CHAPTER THREE

Plastid Genomes in the Myzozoa

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Abstract

The myzozoa encompasses quite disparate protists, like the infamous apicomplexan parasites, or the famous dinoflagellate phytoplankton. Collectively, myzozoans display a wide diversity of plastids; they all most likely descended from a common myzozoan plastid ancestor. Some myzozoan plastids are photosynthetic whereas others are not; some have plastid genomes (plastomes) but others have lost them. The only two eukaryotes known to have lost plastids altogether are myzozoans. In this chapter, we explore the diversity and evolution of myzozoan plastids and plastomes, and compare them to those of other

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photosynthetic eukaryotes. Myzozoan plastomes are remarkable for encompassing the smallest photosynthesis-supporting plastomes known (in peridinin dinophytes) and for having the lowest GC content of all plastomes (in sporozoans). Myzozoan plastomes also have the smallest gene repertoires among red lineage plastomes, and such a state seems to have been reached through at least four episodic events of plastome reduction; two of these episodes appear to be associated with symbiogeneses. Myzozoans have played an important role in our understanding of plastid and plastome reduction among eukaryotes. Future discoveries of 'environmental' plastomes will allow us to increase the diversity and better reconstruct the diversification of myzozoan plastomes.

1. INTRODUCTION

Myzozoans comprise a group of protists that is remarkable for displaying a great diversity of plastids. The reason for this is that their evolutionary diversification has produced parasites, mutualistic endosymbionts, predators, algae (strict photosynthesizers), and mixotrophs (cells capable of predatory heterotrophy but also photosynthesis). Most myzozoans are heterotrophic (sporozoans, colpodellids, perkinsozoans, and half of dinoflagellates), but they are ancestrally plastid-bearing mixotrophs. Conveniently for us, the Myzozoa also turns out to be one of the best sampled groups in terms of plastid diversity. Myzozoans are named after their inferred ancestral capacity to feed by myzocytosis (Cavalier-Smith & Chao, 2004). Myzocytosis is a feeding mode in which the cytoplasmic contents of the prey cell are sucked leaving the plasmalemma outside—this contrasts with phagocytosis in which the whole prey cell is ingested (Schnepf & Deichgräber, 1984). The most commonly known myzozoan protists are apicomplexans and dinoflagellates. The formers are known to be deadly parasites of animals (e.g. malaria), whereas the latter are known as important primary producers or to cause harmful algal blooms (e.g. red tides) in waters. But apicomplexans are not the direct sisters to dinoflagellates; each lineage has closer but less diverse myzozoan relatives. Recent discoveries of algae on the apicomplexan side of the Myzozoa tree have given us more confidence in reconstructing the early steps in the evolution of plastids in this group. In this chapter, we explore the diversity and evolution of myzozoan plastid genomes or plastomes.

2. THE MYZOZOA

2.1 What Are Apicomplexans?

Apicomplexans are eukaryotic unicells (protists) that, in the broad sense, comprise both intracellular and extracellular endosymbionts (or individuals

living inside another that can be commensals, parasites, or even mutualists; apicomplexans in the strict sense), as well as their closest relatives (free-living predators as well as free-living and endosymbiotic photosynthesizers). Classical apicomplexans (parvphylum Sporozoa, see below for a reference taxonomic scheme) are infamous parasites of animals. Some examples are *Plasmodium falciparum*, the cause of malaria in humans, *Cryptosporidium* and *Cyclospora*, causes of gastrointestinal diseases with diarrhoea in humans, and *Babesia* and *Theileria* that infect domestic animals like cattle. Gregarines are parasites, perhaps commensals, of invertebrates. It is believed that every animal species serves as a host for a corresponding coevolved sporozoan parasite. In tropical forests, apicomplexan parasites are the most abundant and diverse protists, at least matching the diversity of vertebrates and invertebrates (Mahé et al., 2017). This makes of apicomplexan parasites perhaps the most diversified and successful group of parasites on Earth.

Apicomplexans are a phylogenetically cohesive group. This has been conclusively shown by several single-gene and multigene phylogenies (e.g. Fast, Xue, Bingham & Keeling, 2002; Harper, Waanders & Keeling, 2005). The group is also united ultrastructurally by possessing a rostrum made of cystoskeletal structures (e.g. a pseudoconoid) and endomembranes (e.g. micronemes) at their cell apex, the so-called more developed apical complex in sporozoans, that is used for attachment and invasion (in gregarines), penetration of host cells (in haematozoans, piroplasms, and some coccidians), or feeding through myzocytosis (in colpodellids). They have also retained ancestral features shared with other myzozoans or alveolates such as cortical alveoli and micropores. Apicomplexans are inferred to have evolved from a plastid-bearing photosynthetic and flagellated myzozoan ancestor that had a precursor apical rostrum, was able myzocytose, made cysts, and reproduced by schyzogony (Cavalier-Smith & Chao, 2004).

2.2 Taxonomy of the Apicomplexa

The latest evolutionary taxonomic scheme for the group has the infraphylum Apicomplexa subdivided into two parvphyla: Apicomonada and Sporozoa (Fig. 1; Cavalier-Smith, 2017; see also Votýpka et al., 2016 for a compatible non-Linnean scheme). The Sporozoa comprises gregarines, the probably mutualistic *Nephromyces*, and classical apicomplexans such as coccidians, piroplasms, and haemosporidians; whereas the Apicomonada comprises the free-living and heterotrophic colopodellid predators, and the chromerid algae which are intracellular photosynthetic endosymbionts of, or free-living and associated to, corals (Fig. 1). A great diversity of

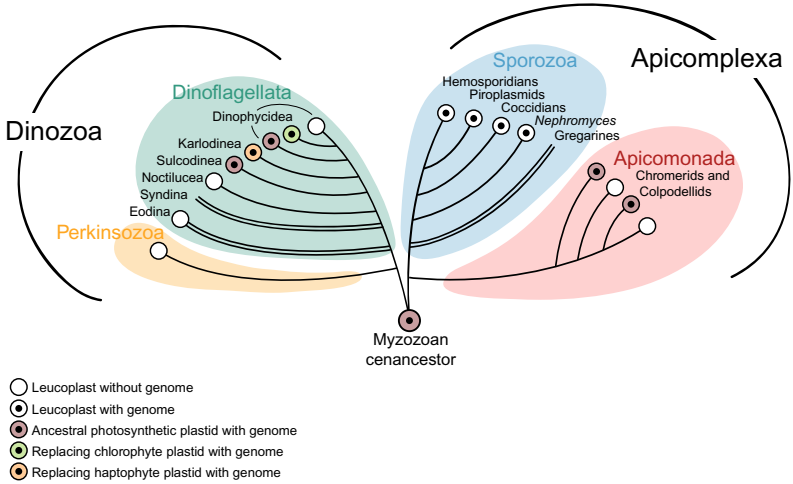

Fig. 1 A schematic phylogeny of the Myzozoa. The diagram summarizes and synthesizes the phylogenetic relationships among myzozoans based on Adl et al. (2012), Votýpka, Modrý, Oborník, Šlapeta, and Lukeš (2016), Janouškovec et al. (2015, 2017), and Cavalier-Smith (2017). For dinozoans, the evolutionary taxonomic scheme and taxon names of Cavalier-Smith (2017) are adopted. For apicomplexans, informal names are used for the particular major lineages discussed within the text, but Cavalier-Smith (2017) is followed for taxa above the parvphylum level. The distribution of plastids and their genomes is shown by different combinations of *coloured* and *inside circles*. Dinophytes with barely reduced ochrophyte endosymbionts (dinotoms), as well as cryptophyte-derived kleptoplastids in the dinophyte *Dinophysis* are not shown. The aplastidic myzozoans *Cryptosporidium* and *Haematodinium* are phylogenetically contained within gregarines and Syndina, respectively. *Double branches* denote paraphyly.

apicomplexans is known only from environmental surveys, apicomplexan-related lineages I–VIII (ARLs), or environmental clades I–XI (Janouškovec, Horák, Barott, Rohwer, & Keeling, 2013; Janouškovec et al., 2015). But these novel species have yet to be cultured and further studied so to be incorporated into formal classification schemes. Another more phylogenetic and cladistic scheme restricts the Apicomplexa clade to the classical endosymbiotic apicomplexans (*sensu stricto*), excluding their free-living and photosynthetic relatives (Adl et al., 2012). We will here refer to apicomplexans in their broadest sense (*sensu lato*) which also includes their free-living and photosynthetic relatives.

2.3 What Are Dinozoans?

Dinozoans encompass a great diversity of protists. About half of them are heterotrophic (either predatory or parasitic), whereas the other half are

photosynthetic (either obligate or mixotrophic). They are all aquatic, and live in both marine and freshwaters. Some dinozoans are popularly known as bioluminescent plankton in seas (*Noctiluca*), algal endosymbionts of corals (*Symbiodinium*), and makers of red tides (*Alexandrium*). Most dinoflagellates, and specially the dinokaryotes, have their cell bodies divided into two parts, the episome and the hyposome (see Janouškovec et al., 2017 for character evolution mapped onto an updated phylogeny). Some 'basal' dinoflagellates, like *Perkinsus* and *Psamossa* (see below for their taxonomy), have apical rostra that are homologous to the specialized apical complex of sporozoans (e.g. see Okamoto & Keeling, 2014). Many also move by means of two flagella, a longitudinal flagellum that sticks out of the cell and propels it, and a ribbon-like transversal flagellum that wraps around the cell and makes it rotate as it swims forward. A subgroup of dinozoans (within the Dinophycidae) evolved heavily armoured cells by building thick cellulose thecal plates within their alveoli (the so-called thecate dinoflagellates). Some dinoflagellates evolved extraordinary structures for predatory feeding, like the peduncle (a flexible tube for sucking up on prey cells) and the pallium (a cytoplasmic veil that entirely covers prey cells). Most photosynthetic dinoflagellates (here informally called dinophytes) are also active predators (i.e. mixotrophs) and this ultimately allowed some dinophyte groups to replace their ancestral peridinin plastids for others of chlorophyte or haptophyte origin. Another very unusual feature found among dinoflagellates (in the Dinokaryota, which covers the Noctilucea, Sulcodinea, and Peridinea) is the 'dinokaryon', a nucleus which has permanently condensed chromosomes, phycodnavirus-like and bacterial histone-like basic proteins instead of proper histones to package bulk DNA, and massive amounts of DNA (Gornik et al., 2012; Janouškovec et al., 2017). Dinozoans are inferred to have descended from a plastid-bearing mixotrophic ancestor quite like that from which apicomplexans (sensu lato) are thought to have evolved. Further aspects of dinozoan biology can be found in Saldarriaga and Taylor (2017).

