Dhaka University scientist and his team *discovered record 41 new strains of the alga *Pyramimonas*, *discovered their significant genetic diversity; *discovered two undescribed subgenera from the Ryukyu Archipelago located in the Pacific Ocean.

The team of scientists included Professor Dr. Mohammad Azmal Hossain Bhuiyan, Department of Botany, University of Dhaka, Bangladesh, Prof. Dr. Shoichiro Suda, Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Japan and Dr. Daphne Georgina Faria, Graduate School of Engineering and Science, University of the Ryukyus, Japan. They discovered record 41 new strains of the genus *Pyramimonas* from the islands located in the Pacific Ocean. They described a big number of strains (41 strains) of the genus *Pyramimonas* with molecular pieces of evidences, electron microscopy, phylogenetic bases for the first time in the world. They discovered significant genetic diversity on the bases of 18S rDNA and *rbc*L genes among the discovered 41 new strains of *Pyramimonas* sp and the results had been published in a peer reviewed *Journal of Marine Science and Technology*, Taiwan. That was the first and biggest sampling on the genus *Pyramimonas* so far in the world perspectives. Dhaka University scientist's team is pioneer in *Pyramimonas* Research in the world.

The genus *Pyramimonas* Schmarda is a group of scaly green flagellates. The taxonomy and phylogeny of the genus *Pyramimonas* were investigated in the present study by analyzing 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 *rbc*L 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*.

The marine ecosystem is the largest aquatic system on the planet. It is a habitat for various types of algae including plankton. 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. Genetic analyses were done on the bases of SSU rDNA and *rbc*L genes and their moleculer data had been deposited in the global GenBank that had enriched the global pool of knowledge on the genus *Pyramimonas* sp. 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.

This study was the first large scale project investigating the full extent of representation of the genus *Pyramimonas* from the Ryukyu Archipelago and to use this as a platform for investigating its phylogeny. This study led to the isolation and establishment of 41 strains, the discovery of new species and the further characterization of spp. with only a light microscope description, all of which contributes considerably to our knowledge of the genus. This work illustrates how a concerted effort addressing one group of organisms in a tightly defined bio-geographical area can contribute to our working knowledge of microalgal diversity and possibly feed into future bio-geographical work. This should therefore be the *modus operandi* for investigating other groups in this geographical area and for this genus in other geographical areas.

Images of the contributors (from left): Professor Dr. Mohammad Azmal Hossain Bhuiyan, Prof. Dr. Shoichiro Suda, and Dr. Daphne Georgina Faria.

Reference: Suda S, **MAH Bhuiyan**, and DG Faria 2013. Genetic diversity of the genus *Pyramimonas* from Ryukyu Archipelago, Japan (Chlorophyceae, Pyramimonadales). Journal of Marine Science and Technology, Taiwan. Vol. **21**: 285-296. DOI: 10.6119/JMST-013-1220-16.

