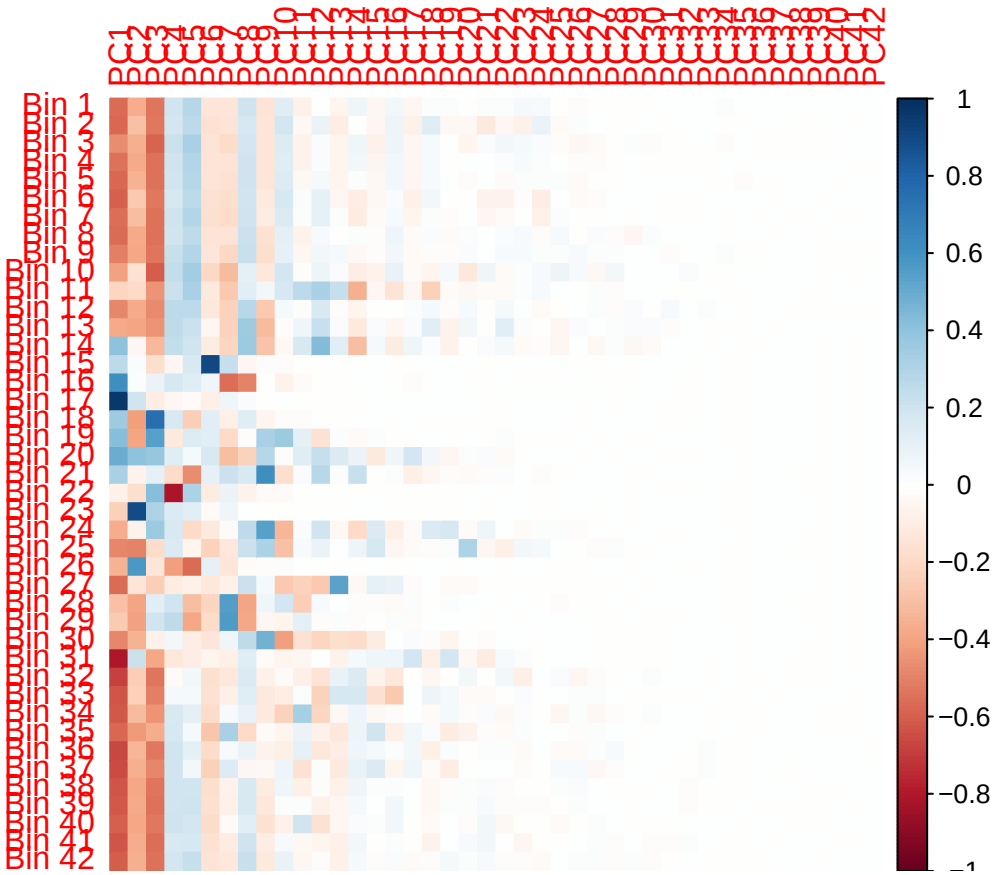
```
#DCM:
pca.result_DCM <- prcomp(binned_matrix_DCM, center = TRUE, scale = FALSE)
#str(pca.result)
PC_DCM = pca.result_DCM$x

F = as.numeric(which(dfAllT_DCM[,3]=="F"))
D = as.numeric(which(dfAllT_DCM[,3]=="D"))
T = as.numeric(which(dfAllT_DCM[,3]=="T"))
```


PCA − 1100 Time Points Bin Width
S. glaucum clade D
S. glaucum clade F
S. trocheliophorum
PC2
0.04
0.02
0.00
−0.02
0.00
0.02
0.04
0.06
PC1



Note the nice separation between Clade F and Clade D given by the PCA plots, even using only the first two principal components.



	Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	Bin 7	Bin 8	Bin 9	Bin 10	Bin 11	Bin 12
PC1	-0.565	-0.577	-0.463	-0.543	-0.573	-0.599	-0.551	-0.562	-0.506	-0.409	-0.218	-0.489
PC2	-0.363	-0.293	-0.354	-0.379	-0.346	-0.263	-0.302	-0.371	-0.384	-0.150	-0.191	-0.371
PC3	-0.536	-0.524	-0.583	-0.541	-0.542	-0.528	-0.550	-0.547	-0.538	-0.605	-0.442	-0.477

