A study of the genus Pyramimonas (Prasinophyceae) from south-eastern Australia

G. I. McFadden, D. R. A. Hill and R. Wetherbee


Eleven marine species of Pyramimonas Schmarda (including eight previously known species and three new species) from south-eastern Australia are examined by light microscopy, thin-section electron microscopy and freeze-etch electron microscopy. The genus is shown to comprise at least three natural sub-groups on the basis of a range of ultrastructural and biochemical characteristics, and three sub-genera are erected. The generic description is emended to incorporate more up-to-date information.

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Introduction

The genus Pyramimonas was first described by Schmarda in 1850, based on material from pools in the Austrian Alps. Stein (1878) and other authors (see Belcher 1969) have used the spelling Pyramidomonas, but Schmarda’s original spelling is now universally accepted. Pyramimonas is a large genus with 78 species described from marine, brackish and freshwater habitats. Belcher (1969) suggested that many of the species are synonymous due to the non-specific variability of the relatively few characters visible to light microscopists, an opinion supported by the fact that 36 species are still known only from their protologue. Butler’s monograph of the genus (1959) is the most recent (examination of 10 marine species from U.K.), but was confined to observations by light microscopy (LM). Norris & Pienaar (1978) have compared the ultrastructure of five marine species from North America.

Electron microscopy (EM) has revealed that the type species, P. tetrarhynchus Schmarda (1850) (Swale 1973), and certain other Pyramimonas spp. (see list in Ettl & Moestrup 1980), have a periplast of non-mineralised scales. Although the composition of Pyramimonas scales is unknown (despite the inclusion of Pyramimonas among organisms with silica scales (Bovee 1981)), they are known to be manufactured within the Golgi apparatus (Manton 1966, Moestrup & Walne 1979). Up to seven varieties may be present on a single cell, arranged in ordered tiers on both the flagellar and cell surfaces. The scales have proved to be a useful diagnostic character at the specific level (eg. Norris & Pienaar 1978; Pennick 1978, 1982a, 1982b, 1983). McFadden & Wetherbee (1984) present preliminary evidence suggesting an exoskeletal role for the scales.

The scaly green flagellates are now generally accommodated in the Prasinophyceae Christensen ex Silva, and Pyramimonas has been removed from its traditional place in the Chlorophyceae Volvocales (family Polyblepharidaceae) to the more primitive Prasinophyceae (Norris 1980, Moestrup 1982). Recently several putative, freshwater species of Pyramimonas were found to lack scales and are now classified as volvocalean flagellates of the genus Hafniaomonas Ettl et Moestrup (Ettl & Moestrup 1980, Ettl 1981). Schmarda’s (1850) original delimitation of Pyramimonas [“Animal e Monadi-
norum familia, pyramidale, lateribus quatuor, in pyramidis basil flagellis instructum" is insufficient to characterize the genus, and it is now desirable that the generic description be amended. In this paper we present detailed observations on the marine Pyramimonas spp. of southern Australia (including eight known and three new species). The generic description is amended to include up-to-date information, and on the basis of both ultrastructural and biochemical characteristics, the genus is shown to comprise at least three sub-genera.

Materials and methods
Since May 1982, a total of 91 samples from 41 localities has been examined for Pyramimonas species (Tab. 1). Samples were concentrated (ca. 200 x) using a continuous centrifuge made from the cup design of Davis (1957) mounted on an electric wood router. Fresh material was examined by LM using an air water immersion lens, and also dried on formvar coated 50 mesh grids for whole-mount EM. Where critical, cells examined by LM were micropipetted onto polylysine/formvar coated grids for comparative analysis by whole-mount EM. Grids were either stained in aqueous uranyl acetate (2 min.) or shadowed with gold/palladium (45° angle). Micrographs of shadowed whole mounts were reverse printed. Trichocysts were stained for fluorescent LM with the fluorochrome dye 4'-6-diamidino-2-phenylindole (DAPI) as outlined by Coleman (1983).

Enrichment cultures were established in MET 44 medium (Schoene & Schoene 1982) and isolates made by micropipetting. Isolations (designated as Melbourne University Culture Collection, MUCC) were maintained in G2 media without soil extract (Jeffrey 1979) at 15°C and a 16:8 h light:dark cycle. Illumination was by cool white fluorescent lights at intensities between 20 and 40 uE m⁻² s⁻¹. A number of strains were obtained from the Cambridge Culture Collection for Algae and Protozoa (CCAP), University of Texas (UTEX), CSIRO Division of Fisheries and Oceanography Cronulla (CS), and the North East Pacific Culture Collection (NEPCC), and are referred to by their designated numbers. All micrographs are from the cultured material except where otherwise designated in the figure legends.

Unfixed samples for freeze-etch were frozen in Freon 22 without any cryoprotectant, then stored in liquid nitrogen prior to preparation in Balzer's 300 apparatus. Cells were fractured at 90°C and etched for four minutes. Etched faces were either shadowed unidirectionally from an elevation of 45° (an encircled arrow-head indicates the direction of shadow in these micrographs), or conically from 25°C at 50 rpm. Conically shadowed freeze-etch micrographs are reverse printed and lack a direction arrow. Material for thin section EM was fixed in several ways: Fix A – 1% glutaraldehyde in media (20 mins), 1% osmium tetroxide in 1M sodium cacodylate (15 mins); Fix B – 1% osmium tetroxide in media (20 mins); Fix C – 0.01% osmium tetroxide (2 H), (working strength after adding 1% osmium tetroxide to unconcentrated culture solution) then transfer to 1M sodium cacodylate; Fix D – 1% glutaraldehyde in sodium cacodylate plus 0.3M sucrose (20 min.), 1% osmium tetroxide (20 min.). Fixed material was dehydrated in a graded ethanol series and embedded in Spurr's via propylene oxide. Photosynthetic pigments were assayed by extraction as per Jeffrey (1980), and thin layer chromatography after the method of Keast & Grant (1976).

Observations
The varieties of scales present on Pyramimonas species have been labelled with a bewildering array of terms through the short history of their study. For example, the scales comprising the outermost layer on the cell body have been previously known as: corona, lace, type 3, outermost, OBS, and crown scales. While de-
criptive names may be unsuited to scales of some species we feel they are the least cumbersome. We have therefore followed a similar terminology for scales as McFadden et al. (1982). We have given scales considered to be homologous the same name. All of our records are verified by EM. Isolated scales and species found in enrichment cultures are considered to indicate the presence of that species in the sample. Box scales proved to be the variety most easily observed and are generally the most diagnostic. Stained whelmouts proved superior to shadowed material for visualization of smaller scales and fine perforations. Material fixed initially in glutaraldehyde loses the crown scales, so osmium fixations are necessary to examine the periplast in detail.

Circumscription of the genus Pyramimonas

The type species of Pyramimonas, *P. tetrahynchus*, is a freshwater species and was not observed in this study, but thorough observations (Manton 1968, Swale & Belcher 1968, Belcher 1969, Swale 1971, Moestrup & Walshe 1979) show that it epitomises the shape, internal organisation, and general periplast features of the genus as circumscribed below.