2.4 Taxonomy of the Dinozoa

Here we follow the updated scheme of Cavalier-Smith (2017) for Dinozoa taxonomy, which is largely in agreement with the latest phylogeny of Janouškovec et al. (2017). The dinozoans comprise both the Perkinsozoa and the Dinoflagellata (Fig. 1). Within the Perkinsozoa, we so far only find intracellular parasites of animals (*Perkinsus*), dinoflagellates (*Parvilucifera*), or cryptophyte algae (*Rastrimonas*) (Reñé, Alacid, Ferrera, & Garcés, 2017).

The Dinoflagellata comprises the rest of dinozoan diversity (see above for some examples). The 'basal groups' of dinoflagellates include predatory flagellates (like *Oxyrrhis* and *Psamossa*; the Eodina) and diverse intracellular parasites (the Syndina). Both groups are probably paraphyletic, and the Syndina includes the marine alveolate groups (MAGs) I and II. The Dinokaryota, informally known as the 'core dinoflagellates', contains the bioluminescent and the giant predatory *Noctiluca* (Noctilucea), and all other dinoflagellates (Sulcodinea, Karlodinea, and Dinophycidea in Fig. 1) among which we first find examples of the ancestral photosynthetic peridinin plastid. All nondinokaryote dinozoans are heterotrophic and some, like *Oxyrrhis* and even the perkinsozoan *Perkinsus*, have relicts of the peridinin plastid found among dinophytes (see Fig. 1). Sister to the Noctilucea is a large group of dinoflagellates that comprises such diverse unicells as all photosynthetic dinozoans (i.e. dinophytes), armoured (thecate) dinoflagellates, ocelloid-guided predators, and kleptoplastidic mixotrophs and among others. Within the Dinophycidea are the common orders of the Gymnodiniales, Gonyaulacales, Peridiniales, Prorocentrales, Dinophysiales, and Suessiales (≈Symbiodiniaceae). For a conservative and more informal scheme, see Saldarriaga and Taylor (2017). For a morphology-based scheme, see Hoppenrath (2017).

3. THE ORIGIN OF MYZOZOAN PLASTIDS

There are currently two main competing groups of ideas about how myzozoans came to have plastids. The first group views the origin of myzozoan plastids as direct vertical descendants from a plastid common ancestor shared with all other red meta-algae. This view is epitomized by the chromalveolate hypothesis that states that the plastids of red meta-algae (i.e. cryptophytes, haptophytes, ochrophytes, dinophytes, chromerid algae, and their nonphotosynthetic apicomplexan descendants) were inherited vertically from a single and ancestral secondary endosymbiosis between a protozoan and a red algal unicell (Cavalier-Smith, 1999). The second group views the origin of plastids in red meta-algae by a succession of higher-order endosymbiosis (lateral spreading), usually starting with a secondary endosymbiosis with a red alga to give rise to the plastids of cryptophytes.

Different hypotheses exist on how secondary red plastids were transferred among red meta-algae. In regard to myzozoans, Sanchez-Puerta and Delwiche (2008) first suggested that myzozoans acquired their plastids from a single (tertiary) endosymbiosis with either a haptophyte or a

hacrobian ancestor (of both cryptophytes and haptophytes). Bodyl, Stiller, and Mackiewicz (2009) suggested a haptophyte origin of the dinophyte peridinin-containing plastid, but remained vague about the precise origin of the apicomplexan plastid. Dorrell and Smith (2011) more generally suggested a haptophyte origin of the myzozoan plastid. Petersen et al. (2014) postulated independent origins for the apicomplexan and dinozoan plastids without specifying donors. More recently, and based on new plastid phylogenies, Ševčíková et al. (2015) suggested that apicomplexan plastids evolved from an ochrophyte most closely related to a limnistan (eustigamtophycean or chrysophycean) alga. However, the support for this phylogenetic association was equivocal and might stem from artefacts in tree reconstruction due to the high divergences (long stems in trees resulting in long-branch attraction artefacts) of apicoplast and eustigmatophycean plastid genomes. Based on the findings of Ševčíková et al. (2015), Füssy and Oborník (2017) argued that it is possible that, early in their evolution, apicomplexans replaced an ancestral myzozoan plastid with one of ochrophyte origin. Bodył (2017) now postulates that myzozoan plastids evolved from a quaternary endosymbiosis with an ochrophyte, but dinophytes later replaced this ancestral plastid with another one of haptophyte origin to give rise to the typical peridinin plastid.

In summary, four possibilities have been imagined (almost every possibility) for the origin and evolution of myzozoan plastids: (1) myzozoans ancestrally had a plastid that has been inherited vertically from a distant ancestor (i.e. a single ancestral secondary endosymbiosis, the chromalveolate hypothesis); (2) myzozoans ancestrally had a plastid, but it was acquired through a higher-order endosymbiosis (from a haptophyte or an ochrophyte) before their diversification; (3) myzozoans ancestrally had a plastid (by either 1 or 2), but dinozoans (or apicomplexans; Füssy & Oborník, 2017) replaced this ancestral plastid to give rise to their divergent perdinin plastid; or (4) the taxa Apicomplexa and Dinozoa acquired their plastids independently from each other after their divergence from a common nonphotosynthetic myzozoan ancestor (Waller & Kořený, 2017). The most parsimonious views, in our opinion, assume a single ancestral myzozoan plastid that was inherited vertically by both dinozoans and apicomplexans (compatible with 1 or 2).

4. DIVERSITY OF PLASTIDS IN THE MYZOZOA

Many plastid types arose from the diversification of the ancestral myzozoan plastid. Photosynthetic plastids (sometimes referred to as

chloroplasts) are found on both sides of the Myzozoa tree: in many dinokaryotes (Dinozoa) and in some apicomonads (chromerid algae; Apicomplexa). All other plastids found in the Myzozoa are nonphotosynthetic; these are called leucoplasts. Some leucoplasts have plastomes (like in sporozoanas) but others have lost them (like colpodellids and perkinsozoans). We now know that leucoplasts were lost at least twice in the Myzozoa; once in the Dinozoa (*Hematodinium*) and once in the Apicomplexa (*Cryptosporidium*). See Fig. 1 for a distribution of different plastid types across the major myzozoan lineages.

4.1 Sporozoan Leucoplasts Still Retain Plastomes

Sporozoans have small genomes (plastomes) in the stroma of their biosynthetic relict plastids. Actually, sporozoan plastids are the only myzozoan leucoplasts with plastomes (see Fig. 1; but see Gavelis et al., 2015 and Fawcett & Parrow, 2014 for the description of two understudied dinoflagellates that might have also retained plastomes in their leucoplasts, *Nematodinium* sp., and one strain of *Esoptrodinium* sp, respectively). The plastid DNA (ptDNA; a 35-Kb circular DNA molecule) was first identified in 1975, but it was first thought to be mitochondrial DNA (Kilejian, 1975). Only later was the true mitochondrial DNA identified (a 6-Kb linear DNA molecule; Suplick, Akella, Saul, & Vaidya, 1988; Vaidya, Akella, & Suplick, 1989), and the real ptDNA localized to spherical bodies (Köhler et al., 1997; McFadden, Reith, Munholland, & Lang-Unnasch, 1996). The plastidic nature of the ptDNA was confirmed by restriction mapping and sequencing of some of its genes (Gardner, Feagin, et al., 1991; Gardner, Williamson, & Wilson, 1991). Spherical bodies were then renamed 'apicoplasts' for *apico*mplexan *plast*id (Köhler et al., 1997). Apicoplasts turned out to be surrounded by four membranes and because of their nonphotosynthetic nature they lack all pigments and thylakoids. The presence of derived plastids within apicomplexan parasites immediately pointed to their algal ancestry.

4.2 Apicoplasts Have a Red Algal Ancestry

The first attempts to decipher the phylogeny of apicoplasts debated the origin of apicoplasts from either a red or a green alga (Funes, Reyes-Prieto, Pérez-Martínez, & González-Halphen, 2004). Conflicting evidence fuelled this controversy (Arisue & Hashimoto, 2015). Support for a green algal origin of apicoplasts came from some single-gene (Funes et al., 2002; Köhler

et al., 1997) and multigene phylogenies (Cai, Fuller, McDougald, & Zhu, 2003; Lau, McElwain, Brayton, Knowles, & Roalson, 2009), but also from a rare split of the mitochondrial *cox2* gene that is shared between apicomplexans and green algae (Funes et al., 2002). In contrast, support for a red algal origin of the apicoplast came from phylogenies of the plastid 16S rRNA gene (Zhang, Green, & Cavalier-Smith, 2000), the plastid but nucleus-encoded GAPDH gene (Fast, Kissinger, Roos, & Keeling, 2001; Harper & Keeling, 2003), and similarities in the organization of apicoplast and red algal plastomes (Blanchard & Hicks, 1999). Today, it is well accepted that apicoplasts ultimately descended from a red alga. The phylogenetic affiliation of apicoplast genes to those of green plastids was shown to be artefactual, and the rare split in the *cox2* gene was found to be convergent (Waller & Keeling, 2006; Waller, Keeling, van Dooren, & McFadden, 2003). The evidence also seems to be strong enough to view apicoplasts as sisters to the peridinin-containing plastids of dinoflagellates, and chromerid plastids as links between the two; all of them having descended vertically from a common myzozoan plastid ancestor (Janouškovec, Horák, Oborník, Lukes, & Keeling, 2010).

