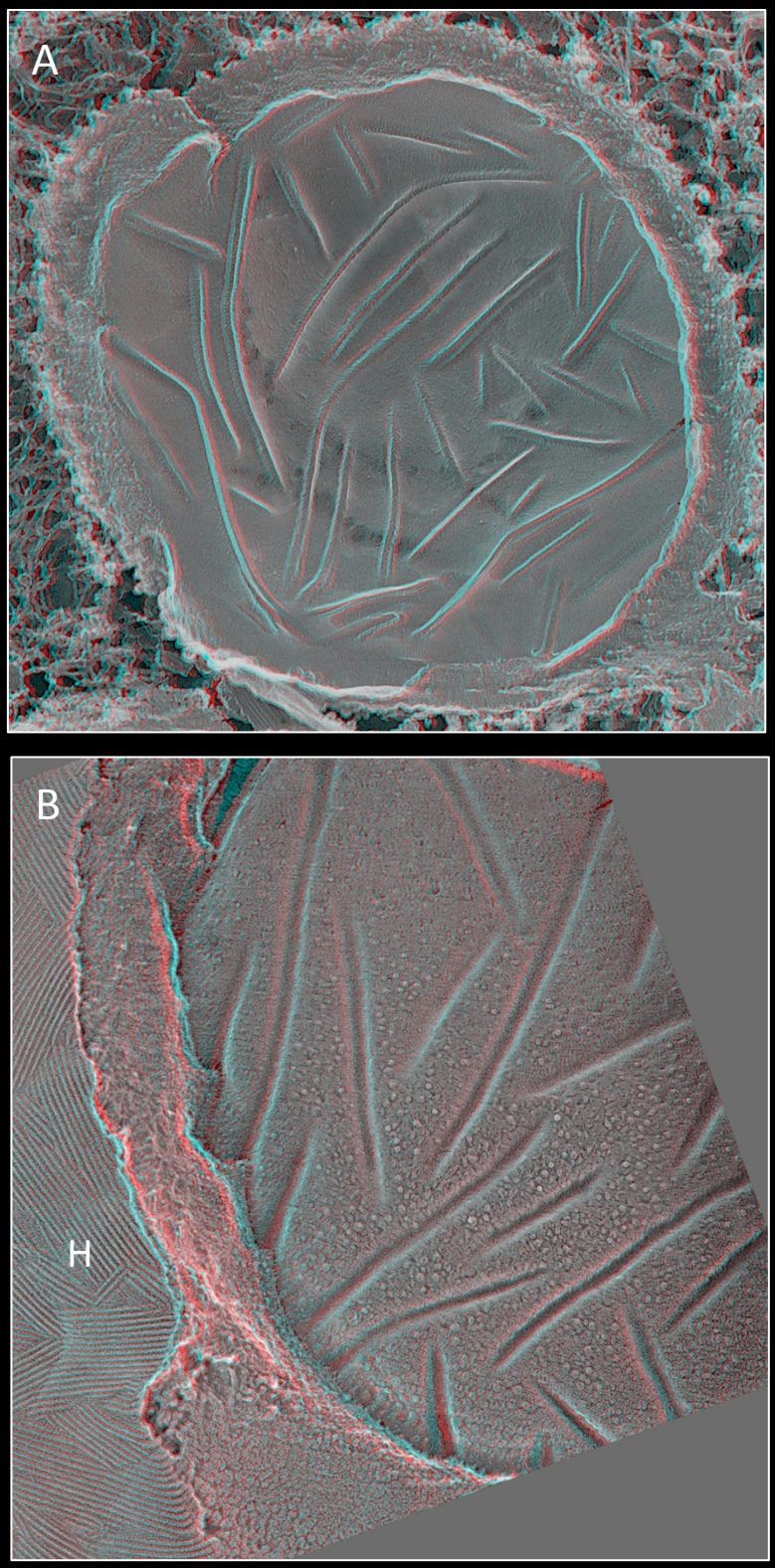
FIG S1 Convex (A) and concave (B) eisosome faces of *Penicillium* sp. isolate K17. Stereo images (use red/green glasses)



H, hydrophobin layer of cell wall

FIG S2 *Candelaria concolor* mycobiont:
(A) and (B) convex faces, (C) concave face

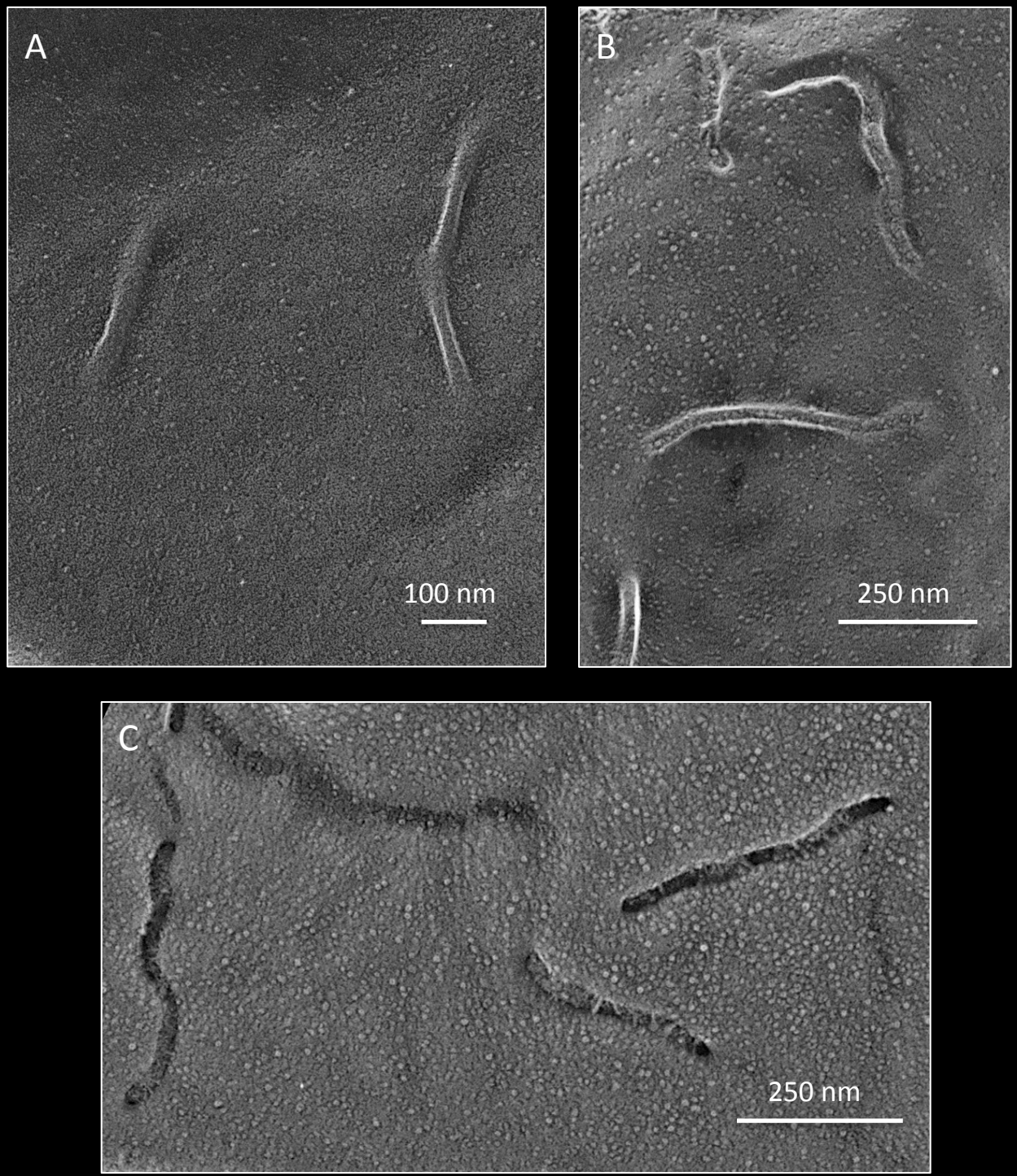


FIG S3 *Cladonia grayi* mycobiont: convex face

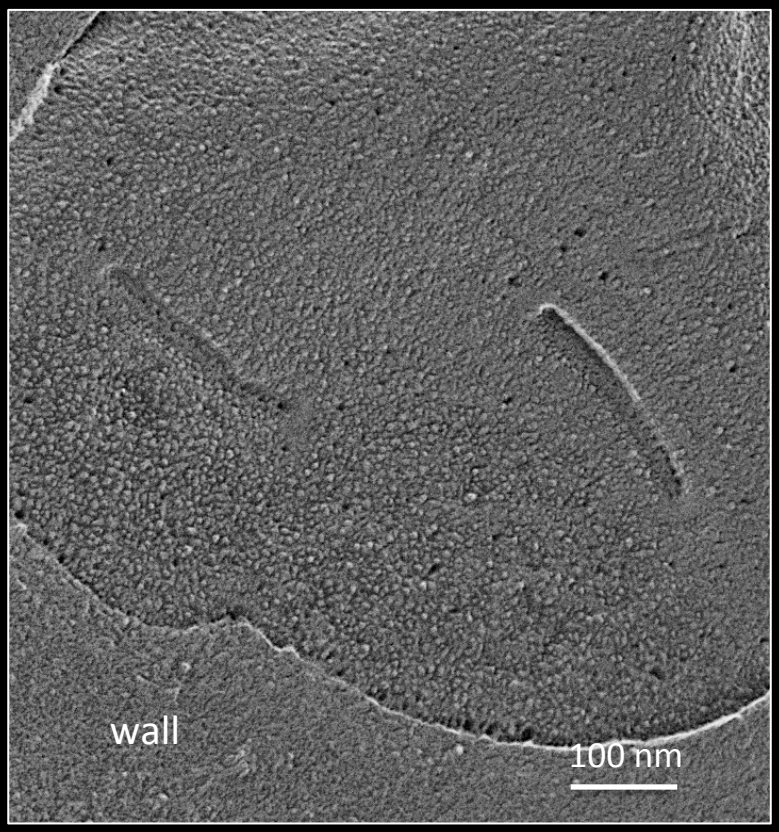
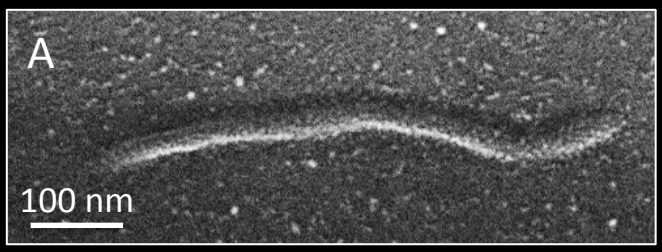


FIG S4 *Cladonia grayi* on agar:
(A) convex face
(B) concave face



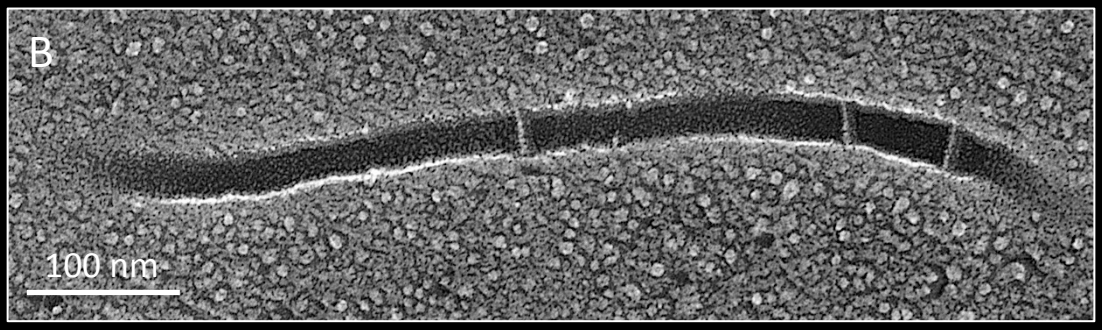
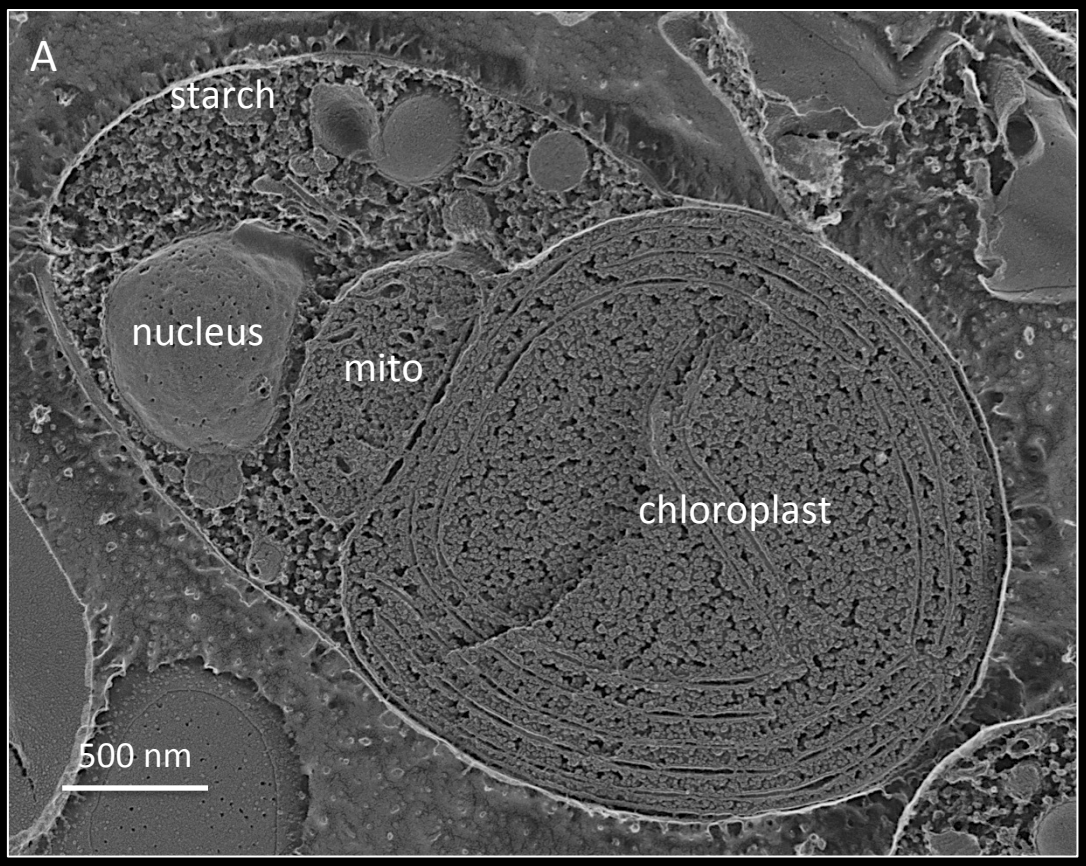