**Pyramimonas Schmarda 1850 emend. McFadden**

Green flagellates (containing chlorophyll a and b, β carotene, neoxanthin, violoxanthin, and either lutein or siphonine) of inverse pyramidal to globular shape, sometimes having four anterior lobes. Four or occasionally eight flagella, approximately equivalent to the cell in length, are inserted in an invagination at the anterior end. Cells swim rapidly, proceeding in a straight line, though occasionally changing direction or remaining quiescent for brief intervals. Reproduction is only known to occur by longitudinal division. Palmelloid phases are known in some species. A periplast consisting of at least five, and up to seven, varieties of scales arranged in ordered tiers covers the flagell and flagellar surfaces. On the flagella are three scale types: an innermost layer of pentagonal scales (Fig. 13 E), with one side so short as to render them almost square, arranged in 24 ascending, helical rows; an outer layer of lumoloid scales are arranged in nine imbricate longitudinal rows; and tubular hairs occurring intermittently along the flagella. On the cell surface the underlayer of scales closest the plasmalemma are square in outline, while the outer layers are of various design, but usually based on a symmetry of four. The scales are produced by the Golgi apparatus. There is a cupulate chloroplast that divides into lobes anteriorly, and a posterior pyrenoid with starch reserves. One or more red stigmata composed of lipid globules are situated laterally within the chloroplast. The transition zone of the flagella usually has a helical fibre around the proximal end of the central pair of microtubules. Mitosis is by an open, persistent, interzonal spindle, and the flagellar apparatus comprises four basal bodies interconnected by a synistosome and peripheral fibres, four cruciate microtubular roots, a thiozoplast, and a microbody.

**Species groups within Pyramimonas**

After appraisal of the available information on scale structure, internal morphology, and biochemical features in *Pyramimonas*, it is apparent that there are at least three groups of mutually related species within the genus. The existence of these groups has been alluded to by previous authors (Melkonian & Robenek 1981, Pennick & Cann 1982, Norris 1980, Bressler & Meuse 1980, Inouye et al. 1983, 1984, Pennick 1984, Melkonian & Robenek 1984), and we now propose the formal recognition of three sub-genera (Tab. 2).

1. **Pyramimonas subg. Vestigifera McFadden, subg. nov.**

Cellulæ quadriflagellata: pyrenoides excentrica, thylacoidibus antice partim invasa, amylosumate cupulato ambitu; stigmata stratia globulorum lipidum duobus composta; trichocystes absentem: squamae substrati umbone magnó medio, aut ad foveam flagellatatem restrictae aut absentem: squamae vestigiformes extra foveam flagellarem praesentem, squamos capsiformes interjectae; corpora basalia 4 in figura rhombi disposita, synistosomat quadrato, sine copulis fibrillaribus. (Latinum: vestigium - a footprint)

This group comprises species with internal organisation similar to *P. gelidicola* McFadden, Moestrup & Wetherbee 1982; *P. gelidicola*, *P. orientalis* Butcher 1959, *P. obovata* Carter 1937, *P. disomatata* Butcher 1959, *P. gurtonatae* Pennick & Cann 1982, *P. occidentalis* Pennick 1982a, *P. spinifera* Pennick 1983, *P. moestrupii* McFadden sp. nov., *P. cordata* McFadden sp. nov., *P. nephiroidea* McFadden sp. nov. Unifying features are: a somewhat excentric pyrenoid, invaded by a few thylakoids anteriorly and with a cup shaped starch grain with two anterior lobes; usually two, bi-layered stigmata; four basal bodies arranged in a diamond configuration and inter-connected by a square synistosome and other fibres as described by Melkonian (1981), McFadden & Wetherbee (1984), and Moestrup & Thomsen (1974); underlayer scales of type 1 (Fig. 13 A) restricted to the flagellar pit, and footprint scales (Fig. 13 D) between box scales outside the flagellar pit (cf. McFadden & Wetherbee 1982). Species examined by ourselves and Ricketts (1970) have lutein as an accessory xanthophyll.

**P. cordata** McFadden sp. nov.

**Diagnosis:** Cellulæ cordatae, 7 μm longae et 5 μm latæ, transsectione subcirculari, stigmata unico; squamae...
Tab. 2. Species groups in Pyramimonas.

<table>
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<tr>
<th>Subgenus</th>
<th>Species</th>
<th>pigment</th>
<th>fibrillar band</th>
<th>trichocysts</th>
<th>starch type</th>
<th>footprint scales</th>
<th>curved synistosome</th>
<th>coiled fibre</th>
<th>underlayer scale type</th>
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<td>Vestigifera</td>
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<td>+</td>
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<tr>
<td></td>
<td>* P. gelidicola *</td>
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<td>+</td>
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<td>* P. nephronea *</td>
<td>lutein</td>
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<td>+</td>
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<tr>
<td>?</td>
<td>* P. virginica *</td>
<td>lutein</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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* species examined by authors.
+ present.
- absent.

capsiformes (280 nm latitudine) base foraminibus parvis et cum quadrato cruceque centraliter parum prominenti, marginibus aequis; squamae coronatue base subcirculari (280 nm diametro), tigilis erectis 4 ad apicem coalitis quo tigillum centrale suspenditur. (Latin: cor - the heart).
Type material collected on 23 May 1981 from warm core eddy Mario, East-Australian Current, Australia. Holotype Fig. 2 D.

Illustrations: Figs 1 D, 2 A–I, 14 A–C.

Typical features: Cells (7 μm × 5 μm) cordate in longitudinal section, sub-circular in transverse section. Four flagella, equivalent to the cell in length, are inserted in an apical pit. A single stigma is situated in a chloroplast lobe adjacent the nucleus toward the anterior. Cells are covered by four types of scales: type 1 underlayer scales cover the plasmalemma in the flagellar pit; box scales (280 nm wide), with a raised quadrate pattern and small perforations on the basal floor, cover the plasmalemma on the outer surfaces of the cell; footprint shaped scales are positioned between the box scales on the outer surfaces of the cell; crown scales (280 nm diameter), comprising a sub-circular base with five uprights that fuse at the apex, cover the box scales.

Previous records: Australia (Hallegraeff 1983 (as Pyramimonas sp. 1)); Denmark (Moesstrup pers. comm.); Sth Africa (Pienaar pers. comm.).

Present findings: Locations 1, 2, 7, 9–11, 15, 18, 23, 25, 26, 28, 29, 36, 39, 40 (24 samples).

Remarks: This species was first brought to our attention by Dr G. Hallegraeff (CSIRO), who provided a culture (CS 140) isolated by Dr S. W. Jeffrey and Dr M. K. Wilson (CSIRO) from a sample taken in the East Australian Current off Sydney. We have since found this species at a number of our sampling sites (see above), and it was especially abundant at locations within Port Phillip Bay during spring. P. cordata most resembles P. janetae Norris (1964) in shape and size, but the latter is distinguished by its reticulated chloroplast which we did not observe in P. cordata. No observations have been made of the scale structure in P. janetae. The scale structure of P. cordata, while reminiscent of other species with footprint scales, is quite unique (in that no species is known to have a similar raised quadrate pattern on the floor of the box scales) and it is therefore described as a new species.

