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Pervasive, genome-wide transcription in the organelle genomes of diverse plastid-bearing protists

Investigation

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Abstract

Organelle genomes are among the most sequenced kinds of chromosome. This is largely because they are small and widely used in molecular studies, but also because next-generation sequencing (NGS) technologies made sequencing easier, faster, and cheaper. However, studies of organelle RNA have not kept pace with those of DNA, despite huge amounts of freely available eukaryotic RNA-sequencing (RNA-seq) data. Little is known about organelle transcription in non-model species, and most of the available eukaryotic RNA-seq data have not been mined for organelle transcripts. Here, we use publicly available RNA-seq experiments to investigate organelle transcription in 30 diverse plastid-bearing protists with varying organelle genomic architectures. Mapping RNA-seq data to organelle genomes revealed pervasive, genome-wide transcription, regardless of the taxonomic grouping, gene organization, or non-coding content. For every species analyzed, transcripts covered at least 85% of the mitochondrial and/or plastid genomes (all of which were ≤105 kb), indicating that most of the organelle DNA—coding and non-coding—is transcriptionally active. These results follow earlier studies of model species showing that organellar transcription is coupled and ubiquitous across the genome, requiring significant downstream processing of polycistronic transcripts. Our findings suggest that non-coding organelle DNA can be transcriptionally active, raising questions about the underlying function of these transcripts and underscoring the utility of publicly available RNA-seq data for recovering complete genome sequences. If pervasive transcription is also found in bigger organelle genomes (>105 kb) across a broader range of eukaryotes, this could indicate that non-coding organelle RNAs are regulating fundamental processes within eukaryotic cells.

Introduction

Mitochondrial and plastid DNAs (mtDNA and ptDNAs) are among the most sequenced and best-studied types of chromosome (Smith 2016). This is not surprising given the widespread use of organelle genome data in forensics, archaeology, phylogenetics, biotechnology, medicine, and other scientific disciplines. Unfortunately, investigations of organelle RNA have not kept pace with those of the DNA, and for most non-model species there are little or no published data on organelle transcription (Sanitá Lima et al. 2016). But this is poised to change.

Next generation sequencing (NGS) technologies, ballooning genetic databanks, and new bioinformatics tools have made it easier, faster, and cheaper to sequence, assemble, and analyze organelle transcriptomes (Smith 2016). The National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), for example, currently houses tens of thousands of freely available eukaryotic RNA sequencing (RNA-seq) datasets (Kodam et al. 2012), hundreds of which come from non-model species and/or poorly studied lineages (Keeling et al. 2014). Among their many uses, these data have proven to be a goldmine for mitochondrial and plastid transcripts (Smith 2013; Shi et al. 2016; Tian and Smith 2016).

Recently, researchers have started mining the SRA for organelle-derived reads, and already these efforts have yielded interesting results, such as pervasive organelle transcription—i.e., transcription of the entire organelle genome, including coding and non-coding regions (Shi et al. 2016; Tian and Smith 2016). This kind of research has been further aided by a range of new bioinformatics tools designed for the assembly,

83 annotation, and analysis of organelle genomes and transcriptomes from NGS data
84 (Castandet et al. 2016; Dierckxsens 2016; Soorni 2017). Nevertheless, most of the
85 eukaryotic RNA-seq data within the SRA have not been surveyed for organelle
86 transcripts, particularly those from plastid-bearing protists, and it is not known if
87 pervasive organelle transcription is a common theme among diverse eukaryotic groups. If
88 it is, then RNA-seq could presumably be used to glean complete or near-complete
89 organelle genomes in the presence *or* absence of DNA data, which would be particularly
90 useful, for example, in cases where there are abundant RNA-seq data but no available
91 DNA information.

92 It goes without saying that the complexities of organelle transcription cannot be
93 unravelled solely via *in silico* RNA-seq analyses (Sanitá Lima et al. 2016). Indeed,
94 organelle gene expression is surprisingly complex and often highly convoluted (Moreira
95 et al. 2012), as anyone who has studied the mtDNA of *Trypanosome* spp. (Feagin et al.
96 1988) or the ptDNA of *Euglena gracilis* (Copertino et al. 1991) can attest. If organelle
97 transcriptional research has taught us anything over the past few decades, it is that even
98 the seemingly simplest mtDNAs and ptDNAs can have unexpectedly complicated
99 transcriptomes and/or modes of gene expression (Feagin et al. 1988; Copertino et al.
100 1991; Marande and Burger 2007; Masuda et al. 2010; Vlcek et al. 2011; Lang et al. 2014;
101 Valach et al. 2014; Smith and Keeling 2016). Moreover, accurately and thoroughly
102 characterizing organelle transcriptional architecture can take years of detailed laboratory
103 work using an assortment of techniques (Marande et al. 2005; Nash et al. 2007; Barbrook
104 et al. 2012; Feagin et al. 2012; Jackson et al. 2012; Mungpakdee et al. 2014; Dorrell and
105 Howe 2015). That said, RNA-seq is a quick and cost-effective starting point for early

exploratory work of organelle transcription, and it can help identify lineages or species with particularly bizarre or unconventional transcriptional architectures.

Here, we use publically available RNA-seq data to survey mitochondrial and plastid transcription in a variety of eukaryotic algae. To streamline and simplify our analyses, we focus specifically on species for which the mitochondrial and/or plastid genomes have been completely sequenced and are not overly long (≤105 kb). Our explorations reveal pervasive, genome-wide organelle transcription among disparate plastid-bearing protists and highlight the potential of publically available RNA-seq data for organelle research.