4.3 Dinozoans Exhibit a Great Diversity of Plastids

Only half of the known species of dinozoans have photosynthetic plastids (Fig. 1; Saldarriaga, Taylor, Keeling, & Cavalier-Smith, 2001). Of these, most have a type of plastid that is thought to be ancestral to dinozoans, the peridinin plastid, and which is likely to be a divergent descendant of the ancestral myzozoan plastid (see discussion on the origins of myzozoan plastids above). This peridinin plastid was early on shown to be of red algal origin and to be related to those of other red meta-algae (Zhang et al., 2000). But the peridinin plastid has some unique features that distinguish it from those of all other red meta-algal plastids. Besides the accessory carotenoid pigment peridinin, the archetypical dinophyte plastid also has chlorophyll *a* and c_2, a three-membraned envelope and a greatly divergent plastome. Another bizarre feature of dinophyte peridinin plastids is their RuBisCO type II (to fix CO_2) of proteobacterial rather than cyanobacterial origin. This ancestral replacement by lateral gene transfer was first thought to be a unique and defining feature of peridinin plastids, but is now also known to be shared with apicomonad algae—a laterally acquired RuBisCO was present in the ancestral myzozoan (Janouškovec et al., 2010).

Some groups of dinophytes have replaced their ancestral peridinin plastid (Fig. 1). In some, the newly acquired plastid or endosymbiont could be alongside a no longer photosynthetic peridinin plastid (this is clearly the case in the 'dinotoms' and *Dinophysis*). There are two clear examples of replacing plastids among dinophytes. The first involves some members of the Gymnodiniaceae (*Lepidodinium chlorophorum* and *Lepidodinium viridae*) which have (secondary) green plastids with chlorophyll *a*, *b* but no peridinin. These green plastids have a pigment composition typical of green algae, and they are also surrounded by four membranes; they also have a 'nucleomorph'. Moreover, their green algal affinity has been confirmed by ultrastructure, biochemistry, and phylogeny (Matsumoto et al., 2011; Matsumoto, Kawachi, Miyashita, & Inagaki, 2012). The specific green algal donor of the green plastid of *L. chlorophorum* was shown to be a pedinophyte (a chlorophyte) based on plastome phylogenies (Kamikawa et al., 2015). The second example is that of *Karenia*, *Karlodinium*, and *Takayama* (Kareniacea) which now have a so-called (tertiary) fucoxanthin plastid. This plastid has the typical pigment composition of a haptophyte plastid (e.g. chlorophyll c_1, c_2, and fucoxanthin but no peridinin) and is also surrounded by four membranes (but no nucleomorph). The origin of the fucoxanthin plastid in the Kareniaceae has also been strongly demonstrated based on phylogenies of plastid- and nucleus-encoded genes for plastid proteins (Gabrielsen et al., 2011; Tengs et al., 2000; Yoon et al., 2005).

A subgroup in the Peridinales, the so-called 'dinotoms', has recently acquired tertiary diatom (Ochrophyta) endosymbionts, which have plastids of red algal origin themselves. These endosymbionts are barely reduced (only the diatom outer shell or frustule seems to have been lost) and thus are not properly called organelles yet. Indeed, the dinotoms *Kryptoperidinium foliaceum* and *Durinskia baltica* derive photosynthate from their endosymbionts (Hehenberger, Burki, Kolisko, & Keeling, 2016). Even though 'dinotoms' are a monophyletic group within the Peridiniales, their diatom endosymbionts have been acquired multiple times independently. Indeed a remarkable example of endosymbiotic convergence likely facilitated by some sort of a constraint (Yamada, Sym, & Horiguchi, 2017). Many other diverse dinoflagellates are also known for engaging in kleptoplastidy, or the stealing of prey's plastids to temporarily tap on them (Waller & Kořený, 2017). A classic example of a kleptoplastidic dinoflagellate is *Dinophysis* which harbours kleptoplastids of cryptophyte origin that are acquired indirectly through the ciliate *Mesodinium rubrum*.

4.4 Apicomonads Have Ancestral-Type Plastids

The closest photosynthetic relatives of sporozoans are the chromerids or apicomonad algae *Chromera velia* and *Vitrella brassicaformis* (Moore et al., 2008; Oborník et al., 2012). *Chromera* and *Vitrella* are not each other's closest relatives but are more closely related to free-living heterotrophic myzozoan predators called colpodellids (Fig. 1; Janouškovec et al., 2015). Apicomonad photosynthetic plastids constitute 'missing links' between sporozoan and dinophyte plastids by possessing features that are present in either one or the other. For example, apicomonad plastids are photosynthetic like dinophyte plastids, but are surrounded by four membranes like the leucoplasts of sporozoans and the dinozoan *Perkinsus*. Despite being more closely related to sporozoan apicoplasts, chromerid plastids share several features with dinophyte perdinin plastids like thylakoids stacked in triplets, a type II RuBisCO, and polyuridinylated plastome transcripts. In terms of major photosynthetic pigments, chromerids have chlorophyll *a* but no chlorophyll *c*, unlike peridinin dinophytes that have both (chlorophyll *c* which is the hallmark pigment of red meta-algae; Janouškovec et al., 2010; Moore et al., 2008). The plastomes of apicomonad photosynthetic plastids have gene contents that encompass the nonoverlapping sets found in both sporozoan and dinophyte plastids. Phylogenies of plastomes have also confirmed that chromerid plastids are more closely related to apicoplasts and perdinin plastids than to other red meta-algae (Janouškovec et al., 2010).

4.5 Why Do Myzozoans Retain Leucoplasts?

The reason why leucoplasts, like apicoplasts, are retained by many myzozoans (and some other ancestrally but no longer photosynthetic groups) is that plastids have become highly integrated with the overall cytosolic metabolism of their host cells. Host cells came to rely on plastids not only for photosynthesis, which is dispensable depending on life style, but also for the biosynthesis of fatty acids, isoprenoids, haeme, and iron–sulfur (Fe–S) clusters. For example, apicomplexans plastids export fatty acids, isoprenoids, and haeme to the cytosol (or mitochondrion), whereas iron–sulfur clusters are required for the biogenesis of plastid enzymes involved in the biosynthesis of fatty acids and isoprenoids (van Dooren & Hapuarachchi, 2017).

The ultimate evolutionary answer to the issue of leucoplast retention, though, might be a combination of historical constraints and efficiency through compartmentalization (selective constraints). Even if the leucoplast plastome is lost by transferring its remaining genes to the nucleus,

leucoplasts remain a place for important metabolic pathways (e.g. isoprenoid biosynthesis) on which the cytosolic metabolism relies—some myzozoan have plastome-less leucoplasts. And whole pathways might not be easy to relocate to the cytosol. For this to happen, all plastid-targeted enzymes should lose their plastid localization simultaneously. So there has been strong phylogenetic inertia for the location of this plastid enzymes, i.e., their relocation to the cytosol would require multiple improbable changes whose intermediate states would be detrimental. On the other hand, it is also possible, but less plausible, that there is an adaptive value in compartmentalizing plastid biosynthetic pathways in a small compartment like the sporozoan apicoplast. Metabolic compartmentalization improves efficiency (by increasing concentrations of metabolites and enzymes) and might contain potential toxic metabolic intermediates.

The first myzozoan ancestor (or an earlier ancestor) was a chimeric cell with redundant metabolism as a result of both plastid and cytosolic pathways for the synthesis of haeme (tetrapyrroles), isoprenoids, and fatty acids. But during myzozoan diversification, metabolic redundancy allowed for the chancy loss of cytosolic pathways, leaving the cell dependent on plastid pathways. Isoprenoid biosynthesis appears to be the most indispensable plastid pathway because it is conserved by every myzozoan that has retained a plastid organelle (Janouškovec et al., 2015; Waller, Gornik, Koreny, & Pain, 2016).

4.6 Why Do Some Sporozoan Leucoplasts Retain Plastomes?

Numerous hypotheses have been formulated to explain why endosymbiotic organelles retain genomes. However, only few of them apply to non-photosynthetic plastids, as they have dispensed with an electron transport (photosynthetic) chain and their plastomes do not encode particularly hydrophobic proteins (Barbrook, Howe, & Purton, 2006). Why do some sporozoans keep their apicoplast plastomes? Apicoplasts are the only non-photosynthetic plastids among myzozoans that are known to retain plastomes. Most of the genes encoded by the apicoplast plastome are transcription and translation genes such as ribosomal proteins, tRNAs, and a RNA polymerase. The only apicoplast plastome-encoded genes that fall outside these categories are *sufB*, *clpC*, and *ycf93*. Therefore, all other apicoplast plastome-encoded genes are there to support the expression of *sufB*, *clpC*, and *ycf93*. The 'limited transfer window' hypothesis best explains the persistence of a plastome among myzozoan leucoplasts (Barbrook, Howe, et al., 2006). The 'limited window transfer' hypothesis states that species with

few or one plastid per cell have extremely low rates of gene transfer (or endosymbiotic gene transfer, EGT) from the plastome to the nuclear genome (Barbrook, Howe, et al., 2006). EGT is primarily driven by the release of ptDNA from lysed organelles that get incorporated into nuclear genomes. If the single apicoplast of a sporozoan cell lyses there is no way to regenerate this organelle and the cell would die. This in turn suggests that the reason why plastomes remain in apicoplasts is simply because some genes like *sufB*, *clpC*, or *ycf93* have not had a chance to be successfully transferred to the nuclear genome. Because examples of successful transfers of *sufB* and *clpC* to the nucleus of some myzozoans are known (see Janouškovec et al., 2015), the retention of plastomes in sporozoans is best seen as a simple historical accident. The adaptationistic alternative, the 'essential tRNA' hypothesis, runs into important counterexamples among sporozoans (see Janouškovec et al., 2015 for a discussion).