Abbreviations used in figures

C – chloroplast
E – stigma
G – Golgi body
M – mitochondrion
MB – microbody
N – nucleus
P – pyrenoid
PF – proximal fibre
S – starch
SR – scale reservoir
SY – synistosome

Nord. J. Bot. 6 (2) 1986
Fig. 1. Drawings of Pyrannomonas spp. (scale bar = 5 μm). - A & B: Two forms of P. disomata showing dark granules in an acute posterior in one form (A) and the large bi-lobed starch grain in both forms. - C: P. cirrolineae showing two dome-shaped starch grains and trichocysts in the anterior. - D: P. cordata showing heart-shaped cell and eccentric pyrenoid. - E: P. amylophora showing eight flagella, several starch grains, and tapering of the cell at the mid-region. - F: P. virginica showing relatively small size, one stigma, and central pyrenoid. - G: P. grossit showing trichocysts and two dome-shaped starch grains. - H: P. longicauda showing long tail containing dark granules and four anterior lobes also containing dark granules. - I: P. parkeae showing egg-shaped cell, trichocysts, posterior pyrenoid surrounded by two dome-shaped starch grains. - J: P. orientalis showing eccentric pyrenoid and starch grain, two stigmata and red globules in the posterior. - K: P. nephroida showing kidney-shaped cell. - L: P. moestrupii showing eccentric pyrenoid.
Fig. 2. *Pyramimonas cordata* – A: Longitudinal section (LS) of cell (Fix A) showing nucleus, stigma, pyrenoid and starch grain (scale bar = 1 μm). – B: Transverse section (TS) at level of flagellar pit (Fix A) showing nucleus, scale reservoir, Golgi apparatus, four chloroplast lobes and stigma (scale bar = 1 μm). – C: Freeze-etch (ES) in flagellar pit region showing Type I underlayer scales and overlying box scales (scale bar = 300 nm). – D: Perpendicular section through periplast (Fix B) showing box scales, footprint scales and one crown scale (scale bar = 300 nm). – E: Light micrograph (Nomarski) (scale bar = 6 μm). – F-H: Stained whole-mounts of large scales (scale bars = 100 nm). – F: Box scale. – G: Crown scale. – H: Limuloid scale. – I: Freeze etch (ES) of flagellum showing pentagonal scales toward proximal end (left hand side) where the limuloid scales are absent (scale bar = 300 nm).
Fig. 3. *Pyramimonas disomata* - A: LS (Fix A) showing nucleus, chloroplast, pyrenoid, starch grain, and mitochondrion (scale bar = 1 μm). - B: TS at level of flagellar pit (Fix A) showing nucleus, scale reservoir, two chloroplast lobes, stigma, mitochondrion and Golgi bodies (scale bar = 1 μm). - C: Freeze-etch (ES) of flagellum showing pentagonal scales and limuloid scales. The proximal end is toward the left side of the micrograph (scale bar = 300 nm). - D: Freeze-etch (ES) showing box scales beneath crown scales (scale bar = 300 nm). - E: Freeze-etch (ES) in flagellar pit region showing Type 1 underlayer scales (scale bar = 300 nm).
Fig. 4. *Pyramidionas disomata* – A–E: Stained wholmounts of large scales. – A: Box scale (MUCC PRA 15) with perforations at base margins (scale bar = 100 nm). – B: Box scale (wild material) showing central spine and pattern of concentric squares on the base (scale bar = 100 nm). – C: Crown scale (wild material) (scale bar = 100 nm). – D: Box and crown scales from a single cell (wild material) showing some box scales with spines and some without (scale bar = 300 nm). – E: Limuloid flagellar scales (scale bar = 100 nm). – F: Perpendicular section of periplast (Fix B) showing box and crown scales (scale bar = 300 nm). – G: Light micrograph (bright field) (scale bar = 5 μm). – H: Thin section (Fix A) of anterior lobe beside flagellar pit showing Type 1 underlayer scales in pit (left hand side of lobe) and footprint scales between box scales (arrow) on outer cell surface (right hand side) (scale bar = 300 nm). – I: Tangential section of periplast (Fix B) showing box and crown scales (scale bar = 300 nm). – J: LS of flagellar bases (Fix A) showing nucleus, microbody, pyrenoid, two basal bodies, striated proximal fibre and synistosome (scale bar = 300 nm).

*P. disomata* Butcher 1959: 27

*Lectotype*: Pl. VII, Fig. 7 Butcher (1959).

*Syntype localities*: Pyefleet, Essex, Norfolk and Is. of Cumbria, UK.

*Previous records*: UK (Butcher 1959); New Zealand (Norris 1964, Moestrup pers. comm.); Norway (Thornsen 1969); West Coast USA (Norris & Pienaar 1978); Vancouver, Canada (NEPCC Nos. 192 and 36, *vivi vivam*); Japan (Adachi 1972).

*Present findings*: Locations 1, 5, 7, 9–15, 17, 21, 25, 27, 32, 33, 37 (37 samples). This species was commonly found in brackish water samples and bloomed in conjunction with *P. ciroleanae* at Patterson Lakes (Location 8), with combined cell density exceeding $3 \times 10^9$ cells/litre. Isolated (MUCC PRA 15) from location 1.

*Illustrations*: Figs 1 A & B, 3 A–E, 4 A–J, 14 D–F.

*Typical features*: Cells (6–12 μm × 4–5 μm) are of variable shape, ovoid to cuneiform. There are two stigmata at the mid-anterior, and in a few cells an accumulation of red granules in an acute posterior. Cells in older cultures appear by LM to have two large longitudinal starch grains surrounding the pyrenoid; the conspicuous feature for which the species was named. However, there is in fact only a single bi-lobed starch grain which cleaves during cytokinesis. This species often lost one or more of its flagella during observation by LM, and spun around in the manner of a catherine wheel. Regarding the larger scales, the situation is very
complex. All strains and wild material examined by us have similar crown scales (Figs 3D; 4C, F & I). However, a number of different box scales are observed. The central spike (Fig. 4B) and the small notches at the edges of the base plate (Fig. 4A) are inconstant features, and we have even observed single cells of P. disomata in which only some box scales have a central spike (Fig. 4D).

Remarks: After appraisal of the available evidence, we believe that P. disomata may prove to be a synonym of P. obovata. Norris (1980: 97) lists as P. obovata a strain previously examined by Norris and Pienaar (1978) and then designated P. aff. disomata. The material examined by us is apparently the same species as examined by Norris and Pienaar (1978), although we do not occur exactly with Norris and Pienaar's interpretation of the scales. Butcher (1959) admitted difficulty in distinguishing the two species, and our observations by LM on a range of wild and cultured material suggest that the two species might be synonymous. The major distinguishing feature of P. disomata is the acute posterior containing red granules (Butcher 1959), but this was not a constant feature, even within a clone, and may be dependent on growing conditions. No "type" cultures exist of either species, but Manton et al. (1963), Pennick et al. (1976) and Melkonian et al. (1981) have examined the scales of CCAP LB 67/6 (Plymouth 280) which apparently resembles Carter's (1937) description of P. obovata. The scales of CCAP 67/6 have now been accepted as typical of P. obovata (Manton & Leadbeater 1974, Thomsen 1982), but comparison of our strains with CCAP 67/6 (Pennick et al. 1976) shows intriguing similarities. Both types of box scales can bear the distinctive pattern of concentric squares on the base which surround a raised protrusion. The crown scales are, at first comparison, quite different, but similarities are present in the arrangement of the basal elements and the presence of long spines.

The finding that a given type of scale can vary in structure on a single cell is at odds with previous workers' findings, but may explain why we have observed a wide range of scale morphology in what we suspect to be a single species. Careful comparison of a range of wild material and CCAP 67/6 will be necessary to clarify the taxonomic situation in this assemblage.

P. moestrupii McFadden sp. nov.

Diagnosis: Cellulacae pyramidales ad obovatae, 7 µm longae et 5 µm latae, stigmatibus dubius; squamae capsiformes (300 nm latitudine) base cum foraminibus parvis et cum quadrato circum obtusum cavum centralem, marginibus acutis ad angulos superius plerumque rima- tis; squamae coronatae base octogona (260 nm diametro) cum lateribus 4 per crucem centralem junctis, tigillis erectis 5 (170 nm altitudine) ad apicem coalatis.

Type material collected on 19 May 1982 from Corio Bay, Australia. Holotype Fig. 5F.