Materials and Methods

By scanning the SRA (using NCBI's Taxonomy Browser), we identified 30 plastid-bearing species for which there are complete mitochondrial and/or plastid genome sequences and abundant RNA-seq data. We downloaded the RNA-Seq reads from the SRA (https://www.ncbi.nlm.nih.gov/sra) and the organelle DNAs from the Organelle Genome Resources section of NCBI (https://www.ncbi.nlm.nih.gov/genome/organelle/) or GenBank (https://www.ncbi.nlm.nih.gov/genbank/). See Table S1 for detailed information on the RNA-seq and organelle genome data we downloaded, including accession numbers, sequencing technologies, read counts, organelle DNA features, and the strains used for genome and RNA sequencing.

We mapped the RNA-Seq reads to the corresponding organelle genomes using Bowtie 2 (Langmead and Salzberg 2012) implement through Geneious v9.1.6 (Biomatters Ltd., Auckland, NZ), a user-friendly, commercial bioinformatics software

suite, which contains a graphical user interface (Kearse et al. 2012). All mapping experiments were carried out using default settings, the highest sensitivity option, and a min/max insert size of 50 nt/750 nt; we also allowed each read to be mapped to two locations to account for repeated regions, which are common in organelle genomes (Smith and Keeling 2015). The mapping histograms shown in Figures 2–4 were extracted from Geneious.

Data availability

The datasets analysed in this study are available in the SRA – Sequence Reads Archive – database (https://www.ncbi.nlm.nih.gov/sra/) and their respective accession numbers are listed in Table S1. Figure S1 depicts transcription maps for all 30 species analysed.

Results and Discussion

Little genome, big RNA: genome-wide, polycistronic transcription in algal organelle DNAs

After an exhaustive search of GenBank and the SRA, we identified 30 plastid-bearing protists for which there were abundant RNA-seq data as well as complete mtDNA and/or ptDNA sequences with lengths of ~100 kb or smaller. We did not include larger organelle DNAs because we wanted to reconstruct entire organelle genomes from the transcript data alone and assumed that it would be easier to do so using RNA from small to moderately sized organelle genomes. Moreover, organelle DNAs greater than 100 kb are typically repeat rich (Smith and Keeling 2015), making RNA-seq mapping much more challenging and error-prone (Treangen and Salzberg 2011). Nonetheless, the

30 species we analyzed span the gamut of plastid-containing eukaryotic diversity, and include taxa with primary plastids and eukaryote-eukaryote-derived (i.e., “complex”) plastids (Keeling 2013) as well as those with ptDNA-containing nonphotosynthetic plastids, such as apicomplexan parasites (Table 1, Figure 1, Table S1 and Figure S1). The organelle genomic architectures of these species vary in structure (e.g., linear- vs. circular-mapping), size (5.8–105 kb), gene repertoire (e.g., gene rich vs. gene poor), gene arrangement (e.g., intact vs. fragmented genes), and coding content (e.g., ~7.5-95%) (Table 1, Figures 2–4, Table S1 and Figure S1). We made sure that the RNA-seq and corresponding organelle genome data always came from the same species, but, in a few instances, they were from different strains of the same species (Table S1). It should be stressed that most of the RNA-seq experiments we sourced were generated under stress-related conditions and often using very different protocols (Table S1). But these caveats did not seem to impede the mapping experiments.

Indeed, for each of the species and genomes we explored, the raw RNA-seq reads covered the entire or nearly entire organelle DNA, regardless of taxonomic grouping, organelle type (i.e., mtDNA vs. ptDNA), or underlying genomic architecture (Table 1, Figure 1, Table S1 and Figure S1). Not only was the overall read coverage high across the various mitochondrial and plastid genomes (85-100%), but the mean read depth (reads/nt), with few exceptions, was consistently high, ranging from 5 to >23,000 (Table 1). Assuming the RNA-seq reads that mapped correspond to bona fide organelle-derived transcripts (see below), these findings suggest that transcription is pervasive, spanning most or all of the organelle genome, including non-coding regions, in a diversity of plastid-bearing protists.

Close inspection of the RNA-seq mapping results revealed some interesting trends within and among the various lineages and genomes (Figures 2–4). As expected, the overall RNA read coverage was particularly high (93–100% of the reference genome) for the miniature and highly compact mtDNAs of the five apicomplexan parasites in our dataset (Figure 2), and when applicable (e.g., *Babesia bovis*) it extended into and encompassed the entire mitochondrial telomeres, as has been observed for linear mtDNAs from other lineages (Tian and Smith 2016). These results are consistent with earlier work on apicomplexans showing that their mitochondrial genomes are transcribed in a polycistronic manner (Ji et al. 1996; Rehkopf et al. 2000), and reinforce the notion that mitochondrial telomeres are involved in gene expression.

The RNA-seq data of the circular-mapping mtDNAs from the green alga *Chlamydomonas moewusii*, the glaucophyte alga *Cyanophora paradoxa*, and the stramenopile alga *Heterosigma akashiwo* are also consistent with a polycistronic mode of transcription, revealing deep, genome-wide RNA coverage across most of the chromosomes, including intergenic regions (Figure 3). Full transcription also appears to be occurring in the mtDNAs from other major algal groups, including brown algae (e.g., *Fucus vesiculosus*), red algae (e.g., *Porphyra purpurea*), dinoflagellate algae (e.g., *Symbiodinium minutum*), and diatom algae (e.g., *Pseudo-nitzschia multiseries*), as well as in both compact and moderately bloated mtDNAs (57–90% coding) (Table 1, Table S1 and Figure S1).

