



Title	Taxonomy and molecular phylogeny of some isolates of the genus <i>Pyramimonas</i> from the Ryukyu Islands, Japan (Pyramimonadales, Chlorophyta)( Digest_要約 )
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## Abstract

The genus *Pyramimonas* Schmarida is a group of scaly green flagellates. The taxonomy and phylogeny of the genus *Pyramimonas* were investigated in the present study by analysing morphological and molecular data.

Phylogenetic studies using both the morphological and molecular characteristics are very important to know its placement among all organisms. From the morphological and molecular data (SSU rDNA and *rbcL* genes) it is understood that Pyramimonadales showed early divergences of the Chlorophyta, hence *Pyramimonas*, as a member of the Pyramimonadales, is considered a key organism in the evolution of Chlorophyta or even green plants.

The Ryukyu Archipelago is a chain of islands located to the south west of Japan in the western Pacific Ocean. The islands lie in the direct path of the Kuroshio Current, a body of warm equatorial water that sustains rich and diverse coral reefs, mangrove swamps and many diverse marine organisms. The Ryukyu Islands are, therefore, hotspots for their diversity of flora and fauna; but the full extent of the biodiversity including microalgae is still unknown. Thus the focus of this study lay in addressing some of the gap in our knowledge of microalgal biodiversity, by addressing the morphological and genetic diversity, taxonomy and phylogeny of the genus *Pyramimonas*.

To evaluate the genetic diversity and reconstruct the phylogeny of *Pyramimonas*, samples were collected from different locations in the Ryukyu Archipelago. A total of 41 strains were isolated and cultured under laboratory conditions. Phylogenetic analyses revealed that the strains were segregated into six clades, four of which represented existing subgenera: *Pyramimonas sensu stricto*, *Vestigifera* McFadden, *Trichocystis* McFadden, and *Punctatae* McFadden, and two of which represented undescribed subgenera. Although, many of the newly isolated strains were closely related to *Pyramimonas aurea* and *P. olivacea*, the nuclear SSU rDNA phylogenetic trees clearly showed that they are genetically different and can be divided into two subgenera. Moreover, many different and un-reported sequences were

obtained. These data suggested that many undescribed *Pyramimonas* species are there. Since a wide range of genetic diversity was currently observed for *Pyramimonas* in the Ryukyus, this location can be considered a biodiversity hotspot for the genus, a trend that is likely to be mirrored for other forms of microalgae.

The first undescribed species, *Pyramimonas vacuolata* Suda, Horiguchi et Sym is from Maeda, Onna and Bise Motobu, Okinawa, Japan; cell size, posterior vacuole, pyrenoid type, scale morphology and molecular sequences were different from the other species of *Pyramimonas*. Among all morphological characters, the large posterior vacuole was the characteristic and its specific epithet, 'vacuolata' has been given after it.

The second undescribed species, *Pyramimonas okinawaensis* sp. ined. is described from Teniya and Nago, Okinawa, Japan; cell morphology, pyrenoid structure, scale morphology, eyespot structure and position, and molecular phylogenetic position were distinctive from previously described *Pyramimonas* species, and its identifiable especially by its unique type of box and crown scales of the body.

Material collected from Tanegashima Island, Kagoshima, Japan (strains TU2 and TUP12) has been described at the light and electron microscope level and this, together with its molecular phylogeny, indicate that it is previously unknown. However, its light microscope features (cell length and width, paired, slightly-anterior eyespots each of which lying on the edges of two adjoining chloroplast lobes (B & C), longitudinal series of puncta, a single large pyrenoid covered by two vertical, saucer-shaped starch plates in addition to numerous other scattered starch grains) agrees well with the original description of *Pyramimonas angulata* Carter (Carter 1937). The box and crown scales of *P. angulata* are quite distinct from those of all other *Pyramimonas* species. The flagellar apparatus is a typical 3-1 type. One of the most distinctive LM features of this species, namely a pyrenoid surrounded by two vertical starch domes, was corroborated at EM level. Furthermore, the pyrenoid lacks any thylakoid invasion, there are, numerous additional starch grains. The cells also have muciferous bodies.

This study was the first of its kind to understand the taxonomy and phylogeny of *Pyramimonas* from the Ryukyu Islands. This study led to the the isolation and establishment of 41 newly isolated strains (Chapter 2), discovery of two new species (Chapter 3 and 4) and one redescribed species (Chapter 5) that contributes considerably to our knowledge of the genus.