Named in recognition of Dr Øjvind Moestrup's important contributions to our knowledge of this genus.

Previous findings: New Zealand (Moestrup pers. comm.).

Illustrations: Figs 1L, 5A–K, 14G–I.

Typical features: Cells (7 µm long × 5 µm wide) pyramidal to obovate in shape. Four flagella, slightly longer than the cell in length, are inserted into a wide apical pit. There are two stigmata (closely appressed) in adjacent chloroplast lobes beside the nucleus. Cells are covered by four types of scales: type 1 underlayer scales cover the plastanelmma in the flagellar pit; box scales (300 nm wide), with a hollow central obelisk and radiating perforations in the basal floor, cover the plastanelmma on the outer surfaces of the cell; footprint shaped scales are positioned between the box scales on the outer surfaces of the cell; crown scales (260 nm wide, 170 nm high) with an octagonal base divided by a cross and having five uprights that fuse at the apex, cover the box scales.

Present findings: Locations 1, 2, 5, 7, 9, 10, 15, 23–25, 29, 37 (23 samples). Isolated (MUCC PRA 00) from location 1 where it is common during the spring.

Remarks: This species is similar to P. orientalis when examined by LM, but the scale structure, particularly the box scales with the distinctive central obelisk and perforation pattern (cf. Figs 5G & 7C), clearly distinguishes P. moestrupii.

P. nephroidea McFadden sp. nov.

Diagnosis: Cellulacae nephroideae, 8–9 µm longae et 9–10 µm latae, antice latitabae, stigmatibus dubius; squamae capsiformes (240 nm latitudine) base aequa, marginibus altis (300 nm) gracilibus semiesperatis; squamae coronatae base circulares (350 nm diametro), tigillis erectis spinosis 4 ad apicem coalitis quo tigillum centrale suspenderit (Greek: nephros – kidney).

Type material collected on 15 June 1983 from Corio Bay, Australia. Holotype Fig. 6E.

Previous findings: New Zealand (Moestrup pers. comm.).

Illustrations: Figs 1K, 6A–J, 14J–L.

Typical features: Cells (8–9 µm long × 9–10 µm wide) are kidney shaped and broadly lobed at the anterior. Four flagella, slightly less than the cell in length are inserted into a wide apical pit. Two stigmata are located in separate chloroplast lobes adjacent the nucleus. Cells are covered by four types of scales: type 1 underlayer
scales cover the plasmalemma in the flagellar pit; box scales (240 nm wide, 300 nm high) with a smooth basal floor and high semi-detached rims, cover the plasmalemma on the outer surfaces of the cell; footprint shaped scales are positioned between the box scales on the outer surfaces of the cell; crown scales with a ring shaped base (350 nm diameter) and four spiny uprights that fuse at the apex, from which is suspended a central strut, cover the box scales.

Present findings: Location 1, 2, 23, 33 (6 samples). Isolated (MUCRA PRA 07) by Mr P. Beech (Univ. of Melbourne) from location 23.

Remarks: This species is similar in shape and approximate dimensions to P. adriaticus Schiller (1913) and P. lunata Inouye et al. (1983). However, P. nephroidea differs from P. adriaticus in having two roundish stigmata (rather than a single elongate one) and considerably shorter flagella. Nothing is known of the scale structure in P. adriaticus, since it has only been observed by Schiller (1913, 1925). Pyraminonas nephroidea is readily distinguished from P. lunata by differences in scale structure (cf. Inouye et al. 1983) and the presence of trichocysts in the latter. The scales of P. nephroidea differ from other known species of Pyraminonas in the box scales having very high, thin, semi-detached rims, while the crown scales are peculiar in lacking cross members in the base.

Typical features: The cell shape is variable, generally ovoid to pyramidal (4–6 μm × 4–5 μm). Four flagella equivalent in length to the cell are inserted in an apical pit. There are two mid-anterior stigmata and in some cells an accumulation of red globules in the posterior end. A detailed account of the internal structure, including the flagellar apparatus, is given by Moestrup and Thomsen (1974). Morphology of the larger scale varieties is given in Norris and Pienaar (1978).

Remarks: Our strains of P. orientalis have identical scale morphology to those of Moestrup and Thomsen (1974), Norris and Pienaar (1978), NEPCC No. 234 (vidi vivam) and CCAP LB 67/13 (Penick et al. 1978, vidi vivam), and we would regard this as the typical form of P. orientalis. Since our strains occasionally developed posterior red globules, they could not be distinguished from P. piaroculata as described by Butcher (1959). We thus believe the two species to be synonymous. Norris and Pienaar (1978) have made observations by EM on a strain that they designated P. aff. piaroculata. Although they were not able to fully characterize the scales of their organism, the box scale structure is different to P. orientalis and other species known by EM, and the strain examined by Norris and Pienaar (1978) is therefore of uncertain affinity at present.

Penick et al. (1978) have made a study of a number of strains resembling P. orientalis (several of which were actually designated as P. orientalis by Butcher) which have distinct scale morphology. Some of these strains have since been removed to new species based on scale differences (Penick 1982a, Penick & Cann 1982, Penick 1983), but a number of strains with different scale morphology (CCAP LB 4/1, 67/14, 67/12, 67/8, 11/2) are still allied to P. orientalis (see Penick et al. 1978).

Pyraminonas subg. Trichocystis McFadden, subg. nov.

Cellulina quadriflagellata; pyrenoides centrals, amylo- somibus tholiformibus duobus ambita; stigmata stratis globulorum lipidum uno pluribuscomposita; trichocystes praesentes; squamae substrati sine umbone medio, paginam cellulare totum tegentes; squamae vesti- giformes absentes; corpora basalia 4 dispositione “3- super-1”, syninostomate arcauto et copulis fibrillarisbus.

This group comprises all species having trichocysts: P. parkeae, P. grossii, P. cirolanae, P. lunata, P. virginica P. orientalis Butcher 1959: 31

Leviotype: Pl. VII. Fig. 12. Butcher (1959).

Synotypes: Essex, Suffolk, and Is. of White, U.K.

Synonym: P. piaroculata Butcher 1959.

Previous records: UK (Butcher 1959, Lackey & Lackey 1963 (as P. piaroculata), Penick et al. 1978); Norway (Thordsen 1969, Leadbeater 1972); Arctic (Thordsen 1970); Denmark (Moestrup & Thomsen 1974, Manton & Leadbeater 1974); Mediterranean (Leadbeater 1974); West Coast USA (Norris & Pienaar 1978); New Zealand (Moestrup 1979); British Columbia (NEPCC No. 234, vidi vivam); Israel/Egypt (Thomsen 1978); Greenland and Finland (Thomsen 1979, 1982).

Present findings: Locations 1, 15, 23, 34 (9 samples). Isolated (MUCRA PRA 012) from location 12.

Illustrations: Figs 1J, 7A–E, 14M–O.

Fig. 5. Pyraminonas moestrupii – A: LS (Fix D) showing nucleus, chloroplast, pyrenoid, starch and mitochondrion (scale bar = 1 μm). – B: TS (Fix D) at level of flagellar pit showing nucleus, scale reservoir, Golgi apparatus, four chloroplast lobes and two stigmata (scale bar = 1 μm). – C: TS of basal bodies (Fix D) in pre-prophase cell showing four daughter basal bodies (arrows) and four parent basal bodies in diamond configuration. Note the square syninostome and other connecting fibres (scale bar = 300 nm). – D: Freeze-etch (ES) showing box scales (scale bar = 300 nm). – E: Glancing section of periplast (Fix C) showing box scales with central obelisks (arrows), and octagonal crown scales overtopping corners (scale bar = 300 nm). – F: Perpendicular section of periplast (Fix C) showing box scales and one crown scale (arrow) (scale bar = 300 nm). – G: Box scales, footprint scales (arrow) and one crown scale. – H: Box scale. – I: Crown scale. – J: Limuloid scale from flagellum.