Almost identical trends were observed for the plastid genome data, all of which showed 85.5–100% RNA coverage and a mean read depth of 72–5,524 (Table 1, Figure 4). Like with the mtDNAs, the overall RNA-seq read coverage was especially high for

small, compact ptDNAs, such as those from apicomplexan parasites (e.g., *Toxoplasma gondii*) (Table 1) and that of the nonphotosynthetic green alga *Helicosporidium* sp. (~37 kb; ~95% coding), 98% of which was represented at the RNA level (Figure 4). The secondary, red-algal-derived plastid genomes of the photosynthetic chromerid *Vitrella brassicaformis* and the haptophyte *Emiliana huxleyi* were also well represented in the RNA reads (100% and 97% coverage, respectively – Figure 4), as were those of *C. moewusii* and *H. akashiwo* (Table 1, Table S1 and Figure S1). Overall, these data, alongside previous experiments (Mercer et al. 2011; Zhelyazkova et al. 2012; Shoguchi et al. 2015; Shi et al. 2016; Tian and Smith 2016), show that pervasive polycistronic transcription is the norm rather than the exception among mtDNAs and ptDNAs, and underscore the usefulness of RNA-seq for recovering whole organelle genomes, which can then be used in an array of downstream applications, such as for phylogenetic analyses, barcoding, or measuring nucleotide diversity within and among populations.

RNA-seq: an untapped resource for organelle research

None of the RNA-seq datasets employed here were initially generated with the intent of studying organelle transcription, and to the best of our knowledge we are the first group to mine organelle transcripts from these experiments. Most, if not all, of the NGS data used here were produced for investigating nuclear gene expression. For instance, the stramenopile alga *Nannochloropsis oceanica* is a model candidate for harvesting biofuels and, thus, the currently available RNA-seq experiments for this species are aimed at better understanding its growth and lipid production, and maximizing its economic potential (Li et al. 2014). The same can be said for many of the other species we investigated, such as the seaweeds *Undaria pinnatifida* and *Saccharina*

219 *japonica*, which are harvested for food (Shan et al. 2015, Ye et al. 2015), and the
220 apicomplexans *Babesia* sp. and *Theileria* sp., which parasitize livestock (Gardner et al.
221 2005; Brayton et al. 2007).

222 Most scientists do not have the time, resources, or expertise to explore every
223 aspect of an NGS dataset, especially when considering the prodigious amount of
224 information that can be contained within one. But if more scientists knew how easy it was
225 to mine organelle transcriptomes from RNA-seq data, they might be more inclined to
226 study various aspects of organelle genetics, even if it was merely collecting a few
227 sequences for building a phylogenetic tree or for barcoding (Smith 2013). And one
228 cannot forget that organelle biology is intimately tied to that of the nucleus—to fully
229 understand the latter one needs to study the former, and vice versa (Woodson and Chory
230 2008).

231 As shown here, and elsewhere (Shi et al. 2016; Tian and Smith 2016), complete
232 organelle genomes can be easily and quickly reconstructed from NGS experiments,
233 provided that these experiments were generated in a way that did not exclude organelle
234 transcripts from the sequencing libraries. In some instances, only a single RNA-seq
235 dataset was needed to successfully recover an entire organelle transcriptome—we
236 recovered 99.4% of the *Pavlova lutheri* plastid genome from one 6.7 Gb paired-end
237 RNA-seq experiment. In other cases, we had to source multiple transcriptomic
238 experiments to recover the complete organelle genome (Table S1), suggesting that the
239 libraries used for the cDNA sequencing were depauperate in organelle-derived
240 transcripts. This could be because RNA-seq libraries are often filtered for polyadenylated

241 transcripts (mRNA) and in some lineages organelle RNA can become unstable upon
242 polyadenylation (Rorbach et al. 2014). Other library preparation techniques, however, are
243 much more organelle friendly, including those that target non-coding nuclear RNAs (Di
244 et al. 2014) as well as those catered to total cellular RNA (Hotto et al. 2011).

245 One must be careful not to overstate or exaggerate the usefulness of online RNA-
246 seq data for organelle research. There are limitations to what can be deduced about gene
247 expression from the mapping or *de novo* assembly of sequencing reads. Moreover, NGS
248 data downloaded from public databanks can have little or no accompanying information
249 about how they were generated, leaving users guessing about the underlying experimental
250 conditions. And this is to say nothing about the problems of combining and comparing
251 RNA-seq data that were generated by different laboratory groups and/or using different
252 protocols. These factors prevented us from carrying out experiments comparing the
253 mapping rates among datasets with different RNA-selection protocols (e.g. poly-A versus
254 rRNA depletion). There is also a danger of confusing the transcripts of nuclear
255 mitochondrial-like sequences (NUMTs) and nuclear plastid-like sequences (NUPTs) for
256 genuine organelle RNA, but this is less of an issue for protists than it is for animals and
257 land plants (Smith et al. 2011). Finally, there is always the possibility of genomic DNA
258 contamination within the cDNA library, even after multiple rounds of DNase treatment
259 (Haas et al. 2012), but this is an issue affecting all types of RNA-seq analyses, not just
260 those exploring organelle RNA.

261 Despite these drawbacks, scouring RNA-seq databases can reveal important
262 features about organelle transcriptional architecture, such as splice variants, post-

263 transcriptional processing, and RNA editing (Castandet et al. 2016) — or the absence of
264 such features. For example, there were no signs of substitutional or insertion/deletion
265 RNA editing in any of the organelle genomes we investigated, but we did detect putative
266 polycistronic processing sites (Figure 3 and Figure 4). RNA-seq has also helped identify
267 transcriptional start sites in the plastid genome of barley (Zhelyazkova et al. 2012) and
268 whole-genome transcription in land plant ptDNAs (Shi et al. 2016). Although not
269 employed in this study, differential (d)RNA-seq and strand-specific (ss)RNA-seq can
270 provide an even deeper resolution of organelle transcription, exposing antisense RNAs
271 and small non-coding RNAs (Mercer et al. 2011; Zhelyazkova et al. 2012). As more
272 dRNA-seq and ssRNA-seq experiments are deposited in the SRA (mostly from model
273 species), they can be used to examine fine-tuned features of organelle gene expression
274 using a similar approach to that taken here.

