



Title	Taxonomy and molecular phylogeny of some isolates of the genus <i>Pyramimonas</i> from the Ryukyu Islands, Japan (Pyramimonadales, Chlorophyta)(Abstract_論文要旨)
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Title: Taxonomy and molecular phylogeny of some isolates of the genus *Pyramimonas* from the Ryukyu Islands, Japan (Pyramimonadales, Chlorophyta)

The Prasinophycean genus *Pyramimonas* Schmarda is a group of scaly green flagellates. Cells of *Pyramimonas* are inversely pyramidal to globular in shape, varieties of scales covers the flagella and cell surfaces, swim rapidly to forward direction, chloroplast cup-shaped and divides into lobes anteriorly and a posterior pyrenoid with starch reserves, one or more eyespots are situated laterally within the chloroplast. The taxonomy and phylogeny of the genus *Pyramimonas* was investigated in the present study using morphological and molecular analyses.

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

The Ryukyu Islands are a chain of islands located southwestern Japan in the western Pacific Ocean. The islands are in the direct path of the Kuroshio Current that sustains rich and diverse coral reefs, mangrove swamps and many diverse marine organisms including microalgae. Ryukyu islands ecosystems are hot spots for their diversity of flora and fauna; but total biodiversity including microalgae is still unknown. To study the genetic diversity, taxonomy and phylogeny of microalgal diversity from these ecosystems, it was emphasised on the genus *Pyramimonas* based on its cells characteristics.

For evaluating the genetic diversity and phylogeny of *Pyramimonas*, samples were collected from different locations of the Ryukyu Archipelago. Then, 41 strains were isolated and cultured under laboratory conditions. Phylogenetic analyses revealed that strains could be segregated into six clades, four of which represented existing subgenera: *Pyramimonas*, *Vestigifera* McFadden, *Trichocystis* McFadden, and *Punctatae* McFadden, and two undescribed subgenera. Although, many of the newly isolated strains were closely related with *Pyramimonas aurea* and *P. olivacea*, but nuclear SSU rDNA phylogenetic trees clearly showed that they are genetically different and can be divided into two subgenera. Since, a wide range of genetic diversity was observed for *Pyramimonas*, hence the present study location can be considered a hotspot for wide range of diversity of microalgae.

The first undescribed species, "*Pyramimonas vacuolata*" sp. nov. is from Maeda, Onna and Bise, Motobu, Okinawa, Japan; cell's 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. nov. is described from Teniya, Nago, Okinawa-jima Island, Japan, cell's morphology, pyrenoid structure, scale morphology eyespot structure and position, molecular structure were distinctive from previously described *Pyramimonas* spp, and its easily identifiable especially by its unique types of box and crown scales of the body.

Pyramimonas angulata is found in Tanegashima Island, Japan and reinvestigated here after a long time since its first report by Carter in 1937. This species had been described based on light and electron microscopy and molecular analyses and an elaborate description of the species were given. Since the morphological characters - cell length and width, two eyespots and their position, longitudinal series of puncta, single large pyrenoid covered by two saucer shaped starch plates, numerous scattered starch grains agreed well with the original description by Carter (1937), strains of TU2 and TUP12 isolated from Tanegashima Island were identified as *P. angulata*. The box and crown scales of *P. angulata* are quite distinct than that of all other *Pyramimonas* spp. in the subgenus *Punctatae* as well as all *Pyramimonas* spp. of other subgenus groups. The flagellar apparatus was typical 3-1 type. In addition, cells contained a single large pyrenoid with two saucer-shaped starch plates that was not invaded by thylakoids, paired eyespots are located slightly anterior and laying in adjoin in the edges of chloroplast lobes, numerous starch grains and 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 discovery of two new (Chapter 3 and 4) and one redescribed species that contributed a lot to know about Pyramimonadales, Pyramimonadales, Chlorophyta (Chapter 5). In order to further validate the microalgal diversity and biogeography concepts more investigation on all floral studies along with molecular studies is required to strengthen our knowledge of microalgal diversity in Ryukyu Islands.

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