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BIZONOPLAST, A UNIQUE CHLOROPLAST IN THE EPIDERMAL CELLS OF MICROPHYLLS IN THE SHADE PLANT *SELAGINELLA ERYTHROPUS* (SELAGINELLACEAE)[1]

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Study of the unique leaf anatomy and chloroplast structure in shade-adapted plants will aid our understanding of how plants use light efficiently in low light environments. Unusual chloroplasts in terms of size and thylakoid membrane stacking have been described previously in several deep-shade plants. In this study, a single giant cup-shaped chloroplast, termed a bizonoplast, was found in the abaxial epidermal cells of the dorsal microphylls and the adaxial epidermal cells of the ventral microphylls in the deep-shade spike moss *Selaginella erythropus*. Bizonoplasts are dimorphic in ultrastructure: the upper zone is occupied by numerous layers of 2–4 stacked thylakoid membranes while the lower zone contains both unstacked stromal thylakoids and thylakoid lamellae stacked in normal grana structure oriented in different directions. In contrast, other cell types in the microphylls contain chloroplasts with typical structure. This unique chloroplast has not been reported from any other species. The enlargement of epidermal cells into funnel-shaped, photosynthetic cells coupled with specific localization of a large bizonoplast in the lower part of the cells and differential modification in ultrastructure within the chloroplast may allow the plant to better adapt to low light. Further experiments are required to determine whether this shade-adapted organism derives any evolutionary or ecophysiological fitness from these unique chloroplasts.

Key words: bizonoplast; chloroplast; iridescence; iridoplast; *Selaginella*; shade plant; structure; thylakoid stacking.

Several structural features of chloroplasts typically found in deep-shade plants were once thought to be representative of all the terrestrial shade plants (Anderson et al., 1973; Boardman, 1977; Sarafis, 1998). Many shade plants have large chloroplasts with numerous thylakoids per granum positioned at the base of conical chlorenchyma (Nasrulhaq-Boyce and Duckett, 1991; Sarafis, 1998; Sheue et al., 2003). Massive grana with large diameters were reported in the gametophytes of liverworts *Dumortiera hirsuta* (Sw.) Nees (50–100 thylakoids per stack, 2–3 μm diameter) (Duckett and Ligrone, 1993) and *Cyathodium foetidissimum* Schiffn. (Duckett and Ligrone, 2006a), the moss *Atrichum undulatum* (Hedw.) P. Beauv. (Geitler, 1937), the tropical fern *Teratophyllum rotundifoliatum* (Bonap.) Holttum [(22–)86(–280) thylakoids per stack] [(minimum–)average(–maximum)] (Nasrulhaq-Boyce and Duckett, 1991), and a range of vascular plants (Sheue et al., 2003) from deeply shaded habitats. The submerged water plant *Synnema triflorum* Kuntze also possesses shade chloroplasts with well-defined granal stacks arranged vertically like a pile of coins (van Spronsen et al., 1989). These unique structural modifications of chloroplast are thought to have adaptive significance for the plants in low light environments (Anderson, 1999).

Recent studies with a range of shade plants, however, indicate that no chloroplast structure universally applies to all shade plants. For example, in deeply shaded liverworts, *Neohodgsonia mirabilis* H. Perss and *Marchantia foliacea* Mitt., grana have only 5–10 thyakoids (Duckett and Ligrone, 2006b), and a *Cyathodium* species collected from Singapore

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has a similar range of granal stacking and size (C. R. Sheue and V. Sarafis, unpublished observation). Similarly, many bryophytes from shady environments lack massive grana and stacking (Duckett and Renzaglia, 1988), including the shining moss *Schistostega pennata* (Hedw.) Web. & Mohr that normally inhabits unusually low light habitats in rock cracks, caves, and holes (Glime, 2006). This moss species normally has several large chloroplasts (Duckett et al., 2004) and normal-sized grana in the aerial part of its protonema (V. Sarafis, unpublished observation). However, Makgomol and Sheffield (2001) concluded that the success of *Trichomanes speciosum* Willd. in deep shade can be attributed to its low metabolic rate because neither gametophyte filaments nor sporophyte leaves have chloroplasts with extreme forms.

Iridescence is defined as variation in color when viewed from different angles. Iridescent blue leaves have been associated with some deep-shade plants, and the ultrastructural basis of this iridescence has been investigated. Two ultrastructural features were reported to produce this feature: the multiple layers of cellulose microfibrils in the external cell walls of the adaxial epidermis in *Selaginella uncinata* and *S. willdenowii* (Desv.) Baker (Hébant and Lee, 1984; Lee, 2001) and the presence of unusual "iridoplasts"—highly modified plastids characterized by equidistant layering of a small number of thylakoid membranes per layer (Gould and Lee, 1996; Lee, 1997, 2001). However, whether both ultrastructural features are required for iridescence is unclear.

The genus *Selaginella*, sometimes called the spike moss (Bold et al., 1987), includes some 750 species occurring mainly in tropical zones (Jermy, 1990). Dimorphic microphylls are characteristic of the dorsiventral species, with two rows of dorsal microphylls and another two rows of ventral microphylls (Bold et al., 1987). Haberlandt (1888) might have been the first to report the large cup-shaped chloroplasts in the funnel-shaped photosynthetic cells of several species of *Selaginella*. Jagels (1970a) showed that such cup-shaped chloroplasts are restricted to the adaxial epidermal cells of *S. apoda* (L.) Spring, *S. martensii* Spring, *S. serpens* (Desv.) Spring, and *S. uncinata* (Desv.) Spring and to the subepidermal cells in *S. kraussiana* (Kunze) A. Braun, while smaller disk-shaped chloroplasts are found in other cells. Moreover, Jagels (1970b) indicated that the ultrastructural details of the chloroplasts of *Selaginella* are identical to those of higher vascular plants.

