

Minireview

The genus *Pyramimonas* (Prasinophyceae) from southern African inshore waters

RN Pienaar* and SD Sym

School of Animal, Plant & Environmental Sciences, University of the Witwatersrand, Private Bag 3 WITS, Johannesburg 2050, South Africa

* Present Address: Deputy Vice Chancellor (Academic: Internal), University of the Witwatersrand, Private Bag 3 WITS, Johannesburg 2050, South Africa

* Corresponding author, e-mail: pienaarr@vco.wits.ac.za

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Some seventeen species of the genus *Pyramimonas* from southern African inshore waters were encountered, isolated and cultured. Only two of these had been found in this locality prior to this study, and four were new to science. Details of their features observable at the light and electron microscope level were recorded and used in a cladistical analysis which yielded two most parsimonious trees. Most ultrastructural features were found to be useful, with the exception of the eye-

spot groupings suggested by Hori *et al.* (1995), which are afforded additional attention. The subgenera *Pyramimonas* and *Punctatae* are not resolved and appear basal. The cladogram supports the notion of very advanced vestigiferans whose sister group interestingly is formed by the trichocystin cluster of *P. grossii*/*P. cirolanae*. The results are compared with a cladogram derived from molecular data, and possible evolutionary trends are presented.

Introduction

The microalgal component is frequently a neglected aspect of many marine floral investigations, principally because they are often not obvious, but also because of the need for tedious and highly specialised techniques and/or equipment for their identification. This is exacerbated by a general lack of reference material on our local waters, which makes it all the more difficult to positively identify an organism. The end result is that little known organisms are frequently and deliberately overlooked, and this means that much of the African marine microalgae remain in obscurity, no less so in South Africa. Thus, although ecological investigations of phytoplankton are of primary importance, one of the greatest shortcomings has been the rather skewed reports of the more readily identifiable components, particularly the Bacillariophyta or the more troublesome Dinophyta. It thus becomes imperative that these shortcomings not be perpetuated and that a fuller knowledge of our flora is established, so that the work of those that follow may be more comprehensive and not exclude forms difficult to identify.

This has been a principal objective of much of the marine microfloral work at our institute, and thus it was decided to improve the database on a frequently encountered genus, *Pyramimonas* Schmarda, in South African marine waters.

This genus is an important contributor to the phytoplankton and some species have even been implicated in 'red' tides in Japan (Inouye *et al.* 1983). It is of further interest as it is a member of the Pyramimonadales (Melkonian 1990), an assemblage which is believed to be fairly representative of the ancestral stock of other green algal groups and of higher plants (Inouye *et al.* 1985). This belief is supported by cladistic analysis of molecular data which indicates that the group is deeply rooted in the chlorophyte lineage of the Viridiplantae (Marin and Melkonian 1999).

Based primarily on ultrastructural data, the genus has been divided into four subgenera: *Pyramimonas* McFadden, *Vestigifera* McFadden, *Trichocystis* McFadden (McFadden *et al.* 1986) and *Punctatae* McFadden (McFadden *et al.* 1987). More recently the distinction between the subgenera *Trichocystis* and *Punctatae* has been questioned, and a further two monospecific subgenera *Hexacta* Hori, Moestrup *et Hoffmann* and *Macrura* Hori *et Moestrup* have been constructed (Hori *et al.* 1995).

Prior to this investigation, only three species of *Pyramimonas* from southern Africa had been reported in the literature, viz. *P. pseudoparkeae* Pienaar *et Aken* (Pienaar and Aken 1985), *P. propulsa* Moestrup *et Hill* (Pienaar

1978), *P. orientalis* Butcher ex McFadden Hill et Wetherbee and *P. cordata* McFadden (as *P. species*, Glen 1980). Four additional species, *P. chlorina* Sym et Pienaar (Sym and Pienaar 1997), *P. formosa* Sym et Pienaar (Sym and Pienaar 1999), *P. mucifera* Sym et Pienaar (Sym and Pienaar 1991b) and *P. robusta* Pienaar et Sym (Pienaar and Sym 1997), have more recently been reported from South African waters. With the exception of *P. cordata*, all these species and additional ones have been encountered, isolated and cultured.

Some of the objectives of this study have been the subject of other publications (Pienaar and Sym 1997, Sym and Pienaar 1991a, 1991b, 1993, 1995, 1997, 1999). This paper will thus concentrate on providing an overview of the diversity in southern African waters and will use ultrastructural features of these organisms to define evolutionary trends within the genus.

Materials and Methods

Opportunistic rather than intensive sampling was undertaken throughout the duration of this study and ranged geographically around the southern African coast from Maputo, Mozambique in the east to Lüderitz, Namibia in the west (Figure 1).

All isolation, culturing and preparation for light and electron microscopical examination followed the protocol of

Pienaar and Aken (1985). The optimum period for the sub-culturing of *Pyramimonas* species was found to be one month. Living material of all the species except *Pyramimonas cirolanae* has been successfully maintained in this manner for many years.

The cladistic tree presented here was obtained by using the Winclada program (Version 0.9.9+ (Beta), KC Nixon 1999) and uses *Halosphaera* c.f. *minor* Schmitz as the out-group. *Cymbomonas tetramitiformis* Schiller and *Pterosperma cristatum* Pouchet were also included in the analysis.

Results

Seventeen species, many with numerous isolates, were encountered in southern African waters, and their general appearance at the light microscope level is shown in Figure 2. They are representative of all four subgenera of McFadden (McFadden *et al.* 1986, 1987) (Table 1).

The variation in the micromorphology of the scale complement is considered most diagnostic in species identification (Norris and Pienaar 1978) and this is provided on a sub-generic basis in Figures 3–6. With the exception of the limuloid scales of the flagella of *P. parkeae* (Sym and Pienaar 1993), the body box scales of *P. moestrupii* (Sym and Pienaar 1995) and *P. aff. nephroidea* (Sym and Pienaar 1995), the scale micromorphology was found to be stable