Nord. J. Bot. 6 (2) 1986
Fig. 6. *Pyramimonas nephroidea* – A: LS (Fix D) showing nucleus, chloroplast, pyrenoid and starch grain (scale bar = 2 μm). – B: LS (Fix D) of pyrenoid showing several thylakoids penetrating matrix from anterior (scale bar = 1 μm). – C: TS (Fix D) of basal bodies showing diamond configuration, square synistosome and other connecting fibres (scale bar = 300 nm). – D: Freezeetch (ES) showing box scales, Type 1. undierlayer scales (long arrows), and footprint scales (short arrows) (scale bar = 300 nm). – E: Perpendicular section of periplast (Fix C) showing box scales with semi-detached rims and crown scales (scale bar = 300 nm). – F: Stained wholemount of box scale. Note attachment of thin rim (scale bar = 100 nm). – G: Stained wholemount of crown scale. Note large spines and free central strut (scale bar = 100 nm). – H: Section of crown scale base (Fix C) showing hollow central strut (arrow) and lack of cross members (scale bar = 100 nm). – I: Stained wholemount of limuloid flagellar scale (scale bar = 100 nm). – J: Light micrograph (bright field) showing kidney shaped cell (scale bar = 5 μm).
(with the possible exception of *P. virginica* Pennick 1977). The pyrenoid structure observed in this group is somewhat variable, with thylakoids traversing the matrix in three species (*P. grossii* Parke 1949 emend. Manton 1969, *P. cirrolanae* Pennick 1982b, *P. parkeae* Norris & Pearson 1975), and thylakoids penetrating only from the anterior in two species *P. lunata, P. virginica*. Four species have a pair of dome-shaped starch grains encasing the pyrenoid (*P. grossii, P. cirrolanae, P. parkeae, P. lunata*), while *P. virginica* has a cupulate starch grain. In all species except *P. virginica*, the basal bodies are connected by a curved synistosome on the convex side of which are located three basal bodies, with only one basal body on the concave side. Peripheral fibres, termed the “fibrillar band”, are associated with the triple set of basal bodies (cf. Inouye et al. 1983, Norris & Pearson 1975). The entire surface of the cells is covered by smooth underlayer scales of type 2 (Fig. 13 B). One species, *P. parkeae*, has been shown to have B spectrum starch (Bressler & Meeuse 1980).

**P. grossii** Parke 1949: 256 emend. Manton 1969: 381

*Lectotype:* Pl. II, Fig. 17 Parke (1949).
*Type locality:* Plymouth Beach UK, April 1936.

*Previous records:* UK (Parke 1949, Butcher 1959, Lackey & Lackey 1963, Pennick & Clarke 1976); New Zealand (Norris 1964, Moestrup 1979, Chang 1983); Norway (Thordsen 1969, 1970, Leadbeater 1972); Mediterranean (Leadbeater 1974); Denmark (Manton & Leadbeater 1974); Japan (Inouye & Hori 1982); Arctic (Hsiao & Trucco 1980).

*Present findings:* Locations 1, 2, 5, 6, 8–12, 14, 15, 19, 23, 28, 29, 32, 35, 36, 39 (45 samples). Isolated (MUCPRA 05) from location 8. Possibly the most common species of *Pyramidina*, occurring in the majority of our samples and having a worldwide distribution.

*Illustrations:* Figs 1 G, 8 A–I, 14 P–R.

*Typical features:* Cells (4–8 µm × 4–6 µm) are ovoid to pyramidal with four broad anterior lobes. Four flagella, slightly longer than the cell, are inserted in a broad anterior pit. There is a single, small stigma composed of a monolayer of globules situated in the mid-region of the chloroplast. The central posterior pyrenoid is encased in two dome-shaped starch grains and is divided transversely by a pair of thylakoids. Up to four trichocysts may be observed in the anterior end of the cell, causing sudden “jumps” of the cell when they discharge. Details of the scale structure are given by Manton et al. (1963), Pennick and Clarke (1976) and Inouye and Hori (1982). The box scales are flat and the rim has one dentate side that was oriented inwards in the cells examined by us.
Fig. 9. *Pyramimonas parkeae*—A: Freeze-etch (ES) of cell surface showing box scales above Type 2 underlayer scales (scale bar = 300 nm). — B: Freeze-etch (ES) showing box scales above Type 2 underlayer scales (scale bar = 100 nm). — C: Freeze-etch of flagellum showing rows of limuloid scales and also tessellate pentagonal scales where limuloid scales are absent at the right hand side (toward distal end) (scale bar = 300 nm). — D: Light micrograph (bright field) (scale bar = 5 μm). — E & F: Fluorescence micrographs (DAPI) (scale bar = 10 μm). — E: Cells from older cultures showing fluorescent ejected trichocyst (top of micrograph) and un-ejected trichocysts in cell (arrow). The nucleus is also fluorescent. — F: Cells from younger cultures in which staining with DAPI does not reveal any trichocysts. — G-I: Stained wholemounts of larger scales (scale bar = 100 nm). — G: Box scale. — H: Crown scale. — I: Limuloid scale.

(Fig. 8 C). We can find no evidence of basal cross bars in the crown scales and believe they are absent.

**Remarks:** Neither Parke’s description (1949) nor Butcher’s (1959) account of this species included mention of the trichocysts, but based on information obtained from Parke, Manton (1969) emended the description to include the presence of trichocysts. Manton (1969) reported that some cells are devoid of trichocysts and staining with DAPI confirms that some cells from young cultures lack trichocysts.

Fig. 8. A-I: *Pyramimonas grossii*—A: Medial freeze-fracture showing one flagellum inserted in flagellar pit, nuclear envelopes, chloroplast, Golgi body, and pyrenoid (scale bar = 1 μm). — B: Freeze-etch showing three flagella (ES). The limuloid flagellar scales are absent toward the proximal ends (bottom of micrograph) revealing the tessellate layer of subtending pentagonal scales (scale bar = 1 μm). — C: Freeze-etch (ES) showing box scales and Type 2 underlayer scales (scale bar = 100 nm). — D-F: Stained wholemounts (scale bar = 100 nm). — D: Box scale. — E: Crown scale. — F: Limuloid flagellar scale. — G: Shadowed wholemount of box scale showing dentate margin on one side (scale bar = 100 nm). — H: Shadowed wholemount of box scale showing smooth margin on opposite side (scale bar = 100 nm). — I: Stained wholemount of ejected trichocyst showing spiral nature (scale bar = 1 μm). — J: Stained wholemount (*Pyramimonas* sp., wild material) of box scale with form intermediate between *P. grossii* and *P. cirrulata* (cf. D & K) (scale bar = 100 nm). — K-Q: *Pyramimonas cirrulata*—K-M: Stained wholemounts (scale bar = 100 nm). — K: Box scale. — L: Crown scale. — M: Limuloid flagellar scale. — N: Freeze-etch (ES) showing box scales and Type 2 underlayer scales (scale bar = 100 nm). — O & P: Shadowed wholemounts of box scale (O) and crown scale (P) (scale bar = 100 nm). — Q: Light micrograph (bright field) (scale bar = 4 μm).
P. cirolanae Pennick 1982: 91

Holotype: Fig. 1 Pennick (1982b).
Type locality: Norwegian Sea 65.01 N, 08.55 E; April 1973.