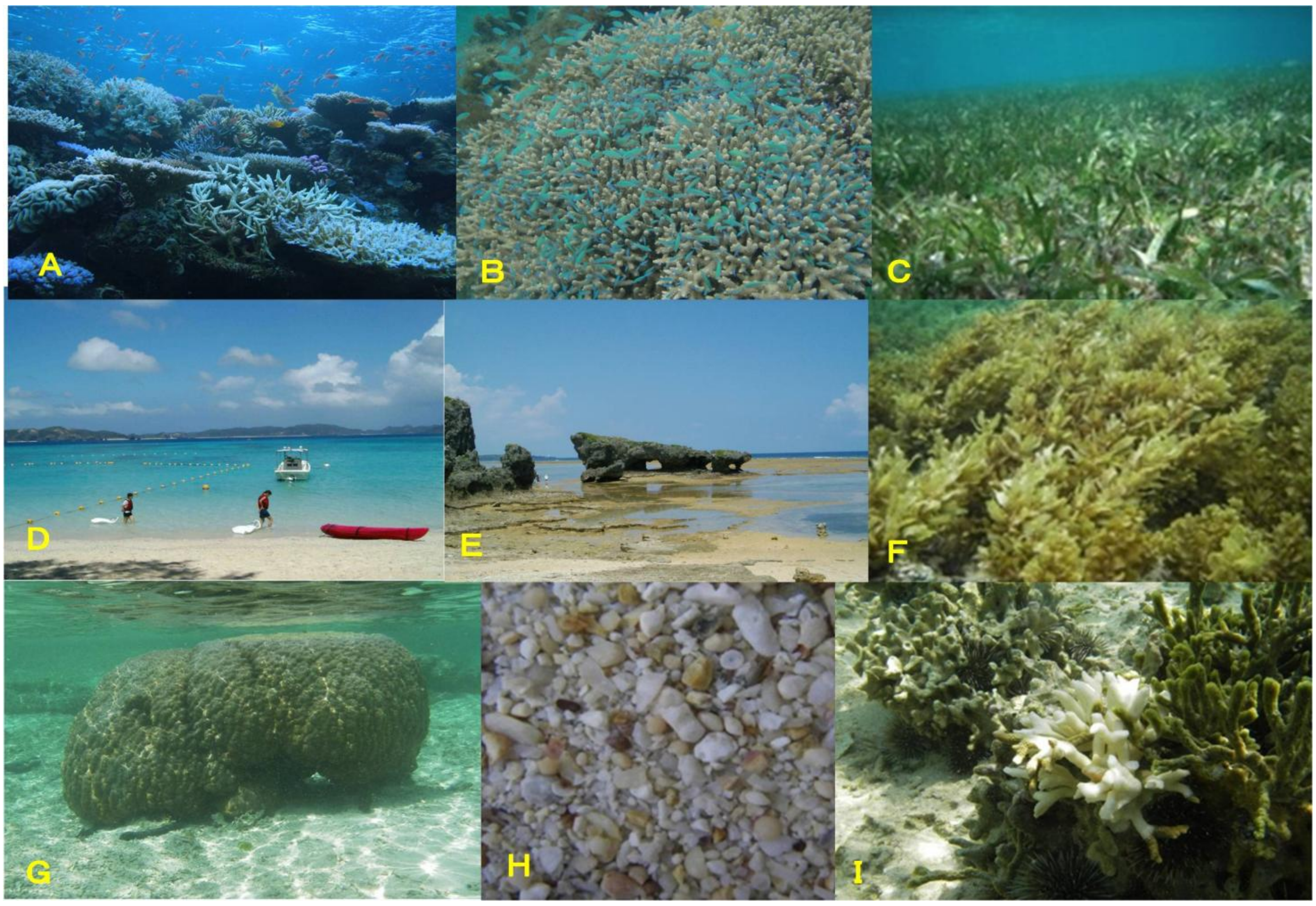

Fig 1: Photographs showing some ecosystems of Ryukyu Islands (A-B, coral ecosystems; C, sea grass turf amongst coral rubbles; D, sandy beach; E, coral lagoon; F, algal turf (sea weeds); G, coral rubbles surrounding coral reefs; H, between stones; I, bleached corals).

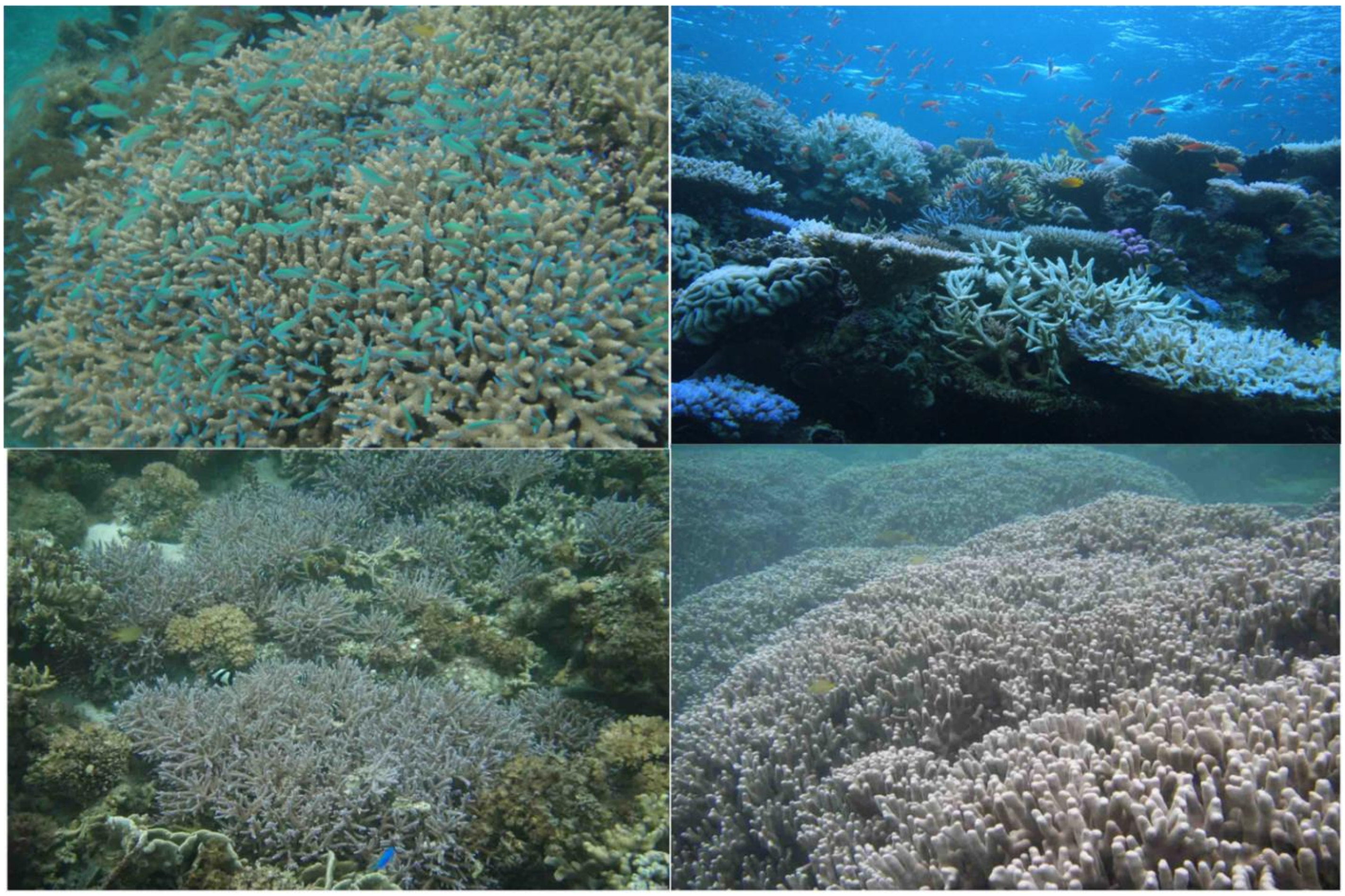

Fig 2: Photographs showing the some ecosystems of Ryukyu Islands.