4.7 Plastome Loss in Some Myzozoans

The most extreme cases of plastome reduction would be exemplified by the outright loss of the plastome in some nonphotosynthetic eukaryotes. Several (nonsporozoan) myzozoans are known to have lost their plastomes but retained their plastid organelles for metabolic functions (e.g. fatty acid and isoprenoid biosynthesis) sustained by plastid-targeted nuclear genes (see Fig. 1). The colpodellids *Alphamonas*, *Colpodella*, and *Voromonas* (Apicomonada; see Fig. 1) seem to have lost their plastomes (Gile & Slamovits, 2014; Janouškovec et al., 2015). Among dinozoans, the perkisozoan *Perkinsus* is also reported to have lost its plastome, and no trace of a plastome has been found in the early-diverging nonphotosynthetic dinoflagellates *Oxyrrhis*, *Noctiluca*, and *Crypthecodinium* (Janouškovec et al., 2017; Sanchez-Puerta, Lippmeier, Apt, & Delwiche, 2007; Slamovits & Keeling, 2008). The more derived dinophyte *Dinophysis* has also retained the ancestral myzozoan plastid, but without its plastome (Janouškovec et al., 2017). The other cases of reported plastome losses among eukaryotes are the green alga *Polytomella* (Smith & Lee, 2014), and the parasitic land plant *Rafflesia lagascae* (Molina et al., 2014), both in the green plastid lineage.

4.8 Plastid Loss Among Myzozoans

The strong metabolic dependency that myzozoan cells have on their plastids makes plastid loss a rare evolutionary event. Only one case on plastid loss has been fully confirmed among apicomplexans: that of the intestinal parasite

Cryptosporidium (more closely related to gregarines; Zhu, Marchewka, & Keithly, 2000). The only other case of outright plastid loss documented among all eukaryotes happened in the dinozoan *Hematodinium* (and by extension also in its sister *Amoebophrya* (Syndina); Gornik et al., 2015, and also see Janouškovec et al., 2017). This dinoflagellate seems to have lost its plastid organelle (and biosynthetic pathways therein) before losing any cytosolic pathways for haeme, isoprenoid, or fatty acid biosynthesis. *Hematodinium* retained an ancestral metabolic redundancy by preserving the cytosolic pathways for fatty acid and haeme biosynthesis that allowed this crustacean parasite to dispense with its plastid organelle. And this was complemented by the evolution of isoprenoid scavenging from its animal host. The apicomplexan *Cryptosporidium*, in contrast, appears to have only conserved the cytosolic pathway for fatty acid biosynthesis, but evolved means to steal haeme and isoprenoids from its animal host cells. Because of this, *Cryptosporidium* was able to lose its plastid organelle. Knowledge is scarce about the very diverse gregarines, but similarly to *Cryptosporidium*, *Gregarina niphandrodes* might have lost its plastid organelles and genomes (Toso & Omoto, 2007). All studied colpodellids and classical intracellular sporozoans have retained plastid organelles, with or without plastomes (Fig. 1).

5. THE PLASTOMES OF MYZOZOANS

5.1 The Plastid Genomes of Apicomonad Algae

Myzozoans exhibit a great diversity of plastomes. Among apicomplexans, only apicomonad plastomes (those of chromerid algae) support photosynthesis. The plastomes of the chromerids *C. velia* (Moore et al., 2008) and *V. brassicaformis* (CCMP3315; Oborník et al., 2012) have the largest sizes and gene repertoires among all myzozoans (with the exception of tertiarily acquired plastids by some kareniacean dinophytes; Figs 2 and 3). Their gene repertoires encompass the reduced set of 12 photosynthetic genes found in most dinophyte plastomes, but also the translation and transcription genes found in sporozoan plastomes. But the gene content of apicomonad algae is still smaller than those of other red meta-algae such as haptophytes, cryptophytes, and ochrophytes (Fig. 3; see Janouškovec et al., 2010). This suggests that some degree of genome reduction through gene loss had already happened before the diversification of modern myzozoans. Because of their relatively big sizes and gene repertoires, chromerid plastomes are the most similar to the ancestral myzozoan plastome.

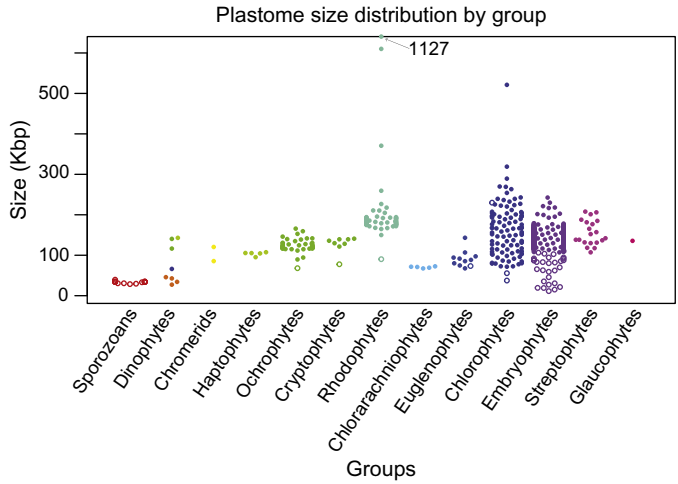

Fig. 2 The distribution of plastome sizes among all eukaryotic algal groups. The rhodophytes have recently been found to comprise the most extraordinarily large plastomes known, but this is a derived condition. In stark contrast, myzozoans, i.e., sporozoans and dinophytes, possess some of the smallest plastomes across eukaryotic algae. But some embryophytes (land plants) hold the record for the most reduced plastomes. The ancestral peridinin plastid of dinophytes can reach sizes smaller than those of the Sporozoa (e.g. in *Symbiodinium*). Derived plastids among dinophytes have larger plastomes and are *coloured* according to their provenance; the plastomes of peridinin dinophytes are coloured in orange. The plastomes of nonphotosynthetic plastids, or leucoplasts, are represented by *empty circles*. The database of plastome sizes used to make this figure can be found at: https://doi.org/10.17632/frxt79djmr.1.

The plastomes of the apicomonad algae *Chromera* and *Vitrella* are also considerably divergent relative to each other. Whereas *Vitrella* has a compact plastome with a size of only 85.5 Kbp, *Chromera*'s plastome is 121.2 Kbp in size. Despite this difference in size, *Vitrella*'s plastome encodes more genes than *Chromera*'s (81 vs 74 genes; Janouškovec et al., 2010; Oborník & Lukeš, 2015). The plastome of *Chromera* is also unusually divergent in (i) being considerably rearranged in comparison to those of sporozoans and *Vitrella*, (ii) possessing genes with long extensions, (iii) having split genes encoding for separately translated protein fragments, and (iv) being a noncircular-mapping linear ptDNA with terminal repeats (Janouškovec et al., 2013). *Vitrella*'s plastome, in contrast, lacks all these divergent oddities seen in *Chromera*'s plastome. The plastome of *Vitrella* has a canonical quadripartite organization shared with most apicomplexans (see below), has retained a 5S rRNA gene (unlike *Chromera*'s, dinoflagellate, and sporozoan plastomes), and also has a one of the highest GC

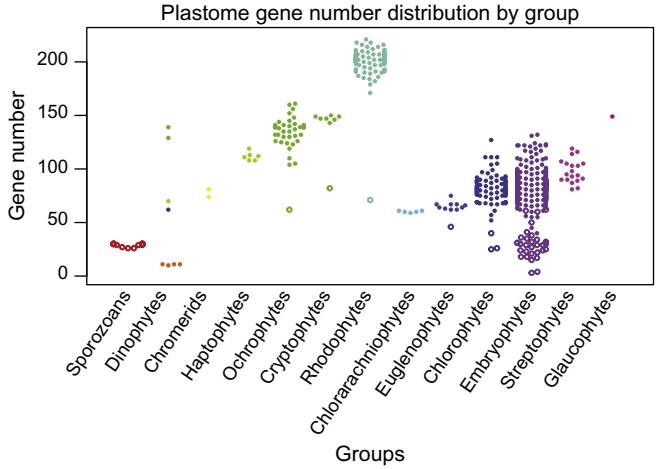

Fig. 3 The distribution of plastome gene repertoires among all eukaryotic groups. Myzozoans ancestrally have plastomes with smaller gene repertoires than other red meta-algae, as seen in *Chromera* and *Vitrella*. The origin of the Dinozoa and Apicomplexa further led to independent episodes of gene loss in the plastomes of each lineage. Derived plastids among dinophytes have larger gene repertoires than peridinin plastids and are *coloured* according to their provenance (see main text for discussion); the plastomes of peridinin dinophytes are coloured in orange. The plastomes of non-photosynthetic plastids, or leucoplasts, are represented by *empty circles*. Gene repertoires reported here are only based on the number protein-coding genes as reported primarily by NCBI (and some other minor sources). RNA-specifying genes and pseudogenes were ignored. Because the gene number reported depends on annotations and these are not unified, there is some variability in the numbers, but all obvious outliers were manually removed. The database of plastome gene numbers used to make this figure can be found at: https://doi.org/10.17632/frxt79djmr.1.

contents (47.7%) among eukaryotes (Fig. 4; Janouškovec et al., 2010, 2013). These features point to the less divergent nature of *Vitrella*'s plastome and suggest that the myzozoan cenancestor had a plastome more similar to *Vitrella*'s than to any other modern myzozoan currently known.

5.2 The Plastid Genomes of Sporozoans and Dinophytes

Dinophyte and sporozoan plastomes are considerably divergent from those of chromerids. Both groups have quite reduced plastomes, but in very different ways. Sporozoan plastomes have dispensed with all photosynthetic genes, whereas dinophyte plastomes have essentially only retained photosynthetic genes. The sporozoan plastome is contained within a single DNA molecule that is circular, but the dinophyte peridinin plastid genome has been fragmented into several 'minicircles', most of which encode one single gene.