Previous records: Norway, Barents Sea, English Channel (Pennick 1982b).

Present findings: Location 1, 8, 10, 13, 18, 33 (11 samples). Isolated (MUCC PRA 03) from location 8 where it blooms occasionally (see P. disomata).

Illustrations: Figs 1C, 8K–Q, 15A–C.

Typical features: Essentially indistinguishable from P. gossii by LM. P. cirolanae shares the general morphological features of the former, including the presence of trichocysts. Cells (4–8 μm × 4 μm) are pyramidal with a blunt posterior and one antero-lateral stigma composed of a monolayer of globules. The posterior pyrenoid is bisected by a pair of longitudinal thylakoids and is encased in two-dome shaped starch grains. The scales have been illustrated by Pennick (1982b) and are similar to P. gossii, excepting that the crown scales have a central spike and the box scales are divided into four squares in P. cirolanae, whereas in P. gossii there is a radial pattern of eight perforations (cf. Fig. 8D & K). One side of the box scale is dentate; the orientation the scales varied from cell to cell. We could find no evidence of cross-bars in the crown scales.

Remarks: Pyramimonas cirolanae has probably been mistaken for P. gossii prior to the advent of EM, and we were not able to reliably distinguish the two species by LM of wild material. Indeed, even the distinction by EM becomes less marked on the examination of box scales of intermediate from (cf. Fig. 8D, J, K).

P. parkeae Norris et Pearson 1975: 209

Holotype: Fig. 5 Norris and Pearson (1975).
Type locality: Tide pools, Hopkins Marine Station, Pacific Grove, California USA; 13 April 1966.

Previous records: West Coast USA (Gibbs 1962 as Pyramimonas sp., Norris & Pearson 1975, Norris & Pienaar 1978); Japan (Inouye & Horiguchi 1982a, Inouye & Chihara 1983); UK (Asher & Spalding 1982).

Present findings: Locations 1, 5, 7 (6 samples). Isolated (MUCC PRA 08) from location 1.

Illustrations: Figs 11, 9A–I, 15D–F.

Typical features: Cells are large (12–20 μm × 8–10 μm) and egg-shaped with 4 flagella slightly shorter than the cell in length. The pyrenoid is central, bisected by several transverse thylakoids and encased in two dome-shaped starch grains. In older cultures cells may have further four oblong starch grains situated in each anterior lobe of the chloroplast. One mid-posterior stigma composed of two or more globule layers is situated in a chloroplast lobe adjacent the nucleus. Trichocysts are usually present within the flagellar pit and also between the chloroplast lobes down the sides of the cell (Fig. 9E). Some cells in young cultures lack trichocysts when stained with DAPI (Fig. 9F). The internal fine structure is reported in detail by Norris and Pearson (1975), and cell division is described by Pearson and Norris (1975). Reports of scale structure are found in Norris and Pearson (1975), Norris and Pienaar (1978), and Inouye and Horiguchi (1982a). Our strain differed slightly from others in that the box scales had, on occasions, larger irregular perforations (e.g. Fig. 9A) and the limuloid scales were distinctly striated longitudinally. Morall and Greenwood (1980) compared the trichocysts of P. parkeae with those of cryptomonads but did not find homology.

Remarks: Though large and distinctive by LM, this species was only first reported in 1975 (Norris & Pearson 1975), and infrequently since. We have encountered it only rarely.

Pyramimonas subg. Pyramimonas

This group contains species having pyrenoids penetrated by convoluted thylakoids and surrounded by numerous starch grains: P. tetrahyphimus, P. amylyfera, P. octopus. At least one species (P. amylyfera Conrad 1939) can be octoflagellate and P. octopus Moestrup sp. indet. is octoflagellate. The central four basal bodies are arranged in a diamond configuration, with additional basal bodies in octoflagellates being positioned along the sides of the diamond in a manner similar to pro-

Fig. 10. Pyramimonas amylyfera – A: Freeze-etch (ES) showing crown scales rather haphazardly arranged over box scales (scale bar = 300 nm), – B: Freeze-etch (ES) showing box scales overlying Type 3 underlayer scales (scale bar = 300 nm), – C: Freeze-etch (ES) showing Type 3 underlayer scales. The individual scales cannot be distinguished due to the protruding sub-units of the rims (cf. Fig. 15C) (scale bar = 100 nm), – D: Tangential section of periplast (Fix D) showing box scales and underlayer scales (scale bar = 100 nm), – E: Freeze-etch of flagellum showing pentagonal scales where limuloid scales are absent (scale bar = 100 nm). – F: Section of pyrenoid (Fix D) showing thylakoids (scale bar = 1 μm), – G: Light micrograph (bright field) showing cell shape (scale bar = 10 μm), – H: Light micrograph (Nomarski) from anterior showing eight flagella (scale bar = 10 μm), – I: Perpendicular section of periplast (Fix B) showing hollow dome shaped crown scales with pendulous central pillar (arrow), box scales and Type 3 underlayer scales adjacent plasmalemma (scale bar = 300 nm), – J-L: Stained whole mounts of larger scales (scale bar = 100 nm), – K: Limuloid scale, – L: Crown scale.
phase quadriflagellates (cf. Figs 5 C & 12 D; Moestrup 1982, Fig. 90). Fibrillar bands interconnect certain basal bodies (cf. Moestrup 1982, Manton 1966, 1968). Underlayer scales (Type 3, Fig. 13 C) with high rims, composed of eight sub-units, and a small central boss occupy the entire surface of the cell. The only species thus far analysed for pigment composition and stalk type (P. amylifera) has siphonin as an accessory xanthophyll rather than lutein (see also Ricketts 1970), and A spectrum stalk (Bressler & Meuse 1980).

P. amylifera Conrad 1939: 1

Lectotype: Fig. 3 Conrad (1939).

Type locality: Maritime canal connecting Bruges and its seaport Zeebrugge, Belgium; 18 April 1939.


Previous records: Belgium (Conrad 1939); UK (Butcher 1959 as A. amylifera and A. propulsus, Manton et al. 1963); West Coast USA (Norris & Pienaar 1978); East Coast USA (Hulburt 1965, Gardiner & Hargraves 1979); Sth. Africa (Pienaar 1978); Japan (Inouye & Horiguchi 1962b, Inouye & Chihara 1963, Adachi 1972 as A. propulsus); Vancouver (NEPCC No. 367, vidi vivant); Norway (Moestrup pers. comm.).

Present findings: Locations 1, 3, 4, 12, 23, 30, 39, 40 (10 samples). Isolated (MUCF PRA 16) from location 4.

Illustrations: Figs 1 E, 10 A–L, 15 G–I.

Typical features: Cells are large (18–20 μm × 10 μm), elongate, pyramidal, and tapering in the mid-region. Cells sometimes have four narrow, longitudinal lobes, previously described as “wings or keels” (Butcher 1959). There are 4 or 8 flagella, one and a half times the cell length and of delicate appearance. The chloroplast appears olive or yellow in colour with one mono-layered stigmata in the mid-region. Ricketts (1970) observed siphonin in this species, and we also found siphonin in our strains. Small stalk grains surround the pyrenoid which is invaded by convoluted thylakoids. Underlayer scales of type 3 (cf. Figs 10 C & 13 C) cover the entire plasmalemma. The scale morphology has been reported by several authors (Manton et al. 1963, Norris & Pienaar 1978, Pennick 1978, Pienaar 1978, Inouye & Horiguchi 1982b) and studies of the internal fine structure and mitosis can be found in Manton (1966) and Woods and Triemer (1981) respectively. Hargraves and Gardiner (1980) report on the life history of this species and Gardiner and Hargraves (1979) analyse the seasonal dynamics.