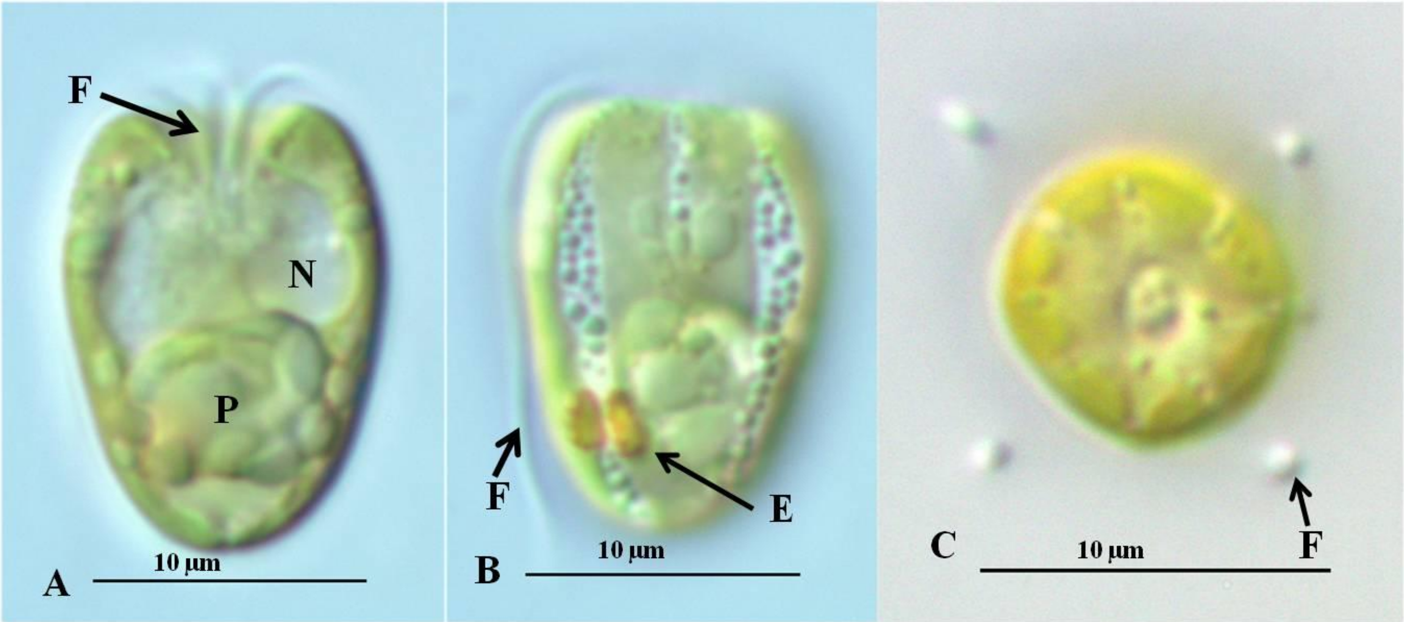

Fig. 3: LM images of a member of 4 flagellate *Pyramimonas* species (*P. okinawaensis* sp. ined.). F, flagella; N, nucleus; P, pyrenoid

