GigaScience

Data For “One Thousand Plant Transcriptomes Elucidate Green Plant Phylogenomics”

--Manuscript Draft--

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Abstract:	The 1000 Plants (1KP) initiative explored the genetic diversity of green plants (Viridiplantae) by sequencing RNA from 1,342 samples representing 1,173 species. All of the analyses done for the 1KP capstone, and previous studies on subsets of these data, are based on a series of de novo transcriptome assemblies and related outputs that will be described in this publication. We also describe assessments of the data quality and an analysis to remove cross-contamination between the samples. These data will be useful to researchers with interests in specific gene families, either across the green plant tree of life or in more focused lineages.
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Response to Reviewers:

Response to Reviewers Comments:

Reviewer #1:

1, phylogenomic analyses need alignments of orthologous genes, but this
data note didn't provide them. Can this dataset be used in phylogenomic
analysis?

This paper is a companion to Ref. 1, which deals with the phylogenomic analyses. Specifics of the phylogenomic analysis including the process of generating alignments between orthologous genes are more properly discussed in Ref. 1 and it's online supplements and are not discussed here.

2, please explain the tables in Line109, 282 and 317.

all three tables now have a title and legend and are referenced from the body text

3, for many species selected here, their transcriptomes had been
sequenced before. Why don't use these pre-existing data? How to
determine the superiority of the data provided in this paper?

This paper describes a data set that was generated some time ago, primarily for a complex phylogenomics analysis just accepted for publication in a major journal. We are not claiming our data is the best available for any given species. Although we tried to avoid overt duplication, considering the time involved, it should come as no surprise that other groups may have also sequenced the same species.

4, evolutionary complexity includes many aspects, including variation in
chromosomal structures and the numbers. Can the transcriptomic sequences
capture the substantial phylogenomic signals of so many plants? Why?

Again, the phylogenomics was reviewed in detail for the capstone paper. This is just a paper to describe the data set used.

Reviewer #2

-Table legends are missing and needs to be added. Also be consistent
using "th" percentile throughout the three tables.

legends have been added and the th suffix is used in all three tables

-line 112: quantify "excessive", level of reads removed?

"excessive" is not the correct word and has been removed for clarity. We do not have data on how extensive this removal was. We expect that it should have been only a small fraction of the total reads sequenced.

-line 125: what is the dataset AEPI?

Discussion of the dataset ID codes used has been added. Dataset AEPI was selected as an example.

-line 142 [cite]?
A placeholder reference to the protocols.io entry is now present.

-line 146 I find the title "Protein translation" a bit strange since it is prediction of coding regions it refers to

This title has been adjusted to better match the material.

-line 153 "those" what?

those codons - text changed

-line 154 Sentence "Outputs…" remove or point at where the output files are

reference to the associated data added

-line 165 and 166: maybe a miss something but the nucleotide sequences are 1/5 of the predicted protein sequences after Transpipe…?

This is correct. Some description has been added to the previous paragraph to help emphasize that the process only translates a portion of the material. (Those assemblies with sufficient similarity the the Phytozome reference sequences.)

-line 175: this statement needs a reference

No reference is available. We have removed the comment.

-line 193: "these problems", please be more specific, and clearly list which ways tried

The other methods are ad hoc analyses and are not as universally applicable as the 18S based analyses. We do not want to waste time/space with detailed discussion of them. The text is rewritten to remove the references to them.

-line 237: can't access github page!

GitHub has been contacted about this and the issue seems to be fixed.

-line 307-308: I don't follow the last part of the argument as BUSCO - Emboryophyta looks fairly linear to number of assembled scaffolds and non-phylum samples should fall outside this linearity (which they might - can they be marked in any way)

After consideration the wording has been changed to make the weaker statement that the difference remains if only the embryophyte samples are considered.

In fig 1 panel B: what are the 4-letter abbreviation before the species ntainingi names?

Discussion of the 4-letter codes has been added to the main text of the paper.

Additional Information:	
Question	**Response**
Are you submitting this manuscript to a special series or article collection?	No
Experimental design and statistics	Yes

Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends. Have you included all the information requested in your manuscript?	
Resources A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible. Have you included the information requested as detailed in our Minimum Standards Reporting Checklist?	No
If not, please give reasons for any omissions below. as follow-up to "**Resources** A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible. Have you included the information requested as detailed in our Minimum	The data is derived from plant samples for which no attempt was made to identify an age or sex for the source.

Standards Reporting Checklist? "	
Availability of data and materials All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript. Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist?	No
If not, please give reasons for any omissions below. as follow-up to "**Availability of data and materials** All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript. Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist? "	Additional data (contamination analysis, etc) will be submitted to GigaDB after this online process, as per the journal instructions.

