Cryptogamie, Bryologie, 2006, 27 (1): 75-83

Novel features of the plastids in some deep-shade, antipodean thalloid liverworts

Jeffrey G. DUCKETT [a*] *& Roberto LIGRONE* [b]

[a] *School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS (England)*

[b] *Dipartimento di Scienze ambientali, Seconda Università di Napoli, via A. Vivaldi 43, Caserta (Italy)*

(Received 30 September 2005, accepted 17 November 2005)

Abstract – The chloroplasts in the inner thallus cells of *Monoclea forsteri* and *Verdoornia succulenta* contain giant grana often with over 100 thylakoids, a feature shared with another deep-shade liverwort *Dumortiera hirsuta* and with numerous extreme-shade vascular plants. In contrast, thylakoid architecture in *Neohodgsonia mirabilis* and *Marchantia foliacea* with small grana comprising only 5-10 thylakoids, is typical of that in most liverworts and mosses. In both the last two taxa and particularly *Marchantia*, bands of filaments, 15 nm in diameter, are closely associated with the thylakoids. These are most likely skeletal elements maintaining the spacial disposition of the internal membrane system. In *Neohodgsonia* dilated outer grana thylakoids contain bundles of tubules 35 nm in diameter. These are most conspicuous in the plastids in the stalks of the carpocephala.

Thalloid liverworts / plastids / Marchantiales

INTRODUCTION

It is with particular pleasure that we dedicate this article to Helene Bischler with whom we shared a lifelong interest in thalloid hepatics. It is also singularly appropriate that we are able to present new information on the plastids of *Marchantia* and *Neohodgsonia,* two genera with which Helene was particularly well aquainted (Bischler, 1998; Bischler-Causse *et al.,* 1995; Boisselier-Dubayle *et al*., 2002).

Previous studies on the ultrastructure of liverwort plastids are limited to a handful of taxa and, within the Marchantiales, developmental data are confined to *Marchantia* and *Blasia* (Apostolakos & Galatis, 1985a,b,c; Berrie & Webster, 1982; Duckett & Renzaglia, 1988) .The occurrence of chloroplasts in both meristematic and mature thallus cells suggests that, in contrast to angiosperm apices, plastid ontogeny from proplastids does not take place during thallus differentiation. Chloroplast shape, size and thylakoid architecture in the majority of liverworts studied to date are remarkably uniform and closely similar to their typical

* Correspondence and reprints: j.g.duckett@qmul.ac.uk

counterparts in higher plants: they are usually oval or disc-shaped, 3-5 μm in diameter and contain regularly - distributed, compact grana 0.5-1.0 μm in diameter each containing from between 3- 5 to 8-15 thylakoids. Noteable departures from this common pattern include the regression of the thylakoid network into scattered vesicles in mucilage papillae (Galatis & Apostolakos, 1977), diverse pleomorphic plastids in placental cells (Carafa *et al*., 2003; Ligrone *et al.,* 1993a) prolamellar bodies in *Cryptothallus* (Duckett & Renzaglia, 1988) and giant grana in the extreme-shade species *Dumortiera hirsuta* (Duckett & Ligrone, 1993). Apart from starch, in varying amounts, and scattered osmiophilic globuli, other stromal inclusions are the occasional crystals of phytoferritin and possible RUBISCO aggregations (Duckett & Renzaglia, 1988; Duckett & Ligrone, 1993).

The aims of the present account are to extend knowledge of unusual thylakoid systems in liverworts (in *Monoclea* and *Verdoornia*) and to describe novel fibrillar and tubular elements in the plastids of *Marchantia* and *Neohodgsonia.* All of these four taxa grow in deep shade on the floors of continuously wet evergreen forests in irradiances typically less than 10%, and sometimes down to only 1%, of those in the open.