275 An emerging and recurring theme from organelle transcriptional studies
276 (including this one) is that mitochondrial and plastid genomes are pervasively transcribed
277 (Mercer et al. 2011; Zhelyaskova et al. 2012; Dietrich et al. 2015; Shoguchi et al. 2015;
278 Shi et al. 2016; Tian and Smith 2016). This is also true for the genomes of
279 alphaproteobacteria and cyanobacteria (Landt et al. 2008; Schlüter et al. 2010; Mitschke
280 et al. 2011; Mitschke, Vioque et al. 2011; Shi et al. 2016), suggesting that pervasive
281 organelle transcription is an ancestral trait passed down from the bacterial progenitors of
282 the mitochondrion and plastid (Shi et al. 2016). Many nuclear genomes also show
283 pervasive transcription (Berretta and Morillon 2009), including those of *Saccharomyces*
284 *cerevisiae* (David et al. 2006), *Drosophila melanogaster* (Stolc et al. 2004), *Oryza sativa*
285 (Li et al. 2006), and *Mus musculus* (Carninci et al. 2005). It is estimated that up to ~75%

286 of the human nuclear genome can be transcriptionally active when looking across tissues
287 and subcellular compartments (Djebali et al. 2012). In fact, the more we study genome-
288 wide transcription, the more we realize that few regions in a genome are entirely exempt
289 from transcription and that genomes are veritable 'RNA machines', producing multiple
290 types of RNA from end to end (Amaral et al. 2008; Wade and Grainger 2014). Some
291 have suggested that pervasive transcription can provide raw RNA material for new
292 regulatory pathways (Libri 2015). However, certain bacteria can repress pervasive
293 transcription (Lasa et al. 2011; Singh et al. 2014), so obviously it is not a good strategy
294 all of time, at least in some systems.

295 It remains to be seen if big (>>100 kb) organelle genomes, such as land plant
296 mtDNAs (Sloan et al. 2012) and chlamydomonadalean ptDNAs (Featherston et al. 2016),
297 are fully transcribed, but preliminary work suggests that they are. RNA-seq analyses
298 revealed complete transcription of the *Symbiodinium minutum* mtDNA (~327 kb)
299 (Shoguchi et al. 2015), *Chlamydomonas reinhardtii* ptDNA (~204 kb), and other bloated
300 organelle DNAs (Shi et al. 2016). Therefore, unravelling pervasive transcription in small
301 and giant organelle genomes across eukaryotes could indicate that non-coding organelle
302 RNAs actually have important, undescribed functions. One should be careful not to
303 mistake transcription for function (Doolittle 2013) and not underestimate transcriptional
304 noise (Struhl 2007), but non-coding organelle RNAs (both long and short) are known to
305 carry out crucial regulatory functions (Hotto et al. 2011; Small et al. 2013; Dietrich et al.
306 2015). Perhaps having more non-coding DNA and therefore more non-coding RNA leads
307 to increased regulatory control of certain metabolic pathways within organelles (e.g.,
308 those for the development of different plastids in land plants [Jarvis and López-Juez

2013]) or more fine-tuned responses to environmental conditions (e.g., changing trophic strategies in mixotrophic algae [Worden et al. 2015]). But if so, why is there such a massive variation in organelle genome size (and transcriptome size) within and among lineages (Khaitovich et al. 2004; Lynch et al. 2006; Smith and Keeling 2015; Smith 2016; Figueroa-Martinez et al. 2017a; Figueroa-Martinez et al. 2017b)? Alas, there is still a lot to be learned about organelle gene expression, and thankfully online RNA-seq data are here to help pave the way.

Conclusions

The primary goal of this study was to show that entire organelle genome sequences from diverse plastid-containing species can be reconstructed from publically available RNA-seq datasets within the SRA, as has been previously argued (Smith 2013). On this front, we were successful: algal mtDNAs and ptDNAs from disparate lineages consistently undergo full or nearly full transcription. Thus, available RNA-seq data are an excellent starting point and an untapped resource for exploring transcriptomic and genomic architecture from poorly studied species. Nevertheless, online RNA-seq experiments have their limitations and drawbacks, and one should be mindful when employing such data. It will be interesting to see if the major trends reported here will be borne out by future investigations, specifically those of larger organelle genomes. Ultimately, a deep understanding of organelle gene expression requires a multi-pronged approach, employing both traditional molecular biology techniques as well as more modern high-throughput methods (Sanitá Lima et al. 2016).

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Figure Legends

Figure 1. Pervasive organelle genome transcription across the eukaryotic tree of life.
Organelle genomes ≤105 kb are fully or almost fully transcribed in diverse eukaryotic groups, regardless of their coding content and structure. Outer dashed boxes summarize the breadth of organelle genomes analysed within each major eukaryotic group. Representation of organelle genomes and organelles are not to scale. Refseq coverage represents the percentage of the reference genome sequence that was covered by one or more RNA-seq reads in the mapping analyses. Phylogenetic tree is adapted from (Burki 2014) for the relationships among major groups; branches within groups are merely illustrative and not based on sequence analyses. The tree was generated using the NCBI Common Tree taxonomy tool (Federhen 2012) and iTOL v3.4.3 (Letunic and Bork 2016).