	Bin 13	Bin 14	Bin 15	Bin 16	Bin 17	Bin 18	Bin 19	Bin 20	Bin 21	Bin 22	Bin 23	Bin 24
PC1	-0.374	0.402	0.263	0.618	0.969	0.355	0.427	0.496	0.322	-0.073	-0.238	-0.366
PC2	-0.393	-0.048	0.027	0.012	0.197	-0.413	-0.397	0.406	-0.063	-0.173	0.898	-0.066
PC3	-0.441	-0.325	-0.173	0.080	-0.092	0.758	0.543	0.381	0.108	0.427	0.302	0.361

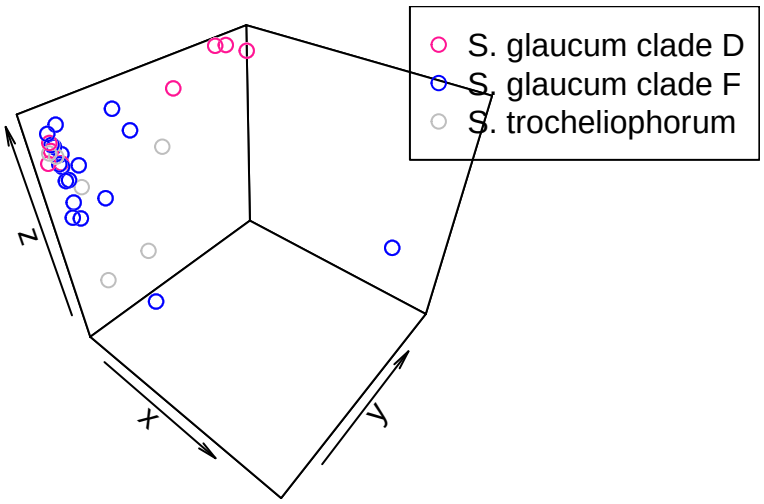
	Bin 25	Bin 26	Bin 27	Bin 28	Bin 29	Bin 30	Bin 31	Bin 32	Bin 33	Bin 34	Bin 35	Bin 36
PC1	-0.486	-0.345	-0.566	-0.299	-0.250	-0.485	-0.802	-0.687	-0.624	-0.614	-0.571	-0.667
PC2	-0.497	0.578	-0.138	-0.394	-0.410	-0.343	0.217	-0.245	-0.238	-0.318	-0.425	-0.337
PC3	-0.185	-0.136	-0.246	0.128	0.195	-0.077	-0.381	-0.534	-0.503	-0.441	-0.363	-0.527

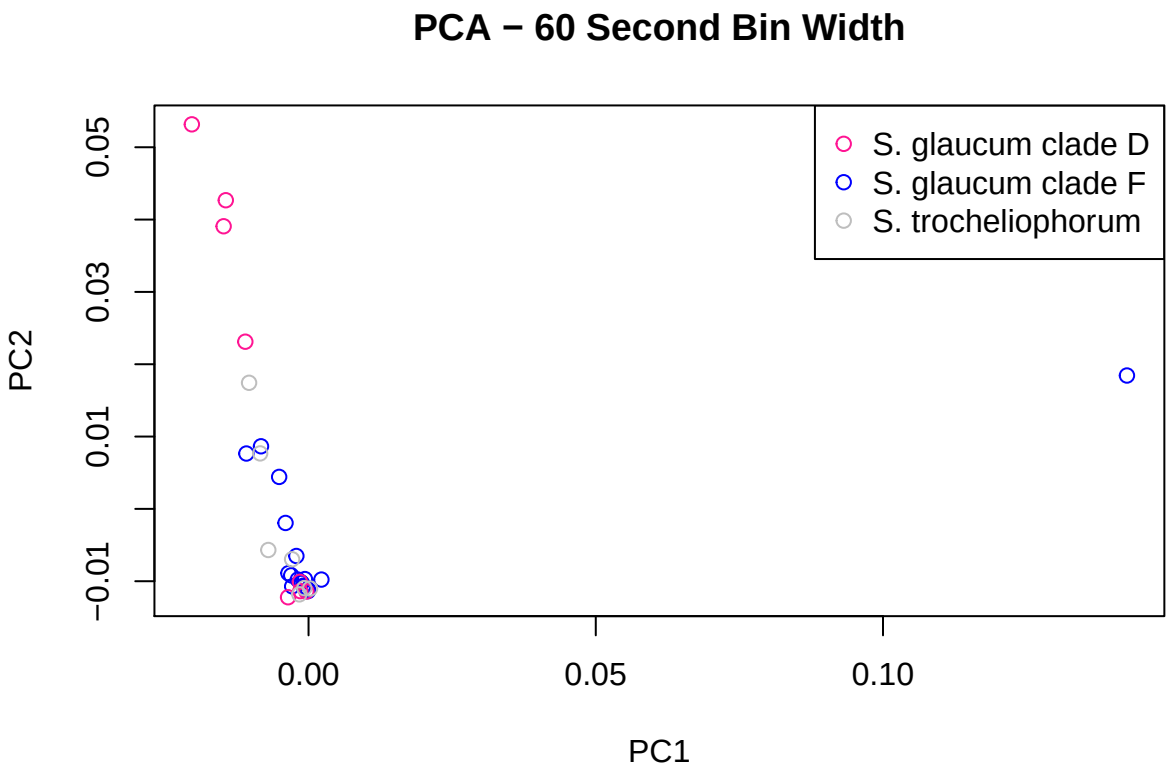
	Bin 37	Bin 38	Bin 39	Bin 40	Bin 41	Bin 42
PC1	-0.650	-0.620	-0.616	-0.593	-0.623	-0.606
PC2	-0.360	-0.388	-0.376	-0.381	-0.360	-0.356
PC3	-0.481	-0.540	-0.550	-0.521	-0.557	-0.541