The aim of this study was to extend the current knowledge of the unusual chloroplasts of *Selaginella* and to describe the novel structure of a new type of chloroplast in the deep-shade-adapted plant *S. erythropus* (Mart.) Spring. We propose calling this type of unique chloroplast as a "bizonoplast", a giant chloroplast with dimorphic ultrastructures separated into zones: an iridoplast-like upper zone without grana and a lower zone with normal grana thylakoid structure. This unusual chloroplast is found in the abaxial epidermal cells of the dorsal microphylls and the adaxial epidermal cells of the ventral microphylls of *S. erythropus*. This deep-shade *Selaginella* species occurs in tropical South America, including Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, and probably Panama (Svenson, 1946; Valdespino, 1993). Collectively, the modifications in chloroplast size, location, and ultrastructure in diverse photosynthetic cells of microphylls may allow *S. erythropus* to use light more efficiently. No iridescent blue color has been observed for the plant either in natural habitats or in cultivation.

MATERIALS AND METHODS

Branches with microphylls from three individual plants of *S. erythropus* (probably from the same colony, voucher specimen number *RK 5303*, SING), grown in the shade in the Singapore Botanic Gardens, were sampled and investigated for anatomical features (Fig. 1A). Incident light (PAR, photosynthetic active radiation) was 10–60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at mid-day (never exceeding 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for any extended period), as measured using a portable LICOR quantum sensor model LI-190 (Lincoln, Nebraska, USA).

Both surfaces of live aerial branches were examined with a stereoscope (Zeiss Stemi SV11, Germany) before and after submersion in water (Fig. 1B, C). We found that submerging the specimens improved the quality of the photographs by excluding stray light reflections without affecting the color of the microphylls but slightly increasing the brightness.

Three dorsal and three ventral microphylls from each of three individual plants were sampled for anatomical and chloroplast ultrastructure investigation. Aerial branches (Fig. 1A), harvested between 1330–1730 hours, were cut into small pieces ($2.0 \times 2.0 \times 0.5$ mm) and fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.3) for 4 h at room temperature. After three washings in buffer for 30 min each, the specimens were postfixed in 1% OsO_4 in the same buffer for 4 h. After dehydration through an ethanol series, the material was infiltrated for 3 d and embedded in Spurr's resin (DER = 6.0) (Spurr, 1969). The embedded material was then polymerized in an oven at 70°C for 12 h. Semithin sections (1 μm) were cut with an Ultracut E Microtome (Reichert-Jung, Wien, Austria) or MTX Ultramicrotome (RMC, Tucson, Arizona, USA) and stained with 1% toluidine blue for observation with a light microscope (Olympus, BH-2, Tokyo, Japan). Ultrathin sections (about 75 nm) were cut and stained with uranyl acetate (5% in 50% methanol) and lead citrate (1% in water) for examination with either a Hitachi H 600 (Tokyo, Japan) or a JEOL (JEM-2000 EXII, Tokyo, Japan) transmission electron microscope (TEM).

Chloroplast size and number per cell were estimated from three replicate cells each from the dorsal-facing epidermis, mesophyll, and elongated ventral-facing epidermis. Therefore, a total of 27 cells were measured for each cell type in the dorsal or ventral microphylls. The excitation wavelength was 633 nm and the emission wavelength was 649–719 nm for observation with a confocal scanning light microscope (CSLM, Leica TSC-SP5, Wetzlar, Germany) using a 63× objective. Three-dimensional-like images with 40 iterations were reconstructed using the program MetaMorph 7.0 (AQI 3D) (Molecular Devices, Downingtown, Pennsylvania, USA). The number of thylakoids per granum and the grana diameter of the different chloroplast types in the cells of conical dorsal-facing epidermis, mesophyll, and elongated ventral-facing epidermis were counted and measured from TEM micrographs taken at high magnifications (60 000× for counting number of thylakoids, 12 000× for measuring grana diameter). For grana characteristics, three grana from each chloroplast type of the 27 replicate cells were measured (81 replications).

RESULTS

Two rows of small dorsal microphylls and two rows of large ventral microphylls occur on each branch of *Selaginella erythropus* (Fig. 1). The abaxial surface of the dorsal microphyll and the adaxial surface of the ventral microphyll are green (Fig. 1B). No iridescent blue color was observed in the plants cultivated in the Singapore Botanic Gardens or in the natural habitats of the species (Fig. 1A, B). The adaxial surface of the dorsal microphylls, which cannot be easily viewed from either the dorsal or ventral surface of the shoot, is green except for the red margins. The abaxial surface of all ventral microphylls on the branches is red on the plants used in our investigation (Fig. 1C), although the upper branches may vary from red to green in natural habitats (Valdespino, 1993).

