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Revision of the Genus *Micromonas* Manton et Parke (Chlorophyta, Mamiellophyceae), of the Type Species *M. pusilla* (Butcher) Manton & Parke and of the Species *M. commoda* van Baren, Bachy and Worden and Description of Two New Species Based on the Genetic and Phenotypic Characterization of Cultured Isolates

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The green picoalgal genus *Micromonas* is broadly distributed in estuaries, coastal marine habitats and open oceans, from the equator to the poles. Phylogenetic, ecological and genomic analyses of culture strains and natural populations have suggested that this cosmopolitan genus is composed of several cryptic species corresponding to genetic lineages. We performed a detailed analysis of variations in morphology, pigment content, and sequences of the nuclear-encoded small-subunit rRNA gene and

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the second internal transcribed spacer (ITS2) from strains isolated worldwide. A new morphological feature of the genus, the presence of tip hairs at the extremity of the hair point, was discovered and subtle differences in hair point length were detected between clades. Clear non-homoplasious synapomorphies were identified in the small-subunit rRNA gene and ITS2 spacer sequences of five genetic lineages. These findings lead us to provide emended descriptions of the genus *Micromonas*, of the type species *M. pusilla*, and of the recently described species *M. commoda*, as well as to describe 2 new species, *M. bravo* and *M. polaris*. By clarifying the status of the genetic lineages identified within *Micromonas*, these formal descriptions will facilitate further interpretations of large-scale analyses investigating ecological trends in time and space for this widespread picoplankter.

Key words: *Micromonas*; Mamiellophyceae; Chlorophyta; green algae; ITS2; molecular signature.

Introduction

Micromonas pusilla (Butcher) Manton & Parke, a motile marine microalga of very small size (1–3 μm), was first described by Butcher (1952) as *Chromulina pusilla*, based on material from the Conway estuary (North Wales) and initially classified, using light microscopy, as a member of the Chrysophyceae. This species was also identified as a dominant member of the ultraplankton and probably the most abundant organism on the British Islands list by Knight-Jones and Walne (1951). Ultrastructural and biochemical characteristics of the original isolate as well as of other strains originating from the English Channel, led Manton (1959) and Manton and Parke (1960) to classify *M. pusilla* within the green algae (Chlorophyceae). This species was further classified within the Prasinophyceae Christensen based on analogies between its light harvesting complexes and those of *Mamiella* Moestrup and *Mantoniella* Desikachary (Fawley et al. 1990). Phylogenetic analyses confirmed the affiliation of *Micromonas* within the order Mamiellales, sometimes termed prasinophyte clade II (Fawley et al. 2000; Guillou et al. 2004; Nakayama et al. 1998), that was raised to class status (Mamiellophyceae) by Marin and Melkonian (2010). *Micromonas* is also the 'type' of a previously described class, the Micromonadophyceae (Mattox and Stewart 1984), introduced to replace the name Prasinophyceae by excluding *Tetraselmis.* The class Micromonadophyceae was declared invalid by Marin and Melkonian (2010).

In the diagnosis by Manton and Parke (1960), based on a neo-type culture isolated off Plymouth in the English Channel, *M. pusilla* is described as a pear-shaped naked cell 1–3 μm long and 0.7–1 μm broad, with a single mitochondrion, nucleus, Golgi body and chloroplast. The single flagellum is laterally attached and includes a 1 μm long basal part (the flagellum proper) and a slender hair-point (ca 3 μm long according to Manton and Parke 1960). In addition to these characteristics, a distinctive swimming behaviour (Manton and Parke 1960) allows identification using light microscopy. The pigment suite of *Micromonas* is typical of members of the Mamiellales (Mamiellophyceae, see above) (Latasa et al. 2004). A pigment named Chl $c_{CS\text{-}170}$, first detected in the tropical *Micromonas* strain CS-170 by Jeffrey (1989), has been reported to occur in other *Micromonas* strains as well as in strains of other green algal genera (such as *Ostreococcus* and *Prasinococcus* respectively members of the Mamiellophyceae and Palmophyllaceae) isolated from the deep sea (Latasa et al. 2004). The life cycle of *Micromonas* has not yet been elucidated, but a palmelloid phase with cells 2.5–5 μm long was reported in the original descriptions (Butcher 1952; Manton and Parke 1960) but apparently not observed since. The presence in the genome sequence of *Micromonas* isolates of meiosis-related genes, low GC regions with features of sex chromosomes, and genes coding for cell wall components suggest that sexual differentiation and formation of a resistant life-cycle stage may occur (Worden et al. 2009) as in other Chlorophyta (e.g. some members of the Pyramimonadales, Nephroselmidophyceae and Chlorophyceae) (Graham et al. 2009; Leliaert et al. 2012).

Micromonas has a worldwide distribution (Thomsen and Buck 1998) and is of major ecological importance in temperate coastal waters (Not et al. 2004, 2005; Throndsen and Kristiansen 1988) as well as polar oceanic waters (Balzano et al. 2012b; Lovejoy et al. 2007; Throndsen and Kristiansen 1991). Evidence of phagotrophy has been recently reported for an arctic strain of *Micromonas*, suggesting that in addition to contributing significantly to primary production,