Remarks: Butcher (1959) transferred the quadriflagellate form of P. amylifera to the genus Asteromanae due to the presence of “wings or keels” and created a new species, A. propulsus, to accommodate the octoflagellate form. Recently, Ettl (1981) transferred P. amylifera to the genus Polyblepharides due to the occurrence of eight flagella. However, it is known that this species can interconvert from the quadriflagellate to the octoflagellate form, for which it has a propensity in culture (Hargraves & Gardiner 1980, Manton 1966). The accumulated ultrastructural evidence indicates that this species is a true member of the genus Pyraminonas.

An interesting situation of scale variation occurs in this species in that some strains, appearing otherwise identical, have a notably distinct crown scale (Pienaar 1978, Inouye & Horiguchi 1982b, see also Figs 10 A, 1 & K this communication). Pienaar (1978) suggested that this may represent a distinct form of the species confined to the Southern Hemisphere but this form has now been observed in Japan (Inouye & Horiguchi 1982b) and Vancouver (vidi vivant).

The occurrence of siphonin could indicate adaptation of P. amylifera to low light environments (see Yokohama 1983). Study of the ecophysiology of this species may prove rewarding.

Species Inc. sedis

P. longicauda Van Meel emend. Inouye et al. 1984

Holotype: Pl. I, Fig. N.

Type locality: Bassin du Commerce, Ostende, Belgium; 16 November 1967.

Fig. 11. Pyraminomas longicauda (wild material) – A: Shadowed whole mounts of intermediate square scale viewed from underside (scale bar = 300 nm). – B: Shadowed whole mounts of square basket scale. Several pyrmenesophylic scales impinge on the edges of the micrograph (scale bar = 300 nm). – C: Stained whole mounts of intermediate square scale (P. longicauda) with crown scale and box scale of P. amylifera (arrows) for comparison of size (scale bar = 300 nm). – D: Stained whole mounts of three limuloid scales (scale bar = 250 nm). – E: Perpendicular section (Fix E) of periplast showing intermediate square scales with sloping perforate rims and supporting buttresses (arrows). The basket shaped scales were lost in embedded material (scale bar = 1 μm). – F: Tangential section of intermediate square scale base (Fix D) showing central perforate base and outer meshwork margin with buttress attachments (arrows) (scale bar = 300 nm). – G: Light micrograph (bright field) showing conical shape and large anterior lobes (scale bar = 10 μm). – H: Light micrograph (Nomarski) of cell with long caudal appendage (scale bar = 10 μm). – I: LS (Fix D) showing a number of intermediate square scales on plasmalemma, chloroplast and nucleus (scale bar = 4 μm). – J: LS pyrenoids and microbody (Fix D) showing thylakoids invading matrix from anterior (scale bar = 1 μm). – K: TS of pyrenoid (Fix E) showing convolution of thylakoids in matrix (scale bar = 1 μm). – L: Section of Golgi cisternae showing nascent intermediate square scales (scale bar = 300 nm). M: Glancing section of periplast (Fix D) showing underlayer scales, probably similar to Type 1. One scale is shown in perpendicular section (arrow) (scale bar = 100 nm). – N: Glancing section of proximal end of flagellum (Fix D) showing pentagonal scales (scale bar = 100 nm).
**Synonym:** *P. ostendensis* Van Meel 1969.

**Previous records:** Belgium (Van Meel 1969); Japan (Inouye & Chihara 1983, Inouye et al. 1984).

**Present findings:** Locations 1, 23, 30, 31 (6 samples). This species occurred rarely, and attempts to establish cultures were unsuccessful. Enough material was collected from Hobson’s Bay on 17 January 1984 (Location 1) to prepare thin-embedded specimens for transmission EM.

**Illustrations:** Figs 1 H, 11 A–N, 15 L–N.

**Typical features:** Cells are large (18–28 μm × 10–12 μm) and distinctive in shape. The overall outline is conical with a long produced tail at the posterior of some cells. The anterior view shows four large lobes that create a profile resembling a four leaf clover. The tail was observed to be colourless except for the occasional presence of dark red granules. Two to three small red/black dots, similar to those in the tail, were seen in each of the anterior lobes. Two stigmata are situated medially in adjacent chloroplast lobes. The thylakoids that penetrate the basal pyrenoid from the anterior are somewhat convoluted within the pyrenoid matrix. Two pyrenoids were present in some cells sectioned by us (Fig. 11 J), but these cells were in early stages of division and had more than four flagella. The stalk reserve consists of two separate grains, each in the shape of a wishbone. The flagellar scales of this species are similar to those observed on other species of *Pyramimonas* (Fig. 11 D & N). On the cell surface the plasmalemma is entirely covered by underlayer scales that are probably the same as type I (cf. Figs 11 M & 13 A). No footprint scales were observed. The two other body scales are extremely large with an intermediate layer of square scales measuring approximately 800 nm across (Fig. 11 A, C & F) that somewhat resemble the "basket" scales of *Mesostigma* Lauterborn (cf. Manton & Ettl 1965), and an outer layer of square, basket like scales that are ca. 1000 nm in width (Fig. 11 B).

**Remarks:** Van Meel (1969) described *P. ostendensis* and *P. longicauda* from brackish waters in Belgium, but Inouye and Chihara (1983) and Inouye et al. (1984) reported that cultured material from Japan indicates the two species to be synonymous. Since certain of the specimens examined by us could be aligned to either *P. longicauda* or *P. ostendensis* when examined by LM, yet always had identical scale morphology, we agree that these two species are synonymous. Our observations by LM agree well with Van Meel’s (1969) description, although he did not record any red/black granules in the tail and anterior lobes. The body scales are almost four times larger than in other species of *Pyramimonas*, however, this is not considered of enough significance to warrant the removal of *P. longicauda* from the genus at present. *P. longicauda* shows affinity with those species having footprint scales; both have type 1 underlayer scales, similar basal body arrangement, and pyrenoids penetrated by thylakoids from the anterior only. However, *P. longicauda* lacks footprint scales and the underlayer scales cover the entire plasmalemma. The convolution of thylakoids within the pyrenoid matrix is similar to *P. amylifera*.

**P. virginica** Pennick 1977: 245

**Holotype:** Fig. 11 Pennick (1977).

**Type locality:** York River, Gloucester Pt., Virginia, USA; 18 March 1972.

**Previous records:** East Coast USA (Pennick 1977). Greenland (Thomsen 1982).

**Present findings:** Locations 1 and 23 (2 samples). An isolate (the type strain) was provided by UTEX (LB 1997).

**Illustrations:** 1 F, 12 A–J, 15 J & K.

**Typical features:** Cells are very small (2–3.5 μm × 2 μm), pyramidal, with four flagella (equal to the cell in length) that often project directly anteriorly from the pit at rest. Several trichocysts, located in the anterior lobes, can be seen to evert during LM. There is one stigma composed of a single layer of lipid globules in a chloroplast lobe adjacent the nucleus. The nucleus has a peculiar invagination into which the cytoplasm protrudes. The central, posterior pyrenoid is invaded from the anterior by a distinctive pattern of thylakoids. The pyrenoid is surrounded by a single cup shaped starch grain.