MATERIALS AND METHODS

Thalli of *Monoclea forsteri* Hook., *Verdoornia succulenta* R.M.Schuster, *Marchantia foliacea* Mitt. and *Neohodgsonia mirabilis* H.Perss. were collected from deeply shaded banks in *Nothofagus* and mixed podocarp forests at Arthur's Pass, Kelly Creek near Otira, Mount Brown, east of Lake Kaniere and on the track to Flora Saddle, Mount Arthur in the South Island of New Zealand. Irradiances at the collecting sites ranged from 10-50Wm^{-2} compared with values of 500-600Wm^{-2} in the open. The plants were immediately prepared for electron microscopy as described previously (Carafa *et al*., 2003) and observed in a Jeol 100C transmission electron microscope**.**

RESULTS

The outer thallus cells of *Monoclea forsteri* contain unremarkable ovoid chloroplasts 4-5 μm in diameter with small grana (1-1.5 μm diameter) comprising 5-10 thylakoids (not illustrated). In contrast those in the inner cells are characterized by a single massive central granum 2-3 μm in diameter (Fig. 1A). These stacks usually comprise between 50-80 thylakoids and have a very distinctive diagonal stacking pattern. Stacks with between 120 and 160 thylakoids are also not uncommon.

While the cells in the dorsal epidermis of *Verdoornia succulenta* contain chloroplasts (not illustrated) identical to those described previously in *Aneura* (Ligrone *et al*., 1993b), viz. small regular grana with between 10-20 thylakoids, in the inner cells the thylakoid arrangement is very different. Up to 70% of the cross-sectional area is occupied by massive grana (Fig. 1B). These frequently extend for up to 4 μm across the whole diameter of the ovoid plastids and frequently contain