Data For “One Thousand Plant Transcriptomes Elucidate Green Plant Phylogenomics”

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Abstract

The 1000 Plants (1KP) initiative explored the genetic diversity of green plants (Viridiplantae) by sequencing RNA from 1,342 samples representing 1,173 species. All of the analyses done for the 1KP capstone, and previous studies on subsets of these data, are based on a series of de novo transcriptome assemblies and related outputs that will be described in this publication. We also describe assessments of the data quality and an analysis to remove cross-contamination between the samples. These data will be useful to researchers with interests in specific gene families, either across the green plant tree of life or in more focused lineages.

Keywords

RNA, plants, assemblies, genes, contamination, completeness

Data Description

1KP has sequenced RNA from 1,342 RNA samples of 1,173 green plant species representing all major taxa within the Viridiplantae, including streptophyte and chlorophyte green algae, bryophytes, ferns, angiosperms, and gymnosperms. Importantly, our selection criteria eschewed the model organisms and crop species where other plant sequencing efforts have historically been concentrated. While many of the samples were selected for the phylogenomic analyses, others were motivated by different subprojects.

Major papers describing the project have been published elsewhere [1, 2]. The most recent papers are focused on phylogenic analyses. This Data Note describes the sequence data set and provides additional details on the sample and sequence processing as well as quality assessments of these data.

Methods

Sampling strategy

Because of the diversity and the number of species analyzed, no one source could be used. Samples were provided by a global network of collaborators who obtained materials from a variety of sources, including field collection of wild plants, greenhouses, botanical gardens, laboratory specimens, and algal culture collections. To ensure an abundance of expressed genes, we preferred live growing cells, e.g. young leaves, flowers, or shoots, although many samples were also from roots, or other tissues. Because of the sample diversity, we did not attempt to define specific standards on growth conditions, time of collection, or age of tissue. For more details, see the supplemental methods in the capstone paper [1].

RNA extraction

Given the biochemical diversity of these samples, no one RNA extraction protocol was appropriate for all samples. Most samples were extracted using commonly known protocols or using commercial kits. For complete details of the many specific protocols used, please see Appendix S1 of Johnson et al. [3] and Jordon-Thaden et al. [4]. These protocols are also available in a protocols.io entry [5]. Depending on the sample, RNA extractions might have been done by the sample provider, a collaborator near the provider, or the sequencing lab (BGI-Shenzhen).

Sequencing at BGI

Samples of extracted RNA or frozen tissues were sent to the sequencing lab, BGI-Shenzhen. Prior to library construction, RNA samples were screened by Agilent Bioanalyzer RIN scores [6] and basic photometry; obvious low-quality outliers (e.g., RIN scores less than 6 and/or loss of distinct electropherogram peaks) were excluded. Libraries for Illumina sequencing were constructed using

Illumina’s standard procedure. Some samples for which only a small amount of RNA was available were processed using TruSeq kits.

Initially, sequencing was done on the Illumina GAII platform, but later samples were run on the HiSeq platform. Associated with this change was a shift from ~72 bp read lengths to 90 bp read lengths (both cases paired-end). Libraries were indexed and multiplexed in the sequencer lanes to a target sequencing depth of 2 Gbp per sample. Average depth achieved was 1.99 Gbp of sequence of better than Phred quality 30 (1 error per thousand bases). This size slightly increases when more error prone bases are counted, and varies across samples with half of samples in the 1.9–2.5 Gbp range as summarized in Table 1.

Table 1 Distribution in amount of sequence data per sample library. Summary percentiles characterising the sizes of the datasets in giga-basepairs of sequence.

Percentile	Dataset Size (all base qualities)
5th	1.3 Gbp
25th	1.9 Gbp
50th	2.2 Gbp
75th	2.5 Gbp
95th	3.0 Gbp

.

The data was cleaned by eliminating reads containing adapter-primer sequences or high numbers of low quality bases (i.e. more than half of Phred quality 5 or lower [32 % error rate] or more than 10% uncalled).

De novo assembly

Once the data was transferred from BGI, the FastQ files were given a uniform name based on a quasi-random four-letter identification code. A list of all the samples and their ID code is included in the associated data. These identifiers also distinguish otherwise identical repeated samples, and provide a stable reference when a sample's species identification was changed.

Quality filtered reads were assembled using the SOAPdenovo-Trans transcript assembler (version 2012-04-05) [7]. No additional pre-processing of the data was performed. This largely used the program defaults, with the slight modification of increasing the *k*-mer length to 25 bp and reducing the number of processor threads to one. This reduced thread count allowed us to more efficiently use our computer resources. Both the internal FillGap module and the external GapCloser post-processor (supplied with SOAPdenovo-Trans) were run. An example of the commands used for one of the assemblies (dataset AEPI, *Lineum leonii*):

```
SOAPdenovo-Trans-31kmer all -s config -p 1 -K 25 -e 2 -F -L 100 -t 5 -o AEPI
GapCloser -a AEPI.scafSeq -b config -o AEPI.GapCloser.fa -l 100 -p 25 -t 1
```

These commands refer to a configuration file named config, which specified the expected insert size, maximum read length, and read-sequence filenames. The contents of this file were:

```
max_rd_len=120
[LIB]
avg_ins=200
rank=1
```

```
q1=AEPI-read_1.fq
q2=AEPI-read_2.fq
```

When multiple samples from the same species were co-assembled, the last five lines were repeated for each data source with the appropriate filenames. Such assemblies were also assigned unique four-letter identifiers. After assembly the output contigs/scaffolds were renamed giving each a unique name containing the assembly's four-letter identifier, a number within the assembly, and as a descriptive name the species, with additional description of the tissue or other identifier when multiples samples of the same species where sequenced.