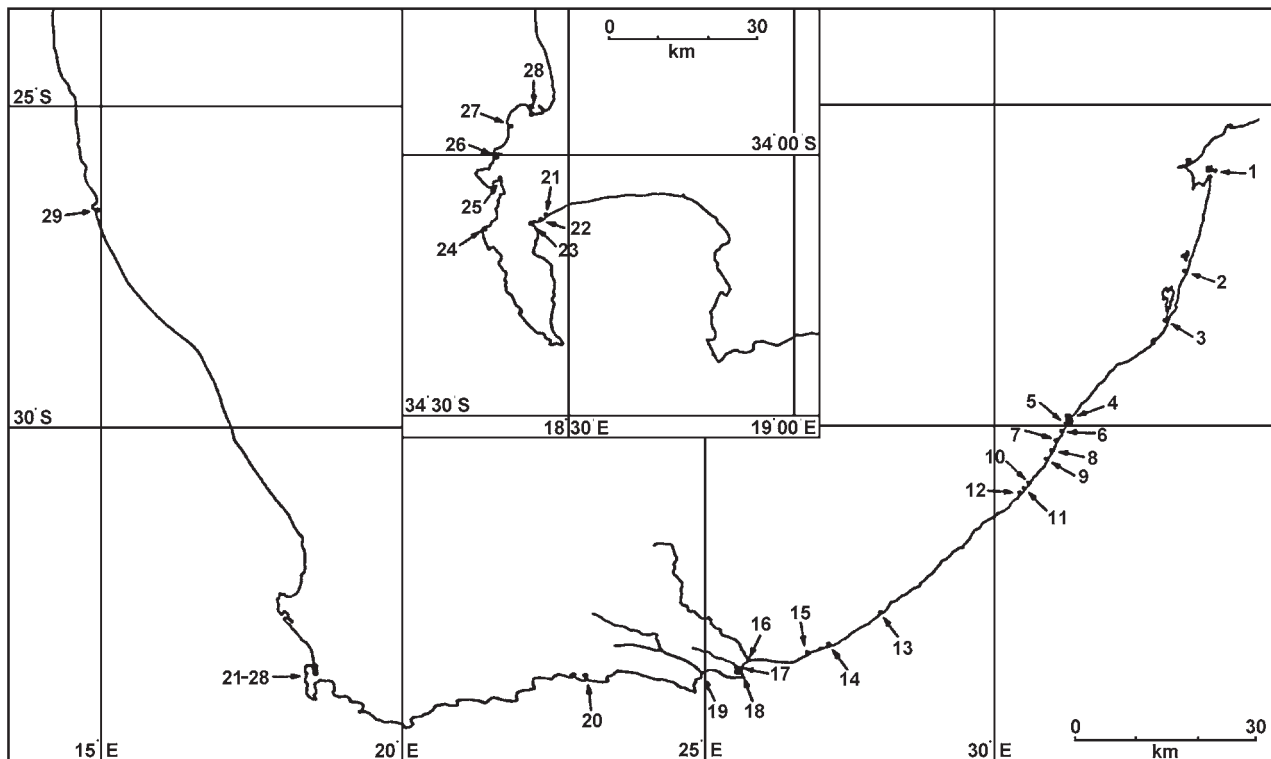


Figure 1: Map of southern Africa and details of the Cape Peninsula (inset) to show the locations of the sample sites. 1 – Inhaca Island, Mozambique; 2 – Sodwana; 3 – St Lucia; 4 – Durban; 5 – Isipingo; 6 – Amanzimtoti; 7 – Widenham; 8 – Rocky Bay; 9 – Mtwalume; 10 – Marina Beach; 11 – Palm Beach; 12 – Port Edward; 13 – East London; 14 – Port Alfred; 15 – Kenton-on-Sea; 16 – Sundays River mouth; 17 – Swartkops estuary; 18 – Port Elizabeth; 19 – Gamtoos estuary; 20 – Knysna lagoon; 21 – St James; 22 – Kalk Bay; 23 – Fish Hoek; 24 – Kommetjie; 25 – Hout Bay; 26 – Llandudno; 27 – Camps Bay; 28 – Cape Town; 29 – Lüderitz, Namibia

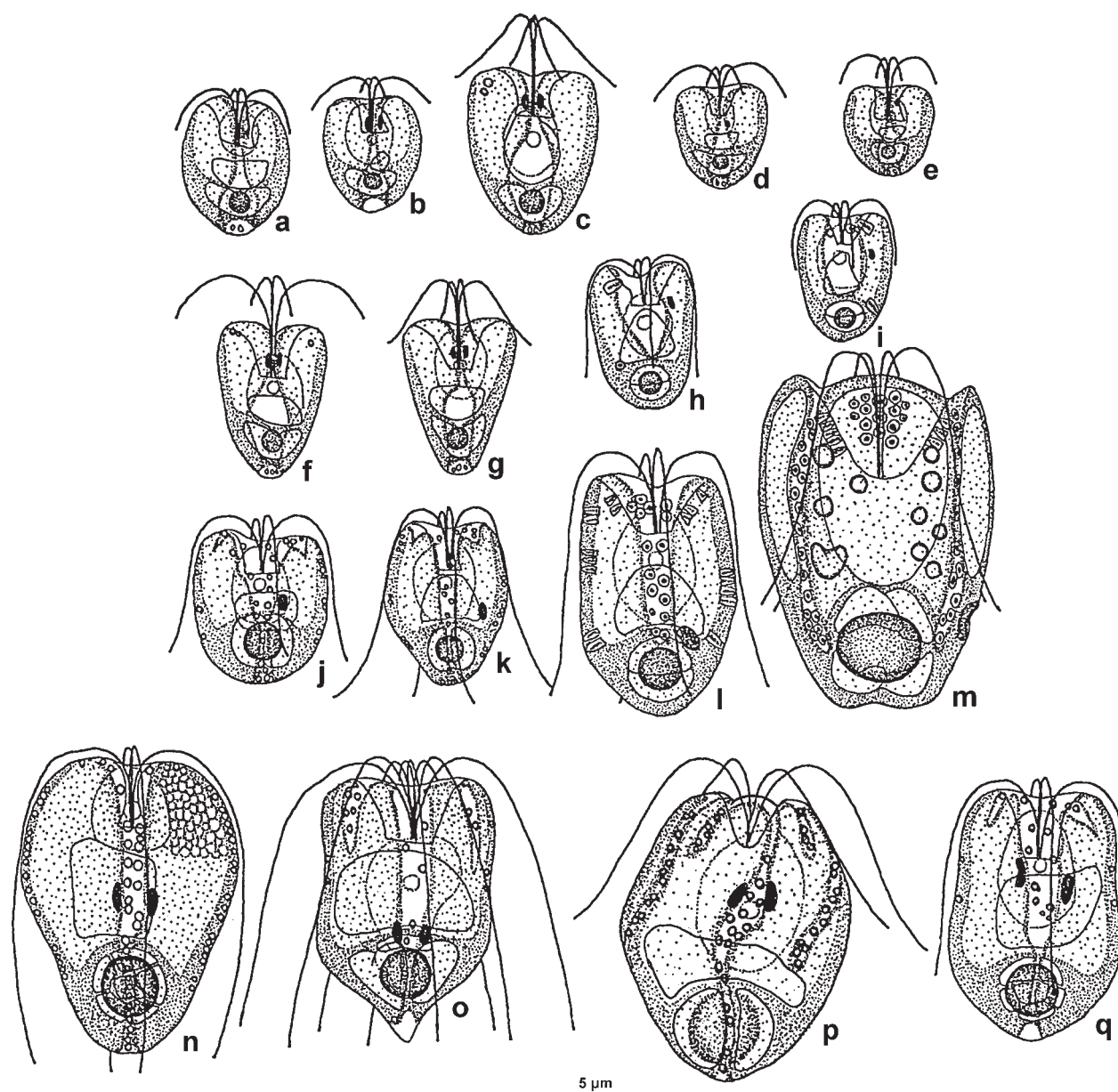


Figure 2: Light microscope drawings of all species of *Pyramimonas* currently encountered. (a) *Pyramimonas mitra*, (b) *P. melkonianii*, (c) *P. moestrupii*, (d) *P. mantonae*, (e) *P. aff. nephroidea*, (f) *P. orientalis*, (g) *P. disomata*, (h) *P. cirolanae*, (i) *P. grossii*, (j) *P. robusta*, (k) *P. olivacea*, (l) *P. parkeae/pseudoparkeae* (T-cell), (m) *P. parkeae/pseudoparkeae* (L-cell), (n) *P. mucifera*, (o) *P. propulsa*, (p) *P. formosa*, (q) *P. chlorina*

and completely in concurrence with previous reports, despite their very divergent origins. Only those species from the subgenus *Punctatae* and *P. propulsa* were found to have distinctly segmented T-hairs (terminology of Marin and Melkonian 1994). Modified T-hairs and P₁-hairs were only encountered in members belonging to the subgenera *Pyramimonas* and *Punctatae*, as well as in the smaller members of the subgenus *Trichocystis* (*P. grossii* and *P. cirolanae*, see Sym and Pienaar 1993, Marin and Melkonian 1994). They were not found in South African isolates of *P. orientalis*, despite the previous reports of such in this species by Belcher *et al.* (1974, as *P. obovata*) and Marin

and Melkonian (1994).

The internal organisation of organelles in all quadriflagellate species (Figures 7–12) was found to be consistent with that described for most other quadriflagellates (with the exception of that of *P. virginica* Pennick, Hori *et al.* 1995). The internal organisation of the only octoflagellate, *P. propulsa*, mirrored that of *P. octopus* (Moestrup *et al.* 1987). The morphology of the chloroplast was found to be quite variable in the genus but uniform within the subgenera. The subgenera *Punctatae* and *Pyramimonas* have a Type 1 chloroplast (Pienaar and Sym 1997, Sym and Pienaar 1997), the subgenus *Trichocystis* has a Type 2 chloroplast with two sub-


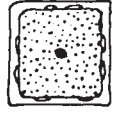

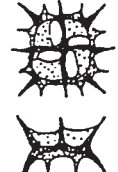


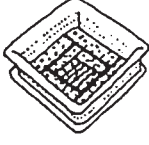






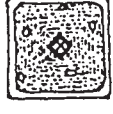











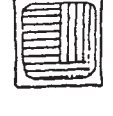

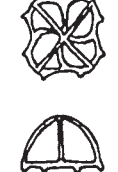



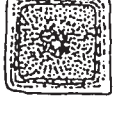
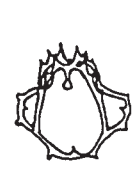



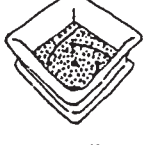
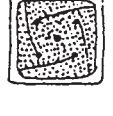




	Body Scales				Flagellar Scales	
	Box		Crown		Limuloid	T-hair
<i>P. disomata</i>						
<i>P. mitra</i>						
<i>P. moestrupii</i>						
<i>P. melkonianii</i>						
<i>P. orientalis</i>						
<i>P. aff. nephroidea</i>						
<i>P. mantonae</i>						

Figure 3: A comparison of the larger scale types of vestigiferan species presently encountered. (Bar scale = 230nm, except for hair scales = 300nm)