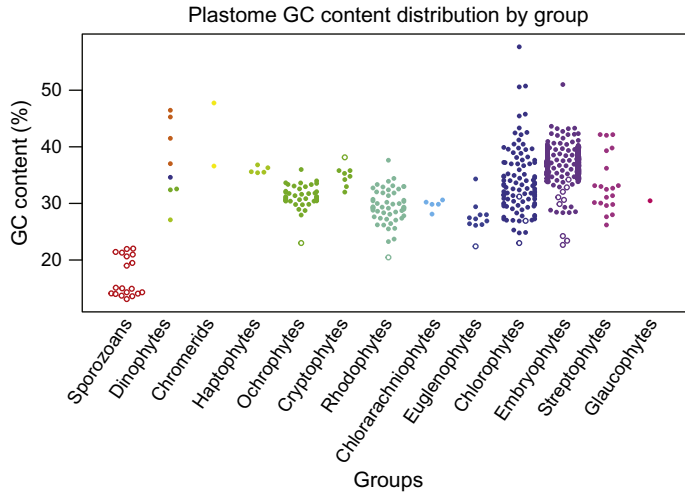

Fig. 4 The distribution of plastome GC content among all eukaryotic algal groups. Sporozoans have plastomes heavily compositionally biased towards low GC contents, and constitute the most extreme example among all eukaryotic algae. Among sporozoans, haemosporidians (e.g. *Plasmodium* and *Leucocytozoon*) have the lowest GC contents, whereas the plastomes of piroplasmids and coccidians have slightly higher GC contents. Among dinophytes, peridinin plastid plastomes have higher GC contents than those more recently acquired through serial secondary or tertiary endosymbiosis. The plastomes of nonphotosynthetic plastids, or leucoplasts, are represented by *empty circles*. Derived plastids among dinophytes are *coloured* according to their provenance; the plastomes of peridinin dinophytes are coloured in orange. The database of plastome GC contents used to make this figure can be found at: https://doi.org/10.17632/frxt79djmr.1.

5.2.1 Plastome Size and Gene Repertoires

Myzozoans are also extraordinary in having some of the smallest plastomes. Sporozoans have apicoplast plastomes that range from 28.6 to 39.5 Kbp in size (see Fig. 2). But even though apicoplast plastomes are incredibly small, some nonphotosynthetic land plants (or embryophytes) have reduced their plastomes even further (Fig. 2). For example, the plastomes of *Pilostyles* and *Epigogium* have sizes of just 11.4 Kbp (Bellot & Renner, 2015) and 19 Kbp (Schelkunov et al., 2015), respectively. The parasitic green alga *Helicosporidium* is another example of plastome reduction within the green plastid lineage (37.4 Kbp in size) and represents another interesting case of convergent evolution with the plastomes of sporozoans (de Koning & Keeling, 2006). These examples are the most extreme, but they are found within the green plastid lineage. Sporozoans, on the other hand, have the smallest plastomes for the red plastid lineage (compare to green plastids in Fig. 2).

Plastome expansion, the opposite to plastome reduction in non-photosynthetic parasites like sporozoans, is seen among primary plastids. Some green and red algae have massively expanded their plastomes by the accumulation of different kinds of noncoding DNA (introns, insertion sequences, or repetitions) and have then reached sizes of up to 1.13 Mbp in the case of the red algal unicell *Corynoplastis japonica* (see distant outlier for rhodophytes in Fig. 2; Muñoz-Gómez et al., 2017). One recent example also shows that leucoplast plastomes (like those of apicoplast's) are not immune to expansion. Even though it has lost all photosynthetic genes, the plastome of the heterotrophic green alga *Polytoma uvella* has inflated to a size of 230 Kbp, 75% of which is noncoding DNA (Fig. 2; Figueroa-Martinez, Nedelcu, Smith, & Reyes-Prieto, 2017). It has been suggested that the reason for this lies in that *Polytoma* is a free-living unicell, and so it does not necessarily experience the evolutionary forces that drive genome compaction in parasites like sporozoans (Figueroa-Martinez, Nedelcu, Reyes-Prieto, & Smith, 2017).

The current sizes and gene repertoires of dinoflagellate and apicomplexan plastomes seem to have been achieved through at least four episodic events of plastome reduction, the first two of which are associated to symbiogeneses (Oborník, Janouškovec, Chrudimský, & Lukeš, 2009). The largest and most ancestral plastome gene repertoires are found among the red algae (Fig. 3), but they only represent a fraction of the total gene number of their cyanobacterial genome progenitors. The progenitor of all plastomes was probably a cyanobacterial genome of only about 3.05 Mbp in size (2929 protein genes; Ponce-Toledo et al., 2017), and the ancestral plastome was about 200 Kbp in size (≈200 protein genes; Figs 2 and 3), most similar to those of modern red algae like bangiophyceans and florideophyceans. The symbiogenetic origin of primary plastids was then the first episode of drastic plastome reduction. The gene repertoires of most red meta-algal groups reflect their red algal ancestry: they have, on average, larger gene repertoires than most green plastids (both primary and secondary), but still smaller than those of red algae (Fig. 3). Thus, the secondary symbiogenesis that led to the origin of red meta-algae was the second episode of plastome reduction. Chromerid plastomes most resemble the ancestral myzozoan plastome (74–81 protein genes), but they are notoriously reduced in comparison to those of other red meta-algae, i.e., cryptophytes (≈147 protein genes), haptophytes (≈111 protein genes), and ochrophytes (≈134 protein genes); the third episode of plastome reduction (see Fig. 3). Dinophyte plastomes have the smallest gene set for any algal group. Sporozoan plastomes are also considerably reduced in terms of gene repertoires (≈29 protein

genes), but are still larger than their dinophyte sisters (≈11 protein genes). Both dinophyte and sporozoan plastomes greatly reduced after their divergence from a common ancestor, but they followed quite different evolutionary trajectories; the fourth episode of genome reduction.

5.2.2 Plastome GC Content

Another extreme feature of apicoplast plastomes is their strong nucleotide compositional bias. Indeed, their GC content is the lowest among all plastomes (see Fig. 4). Some apicoplast genes, like *rpl11*, are 95% AT. There is a general correlation between plastome size and GC content: the smallest plastome sizes have the lowest GC contents (compare leucoplast plastomes in Figs 2 and 3). It is a little surprising then that apicoplast plastomes have such strong compositional bias towards AT given that other plastomes, such as those of some heterotrophic land plants, are more reduced in size (see embryophytes in Fig. 2). Indeed, the correlation between plastome size and GC content is not perfect. Some exceptions are the leucoplasts of the cryptomonad *Cryptomonas paramecium* (38.14% GC) and the chlorophyte *Prototheca wickerhamii* (31.2% GC), whose plastomes are smaller but have higher GC contents than some of their photosynthetic relatives. Some peridinin dinophytes, which have the smallest plastomes known, have minicircles whose GC composition is relatively high (Fig. 4). In the case of myzozoans, evolutionary forces driving plastome reduction and high AT bias have been linked for apicoplast genomes, but it appears that the two trends have been unlinked during the evolution of peridinin plastomes. The comparatively high GC contents of the plastomes of apicomonad algae (*Vitrella* in particular) and many 'basal' dinophytes (as suggested by the third codon position in protein-coding genes; Dorrell et al., 2017) suggest that the ancestral myzozoan plastome was GC-rich.

5.2.3 Plastome Organization in Sporozoans

The most common and therefore ancestral plastome architecture among sporozoans corresponds to a circular-mapping DNA molecule with a quadripartite organization (Fig. 5). Inverted repeats (IRs) containing the small subunit (SSU) and large subunit (LSU) rRNA genes, as well as many tRNAs, divide the plastomes into a large single copy (LSC) region which virtually encompasses all other genes, and an extremely shrunk small single copy (SSC) region that contains no genes at all (Arisue & Hashimoto, 2015). This genome architecture is shared among haemosporidians, coccidians, and *Nephromyces*. A quadripartite organization of the plastome seems to be an

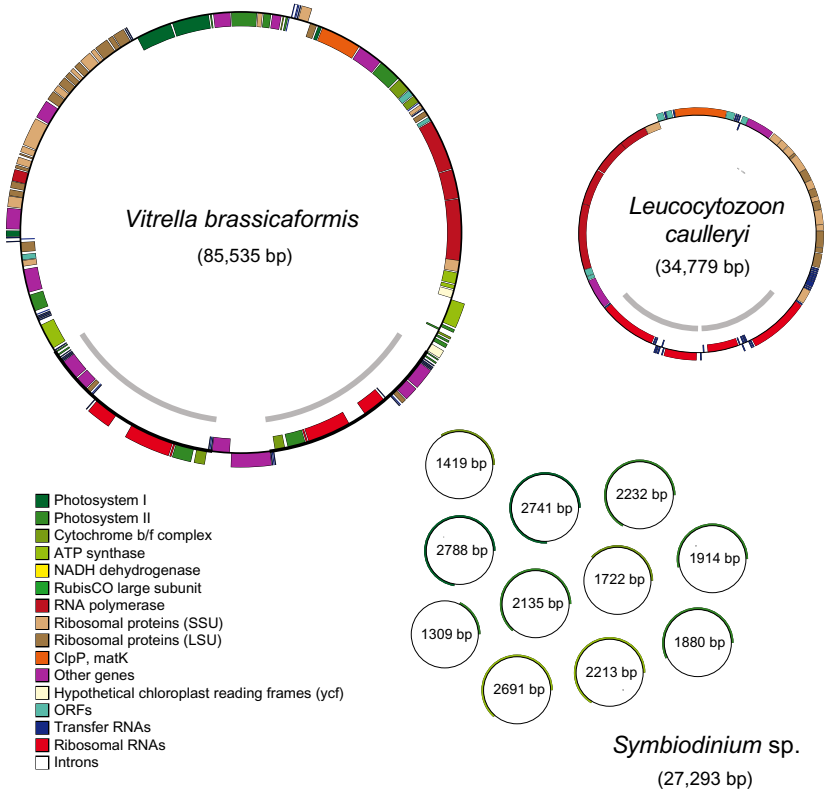

Fig. 5 Plastome organizations and structures among myzozoans. The plastomes of the apicomonad alga *Vitrella brassicaformis*, the haemosporidian sporozoan *Leucocytozoon caulleryi*, and the dinophycean *Symbiodinium* sp., are used as representatives for their groups. Apicomonad algae have ancestral-like plastomes that support photosynthesis and have the largest gene repertoires among myzozoans. Dinophytes have highly divergent plastomes that still support photosynthesis but are fragmented into plasmid-like minicircles, each encoding one to few genes. Sporozoans have small plastomes that do not support photosynthetic plastids but metabolic apicoplasts that make fatty acids, isoprenoids, and haeme.

ancestral feature to all plastids, although it also seems to be a feature prone to be lost or evolved convergently. There is also a strong strand polarity in the apicoplast plastomes of haemosporidians, coccidians, and *Nephromyces*, with half of the plastome having genes on one strand, whereas the other half having genes on the opposite strand (Fig. 5).