Dorsal and ventral microphylls of *S. erythropus* are six cells thick in the vein region, with microphyll thickness gradually reduced to two layers (the upper and lower epidermis) toward the margin (Fig. 2). Five types of chloroplasts, based on size and number, can be recognized from the dorsal and ventral microphylls in association with different cell types: (1) cuplike

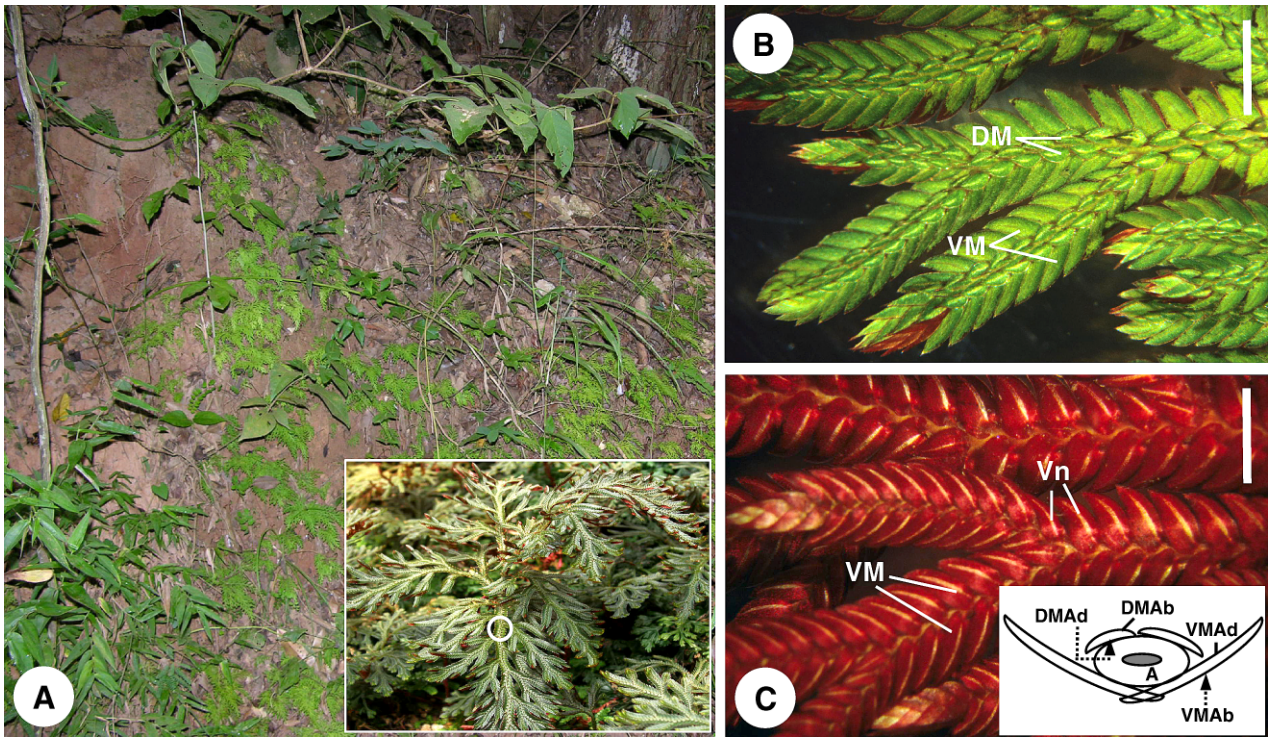

Fig. 1. Habitat and morphology of *Selaginella erythropus*. (A) Plants grown in the natural habitat of deep-shade forest understory in Brazil and in the Singapore Botanic Gardens; the circled area in the inset shows the part of microphylls sampled for structure investigation. (B) Aerial branches showing arrangement and dimorphism of microphylls, the abaxial surface of the two small dorsal microphylls, and the green adaxial surface of the two large ventral microphylls. (C) The abaxial surface of the ventral microphylls showing the distinct red color of the ventral microphylls. The inset shows a transverse view of a shoot of anisophyllous microphylls and surfaces of microphylls. B and C were photographed under water to improve image quality by excluding stray light reflection. Scale bars: B, C = 1 mm. A: axis, Ab: abaxial surface, Ad: adaxial surface, DM: dorsal microphyll, VM: ventral microphyll, Vn: vein.

chloroplasts (14.0–22.7 μm long), termed bizonoplasts in this study (see next paragraph), in the abaxial, conical epidermal cells of the dorsal microphylls and the adaxial conical epidermal cells of the ventral microphylls; (2) disk-shaped chloroplasts (6.4–15.0 μm long) in the mesophyll; (3) elongated or beadlike chloroplasts (5.8–10.0 μm long) arranged as a chain in the elongated, adaxial epidermal cells of the dorsal microphylls and the abaxial epidermal cells of the ventral microphylls; (4) trichome chloroplasts; and (5) stomatal chloroplasts. The specific features of chloroplast types 1–3 are described in Table 1.

Adjacent to the anticlinal walls in each conical epidermal cell is a single large bizonoplast (Fig. 2), with mean size of 19.6 × 13.4 × 17.0 μm (Table 1). The bizonoplasts are localized in the lower part of the epidermal cells. Each bizonoplast is elongated vertically and is cuplike in structure, with a wide and concave top as seen from the reconstructed 3D-like images (Fig. 3) and the real 3D images (see Supplemental Video with the online version of this article). Starch grains are predominantly located in the upper central region of the lower zone, but few large starch grains are found in the external region of the upper zone (Figs. 2B, 4A, 5A–C). The external wall of the conical epidermal cells is convex (Fig. 2) and lacks the structure of multilayered cellulose microfibrils (Fig. 4A), such as that found in *S. willdenowii* (Hébant and Lee, 1984).

Within the mesophyll cells, the chloroplasts are smaller, and each cell has 3–5 chloroplasts (Table 1), with mean size of 10.0 × 6.5 × 8.5 μm. In the elongated epidermal cells are 6–8 small chloroplasts, with mean size of 7.3 × 3.9 × 7.7 μm, arranged in a chain (Table 1; figure not shown).