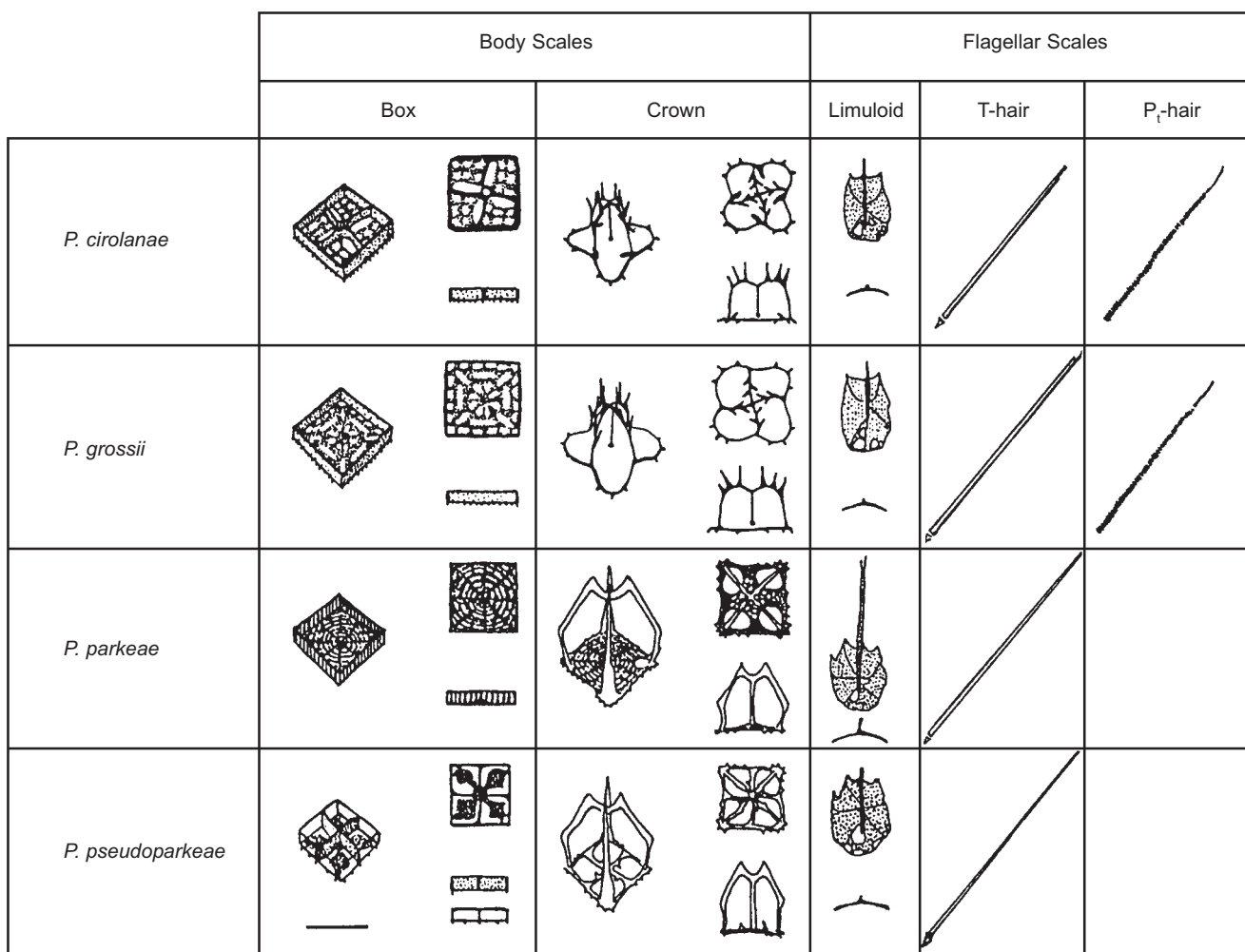


Figure 4: Larger scale types of presently-encountered trichocystin species. (Bar scale = 230nm, except for hair scales = 300nm)

Table 1: Species of *Pyramimonas* encountered in southern Africa and the subgenera with which they are most closely affiliated

Subgenus	Species
<i>Pyramimonas</i>	<i>P. propulsa</i> Moestrup & Hill
	<i>P. chlorina</i> Sym & Pienaar
<i>Trichocystis</i>	<i>P. cirolanae</i> Pennick
	<i>P. grossii</i> Parke
	<i>P. parkeae</i> Norris & Pearson
	<i>P. pseudoparkeae</i> Pienaar & Aken
<i>Punctatae</i>	<i>P. formosa</i> Sym & Pienaar
	<i>P. mucifera</i> Sym & Pienaar
	<i>P. olivacea</i> N. Carter
	<i>P. robusta</i> Pienaar & Sym
<i>Vestigifera</i>	<i>P. disomata</i> McFadden, Hill & Wetherbee
	<i>P. mitra</i> Moestrup & Hill
	<i>P. mantonae</i> Moestrup & Hill
	<i>P. moestrupii</i> McFadden
	<i>P. melkonianii</i> Sym & Pienaar
	<i>P. aff. nephroidea</i> McFadden
	<i>P. nephroidea</i> McFadden
<i>P. orientalis</i> McFadden, Hill & Wetherbee	

Table 2: Types of configurations of flagellar apparatus found in the various subgenera of *Pyramimonas*

Subgenus	Type of Flagellar Apparatus Configuration
<i>Pyramimonas</i>	3-1; shifted
<i>Punctatae</i>	3-1
<i>Trichocystis</i>	3-1
<i>Vestigifera</i>	rhombic ('diamond')

Table 3: Microtubular root complement in South African representatives of *Pyramimonas*

Subgenus	Species	Microtubular Roots			
		1s	1d	2s	2d
<i>Pyramimonas</i>	<i>P. propulsa</i>	4(3/1)	3	4(3/1)	3
	<i>P. chlorina</i>	4(3/1)	4	3(2/1)	2
<i>Punctatae</i>	<i>P. formosa</i>	4(3/1)	4	3(2/1)	2
	<i>P. mucifera</i> / <i>robusta</i>	3(2/1)	4	3(2/1)	2
	<i>P. olivacea</i>	3(2/1)	3	3(2/1)	2
<i>Trichocystis</i>	<i>P. cirolanae</i> / <i>grossii</i>	3(2/1)	3	3(2/1)	2
	<i>P. parkeae</i> / <i>pseudoparkeae</i>	4(3/1)	3	3(2/1)	2
<i>Vestigifera</i>	All species	4(3/1)	2	3(2/1)	2



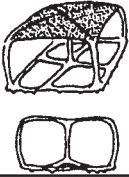

















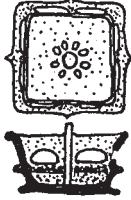




	Body Scales				Flagellar Scales		
	Box		Crown		Limuloid	T-hair	Modified T-hair
<i>P. mucifera</i>							
<i>P. olivacea</i>							
<i>P. robusta</i>							
<i>P. formosa</i>							

Figure 5: The larger scale types of members of the subgenus *Punctatae*. (Bar scale = 230nm, except for *P. formosa*, = 300nm)