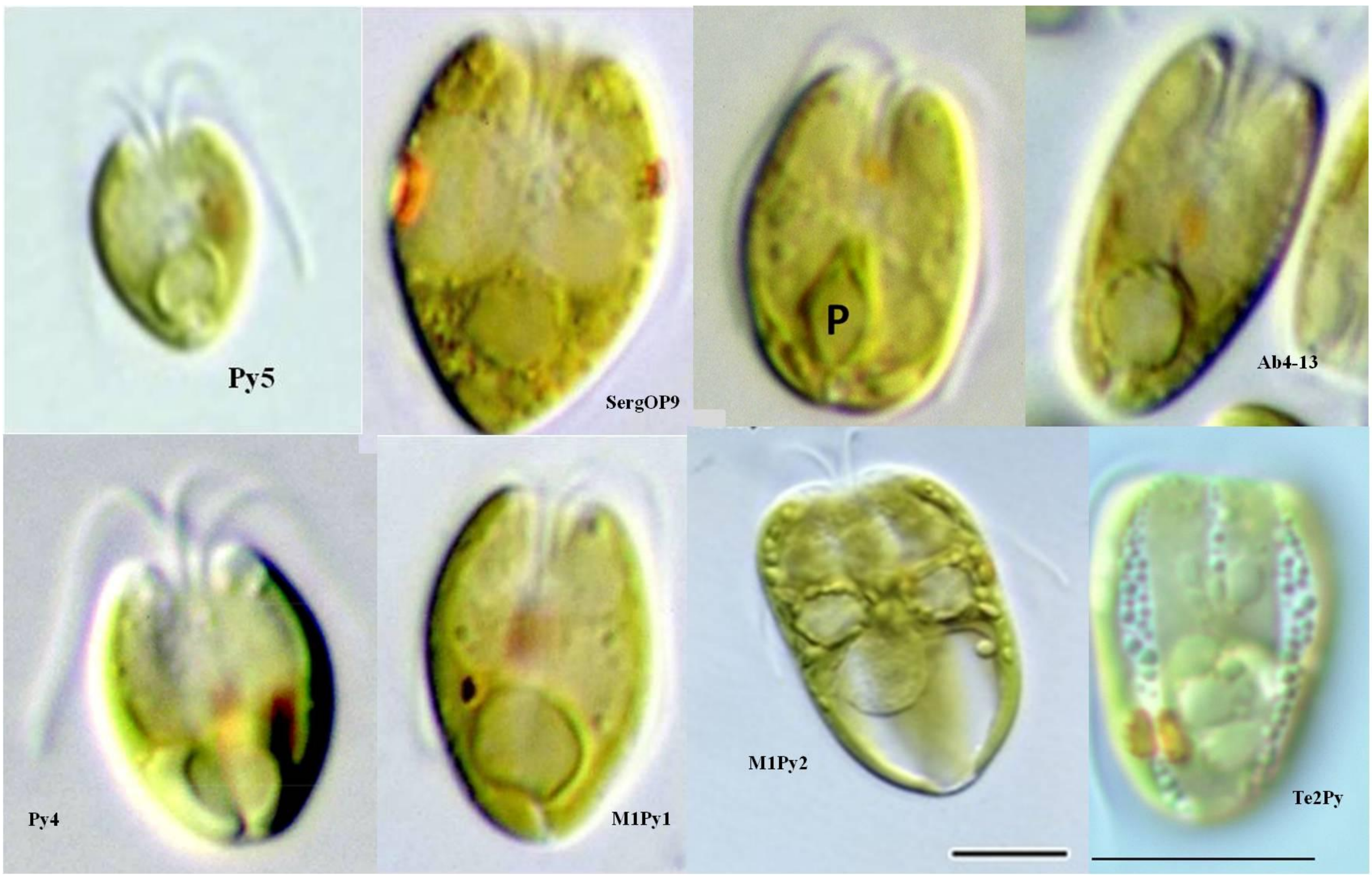

Fig. 4 : LM images of the genus *Pyramimonas* strains.

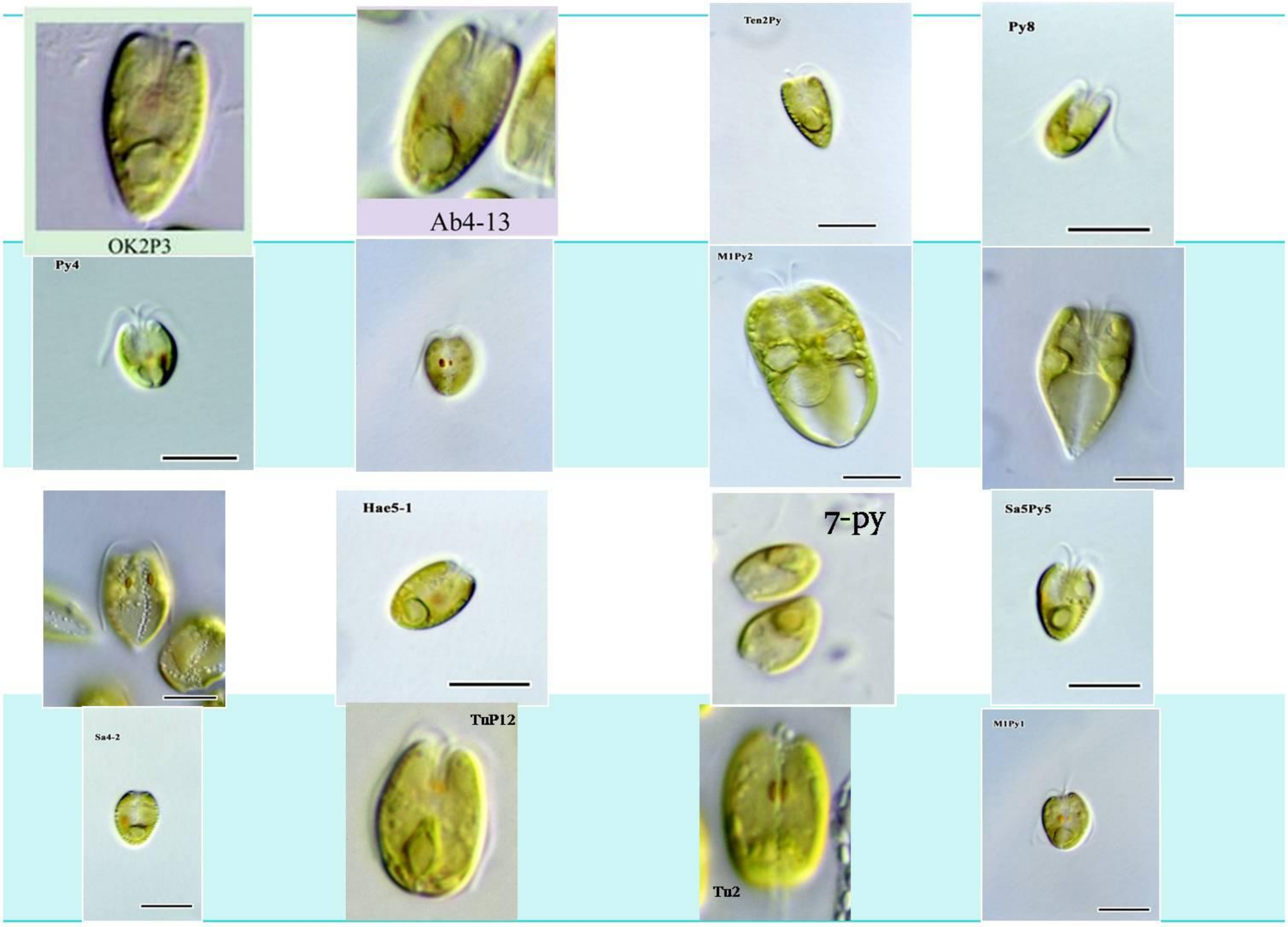

Fig. 5 : LM images of the genus *Pyramimonas* strains.