A striking feature of the large bizonoplasts is that they contain an unusual upper zone without normal grana structure (Figs. 4A, 5A–C) and a lower zone containing normal grana (Figs. 4A, 5A–B). We call these novel chloroplasts bizonoplasts (two-zoned chloroplasts) to describe their variation in spatial structural. The bizonoplasts in the dorsal and ventral microphylls are similar in morphology and structure. The upper zone has 15–25 layers of stacked membranes (2–4, rarely 5 thylakoids), and these layers run conspicuously parallel to one another (Figs. 4A, 5A–C), nearly always ~60–90 nm apart (Fig. 5D). No normal grana are observed in this zone, but instead, 1–3 large starch grains and some plastoglobuli occur (Fig. 5). The smaller starch grains in the upper central region of the lower zone of the bizonoplast are surrounded by unstacked and stacked granal thylakoid membranes (Figs. 4A, 5A–B).

The number of thylakoid membranes per granum in the lower zone of the bizonoplast in the conical epidermal cells of both the dorsal and ventral microphylls is similar to that of the chloroplasts in the mesophyll cells, with (4–)18(−44) [(min.) mean (max.)] and (6–)20(−35) membranes per granum, respectively (Table 1). The smaller chloroplasts of the elongated, adaxial epidermal cells of the dorsal microphylls and the abaxial epidermal cells of the ventral microphylls have fewer thylakoidal stacks per granum (Fig. 4C)—only (4–)8(−16) membranes per granum (Table 1). The grana of the

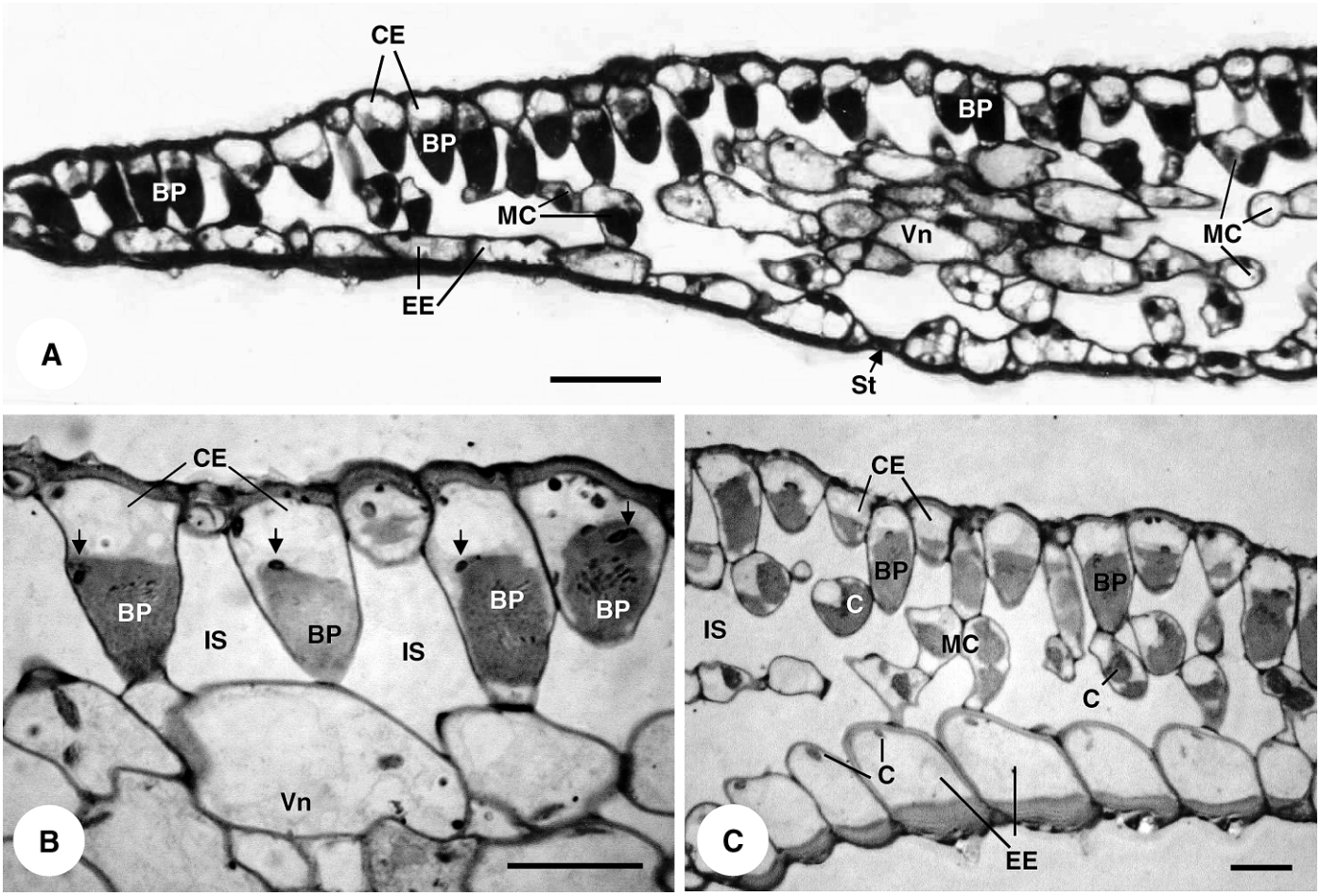

Fig. 2. Light micrographs of ventral microphyll of *Selaginella erythropus*. (A) Transverse section of microphyll, six cells thick in the vein region with thickness gradually reduced to two epidermal layers toward the margin. Note distinctive giant bizonoplasts positioned at the base of each adaxial conical epidermal cell. (B) Close-up of conical epidermal cells. Note the larger starch grains located in the upper zone (arrows). (C) Transverse section of ventral microphyll toward the margin showing epidermal and mesophyll cells. Note the various sizes of chloroplasts in the adaxial conical epidermal cells, mesophyll cells, and abaxial elongated epidermal cells. Scale bars: A = 30 μm; B, C = 10 μm. BP: bizonoplast, C: chloroplast, CE: conical epidermal cell, EE: elongated epidermal cell, IS: intercellular space, MC: mesophyll cell, St: stoma, Vn: vein.