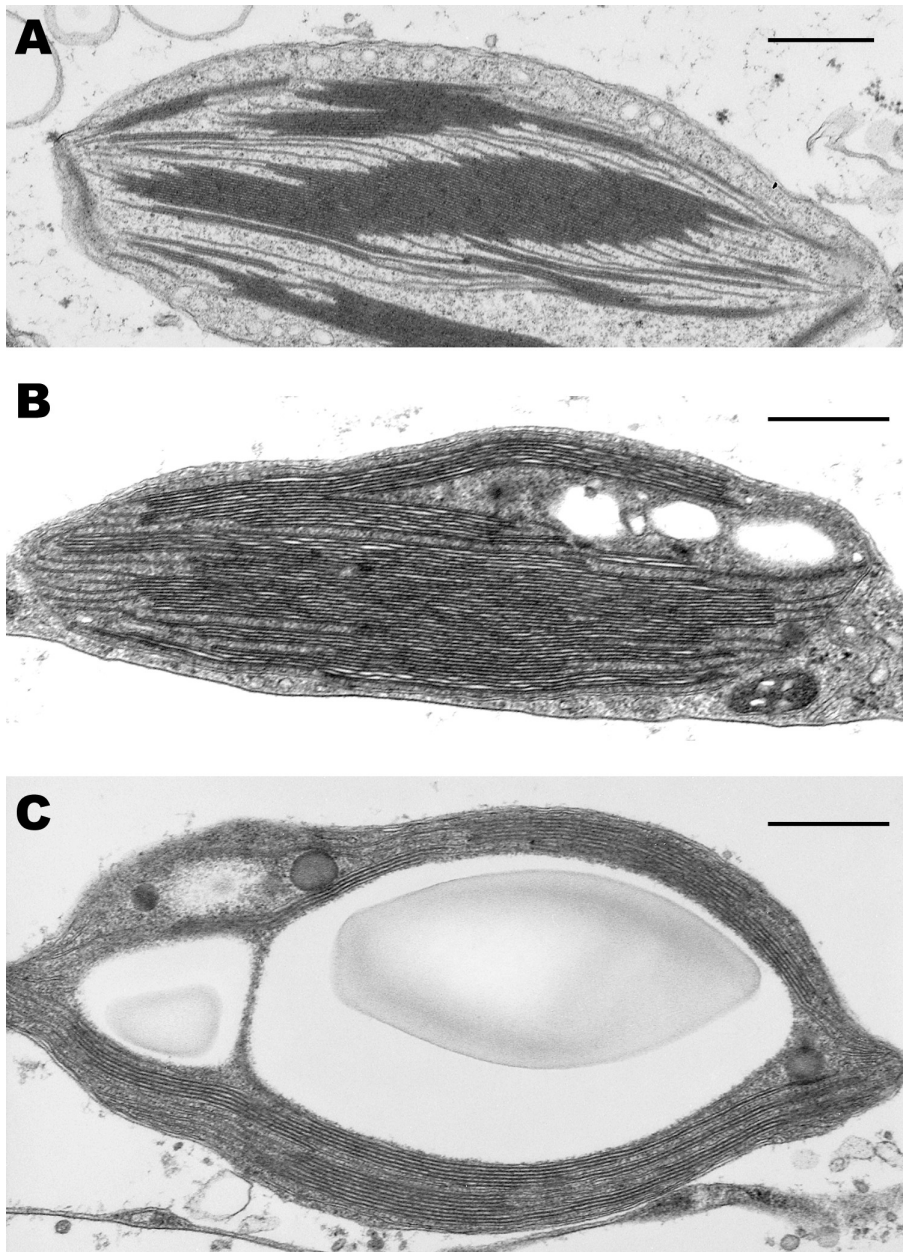


Fig. 1. **A**: *Monoclea forsteri*. Chloroplast from an internal thallus cell showing a massive granum with diagonally-stacked thylakoids. **B, C**: *Verdoornia succulenta*. B: A massive granum stretched across a chloroplast. C: Elongate grana around large starch grains. Scale bars = 0.5 μm.