FIG S5 (A) *Cyanidioschyzon merolae*
(B) *Cyanidioschyzon* YNP 1A



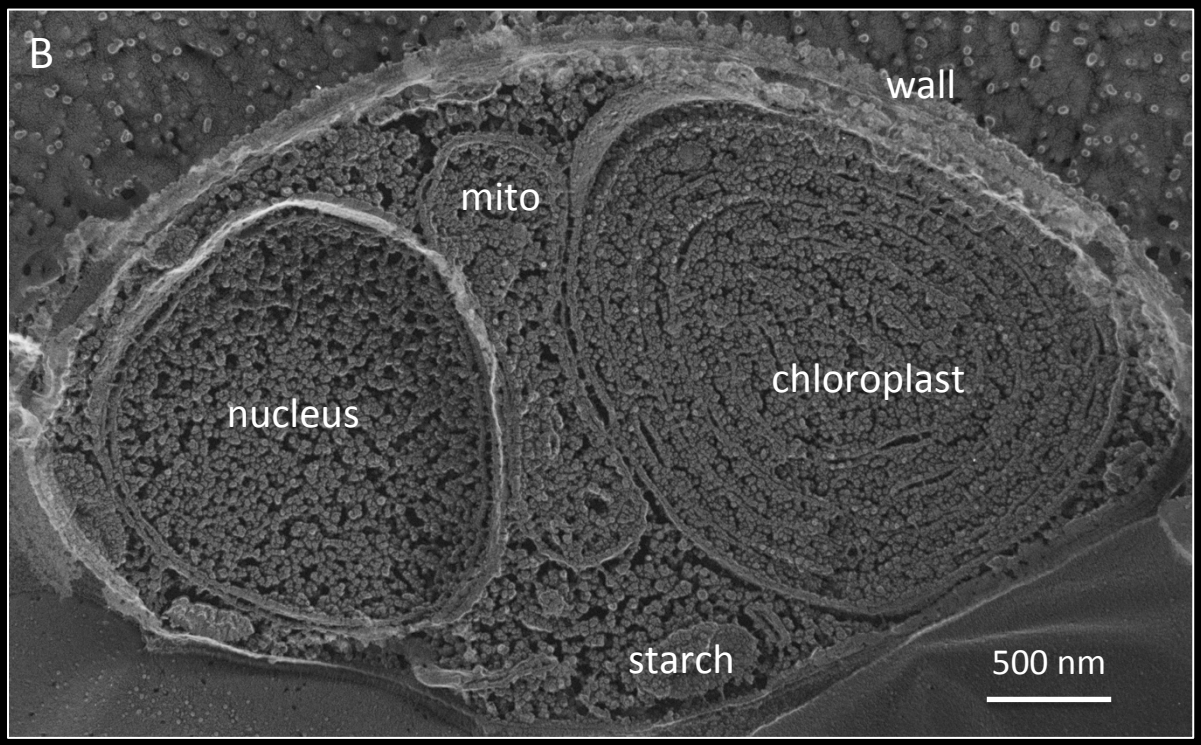
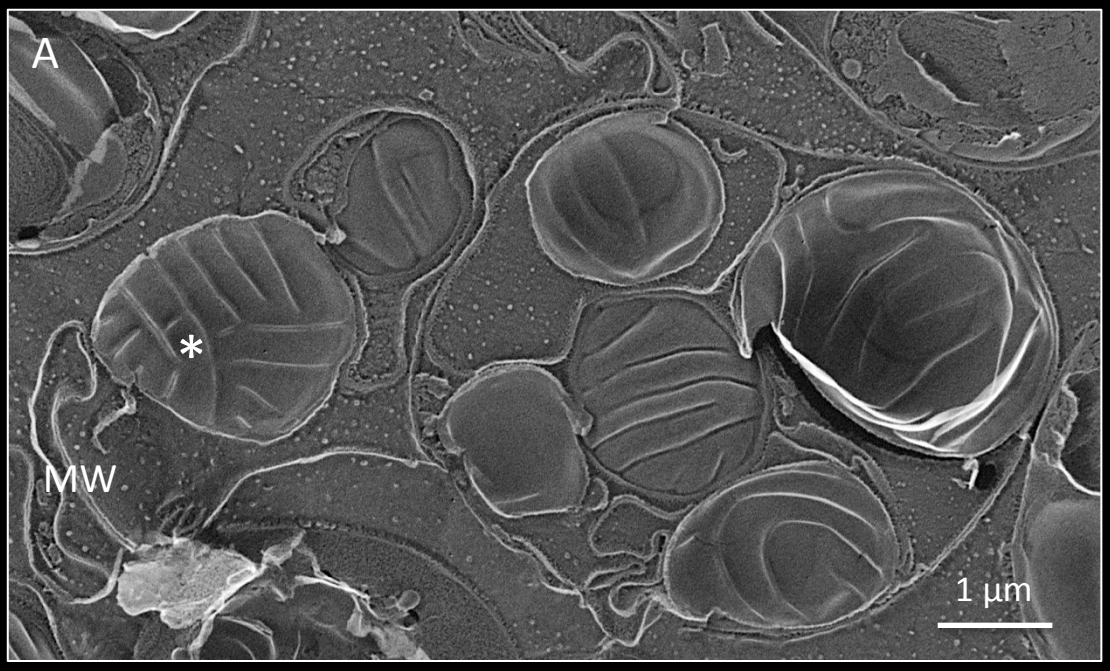
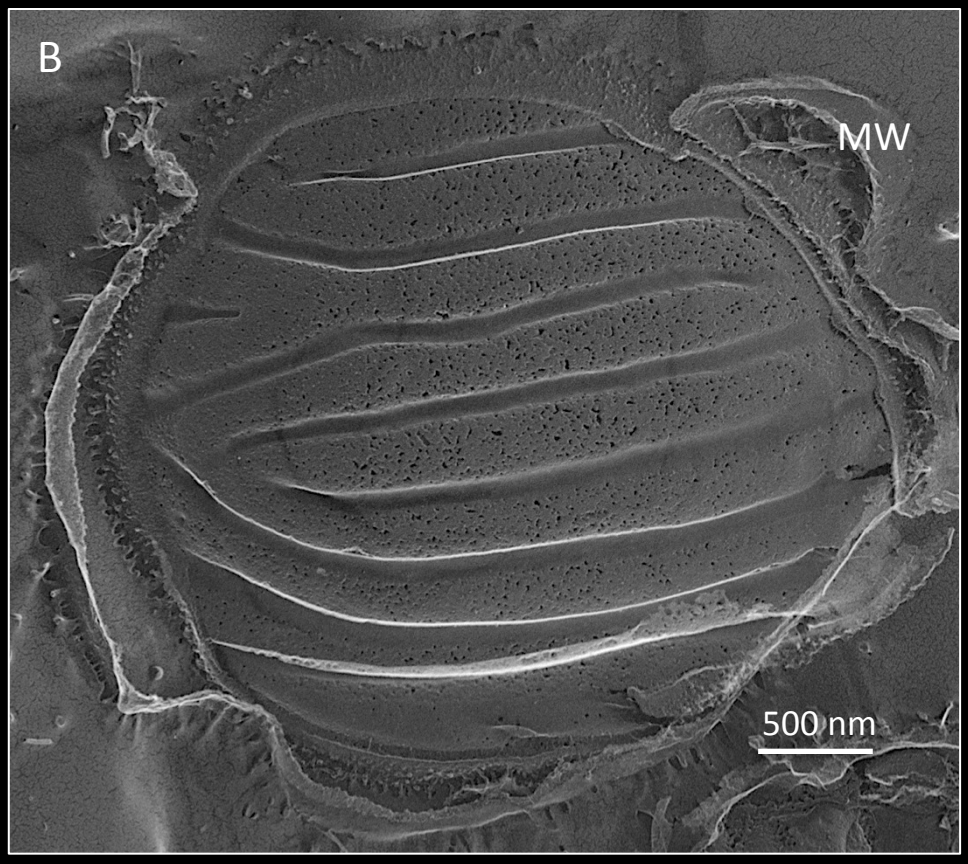


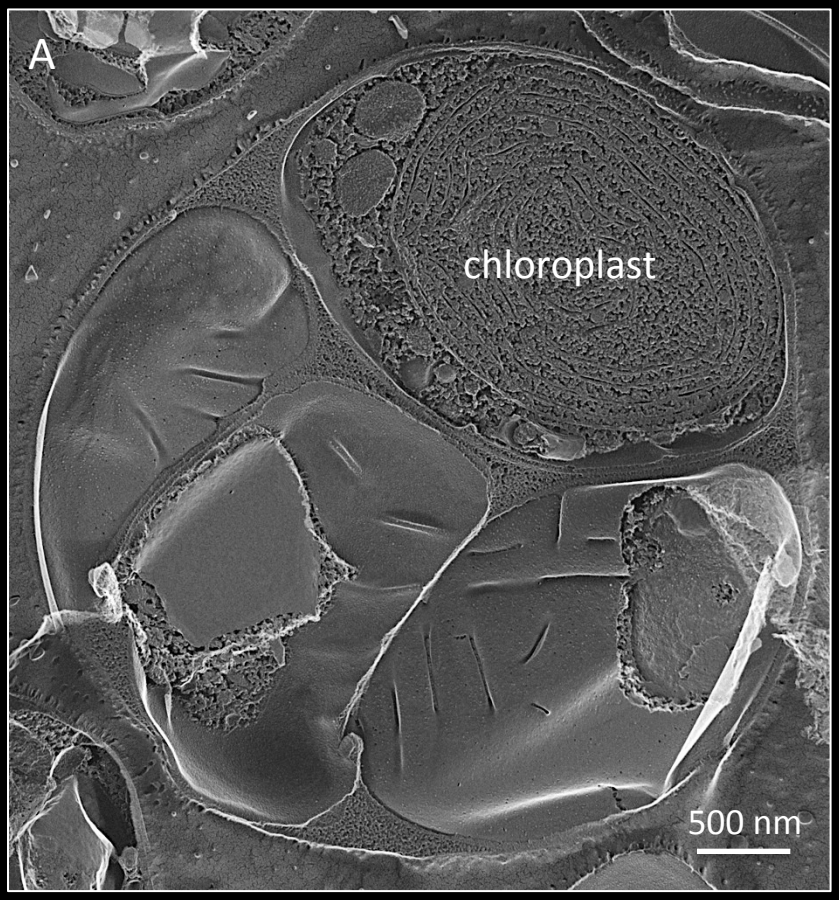
FIG S6 *Cyanidioschyzon* YNP 1A:
Eisosomes in mature and daughter cells





The eisosomes are generally circumferential, but exceptions occur (asterisk). MW, discarded mother walls.

FIG S7 *Cyanidioschyzon* YNP 1A:
Eisosomes in mature and daughter cells



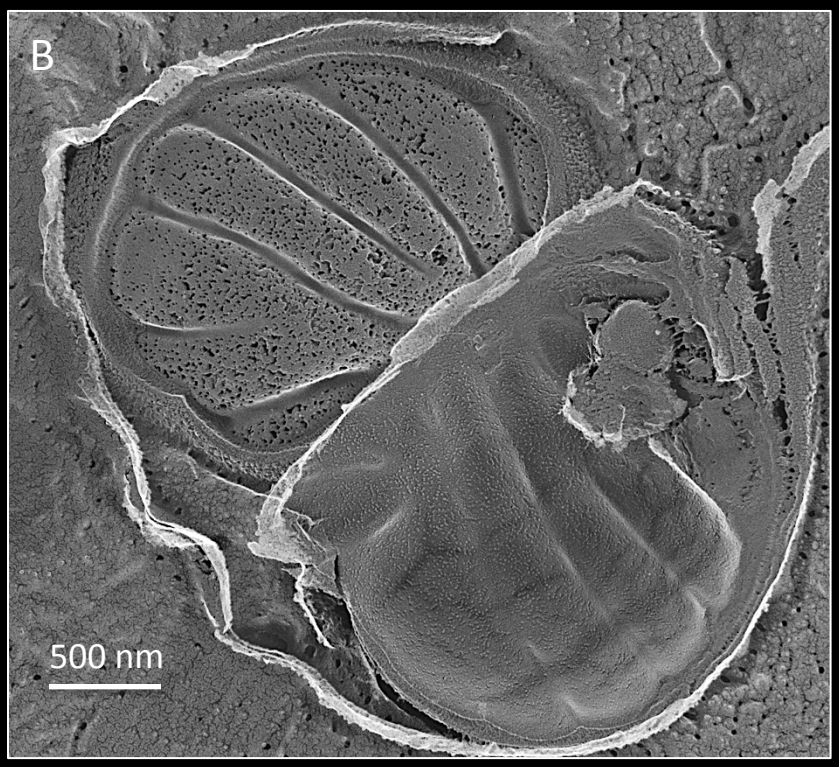
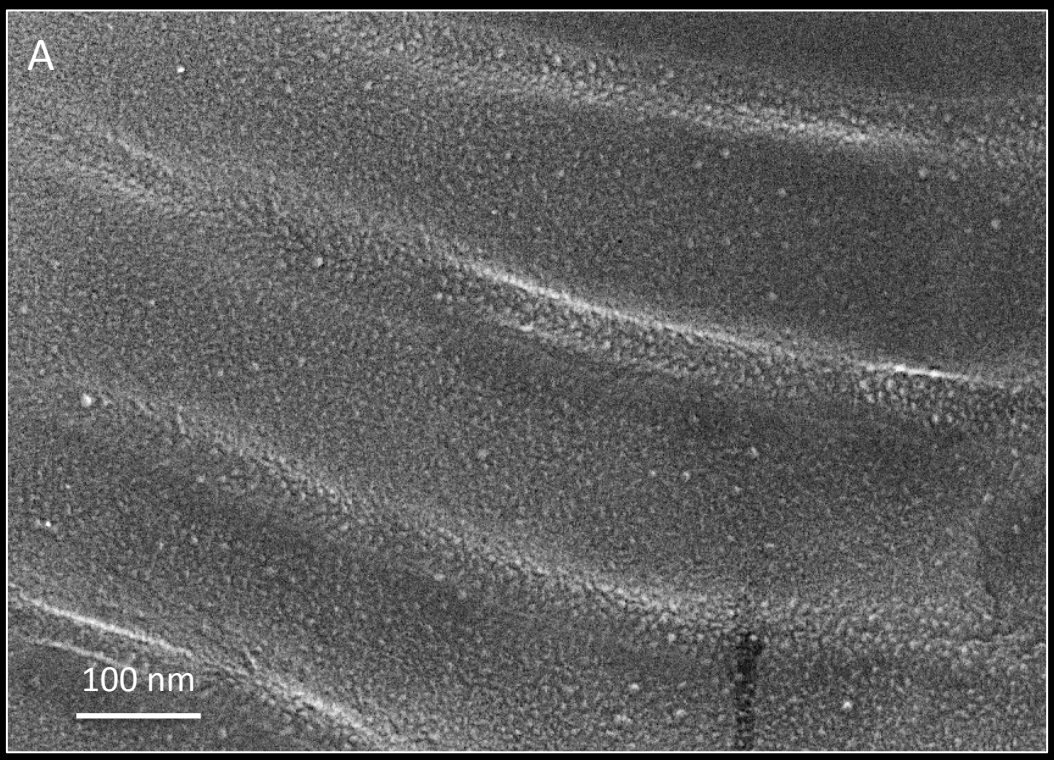


