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Goniomonas: rRNA sequences indicate that this phagotrophic flagellate is a close relative of the host component of cryptomonads

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The nucleotide sequence of the polymerase chain reaction (PCR)-amplified small subunit ribosomal RNA gene of *Goniomonas truncata* was determined. Addition of the *Goniomonas* sequence to the eukaryotic phylogenetic tree revealed this heterotrophic flagellate to be the sister taxon of photosynthetic cryptomonads. The molecular phylogeny supports morphological data suggesting that *Goniomonas* diverted from the cryptomonad lineage prior to their acquisition of a plastid through endosymbiosis of a eukaryote. *Goniomonas*, which is phagotrophic, may thus represent an extant relative of the host component of cryptomonad algae.

Keywords: Cryptomonads, Endosymbiosis, *Goniomonas*, Plastid origins, rRNA phylogeny.

Introduction

Cryptomonads are chimeric cells composed of two different eukaryotes (see McFadden, 1993, for review). A biflagellate host harbours a photosynthetic endosymbiont (Maier *et al.*, 1991; Douglas *et al.*, 1991*b*). Although much reduced, the eukaryotic endosymbiont retains its nucleus and cytoplasm (McFadden *et al.*, 1994). The endosymbiont nucleus, known as the nucleomorph (Greenwood, 1974), encodes rRNAs (Douglas *et al.*, 1991*b*; Maier *et al.*, 1991) which are apparently incorporated into ribosomes in the endosymbiont cytoplasm (McFadden *et al.*, 1994). Genes for both the endosymbiont and host rRNAs have been cloned and sequenced (Maier *et al.*, 1991; Douglas *et al.*, 1991*b*), and the sequences have been added to the eukaryotic phylogenetic tree to identify the evolutionary relationships of the two components making up cryptomonads (Maier *et al.*, 1991; Douglas *et al.*, 1991*b*). These trees suggest that the endosymbiont is most closely related to extant red algae, which is also consistent with the presence of phycobilin pigments and the site of starch storage in the endosymbionts and red algae (see McFadden, 1993, for review). In addition, phylogenetic trees inferred from sequences of plastid genes such as *rbcL* and 16S rRNA also suggest that the endosymbiont could share a common ancestry with red algae (Douglas *et al.*, 1991*a*; Douglas & Turner 1991; Maerz *et al.*, 1992).

While the endosymbiont is thus widely held to be a red-algal-like organism (but see Cavalier-Smith, 1989, for an argument against a red algal endosymbiont), the identity of the cryptomonad host is not at all clear. Phylogenetic trees group the cryptomonad hosts with the rhizopods *Acanthamoeba* and *Hartmanella* (Maier *et al.*, 1991; Douglas *et al.*, 1991*b*). However, cryptomonads and rhizopods share few morphological features. Cryptomonads are biflagellates with semi-rigid periplasts, ejectisomes and a gullet, while *Acanthamoeba* and *Hartmanella* are naked amoebae, making it difficult to envisage a rhizopod as the original host for the symbiosis. A far more likely candidate for the cryptomonad host is the phagotrophic flagellate *Goniomonas*. This plastid-lacking organism is recognised as sharing a number of ultrastructural features with the plastid-containing cryptomonads, and, on the basis of these ultrastructural similarities, *Goniomonas* has been proffered as a possible candidate for the cryptomonad host (Kugrens & Lee, 1991). To test the likelihood that *Goniomonas* is related to the cryptomonad host, we amplified the small subunit ribosomal RNA (srRNA) gene of this organism and used the nucleotide sequence from the polymerase chain reaction (PCR)-amplified gene to infer phylogenetic trees.