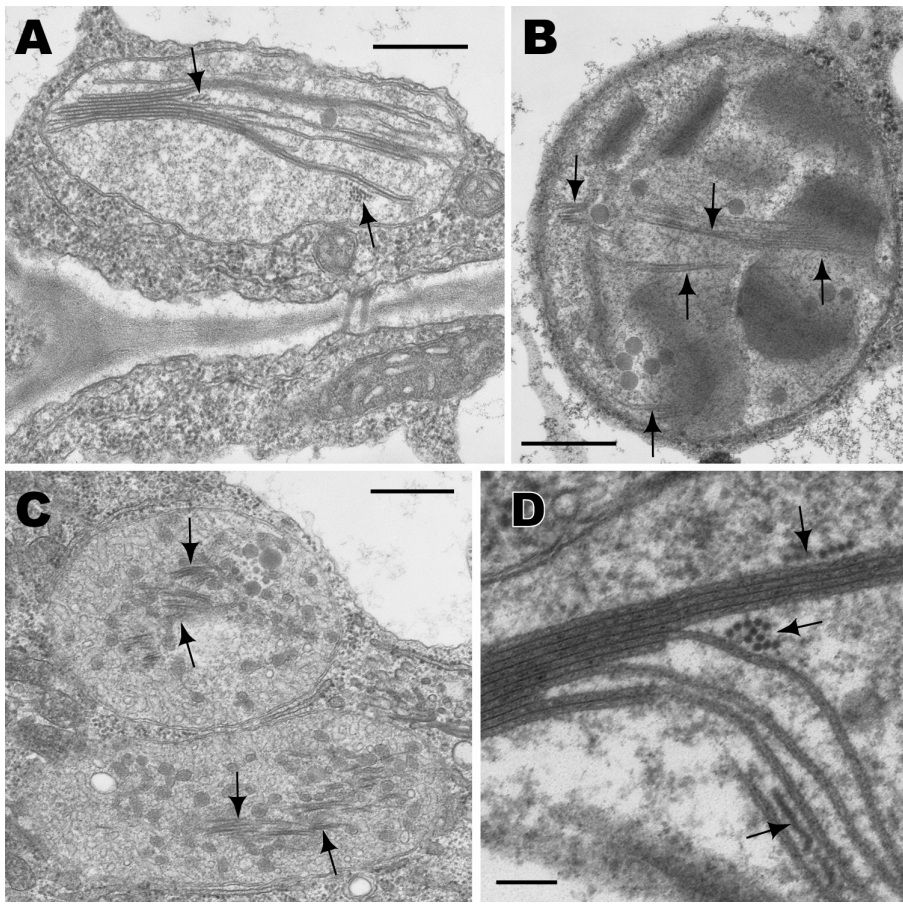


Fig. 2. *Marchantia violacea*. **A**: Undifferentiated plastid in a cell near the thallus apex with small grana and 2 groups of stromal fibres (arrowed) in a cell near the thallus apex. **B**: Young plastid showing several groups of parallel fibres (arrowed) in the stroma. **C**: Plastids in a mucilage papilla with groups of stromal fibres (arrowed) amidst scattered vesiculate thylakoids with granular contents. **D**: Stromal fibres (arrowed) at high magnification. Scale bars = A-C 0.5 μm, D 0.1 μm.

more than 50 thylakoids. Where starch grains are present (Fig. 1C) these occupy a central location closely surrounded by elongate grana.

The ultrastructure of the plastids of *Marchantia violacea* conforms very closely to that described previously in *M. paleacea* and *M. polymorpha* (Apostolakos & Galatis, 1985a,b,c; Berrie & Webster, 1982). Each organelle is 2-3 μm in diameter and contains small grana 1-1.5 μm in diameter comprising 5-10 thylakoids. However a ubiquitous feature, not noted previously, is the presence of clusters and rows of filaments lying adjacent to the thylakoids (Fig. 2A). In longitudinal profiles (Fig. 2B) these can be seen to form several distinct bands running in different directions across the plastids. Similar filaments can also be

seen amidst the rudimentary thylakoid systems comprising scattered vesicles in the plastids of mucilage papillae (Fig. 2C). At high magnification the filaments have a circular outline, a diameter of 12-15 nm and a regular centre-centre spacing of approximately 25 nm.