FIG S8 *Cyanidioschyzon* YNP 1A:
(A) convex face, (B) concave face



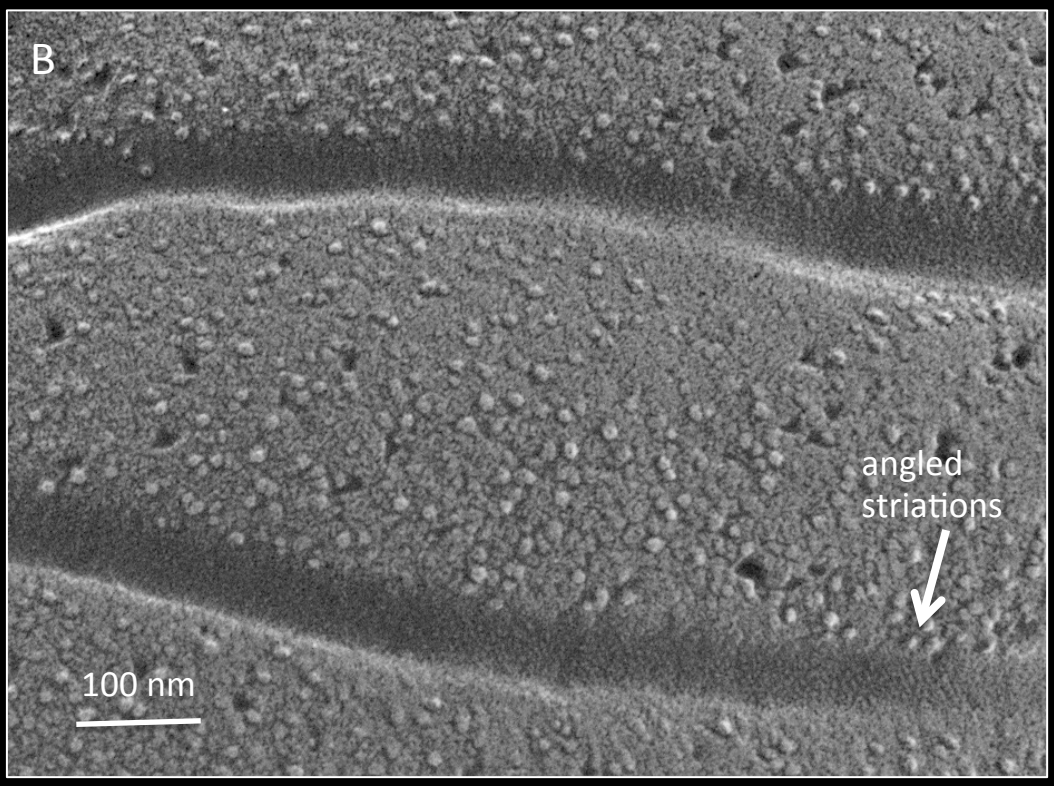
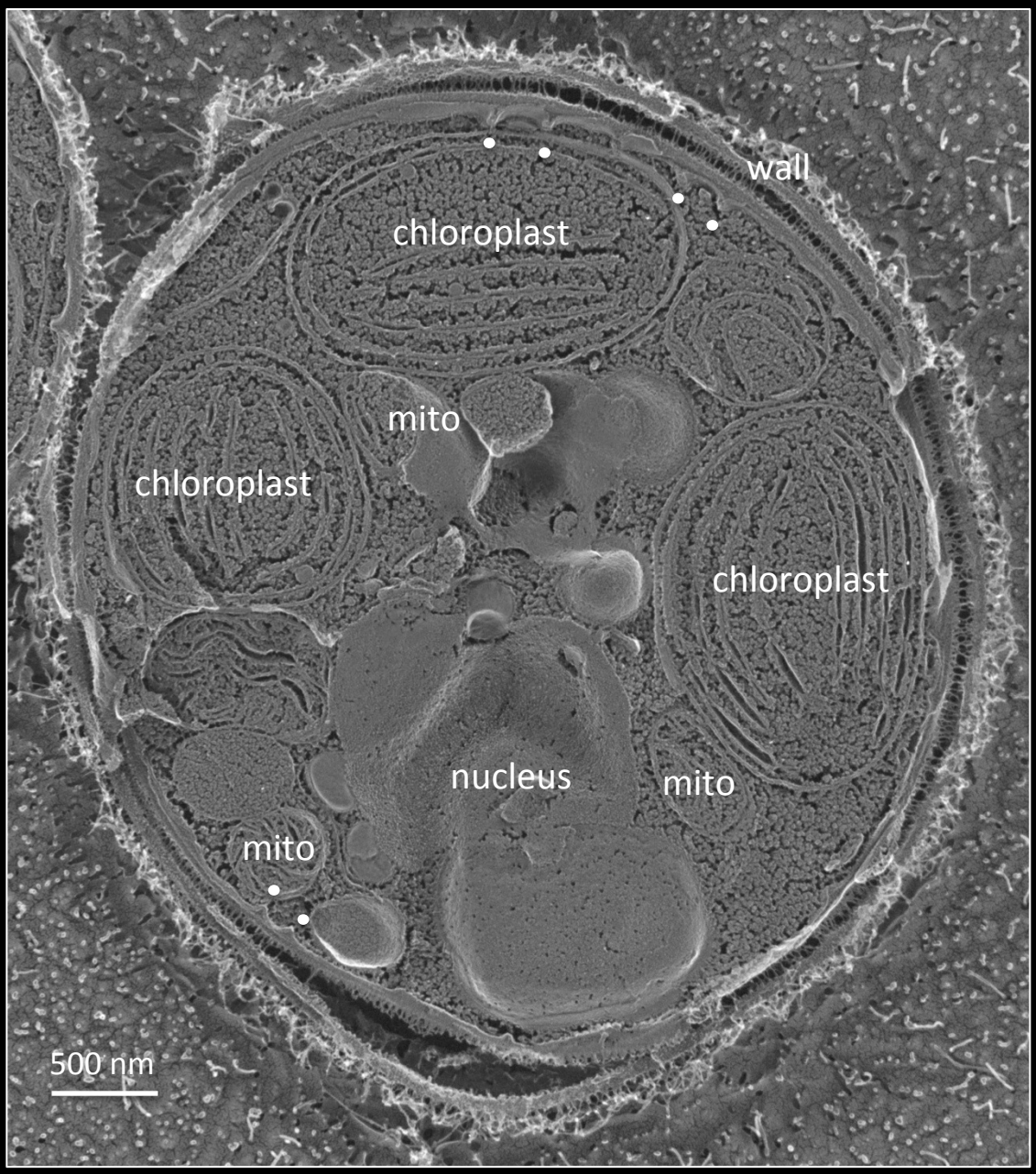
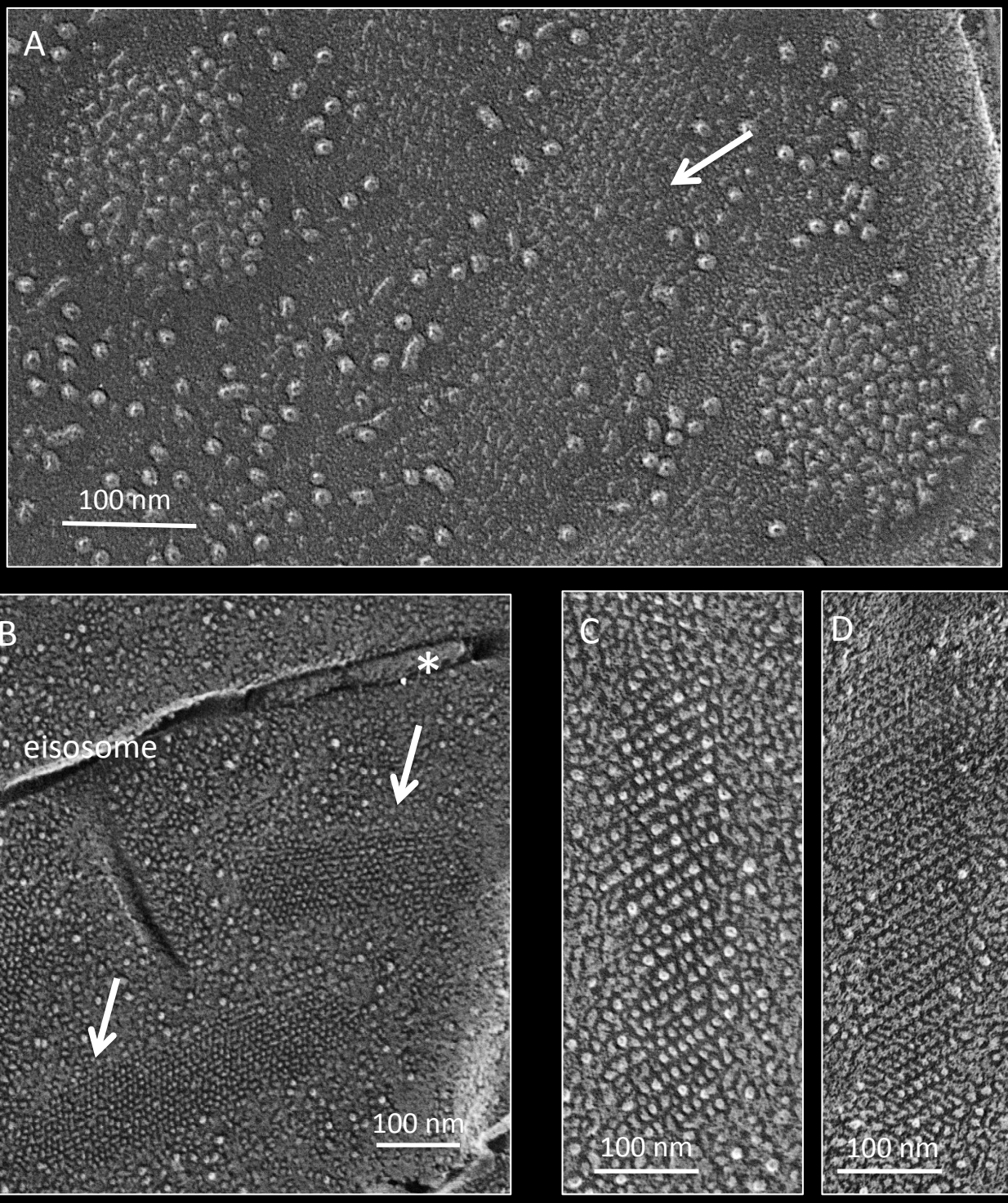


FIG S9 *Galdieria sulphuraria*



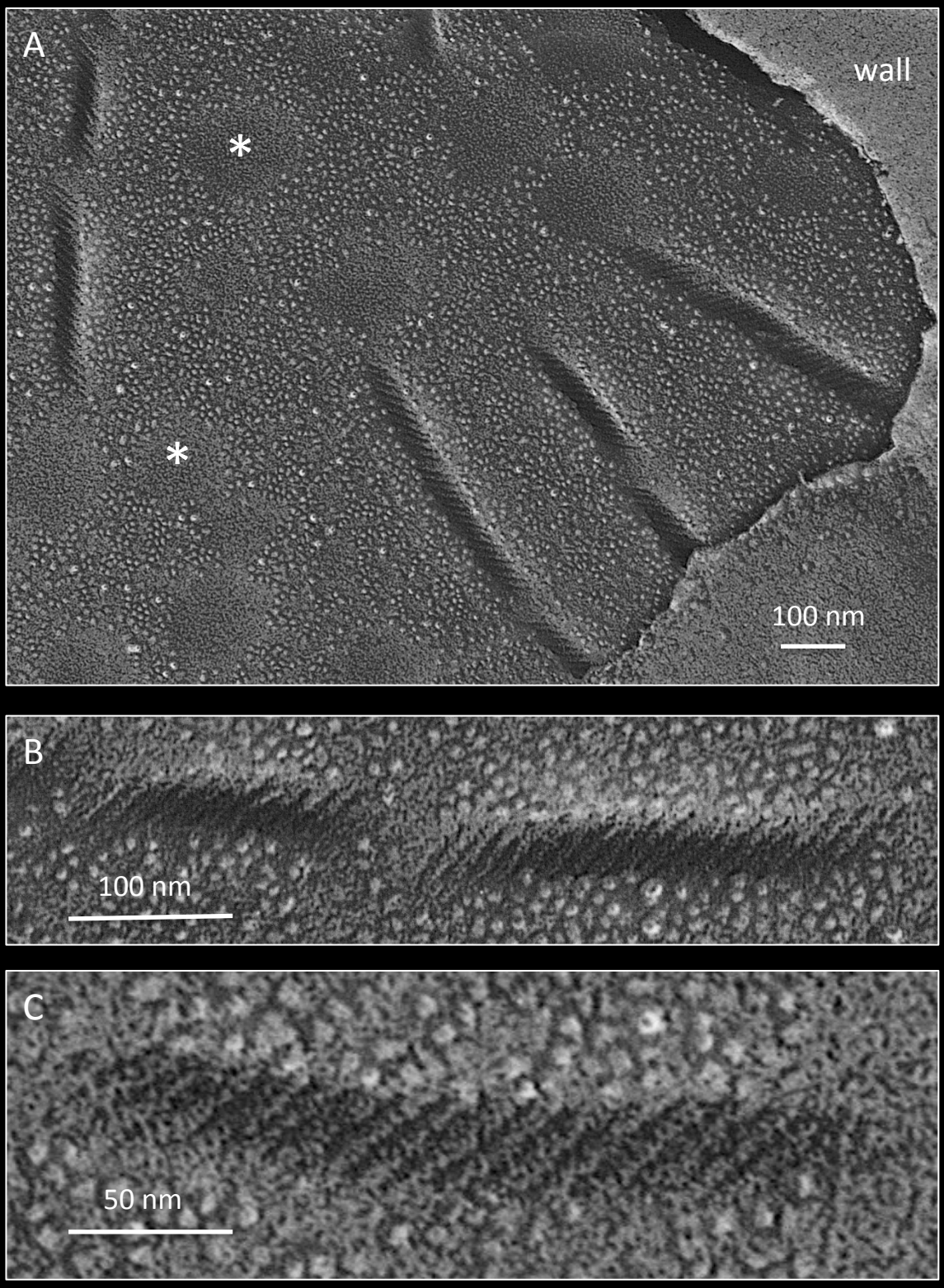
G. sulphuraria cell with several chloroplast profiles, several mitochondria, and no cytoplasmic starch. Eisosomes at white dots.

FIG S10 *Galdieria sulphuraria*:
Non-etched plasma membrane differentiations



(A) Circular IMP "islands" and parallel rectangular arrays (arrow).
(B) Parallel rectangular arrays (arrows) and eisosome with cell-wall inclusion (asterisk). (C and D) Rectangular arrays of angled IMPs.

FIG S11 *Galdieria sulphuraria*
Non-etched concave eisosomes



Asterisks: P-faces of circular IMP “islands” (c.f. Fig. S10)

FIG S12 *Galdieria sulphuraria*: non-etched convex faces.
Stereo image (use red/green glasses)