Figure 2. Full transcription of small mitochondrial genomes in Apicomplexa.
Mapping histograms (or transcription maps) depict the coverage depth – number of transcripts mapped per nucleotide – on a log scale. We used the organelle genome annotations already present in the genome assemblies deposited in GenBank (accession numbers provided in Table 1 and Table S1). Mapping contigs are not to scale and direction of transcription is represented by the direction of the arrows – annotated genes. Mapping histograms were obtained from Geneious v9.1.6 (Kearse et al. 2012).

Figure 3. Polycistronic transcription in mitochondrial genomes of chlorophytes, raphidophytes, and glaucophytes.
Chlamydomonas moewusii (Chlorophyta), *Heterosigma akashiwo* (Raphidophyta) and *Cyanophora paradoxa* (Glaucophyta) exhibited clear drops of transcript coverage in some potentially non-coding regions (intergenic regions, intros and hypothetical proteins). Mapping histograms follow the same structure as in Figure 2 and mapping contigs are not to scale.

Figure 4. Entire and near entire transcriptional coverage of diverse plastid genomes.
Vitrella brassicaformis (Chromerida) exhibited entire genome transcription, whereas *Helicosporidium* sp (Chlorophyta) and *Emiliana huxleyi* (Haptophyta) had near entire genome transcriptional coverage. Drops in coverage happened mostly in intergenic regions of the *E. huxleyi* plastid genome. Mapping histograms follow the same structure as in Figure 2 and Figure 3; mapping contigs are not to scale.

Table 1 Diverse organelle (mitochondrial and plastid) genomes and their respective transcription rates (mean and percent coverage).

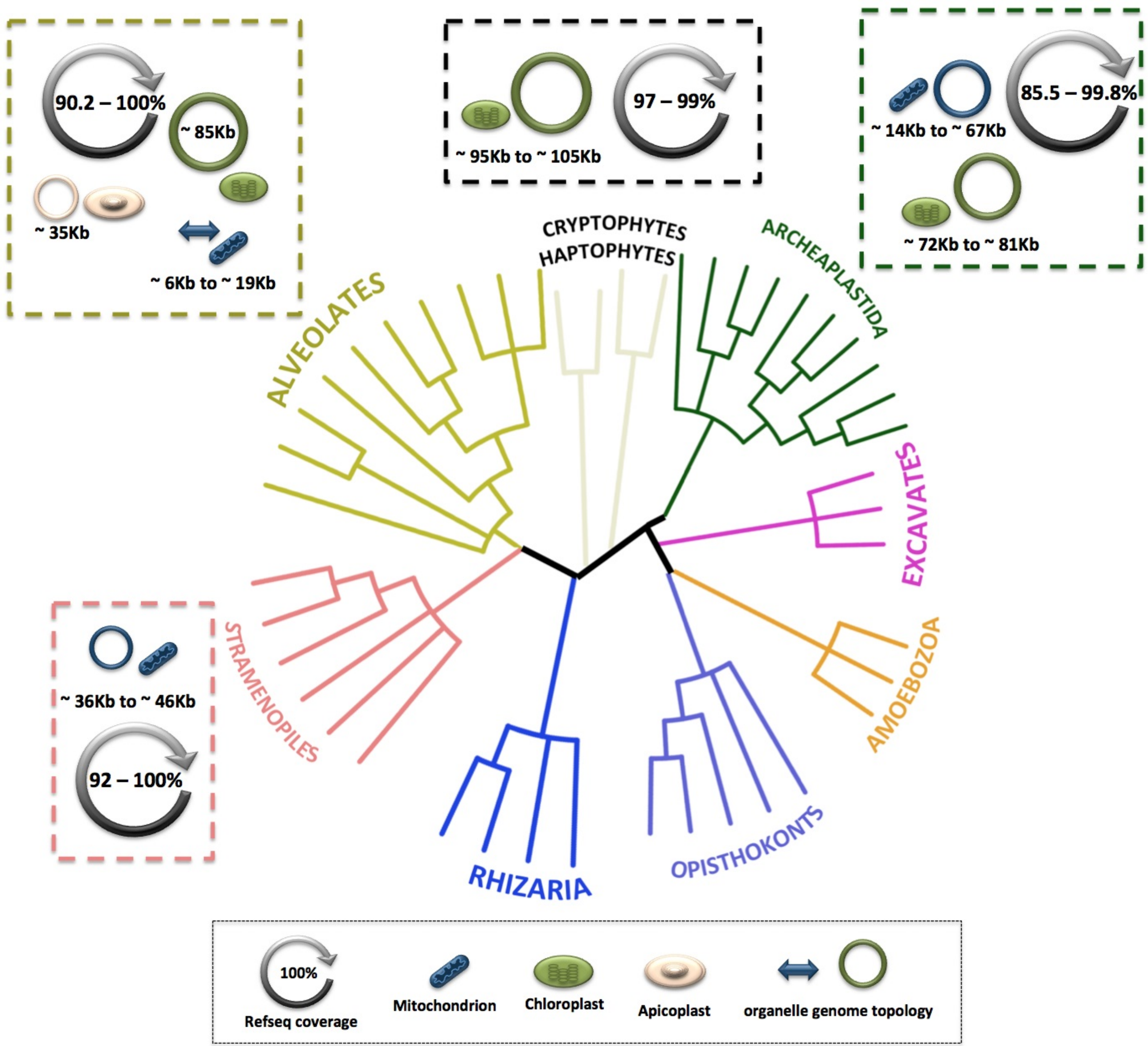
TAXONOMIC GROUP AND SPECIES	ORGANELLE	GENBANK ENTRY	GENOME SIZE (bp)	MEAN COVERAGE (reads/nt)	% REFSEQ[a]	% CODING[b]
API - *Theileria parva*	mt	NC_011005.1	5,895	710.934	99.7	67.5
API - *Plasmodium berghei*	mt	LK023131.1	5,957	3,111.87	100	92.4
API - *Plasmodium falciparum*	mt	AY282930.1	5,959	368.286	100	55.7
API - *Plasmodium vivax*	mt	NC_007243.1	5,990	693.631	100	56.3
API - *Babesia bovis*	mt	NC_009902.1	6,005	614.848	99.9	63.5
	api	NC_011395.1	35,107	71.60	90.2	54.1
API - *Babesia microti*	mt	LN871600.1	10,547	5.188	93.4	37
CP - *Chlamydomonas leiostraca*	mt	NC_026573.1	14,029	136.967	95.8	86.4
DF - *Symbiodinium minutum*	mt	LC002801	19,577	2,763.05	100	7.43
CP - *Chlamydomonas moewusii*	mt	NC_001872.1	22,897	59.767	86.7	55.4
CP - *Pycnococcus provasolii*	mt	GQ497137	24,321	2,942.35	99.8	87.7
PP - *Fucus vesiculosus*	mt	NC_007683.1	36,392	98.866	97.9	90
RP - *Porphyra purpurea*	mt	NC_002007.1	36,753	1,250.44	98.7	81.5
RP - *Pyropia haitanensis*	mt	NC_017751.1	37,023	24.413	85.6	63.2