Sporozoan plastomes ancestrally support nonphotosynthetic plastids and therefore have reduced by losing all genes for photosynthetic proteins.

The protein-coding gene content of the sporozoan plastome is reduced to a set of translation (*rps*, *rpl*, and *tufA*) and transcription (*rpo*) genes, the chaperone *clpC*, the iron–sulfur cluster biogenesis protein *sufB*, and the unknown but conserved *ycf93* gene. ClpC is a plastid chaperone required to properly deliver unfolded proteins to the ClpP proteases. SufB is a protein required for the biogenesis of iron–sulfur-containing proteins (like fatty acid and isoprenoid biosynthetic enzymes). Ycf93 seems not to be a ribosomal protein, but a membrane protein whose exact function remains unknown (Goodman & McFadden, 2014). There are also some ORFs encoded by apicoplast plastome whose functions remained unknown, but many of them might be divergent ribosomal proteins—our own searches reveal that most of the unknown ORFs have remote similarities to ribosomal protein genes, namely *rps13*, *rps16*, *rps17*, *rps18*, *rpl11*, *rpl19*, and *rpl20*. The gene repertoires of apicoplast plastomes are fairly stable and comprise about 30 protein-coding genes with only sporadic gene losses in some species (Fig. 3). The RNA-specifying gene content of apicoplast plastomes includes 24 tRNAs and 2 rRNA genes (the '16S' SSU and '23S' LSU rRNA genes); there is no trace of a '5S' rRNA gene. Apicoplast plastomes are also extremely compact with insignificant intergenic regions (i.e. gene dense), and many instances of overlapping genes. Another intriguing property of the apicoplast plastomes of coccidians and *Nephromyces* is that they use the stop codon UGA for tryptophan instead (Oborník & Lukeš, 2015). This alternative genetic code is also observed in *Chromera*, but not in *Vitrella*, and is therefore assumed to be ancestral to all apicomplexans but to have been lost in the plastomes of *Vitrella*, haemosporidians and piroplasmids (which together form a clade, see Fig. 1).

Piroplasmids, like *Babesia* and *Theileria*, possess the most divergent apicoplast genomes. Even though their apicoplast genomes are circular mapping, they have no IRs and the rRNA genes are thus found as single copy. Piroplasmid plastomes are considerably more rearranged than those of other sporozoans and have repetitive unknown ORFs with varying degrees of similarity to each other. All protein-coding genes are encoded on the same strand, i.e., there is absolute strand polarity in their plastomes (Sato, 2011). They also have duplicated *clpC* genes and have lost *sufB*—the latter suggests plastome loss would be easier in piroplasms. All of these divergent features were gained early in the evolution of piroplasmids and therefore are also derived among sporozoans.

5.2.4 Nephromyces *Is the Deepest-Branching Sporozoans With a Plastome*

We have recently performed a genomic survey of *Nephromyces*, a probably mutualistic apicomplexan. *Nephromyces* is an endosymbiont of molgulid tunicates, where it is found infecting the tunicate's renal sac (Saffo, McCoy, Rieken, & Slamovits, 2010). This unusual apicomplexan has a complex life cycle composed by a succession of disparate stages, several of which are extracellular (Saffo & Nelson, 1983). Our survey revealed that *Nephromyces* contains a typical sporozoan plastome, quite similar in structure to those of coccidians. In preliminary trees, *Nephromyces* appears as the deepest-branching sporozoan known with an apicoplast genome.

5.2.5 *Plastome Organization in Dinophytes*

The plastomes of dinophyte peridinin plastids are unlike any other plastome known. On one side, these plastomes encode fewer proteins than any other photosynthetic or nonphotosynthetic plastome; they are also the smallest photosynthetic plastomes known, being only between 27.2 and 45.8 Kbp in size (Barbrook, Voolstra, & Howe, 2014; Howe, Nisbet, & Barbrook, 2008). The genes encoded in 'peridinin' plastomes are considerably divergent in comparison to their homologues in other eukaryotic algae, having accumulated many nonsynonymous substitutions, indels, unusual codon-usage preferences and alternative translation initiation codons (Dorrell et al., 2017). On the other side, the genes are not arranged collinearly in a circular-mapping molecule as usual, but they are split into very small circular DNA molecules termed minicircles (Zhang, Green, & Cavalier-Smith, 1999). Most minicircles contain one gene (protein-coding, tRNA-, or rRNA-specifying), and a few have been found to contain two genes. The largest number of genes in a single minicircle was recorded for *Amphidinium carterae* (Sulcodinea), where the largest minicircle carries three identified (*psbD*, *psbE*, and *psbI*) and one unknown ORF (Barbrook, Santucci, Plenderleith, Hiller, & Howe, 2006). This, however, appears to be an exceptional situation, likely resulting from fusion of otherwise single-gene minicircles (Howe et al., 2008). In addition to the coding region, minicircles include a noncoding element termed 'core' (Howe et al., 2008) or 'conserved noncoding sequence' (CNS) (Mungpakdee et al., 2014). This element is found in all minicircles and it is likely to have a regulatory function by driving transcription of the gene (Mungpakdee et al., 2014). While highly similar among the minicircles in one given species, CNSs are species specific, although some similarity between strains of the C phylotype of *Symbiodinium*

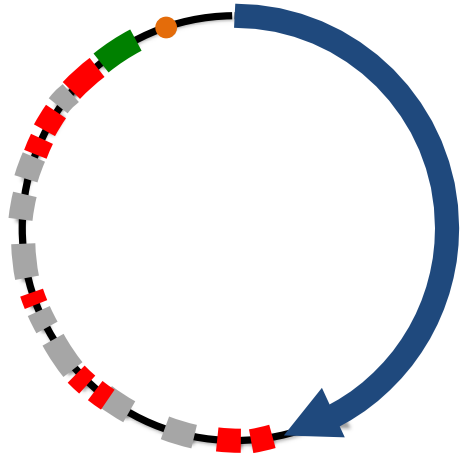

Fig. 6 Structure of a minicircle from the dinophyte *Symbiodinium* sp. The schematic representation of a single-gene minicircle shows the organization of the various elements found in most minicircles as determined in the most detailed analysis of a peridinin plastid genome conducted to date (Mungpakdee et al., 2014). The *blue arrow* represents either an ORF (if a protein-coding minicircle) or an rRNA gene. Upstream of the gene is the regulatory region consisting of a promoter (*green*) and a putative site for a pentatricopeptide RNA-binding protein (*orange circle*). *Minicircles* also contain a high density of conserved noncoding elements (*red*) and short repeats (*grey*).

sp. has been observed (Barbrook et al., 2014; Howe et al., 2003; Mungpakdee et al., 2014; Zhang et al., 1999). Aside from the coding region and the CNS core, small blocks of inverted and direct repeats are found throughout (Fig. 6; Barbrook et al., 2014; Mungpakdee et al., 2014). No function has been assigned or suggested for these small elements. It is possible that they constitute 'hot spots' of recombination. Several studies noticed certain level of heterogeneity in the composition of minicircles encoding a particular gene, and often the differences between different variants are due to small deletions spanning a few dozen base pairs (Santos, Gutierrez-Rodriguez, & Coffroth, 2003; Zhang et al., 1999). Though not yet experimentally studied, it is easy to envision that the abundance of small repeats throughout the minicircles can promote intermolecular recombination, resulting in a variety of rearranged forms. Other types of minicircle variants consistent with the occurrence of recombinational exchanges have been observed, including empty minicircles (Barbrook, Symington, Nisbet, Larkum, & Howe, 2001; Hiller, 2001), jumbled minicircles (Zhang, Cavalier-Smith, & Green, 2001), and microcircles (Nisbet, Koumandou, Barbrook, & Howe, 2004). Under closer scrutiny, some 'empty' circles were found to encode

tRNA genes, although very few have been identified. In *A. carterae* and *Amphidinium operculatum* only one tRNA (formyl-methionine) appears to be encoded in the plastome. The tRNAs for proline and tryptophan (but not formyl-methionine) were found in *Heterocapsa triquetra* and *Heterocapsa pygmaea*. In contrast, no tRNAs were found in *Symbiodinium* spp., in spite of thorough examination in several species or isolates (Barbrook & Howe, 2000; Mungpakdee et al., 2014; Nelson et al., 2007; Nisbet et al., 2004; Zhang et al., 2001, 1999), therefore, the plastid must rely on tRNA molecules imported from the cytosol for protein synthesis.

Other unusual features reported for peridinin dinophytes include the possible nuclear localization (rather than plastidic) of the plastome minicircles in *Ceratium horridum* (Laatsch, Zauner, Stoebe-Maier, Kowallik, & Maier, 2004; plastome minicircles have nevertheless been experimentally shown to localize to the peridinin plastid stroma in *Amphidinium massartii*; Owari, Hayashi, & Ishida, 2014), and the possible lateral transfer of genes from nonphotosynthetic eubacteria to the plastomes of *Ceratium horridum* and *Pyrocystis lunula* (Mackiewicz, Bodył, & Moszczyński, 2013; Moszczyński, Mackiewicz, & Bodył, 2012; these reported laterally transferred genes are likely contaminants because they are not found in the close relatives of *Ceratium* and *Pyrocystis* (Dorrell et al., 2017).