Materials and methods

Goniomonas truncata (Fresenius) Stein [synonym=*Cyathomonas truncata* (Fresenius) Fisch] was isolated into unialgal culture by micropipetting from a water sample collected from the Yarra River, Ivanhoe, Victoria, Australia. Cells were maintained in WARIS-H medium (McFadden & Melkonian, 1986) to which was added one autoclaved

wheat grain for every 5 ml of medium just prior to subculturing. Growth temperature was 20°C. DNA was isolated as described by Rochaix *et al.* (1988), and universal eukaryotic primers ($^{5'}$TACCTGGTGGATCCTGCCAG$^{3'}$ and $^{5'}$TGATCCTTCTGCAGGTTCACCTAC$^{3'}$) were used to amplify the small subunit rRNA gene by PCR. After an initial 3 min denaturation at 94 °C, a 50 μl reaction mix containing 100 ng template DNA, 1 U AmpliTaq (Perkin Elmer/Cetus), 0·25 mM dNTPs, 100 pmol of each primer, and 5 μl of ×10 reaction buffer (Perkin Elmer/Cetus) was subjected to 35 cycles comprised of 30 s at 48 °C, 2 min at 72 °C and 30 s at 94 °C in a TC1 cycler (Perkin Elmer). The extension time in the final cycle was increased to 6 min. PCR reactions were electrophoresed in 0·8% agarose containing 100 μg/ml ethidium bromide and the amplified product purified using Prep-A-Gene matrix (Bio-Rad).

The purified PCR product was digested with the enzymes *Bam*HI and *Pst*I, which digest restriction endonuclease sites incorporated within the amplification primers, for sticky-ended cloning into a plasmid sequencing vector. As the PCR-amplified *Goniomonas* srRNA gene was found to contain two internal *Bam*HI sites, it was cloned in three fragments. Both strands of the plasmid inserts were sequenced on an ABI 373a Automated Sequencer using either dye-labelled vector primers or universal srRNA gene primers (Saunders & Druehl, 1992) in conjunction with dye-labelled dideoxy terminators (Applied Biosystems). Contiguous sequences were assembled using SeqEd 1.03 (Applied Biosystems). The *Goniomonas* srRNA gene sequence (GenBank accession number U03072) was aligned to 51 other eukaryote srRNA sequences that were selected from a published alignment containing 1396 srRNA gene sequences with 4371 positions based on both primary and secondary structure (De Rijk *et al.*, 1992). Gaps common to the selected sequences were removed with SeqApp (version 1.8α154 for Apple/Macintosh written by Don Gilbert, Indiana University, and available by anonymous ftp from ftp.bio.indiana.edu/molbio/seqapp/seqapp.hqx), leaving 3080 positions. The *Goniomonas* sequence was aligned to the large alignment using the profile option in Clustal V (Higgins *et al.*, 1992). Minor adjustments to the alignment were made using SeqApp, resulting in an alignment of 3164 positions. Bootstrap replicates, Jukes–Cantor distance matrices, neighbour-joining, consensus trees and parsimony analysis were done with routines in Phylip 3.41 (Felsenstein, 1989) using 1788 positions judged to be aligned unambiguously.

Results and discussion

PCR amplification of srRNA genes using universal eukaryotic primers with *Goniomonas* DNA as template yielded a single product 1988 base pairs in length. When plastid-containing cryptomonads were used in similar PCRs, two nuclear-type srRNA gene products were recovered (Douglas *et al.*, 1991*b*; McFadden *et al.*, 1994). One product is from the host nuclear srRNA genes, while the other is from the endosymbiont nucleomorph genome (Douglas *et al.*, 1991*b*; Maier *et al.*, 1991; McFadden *et al.*, 1994). Previous electron microscopy analyses found no trace of any endosymbiont in *Goniomonas* (Kugrens & Lee, 1991), and the PCR analysis undertaken here indicates that no endosymbiont srRNA genes are present in *Goniomonas*.

The phylogenetic tree inferred from the srRNA gene sequences identifies *Goniomonas* as a sister to the host component of cryptomonads (Fig. 1). Bootstrap values of 100% indicate that this is very robust grouping (Fig. 1). Since parsimony analysis also grouped *Goniomonas* as a sister to the host component of cryptomonads (not shown), we believe the relationship is firm. The grouping of *Goniomonas* with cryptomonads is congruent with available morphological data. *Goniomonas* and cryptomonads are ultrastructurally similar in that both are biflagellate, have an anteriolateral furrow and vestibulum, possess ejectisomes, have mitochondria with flattened cristae, contain doublet septa in the flagellar transition region, and have a semi-rigid periplast (Mignot, 1965; Mignot *et al.*, 1968; Schuster, 1968; Hausmann, 1979; Hill, 1991*a*; Kugrens & Lee, 1991). However, *Goniomonas* differs from the other cryptomonads in several ways: the flagella lack typical cryptomonad mastigonemes; the large ejectisomes are arranged in a band around the furrow; the flagella are inserted on the dorsal side of the vestibulum rather than the right side; and it possesses a cytopharynx or infundibulum, through which it is believed to ingest food particles (Mignot, 1965; Schuster, 1968; Mignot *et al.*, 1968; Hausmann, 1979; Kugrens *et al.*, 1987; Hill, 1991*a*; Kugrens & Lee, 1991).