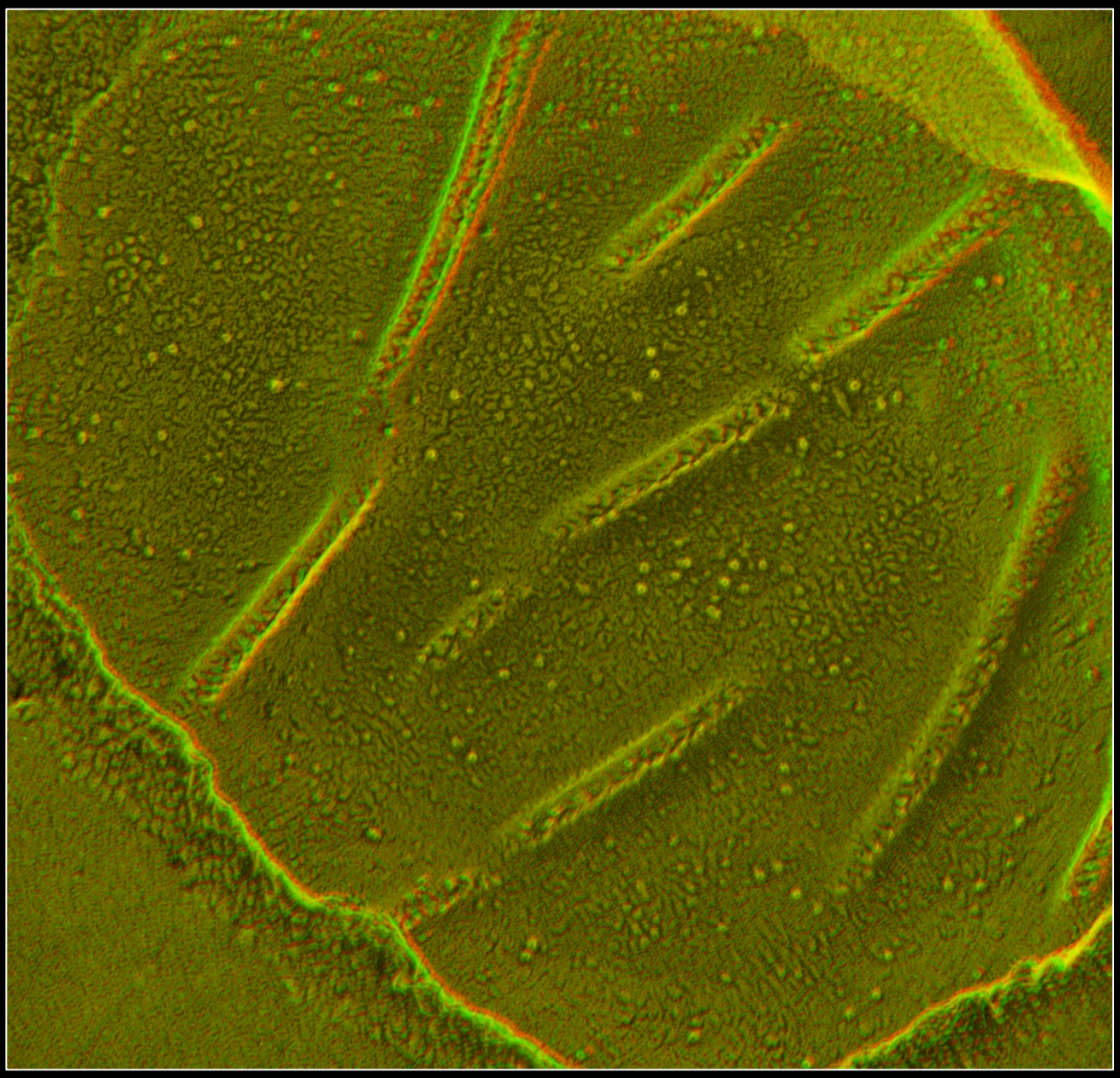
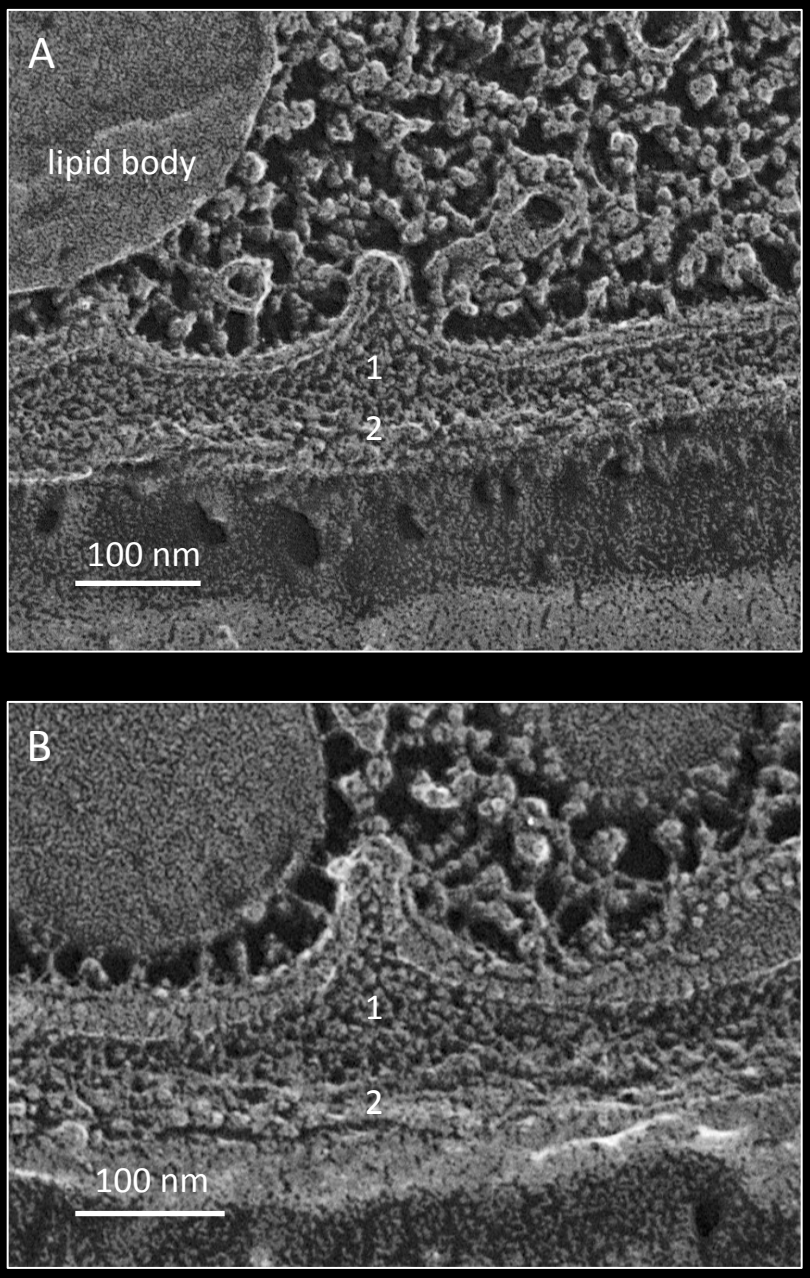


FIG S13 *Galdieria sulphuraria*: non-etched concave faces (arrows)
Stereo image (use red/green glasses)

FIG S14 *Auxenochlorella protothecoides*:
eisosomes and walls



The inner granular layer 1 penetrates the eisosome furrow; the outer denser layer 2 does not.

FIG S15 *Auxenochlorella protothecoides* eisosomes:
Concave faces (most elongated)

Arrows, wall entering eisosomes; cpst, chloroplast

FIG S16 *Auxenochlorella protothecoides* eisosomes:
Convex faces (punctate)

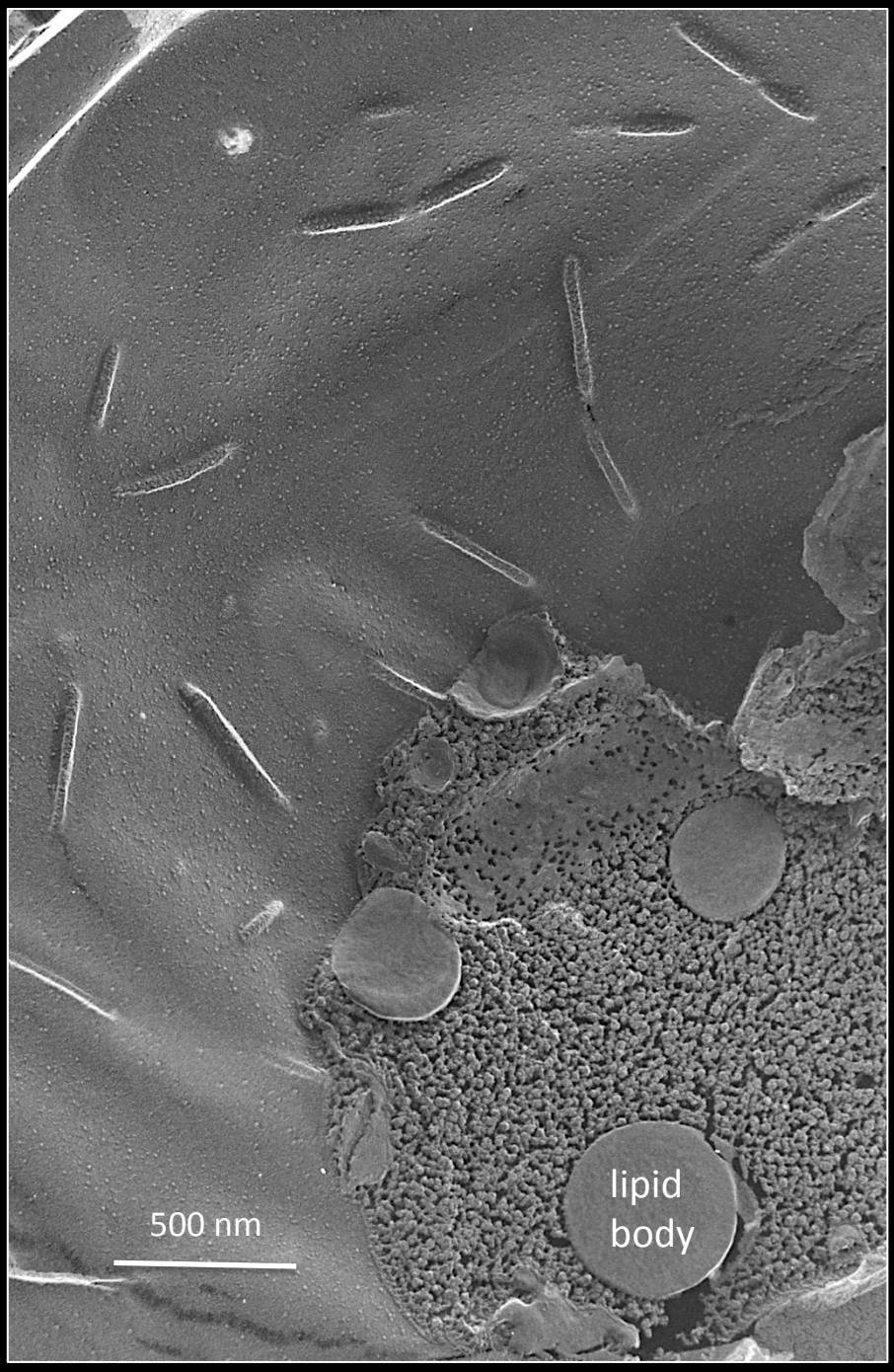


FIG S17 *Auxenochlorella protothecoides* eisosomes:
Periodicity of angled striations

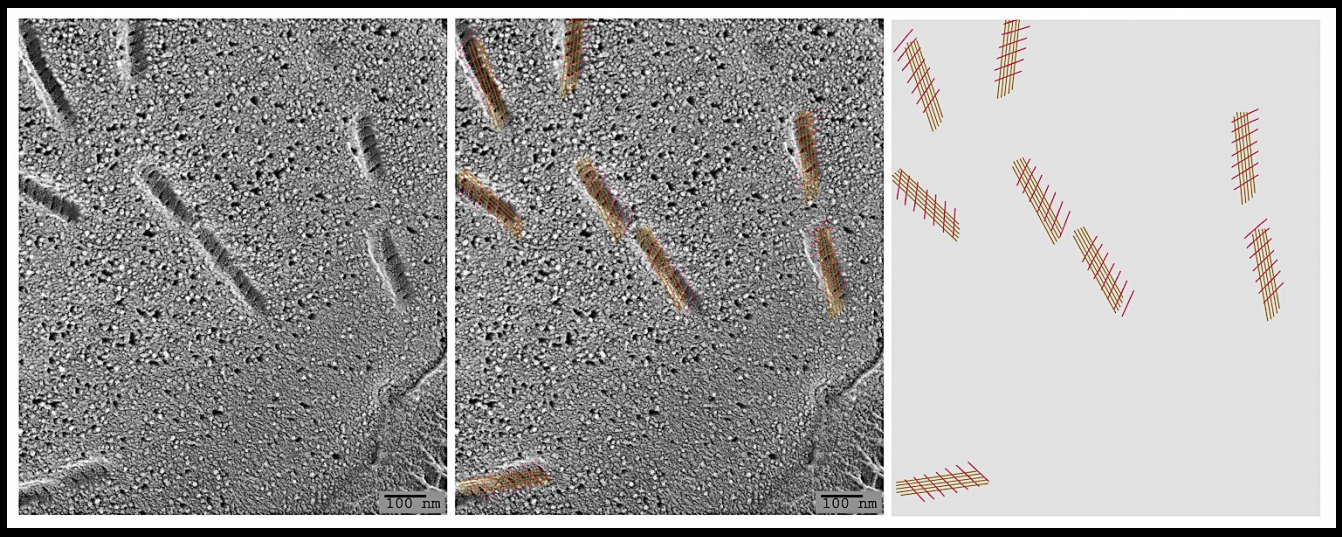
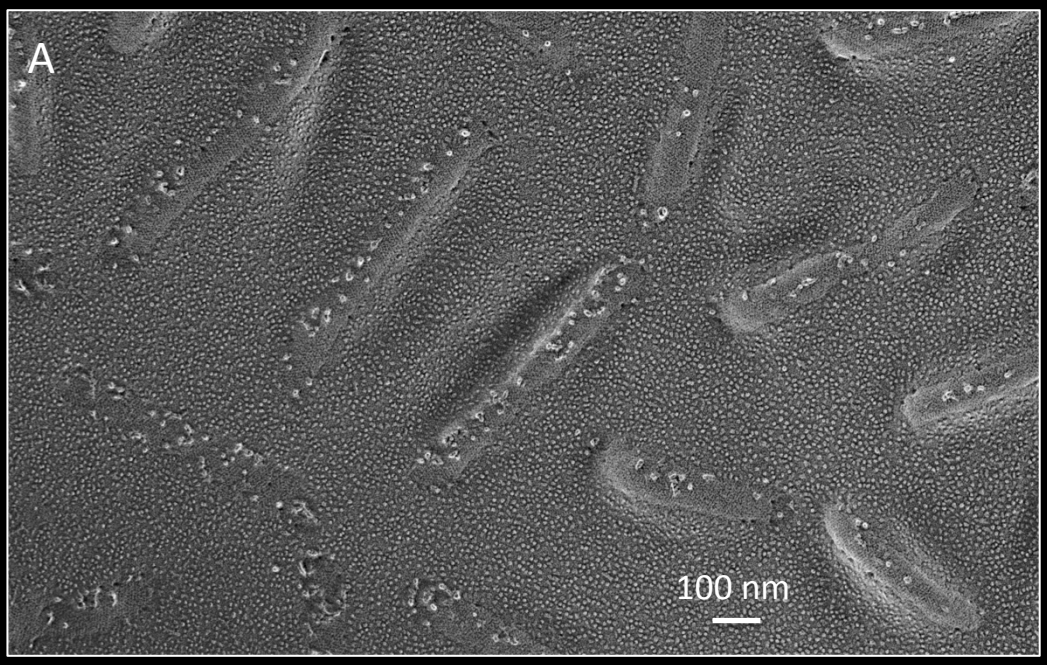


FIG S18 *Chlamydomonas monoica*:
Convex faces with irregularly shaped and distributed particles



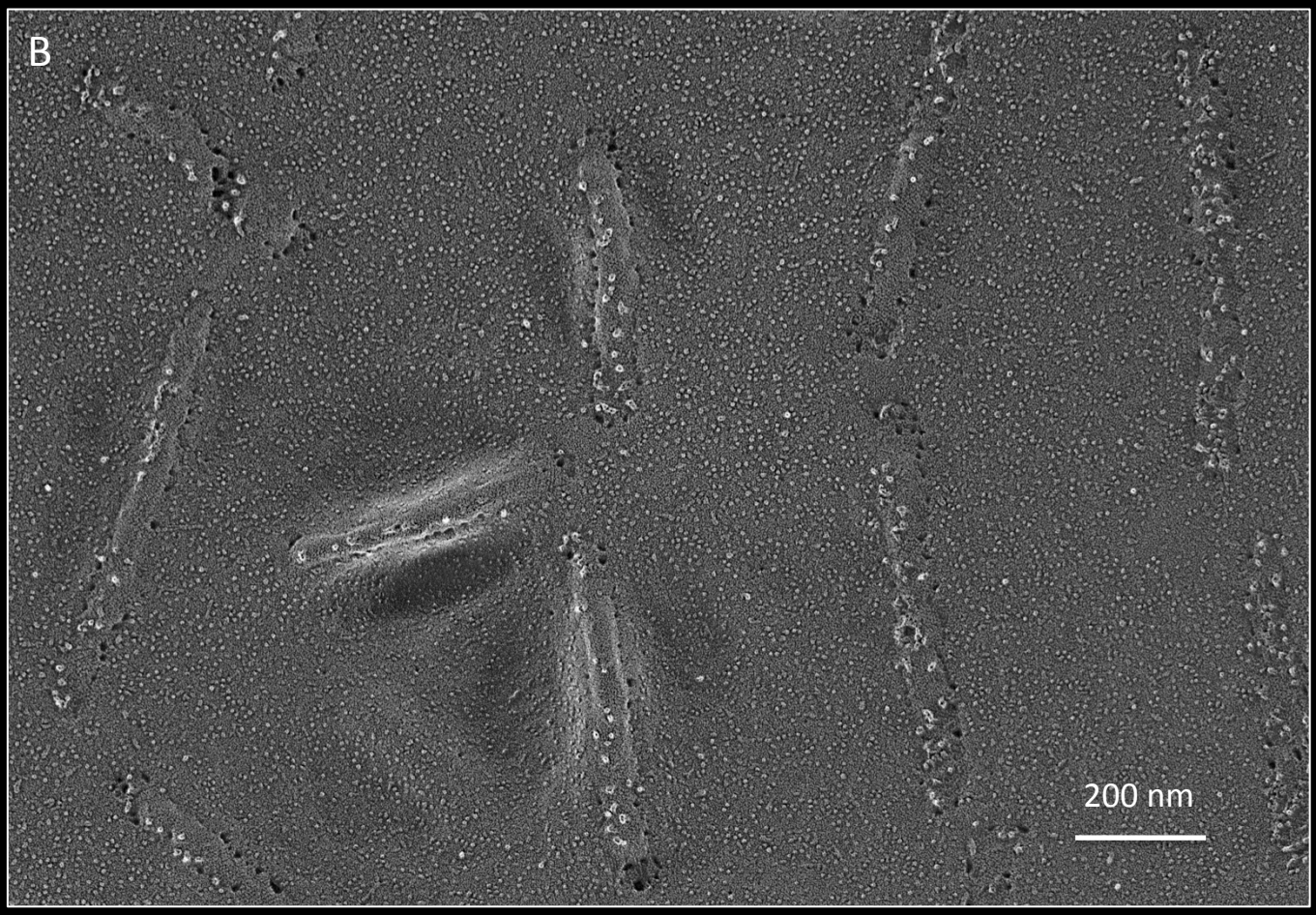


FIG S19 *Chlamydomonas monoica* concave faces.
Stereo image (use red/green glasses)

