

## NUCLEOMORPH KARYOTYPE DIVERSITY IN THE FRESHWATER CRYPTOPHYTE GENUS *CRYPTOMONAS*<sup>1</sup>

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Cryptophytes are unicellular, biflagellate algae with plastids (chloroplasts) derived from the uptake of a red algal endosymbiont. These organisms are unusual in that the nucleus of the engulfed red alga persists in a highly reduced form called a nucleomorph. Nucleomorph genomes are remarkable in their small size (<1,000 kilobase pairs [kbp]) and high degree of compaction (~1 kbp per gene). Here, we investigated the molecular and karyotypic diversity of nucleomorph genomes in members of the genus *Cryptomonas*. 18S rDNA genes were amplified, sequenced, and analyzed from *C. tetrapyrenoidosa* Skuja CCAP979/63, *C. erosa* Ehrenb. emmend. Hoef-Emden CCAP979/67, *Cryptomonas* sp. CCAP979/52, *C. lundii* Hoef-Emden et Melkonian CCAP979/69, and *C. lucens* Skuja CCAP979/35 in the context of a large set of publicly available nucleomorph 18S rDNA sequences. Pulsed-field gel electrophoresis (PFGE) was used to examine the nucleomorph genome karyotype of each of these strains. Individual chromosomes ranged from ~160 to 280 kbp in size, with total genome sizes estimated to be ~600–655 kbp. Unexpectedly, the nucleomorph karyotype of *Cryptomonas* sp. CCAP979/52 is significantly different from that of *C. tetrapyrenoidosa* and *C. lucens*, despite the fact that their 18S rDNA genes are >99% identical to one another. These results suggest that nucleomorph karyotype similarity is not a reliable indicator of evolutionary affinity and provides a starting point for further investigation of the fine-scale dynamics of nucleomorph genome evolution within members of the genus *Cryptomonas*.

**Key index words:** *Cryptomonas*; cryptophyte; genome evolution; nucleomorph; secondary endosymbiosis

**Abbreviations:** GTR, general time reversible; ML, maximum likelihood; PFGE, pulsed-field gel electrophoresis

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Eukaryotic cells first acquired photosynthesis through the engulfment and retention of a cyanobacterial endosymbiont >1 billion years ago (Yoon

et al. 2004). This primary endosymbiotic event produced the first primary plastid-containing algae, which ultimately gave rise, through vertical evolution, to the red, green, and glaucophyte algae (Archibald and Keeling 2002, 2005, Palmer 2003, Keeling 2004). More recently, plastids have moved horizontally across the eukaryotic tree by the process of secondary endosymbiosis, in which a primary plastid-containing eukaryote is taken up by a non-photosynthetic eukaryotic host cell (Archibald and Keeling 2002, Palmer 2003, Keeling 2004). The exact number of secondary endosymbioses that have occurred during eukaryotic evolution is contentious. However, it is thought that secondary endosymbiosis has occurred at least twice and involved the uptake of both red and green algal endosymbionts (Archibald and Keeling 2002, Palmer 2003, Keeling 2004, Bodyl 2005).

The cryptophytes are an important lineage of secondary plastid-containing algae whose plastid is derived from a captured red alga (McFadden 1993). Cryptophytes are unusual in that they still possess the vestigial nucleus of their eukaryotic endosymbiont, referred to as a nucleomorph (McFadden 1993, Archibald 2007). Molecular and karyotypic analyses have revealed that the nucleomorphs of cryptophytes, as well as those of an unrelated group of algae called chlorarachniophytes, possess the smallest eukaryotic genomes reported thus far, with those in cryptophytes ranging from ~450 to 845 kbp in size (Eschbach et al. 1991, Rensing et al. 1994, Lane et al. 2006). All known nucleomorph genomes comprise three miniature chromosomes (Gilson and McFadden 2002, Archibald 2007), which can be resolved using PFGE (Eschbach et al. 1991, Rensing et al. 1994, Lane and Archibald 2006, Lane et al. 2006). The nucleomorph genome of the model cryptophyte *Guillardia theta* has been sequenced and is 551 kbp in size, with a gene density of ~1 kbp per gene and 44 instances of overlapping genes (Douglas et al. 2001). Each chromosome end is capped with an atypical telomere and subtelomeric rDNA operons. These unusual features make cryptophyte nucleomorphs an appealing subject for the study of eukaryotic genome evolution and, in particular, the process of genome reduction and compaction.