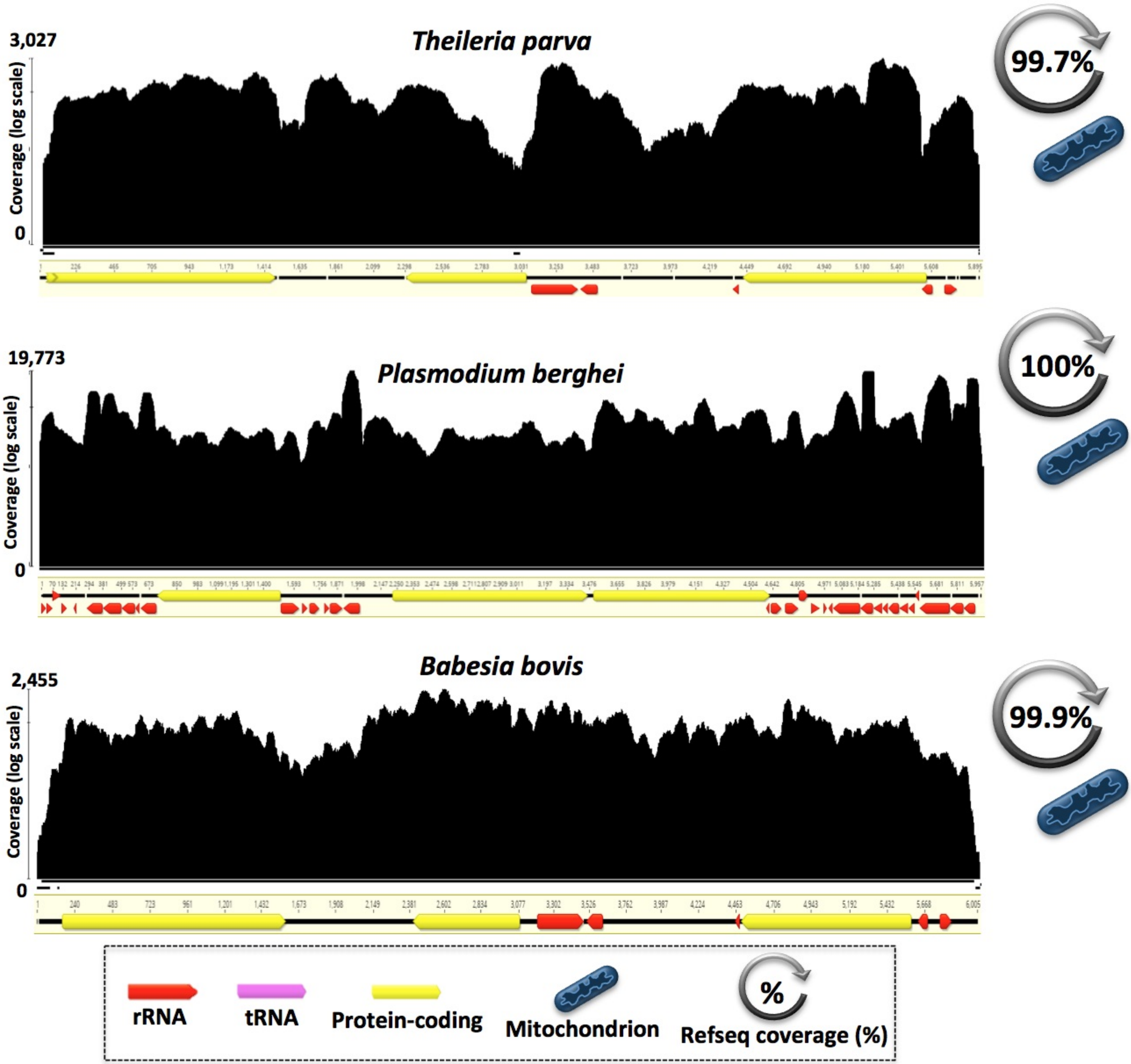
PP - *Undaria pinnatifida*	mt	NC_023354.1	37,402	165.098	92.8	89.9
PP - Saccharina japonica	mt	NC_013476.1	37,657	145.915	100	89.4
EP - *Nannochloropsis oceanica*	mt	NC_022258.1	38,057	118.754	95.8	88.8
RH - *Heterosigma akashiwo*	mt	NC_016738.1	38,690	205.219	98.5	81.3
RP - *Pyropia yezoensis*	mt	NC_017837.1	41,688	16.205	88	56.6
DT - *Pseudo-nitzschia multiseries*	mt	NC_027265.1	46,283	1,261.27	96.4	71.5
CP - *Micromonas commoda*	mt	NC_012643.1	47,425	180.623	94	82.5
CP - *Helicosporidium sp.*	mt	NC_017841.1	49,343	147.453	94.7	65
	pt	NC_008100.1	37,454	103.633	98	94.9
GP - *Cyanophora paradoxa*	mt	NC_017836.1	51,557	3,355.88	94.6	58.9
CP - *Chlorella sorokiniana*	mt	NC_024626.1	52,528	23,494.23	86.6	63
CA - *Chara vulgaris*	mt	NC_005255.1	67,737	24.862	94.2	52.3
CP - *Micromonas commoda*	pt	NC_012575.1	72,585	2,854.087	93.7	67.8
CP - *Picocystis salinarum*	pt	NC_024828.1	81,133	142.060	85.5	90.6
CR - *Vitrella brassicaformis*	pt	HM222968	85,535	5,523.59	100	88.5
HP - *Emiliana huxleyi*	pt	NC_007288.1	105,309	789.915	97	85.8
HP - *Pavlova lutheri*	pt	NC_020371.1	95,281	2,771.83	99.4	81
API - *Toxoplasma gondii*	apic	NC_001799.1	34,996	1,501.45	95	80.7

