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Diversity of the Photosynthetic *Paulinella* Species, with the Description of *Paulinella micropora* sp. nov. and the Chromatophore Genome Sequence for strain KR01

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The thecate filose amoeba *Paulinella chromatophora* is a good model organism for understanding plastid organellogenesis because its chromatophore was newly derived from an alpha-cyanobacterium. *Paulinella chromatophora* was the only known photosynthetic *Paulinella* species until recent studies that suggested a species level of diversity. Here, we described a new photosynthetic species *P. micropora* sp. nov. based on morphological and molecular evidence from a newly established strain KR01. The chromatophore genome of *P. micropora* KR01 was fully determined; the genome was 976,991 bp in length, the GC content was 39.9%, and 908 genes were annotated. A pairwise comparison of chromatophore genome sequences between strains KR01 and FK01, representing two different natural populations of *P. micropora*, showed a 99.85% similarity. Differences between the two strains included single nucleotide polymorphisms (SNPs) in CDSs, which resulted in 357 synonymous and 280 non-synonymous changes, along with 245 SNPs in non-coding regions. Indels (37) and microinversions (14) were also detected. Species diversity for photosynthetic *Paulinella* was surveyed using samples collected from around the world. We compared our new species to two photosynthetic species, *P. chromatophora* and *P. longichromatophora*. Phylogenetic analyses using four gene markers revealed three distinct lineages of photosynthetic *Paulinella* species including *P. micropora* sp. nov.

Key words: Cyanobacteria; endosymbiosis; inverted repeat; microinversion; primary plastid; species diversity.

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Introduction

Paulinella is a thecate filose amoeba that belongs to the Cercozoa (Rhizaria) (Adl et al. 2012; Bhattacharya et al. 1995). *Paulinella chromatophora* Lauterborn, the first described autotrophic species, has two sausage-shaped plastids termed chromatophores that were derived from an alpha-cyanobacterium (Kies 1974; Marin et al. 2005, 2007; Yoon et al. 2006, 2014). While *P. chromatophora* is a photosynthetic organism (Kies and Kremer 1979), nine heterotrophic sister species lack chromatophores and feed by phagocytosis on bacteria (Bhattacharya et al. 2012; Hannah et al. 1996; Johnson et al. 1988; Nicholls 2009; Vørs 1993). The phagocytotic capability within the genus suggests that the ancestor of *P. chromatophora* acquired the ability for photosynthesis after capturing a cyanobacterium and enslaving it as a photosynthetic organelle (Bodył et al. 2012; Nowack 2014; Nowack et al. 2008; Yoon et al. 2006, 2014). This event was completely distinct from the major plastid organellogenesis, or primary plastid endosymbiosis, that gave rise to the Archaeplastida (=Supergroup Plantae). The only other primary endosymbiotic event that is recognized is that which led to the photosynthetic *Paulinella.* Furthermore, the primary endosymbiosis of *P. chromatophora* occurred relatively recently (90-140 Mya) (Delaye et al. 2016), whereas the primary endosymbiosis that gave rise to the Archaeplastida occurred before 1,500 Mya (Yoon et al. 2004). Thus, the study of *P. chromatophora* should provide genetic clues concerning early plastid establishment. Due to these features, *P. chromatophora* has received considerable attention from researchers in diverse areas. To date, endosymbiotic gene transfer from the chromatophore to host nucleus (Nakayama and Ishida 2009; Nowack et al. 2011; Reyes-Prieto et al. 2010), protein trafficking into the chromatophore (Bodył et al. 2010; Gagat and Mackiewicz 2014; Mackiewicz et al. 2012a, b; Nowack and Grossman 2012), enzyme activity (Bernal-Bayard et al. 2014), and siliceous scale production and test assembly (Nomura et al. 2014; Nomura and Ishida 2016) have been studied.

Paulinella chromatophora was first described by Robert Lauterborn after he collected the organism from a backwater of the River Rhine near Neuhofen (Lauterborn 1895; Melkonian and Mollenhauer 2005). Subsequently, *P. chromatophora* has been reported from various parts of the world including Great Britain (Brown 1910), Switzerland (Chodat 1920; Penard 1905), the Netherlands (Hoogenraad 1927), Austria (Geitler 1927), the Baltic Sea (Pankow 1982), the Czech Republic (Lukavský and Cepak 1992), Ukraine (Kapustin 2012), the United States (Kepner 1905; Lackey 1936), Canada (Nicholls 2009), New Zealand (Qiu et al. 2012), Japan (Yoon et al. 2009), and Korea (Qiu et al. 2012). One Swiss *Paulinella* was described as a new euglenoid genus (*Cyanospira aeruginosa* Chodat), but it has a morphology that is consistent with *Paulinella chromatophora* (Chodat 1920). The photosynthetic *Paulinella* were known as a single species, *P. chromatophora* until recent studies (Kim and Park 2015; Qiu et al. 2012; Yoon et al. 2009) demonstrated that there was greater diversity. These studies showed that strain FK01 (sampled from Japan) was genetically divergent when compared to strain CCAC 0185, which was obtained within ~100 km of Lauterborn's type locality. Differences in test size, number of collar scales, number of scales per column, and fine structure of the silica scales also supported the genetic divergence (Yoon et al. 2009). In addition, the coexistence of two photosynthetic *Paulinella* lineages was reported from Japan, Korea, and New Zealand (Qiu et al. 2012). Recently, a marine photosynthetic species, *P. longichromatophora* Kim & Park, was reported from the benthic sandy sediment of a beach in Korea (Kim and Park 2015). Therefore diversity of the photosynthetic species of *Paulinella* is greater than originally envisaged.

The complete chromatophore genome sequences of two strains, CCAC 0185 and FK01, have been published (Nowack et al. 2008; Reyes-Prieto et al. 2010). When compared to *Synechococcus* sp. strain WH5701 (3.04 Mbp with 3,346 protein-coding genes), a free-living close relative of the chromatophore, *Paulinella* strain CCAC 0185 retained only 26% of the original gene content. Eleven putative pseudogenes in the chromatophore genome suggested that the genome reduction was still ongoing, and the *Ka/Ks* ratios for a majority of these genes were below one, suggesting a purifying selection effect (Reyes-Prieto et al. 2010). In addition, there were five inversions and one translocation between the two chromatophore genomes, and independent gene losses were found (i.e., 27 genes in FK01 and 39 genes in CCAC 0185). The chromatophore genome data provided a detailed evolutionary trajectory that started from an alpha-cyanobacterium in the common ancestor of the photosynthetic *Paulinella* spp.

Although Yoon et al. (2009) suggested a possible new photosynthetic lineage (i.e., an undescribed species) based on morphological and molecular phylogenetic evidence, the organism was not