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<sup>1</sup>Received 12 September 2007. Accepted 28 September 2007.

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The genus *Cryptomonas* is unique among cryptophyte genera in that it includes exclusively freshwater species, as well as the only species containing a leucoplast (i.e., a nonphotosynthetic plastid). *Cryptomonas paramecium* has been shown to possess the smallest nucleomorph genome of known cryptophytes (~450 kbp; Rensing et al. 1994) and resides within a clade of species that appear to have elevated rates of nucleomorph sequence evolution (Hoef-Emden et al. 2002, Hoef-Emden and Melkonian 2003, Hoef-Emden 2005). These features make *Cryptomonas* an intriguing group for the comparative study of genome reduction and gene transfer from the nucleomorph to the host genome. Here, we present the first survey of nucleomorph karyotype diversity within the genus *Cryptomonas*. Our results indicate that nucleomorph genome size varies considerably within this lineage, even among closely related species.

Cryptophyte cultures were obtained from the Culture Collection of Algae and Protozoa (CCAP; Dunbeg, Argyll, UK). Nucleomorph-specific primers were used to amplify, clone, and sequence 18S rDNA genes from four cryptophyte species (as in Lane et al. 2006)—*C. tetrapyrenoidosa* CCAP979/63 (GenBank accession EU135976), *Cryptomonas* sp. 979/52 (EU135977), *C. lundii* CCAP979/69 (EU135975), and *C. lucens* CCAP979/35 (EU135974)—whereas data from *C. erosa* CCAP979/67 (AM396361) were already available. A maximum-likelihood (ML) phylogenetic tree was constructed using the general-time-reversible (GTR) model in PhyML (Guindon and Gascuel 2003) with four rate categories and an additional invariable-rates category. Parameters were estimated from the data, and 1,000 bootstrap replicates were performed to assess the reliability of the resulting topology.

Phylogenetic analysis of these sequences in the context of a range of cryptophyte 18S rDNA sequences obtained from GenBank revealed that *C. tetrapyrenoidosa*, *Cryptomonas* sp. 979/52, *C. lundii*, and *C. lucens* group within a closely related clade, supported by a bootstrap value of 99% (Fig. 1) and an average nucleomorph 18S rDNA sequence identity of 99.5%. Of all five strains examined here using PFGE, only *C. erosa* was resolved outside of the main *Cryptomonas* clade, sharing ~93% sequence identity with the other four strains. Collectively, all five sequences were distinct from the nonphotosynthetic members of the genus such as *C. paramecium* (Fig. 1).

PFGE was used as described previously (Lane et al. 2006) to separate the nucleomorph chromosomes of the five *Cryptomonas* strains. PFGE-separated chromosomes were transferred to nylon membranes and hybridized against an 18S rDNA probe created from *Hemiselms anderseni* (CCMP644) nucleomorph DNA as in Lane and Archibald (2006). As has been observed for other species, distinct nucleomorph chromosomal bands were not

visible under ethidium bromide staining for most of the *Cryptomonas* strains examined here (data not shown), likely due to the exceedingly small fraction of total cellular DNA that is of nucleomorph origin. However, Southern hybridization with an 18S rDNA probe produced positive hybridization signals from the nucleomorph chromosomes, due to the fact that rDNA operons are a characteristic feature of nucleomorph chromosome ends (Fig. 2). Extensive cross-hybridization to the nuclear chromosomes of most species was also apparent, as seen previously (Rensing et al. 1994, Lane et al. 2006).