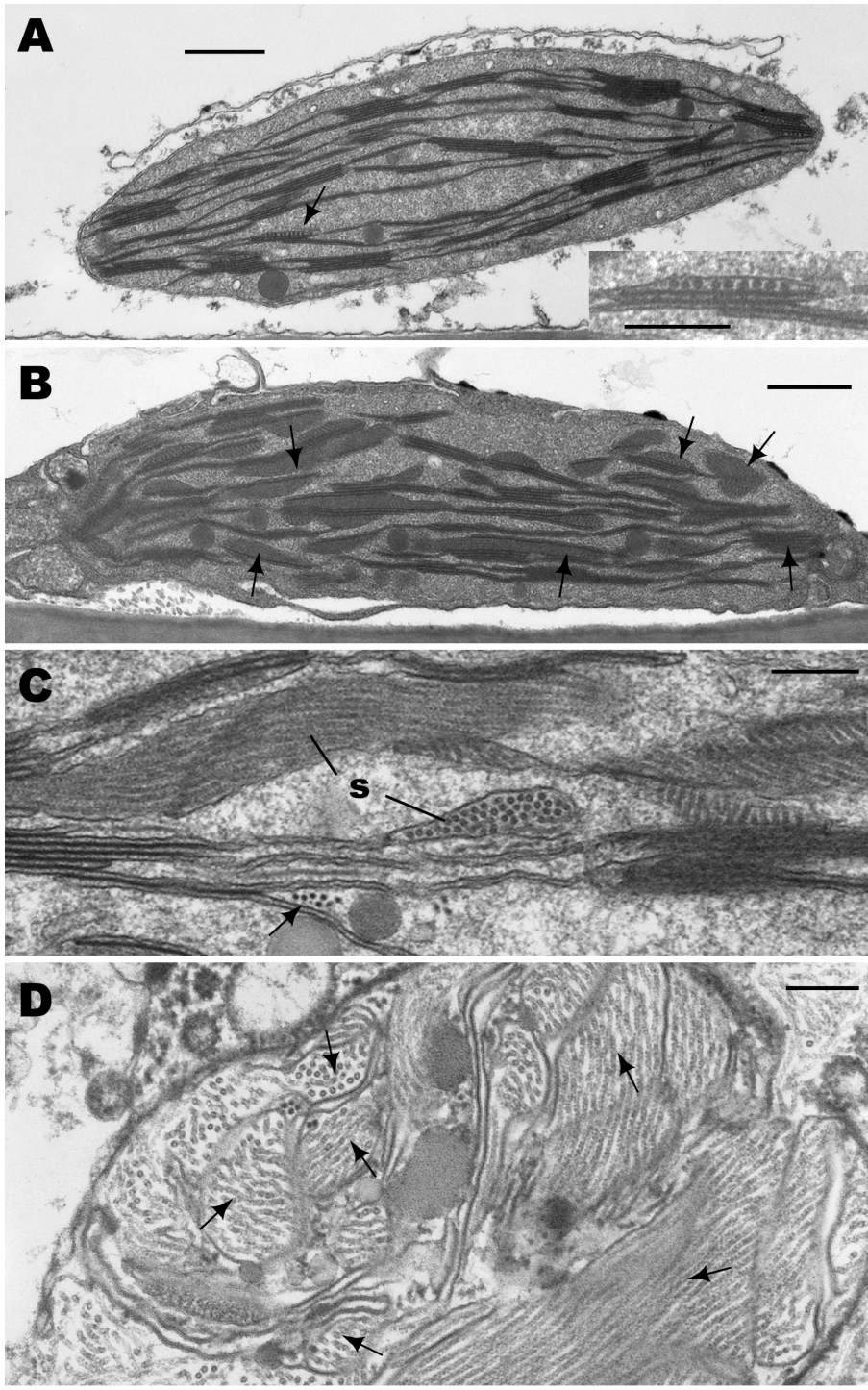
At first sight the chloroplasts in the thallus apices of *Neohodgsonia mirabilis* are unremarkable with several regularly distributed small grana 0.5-1.0 μm in diameter and each containing 5-10 thylakoids (Fig. 3A). Closer scrutiny however, reveals that the outermost thylakoid in some of the stacks is slightly swollen and contains rows of tubules approximately 30 nm in diameter (Fig. 3A and inset). Progressing rearwards along the thalli swollen outer grana thylakoids become increasingly frequent and contain either granular material or tubules (Fig. 3B). Tubule-containing thylakoids are even more conspicuous in the plastids of young carpocephala stalks (Fig. 3C) where stromal filaments, like those described above in *Marchantia,* are also visible (Fig. 3C). In longitudinal profiles the tubules have a regular repeating substructure. In fully extended carpocephala stalks thylakoid organization breaks down and the plastids become filled with swollen thylakoids each packed with tubules (Fig. 3D).

DISCUSSION

The massive grana, both in terms of their dimensions and numbers of thylakoids, described here in the chloroplasts of *Monoclea* and *Verdoornia*, are closely similar to those in two other deep-shade hepatics *Dumortiera* (Duckett & Ligrone, 1993) and *Cyathodium* (Duckett & Ligrone, 2005) not to mention numerous deep-shade angiosperms, cycads,ferns and *Selaginella* (Nasrulhaq-Boyce & Duckett, 1991; Graham *et al*., 1993; Lee, 1997, 2001). The diagonally-slanted stacks (Fig. 1A) however, appear to be peculiar to *Monoclea*. It should also be noted that *Monoclea, Verdoornia* and *Dumortiera* have numerous small chloroplasts in their principal photosynthetic cells whilst in *Cyathodium* (Duckett & Ligrone, 2006), some deep shade ferns, *Selaginella* (Jagels, 1969; Nasrulhaq-Boyce & Duckett, 1991) and in the protonema of *Schistostega* (Duckett *et al.,* 2004) these contain small numbers of unusually large chloroplasts.

