Plate 1

Figs. 1-8. Light microscopic images of *Anthophysa vegetans* colonies and individual motile cells. Figs 1-5. Stalked (arrow; Figs 1,2) or unstalked (Figs 3-5) colonies consisting of *ca*. 10-20 spherical (carat), pyriform (asterisks) or ovoid cells. The monadoid nature of the cells composing colonies is demonstrated in Fig. 4 in which flagella are visible (chevron). Figs 6-8. *A. vegetans* motile single cells. Spherical (Figs 6, 7) to ovoid (Fig 8) motile unicells diassociated from colonies bearing apically inserted unequal length flagella. The short posterior (mature) flagellum is noted by arrowheads, whereas the long, anterior flagellum is indicated with a chevron. (Scale = 10 μm)

Plate 2

Figs. 9-15. Vegetative cells of *Polykaryon pyrenoidosum gen. et comb. nov.* Note the variation in cell sizes observed. Cells are characterized by the possession of numerous lenticular or polygonally shaped chloroplasts (Figs 9-12) that often become difficult to discriminate in older, larger cells (Figs 12, 13). Cells are bounded by a thin but distinct cell wall (Figs 11-13). Figs 14, 15. *P. pyrenoidosum* vegetative cells stained with the DNA-binding fluorochrome DAPI demonstrating the multinucleate condition observed in most cells. Note that the many nuclei are elongate and paired (arrowheads).

Fig. 16. Majority rule consensus tree depicting phylogenetic relationships inferred among 74 species of heterokont algae. Note that *A. vegetans* is placed sister to *O. danica* in a strongly supported clade (BP=99%) including species placed in the Chrysophyceae and Synurophyceae. The position of *P. pyrenoidosum* is unresolved.

Fig. 17. Maximum likelihood tree including 74 species of heterokont algae. *A. vegetans* is placed within the Chrysophyte clade, whereas the position of *P. pyrenoidosum* is unresolved.

Fig. 18. Majority rule consensus tree depicting evolutionary relationships inferred among 44 species belonging to the classes Chrysophyceae and Synurophyceae. The outgroup is comprised of members of the class Eustigmatophyceae. This analysis implies that *A. vegetans* is most closely related to *Poterioochromonas* spp. (BP=75%). Black bars indicate taxa in which photosynthesis and/or chloroplasts have been lost.

Fig. 19. Maximum likelihood tree inferred based upon an analysis of 18S rRNA gene sequences for 44 species placed within the Chrysophyceae and Synurophyceae. *A. vegetans* is resolved within a clade including the chrysophytes *Ochromonas sphaerocystis* and species of *Poterioochromonas*. Black bars denote loss of photosynthesis and/or chloroplasts.

Plate 3:

Figs. 20-22. Micrographs of *P. pyrenoidosum* whole cells with prominent cell walls (CW), nuclei (N), peripheral chloroplasts (C) with girdle lamellae (gl), mitochondrial profiles (M), vacuoles (V), and fringe (F). Note senescent mitochondria (*) Fig. 20, 21 and a putative lipid droplet (L) shown in Fig 22. A bulging pyrenoid (P) and possible evidence for adherence of separate cells via the glycocalyx are demonstrated in Fig. 22 (arrowhead, lower left).

Figs. 23-25. Whole cell micrographs showing multinuculate profiles (N) and chloroplasts (C). The nucleolus, when present, is marked with an arrowhead in Figs. 23, 24. Note osmiophilic droplets of unknown composition located beneath the plasmalemma observed in Figs. 24, 25.

Plate 4:

Fig. 26, 27. Cytoplasmic section of *P. pyrenoidosa* cell containing several vacuoles (V), portion of a chloroplast (C), and numerous senescent mitochondria (*). High magnifactaion image of senescent mitochondria showing the double membranes (arrowheads).

Figs. 28, 29. High-resolution images of the cell wall (CW) and fringe (F). A chloroplast (C) containing a pyrenoid (P) located beneath the outer chloroplast membranes and plastoglobuli (arrowhead) is shown in Fig. 28. Note fringe appears to be an extension of cell wall (arrowhead) Fig. 29.

Figs. 30, 31. Non-serial sections of *P. pyrenoidosa* cell wall (CW), fringe (F) and chloroplasts (C). Note the four membranes surrounding the chloroplasts (arrowheads) and the presence of girdle lamellae (gl).

Plate 5:

Fig. 32. Section showing the nucleus (N), nucleolus (nu), vacuoles (V) and Golgi apparatus (G). The outer chloroplast and outer nuclear membranes are confluent with one another (arrowheads) indicating the presence of a chloroplast-endoplasmic reticulum, or CER.

Fig. 33. Cross-section of chloroplast thylakoid membranes (T) that are adpressed in groups of three or four. The outer chloroplast membrane (arrowhead) is observed forming a compartment or 'out-pouching' containing putative flagellar hairs (pfh).

Fig. 34. Section through the Golgi apparatus (G), mitochondria (M) with tubular cristae, and chloroplast with several plastoglobuli (arrowheads).

Fig. 35. Section showing the nucleus (N) and an active Golgi apparatus (G) with approximately ten individual cisternae. Note the vessicles derived from the *trans*- face of the Golgi (arrowheads).

Figs. 36, 37. Sections of *P. pyrenoidosum* cells with a single nucleus (N), nucleolus (nu), and Golgi apparatus (G) but multiple chloroplasts (C), mitochondrial profiles (M), and vacuoles (V). Note that cells observed contained a single Golgi body always situated near the nucleus (cf. Figs 35-37). Trace of a single basal body (B) is apparent in Fig. 37.

Plate 6:

Figs. 38, 39. Basal bodies in vegetative cells of *P. pyrenoidosum*. Fig. 34. Tangential section through a cell containing a basal body (B); arrow denotes the presence of possible basal body-associated connecting fiber(s). Fig. 39. Cross-section through a basal body. Note 9 +2 arrangement of microtubules.

Figs. 40-42. Membrane-bound (see arrows) compartments and vesicles containing putative flagellar hairs (pfh). Fig. 40. Cross-section through a compartment formed by the outer membrane of the nucleus containing PFHs. Figs 41, 42. Longitudinal sections of vesicles containing PFHs. Note in Figs 40 and 42 that the sectioned PFHs are oriented in different directions; longitudinal- (or slightly tangential-) and cross-sectional views of PFHs are observed within a single membrane-bound compartment.

Fig. 43. Longitudinal sections of vesicle containing PFHs.

Fig. 44. Section through a chloroplast (C) containing longitudinally oriented PFHs.

Plate 7:

Fig. 45. Computer generated illustration of the section shown in Fig. 44. This schematic demonstrates how a section through a 'sickle shaped' chloroplast with a PFH compartment located in or near the concave side of the chloroplast could be misinterpreted as evidence that PFHs are found inside the chloroplast. It is contended that PFHs are not found inside the chloroplast and are always located (intracellularly) within the endomembrane system.