5.2.6 The Plastomes of Derived Plastids in Dinophytes

There are currently four plastomes sequenced for dinophyte plastids that have replaced the ancestral peridinin plastid. The plastome of the tertiarily acquired plastid of the fucoxanthin dinophyte *Karlodinium veneficum* (Kareniacea) is considerably divergent relative to that of their haptophyte progenitor. *Karlodinium*'s plastome is larger than that of all haptophytes because of the expansion of its intergenic spacers (172.9 vs and average of 103.6 Kbp in size for haptophytes; see Fig. 2). It also encodes for considerably fewer protein-coding genes, only 70 rather than ≈111 like most haptophytes (Fig. 3). Furthermore, the 'fucoxanthin' plastome of *Karlodinium*'s is considerably rearranged and its gene sequences are fast evolving (as seen in phylogenies; Gabrielsen et al., 2011). Most interestingly, this plastome seems to also encode genes in extrachromosomal elements that possibly resemble the minicircles of 'peridinin' plastomes—this points to convergence in plastome organization/structure due to constraints imposed by the genetic environment of the host (Espelund et al., 2012; Richardson, Dorrell, & Howe, 2014). The secondarily acquired plastid of the green dinophyte *L. chlorophorum* is also divergent

relative to its chlorophyte ancestor, but to a lesser degree than in the Kareniaceae. The 'green' plastome of *Lepidodinium*'s is smaller (66.2 vs 98.3 Kbp; Fig. 2), more compact (shorter intergenic spacers; 13% vs 25% of the plastome) and has fewer genes (62 vs 82 protein genes; Fig. 3) than that of its closest chlorophyte relative, *Pedinomonas minor* (Kamikawa et al., 2015). Unlike green and fucoxanthin dinophytes, the dinotoms *Kriptoperidinium* and *Durisnkia* have plastomes which largely fall within the range of variation seen among their ochrophyte progenitors, i.e., they are not considerably divergent (see Figs 2–4; Imanian, Pombert, & Keeling, 2010). The state of dinotome plastomes probably reflects their most recent acquisition. In all the replacing plastids, however, plastome genes appear to now evolve faster relative to their progenitors (or donor groups). In the case of green and fucoxanthin dinophytes, their plastomes have undergone yet another episode of reduction that is associated with new symbiogeneses (on top of the preceding four; Figs 2 and 3).

5.2.7 Expression of Peridinin Plastome Genes in Dinophytes

The fragmented nature of the peridinin plastome is not the only unusual feature of these organelles. Unlike any other plastidic system, the transcripts of protein-coding genes in minicircles are polyuridylylated at their 3′ ends, resulting in a poly(U) tail spanning between 24 and 40 U residues in the mature mRNAs (Nelson et al., 2007; Wang & Morse, 2006). But such postranscriptional modification is also seen in the fucoxanthin plastids of *Karlenia mikimotoi* (Dorrell & Howe, 2012) and *Karlodinium veneficum* (Richardson et al., 2014), and in *Chromera velia* (Janouškovec et al., 2010). The poly(U) tail is not encoded in the minicircle DNA, and therefore it is inferred to be added posttranscriptionally by a yet to be identified enzyme. Transcription of minicircle-encoded genes involves synthesis of a primary RNA via a 'rolling circle' mechanism, which results in a long RNA spanning the minicircle up to several times. This long RNA is then cleaved into smaller pieces (pre-mRNAs) which are then subject to processing at both ends to produce a translatable monocistronic mRNA. Processing of the 3′ terminus involves trimming to a short 3′-UTR and polyuridylylation. Like the poly(A) tail of nuclear transcripts, the poly(U) tail is thought to contribute to stability and protection of the transcripts. The 5′ terminus of the pre-mRNA is also trimmed to a ~40 residue untranslated region (Fig. 7; Barbrook et al., 2012; Dang & Green, 2010). In some species, transcripts of plastid-encoded genes are subject to substitutional editing (i.e. a kind of RNA editing). This is, again, also seen in the

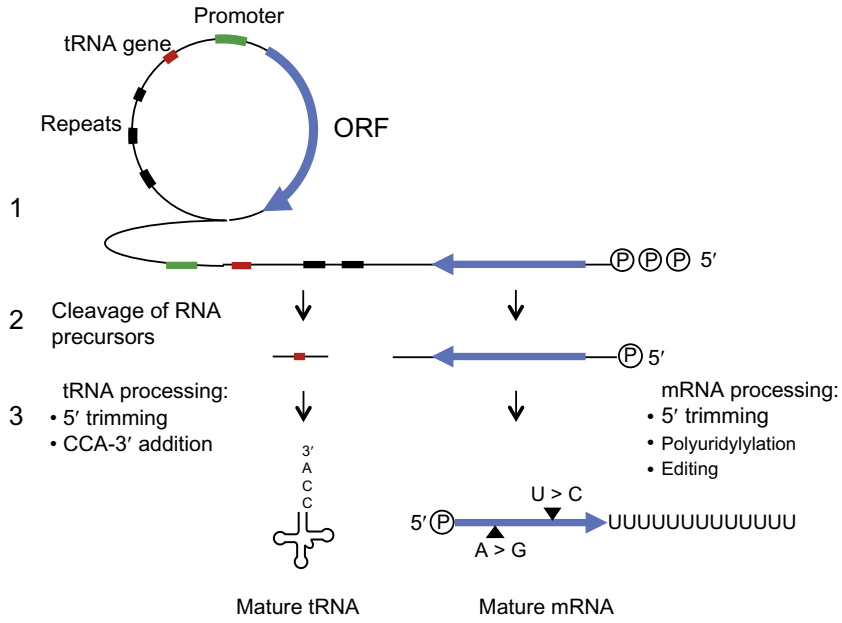

Fig. 7 A summary of the current understanding of gene expression in these organelles. Schematic representation of the steps involved in transcription of plastid-encoded genes in the peridinin plastids: (1) transcription by a yet not identified DNA-dependent RNA polymerase initiates from the 'core' or CNS (promoter) and presumably, RNA synthesis proceeds continuously spanning the entire minicircle more than once (*rolling circle*); (2) it was proposed that RNase Z-type RNases cleave the nascent transcript into 'pre-RNAs', each containing a gene; and (3) each gene is further processed into the mature forms (i.e. tRNA or mRNA).

fucoxanthin plastids of the Kareniaceae, further exemplifying convergent evolution with peridinin plastids (Jackson, Gornik, & Waller, 2013; Richardson et al., 2014). The extent of editing varies from species to species but also between genes. In *Ceratium horridum*, the genes encoding for PsbA, PsbB, and PsbE suffer editing in about 7% of their nucleotide positions, whereas in the SSU rRNA gene the proportion of edited sites was 3.3%. The most frequent substitutions were transitions, being A-to-G and U-to-C the most numerous substitutions, but all possible editing interconversions are known in dinophyte plastome transcripts (Dorrell & Howe, 2015). A similar pattern and proportion of substitutions were observed in *Lingulodinium polyedrum* (Wang & Morse, 2006), *H. triquetra* (Dang & Green, 2010), and in *Symbiodinium minutum* (Mungpakdee et al., 2014). No evidence of editing was found in *A. carterae* (Barbrook et al., 2001). Although editing affects a small proportion of nucleotides, the process seems

to be critical for maintaining the proper performance of the encoded proteins. In *S. minutum*, a large majority of the substitutions resulting from editing (88%) caused amino acid changes (Mungpakdee et al., 2014). Like in other systems were editing happens, such as the mitochondria of plants, kinetoplastids, and other organisms (including dinoflagellates), translation of the DNA-encoded sequence results in loss of conserved and otherwise functionally important amino acid positions, or even in premature stop codons. Editing, therefore, is essential to overcome deleterious substitutions.

5.3 Availability of Myzozoan Plastomes

To date, as of September 2017, numerous apicoplast plastomes have been sequenced (see Table 1). However, many of them have been incompletely sequenced or assembled and are therefore found as partial in public databases. The presence of nearly identical IRs in many apicomplexan plastomes difficult their final assembly and circularization. Only two chromerid plastomes have been sequenced so far (for the only two culturable species), and there are currently eight plastomes sequenced for dinophytes, four for peridinin dinophytes, one for a green dinophyte, one for a fucoxanthin dinophyte, and two for dinotoms. In addition to the four fully (or almost fully) sequenced plastomes for peridinin dinophytes (*Amphidinium carterae* CCAP1102/6, *Amphidinium carterae* CS21, *Heterocapsa triquetra*, and *Symbiodinium* sp. clade C3), there are some few plastome minicircles/genes reported for *Adenoides eludens* (*psbA, psbD*), *Ceratium horridum* (*psaA, psaB, psbB, psbC, psbD, petB, ycf24, ycf16, psbE, psaB, psbC, psbD*), *Heterocapsa niei* (*psbA*, 23S rRNA), *Heterocapsa pygmaea* (*psbA*, 23S rRNA), *Heterocapsa rotundata* (23S rRNA, *psbA, trnW, trnP*), *Protoceratium reticulatum* (23S rRNA), *Pyrocystis lunula* (*rpl28* (?), *rpl33* (?), *psbC*, *psbC*), *Symbiodinium* sp. clade A (*psbA*), and *Symbiodinium* sp. clade B (*psbA*) (see Howe et al., 2008; Moszczyński, Mackiewicz, & Bodył, 2012).

6. GENE TRANSFER IN MYZOZOANS

Gene transfer from organelle to nuclear genomes, or EGT, is a well-known phenomenon (Martin, 2003). The main evolutionary function of EGT in the evolution of plastids has been to transfer genes from the ancestral plastome to the nuclear genome of its host. This has served to integrate plastids within their host cells, and to relieve plastome genes from mutational meltdown due to Muller's ratchet (the accumulation of deleterious mutations in asexual lineages). EGT has been well studied in plants (all eukaryotes

Table 1 Availability of Sequenced Plastomes for Species in the Myzozoa

Species	Accession Number	Source	Completeness	Reference
Sporozoa				
Babesia bovis T2Bo	AAXT01000007	NCBI GenBank	Complete	Brayton et al. (2007)
Babesia microti RI	LK028575	NCBI GenBank	Complete	Garg et al. (2014)
Babesia orientalis Wuhan	KT428643	NCBI GenBank	Complete	Huang et al. (2015)
Babesia sp. Lintan	KX881915.1	NCBI GenBank	Complete	Wang et al. (2017)
Babesia sp. Xinjiang	KX881914.1	NCBI GenBank	Complete	Wang et al. (2017)
Cyclospora cayetanensis CHN HEN01	KP866208	NCBI GenBank	Complete	Tang et al. (2015)
Eimeria tenella Penn State	AY217738	NCBI GenBank	Complete	Cai et al. (2003)
Leucocytozoon caulleryi Niigata	AP013071	NCBI GenBank	Complete	Imura et al. (2014)
Plasmodium berghei ANKA	LK023130	NCBI GenBank	Partial	Aslett et al. (unpublished)
Plasmodium berghei ANKA	AB649421	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium berghei NK65	NC_030892.1	NCBI GenBank	Partial	GenBank (unpublished)
Plasmodium brasilianum Bolivian I	CM007351	NCBI GenBank	Partial	Talundzic (unpublished)
Plasmodium chabaudi AS	AB649423	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium chabaudi chabaudi	HF563595	NCBI GenBank	Partial	Sato, Sesay, and Holder (2013)
Plasmodium coatyeni CDC	AB649420	NCBI GenBank	Partial	Arisue et al. (2012)