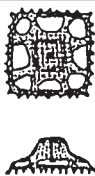

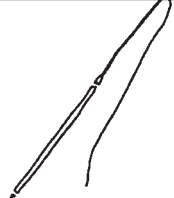








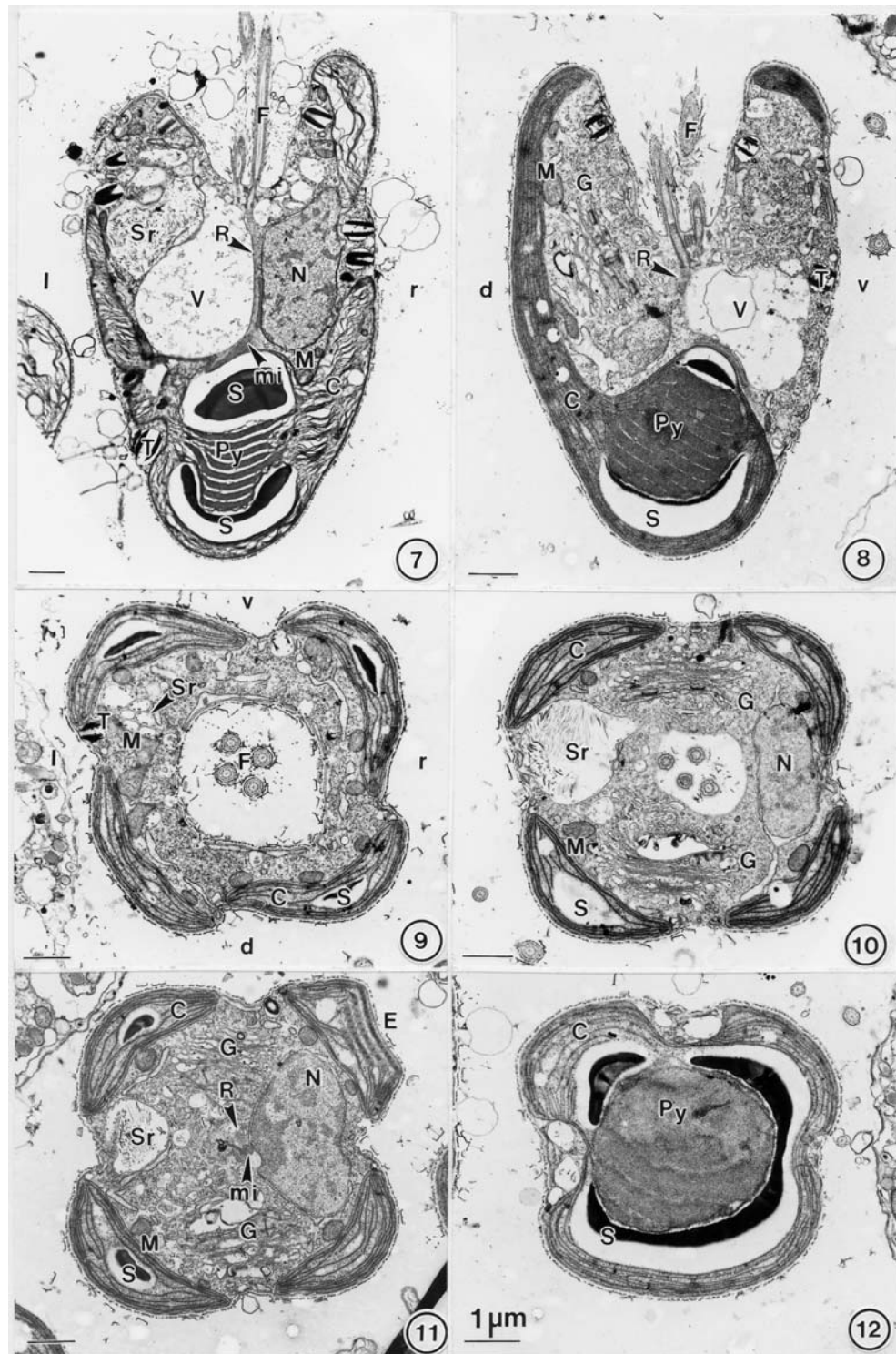
	Body Scales				Flagellar Scales		
	Box		Crown		Limuloid	T-hair	P ₁ -hair
<i>P. propulsa</i>							
<i>P. chlorina</i>							

Figure 6: Larger scale types of the subgenus *Pyramimonas*. (Bar scale = 230nm, except for hair scales = 300nm)



Figures 7–12: Internal cell organisation in *Pyramimonas parkeae* Norris et Pearson. Figures 7–8: Longitudinal sections of cells in a left-right and a dorsi-ventral plane respectively. Figures 9–12: Cross sections in the upper and lower regions of the flagellar pit, the mid region through the rhizoplast, and the pyrenoid region of the cell respectively.

Abbreviations for all figures: C – chloroplast; cp – proximal cartwheel; cpm – central pair microtubules; D – scale reservoir duct; dcf – distal connecting fibre; dc – diagonal connective; dcp – distal cartwheel; ddc – discontinuous distal cartwheel; df – duct fibre; dp – daisy pattern; F – flagellum; fb – fibrillar band; G – dictyosome; lf – longitudinal fibrils; M – mitochondrial profile; mi – microbody; N – nucleus; pb – proximal band; pcf – proximal connecting fibre; pf – prominent proximal fibre; Py – pyrenoid; R – rhizoplast; ri – ribosomes; S – starch, s – synisotosome; sp – stellate pattern; Sr – scale reservoir; tf – transfibre; th – transitional helix; ts – transverse septum; V – vacuole; 1–8 – flagella; 1s, 1d, 2s and 2d – microtubular roots.

types (a and b) (Sym and Pienaar 1993) and the subgenus *Vestigifera* has a Type 3 chloroplast (Sym and Pienaar 1995) (Figure 13). Findings with regard to the positioning of the eyespot/s (Figure 13) were in complete concordance with those of Hori *et al.* (1995).

The configuration of the flagellar apparatus of members of each subgenus basically was found to agree with each circumscription (McFadden *et al.* 1986, 1987) and with one of the three types outlined by Inouye *et al.* (1985) (Table 2). Serial sections (Figures 14–25) through the basal body apparatus of various species provided the details required for three dimensional reconstructions (Figure 26). The inter-

connectives at all levels in all species were found to have an amazing degree of constancy (Figure 27), with only minor variations on the theme in some. Details of these inter-connectives are reported elsewhere (Pienaar and Sym 1997, Sym and Pienaar 1991a, 1991b, 1993, 1995, 1997).

The microtubular root complement of all species investigated is provided in Table 3. The younger roots associated with basal body 2, with the exception of the octoflagellated *P. propulsa*, are constant and most variation (2–4 microtubules) is found in the 1d root.

Basal body structure was also surprisingly variable within the genus (Figure 28). The proximal cartwheel pattern in *P.*