Identification of coding regions and protein translation

To identify likely proteins within the assembled transcripts, sequences were passed through TransPipe [8], which identified reading frames and protein translations by comparison to protein sequences from 22 sequenced and annotated plant genomes in Phytozome [9]. Using BLASTX [10], best hit proteins were paired with each assembled scaffold at a threshold of 1E-10 expectation-value and a minimum length of 100 amino acid residues. Scaffolds that did not have a best hit protein at this level were removed. These removed scaffolds are predominantly from the numerous short and likely fragmentary sequences; however some complete genes will have been lost. To determine reading frames and estimate amino acid sequences, each gene is aligned against its best hit protein by Genewise 2.2.0 [11]. Using the highest scoring Genewise DNA-protein alignments, stop codons and those codons containing ambiguous nucleotides were removed to produce an amino acid sequence for each gene. Outputs in the associated data are paired DNA and protein sequences.

BLAST searches

Thanks to the support of China National GeneBank (CNGB), a BLAST search service (http://db.cngb.org/onekp/) allows public searches against the assemblies and protein translations. CNGB developed the service using NCBI BLAST+ (version 2.6.0) [12]. It integrates all public datasets from CNGB applications, BGI projects and external data sources, and provides a comprehensive and convenient sequence searching. A specialized interface for BLAST searching the 1KP dataset allows limiting the search to specific families, orders, or 25 higher-level clades. For assemblies, there are 21,398,790 nucleotide sequences, 6,188,419,272 bases in total. And for the Transpipe protein translations, there are 103 million protein sequences comprising over 47 billion amino acids in total.

Validation

Purity and contamination

High throughput sequencing methods are always at risk of contamination. In practice, data has been found to often include sequences best attributed to additional contaminating sources [13]. For 1KP, the diversity of sources for the samples, and especially the fact that axenic cultures are not a viable option in most instances, ensures that there will always be some contamination of the plant tissue by other environmental nucleic acids. These can reasonably be expected to include bacterial, fungal, and insect species that live in and on the plant tissues, and more rarely, from contact with larger species such as frogs, mice, birds and humans.

For most analyses, these minor contaminants are not expected to matter, as only the most abundant of such contaminants will be present in sufficient quantities to assemble. In many cases, they are also sufficiently diverged from the intended species that they can be easily recognised as non-plant genes. Unfortunately, this is not always the case. Some analyses are further protected by looking at the whole of the available transcriptome, whereby the many genes from the target species will overpower a few contaminants. Single gene family analyses do not have this advantage and must rely on other methods to reject non-plant genes.

Another possibility is significant contamination during sample processing when plant RNA is transferred between adjacent samples, or when whole samples are accidentally mislabelled.

Given the potential contamination problems, we tried to identify them in the sequence data by comparing the assembled sequences by BLASTn to a reference set of nuclear 18S rRNA sequences from the SILVA SSU rRNA database (http://www.arb-silva.de) [14]. The BLASTn alignment to an assembly with the lowest expectation-value is taken to indicate the assembly has a similar taxonomic origin as the reference sequence. However, alignments of less than 300 bp or expectation-values above 1E-9 often align to several distantly related species and were ignored.

For most samples we found an 18S sequence most-similar to a SILVA sequence from the same taxonomic family as the expected sample species. This is not true for all our samples, and may indicate a failure to assemble the 18S sequence, limitations in the taxonomic identification from the BLASTn results, or mislabelling of sample. In a few cases, additional (and possibly contaminant) 18S sequences were found. Because the 18S rRNA sequence is highly expressed, we expect that this method is likely to be sensitive to low levels of contamination. In a few cases, the taxonomic irregularities were judged sufficiently severe that samples were excluded from various analyses.

The accompanying data includes two accessory files containing details of this SILVA based SSU validation for each sample. The first lists whether the sample is overall judged to be validated as containing the expected taxon, and whether it had alignments to any other plant sequences (described as “worrisome contamination”). The second file, more detailed, lists each scaffold identified as being 18S-like sequence, and which reference sequence it matched against.