Thylakoid architecture in *Marchantia foliacea* and *Neohodgsonia*, in contrast to the aforementioned taxa, is not indicative of deep-shade conditions and hardly differs from that observed previously in two species of *Marchantia* (*M. paleacea, M. polymorpha*) both from open habitats (Apostolalos & Galatis, 1985a,b,c; Berrie & Webster, 1982). The absence of massive grana is however of common occurrence in a range of bryophytes from shaded habitats (Duckett & Renzaglia, 1988) and normal grana also occur in extreme-shade filmy ferns (Makgomol & Sheffield, 2001). It would now be of considerable interest to compare photosynthesis in deep-shade liverworts with contrasting thylakoid systems.

The nature and possible functional significance of the intrathylakoidal tubules in *Neohodgsonia* must, for the present, remain conjectural. We are not aware of any previous descriptions of such features within thylakoids though it should be noted that, in longitudinal profiles (Fig. 3C, D), they are strikingly similar to fibrillar phloem protein (Behnke & Sjolund, 1989) and to a variety of plant viruses (Esau & Hoefert, 1971; Esau, 1979; Kim, 1981; Milne, 1966). Although their


A
B
C
s
D

prevalence in mature and ageing carpocephala stalk cells would not be out of line with the latter possibility, it must be noted that viruses rarely accumulate within plastids. Moreover, the *Neohodgsonia* plants showed no signs of necrosis and the same tubules were found in specimens collected from sites over 200 km apart. Thus they appear to be a normal feature of *Neohodgsonia.*

Superficially, particularly from their transverse profiles and centre-centre spacing (Fig. 3D), the stromal filaments in *Marchantia* and *Verdoornia*, closely resemble the stroma centres (crystalline arrays of RUBISCO) sometimes found in angiosperm plastids after water stress or mineral deficiency (Gunning, 1965: Gunning & Steer, 1975). The following features however, suggest that this is somewhat unlikely. Aside from hornwort pyrenoids, now known to be permanent aggregations of RUBISCO (Vaughn *et al*., 1990, 1992), stroma centres, with the possible exception of the crystalline deposits seen in the archegoniophore plastids in *Dumortiera* (Duckett & Ligrone, 1993), have never been reported in bryophytes even under desiccated conditions (Pressel *et al*., 2006). Moreover stroma centres never form discrete bands running throughout the plastids as do the fibre bundles in *Marchantia* (Fig. 2B). More likely these bands may have some kind of skeletal role in maintaining the integrity of the thylakoid system. With the recent discovery of prokaryotic cytoskeletal analogues to microtubules, actin filaments and particularly intermediate filaments, given their similar dimensions, *Marchantia* could well prove to be ideal material for investigating the nature and role of these elements in the plastids of land plants (Amos *et al*., 2004; Carballido-Lopez & Errington, 2003; Gitaz, 2005; Mayer, 2003).

Acknowledgements. We thank the Department of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand for providing laboratory facilities and the New Zealand Department of Conservation for granting collecting permits. Collection of the specimens used in this study was facilitated by local knowledge of the sites by Professor Brian Butterfield (University of Canterbury) and David Glennie (Landcare, Lincoln, New Zealand). J.G. Duckett was supported in New Zealand by an overseas travel grant from the Royal Society (UK) and by the New Phytologist Trust. R. Ligrone's visit to New Zealand was supported by a short-term stay grant from CNR (Italy). Keith Pell (Queen Mary, University of London) provided skilful technical assistance. This work was funded in part by grants from the Seconda Università di Napoli and MURST, and was performed using facilities at the CISME (University of Naples "Federico II", Italy) and the Centro di Competenza Regionale INNOVA (Campania, Italy).

REFERENCES

AMOS L.A., VAN DEN ENT F. & LOWE J., 2003 — Cytoskeletons in prokaryotes. *Cell biology international* 27: 503-505.