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Fig. 12. *Pyramimonas virginica* – A: LS of cell (Fix D) showing nucleus, chloroplast, stigma, pyrenoid, starch grain and scale reservoir (scale bar = 1 μm). – B: TS (Fix D) at level of flagellar pit showing nucleus, four chloroplast lobes and Golgi apparatus (scale bar = 1 μm). – C: LS of nucleus (Fix D) showing cytoplasmic intrusion (scale bar = 500 nm). – D: TS of basal bodies (Fix D) in prophase cell showing diamond configuration of four parent basal bodies, sinitosome and four daughter basal bodies. Note the exclusion of ribosomes in square zone around basal bodies (scale bar = 300 nm). – E: LS of trichocyst in anterior lobe showing coils of ribbon (scale bar = 300 nm). – F: TS of anterior lobe from dividing cell. A coiled structure, possibly representing a trichocyst in the process of exocytosis is shown (scale bar = 300 nm). – G: Stained wholomount of basket shaped scale from cell periplast (wild material) (scale bar = 100 nm). – H: Stained wholemount of limuloid flagellar scale (scale bar = 100 nm). – I: Freeze-etch (ES) showing one basket shaped scale and underlayer scales of Type 2 (scale bar = 300 nm). – J: Freeze-etch (ES) of flagellum showing imbricate rows of limuloid scales and underlying pentagonal scales at the distal end (right hand side of micrograph) (scale bar = 300 nm).
The basal bodies are arranged in a diamond configuration similar to *P. gelidicola* (cf. McFadden & Wetherbee 1984). We were not able to detect the presence of a transitional helix (coiled fibre). The flagella bear three types of scales resembling those of other species of the genus. Cells are completely covered by underlayer scales of type 2. The only other scale type on the cell surface is neither comparable to box nor crown scales of other species but is basket shaped (200 nm wide, 230 nm high) and has a unique six-fold symmetry (see also Penick 1977). The wild material examined by us, and UTEX LB 1997, had only one rail at the upper rim of the basket shaped scale in contrast to the report of Penick (1977) in which two rails were reported in CCAP LB 67/16 (= UTEX LB 1997).

**Remarks:** This putative species of *Pyramimonas* has four characteristics setting it apart from other members of the genus: diminutive size, a unique body scale type of different symmetry to all other species, the possible absence of a transitional helix, and the cytoplasmic invasion of the nucleus. However, the underlayer scales

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Fig. 13. A–E: Drawings of small scale types (scale bar = 20 nm). – A: Type 1 underlayer scale. – B: Type 2 underlayer scale. – C: Type 3 underlayer scale. – D: Footprint scale. – E: Pentagonal flagellar scale.

and the presence of trichocysts align *P. virginita* to *P. grossii*, *P. cirolanae*, *P. lunata*, and *P. parkeae*. Although *P. virginita* has pyrenoid, starch grain, and basal body configuration different to the above species, the similarities in underlayer scales and trichocysts suggest it is probably closely related to them; it is therefore retained in the genus *Pyramimonas*. Characterization of further material of this species is desirable.

**Discussion**

Of the 78 species described for the genus *Pyramimonas*, one species (*P. hexaciliata* Van Meel 1969) resembles the chrysophyte *Apidinella*, and is mistakenly named, eleven species have been transferred to the genus *Hafnimonas* (Ettl 1981), and *P. ostiendensis* and *P. plurioculata* are here relegated to synonymy with *P. longicauda* and *P. orientalis* respectively. Of the remaining 64 species, 19 have been studied by EM, 11 of which we have found in Australia. Of the species known by EM but not observed here, one is freshwater (*P. tetrahyranchus*), one is benthic (*P. octopus*), one is probably endemic to Antarctica (*P. gelidicola*), and another *P. obovata* is possibly synonymous with a species treated here (*P. disomata*). Of the remaining four species, three (*P. spinifera* (Pennick 1983), *P. occidentalis* (Pennick 1982a), *P. gorlestonae* (Pennick & Cann 1982)) are only known from the UK and one (*P. lunata*) from Japan (Inouye et al. 1983). All species observed here, except *P. cordata* and *P. grossii*, are new distribution records for Australia, and *P. parkeae*, *P. cirolanae*, *P. virginita* and *P. longicauda* are new to the Southern Hemisphere. We have also encountered a number of unidentified species, but since these could not be fully characterized by thin-section and freeze fracture/etch EM they must remain undescribed for the present.

The majority of *Pyramimonas* species that have been described, in particular those observed only by LM, have no subsequent records and there may be many which are synonymous or mis-identifications. The dis-
covery of species-specific scale structures, visible by EM, has greatly assisted the taxonomy. This has lead to the description of a number of new species based almost solely on differences in the scale architecture. The validity of this approach can only be known after further investigations of anomalies such as that reported here for *P. disomata*. Species specificity of scale architecture may imply that some unknown selective pressure is in operation. Alternatively, if *Pyramimonas* is haploid, single point mutations could possibly alter the fine structure of a scale, and non-deleterious variants could be perpetuated as asexual clones.

**Trichocysts and possible relationships within Pyramimonas**

We present evidence that trichocysts may be lost from cells by exocytosis at division. Since Norris and Pearson (1975) suggest that trichocysts originate in the nuclear envelope, it seems reasonable that they participate in net endomembrane flow, eventually being extruded. The function of trichocysts is unknown, but Cavalier-Smith (1982) has suggested that ejective organelles in an anterior evagination are an adjunct in prey capture by primitive phagotrophic flagellates. Phagotrophy is not known in *Pyramimonas*, but has been observed in the closely related *Halosphaera Schmitz* (Parke & Adams 1965), suggesting that trichocysts remain as a relic organelle in *Pyramimonas*. Alternatively, *Pyramimonas* may have acquired trichocysts at a later stage, perhaps by means of a viral vector. The extrusion of trichocysts prior to cytokinesis may represent a retarded version of the usual ejective sequence, now unnecessary. Following the premise that trichocysts are a primitive characteristic (Cavalier-Smith 1982), it can be hypothesised that those species of *Pyramimonas* having trichocysts in the flagellar pit are the more primitive. More advanced species presumably lost their trichocysts as they were no longer useful to autotrophic organisms. This assumption indicates that an entire covering of underlayer scales (as found in *P. subg. Trichocysts*) is the more primitive condition and that *P. subg. Vestigifera* (which lacks trichocysts) has undergone a reduction of underlayer scales and developed footprint scales; or perhaps conversion of underlayer scales into footprint scales. Possibly the footprint scales are involved in interlocking the box scales which now form a continuous, rigid periplast attached to the plasmalemma in *P. subg. Vestigifera*. It has been suggested that the underlayer scales of the *Prasinophyceae* were modified into the crystalline wall of the Chlorophyceae *sensu* Stewart and Mattox (1978) (Donnyczyn et al. 1980, Mattox and Stewart 1977) and perhaps *P. subg. Vestigifera* represents a stage in the depletion of underlayer scales. It has also been proposed that the underlayer scales of type 1 may be homologous to the small pentagonal scales of the flagellum (Melkonian & Robeneck 1981), and comparison between the two types in *P. subg. Vestigifera* (cf. Fig. 3C & E) supports this hypothesis. This introduces the possibility that the restriction of underlayer scales to the pit in *P. subg. Vestigifera* may represent an early stage in the development of underlayer body scales from flagellar pentagonal scales. Regarding *P. subg. Pyramimonas*, it seems most likely that since at least one species has siphonema, it must be derived from a group having lutein, as siphonema is a derivative of lutein in the biosynthetic pathway (Yokohama 1983). Probably *P. subg. Pyramimonas* is derived from *P. subg. Trichocysts* as these groups show some resemblances, having fibrillar bands connecting the basal bodies and an entire covering of underlayer scales. Study of further species should assist in clarifying relationships between the three sub-genera proposed here, and perhaps further sub-groups not yet recognised.

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