Considerable nucleomorph karyotype variation was observed among the five strains, with individual chromosomes ranging from ~160 to 280 kbp (Fig. 2). *Cryptomonas erosa*, *C. lucens*, and *C. tetrapyrenoidosa* were determined to possess similar karyotypes, with three similarly sized chromosomes and total genome sizes ranging from ~635 to 655 kbp (Fig. 2). Interestingly, *Cryptomonas* sp. 979/52 and *C. lundii* possess highly distinct karyotypes, despite the fact that their 18S rDNA genes share 99.5% sequence identity with those of *C. lucens* and *C. tetrapyrenoidosa*. *Cryptomonas* sp. 979/52 contains three very dissimilarly sized chromosomes of ~160, ~200, and ~280 kbp but maintains a total genome size of ~640 kbp. The 18S rDNA and karyotype results for this species were confirmed using two independent cell cultures, ruling out the possibility of DNA contamination. Surprisingly, hybridization to *C. lundii* chromosomal DNA produced a single, slightly diffuse hybridizing band. This finding raises the following possibilities: (i) the *C. lundii* nucleomorph genome has fewer than three chromosomes, (ii) the genome contains chromosomes that lack 18S rDNA genes (as has been reported for members of the genus *Hemiselms* [Lane and Archibald 2006]), or (iii) *C. lundii* has nucleomorph chromosomes of sufficiently similar size so as to be inseparable under the electrophoretic conditions used in this study. Given that all known cryptophyte (and chlorarachniophyte) nucleomorph genomes possess three chromosomes (Archibald 2007), we favor the latter scenario and tentatively assign a nucleomorph genome size of ~600 kbp to *C. lundii* (Fig. 2).

The genus *Cryptomonas* is one of the least understood groups of cryptophytes from the perspective of nucleomorph genome size diversity (Rensing et al. 1994). Considering the nucleomorph 18S rDNA and karyotype analyses presented above, it would appear that nucleomorph karyotype similarity is a poor indicator of phylogenetic relatedness in members of this genus. Very closely related organisms with nearly identical nucleomorph 18S rDNA sequences, such as *Cryptomonas* sp. 979/52 or *C. lundii* compared with *C. lucens*, were shown to have substantially different nucleomorph karyotypes; whereas more divergent taxa, such as *C. erosa* and *C. tetrapyrenoidosa*, show a similar (presumably pleisiomorphic) karyotype (Figs. 1 and 2). These observations