Pairwise Cross-contamination of Assemblies

Cross contamination between the datasets was identified by using a genome-scale sequence search pipeline, adapted from previous studies [15-17]. Briefly, each pair of assemblies (nucleotide) was compared and a threshold identity level established, above which sequences are likely to be contamination between the pair. While best for identifying technical contamination between libraries (e.g. due to mixing of RNA samples), this technique could also detect other biological contamination events (e.g. contamination of pairs of libraries with common commensal organisms). An additional search step, using the entire 1KP sequence library, identified the probable evolutionary origin of each sequences.

The pair-wise comparison used LAST v. 963 [18] using the --cR01 option, and the respective matches were grouped and ordered by similarity. To avoid artifactually excluding sequences between closely related species, which may have very high degrees of similarity [14], pairs of libraries from the same family, along with pairs of libraries separated by two or fewer branches in the consensus 1KP multigene phylogeny, were excluded from the searches [2].

The expected distribution of the matched sequence identities has a maximum at the pairwise identity reflecting the evolutionary distance between the two species [16, 17]. In contrast, a cross-contaminated pair should contain many sequences of near 100% similarity, and the similarity value which has the first minimum number of sequences below this level (i.e. the first inflexion point in a curve plotting the total number of sequences of each percentage similarity value) can be used as a threshold for discriminating contaminating sequences [16, 17]. The code is available at https://github.com/Plant-and-diatom-genomics-IBENS-Paris/Decontamination-pipeline.

The output of this analysis is pairs of apparent orthologs whose sequence similarities are higher than the cut-off in one or both libraries, i.e. potential contamination. To discriminate donors and recipients in each contaminant pair, each of these potential contaminants was searched against all the non-contaminant assemblies by BLASTn, using the option -max_target_seqs 3 [19]. Queries with at least one of the three best alignments against a sequence from the same family, or from a taxon separated by fewer than two branches within the 1kp tree [2], were excluded from the list of potential contaminants; whereas sequences that yielded best hits exclusively against more distantly related taxa, were verified as potential contaminants. Clean and contaminant FASTA sequence files for each library are available in the accompanying data.

An overview of the results is presented in Fig. 1. In total, we identified 79,175 nucleotide sequences (0.3 %) of a total 23,436,405 searched as being clearly of contaminant origin (Fig. 1A). A further 1,477,637 (6.3%) of the sequences might either occur as contaminants in other libraries, or could not clearly be identified as being of vertical origin via the search pipeline used. The results obtained were concordant with the other contamination analyses. For example, libraries known to have aberrant 18S sequences contained a much larger average proportion of contaminant sequences (5.890/217,270 sequences, 2.7 %), but contained very few sequences that were identified as contaminants in other libraries (252 sequences, 0.1%, Fig. 1A). A similar, but smaller enrichment in contaminants was identified in libraries identified through 18S sequences as containing unconfirmed contamination (16,871/ 912139 sequences; 1.8%), suggesting that at least some of these libraries are genuinely biologically contaminated (Fig. 1A).

Specific libraries contained a much larger proportion of contaminant sequences, with 57.8% of the *Deutzia scabia* (OTAN) found to be contaminant (Fig. 1B). These specific contaminations are from *Gunnera manicata* (XMQO) (Fig. 1C), in line with the 18S based finding. Other cross-contamination events found by this method include *Pseudolarix amabilis* found in *Monoclea gottschei* and *Galium boreale* in *Impatien balsamifera*. We also, however, identified examples of widespread contamination in libraries that had previously not been detected, for example over 35% of the sequences detected in two libraries of the green alga *Olltmansiellopsis viridis* (Fig. 1B). These may relate to contaminants that do not produce 18S sequences, as evidenced by the recent detection of Rhodobacteralean commensal sequences in 1kp libraries from *Mantoniella squamata* (QXSZ), *Bathycoccus prasinos* (MCPK) and *Nannochloropsis oculata* (JCFK) [20]. Additional results are provided in the associated data release.

Assembly qualities

We assessed the quality of each assembled scaffold/contig using the read mapping mode of Transrate [21], which detects several classes of common assembly errors and assigns a quality score to each scaffold. Users of the data may choose to omit those portions of the assembly judged as low-quality when doing their own analyses. While the assemblies for each sample vary in assessed quality (Table 2), there are thousands of good scaffolds in even the worst of them.

Table 2. Assembly quality assessment by Transrate. Characteristic percentiles summarising the per sample distributions of high-quality scaffolds for both total counts and fractions of the sample.

Percentile	Good Scaffolds (all sizes)	Good Scaffolds - Percentage
5th	19,355	32.47%
25th	30,755	44.83%
50th	37,983	53.65%
75th	47,608	62.93%
95th	71,368	74.87%

.

Completeness of gene set

Two different approaches were used to estimate transcriptome completeness. Firstly, BUSCO v1 [22] was applied with default settings, using the eukaryote and embryophyte conserved gene data sets (eukaryota_odb9, embryophyta_odb9) as the query databases. Secondly, conditional reciprocal best BLAST (CRBB) hits were calculated using CRB-BLAST [23] with default parameters. The predicted coding sequences were used as queries against the set of 248 core eukaryotic genes (CEGs) distributed with the CEGMA software (Core Eukaryotic Genes Mapping Approach); these 248 genes are highly conserved in eukaryotic genomes [24] and hence should be present in most transcriptomes.