mesophyll chloroplasts are larger (662 ± 108 nm) than the conical epidermal cell bizonoplasts (594 ± 139 nm) and the elongated epidermal cell chloroplasts (469 ± 56 nm) (Table 1).

DISCUSSION

Clearly, the microphylls of the deep-shade plant *Selaginella erythropus* have developed diverse photosynthetic machineries at both the cellular and ultrastructural levels. First, in addition to the mesophyll cells, the epidermal cells are major photosynthetic cells as well. Second, the preferential localization of chloroplasts in the lower part of the funnel-shaped epidermal cells would allow more light to penetrate and reach mesophyll cells. Furthermore, the conical-shaped cells of the abaxial epidermis of the dorsal microphylls and the adaxial epidermis of the ventral microphylls contain a novel and unique chloroplast with a dimorphic internal ultrastructure. This special chloroplast that we called a bizonoplast, is differentiated ultrastructurally into two zones that have never been reported. The upper zone of the chloroplast contains 15–25 layers of 2–4 stacked thylakoids, which are parallel to each other, while the lower zone possesses typical chloroplast ultrastructure in the arrangement of unstacked agranal and stacked granal thylakoid membranes. This unusual ultrastructural arrangement within a single giant chloroplast in the epidermal cells may increase the efficiency of light absorption and utilization via differential photochemical activities in the separated zones. The stacked granal thylakoids in the lower zone will be more effective in absorbing far-red light, which dominates the low light environments (Anderson, 1999). Photosystem I (PSI) and ATPase are almost exclusively localized in the unstacked membrane region, whereas PSII is localized primarily in the stacked regions of thylakoid membrane. Thus, the upper zone of bizonoplasts may be enriched in PSI, whereas the lower zone will be more balanced with both PSI and PSII as in a typical chloroplast.

Although few large starch grains are located in the external region of the upper zone of bizonoplasts, starch grains in the giant chloroplast are predominantly in the upper central region of the lower zone. Jagels (1970b) reported that in the microphylls of *Selaginella* the deposition of starch grains