The principal feature distinguishing *Goniomonas* from the plastid-containing cryptomonads is the complete lack of the plastidial complex, viz. plastid, periplastid rough endoplasmic reticulum, periplastid membrane, periplastidial cytoplasm and nucleomorph (Hill, 1991*a*). There are two possible explanations for the lack of a plastidial complex in *Goniomonas*: either it never possessed one, or it has undergone a secondary loss of the plastidial complex. Secondary loss implies that *Goniomonas* is derived from a plastid-containing cryptomonad, but several lines of evidence make this alternative unlikely. Firstly, plastid loss has never been documented in cryptomonads. Even though *Chilomonas* has apparently lost the ability to photosynthesise and is an obligate heterotroph (Hill, 1991*a*), it retains a complete plastidial complex with a nucleomorph, periplastid membranes and a non-pigmented plastid (Kugrens & Lee, 1991). Ultrastructural comparisons indicate that *Chilomonas* is derived from the pigmented genus *Campylomonas* (Hill, 1991*b*). If *Goniomonas* were indeed derived from a photosynthetic cryptomonad by secondary loss of the plastidial complex, we might expect it to show some morphological and/or molecular relationship to an extant lineage within the radiation of photosynthetic cryptomonads. No such close morphological similarities exist (Kugrens & Lee, 1991; Hill, 1991*a*), and our molecular data firmly position *Goniomonas* as a sister to the photosynthetic cryptomonads – not as a