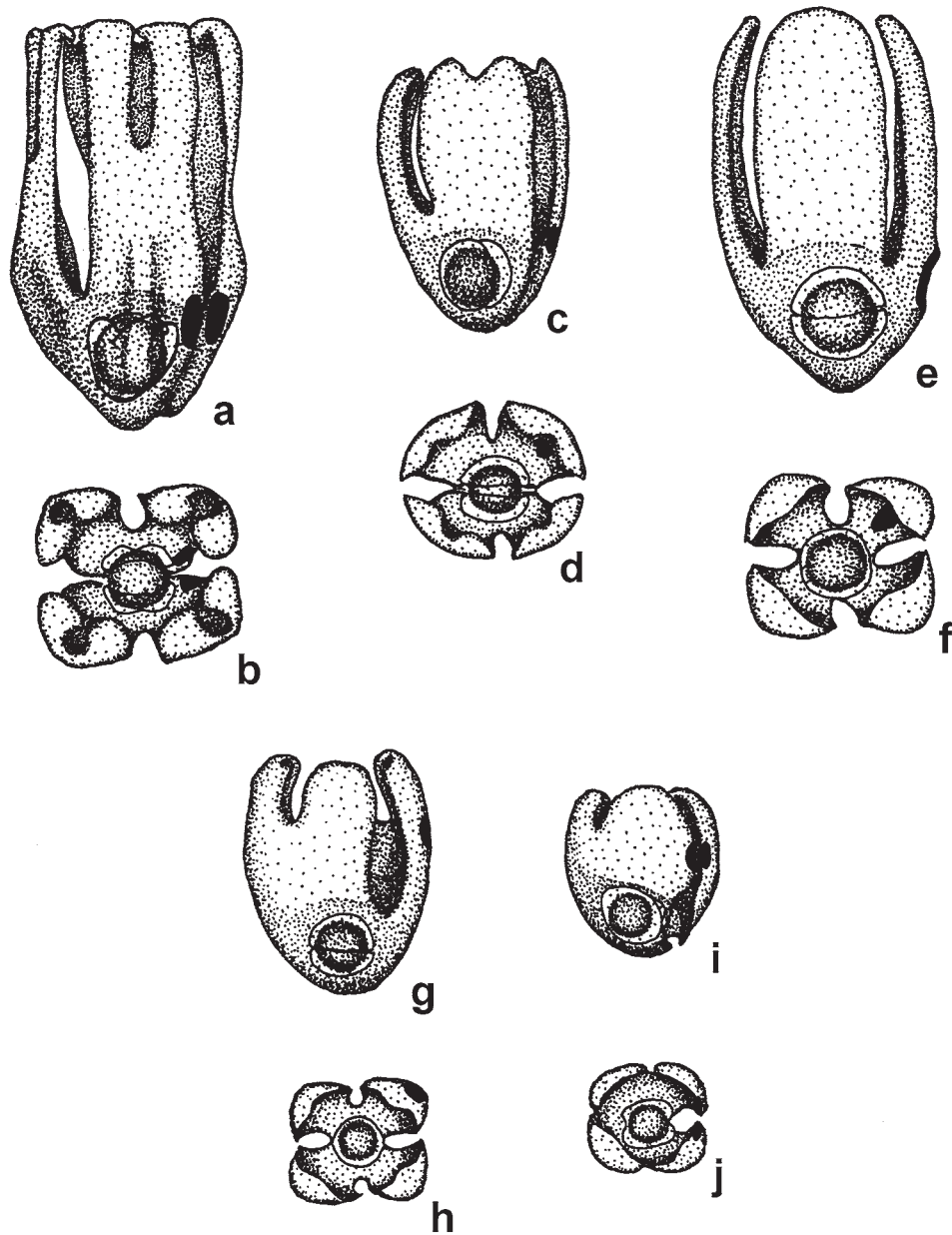
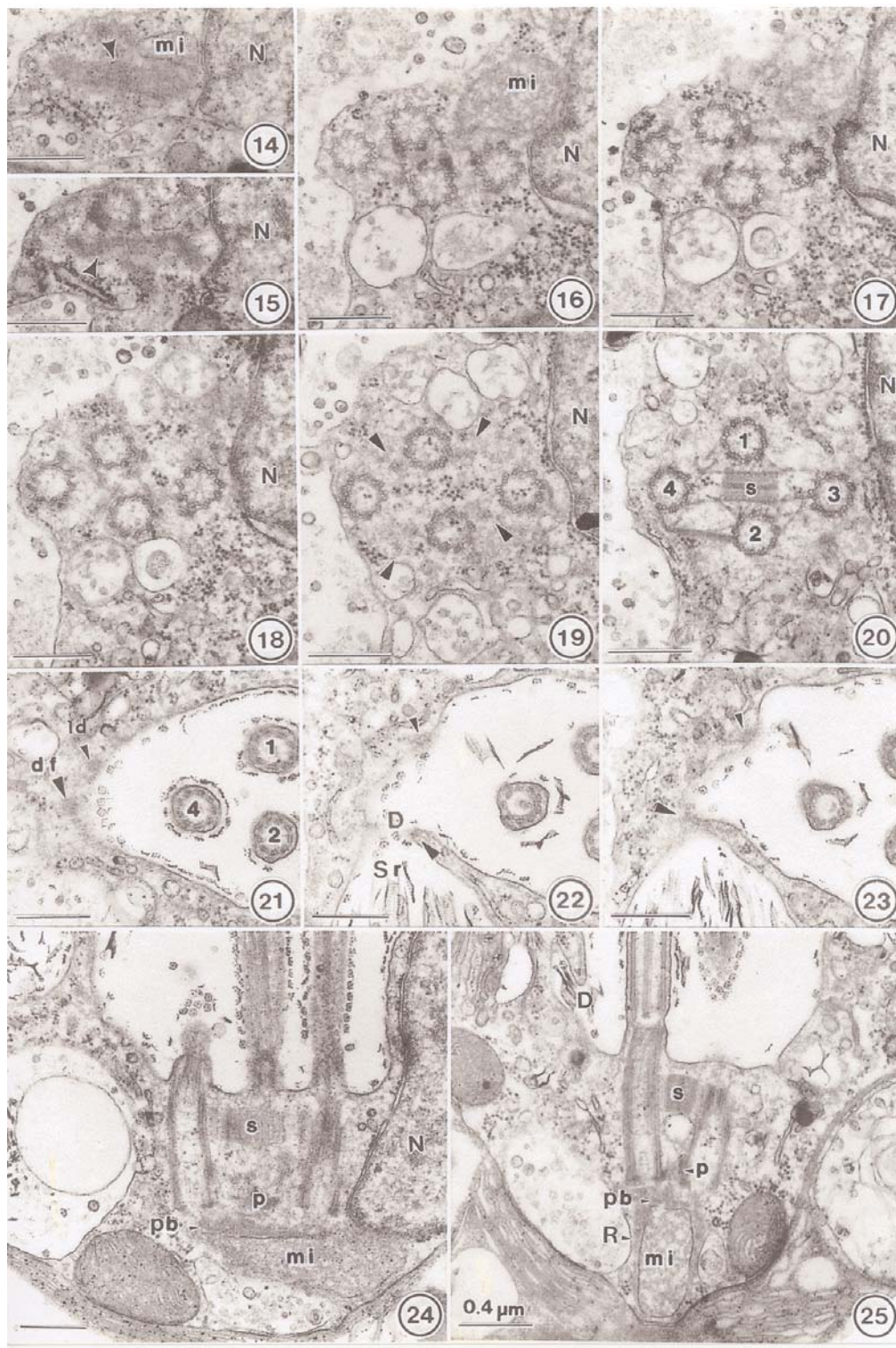


Figure 13: Light microscope drawings of the variation possible in the morphology of chloroplasts in species of *Pyramimonas* as seen in side and apical view respectively. (a) and (b) *P. propulsa* – Type 1 chloroplast; (c) and (d) *P. olivacea* – Type 1 chloroplast; (e) and (f) *P. parkeae* – Type 2a chloroplast; (g) and (h) *P. grossii* – Type 2b chloroplast; (i) and (j) *P. mitra* – Type 3 chloroplast



Figures 14–25: The flagellar apparatus and duct fibre in *Pyramimonas mitra* McFadden. Figures 14–20: Serial, non-consecutive transverse sections of the flagellar apparatus from proximal to distal levels. Figures 14–15: The underlying microbody and the proximal band (arrow-head). Figures 16–17: The prominent central connective, bordering diagonal connectives and more lateral transfibres and proximal connecting fibres. Figures 18–19: The proximal extremes of the microtubular roots (arrowheads) and the intermediate level of the flagellar apparatus; Figure 20: Distal connectives including the synstosome and the elbow-shaped fibre between basal bodies 4 and 2. Figures 21–23: Serial, non-consecutive transverse sections near the base of the flagellar pit to show the 1d root and associated duct fibre encircling the opening of the scale reservoir duct. Figures 24–25: Medial longitudinal sections of the flagellar apparatus in the planes formed by basal bodies 3 and 4 and 1 and 2 respectively

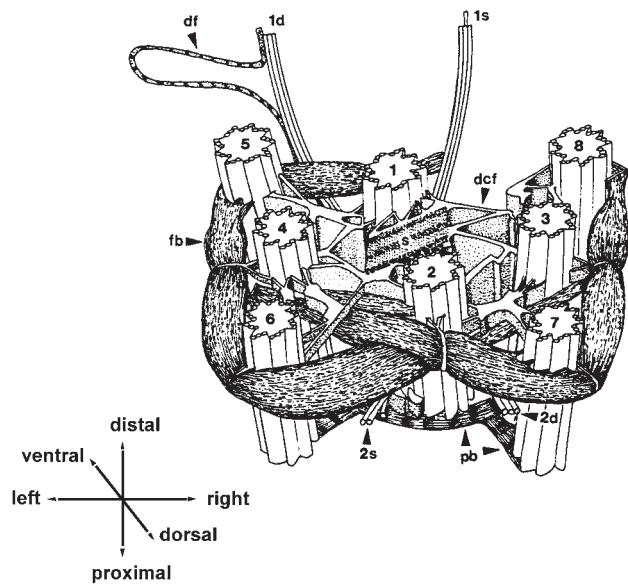


Figure 26: A 3-dimensional reconstruction of the flagellar apparatus of *Pyramimonas propulsa*

parkeae and *P. pseudoparkeae* (Figure 28d), and in all members of the subgenera *Pyramimonas* and *Punctatae* currently investigated (Figure 28e), has a unique series of centripetal dense arcs attached to the A-tubule, a configuration termed the 'daisy pattern' (Sym and Pienaar 1993). The proximal cartwheel pattern of *P. cirolanae* and *P. grossii* (Figure 28c) and of all vestigiferan members investigated (Figure 28a, b) lacks this daisy pattern.

Distal to this cartwheel, the lumen may be free of any substructures apart from ribosomes (most vestigiferans and *P. grossii*). All species investigated had a distal cartwheel structure, always being evident at the point of emergence of the flagellum from the cell, but also possibly extending for some distance into the basal body. This structure, like the proximal cartwheel structure, has a central core and radial spokes extending between the core and the A-tubules of the peripheral triplets. The basal body of *P. orientalis* (Figure 28b) has a discontinuous osmiophilic core, as described in *P. gelidicola* McFadden, Moestrup et Wetherbee (McFadden and Wetherbee 1984), and cross-sections where it is present show its structure to be indistinguishable from a distal cartwheel structure. Some species (*P. grossii* and *P. cirolanae*) have a very weakly developed distal cartwheel structure that can easily be missed (Figure 28c). Others, e.g. *P. parkeae* and *P. pseudoparkeae*, have an extended cartwheel structure stretching throughout much of the length of the basal body (Figure 28d).

The lumina of basal bodies of *P. mucifera* may additionally have longitudinal fibrils, but otherwise longitudinal fibrils and the extended distal cartwheel structure are mutually exclusive (Figure 28e).