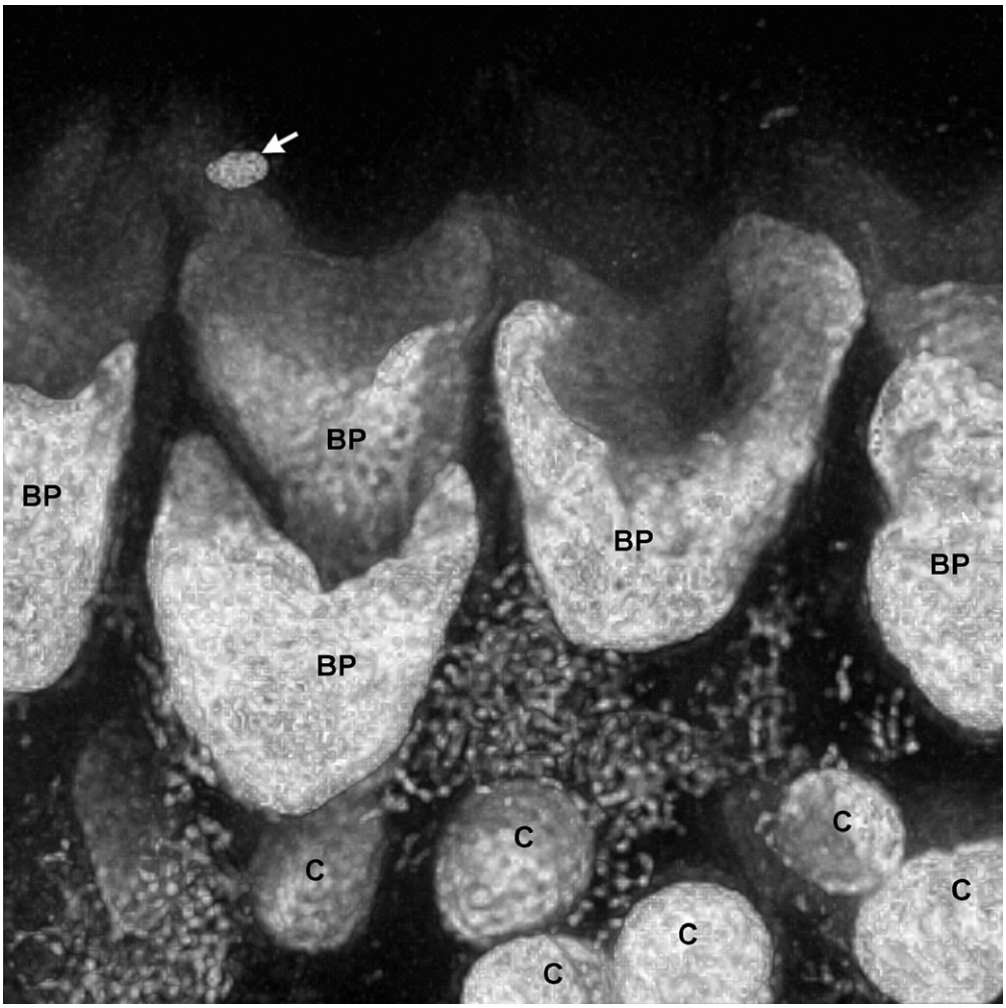

Fig. 3. Deconvoluted projection image reconstructed from 3D-like images of the large cup-shaped bizonoplasts of conical epidermal cells and the chloroplasts of mesophyll cells viewed from transverse section of a ventral microphyll of *Selaginella erythropus* with a confocal microscope. Note the wider concave top end of the bizonoplast. BP: bizonoplast, C: mesophyll chloroplast. The chloroplast of an alga on the microphyll surface is included in this image (white arrow). Scale bar = 10 μm.

TABLE 1. The size, number per cell, shape, and ultrastructure for three major types of chloroplasts in the microphylls of *Selaginella erythropus*. Three dorsal and three ventral microphylls were sampled from each of three individual plants. Three replicate cells for each cell type (conical epidermal, spongy mesophyll, and elongated epidermal cells) were selected for measuring chloroplast size and number per cell (27 replications). For grana characteristics, three grana from each chloroplast type were measured (81 replications).

Characteristic	Bizonoplast (type 1)	Disk-shaped (type 2)	Elongated or beadlike (type 3)
Location	Conical epidermal cells in abaxial epidermis of dorsal microphylls and adaxial epidermis of ventral microphylls	Spongy mesophyll cells of dorsal and ventral microphylls	Elongated epidermal cells in adaxial epidermis of dorsal microphylls and abaxial epidermis of ventral microphylls
Size	Large	Medium	Small
Length × width × depth (μm)	(14–)19.6 ± 3.4(−22.7)[a] × (9.1–)13.4 ± 3.3(−17.5) × (14.4–)17.0 ± 1.9 (−18.5)	(6.4–)10.2 ± 2.7(−15.0) × (4.3–)6.5 ± 1.8(−9.5) × (7.0–)8.5 ± 1.8(−11.1)	(5.8–)7.3 ± 1.2(−10.0) × (3.1–)3.9 ± 0.9(−5.4) × (6.3–)7.7 ± 1.4(−9.6)
No. per cell	1	3–5	6–8
Shape	Cup-shaped	Disklike	Beadlike or elongated, arranged as a chain
Ultrastructure	Unusual, upper zone with parallel layers of 2–4 stacked membranes separated by a consistent distance of c. 60–90 nm, the lower zone with both stacked granal [(4–)18(−44) membranes][1] and unstacked stromal thylakoids	Typical, with stacked granal [(6–)20 (−35) membranes] and unstacked stromal thylakoids	Typical, with stacked granal [(4–)8 (−16) membranes] and unstacked stromal thylakoids
Grana size (nm)	(416–)594 ± 139(−950)[1]	(500–)662 ± 108(−909)	(350–)469 ± 56(−541)

[a] [(min–) average ± SD (–max)].