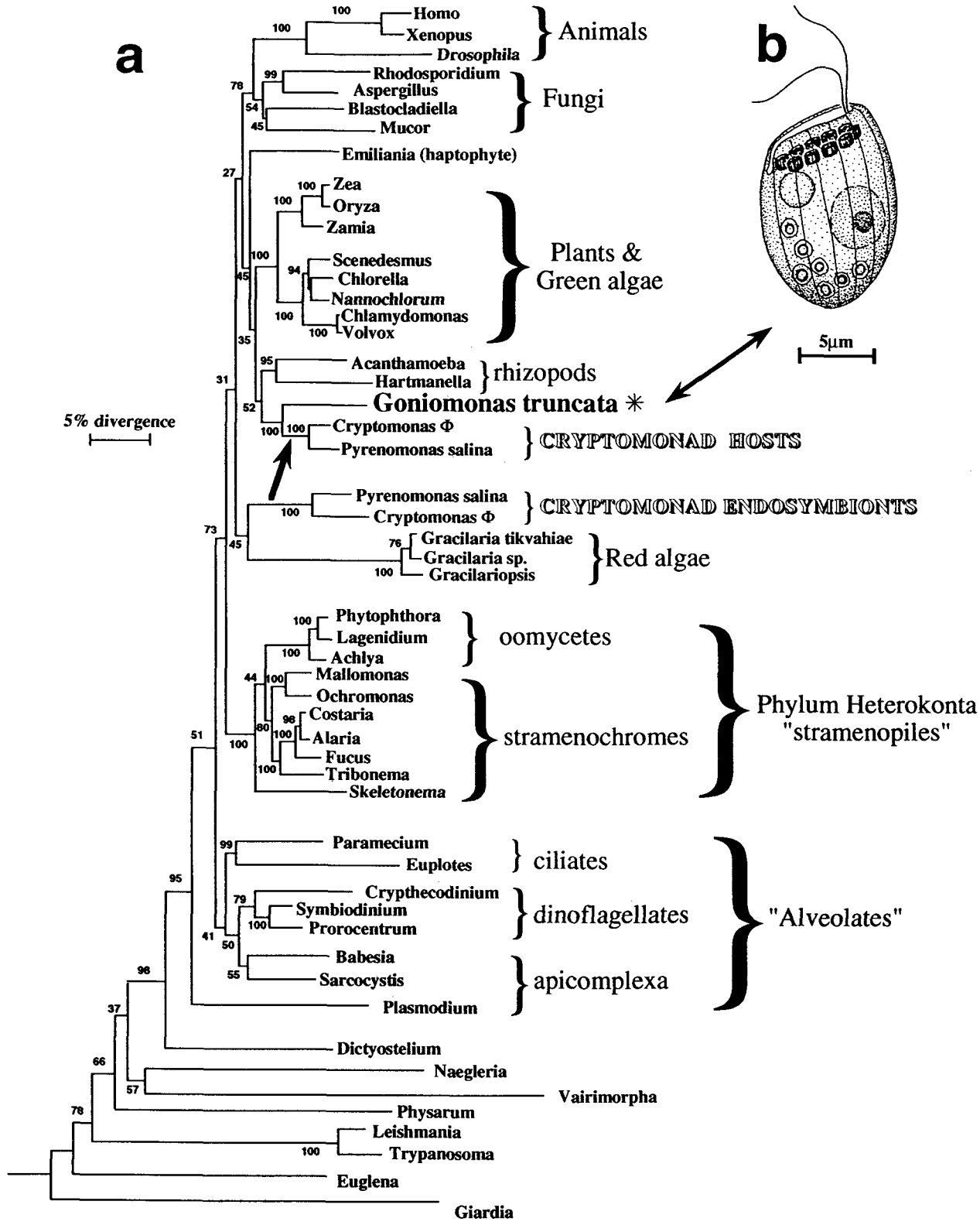

Fig. 1. (*a*) Phylogenetic tree of eukaryotes based on small subunit rRNA sequences incorporating the phagotrophic flagellate *Goniomonas truncata*. *Goniomonas* is firmly positioned as the sister taxon to the host component of photosynthetic cryptomonads. The cryptomonad endosymbionts, which are weakly allied to the red algae, could have been engulfed (arrow) by a *Goniomonas*-like ancestor to create cryptomonads with a plastid acquired through secondary endosymbiosis of a eukaryote. The tree shown was inferred by neighbour-joining from a Jukes–Cantor distance matrix and has the same topology as the consensus of 100 bootstrap replicates. Values at the nodes indicate the percentage of replicates in which that particular node was present. The tree was rooted using *Giardia* as an outgroup. Some principal eukaryotic lineages are indicated by the brackets. Sequences other than *Goniomonas* were taken from De Rijk *et al.* (1992) and the GenBank database. (*b*) Schematic illustration of *Goniomonas truncata*.

species within this lineage (Fig. 1). Admittedly, our phylogeny contains only two representatives of the plastid-containing cryptomonads, but a phylogeny incorporating six genera of plastid-containing cryptomonads also positioned *Goniomonas* as a sister to the photosynthetic cryptomonads (J. Couch & T. Cavalier-Smith, personal communication).

In view of the unique morphology of *Goniomonas*, and the lack of any clear evolutionary relationship between it and any one extant plastid-containing cryptomonad, we do not favour a secondary loss of the plastid complex to create *Goniomonas*. Rather, we believe that *Goniomonas* is a direct descendant of the host cell that gave rise to the plastid-containing cryptomonads. Because *Goniomonas* is phagotrophic, its ancestors potentially had the ability to ingest eukaryotic algal cells that could become endosymbionts.

In conclusion, our rRNA sequence data for *Goniomonas* show that this phagotrophic flagellate is a very close relative of the photosynthetic cryptomonads, confirming earlier alliances deduced from morphological characters (Stein, 1878; Pascher, 1913; Bourelly, 1970; Cavalier-Smith, 1989; Kugrens & Lee, 1991; Hill, 1991*a*). On the basis of phagotrophic capacity, and its close relationship with the host component of photosynthetic cryptomonads, we believe that a *Goniomonas*-like flagellate engulfed a photosynthetic eukaryote and retained the algal cell as an endosymbiont. Such a partnership could have spawned the lineage leading to plastid-containing cryptomonads. The cryptomonads thus represent a unique model for the endosymbiotic origin of organelles in that free-living descendants of both the host and the endosymbiont components are identifiable amongst extant organisms. This system potentially allows us to reconstruct the events, both morphological and molecular, leading to the acquisition of a plastid.

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