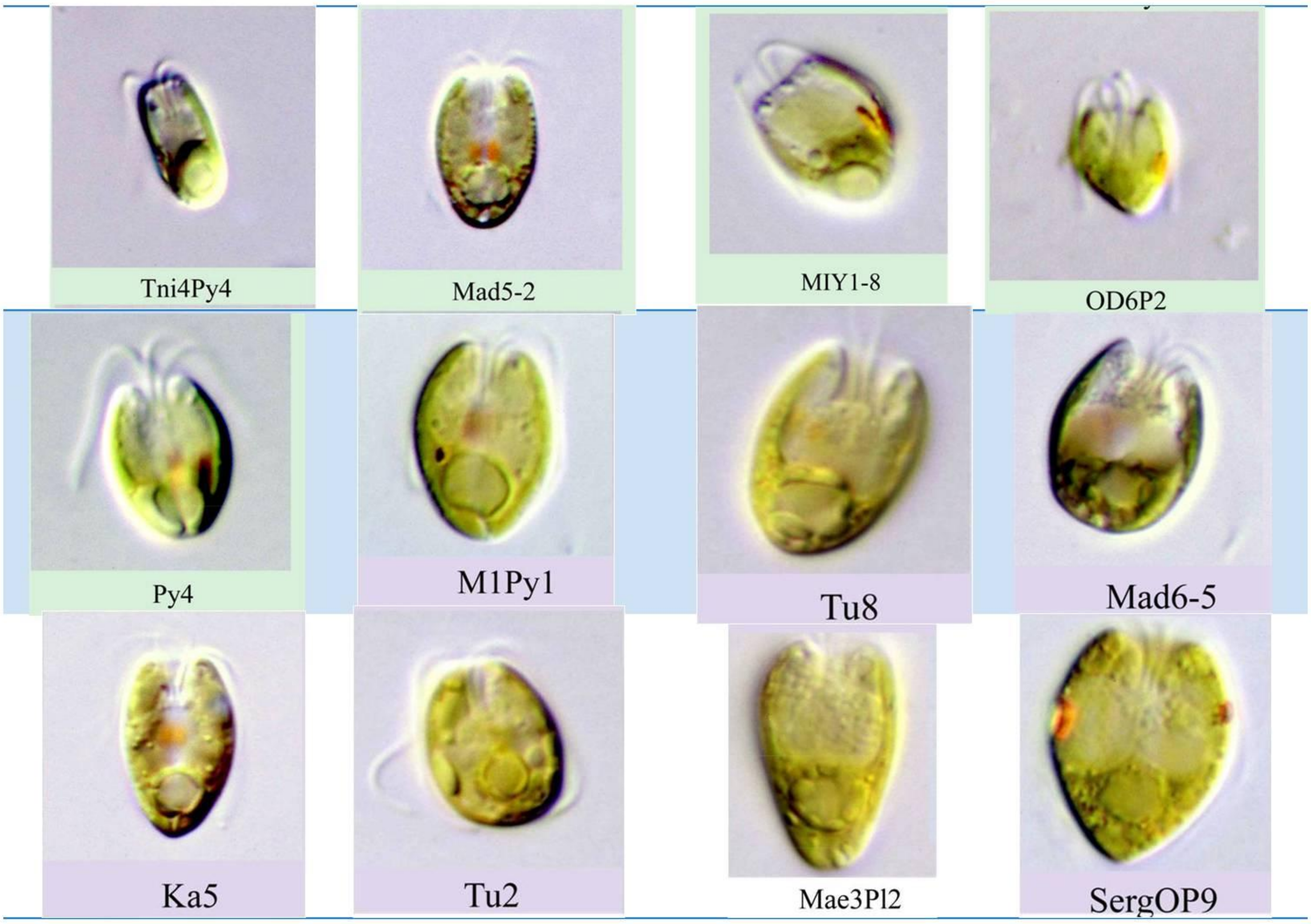

Fig. 6: LM images of Undescribed subgenus 1 & 2 of *Pyramimonas*

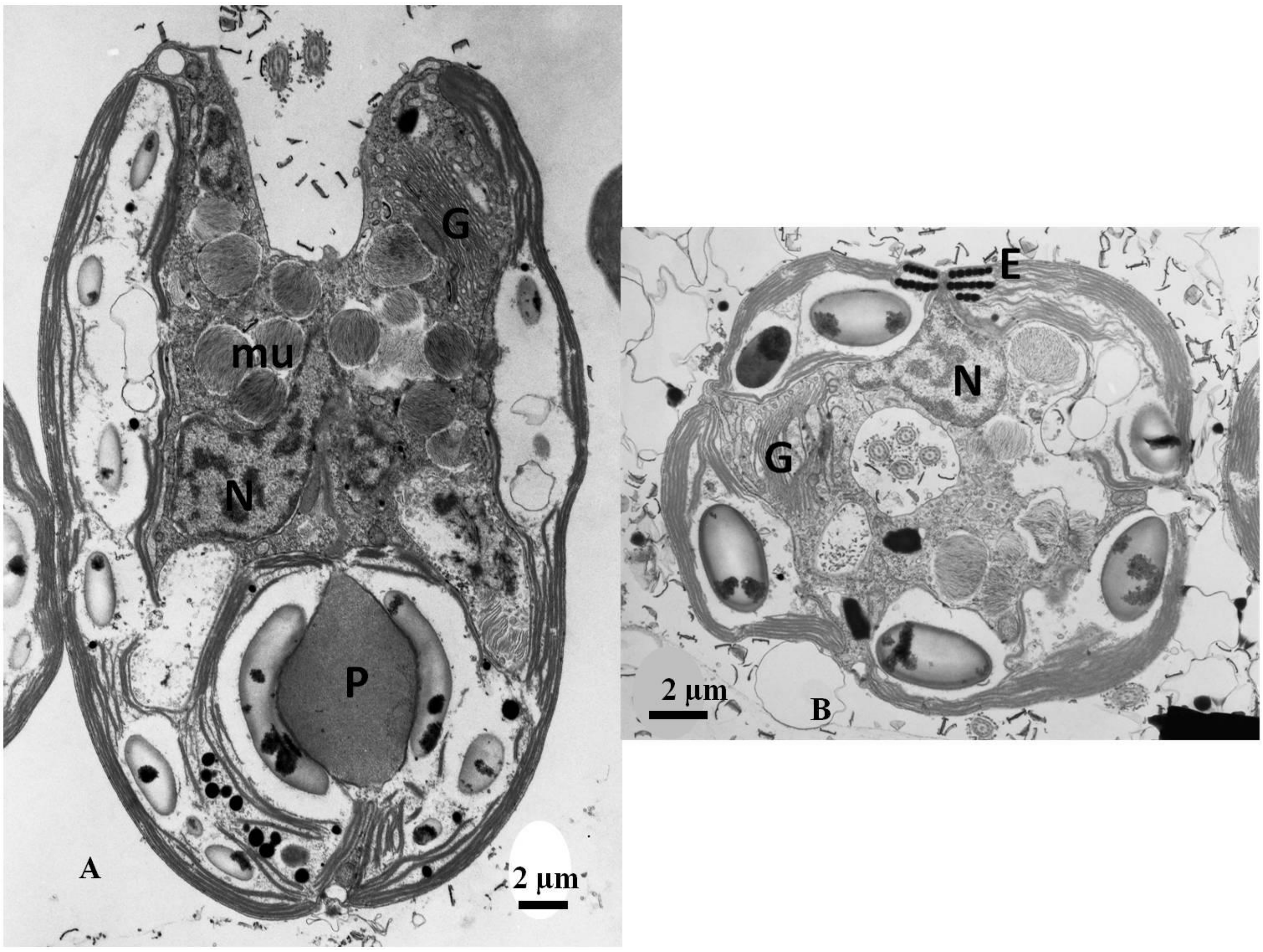

Fig 7: Transmission electron microscopic images of *Pyramimonas* sp. (*P. angulata*). A. Photographs of cell contents in vertical view; B. Photographs of cell contents in side view; Ch, Chloroplast; E, eyespot; G, Golgi body; Mu, muciferous bodies; N, nucleus; P, pyrenoid; S, starch; sr, scale reservoir.

Table 1. Evolutionary models, log likelihood values (-lnL), and model parameters selected by hLRTs results of MrModeltest2 (Nylander 2004) and MEGA 5 (Tamura *et al.* 2011) for nuclear SSU rDNA.

Phylogeny	**Large**	**Small**
Number of taxa	81	62
Model parameter/Data type	Nuclear SSU rDNA	
Model	GTR+I+G	K2+G+I
-lnL	9137.496	2681.572
I	0.5738	0.7023
G	0.5175	0.5540
Base frequencies		
A	0.2531	0.2500
C	0.2097	0.2500
G	0.2710	0.2500
T	0.2662	0.2500
Rate Matrix (G/T=1.0)		
A/C	0.7582	0.0500
A/G	1.7667	0.1400
A/T	0.9723	0.0500
C/G	0.9042	0.0500
C/T	3.8298	0.1400
Characteristics		
Nt	1,617	788
Nc	1,175	660
Nv	434	127
PI	318	81

Abbreviations: -lnL, negative log likelihood; A, C, G and T, frequency of nucleotides; I, fraction of invariant nucleotides; G, Shape parameter of gamma (G) distribution rates; A-C, A-G, A-T, C-G, C-T and G-T, rates of reversible nucleotide substitutions; hLRTs, hierarchical Likelihood Ratio Tests; GTR, General Time Reversible; K2, Kimura 2-parameter; Nt, total number of sites compared; Nc, total number of conserved sites; Nv, total number of variable sites; PI, number of parsimony informative sites.