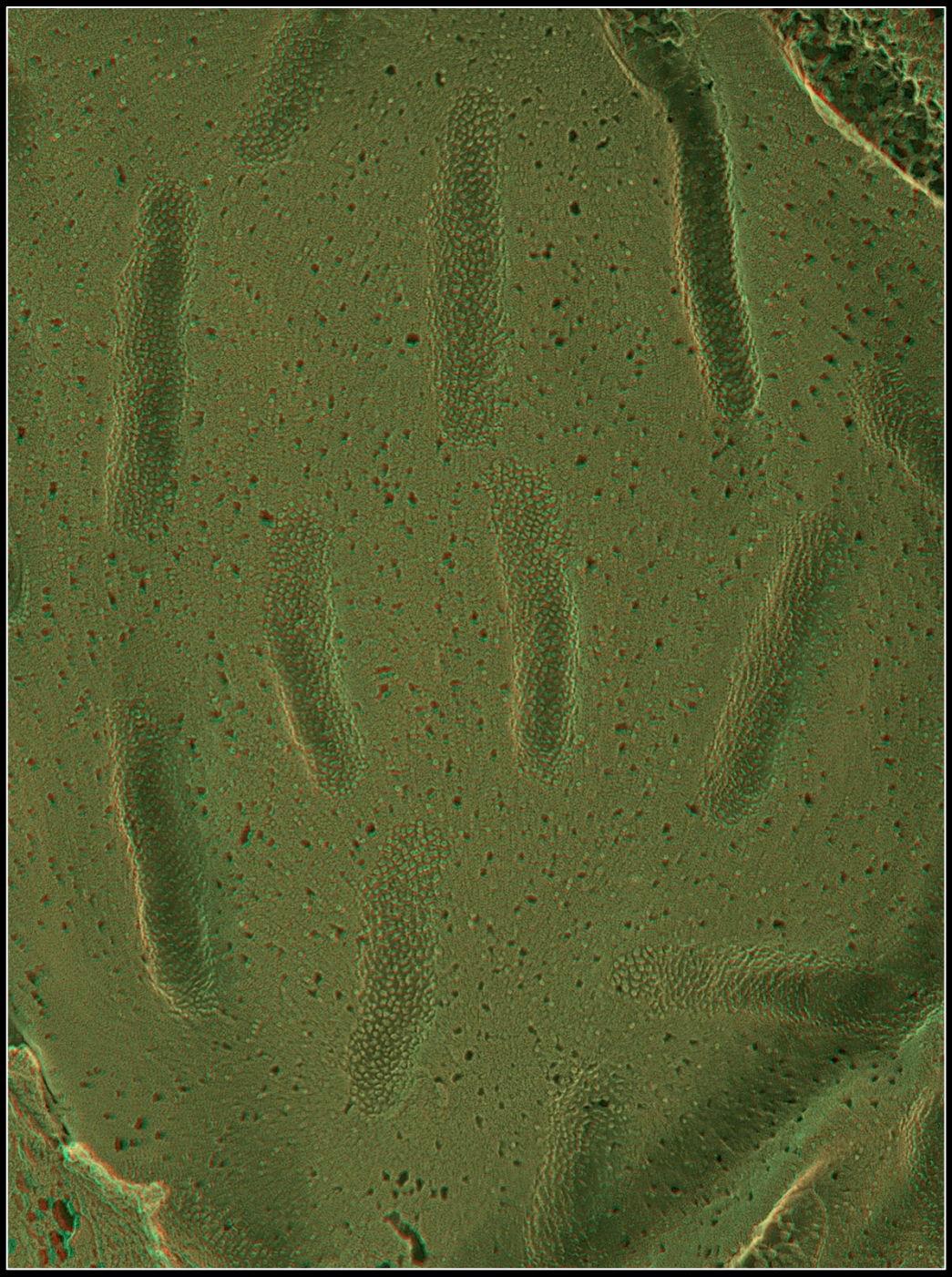


FIG S20 *Chlamydomonas monoica* convex faces
Stereo image (use red/green glasses)

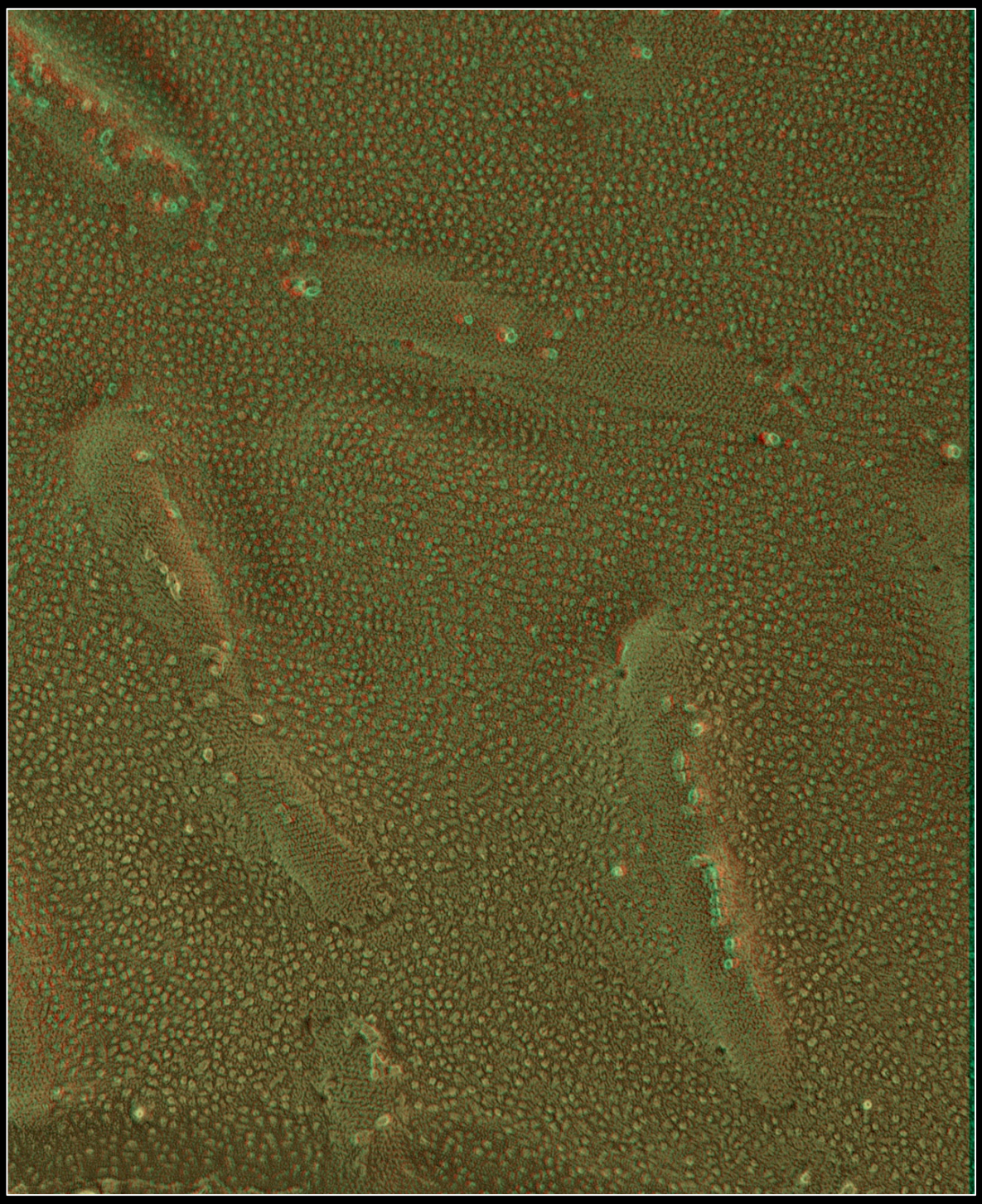


FIG S21 *Chlamydomonas monoica* concave faces.
Stereo image (use red/green glasses) (see Fig. S22 for labels)

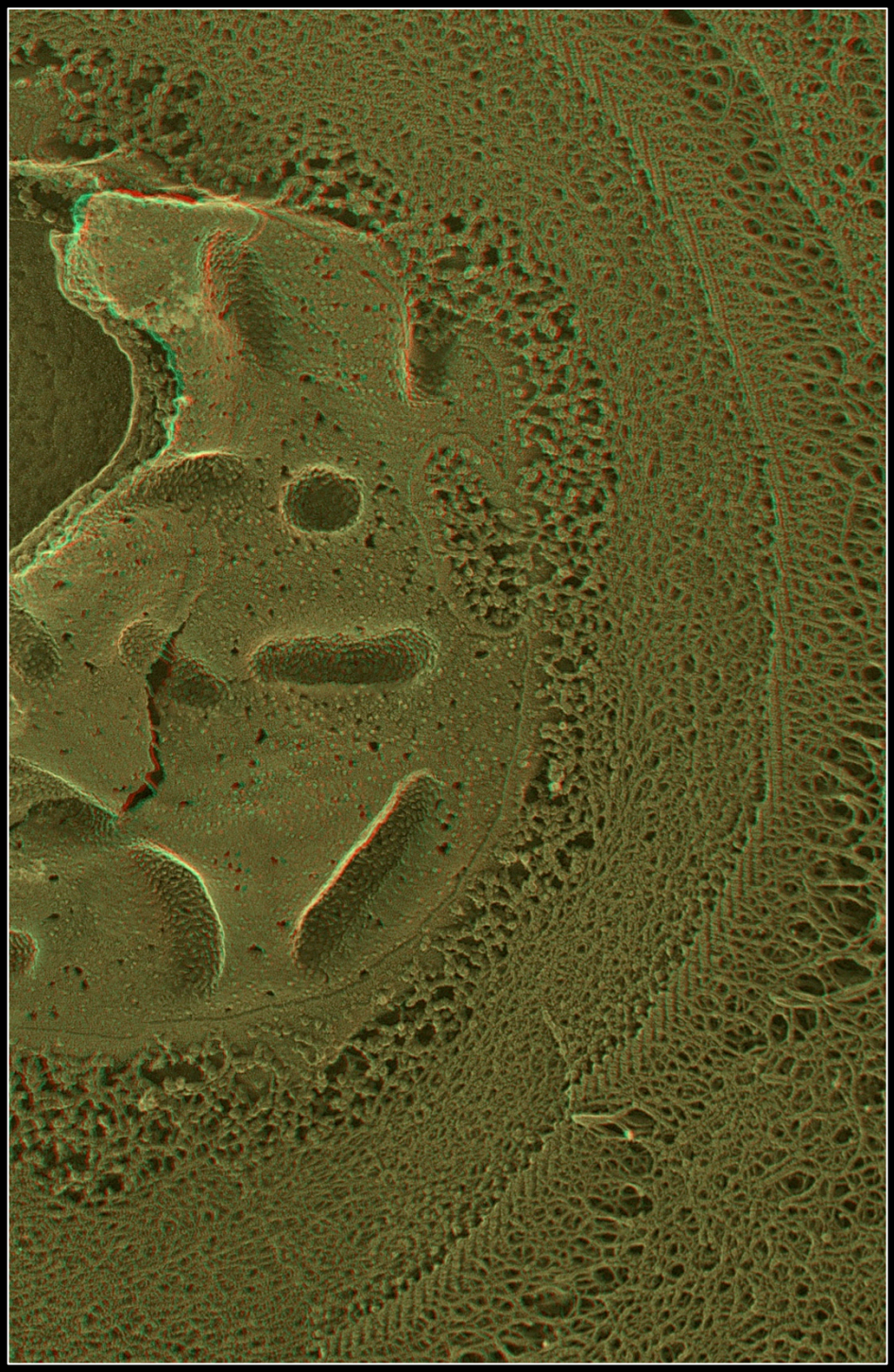
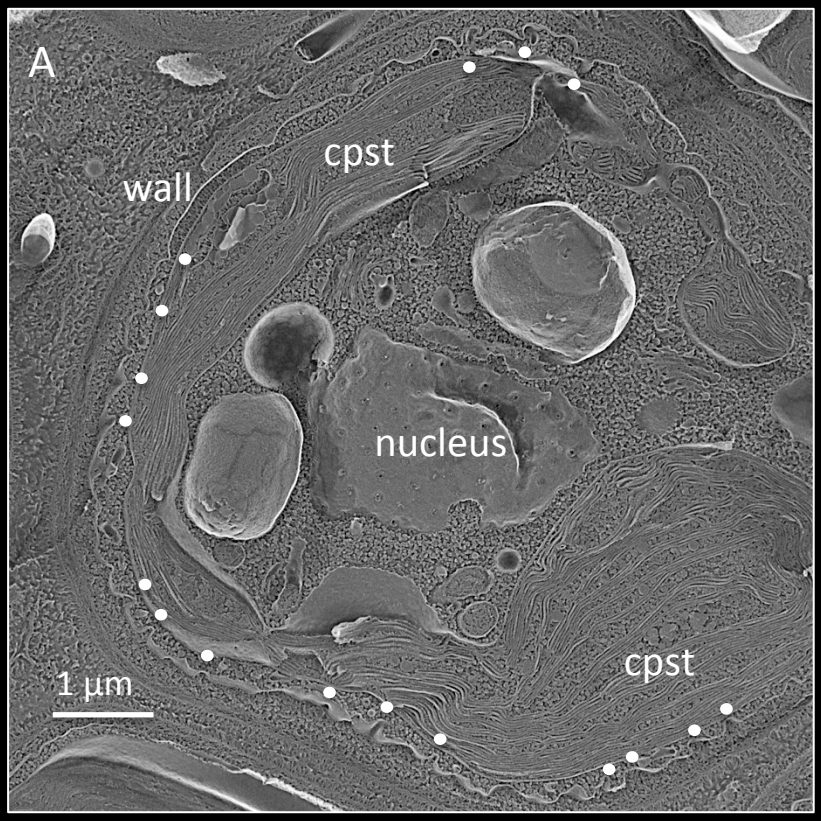