The transition regions are far more uniform. All species have an undivided stellate pattern and a transitional helix with no discernible individual gyres. The transitional helix is markedly longer in some (*P. parkeae*, Figure 28d) or of inter-

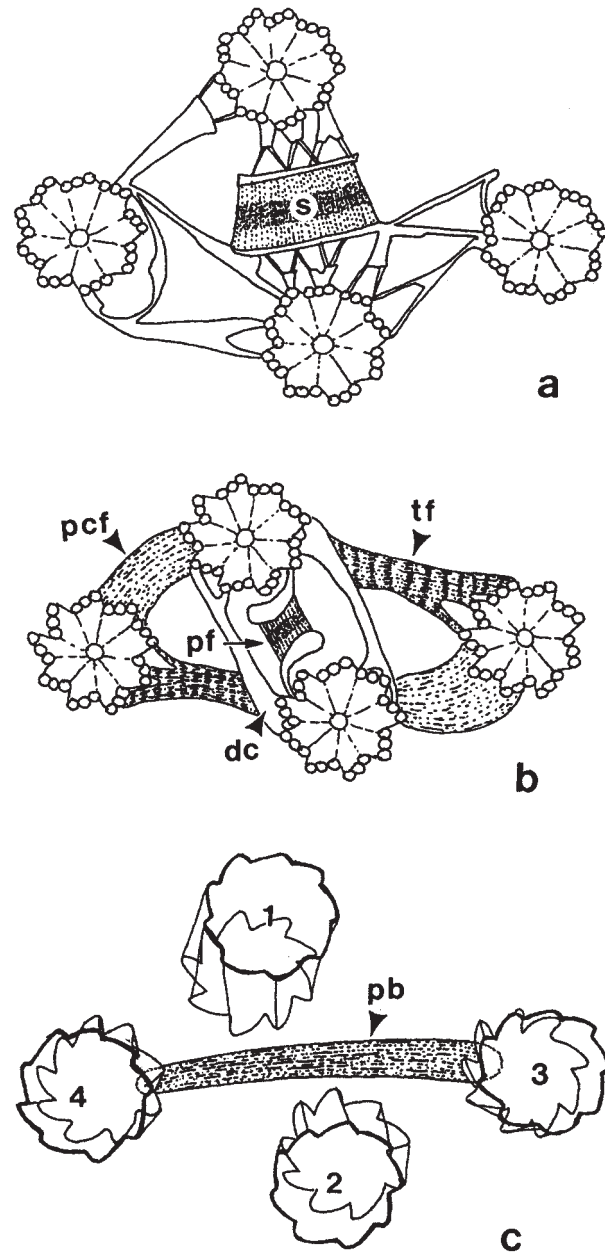


Figure 27: Connectives of the flagellar apparatus of *P. mitra*. (a) Distal level; (b) Proximal level; (c) Entire basal bodies and underlying proximal band

mediate length (*P. propulsa*, Figure 28e). Regression analysis, using presently investigated isolates and prompted by the investigation of Daugbjerg and Moestrup (1992), showed a greater correlation between the length of the cell and the length of the transitional helix ($r^2 = 0.49$) than between cell length and the length of the stellate pattern ($r^2 = 0.16$) or the length of the entire basal body ($r^2 = 0.006$). This trend is similar to their findings but with much lower r^2 values (0.84, 0.36 and 0.013 respectively; Daugbjerg and Moestrup 1992). Surprisingly, the transition region of all vestigiferans investi-

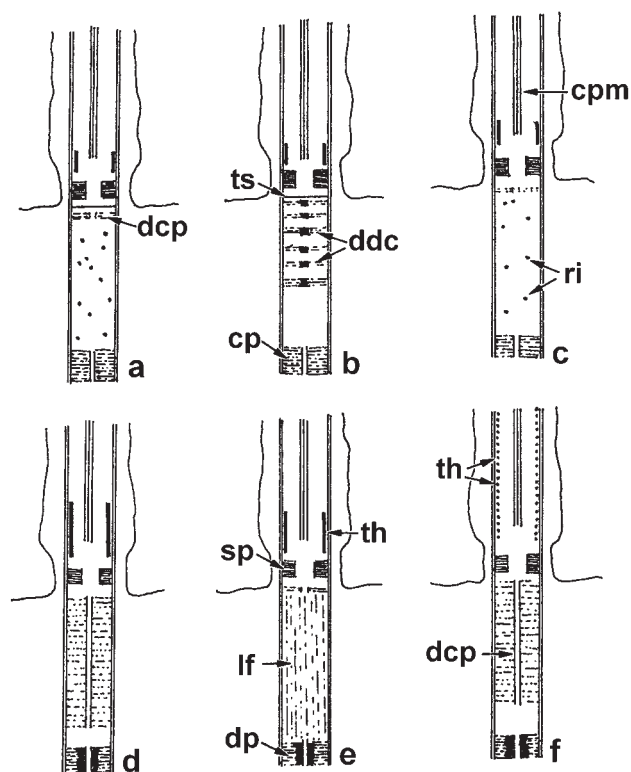


Figure 28: Diagrammatic representation of the possible variation in the ultrastructure of the basal bodies in *Pyramimonas* (a–e) and in the outgroup genera (f) (see text for details)

gated (Figure 28a, b) had a transverse plate-like structure (Sym and Pienaar 1995).

For comparative purposes, the generalised basal body of the outgroup genera (*Halosphaera*, *Cymbomonas* and *Pterosperma*) (Figure 28f) has the following features: A long and separated transitional helix, and extended distal cartwheel pattern and a daisy pattern to the proximal cartwheel.

Cladistic analysis

Features found most useful in separating out the taxa included the benthic nature of certain species; the chloroplast colour and morphology; details of flagellar scales (T-hair scales and limuloid scales); details of the body underlayer scales (presence/absence of high walls and/or central boss); details of the basal bodies (including proximal, distal and discontinuous cartwheel structures, presence/absence of the transitional septum and presence/absence of longitudinal fibrils in the lumina); basal body configuration (including the profile of the synistosome in cross-sections of the cell, presence/absence of the lateral/external fibrillar bands and the number of proximal bands); the number of microtubules comprising the 1d root and the 1s root; and finally, the presence/absence of extrusomes/muciferous bodies and/or trichocysts (Table 4).

Two equally parsimonious trees were recovered (Figure

29), irrespective of the organism used as the ultimate outgroup (*Halosphaera viridis*, *Cymbomonas tetramitiformis* or *Pterosperma cristatum*). The consensus tree (Figure 30) shows clear support for the concept of the subgenus *Vestigifera* (McFadden *et al.* 1986) but members of the subgenus *Trichocystis* form two clades consistent with the two subgroups described by Sym and Pienaar (1993), the subgroup 2 clade rooting more deeply. Some members of the subgenus *Punctatae* (*P. olivacea*, *P. mucifera* and *P. robusta*) form a well-defined clade but collectively are not clearly resolved from *P. propulsa* and the trichocystin/vestigiferan assemblage. Similarly, the anomalous species, *P. formosa* and *P. chlorina*, with characters almost equally shared with the subgenera *Pyramimonas* and *Punctatae* (Sym and Pienaar 1997, 1999), are not clearly resolved from each other or the clade of the remainder of the species investigated, but are most deeply rooted in the tree.

Discussion

The current survey of the genus in southern Africa resulted in the discovery of four new species (Sym and Pienaar 1991b, 1997, 1999, Pienaar and Sym 1997) and five new records from our coastline. It is thus obvious that intensive surveys of this sort are essential if we are to become familiar with phytoplanktonic organisms and obtain baseline data for future comparisons.

Existing subgeneric divisions of the genus rely principally on ultrastructural features (McFadden *et al.* 1986, 1987, Hori *et al.* 1995), but these have not been corroborated by cladistic analyses, despite a call for such by Theriot (1990). Phylogeny within the genus has been investigated using cladistic methods on molecular data (*rbcL* gene sequences; Daugbjerg *et al.* 1994). The inferred topology was shown to be in concordance with McFadden's subgenera rather than those of Hori *et al.* (1995), the latter of whom intimate a merging of the subgenera *Punctatae* and *Trichocystis*. Unfortunately, neither of the representatives of the monospecific subgenera *Hexactis* and *Macrura* were included in Daugbjerg *et al.*'s (1994) study. Although the present cladistic analysis showed a topology more supportive of the subgenera of McFadden (McFadden *et al.* 1986, 1987), considerable uncertainty exists with regard to the delimitation of the subgenera *Pyramimonas* and *Punctatae*, despite good support for three punctate species (including the Type). This lack of clear resolution is somewhat supportive of the concept of a broader circumscription for the subgenus *Pyramimonas* to include the subgenus *Punctatae* (Sym and Pienaar 1999) but it would be far more convincing if all species involved (*P. chlorina*, *P. formosa*, *P. mucifera*, *P. olivacea*, *P. propulsa* and *P. robusta*) had formed a clear clade. Nevertheless, the topology supports the notion that these two subgenera have a predominance of plesiomorphic characters.