Table 2. Comparisons of within subgenera for the genus *Pyramimonas* based on nuclear SSU rDNA gene sequences.

Subgenera	Nuclear SSU rDNA					
	N	Length (bp)	Similarity (%)	Similarity*	*p*-distance	Pairwise differences
Punctatae	14	1,617	97.4-100.0	98.8	0.00-0.03	0-40
Pyramimonas	6	1,617	98.4-100.0	99.0	0.00-0.02	0-28
Trichocystis	6	1,617	97.0-99.7	98.2	0.00-0.03	4-47
Undescribed subgenus 1	17	1,617	98.4-100.0	99.2	0.00-0.02	0-26
Undescribed subgenus 2	6	1,617	99.8-100.0	99.9	0.00-0.00	0-3
Vestigifera	9	1,617	98.0-99.9	99.0	0.00-0.02	1-32

Pair wise (*p*) distances were calculated based on the Kimura two-parameter model in MEGA 5; *N*, number of isolates; * represents average scores

Table 3. Table of distances (below diagonal) and percentage similarity (above diagonal) between *Pyramimonas* subgenera based on nuclear SSU rDNA.

		[1]	[2]	[3]	[4]	[5]	[6]
	Subgenera	[Percentage Similarity]					
[1]	*Punctatae*		97.9	97.4	98.7	97.4	98.2
[2]	*Pyramimonas*	0.02		97.3	98.2	97.3	97.8
[3]	*Trichocystis*	0.03	0.03		97.6	97.4	97.4
[4]	Undescribed subgenus 1	0.01	0.02	0.02		97.7	98.3
[5]	Undescribed subgenus 2	0.03	0.03	0.03	0.02		97.4
[6]	*Vestigifera*	0.02	0.02	0.03	0.02	0.03	
		[Distances]					

Table 4. Percentage difference within *Pyramimonas* subgenera based on nuclear SSU rDNA.