FIG S22 *Chlamydomonas monoica*:
Wall/eisosome relationships



White dots,
eisosomes;
cpst, chloroplast

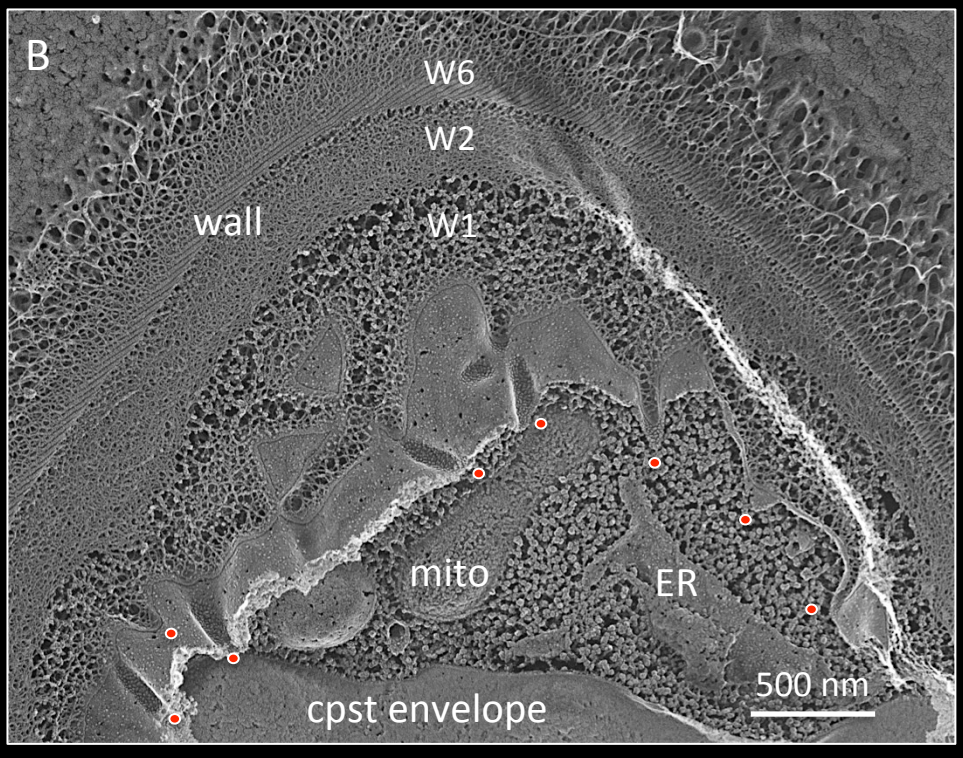
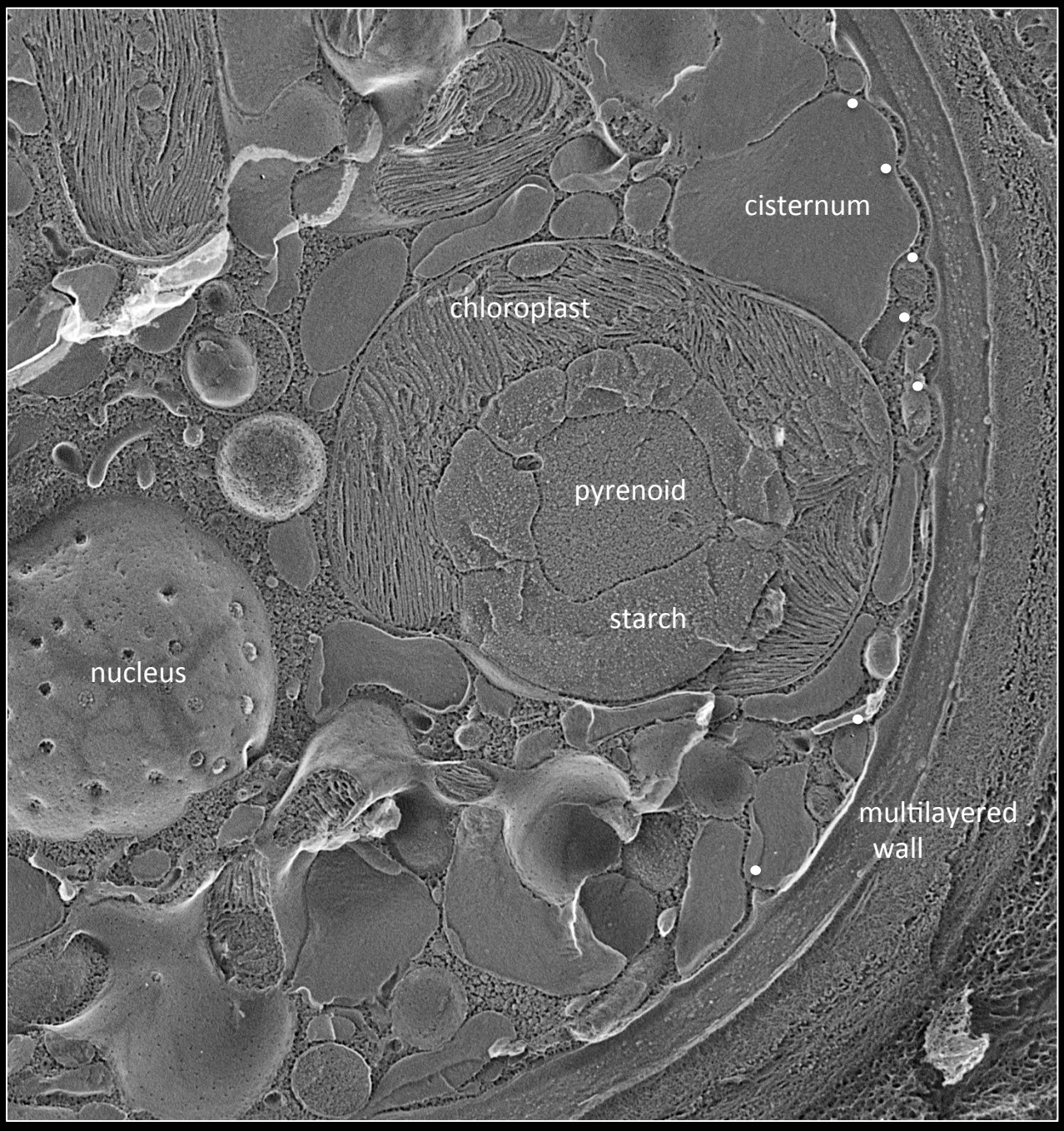


FIG S23 *Chlamydomonas reinhardtii* zygote



White dots, eisosomes

FIG S24 *Chlamydomonas reinhardtii* zygotes:
(A) concave faces (B) convex faces

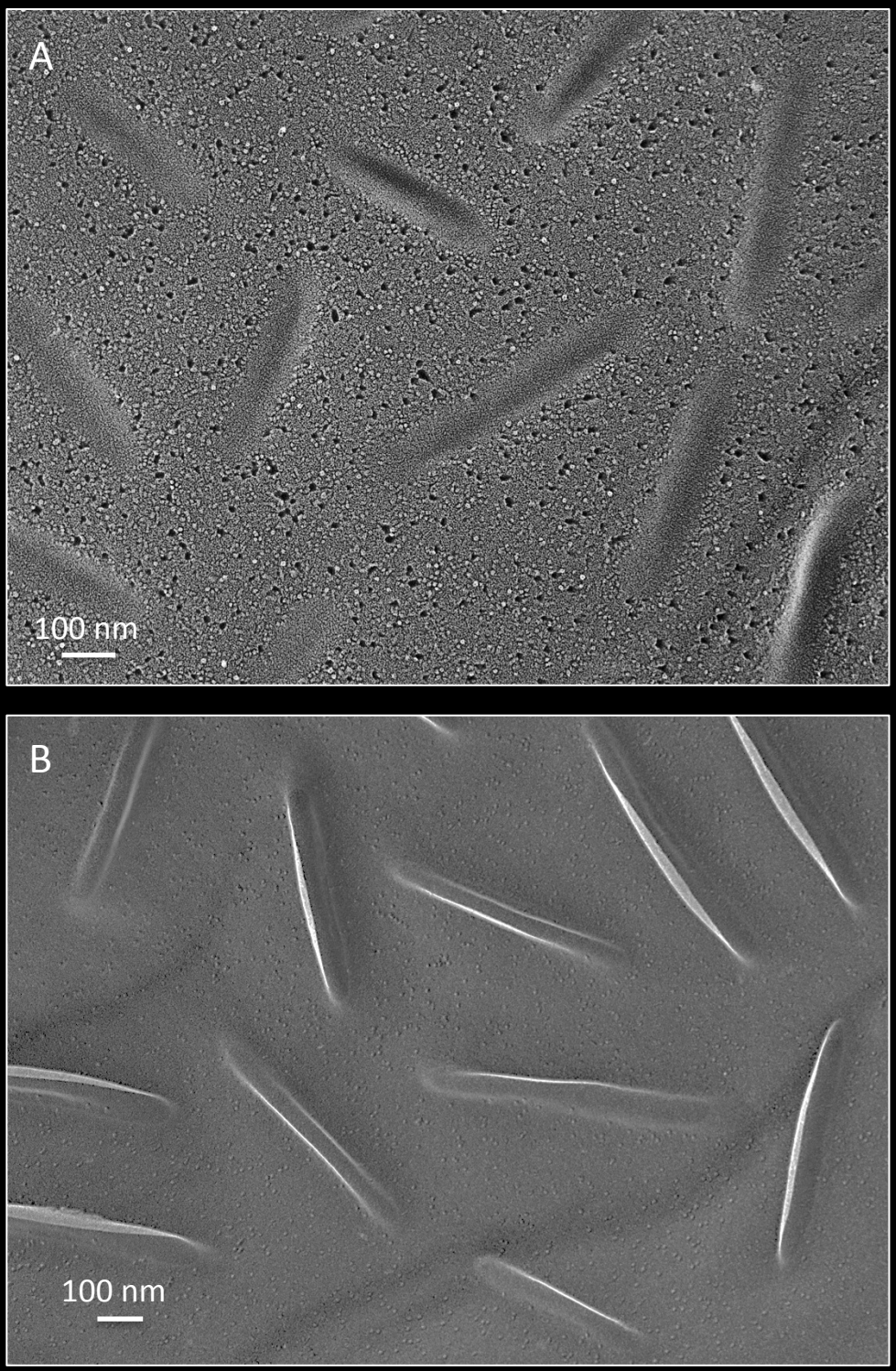
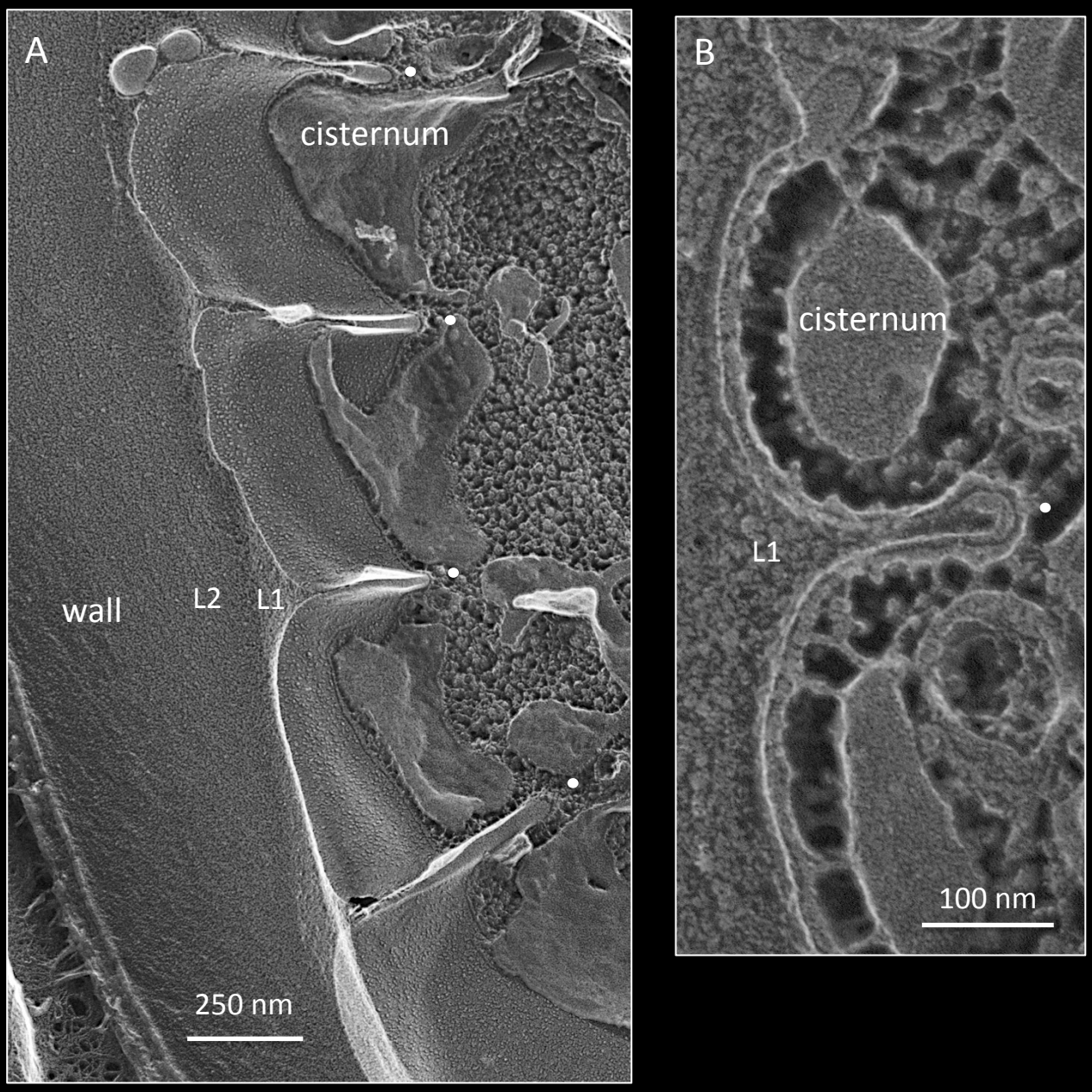
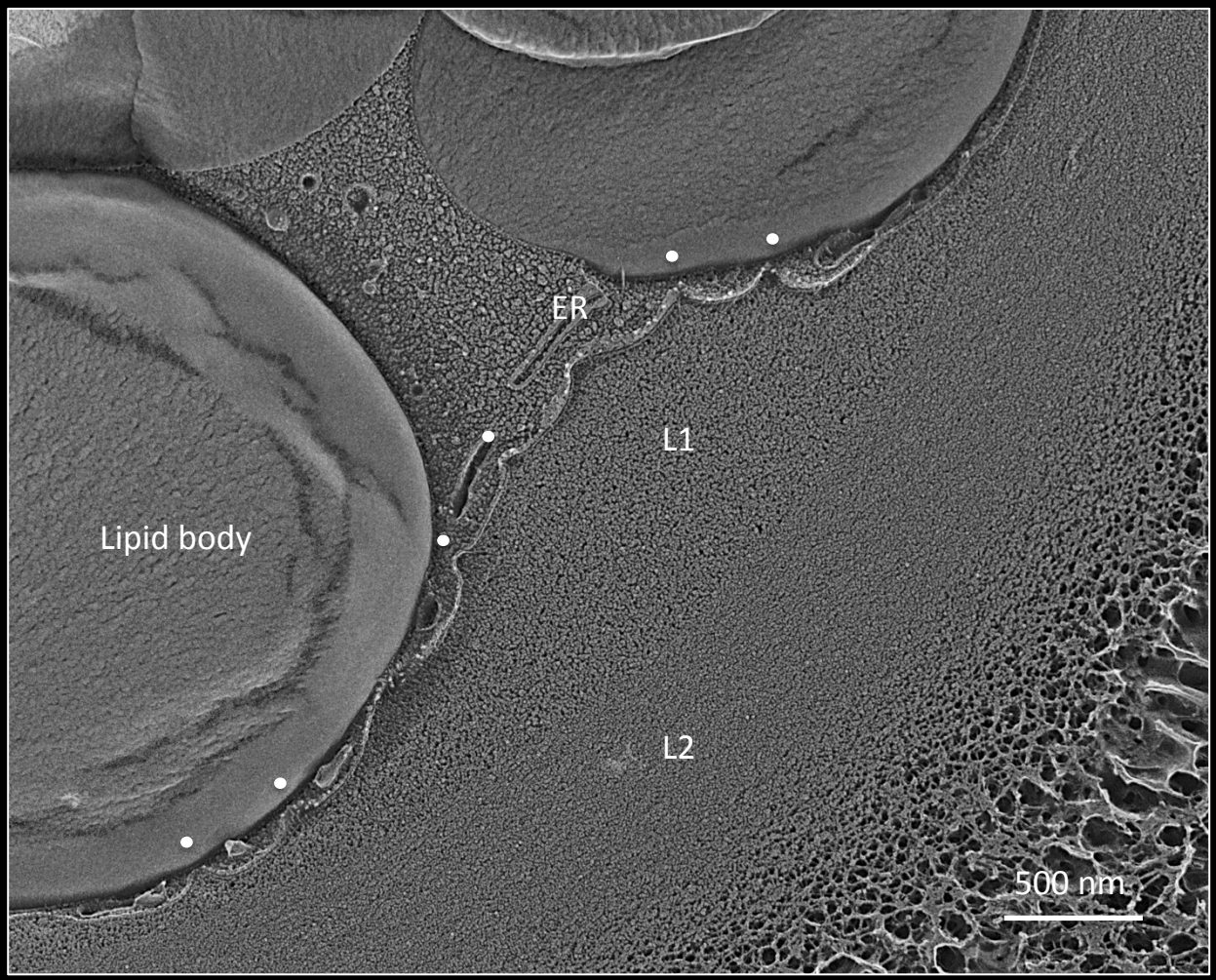


FIG S25 *Polytomella parva* cysts



White dots, eisosomes; L1, granular wall layer; L2 denser wall layer.

FIG S26 *Borodinellopsis texensis* cyst



White dots, eisosomes; L1, granular wall layer; L2 denser wall layer.

