Subject: Responses to Requests in Subaward #SI-1 Termination Memo of 11 September 2018

Date: 24 September 2018

1. Final metadata spreadsheet

See Excel spreadsheet "All-extractions_EMU-LIST_FINAL-DEFINITIVE-VERSION_2018-09-24.xlsx" on hard drive submitted to Gettysburg.

2. Insufficient DNA, Poor DNA Extractions, and/or Lack of Time

See comments in "Tissue Sample Extraction Notes" column of "All-extractions_EMU-LIST_FINAL-DEFINITIVE-VERSION_2018-09-24.xlsx." Sequencing was attempted for ALL samples, but the termination of the subaward prevented gap-filling on all unsuccessful samples.

3. Discrepancy between 2017 barcoding report and 2018 barcoding report

The 2017 report of number of DNA barcode sequences included up to 235 fern barcodes (2.5 plates; see spreadsheet "Fern-extractions_ 2018-09-24.xlsx") for the markers *trnH-psbA* and *rbcL*. These sequences were not included in this 2018 count, which only listed sequences from the original 2,375 extractions in the "All-Extractions" spreadsheet. With the ferns the total sample list includes 2,610 extractions. The total number of sequences completed as of 5 September 2018 should have been recorded as:

rbcL= 1,835 barcode sequences
matK= 1,613 barcode sequences
trnH-psbA= 1,701 barcode sequences

Additionally, within the last year, careful editing of many already generated barcode sequences of the 2,375 original samples revealed a number of low quality sequences. These sequences were temporarily removed from the 2018 total awaiting later resequencing during the "gap-filling" phase of the project. In addition, an entire plate of extractions (#K1314: 95 samples) was a duplicate plate (#K1313; see spreadsheet) included in the 2017 total and these duplicate sequences have now been removed.

With these discrepancies taken into account and including the additional sequences generated over the last month (see #4 below) the total number of sequences completed as of 24 September 2018 are:

rbcL = 1,835 barcode sequences
matK = 1,647 barcode sequences (including 34 sequence gaps filled within the last month)
trnH-psbA = 1,734 barcode sequences (including 33 sequence gaps filled within the last month)

In total, 5,216 DNA barcode sequences across all three markers have been completed as of September 2018, compared to the 3,848 DNA barcode sequences reported in 2017.

4. Additional sequencing attempted between 24 August 2018 and 24 September 2018

An initial three "gap-filling" plates were assembled for sequencing (see spreadsheet "Puebla Gap plates.xlsx" on hard drive submitted to Gettysburg). Plates for *trnH-psbA* (2, 3) and *matK* (4,5) were

partially sequenced (4 sequencing reactions) before time ran out on the subaward. Plate 1 for *rbcL* was not sequenced due to lack of time.

Gap plate 1 rbcL-F (92 samples; no sequencing attempted due to termination of subward)
Gap plate 1 rbcL-R (92 samples; no sequencing attempted due to termination of subward)
Gap plates 2, 3 trnH (58 samples; 33 samples successfully sequenced)
Gap plates 2, 3 psbA (58 samples; 33 samples successfully sequenced)
Gap plates 4, 5 matK-F (xF) (94 samples; 34 samples successfully sequenced)
Gap plates 4, 5 matK-R (MALP) (94 samples; 34 samples successfully sequenced)

Note that sequences obtained through gap-filling are highlighted in red in spreadsheet "All-extractions_EMU-LIST_FINAL-DEFINITIVE-VERSION_2018-09-24.xlsx".

5. All .fasta sequence files are on the hard drive submitted to Gettysburg.

6. All .abi trace files are on the hard drive submitted to Gettysburg.