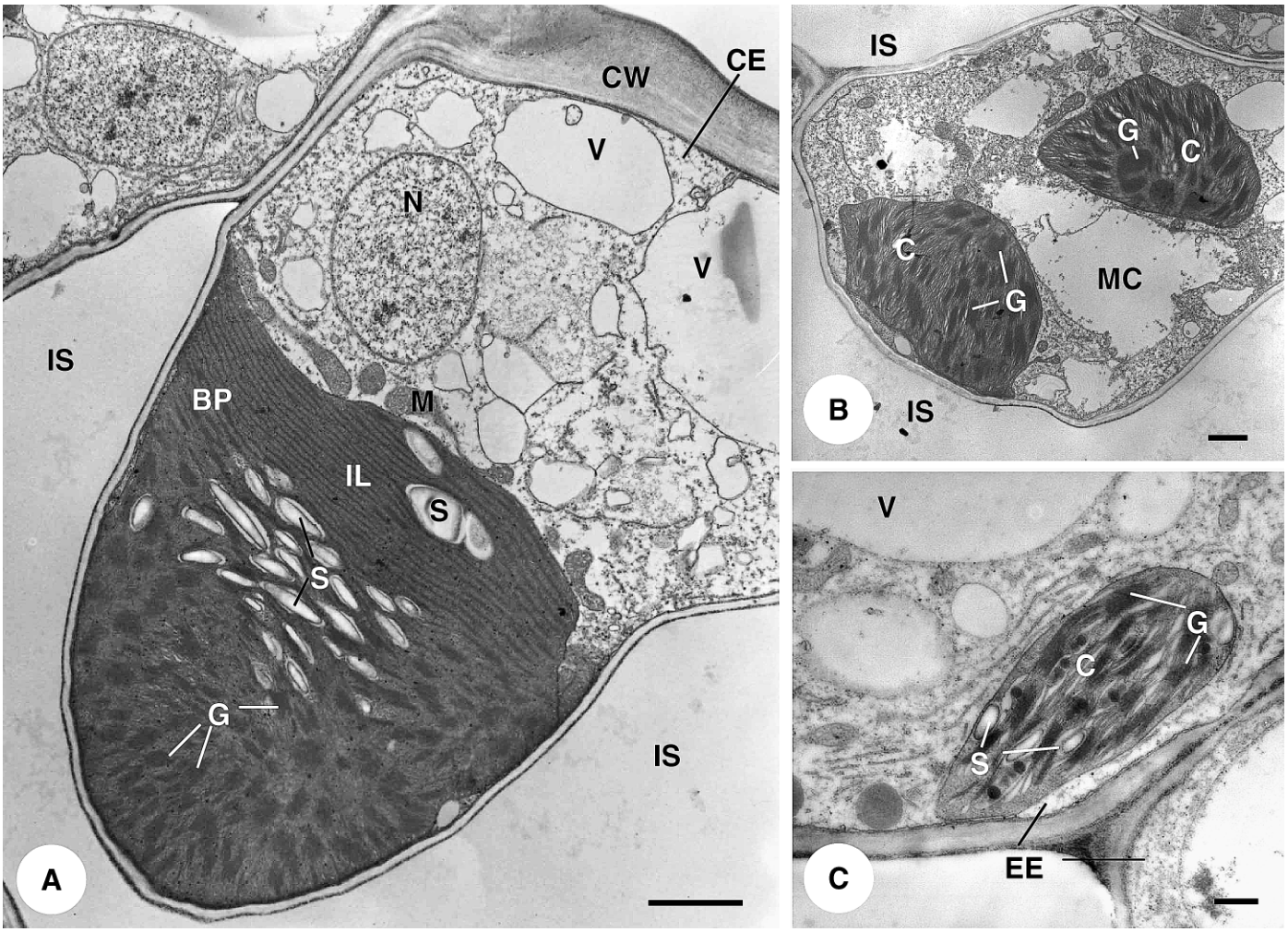

Fig. 4. Transmission electron micrographs of the unusual bizonoplast and typical chloroplasts in microphylls of *Selaginella erythropus*. (A) Conical epidermal cell with a bizonoplast showing two types of organization of thylakoid membranes: the upper zone comprising the iridoplast-like region with groups of parallel thylakoid membranes and the lower zone with typical chloroplast structure. Note the external cell wall of the conical epidermal cell does not have multi-layered structure. (B) Chloroplasts of a mesophyll cell, with typical granal stacking and agranal unstacking of thylakoid membranes. (C) A small chloroplast in an elongated epidermal cell, with fewer granal membranes and smaller granal size. Scale bars: A = 2 μm, B = 1 μm, C = 500 nm. BP: bizonoplast, C: chloroplast, CE: conical epidermal cell, CW: cell wall, EE: elongated epidermal cell, G: granum, IL: iridoplast-like region, IS: intercellular space, M: mitochondrion, MC: mesophyll cell, N: nucleus, S: starch grain, V: vacuole.

varied in different environments (e.g., light intensity and temperature). We noticed that the number of starch grains in the bizonoplast increased when *S. erythropus* was grown in more light, but that these starch grains still were primarily in the upper central region of the lower zone of bizonoplast (C. R. Sheue and colleagues, unpublished data). The specific localization of starch grains in bizonoplasts may be related to energy generation and supply for starch biosynthesis. It will be important to explore further the different photosynthetic functionalities of the upper and the lower zones of bizonoplast in order to understand this unusual distribution.

The upper zone of the bizonoplasts, with layers of 2–4 stacked thylakoids parallel to each other, resembles the structure of the iridoplast, as previously described by Gould and Lee (1996). These authors suggested that the presence of iridoplasts in some deep-shade plants is associated with iridescent blue color. Iridoplasts are found in iridescent leaves of the filmy fern *Trichomanes elegans* Rich. (Lee, 1997, 2001), *Begonia pavonina* Ridl., and *Phyllagathis rotundifolia* Blume (Gould and Lee, 1996; Lee, 1997, 2001). According to Gould and Lee (1996), the presence of the iridoplasts in *B. pavonina* is consistent with the hypothesis that this structure causes blue iridescence via the constructive interference of reflected light, but this hypothesis does not hold for the blue leaves of *P. rotundifolia*. Sheue et al. (2003) found iridoplasts not only in the adaxial epidermal cells, but also in the spongy mesophyll cells of *B. decora* Stapf and *B. sinuata* Meisn, with both lack the blue iridescence. Although iridoplasts have been associated with blue iridescence (Lee, 1997, 2001), the absence of the iridescence in *B. decora*, *B. sinuata* (Sheue et al., 2003), and *S. erythropus* brings into question the connection between this structural chloroplastic component and iridescence.

It is intriguing that patterns similar to the thylakoid membrane stacking of the upper zone of the bizonoplast in *S. erythropus* were also reported in the chloroplasts of the brown algae *Fucus vesiculosus* L., *Chorda filum* (L.) Lam., and