FIG S27 *Euplotes* sp. cysts
(A) Freeze-substituted thin section (B) convex faces
White dots: eisosomes

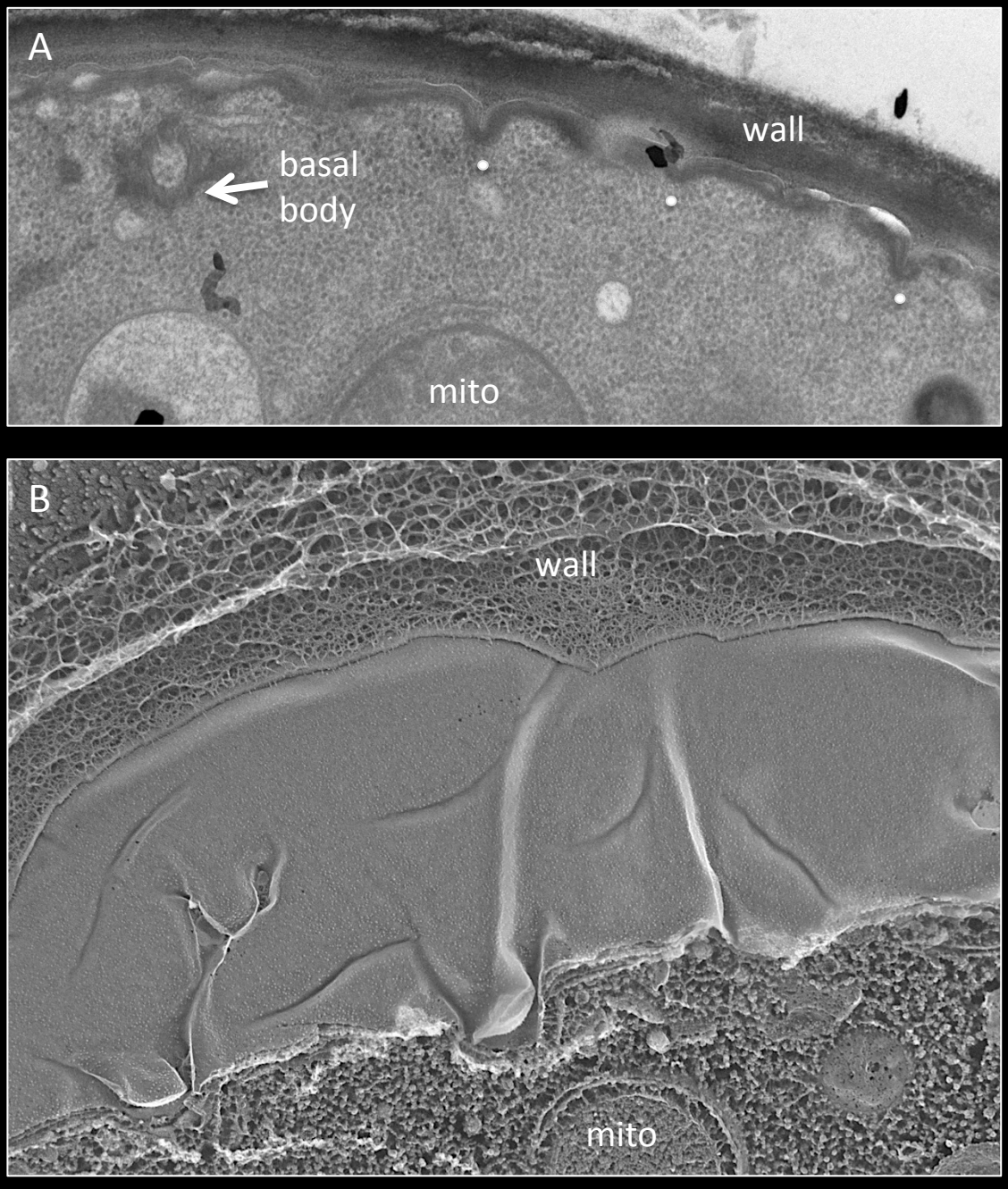
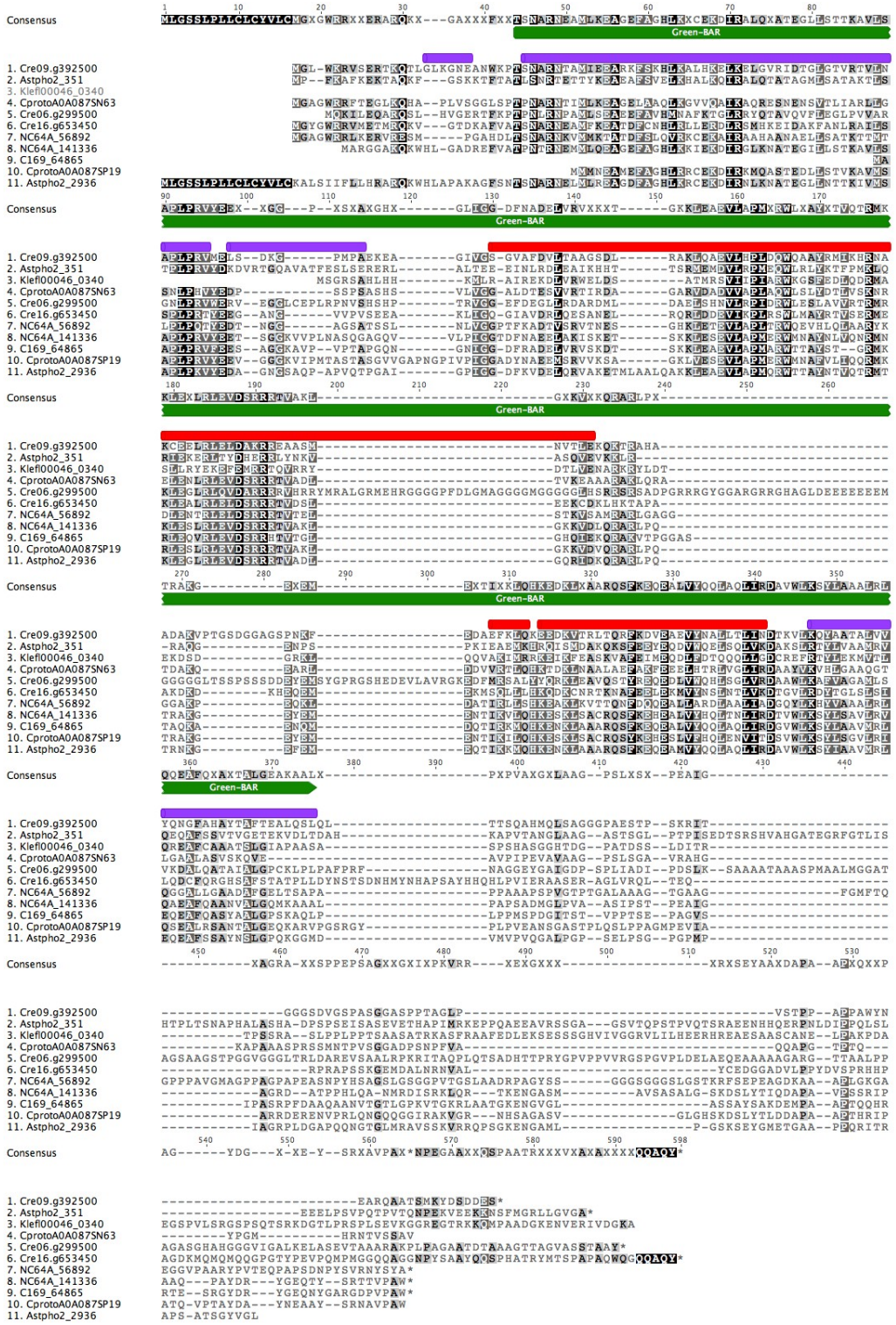


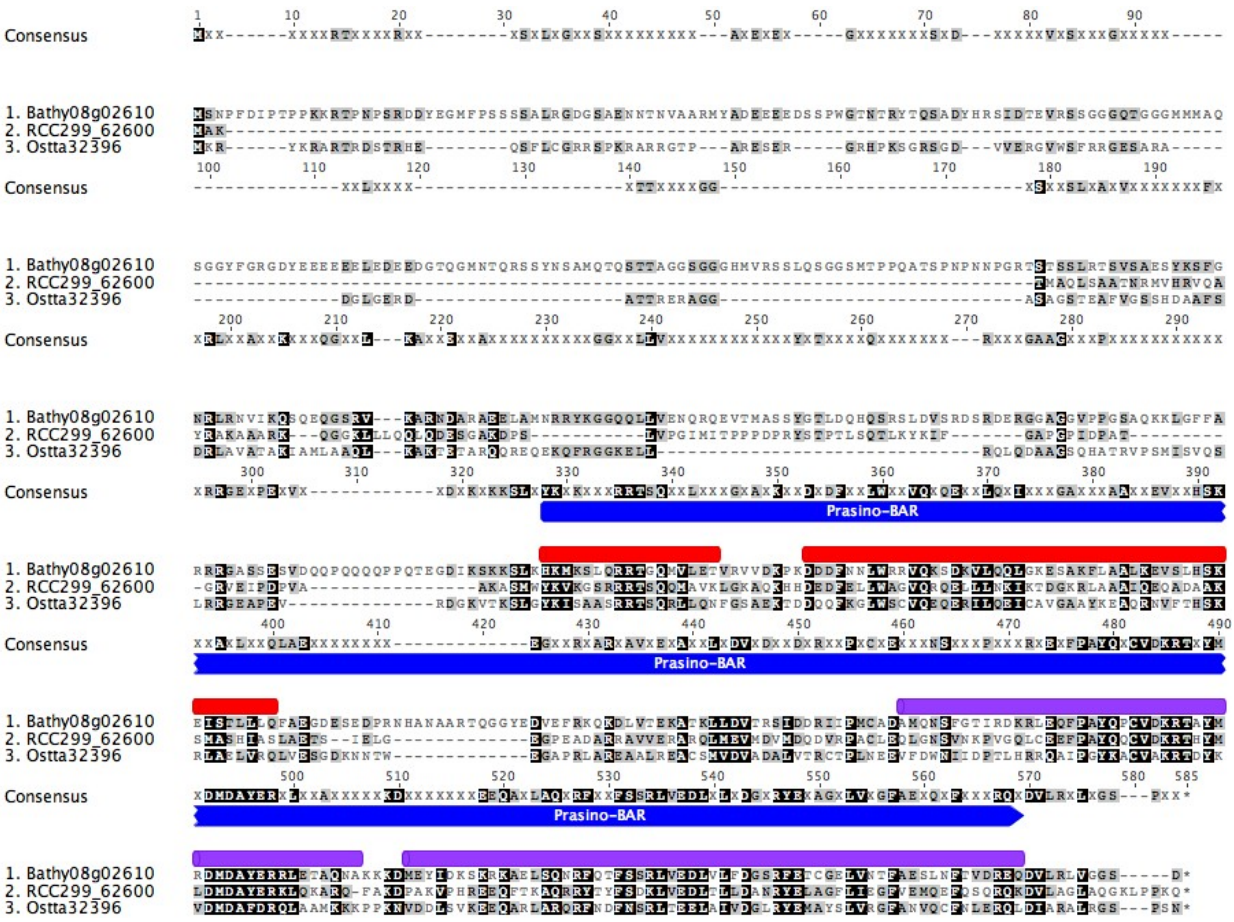
FIG S28 Alignment of Green-BAR proteins.

Red, predicted α-helix; purple, predicted amphipathic α-helix.



Cre, *Chlamydomonas reinhardtii*: Astpho2, *Asterochloris* sp.; Klefl, *Klebsormidium flaccidum*; Cproto, *Chlorella prototheocoides*; NC64A, *Chlorella* sp.; C169, *Coccomyxa subellipsoidea*.

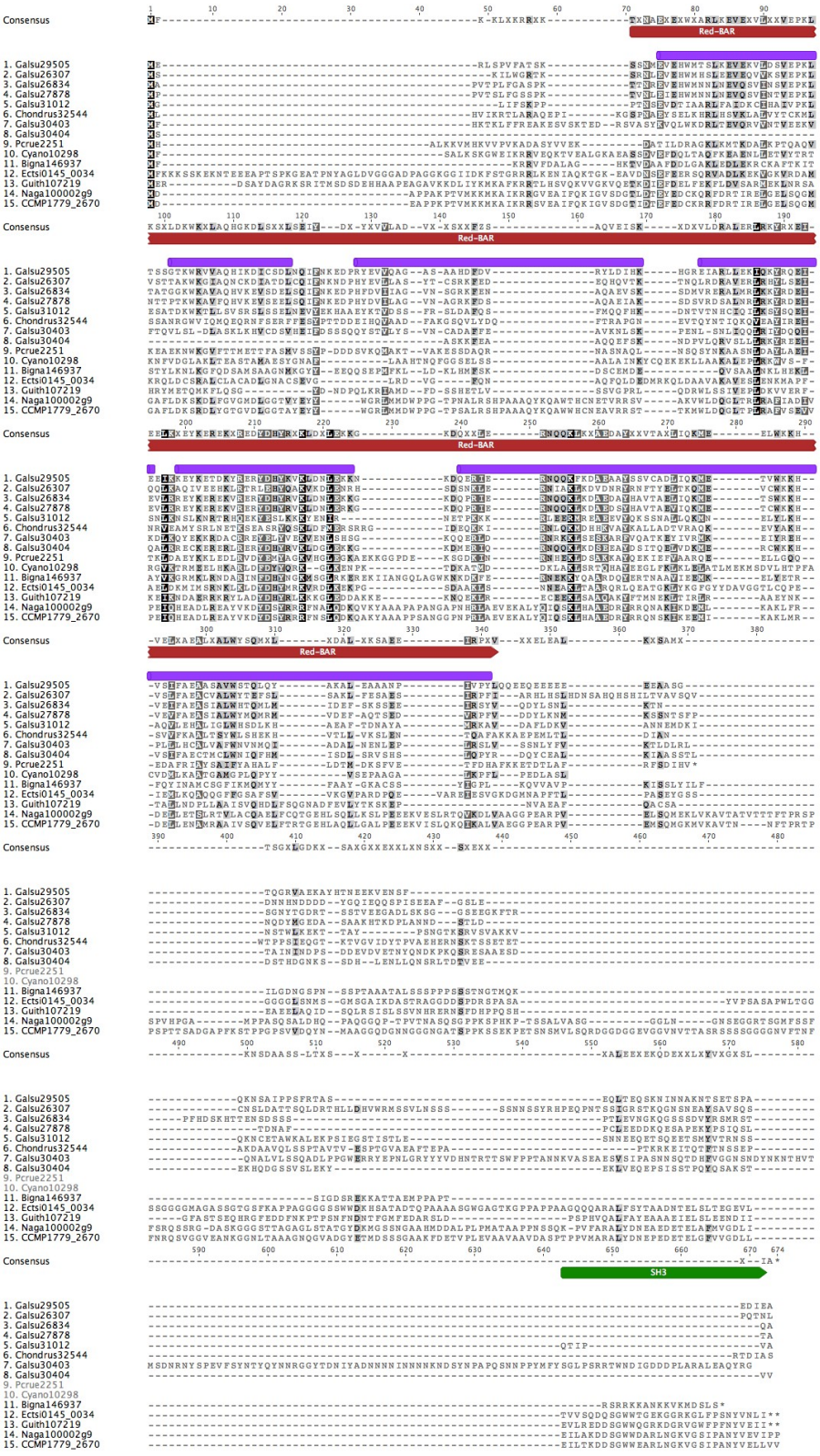
FIG S29 Alignment of Prasino-BAR proteins.
Red, predicted α-helix; purple, predicted amphipathic α-helix



Bathy, *Bathycoccus prasinos*; RCC299, *Micromonas pusilla*; Ostta, *Ostreococcus tauri*

FIG S30 Alignment of Red-BAR proteins.

Purple, predicted amphipathic α-helix



Galsu, *Galdieria sulfuraria*; Chondrus, *Chondrus crispus*; Pcrue, *Porphyridium cruensis*; Cyano, *Cyanophora paradoxa;* Bigna, *Bigelowiella natans*; Ectsi, *Ectocarpus siliculosus*; Guith, Guillardia theta*;* Naga, *Nannochloropsis gaditana*; CCMP, *Nannochloropsis oceanica*.