Subgenera	Within subgenera differences (%)
Punctatae	0-2.6
Pyramimonas	0-1.8
Trichocystis	0.3-3.0
Undescribed subgenus 1	0-1.7
Undescribed subgenus 2	0-0. 2
Vestigifera	0.1-2.0

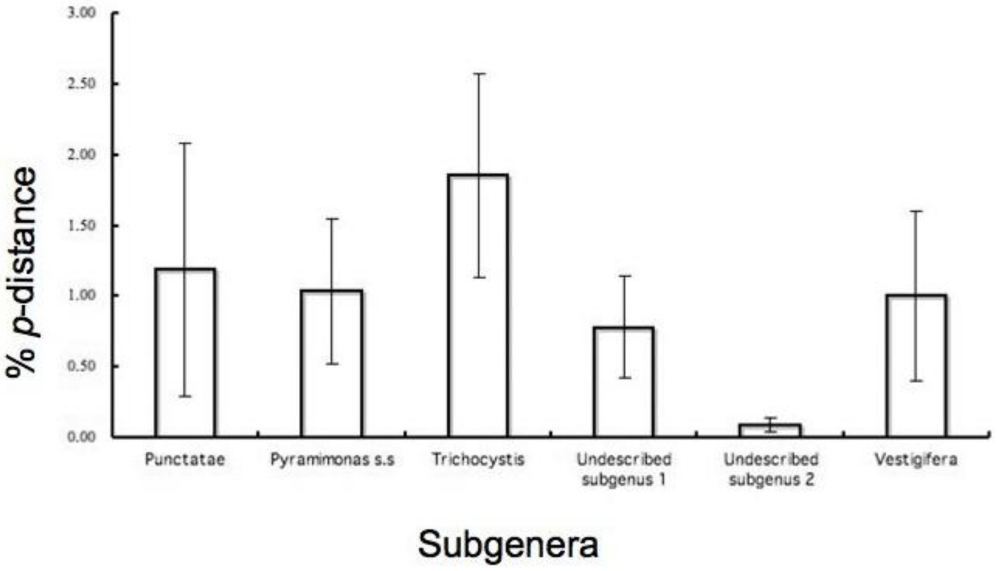

Fig. 8. Genetic divergence of genus *Pyramimonas* nuclear SSU rDNA sequences based on corrected *p*-distances. Genetic distance between each sequence pair was calculated using Kimura two-parameter model, where representative members of the *Pyramimonas* subgenera were compared.