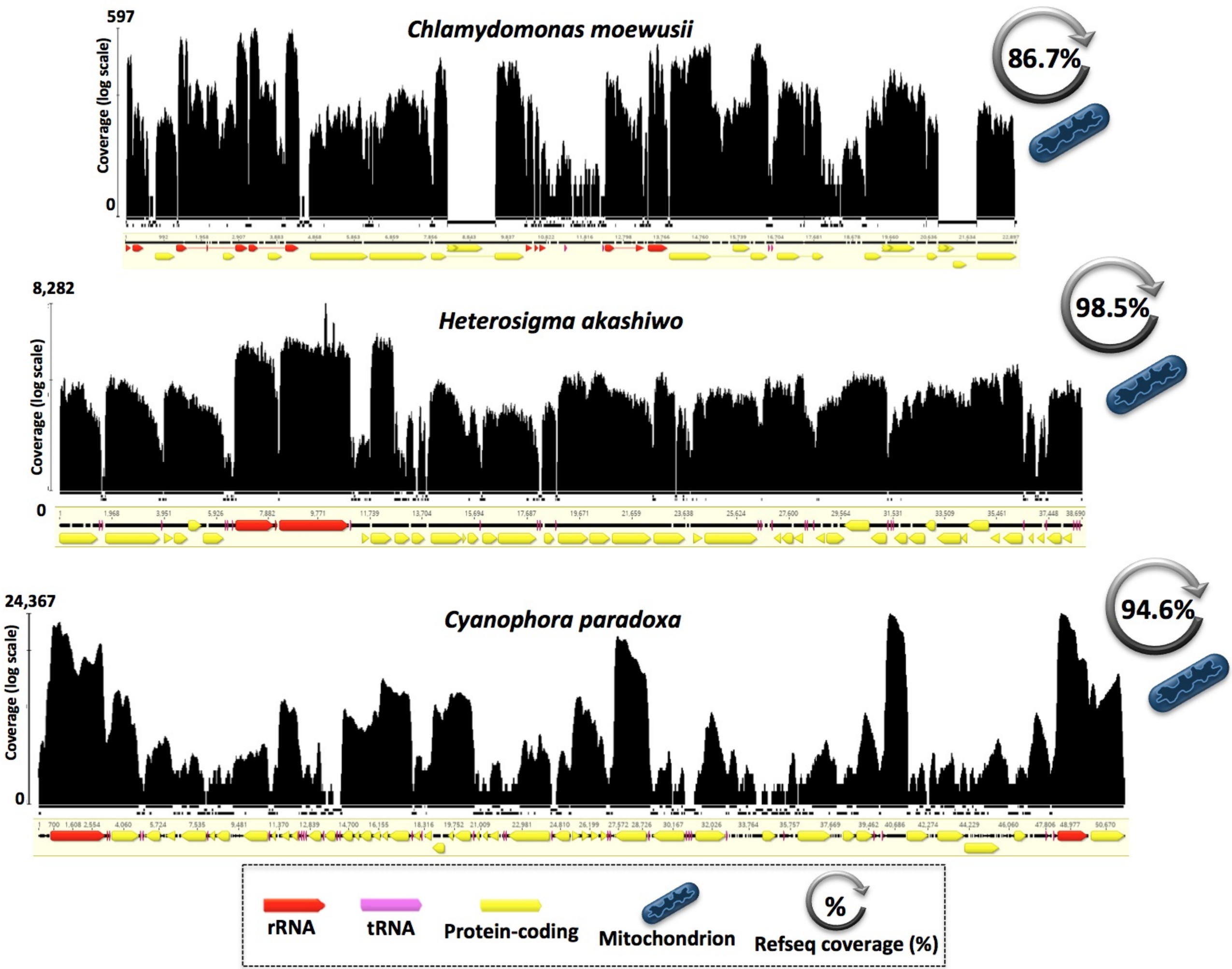
MT, mitochondrion; PT, plastid; APIC, apicoplast API, Apicomplexa; CP, Chlorophyta; DF, Dinoflagellates; PP, Phaeophyta; RP, Rhodophyta; EP, Eustigmatophytes; RH, Raphidophyta; DT, Diatoms; GP, Glaucophyta; CA, Charophyta; CR, Chromerida; HP, Haptophyta.