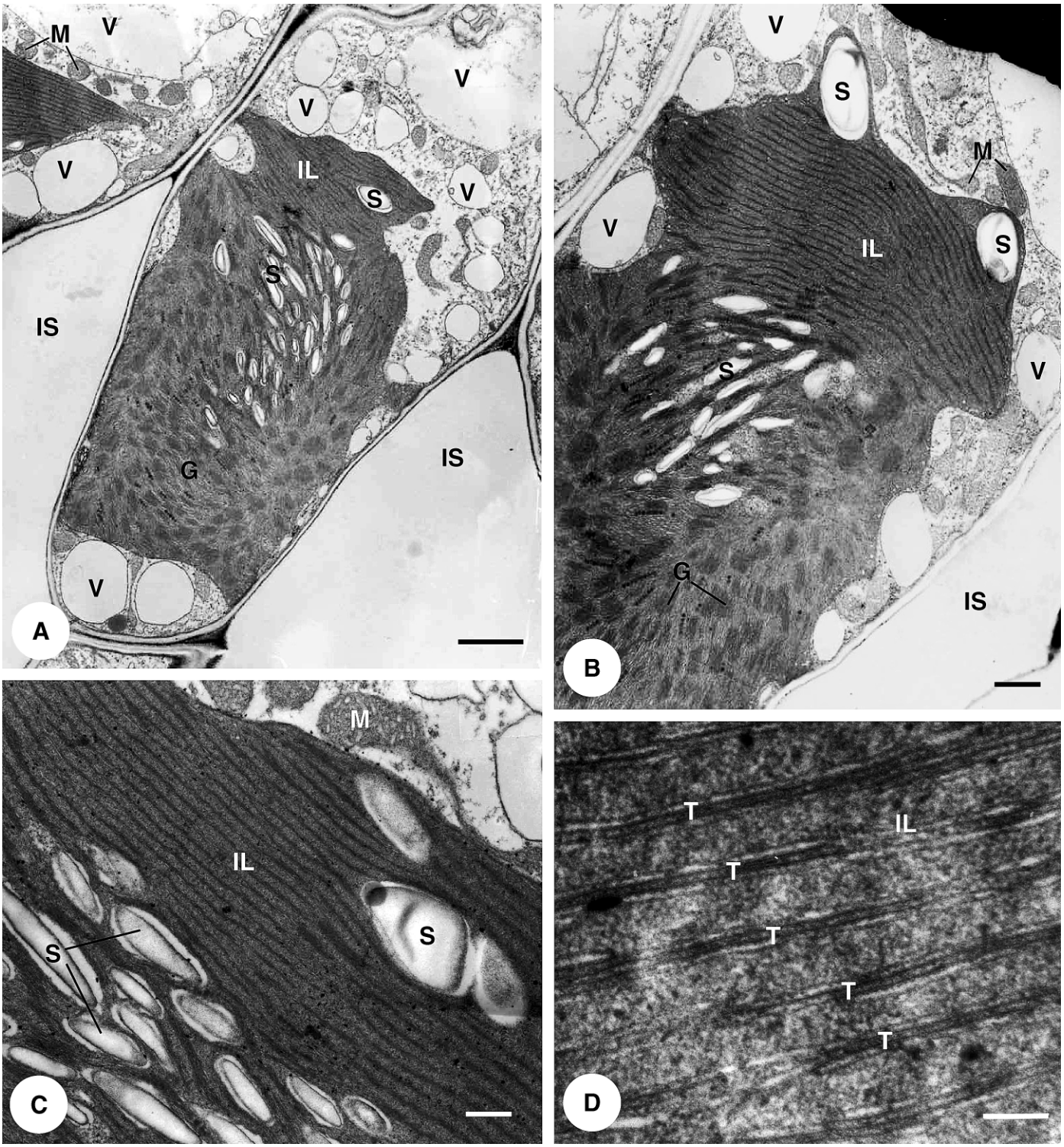

Fig. 5. TEM views of the bizonoplasts from the conical epidermal cells of the microphylls of *Selaginella erythropus*. (A–B) The single giant bizonoplast showing two zones that differ in the organization of thylakoid membranes: the upper iridoplast-like zone with layers of 2–4 stacked thylakoids lying horizontally and parallel to the upper chloroplast boundary and the lower zone with granal and agranal thylakoid membrane stacking. (C) The upper zone of a bizonoplast with iridoplast-like region and large starch grains. (D) Close-up of the iridoplast-like region of the upper zone showing layers of 2–4 stacked thylakoids with an almost consistent spacing (60–90 nm) between each layer. Scale bars: A = 2 μm, B = 1 μm, C = 500 nm, D = 100 nm. G: granum, IL: iridoplast-like region, IS: intercellular space, M: mitochondrion, S: starch grain, T: a group of thylakoid membranes, V: vacuole.

Giffordia spp. (Bouck, 1965). This pattern is also similar to those of the iridoplasts mentioned earlier (Gould and Lee, 1996; Lee, 1997; Sheue et al., 2003), suggesting that the function and evolutionary implications of this unusual structure in these distantly related organisms (brown algae, spike moss, fern and flowering plants) need to be studied further.

The size, number, and ultrastructure of chloroplasts of *S. erythropus* (including the dimension and number of stacks of thylakoids per granum) are distinctively different in the conical epidermial cells, mesophyll cells, and elongated epidermal cells. This pattern of differences is very similar to that reported for the fern *Teratophyllum rotundifoliatum* (Nasrulhaq-Boyce and Duckett, 1991) and somewhat similar to the results obtained with other *Selaginella* species (Jagels, 1970a, b). In *S. apoda, S. martensii, S. serpens,* and *S. uninata,* however, the dimensions of the grana in the small discoid and cup-shaped chloroplasts are virtually identical (Jagels, 1970b).

In summary, this study clearly demonstrated that the chloroplasts in the microphylls of *S. erythropus* possess an even greater diversity (cup-shaped bizonoplast, disklike mesophyll chloroplast, elongated or beadlike chloroplast in chains, trichome chloroplast, and stomatal chloroplast) than that reported by Haberladt (1888). The diversification in chloroplast structure in different cell types and in ultrastructure within a chloroplast in the deep-shade plant *S. erythropus* may represent evolutionary changes in photosynthetic functionality in adaptation to low-light environments. The giant and distinctive bizonoplasts of *S. erythropus* could provide us with novel plant material to further understand the evolutionary development of chloroplasts in a light-limited environment.

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