Fig. 3. *Neohodgsonia mirabilis*; intrathylakoidal tubules. **A**: Chloroplast in an inner thallus cell with small grana and one row of intrathylakoidal tubules (arrowed). Inset: The tubules at high magnification. **B**: Chloroplast from an inner thallus cell with swollen outer grana thylakoids containing dense granular material and tubules (arrowed). **C**: Plastid from a young carpocephalum showing swollen thylakoids (S) filled with tubules sectioned both transversely and longitudinally. In the later profiles note their fibrillar rope-like substructure. A group of stromal fibres is arrowed. **D**: Necrotic plastid packed with intrathylakoidal tubules (arrowed) from a mature carpocephalum stalk Scale bars = A, B 0.5 μm, A (inset), C,D 0.2 μm.

APOSTOLAKOS P. & GALATIS B., 1985a — Studies on the development of air pores and air chambers of *Marchantia paleacea*. II. Ultrastructure of the initial microtubule organizing centers. *Canadian journal of botany* 63: 744-756.

APOSTOLAKOS P. & GALATIS B., 1985b — Studies on the development of air pores and air chambers of *Marchantia paleacea*. III. Microtubule organization in pre-prophase-prophase initial aperture cells-formation of incomplete preprophase microtubule bands. *Protoplasma* 128: 120-135.

APOSTOLAKOS P. & GALATIS B., 1985c — Studies on the development of air pores and air chambers of *Marchantia paleacea*. IV. Cell plate arrangement in initial aperture cells. *Protoplasma* 128: 136-146.

BERRIE G. K. & WEBSTER P.M., 1982 — Ultrastructure of plastids and mitochondria in gemmae of *Marchantia polymorpha* L. *Annals of botany* 50: 199-206.

BEHNKE H.D. & SJOLAND R.D., 1989 — *Sieve Elements.Comparative Structure, Induction and Development.* Berlin, Springer, 460 p.

BISCHLER H., 1998 — Systematics and evolution in the genera of the Marchantiales. *Bryophytorum bibliotheca* 51: 1-201.

BOISSELIER-DUBAYLE M.-C., LAMBOURDIÈRE J. & BISCHLER H., 2002 — Molecular phylogenies support multiple morphological reductions in the liverwort subclass Marchantiidae (Bryophyta). *Molecular phylogenetics and evolution* 24: 66-77.

BISCHLER-CAUSSE H., GLENNY D. & BOISSELIER-DUBAYLE M.-C., 1995 — On *Neohodgsonia* H. Perss. (Marchantiales, Hepaticae). *Cryptogamie, Bryologie-Lichénologie* 16: 235-245.

CARAFA A., DUCKET J.G. & LIGRONE R., 2003 — The placenta of *Monoclea forsteri* Hook. And *Treubia lacunosa* (Col.) Prosk.: insights into placental evolution in liverworts. *Annals of botany* 92: 299-307.

CARBALLIDO-LOPEZ R. & ERRINGTON J., 2003 — A dynamic bacterial cytoskeleton. *Trends in cell biology* 13: 577-583.

DUCKETT J.G. & RENZAGLIA K.S., 1988 — Ultrastructure and development of plastids in the bryophytes. *Advances in bryology* 3: 33-93.

DUCKETT J.G. & LIGRONE R., 1993 — Massive grana and stromal crystals in the chloroplasts of an extreme-shade liverwort *Dumortiera hirsuta* (W.) Nees (Marchantiales, Hepaticae). *Giornale botanico italiano* 127: 320-322.

DUCKETT J.G., BURCH J., FLETCHER P., MATCHAM H.W., READ D.J., RUSSELL A.J. & PRESSEL S., 2004 — *In vitro* cultivation of bryophytes: a review of practicalities, problems, progress and promise. *Journal of bryology* 26: 3-20.

DUCKETT J.G. & LIGRONE R., 2006 — *Cyathodium* Kunze (Cyathodiaceae, Marchantiales), a tropical liverwort genus and family new to Europe, in Southern Italy. *Journal of bryology* (in press).