As with all RNA-Seq data, some genes are more highly expressed than others. While the CEGMA and BUSCO gene sets are intended to demonstrate the completeness of the transcriptomes, they are sensitive to the expression of these genes. Not all these genes will be expressed in the sample’s tissues at sufficiently high levels to be assembled. A plot of the number of assembled scaffolds vs. the fraction of the three gene sets found in the assembled scaffolds shows an increase in the gene fractions found as the number of assembled scaffolds increases (Fig. 2). However, these quickly saturate at 80+% for the

CEGMA and BUSCO-eukaryote sets, with a continuing rise over a larger range for the BUSCO-embryophyte set.

This shows that the three gene sets have somewhat different expression patterns, with the CEGMA and BUSCO-eukaryotic sets comprising genes that are more readily detected in our RNA samples. Some of the weaker sensitivity to the BUSCO-embryophyte set is attributable to our sampling species outside of this phylum, which may not have the homologous genes; however, the difference is present when only the embryophyte samples are considered (not shown).

Percentage CEG abundance was calculated as number of CEGs with a CRBB hit divided by 248, the number of CEGs used. The percentage BUSCO abundance was calculated as 100% minus the missing percentage. Samples with low abundance by these measures should be treated with caution because the observed transcriptome incompleteness may indicate problems in library preparation or other types of poor sample quality. For these reasons the taxonomic analyses in Ref. 1 excluded samples with less than 57.5% BUSCO abundance. The table below shows the percentages of complete genes found for each of the three references at several percentile of the whole dataset.

Table 3. Completeness of gene sets. Characteristic percentiles summarizing the distributions of the CEGMA 248 and BUSCO genome completeness scores. *BUSCO numbers are the sum of the complete and fragment assembly counts reported, with numbers based on the complete sequence numbers alone given in parentheses.

Percentile	CEGMA 248	BUSCO – Embryophyta*	BUSCO – Eukaryota*
5th	79.03	11.2 (8.5)	66.0 (37.3)
25th	89.92	44.1 (29.8)	84.9 (64.4)

50th	92.34	62.5 (48.2)	90.4 (75.9)
75th	93.55	75.2 (59.6)	93.7 (84.1)
95th	94.76	82.6 (73.2)	96.1 (91.0)

Re-use potential

Since many of the samples are from poorly sequenced clades, the Thousand Plant sequence data is the first-large scale sequence data available for many species. We expect these sequences to be of broad interest to the plant sciences community, whether researchers merely use our sequences, supplement them with their own sequences, or develop PCR primer and probe sets to collect entirely new sequence data.

Availability of Supporting Data

Data to be in an associated *Gigascience*/GigaDB submission: [A copy of this is currently available at: https://drive.google.com/drive/folders/175nB8kf1UQushuEzv7UaJLPNNwdOrxh5?usp=sharing]

1. Tables with list of samples/assemblies (Sample-List-with-Taxonomy.tsv) and corresponding ENA/NCBI references (NCBI-ENA-Sequence-Identifiers.csv) and GigaDB links (to be added).

2. The major part of the provided data has for a directory for each assembly. This is named based on the four-letter code and a species name. Within the directory are a FASTA file containing the SOAPdenovo-Trans assembly, translations of the scaffolds to amino acids, the subset of the nucleotide sequence corresponding to the translation, and tab-separated (text) files with tables of Transrate outputs

assessing the assemblies and lists of the reference sequence each translation is based on. These are available for each of the assemblies listed in the supplemental table. (onekp-data directory)

e.g. in directory AALA-Meliosma_cunifolia are AALA-SOAPdenovo-Trans-assembly.fa.bz2, AALA-translated-protein.fa.gz,. AALA-translated-nucleotides.fa.gz, AALA- Transrate-assembly-stats.tsv.gz, and AALA-translated-reference-names.tsv.gz

3. Two accessory tables containing details of the SILVA based SSU validation for each sample. The first (18S-analysis-Sample-Summary.xlsx) lists whether the sample is overall judged to be validated as containing the expected sequence, and whether it had alignments to any other plant sequences (described as worrisome contamination). The second file (18S-analysis-Scaffold-Results.xlsx), has more details listing each scaffold identified as being an 18S sequence, and which reference sequence it matched against.

4. The cross-contamination details. A summary file (Cross-contamination-Details.xlsx) includes a table (sheet Contamination Frequencies) with the number of contaminants, number of non-contaminant sequences, and the number of sequences inferred to be contaminants in other taxa for each sequence library.. Also included (sheet Contaminant Pairs) is a list of each pair of contaminant sequences identified, with the first column showing the contaminant sequence, and the second column the sequence corresponding to the orthologous contaminating partner against which the sequence was identified. Also included is a list of taxonomically close sample pairs which were not compared (sheet Excluded Taxa). Clean and contaminant FASTA sequence files for each library are available in the accompanying data (1kp_decontamination_libraries.gz.zip).