In a bid to merge the subgenera *Punctatae* and *Trichocystis* based on eyespot location, Hori *et al.* (1995) viewed puncta and trichocysts as homologous structures. We concur with this, but differences in their substructure, staining properties (McFadden *et al.* 1986, 1987) and explosive mechanisms (Manton 1969, McFadden *et al.* 1987) must make for significant advances in the formation

Table 4: List of characters and character states used in the construction of Figure 29

Char. No.	Character Description	Character States	
		Plesiomorphic	Apomorphic
0	Colour	Olive-green	Grass green
1	Anterior sinuses in chloroplast lobes	Present	Absent
2	Long left-right sinus in chloroplast	Present	Absent
3	Chloroplast lobes AB and CD	Free	Fused
4	All chloroplast lobes	Largely free	Largely fused
5	Shafts of T-hair flagellar scales	Fused and tubular	Septate and solid
6	P ₁ hairs	Present	Absent
7	Face of limuloid scales	Cob-webbed	Filled
8	Ribs of limuloid scales	5	4
9	Height of body undelayer scales	High	Low
10	Floor of body undelayer scales	cross-like	filled (hatched)
11	Muciferous bodies	Present	Absent
12	Trichocysts	Absent	Present
13	Settling behaviour	Common	Rare
14	Daisy pattern in proximal cartwheel	Present	Absent
15	Distal cartwheel in basal body	Present	Absent
16	Distal cartwheel in basal body	Continuous	Discontinuous
17	Length of distal cartwheel	Long	Short
18	Transitional plate	Present	Absent
19	Longitudinal fibrils in lumen of basal body	Present	Absent
20	Flagellar apparatus configuration	3/1 or variant	Rhombic
21	Synistosome	Curved/Essentric	Straight
22	External fibrillar band	Present	Absent
23	Lateral fibrillar band	Present	Absent
24	Number of proximal bands	More than 1	1
25	Multi-layered structure	Present	Absent
26	Oblique band	Present	Absent
27	1d root	>2 microtubules	2 microtubules
28	1s root	4 (3/1)	3 (2/1)

of trichocysts, from an evolutionary point of view. In this regard, we also view the extrusomes of *Dolichomastix* Manton (pers. obs.), *Mantoniella* Desikachary (Barlow and Cattolico 1980), *Mamiella* Moestrup (Moestrup 1984) and *Pterosperma* (Inouye *et al.* 1990) and the muciferous bodies of *Cymbomonas* (pers. obs.) and *Halosphaera* (Parke and Adams, 1961) as homologues of puncta and trichocysts. *Pyramimonas mucifera*, a somewhat enigmatic species of the genus, is considered a prime example of a link between the subgenera *Punctatae* and *Trichocystis*, and Daugbjerg *et al.* (1994) allude to the need for a new subgenus to accommodate it. While the ultrastructural differences between *P. olivacea*, the Type of the subgenus *Punctatae*, and *P. mucifera* highlighted by these authors are correct, it is interesting that the topology inferred by the majority of other ultrastructural features shows this species as well-seated within the subgenus *Punctatae*. Work on the molecular data of this species is now critical and has been initiated.

Details of the chloroplast morphology are often ignored in taxonomic studies. The chloroplasts of the supposed primitive genera in the order Pyramimonadales (*sensu* Melkonian 1990) are provided in Figure 31. There is no clear trend between the chloroplast morphologies in these genera, but the findings of Inouye *et al.* (1990), amongst others, infer that *Halosphaera* is the closest genus to *Pyramimonas*. The chloroplast of *Halosphaera* is typified by deep fissures on both the anterior and posterior sides of the main body. This

system is most closely mirrored by Type 1 chloroplasts within the genus *Pyramimonas*, a type that therefore may be viewed as plesiomorphic.

The biggest difference in the topology of the currently generated cladogram to that of Daugbjerg *et al.* (1994) is that certain members of the subgenus *Trichocystis* (Group I *sensu* Sym and Pienaar 1993) form a sister group to the subgenus *Vestigifera*. While this provides a novel way of viewing relationships between these two subgenera, we are fully aware of the possible convergence of several features that may arise from a tendency of both forms to be dominated by small cells, particularly with regard to the rhombic configuration of the flagellar apparatus.

Additional similar trends of evolution in Group I trichocystins and vestigiferans, assuming traits of the subgenera *Pyramimonas* and *Punctatae* to be primitive, include a loss of settling behaviour, (possibly concomitant with) an anterior migration of the eyespot, the loss of the external fibrillar band (*sensu* Sym and Pienaar 1993), a decrease in the number of proximal bands, the loss of the daisy pattern in the proximal cartwheel structure of the basal body and the loss of a radial rib and a lateral spine in the limuloid scale.

However, differences are also apparent (Table 5) so, although the present topology shows these two as grouped, we would not be surprised if the relative importance of the presumed evolutionary progression, or of that of the individual ultrastructural features selected, were not overstressed. We are thus not averse to the idea of a deep division

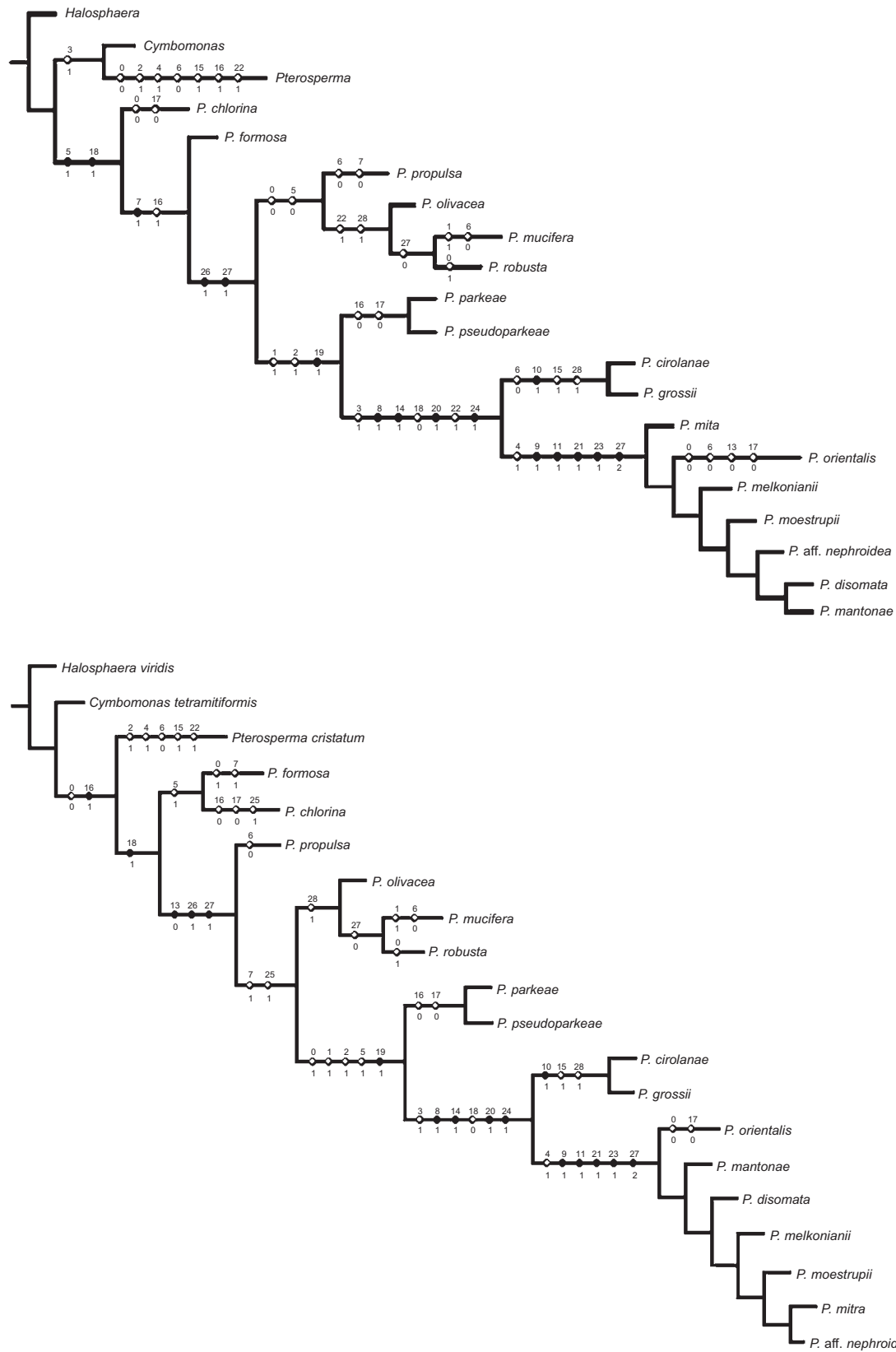


Figure 29: The two most parsimonious trees for South African members of the genus *Pyramimonas* based on ultrastructural data. Length = 60; Consistency index = 50; Retention index=82; ● = autapomorphy; ○ = parallelism/reversal; Superscript digit = character number; subscript digit = character state