ESAU K. & HOEFERT L.L., 1971 — Cytology of beet yellows virus infection in *Tetragonia.*I. Parenchyma cells in infected leaf. *Protoplasma* 72: 255-273.

ESAU K., 1979 — Beet yellow stunt virus in cells of *Sonchus oleraceus* L. and its relation to host mitochondria. *Virology* 98: 1-8.

GALATIS B. & APOSTOLAKOS O., 1977 — On the fine structure of differentiating mucilage papillae of *Marchantia. Canadian journal of botany* 55: 772-795.

GITAZ Z., 2005 — The new bacterial cell biology: moving parts and subcellular architecture. *Cell* 120: 577-586.

GRAHAM R.M., LEE D.W. & NORSTOG K., 1993 — Physical and ultrastructural basis of blue iridescence in two neotropical ferns. *American journal of botany* 80: 198-203.

GUNNING B.E.S., 1965 — The fine structure of chloroplast stroma following aldehyde osmium-tetroxide fixation. *Journal of cell biology* 24: 79-93.

GUNNING B.E.S. & STEER M.W., 1975 — *Ultrastructure and the Biology of Plant Cells*. London, Arnold.

JAGELS R., 1969 — Photosynthetic apparatus in *Selaginella.* I. Morphology and photosynthesis under different light and temperature regimes. *Canadian journal of botany* 48: 1843-1852.

KIM K.S., 1981 — Ultrastructure of bean leaf beetle (*Ceratoma trifurcata*) hemocytes and their phagocytic activities on tobacco mosaic virus, a non-beetle-transmitted virus. *Journal of ultrastructure research* 75: 300-313.

LEE D.W., 1997 — Iridescent blue plants. *American scientist* 85: 56-63.

LEE D.W., 2001 — Leaf colour in tropical plants: some progress and much mystery. *Malayan nature journal* 55: 117-131.

LIGRONE R., DUCKETT J.G. & RENZAGLIA K.S., 1993a — The gametophyte-sporophyte junction in land plants. *Advances in botanical research* 19: 231-317.

LIGRONE R., POCOCK K. & DUCKETT J.G., 1993b — A comparative ultrastructural study of endophytic basidiomycetes in the parasitic achlorophyllous hepatic *Cryptothallus mirabilis* and the closely allied photosynthetic species *Aneura pinguis*. *Canadian journal of botany* 71: 666-679.

MAKGOMOL K. & SHEFFIELD E., 2001 — Gametophyte morphology and ultrastructure of the extremely deep shade fern, *Trichomanes speciosum*. *New phytologist* 151: 243-255.

MAYER F., 2003 — Cytoskeletons in prokaryotes. *Cell biology international* 27: 503-505.

MILNE R.G. 1966 — Multiplication of tobacco mosaic virus in tobacco leaf palisade cells. *Virology* 28:79-89.

NASRULHAQ-BOYCE A. & DUCKETT J.G., 1991 — Dimorphic epidermal cell chloroplasts in the mesophyll- less leaves of an extreme- shade tropical fern, *Teratopyllum rotundifolium* (R.Bonap.) Holtt.: a light and electron microscope study. *New phytologist* 119: 433-444.

PRESSEL S., LIGRONE R. & DUCKETT J.G., 2006 — A cytological study of de- and rehydration of the food-conducting cells in the moss *Polytrichum formosum*. *Annals of botany* (in press).

VAUGHN K.C., CAMPBELL E.O., HASEGAWA J., OWEN H.A. & RENZAGLIA K.S., 1990 — The pyrenoid is the site of ribulose I,5-bisphosphate carboxylase/oxygenase accumulation in the hornwort (Bryophyta: Anthocerotae) chloroplast. *Protoplasma* 156: 117-129.

VAUGHN K.C., LIGRONE R., OWEN H.A., HASEGAWA J., CAMPBELL E.O., RENZAGLIA K.S. & NAJERA J., 1992 — The anthocerote chloroplast. *New phytologist* 120: 169-190.