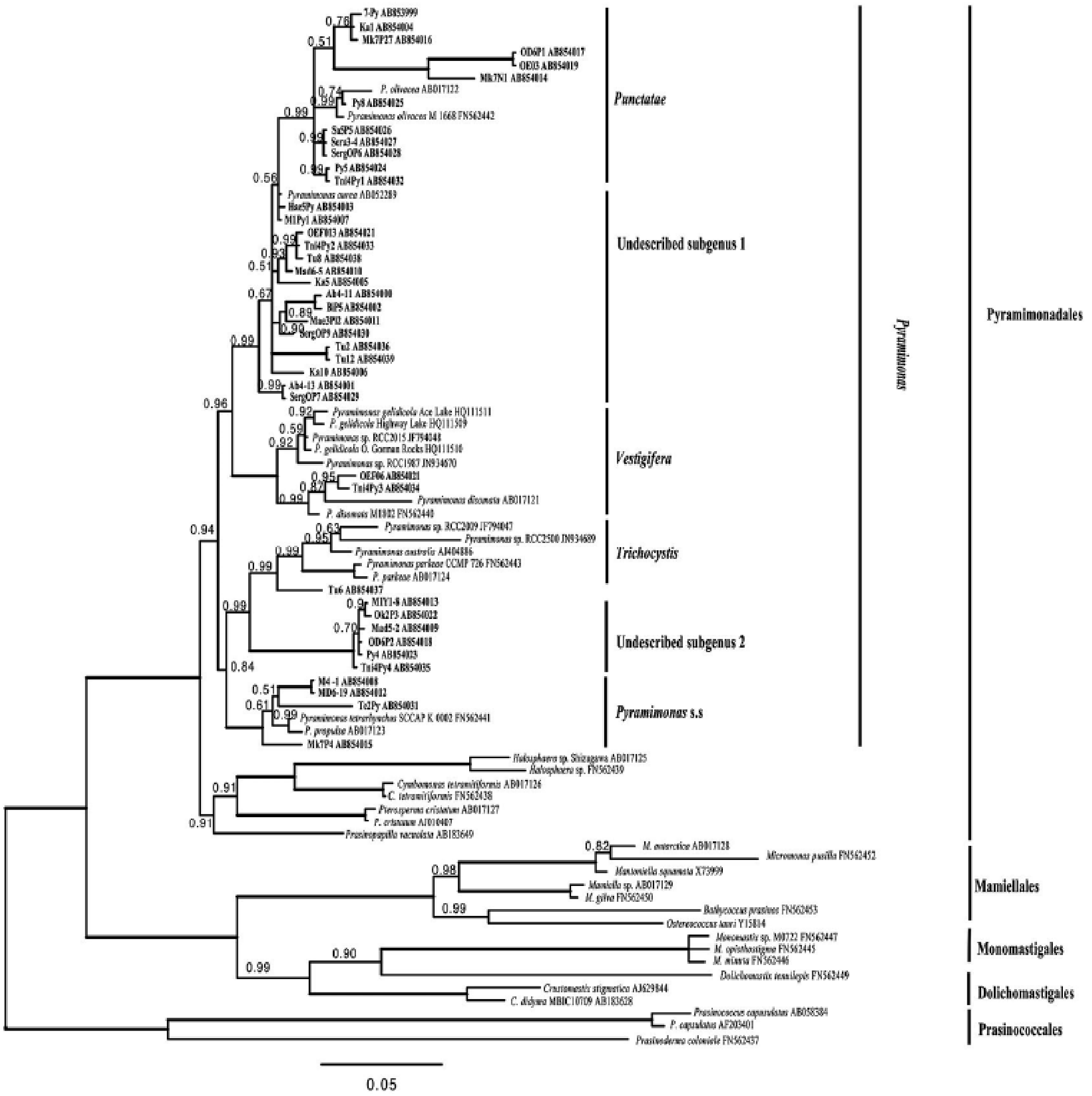

Fig. 9 : Phylogenetic relationships of selected prasinophytes inferred from nuclear SSU rDNA sequences using a Bayesian algorithm, including eighty-one strains. GTR+I+G nucleotide substitution model were used for the analysis. Bayesian likelihood scores for the tree were calculated at –lnL = 9137.4 and 23 strains were used as outgroup (see Table 2 for details). The numbers at the nodes display PP (> 0.50) in Bayesian analysis. Monophylies with posterior probabilities=1.00 are shown as thick branches. Note: Class Mamiellophyceae is divided into three orders: Monomastigales, Dolichomastigales, and Mamiellales (Marin and Melkonian 2010).

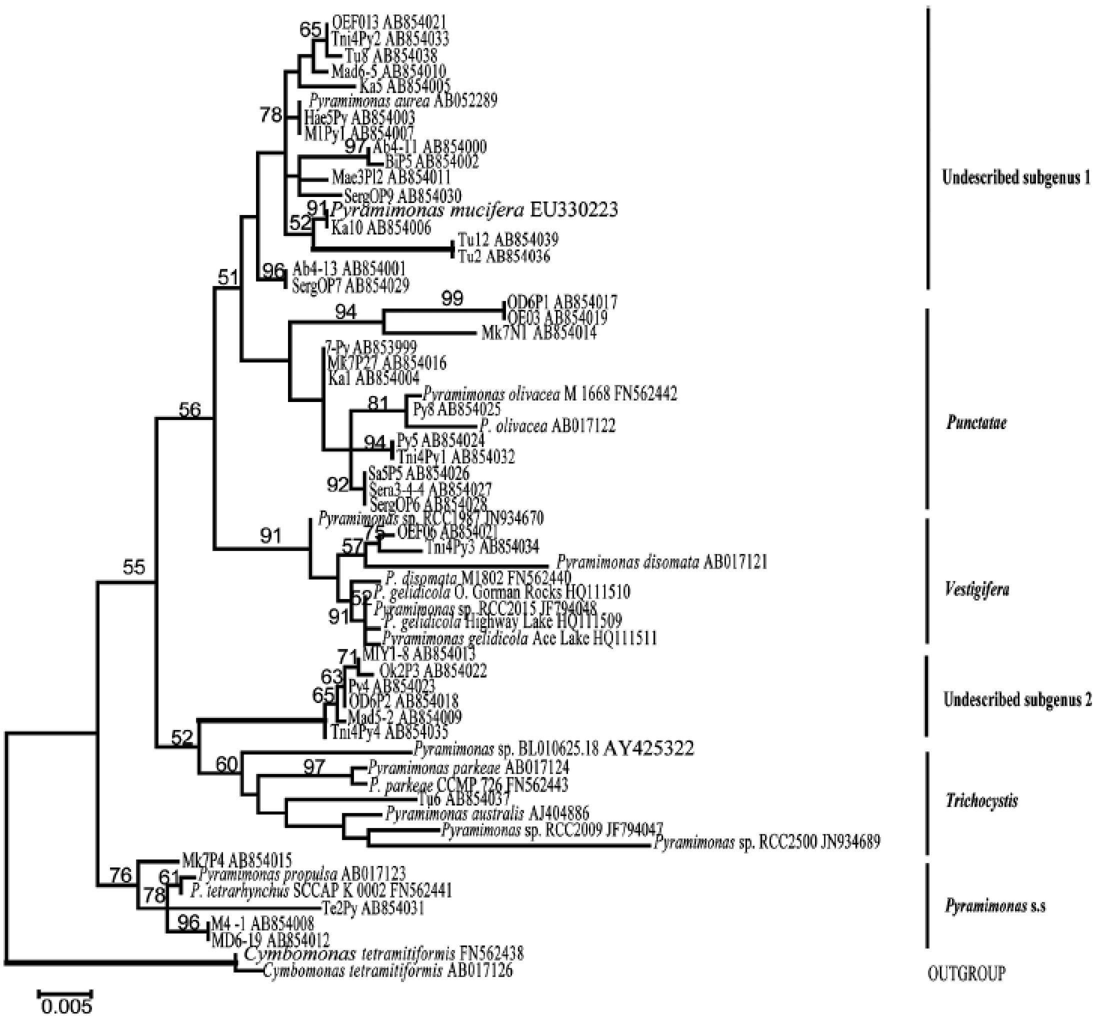

Fig. 10 : Maximum likelihood tree for the genus *Pyramimonas* inferred from nuclear SSU rDNA sequences, including sixty-two strains. K2+G+I nucleotide substitution model was used for the analysis. Maximum likelihood scores for the tree were calculated at –lnL = 2681.6 and 2 strains of *Cymbomonas tetramitiformis* were used as outgroup. The numbers at the nodes display BP (> 50) in likelihood analysis. Monophylies with bootstrap proportions=100 are shown as thick branches.