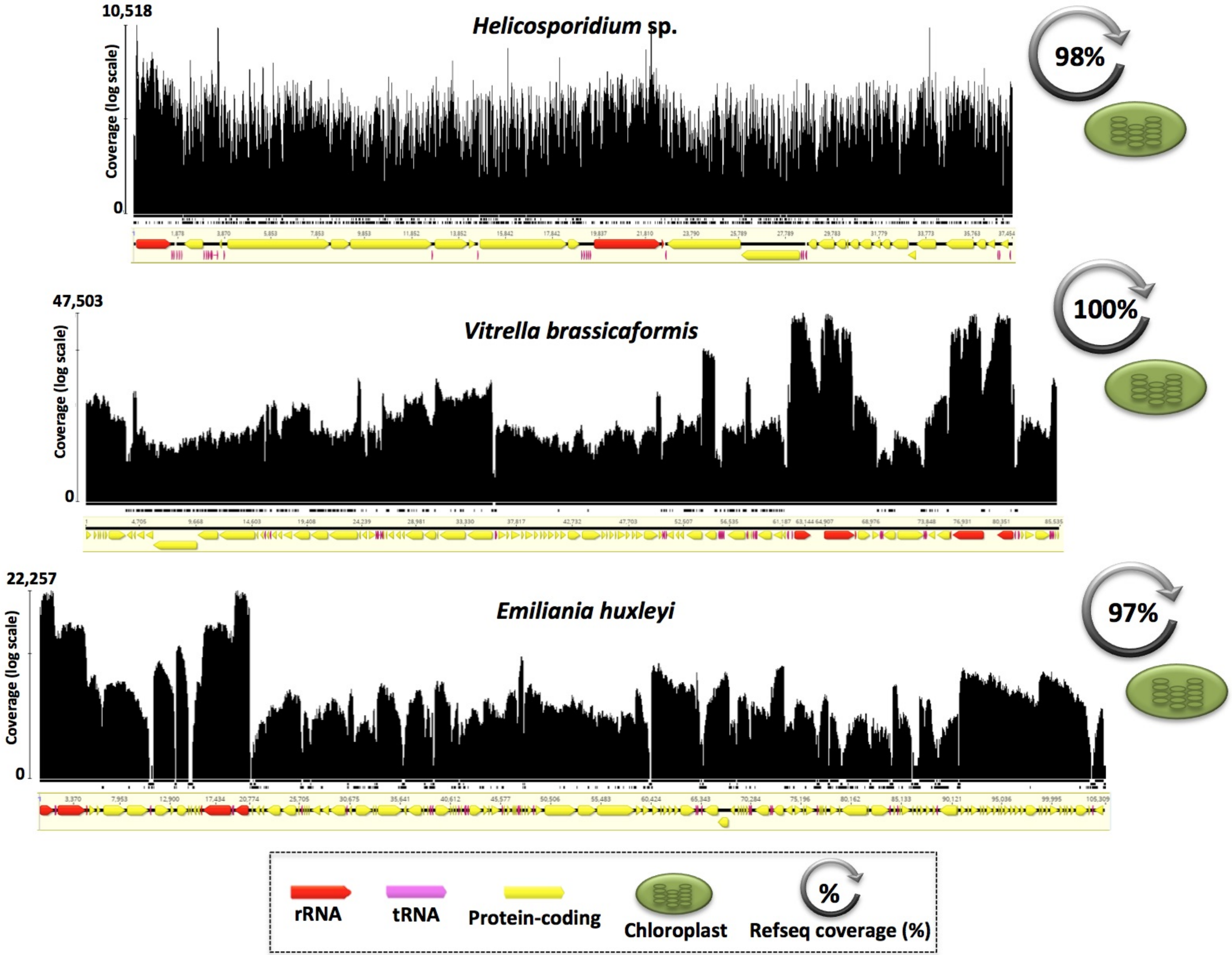
[a] Percentage of the reference genome sequence that is covered by one or more reads in the mapping contig.

[b] Percentage of the coding region (tRNA-, rRNA- and protein-coding genes) in the organelle genome. The "% coding" of each genome was determined for this study using the function "extract annotation" in Geneious. We extracted tRNA-, rRNA- and protein-coding (CDS) gene annotations, then excluded spurious annotations and calculated the final length of coding sequences altogether.


90.2 – 100%
~ 85Kb
~ 35Kb
~ 6Kb to ~ 19Kb
~ 95Kb to ~ 105Kb
97 – 99%
~ 14Kb to ~ 67Kb
85.5 – 99.8%
~ 72Kb to ~ 81Kb
CRYPTOPHYTES
HAPTOPHYTES
ARCHEAPLASTIDA
ALVEOLATES
EXCAVATES
AMOEBOZOA
STRAMENOPILES
RHIZARIA
OPISTHOKONTS
~ 36Kb to ~ 46Kb
92 – 100%
100%
Refseq coverage
Mitochondrion
Chloroplast
Apicoplast
organelle genome topology


Theileria parva
3,027
Coverage (log scale)
0
99.7%
Plasmodium berghei
19,773
Coverage (log scale)
0
100%
Babesia bovis
2,455
Coverage (log scale)
0
99.9%
rRNA
tRNA
Protein-coding
Mitochondrion
%
Refseq coverage (%)


Chlamydomonas moewusii
597
Coverage (log scale)
0
86.7%
Heterosigma akashiwo
8,282
Coverage (log scale)
0
98.5%
Cyanophora paradoxa
24,367
Coverage (log scale)
0
94.6%
rRNA
tRNA
Protein-coding
Mitochondrion
%
Refseq coverage (%)


10,518
Helicosporidium sp.
Coverage (log scale)
0
98%
47,503
Vitrella brassicaformis
Coverage (log scale)
0
100%
22,257
Emiliania huxleyi
Coverage (log scale)
0
97%
rRNA
tRNA
Protein-coding
Chloroplast
%
Refseq coverage (%)