Declarations

The authors declare that they have no conflicting interests, and that they believe that all the plant tissues were collected in accordance with applicable regulations and laws.

References

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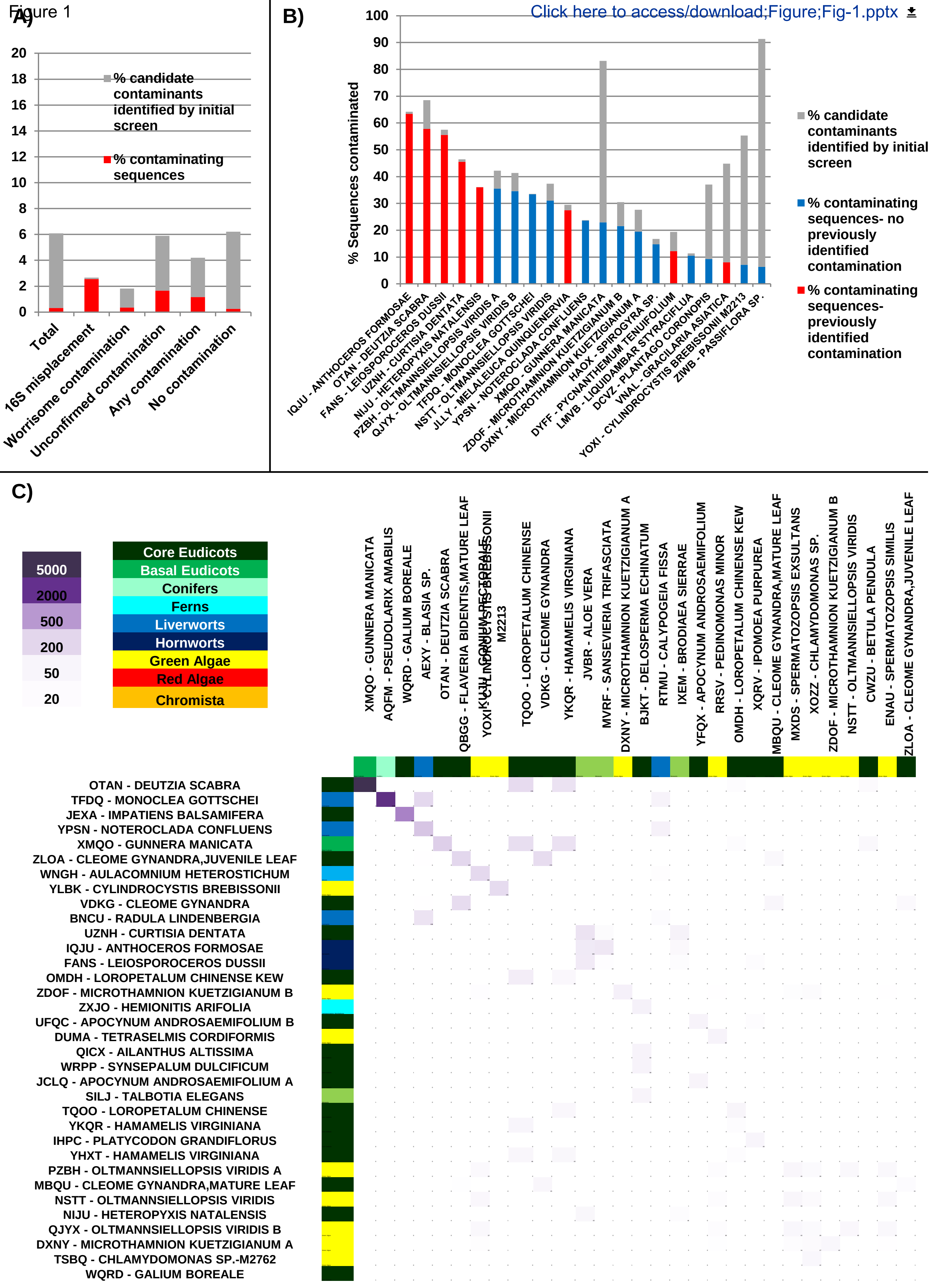
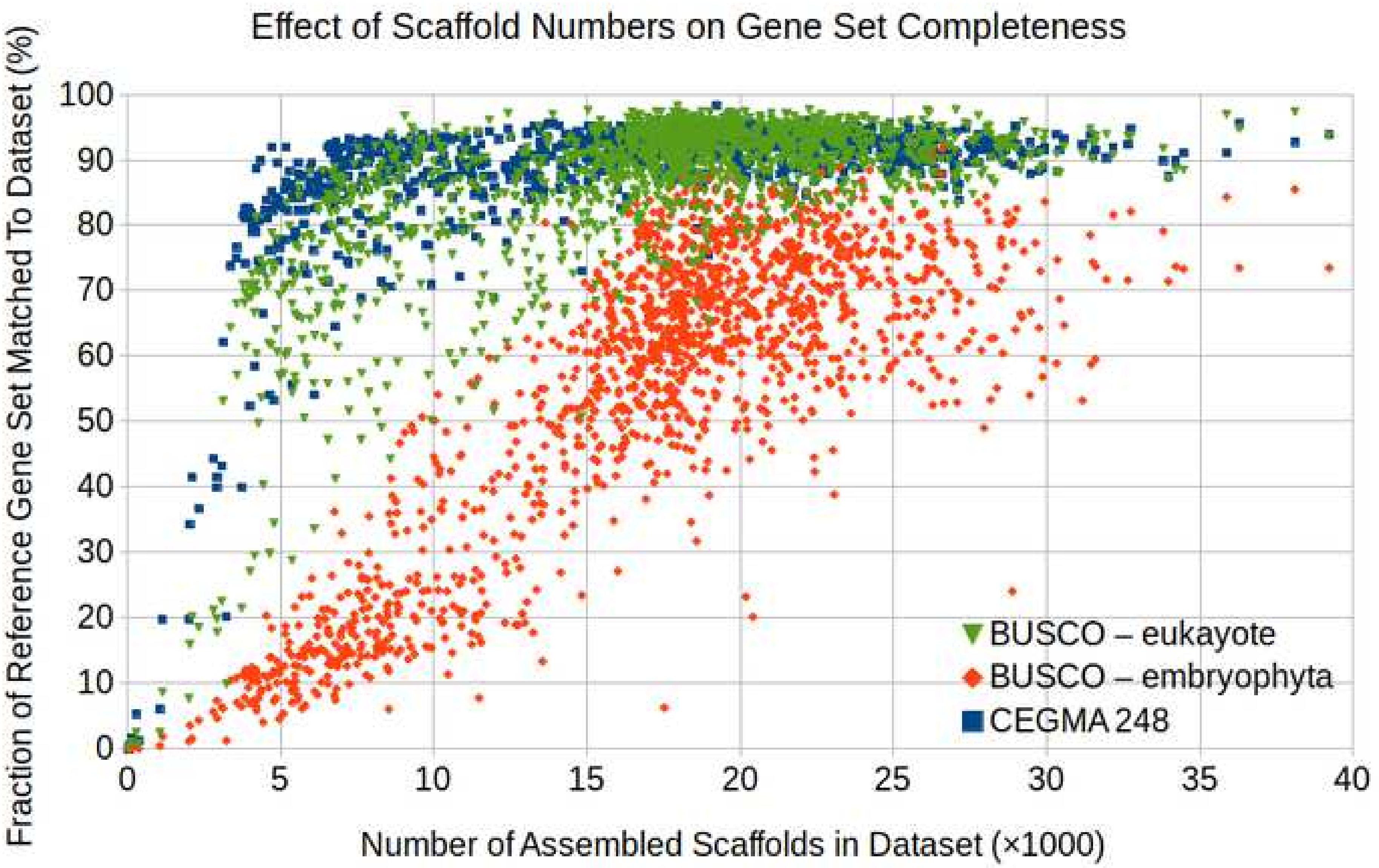
Figure Captions:

Fig. 1. Panel A provides an overview of the total sequence percentage verified to be of contaminant origin (red), or inferred to be possible contaminants in other sequence libraries (grey) in all 1KP libraries, and libraries inferred to be contaminated through the 18S phylogenetic placement. Panel B lists 21 libraries in which more than 6% of the total sequences are potential contaminants. Panel C shows a heatmap of inferred contaminant interactions between pairs of species; contaminated species are shown on the vertical axis, and contaminating species on the horizontal axis.

Fig. 2. Fraction of the gene sets found (complete + fragments) versus the number of scaffolds (300+ bp) in the assemblies. For each sample, the fraction of the eukaryota and embryophyta sets found in the assemblies are calculated with BUSCO and the fraction of the CEGMA 248 set with the CRBB tool. All three sets are more completely recovered at higher scaffold counts, but the BUSCO embryophyta set is less complete in our samples.

Figure 1


A)
20
18
16
14
12
10
8
6
4
2
0
% candidate contaminants identified by initial screen
% contaminating sequences
Total
16S misplacement
Worrisome contamination
Unconfirmed contamination
Any contamination
No contamination
B)
100
90
80
70
60
50
40
30
20
10
0
% Sequences contaminated
IQJU - ANTHOCEROS FORMOSAE
OTAN - DEUTZIA SCABRA
FANS - LEIOSPOROCEROS DUSSII
UZNH - CURTISIA DENTATA
NIJU - HETEROPYXIS NATALENSIS
PZBH - OLTMANNSIELLOPSIS VIRIDIS A
QJYX - OLTMANNSIELLOPSIS VIRIDIS B
TFDQ - MONOCLEA GOTTSCHEI
NSTT - OLTMANNSIELLOPSIS VIRIDIS
JLLY - MELALEUCA QUINQUENERVIA
YPSN - NOTEROCLADA CONFLUENS
XMQO - GUNNERA MANICATA
ZDOF - MICROTHAMNION KUETZIGIANUM B
DXNY - MICROTHAMNION KUETZIGIANUM A
HAOX - SPIROGYRA SP.
DYFF - PYCNANTHEMUM TENUIFOLIUM
LMVB - LIQUIDAMBAR STYRACIFLUA
DCVZ - PLANTAGO CORONOPIS
VNAL - GRACILARIA ASIATICA
YOXI - CYLINDROCYSTIS BREBISSONII M2213
ZIWB - PASSIFLORA SP.
% candidate contaminants identified by initial screen
% contaminating sequences- no previously identified contamination
% contaminating sequences- previously identified contamination
C)
5000
2000
500
200
50
20
Core Eudicots
Basal Eudicots
Conifers
Ferns
Liverworts
Hornworts
Green Algae
Red Algae
Chromista
XMQO - GUNNERA MANICATA
AQFM - PSEUDOLARIX AMABILIS
WQRD - GALIUM BOREALE
AEXY - BLASIA SP.
OTAN - DEUTZIA SCABRA
QBGG - FLAVERIA BIDENTIS,MATURE LEAF
KJJH - GONIUM PECTORALE
YOXI - CYLINDROCYSTIS BREBISSONII M2213
TQOO - LOROPETALUM CHINENSE
VDKG - CLEOME GYNANDRA
YKQR - HAMAMELIS VIRGINIANA
JVBR - ALOE VERA
MVRF - SANSEVIERIA TRIFASCIATA
DXNY - MICROTHAMNION KUETZIGIANUM A
BJKT - DELOSPERMA ECHINATUM
RTMU - CALYPOGEIA FISSA
IXEM - BRODIAEA SIERRAE
YFQX - APOCYNUM ANDROSAEMIFOLIUM
RRSV - PEDINOMONAS MINOR
OMDH - LOROPETALUM CHINENSE KEW
XQRV - IPOMOEA PURPUREA
MBQU - CLEOME GYNANDRA,MATURE LEAF
MXDS - SPERMATOZOPSIS EXSULTANS
XOZZ - CHLAMYDOMONAS SP.
ZDOF - MICROTHAMNION KUETZIGIANUM B
NSTT - OLTMANNSIELLOPSIS VIRIDIS
CWZU - BETULA PENDULA
ENAU - SPERMATOZOPSIS SIMILIS
ZLOA - CLEOME GYNANDRA,JUVENILE LEAF
OTAN - DEUTZIA SCABRA
TFDQ - MONOCLEA GOTTSCHEI
JEXA - IMPATIENS BALSAMIFERA
YPSN - NOTEROCLADA CONFLUENS
XMQO - GUNNERA MANICATA
ZLOA - CLEOME GYNANDRA,JUVENILE LEAF
WNGH - AULACOMNIUM HETEROSTICHUM
YLBK - CYLINDROCYSTIS BREBISSONII
VDKG - CLEOME GYNANDRA
BNCU - RADULA LINDENBERGIA
UZNH - CURTISIA DENTATA
IQJU - ANTHOCEROS FORMOSAE
FANS - LEIOSPOROCEROS DUSSII
OMDH - LOROPETALUM CHINENSE KEW
ZDOF - MICROTHAMNION KUETZIGIANUM B
ZXJO - HEMIONITIS ARIFOLIA
UFQC - APOCYNUM ANDROSAEMIFOLIUM B
DUMA - TETRASELMIS CORDIFORMIS
QICX - AILANTHUS ALTISSIMA
WRPP - SYNSEPALUM DULCIFICUM
JCLQ - APOCYNUM ANDROSAEMIFOLIUM A
SILJ - TALBOTIA ELEGANS
TQOO - LOROPETALUM CHINENSE
YKQR - HAMAMELIS VIRGINIANA
IHPC - PLATYCODON GRANDIFLORUS
YHXT - HAMAMELIS VIRGINIANA
PZBH - OLTMANNSIELLOPSIS VIRIDIS A
MBQU - CLEOME GYNANDRA,MATURE LEAF
NSTT - OLTMANNSIELLOPSIS VIRIDIS
NIJU - HETEROPYXIS NATALENSIS
QJYX - OLTMANNSIELLOPSIS VIRIDIS B
DXNY - MICROTHAMNION KUETZIGIANUM A
TSBQ - CHLAMYDOMONAS SP.-M2762
WQRD - GALIUM BOREALE


Effect of Scaffold Numbers on Gene Set Completeness
Fraction of Reference Gene Set Matched To Dataset (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
Number of Assembled Scaffolds in Dataset (×1000)
▼ BUSCO – eukayote
◆ BUSCO – embryophyta
■ CEGMA 248