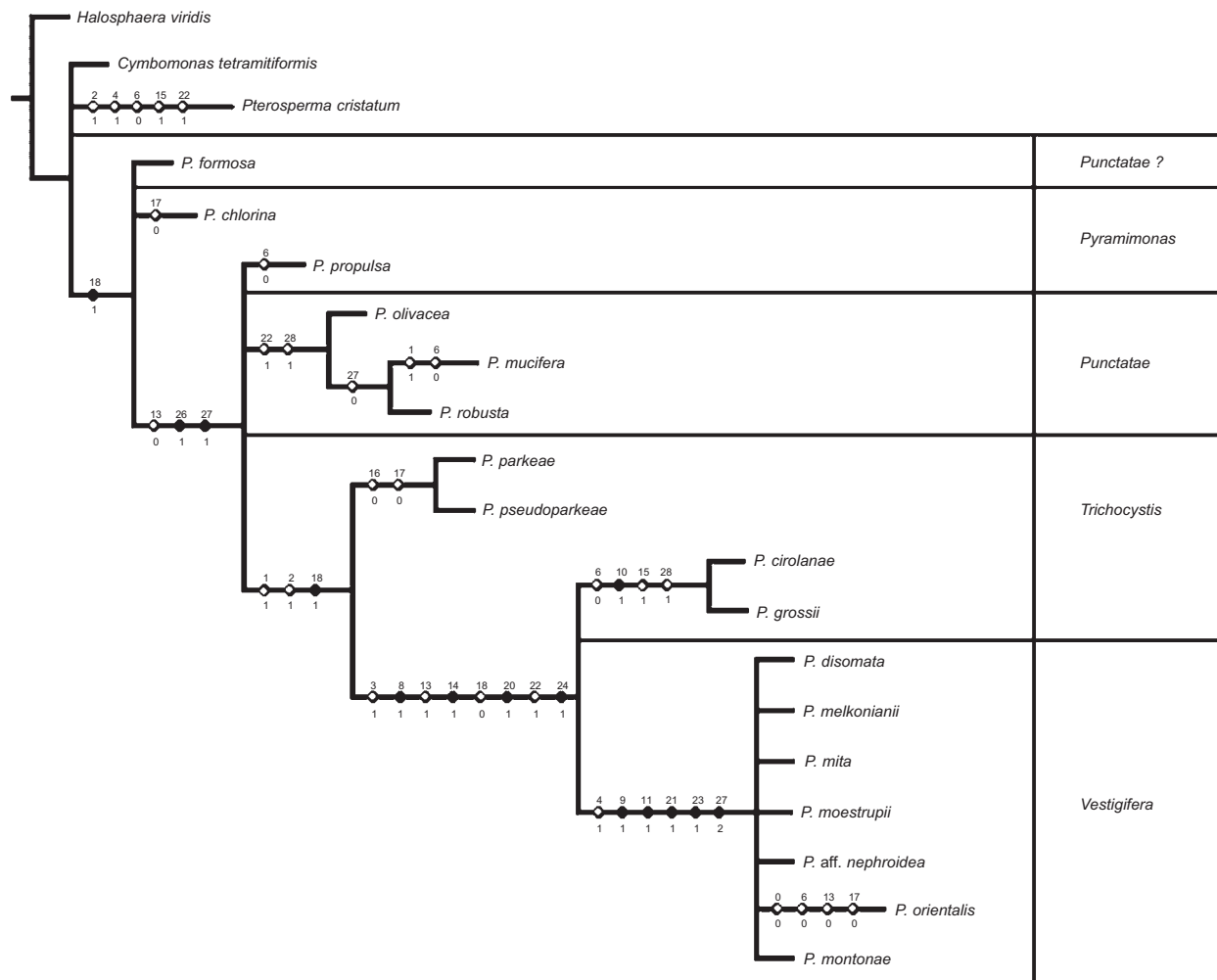


Figure 30: Consensus tree of the trees illustrated in Figure 29. Affiliations of the various species with traditional subgenera are indicated by the table to the right of the cladogram

between the subgenus *Vestigifera* and the remaining subgenera as inferred by *rbcL* gene sequencing (Daugbjerg *et al.* 1994). This would simply mean a considerable degree of convergence which would not be too surprising if they are driven by similar requirements that come together with a reduction in cell size.

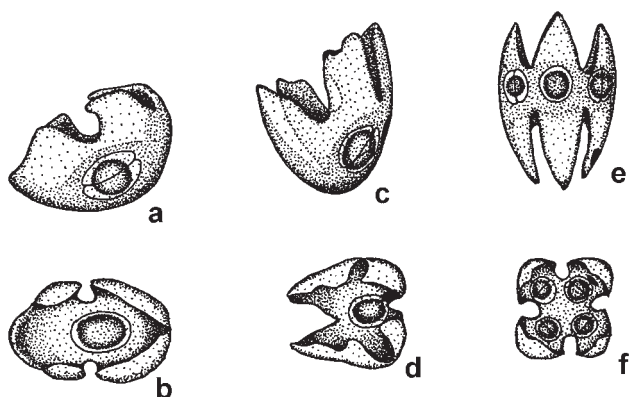
A major concern with the consensus tree is that individuals with a Group II eyespot location (*sensu* Hori *et al.* 1995) are found in two widely-divergent groups; in the presumably most plesiomorphic forms and the apomorphic subgenus *Vestigifera*, with interpolated subgenera predominantly displaying Group II eyespot locations. It seems inconceivable that a structure of such importance to the success of swimming behaviour should be so plastic through evolution. The eyespot arrangement of Hori *et al.* (1995) was not included in this cladistic analysis, as it has no major repercussions for the final topology apart from separating out two basal species of the subgenus *Vestigifera*. Were these features to be accommodated in the cladogram, however, numerous reversals would be incurred, and thus these characters do not enjoy much support in a scheme based on ultrastructur-

al features. In any event, the groups of eyespot arrangement of Hori *et al.* (1995) are again supportive of a deep divide between the subgenera that *Vestigifera* and the remaining subgenera (Daugbjerg *et al.* 1994).

Finally, we would like to pass comment on the findings of Hori *et al.* (1995) with regard to flagellar apparatus orientation in the cell. All quadriflagellate species currently investigated, without exception, have basal bodies 3 and 4 forming a plane that roughly bisects chloroplast lobes A and C (*sensu* these authors). Basal bodies 1 and 2 thus lie on a plane just offset in a counterclockwise direction from one perpendicular to the 3–4 basal body plane and thus just glancing lobes B and D. All also have their eyespot in lobe B or lobes B and C. We were thus surprised when mention was made of the eyespot of *Pyramimonas tetrahynchus* being in lobes A and B. Unfortunately, this material is unavailable to us, but careful scrutiny of the photographic plates of Belcher (1969), assuming his observation of eyespot location being on the plane of future cell division (i.e. a plane bisecting chloroplast lobes B and C and A and D) to be correct, showed that basal bodies 3 and 4 (Plate 3, Figure E

Table 5: Differences in certain ultrastructural features between Group I trichocystins and the subgenus *Vestigifera*

Feature	Subgenus	
	Group I <i>Trichocystis</i>	<i>Vestigifera</i>
1s root	2/1	3/1
1d root	3	2
Distal cartwheel	Absent/reduced	Present
Flagellar apparatus	Weakly curved synstosome, no elbow fibre, 3-1 but rhombic-like	Straight synstosome, elbow fibre, rhombic
Body underlayer scales	Present all around the cell surface	Only present in the flagellar pit
Eyespot location	Group II	Group III

**Figure 31:** Chloroplast morphology (side and apical views respectively) in other members of the order Pyramimonadales *sensu* Melkonian (1990). (a) and (b) *Pterosperma*; (c) and (d) *Cymbomonas*; (e) and (f) *Halosphaera*. (Not drawn to scale)

and H) are similarly oriented. Thus we cannot concur with this species having a Group IV eyespot and would rather view it as having a Group III eyespot. *Pyramimonas virginica* continues to be an enigmatic species in this regard (Group I, Hori *et al.* 1995). From this analysis, the characters of scales within the genus *Pyramimonas* that may be considered plesiomorphic include an absence of footprint scales; a complete covering of square underlayer body scales; limuloid scales with five radial ribs, three subsidiary spines and spider-webbed faces; T hairs with solid, segmented shafts and Pt hairs in tufts at the flagellar tips.

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