We see high (in absolute value) correlations between the first principal component and bins 31 and 17. Note that in the binned barplots clade D exhibits a greater area in bin 31 as compared to surrounding areas whereas no such jump occurs in clade F. Similarly, clade F has greater area in bin 17.

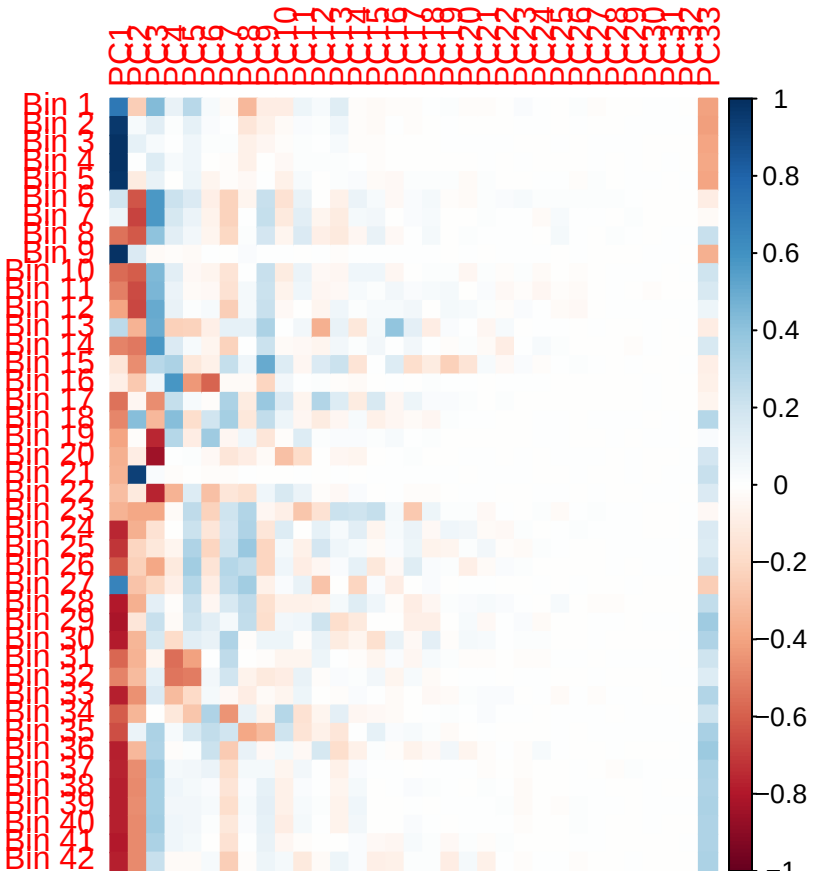
```
#HEX:
pca.result_HEX <- prcomp(binned_matrix_HEX, center = TRUE, scale = FALSE)
#str(pca.result_HEX)
PC_HEX = pca.result_HEX$x

F = as.numeric(which(dfAllT_HEX[,3]=="F"))
D = as.numeric(which(dfAllT_HEX[,3]=="D"))
T = as.numeric(which(dfAllT_HEX[,3]=="T"))
```





We do not see the separation we would hope to using the Hexane samples. Note that we are dealing with a fairly small sample size (just 7 clade D samples and 18 clade F samples), and PCA may be sensitive to any outliers.



	Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	Bin 7	Bin 8	Bin 9	Bin 10	Bin 11	Bin 12
PC1	0.718	0.969	0.987	0.982	0.978	0.196	0.079	-0.544	0.986	-0.561	-0.505	-0.397
PC2	-0.241	0.040	0.103	0.008	-0.108	-0.626	-0.676	-0.615	0.156	-0.602	-0.660	-0.664
PC3	0.432	0.128	0.037	0.145	0.095	0.578	0.575	0.404	-0.030	0.445	0.450	0.510

	Bin 13	Bin 14	Bin 15	Bin 16	Bin 17	Bin 18	Bin 19	Bin 20	Bin 21	Bin 22	Bin 23	Bin 24
PC1	0.261	-0.494	-0.137	-0.086	-0.540	-0.487	-0.398	-0.353	-0.341	-0.303	-0.346	-0.753
PC2	-0.342	-0.526	-0.464	-0.269	-0.046	0.430	-0.010	-0.095	0.939	-0.116	-0.382	-0.351
PC3	0.491	0.574	0.272	0.061	-0.466	-0.321	-0.760	-0.843	0.024	-0.763	-0.389	-0.158

	Bin 25	Bin 26	Bin 27	Bin 28	Bin 29	Bin 30	Bin 31	Bin 32	Bin 33	Bin 34	Bin 35	Bin 36
PC1	-0.711	-0.614	0.663	-0.794	-0.818	-0.780	-0.571	-0.497	-0.779	-0.602	-0.647	-0.771
PC2	-0.216	-0.235	-0.288	-0.356	-0.136	-0.339	-0.341	-0.318	-0.450	-0.339	0.083	-0.329
PC3	-0.129	-0.385	-0.206	0.118	0.222	0.179	-0.053	0.104	0.157	-0.001	0.319	0.305

	Bin 37	Bin 38	Bin 39	Bin 40	Bin 41	Bin 42
PC1	-0.770	-0.777	-0.776	-0.772	-0.793	-0.773
PC2	-0.465	-0.479	-0.470	-0.478	-0.473	-0.479
PC3	0.359	0.336	0.333	0.349	0.312	0.228

We see that many bins correlate highly with our first principal component.

Cluster Analysis - Hierarchial Clustering

```
#samples = rownames(binned_matrix_DCM)
#clades = c(rep("F", 23), rep("D", 11), rep("T", 11))
#for(j in 1:length(clades)){
#  clades[j] = paste(clades[j], samples[j])
#}


#clust_DCM = binned_matrix_DCM
#rownames(clust_DCM) = clades

#distDCM = dist(clust_DCM)
#hcDCM = hclust(distDCM)
#plot(hcDCM)



#clust_DCM_PC = PC_DCM
#rownames(clust_DCM_PC) = clades

#distDCM_PC = dist(clust_DCM_PC)
#hcDCM_PC = hclust(d=distDCM_PC, method="ward.D2")
#plot(hcDCM_PC)



#clust_HEX_PC = PC_HEX
#rownames(clust_HEX_PC) = clades

#distDCM_PC = dist(clust_HEX_PC)
#hcHEX_PC = hclust(d=distHEX_PC, method="ward.D2")
#plot(hcHEX_PC)
```

HC Notes:

- We use euclidian distance as our measure of distance between samples.

Linear Discriminant Analysis

Documentation of Resources

Packages Used for Statistical Analysis:

- stats
  - standard deviation
  - pca
  - qqplots
  - correlation
- MASS
  - lda

```
citation("stats")
citation("MASS")
```

Other Packages Used:

- knitr
  - clean table output in markdown file
- corrplot
  - correlation matrix plots
- plot3d
  - 3d PCA plot

```
citation("knitr")
citation("base")
citation("corrplot")
citation("plot3D")
```

PCA Sources:

- http://setosa.io/ev/principal-component-analysis/
  - Provides nice visualization of dimension reduction
- https://www.unt.edu/rss/class/mike/6810/Principal%20Components%20Analysis.pdf
  - Describes difference between PCA and Factor Analysis in powerpoint
- https://www.cs.princeton.edu/picasso/mats/PCA-Tutorial-Intuition_jp.pdf
  - Provides background on PCA
  - Lists assumptions and limitations of PCA
- http://www.floppybunny.org/robin/web/virtualclassroom/stats/statistics2/pca1.pdf

HC Sources:

- http://www.r-tutor.com/gpu-computing/clustering/hierarchical-cluster-analysis
  - R tutorial for hierarchial clustering analysis