Plasmodium falciparum	X95275–X95276	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium falciparum HB3	DQ642846	NCBI GenBank	Partial	Birren et al. (unpublished)
Plasmodium gaboni SY75	CM003884	NCBI GenBank	Partial	Sundararaman et al. (2016)
Plasmodium gallinaceum A8	AB649424	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium malariae Kisii67	AB649418	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium ovale NIGERIA II	AB649417	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium reichenowi SY75	CM003883	NCBI GenBank	Partial	Sundararaman et al. (2016)
Plasmodium vivax Brazil I	JQ437257	NCBI GenBank	Partial	Neafsey et al. (unpublished)
Plasmodium vivax Mauritania I	JQ437258	NCBI GenBank	Partial	Neafsey et al. (unpublished)
Plasmodium vivax North Korean	JQ437259	NCBI GenBank	Partial	Neafsey et al. (unpublished)
Plasmodium vivax Salvador I	AB649419	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium yoelii 17NXL	AB649422	NCBI GenBank	Partial	Arisue et al. (2012)
Plasmodium yoelii 17X	LM993669	NCBI GenBank	Partial	Aslett et al. (unpublished)
Plasmodium yoelii YM	LK934643	NCBI GenBank	Partial	Aslett et al. (unpublished)
Theileria parva Muguga	AAGK01000009	NCBI GenBank	Complete	Gardner et al. (2005)
Toxoplasma gondii ME49 RH	U87145	NCBI GenBank	Complete	Kissinger et al. (unpublished)
Apicomanda				
Chromera velia CCMP2878	NC_014340.2	NCBI GenBank	Complete	Janouškovec et al. (2010)
Vitrella brassicaformis CCMP3155	HM222968.1	NCBI GenBank	Complete	Janouškovec et al. (2010)

Continued

Table 1 Availability of Sequenced Plastomes for Species in the Myzozoa—cont'd

Species	Accession Number	Source	Completeness	Reference
Dinokaryota				
Amphidinium carterae CCAP1102/6	Many nonconsecutive GenBank entries. See reference for accession numbers.	NCBI GenBank	'Complete'	Barbrook and Howe (2000), Barbrook et al. (2001), Nisbet et al. (2004), and Barbrook, Santucci, et al. (2006)
Amphidinium carterae CS21	Many nonconsecutive GenBank entries. See reference for accession numbers.	NCBI GenBank	'Complete'	Hiller (2001) and Barbrook, Santucci, et al. (2006)
Durinskia baltica CS-38	NC_014287.1	NCBI GenBank	Complete	Imanian et al. (2010)
Heterocapsa triquetra	Many nonconsecutive GenBank entries. See reference for accession numbers.	NCBI GenBank	'Complete'	Zhang et al. (2001, 1999) and Nelson et al. (2007)
Karlodinium veneficum	JN039300.1	NCBI GenBank	Partial	Gabrielsen et al. (2011)
Kryptoperidinium foliaceum CCMP1326	NC_014267.1	NCBI GenBank	Complete	Imanian et al. (2010)
Lepidodinium chlorophorum	NC_027093.1	NCBI GenBank	Complete	Kamikawa et al. (2015)
Symbiodinium sp. clade C3	HG515015–HG515025, HG515027, and HG515028	NCBI GenBank	'Complete'	Barbrook et al. (2014)

that belong to the clade Archaeplastida) where it has been inferred that nuclear genomes have 200–600 genes of cyanobacterial origin (Moustafa & Bhattacharya, 2008; Price et al., 2012). Most of these genes likely have plastid functions (Reyes-Prieto, Hackett, Soares, Bonaldo, & Bhattacharya, 2006). In the case of myzozoans, which acquired their plastid from a red alga, most genes were transferred from the nucleus of the endosymbiotic red alga to the host nucleus (of either an ancestral chromalveolate or myzozoan; see discussion above). Because myzozoan plastomes have smaller gene repertoires than those of other red meta-algae (Fig. 3), some direct gene transfer from the plastome to the host nucleus also happened. The proteome of a photosynthetic plastid in red meta-algae is estimated to be composed of about 800–1000 proteins (Dorrell et al., 2017; Gruber et al., 2007), which means that about 700–900 genes might have been transferred and now reside in the myzozoan nucleus (ancestral myzozoan plastomes encoded only 80 genes). During dinozoan evolution even more genes, about 69 (all ribosomal and many photosynthetic proteins), were transferred from the plastome to the 'dinokaryon'. Some reports have attempted to estimate the impact of EGT in some dinophyte nuclear genomes (e.g. see Hackett et al., 2004; Hehenberger et al., 2016; Nosenko et al., 2006; Patron, Waller & Keeling, 2006; Minge et al., 2010; Burki et al., 2014). Apicomplexans, on the other hand, greatly reduced their plastid proteome when they lost photosynthesis. It is estimated that the apicoplast proteomes has 500 proteins (Ralph et al., 2004), and thus about 470 ancestrally plastome genes now reside in the apicomplexan nucleus. Of course, these are just rough estimates because some ancestral plastid genes could simply have been lost (instead of transferred, i.e. the plastid proteome simplified), and the host could also have retargeted its own new proteins to the plastid.

EGT has also contributed to the accumulation of nonfunctional and noncoding DNA in nuclear genomes. These are called 'NUPT' for nuclear plastid DNA. Analyses of genomes have concluded that sporozoans have relatively low amounts of NUPTs (Smith, Crosby, & Lee, 2011). This is expected according to the 'limited window transfer' hypothesis which proposes low rates of DNA transfer from the apicoplast to the nucleus (because all sporozoans have one single apicoplast). The relatively small nuclear genomes of parasitic sporozoans seem to primarily evolve in a reductive fashion and therefore also make them less prone to accumulate noncoding DNA-like NUPTs (Smith et al., 2011). In some sporozoans, like the piroplasmids *Babesia* and *Theileria*, no NUPTs were found at all; they also have the smallest nuclear genomes among sporozoans. The coccidians

Eimeria and *Toxoplasma*, which have larger nuclear genomes, have 31 and 77 NUPTs reported, respectively. But these numbers are insignificant in comparison to land plants which harbour many plastids per cell, have incredibly bloated nuclear genomes, and can have as many as 2036 NUPTs in the case of *Oryza sativa*. Unfortunately, rates of gene transfer from plastids to the nuclei of dinozoans have not been studied yet. The reason is that those dinozoans for which we have nuclear genomes have lost their plastomes (*Perkinsus* and *Hematodonium*), whereas dinozoans for which we have their plastomes (*Heterocapsa* and *Amphidinium*) do not have their nuclear genomes sequenced (because of their massive proportions). The only exception would be the coral endosymbiont *Symbiodinium* for which there are now both nuclear and plastid genomes available (Aranda et al., 2016; Barbrook et al., 2014; Lin et al., 2015; Shoguchi et al., 2013); however, no search for NUPTs has been done yet. It is expected for dinophytes to have large number of NUPTs because they usually possess numerous peridinin-containing plastids and have easily expandable genomes. Such analyses are also wanting for the apicomonads *Chromera* and *Vitrella*, for which both nuclear and plastid genomes are now available (Janouškovec et al., 2010; Woo et al., 2015).

7. CONCLUSIONS AND FUTURE DIRECTIONS

This chapter has provided a general description of the main features observed among the diversity of myzozoan plastomes. It has also attempted to generally describe the evolutionary trajectories that plastome-bearing myzozoans have followed. We aimed to do both things within a general framework where some of the eccentricities observed among myzozoan plastomes can be compared to all other plastid-bearing eukaryotes.

To summarize, myzozoan plastomes most likely have a most recent common ancestor. But it is more uncertain whether this ancestral myzozoan inherited its plastid vertically from a distant ancestor or laterally from an unrelated alga. Myzozoan diversification produced a great diversity of plastids. Some preserved the ancestral property of performing photosynthesis (like in some apicomonads and dinophytes). But leucoplasts evolved repeatedly among myzozoans. One lineage turned plastids into leucoplasts that retained a plastome (the Sporozoa), whereas many others repeatedly lost the plastome altogether (some apicomonads and dinozoans). The only two examples of outright plastid loss known to date are myzozoans, one dinoflagellate and one sporozoan. The ancestral myzozoan plastome most closely resembled that of *Vitrella*'s among the sampled diversity of modern myzozoans. The other

two plastome-bearing lineages (sporozoans and dinophytes) have followed divergent evolutionary lines and their plastome now virtually have non-overlapping gene repertoires. Peridinin dinophytes have the smallest plastomes among eukaryotes, and yet they support photosynthesis. They are also fragmented into plasmid-like minicircles that generally contain one single gene. Sporozoans have a more typical leucoplast plastome that generally conserves a classical quadripartite organization. Myzozoans plastomes hold records as the smallest plastomes (for dinophytes) and the most GC-rich (for sporozoans). The small sizes of myzozoan plastomes seems to have been achieved through four episodes of genome reduction.

Future sampling will undoubtedly expand the known diversity of myzozoan plastomes. We will most likely find new chromerid plastomes (like ARLs), as well as 'deeply diverging' apicoplast plastomes (like environmental lineages VI–X), and perhaps dinozoan plastomes that are less reduced and fragmented. These will allow us to better reconstruct the changes that gave rise to the reduced plastomes of sporozoans and dinophytes. For instance, the fine-grain sampling among parasitic land plants has unravelled the gradual mode of plastome reduction in different embryophytic lineages. The field of metagenomics promises to make these discoveries soon and to greatly improve our knowledge of plastome diversity in the Myzozoa.

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