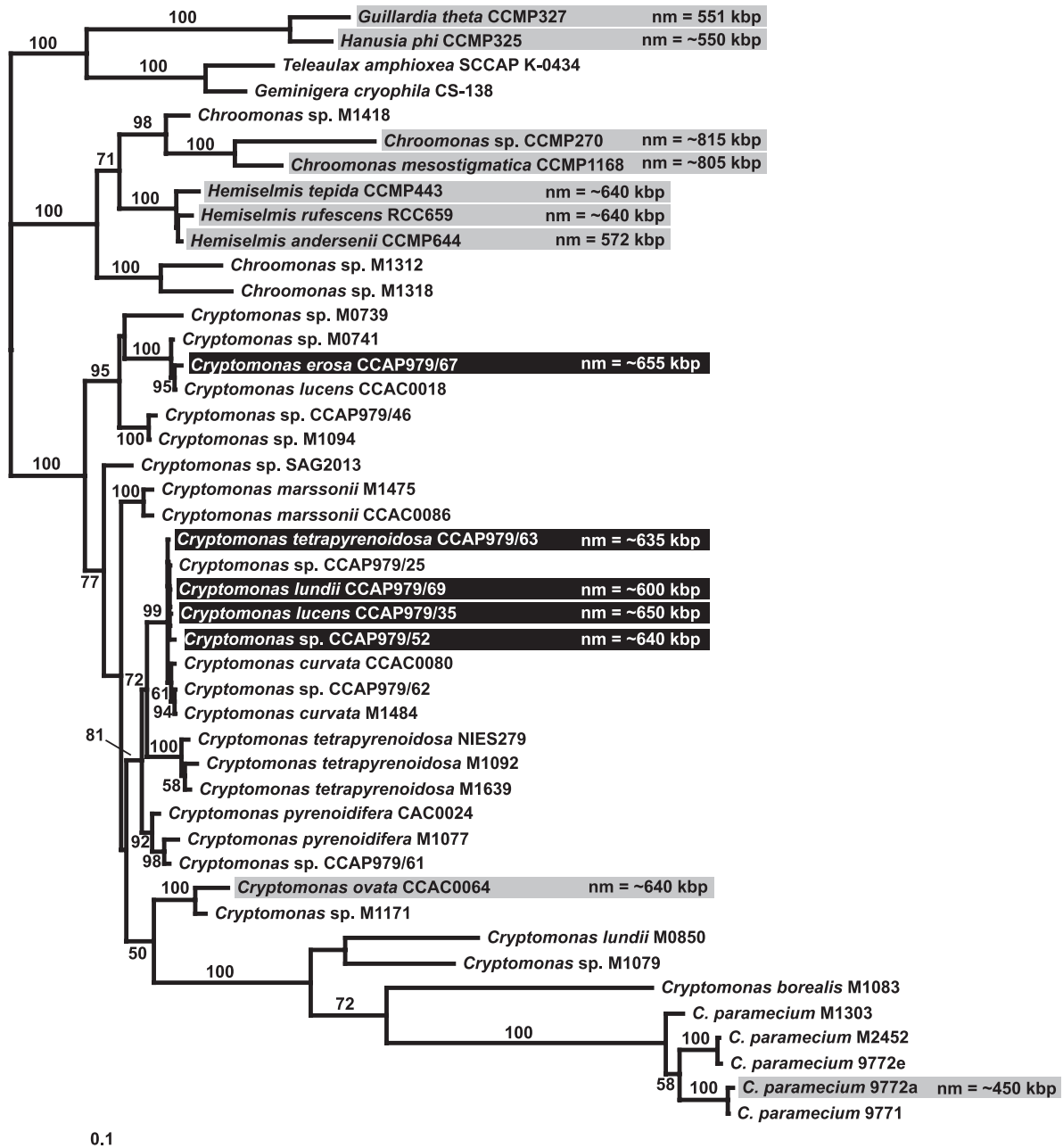


FIG. 1. Maximum-likelihood nucleomorph 18S rDNA phylogeny of diverse members of the cryptophyte genus *Cryptomonas*. The phylogeny is arbitrarily rooted with the clade containing *Guillardia theta* and *Teleaulax acuta*. Taxa for which new nucleomorph 18S rDNA sequence data and genome size estimates are provided are highlighted with a black background, whereas previously published data are highlighted in gray. Genome sizes are estimated from chromosomes separated by pulsed-field gel electrophoresis, except for two sequenced genomes, *G. theta* and *Hemiselmis andersenii*. Bootstrap support values (1,000 replicates) are provided where  $\geq 50\%$ . Scale bar indicates the inferred number of nucleotide substitutions per site. Culture collection abbreviations are as follows: CCAC, Culture Collection of Algae at the University of Cologne, Cologne, Germany; CCAP, Culture Collection of Algae and Protozoa, Dunbeg, Argyll, UK; CCMP, Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Boothbay Harbor, Maine, USA; CS, CSIRO Collection of Living Micro-algae, Hobart, Tasmania, Australia; M, Dr. Michael Melkonian, Universität zu Köln, Köln, Germany; NIES, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan; RCC, Roscoff Culture Collection, Roscoff, France; SAG, Sammlung von Algenkulturen, Göttingen, Germany; SCCAP, Scandinavian Culture Centre for Algae and Protozoa, Copenhagen, Denmark.

indicate the dynamic nature of nucleomorph genome architecture in *Cryptomonas*. However, even in cases such as the highly distinct karyotype observed from *Cryptomonas* sp. 979/52, the overall genome

size (~640 kbp) is very similar to that of its closest relatives based on 18S rDNA phylogenies (*C. lucens* and *C. tetrapyrenoidosa* have estimated nucleomorph genome sizes of ~650 and ~635 kbp, respectively).