FIG S31 Alignment of yeast Lsp1/Pil1 eisosome proteins

Red, predicted α-helix; purple, predicted amphipathic α-helix

FIG S32 Alignment of animal amphypysin proteins

Red, predicted α-helix; purple, predicted amphipathic α-helix

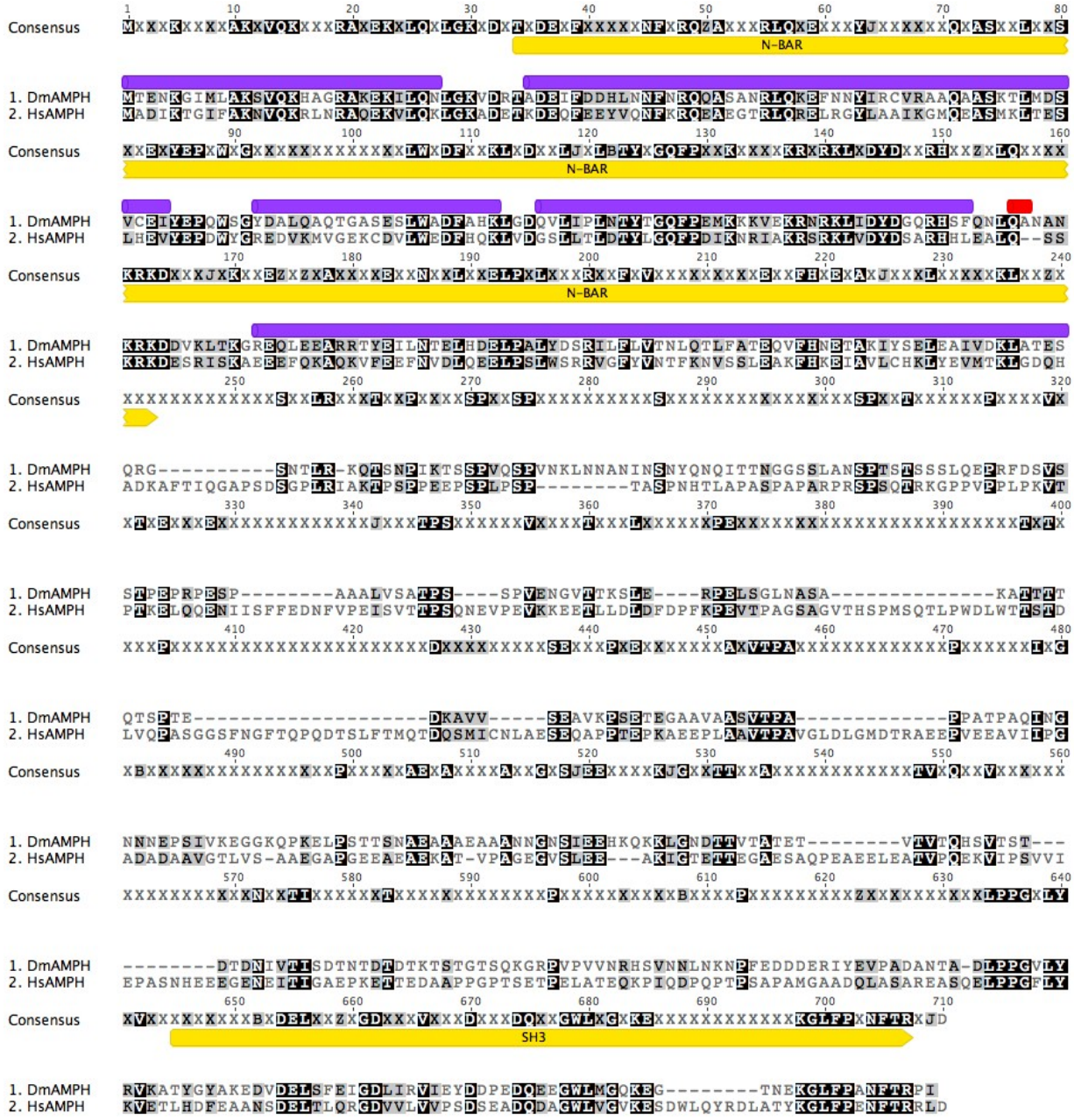


FIG S33 3D structure of the two faces of BAR-domain monomers of yeast Lsp1p (3plt), *Drosophila* amphiphysin (1uru), *Chlamydomonas* Green-Bar (Gbar) and *Galdieria* Red-BAR (3caz).

Surface charge potential: Blue, positive; Red, negative; Yellow, polar; White, hydrophobic
Arrows, N-terminal amphipathic α-helices

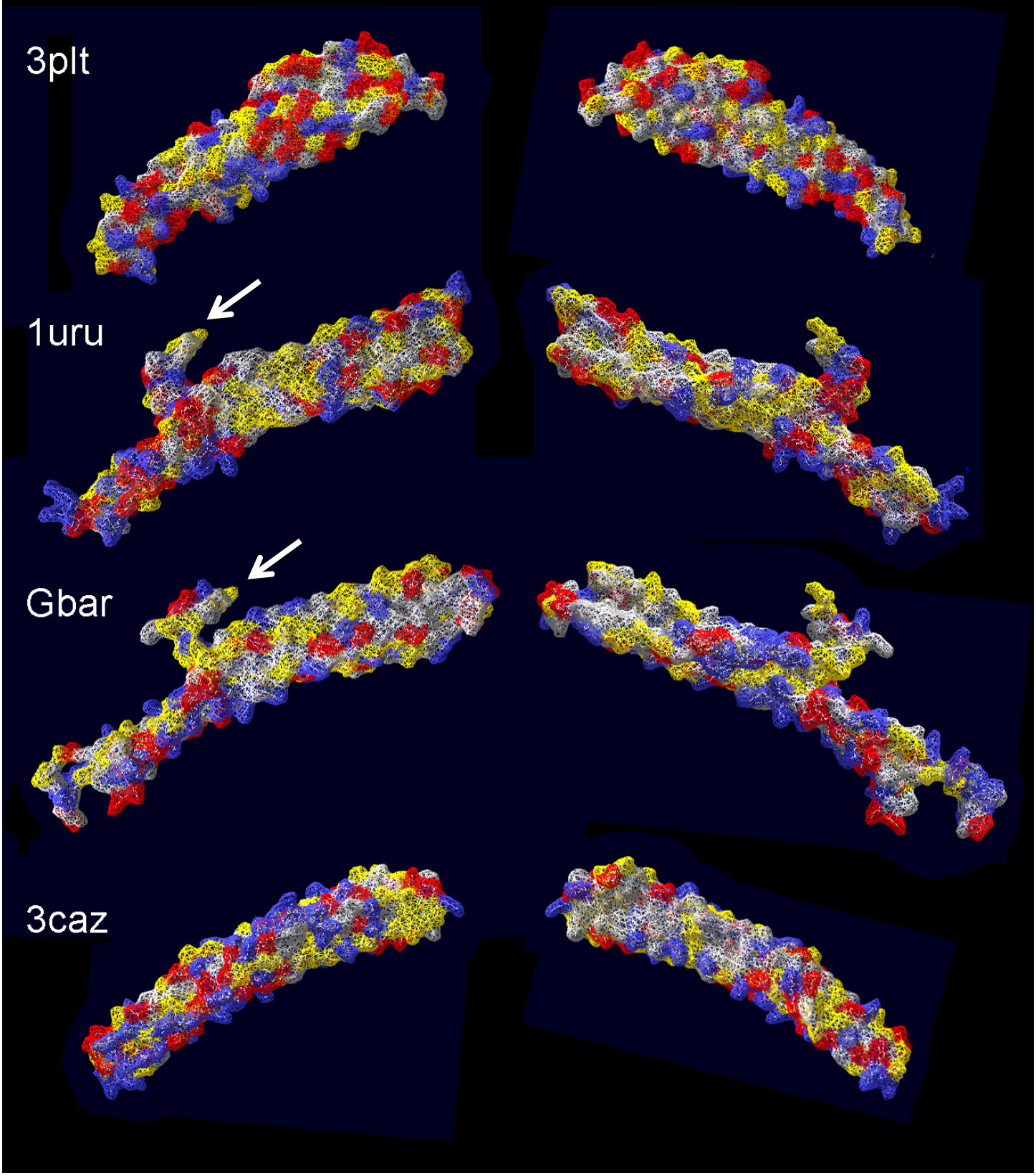


Table S1 Microalgae that do **not** produce eisosomes during vegetative growth.

Class	Genus and species	Cell wall?
Chlorophyceae	*Dunaliella tertiolecta*	No
	Dunaliella salina	No
	Polytomella parva	No
	Chlamydomonas reinhardtii	Yes
	Volvox carteri	Yes
	Haematococcus sp.	Yes
	Borodinellopsis texensis	Yes
Trebouxiophyceae	*Botryococcus braunii* Race A	Yes
	Botryococcus braunii Race B	Yes
	Botryococcus braunii Race C	Yes
Prasinophyceae	*Micromonas* CCMP 1545	No
	Bathycoccus RC 216	No (scales)
Cyanidiophyceae	*Cyanidioschyzon merolae*	No
Eustigmatophyceae	*Nannochloropsis salina*	Yes
	Nannochloropsis oceanica	Yes
	Nannochloropsis gaditana	Yes
	Nannochloropsis occulata	Yes
	Eustigmatos vischeri	Yes
Bacillariophyceae	*Phaeodactylum tricornutum*	Yes
	Thallasiosira pseudonanna	Yes
	Cyclotella cryptica	Yes
Chrysophyceae	*Ochromonas danica*	No
Phaeophyceae	*Pelagomonas* sp. CCMP 1756	Yes
Dinophyceae	*Symbiodinium* sp	Yes

Table S2. BAR-domain containing genes described in this report. Abbreviations: AGD, Arf-GAP; Ank, Ankyrin; PH, Pleckstrin-homology; PX, Phosphinositide-binding structural domain; RNI, RNase-inhibitor; SNX, Sorting nexin; Vps5, Vacuolar protein sorting 5; ext, additional C-terminal sequences with no identified sorting domains.

Species	ID	Domain Structure (protein length)	Family
Chlamydomonas reinhardtii			
	Cre16.g653450	BAR + ext (398 aa)	**Green-BAR**
	Cre06.g299500	BAR + ext (530 aa)	**Green-BAR**
	Cre09.g392500	BAR + ext (340 aa)	**Green-BAR**
	Cre03.g154150	BAR + PH + ArfGAP + Ank	AGD homolog
	Cre06.g281050	PX + Vps5	AtSNX1 homolog
	Cre07.g326450	PX + Vps5	AtSNX2b homolog
	Cre10.g462300	PX+divergent Vps5	AtSNX2b homolog
Chlorella sp. NC64A			
	jgi|ChlNC64A_1|141336	BAR + ext (359 aa)	**Green-BAR**
	jgi|ChlNC64A_1|56892	Divergent BAR + ext (752 aa)	**Green-BAR**
	jgi|ChlNC64A_1|142705	PX + Vps5	SNX homolog
Cocomixa subellipsoidea C-169			
	jgi|Coc_C169_1|64865	Divergent BAR + ext (296 aa)	**Green-BAR**
	jgi|Coc_C169_1|66447	PX + Vps5	SNX homolog
	jgi|Coc_C169_1|11161	PX + Vps5	SNX homolog
Asterochloris sp.			
	Astpho2|2936	BAR + ext (897 aa)	**Green-BAR**
	Astpho2|351	Divergent BAR + ext (465 aa)	**Green-BAR**
	Astpho2|8374	BAR + PH + ArfGAP + Ank	AGD homolog
	Astpho2|2830	PX + Vps5	SNX homolog
Chlorella protothecoides			
	Cproto_A0A087SP19	BAR + ext (347 aa)	**Green-BAR**
	Cproto_A0A087SN63	BAR + ext (315 aa)	**Green-BAR**
	Cproto_A0A087SN62	RabGAP-TBC + BAR	Others
Klebsormidium flaccidum			
	Klefl00046_0340	BAR + ext (323 aa)	**Green-BAR**
	Klefl00290_0020	BAR + PH + ArfGAP + Ank	AGD homolog
	Klefl00109_0220	PX + Vps5	SNX homolog
	Klefl00036_0250	PX + Vps5	SNX homolog
	Klefl00014_0220	BAR + ext (882 aa)	P-rich associated
Micromonas RCC299			
	jgi|MicpuN3|62600|	Ext + BAR (347 aa)	**Prasino-BAR**
	jgi|MicpuN3|89004|	BAR + PH + ArfGAP + Ank	AGD homolog
	jgi|MicpuN3|55855|	BAR + PH + ArfGAP	AGD homolog
	jgi|MicpuN3|62692|	PX + Vps5	SNX homolog

Table S2 (cont.)

Ostreococcus tauri		
jgi|Ostta4|32396|	Ext + BAR (428 aa)	**Prasino-BAR**
jgi|Ostta4|16066|	PX + Vps5	SNX homolog
jgi|Ostta4|35163|	PX + Vps5+P-rich	SNX homolog
jgi|Ostta4|36908|	PX + Vps5	SNX homolog
Bathycoccus prasinos		
Bathy08g02610	Ext + BAR (577 aa)	**Prasino-BAR**
Galdieria sulfuraria		
gi|452824006|gb|EME31012.1|	BAR + ext (303 aa)	**Red-BAR**
gi|452820841|gb|EME27878.1|	BAR + ext (286 aa)	**Red-BAR**
gi|452822486|gb|EME29505.1|	BAR + ext (292 aa)	**Red-BAR**
gi|452819782|gb|EME26834.1|	BAR + ext (297 aa)	**Red-BAR**
gi|452819243|gb|EME26307.1|	BAR + ext (307 aa)	**Red-BAR**
gi|452823393|gb|EME30404.1|	BAR + ext (218 aa)	**Red-BAR**
gi|452824006|gb|EME31012.1|	BAR + ext (303 aa)	**Red-BAR**
gi|452823392|gb|EME30403.1|	BAR + WW	**Red-BAR**
gi|452825554|gb|EME32550.1|	BAR + F-box + RNI	Others
Cyanidioschyzon merolae		
gnl|CMER|CMR296C	BAR only (237 aa)	Others
gnl|CMER|CMS157C	BAR only (243 aa)	Others
Cyanophora paradoxa		
ConsensusfromContig39931	BAR + ext (342 aa)	Others
ConsensusfromContig10298	BAR only (242 aa)	**Red-BAR**
ConsensusfromContig53923	BAR + ext (431 aa)	Others
ConsensusfromContig9140	Divergent BAR (138 aa)	Others
ConsensusfromContig9428	PX+BAR	SNX homolog
ConsensusfromContig48286	Vps5 only	SNX homolog
ConsensusfromContig55813	Vps5 only	SNX homolog
Chondrus crispus		
gi|507108597|emb|CDF32544.1|	BAR + ext (327 aa)	**Red-BAR**
Porphyridium cruensis		
evm.model.contig_2251.3	BAR + ext (250 aa)	**Red-BAR**
evm.model.contig_2493.1	BAR + ext (481 aa)	Others
evm.model.contig_3425.9	BAR + ext (517 aa)	Others
Bigelowiella natans		
jgi|Bigna1|146937|	BAR + ext (309 aa)	**Red-BAR**
Guillardia theta		
jgi|Guith1|107219|	BAR + SH3	**Red-BAR**
Ectocarpus siliculosus		
Esi0145_0034	BAR + SH3	**Red-BAR**
Nannochloropsis gaditana		
Naga100002g9	BAR + SH3	**Red-BAR**
Nannochloropsis oceania		
CCMP1779_2670	BAR + SH3	**Red-BAR**

Table S3 Genomes of algae and protists lacking Green-BAR and Red-BAR homologues.

Aureococcus anophagefferens (Pelagophyte)

Fragilariopsis cylindrus (Bacillariophyte)

Phaeodactylum triconutum (Bacillariophyte)

Thallasiosira pseudonana (Bacillariophyte)

Emiliania huxlei (Bacillariophyte)

Pytophthora sojae (Oomycete)

Oxytricha trifallax (Ciliate)

Ichthyophthirius multifiliis (Ciliate)

Styolnychia lemnae (Ciliate)

Paramecium tetraurelia (Ciliate)

Tetrahymena thermophile (Ciliate)