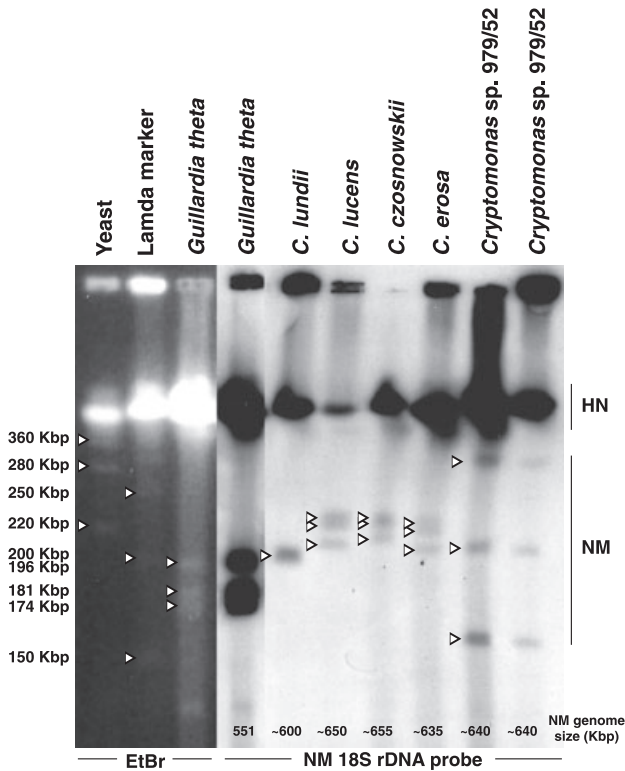


Fig. 2. Nucleomorph karyotype diversity in members of the genus *Cryptomonas*. Left, pulsed-field gel electrophoresis (PFGE) of nucleomorph chromosomes from the model cryptophyte *Guillardia theta* run beside size standards (the yeast *Saccharomyces cerevisiae* and Lambda). The *G. theta* genome has been completely sequenced and is 551 kbp in size. Right, Southern hybridization of PFGE-separated chromosomes from *G. theta* and five different *Cryptomonas* sp. CCAP979/52 lanes correspond to PFGE samples prepared from two cell cultures obtained independently of one another. The precise strain designations are provided in the main text. Hybridization signals corresponding to nucleomorph chromosomes are highlighted with an arrow. A significant amount of cross-hybridization to 18S rDNA loci on host nuclear chromosomes is also apparent. NM, nucleomorph; HN, host nucleus; EtBr, ethidium bromide; kbp, kilobase pair.

This finding suggests that one or more partial nucleomorph chromosome translocations have taken place very recently during the evolution of this genus.

The exceedingly small and gene-dense nucleomorph genomes contained within the cryptophyte algae have the potential to provide considerable insight into the process of genome reduction and compaction underway in intracellular endosymbionts and organelles (Archibald 2007). The nucleomorph karyotype and 18S rDNA sequence data presented in this study indicate that significant chromosomal rearrangements can occur over relatively short evolutionary timescales, even between closely related strains of the same species. Elucidating the processes of gene loss and nucleomorph-to-host-nucleus gene transfer occurring in the more

highly rearranged genomes will be important in understanding the process of genome reduction in these miniature nuclei and may provide clues to the same process in eukaryotes as a whole.

This work was supported by a Discovery Grant awarded to J. M. A. from the Natural Sciences and Engineering Research Council of Canada (NSERC). Much appreciated salary support for K. P. and N. D. was provided by NSERC. NSF and BSA contributed to the dissemination of these data in the systematics and phycology sections of the 2007 Plant Biology and Botany joint congress in Chicago. J. M. A. is a Scholar of the Canadian Institute for Advanced Research, Integrated Microbial Biodiversity Program.

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