Eyelike “ocelloids” are built from different endosymbiotically acquired components as revealed by single-organelle genomics.

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Multicellularity is often considered a prerequisite for morphological complexity, as seen in the camera-type eyes found in several groups of animals. A notable exception exists in single-celled eukaryotes called warnowiid dinoflagellates, which have an eyelike “ocelloid” consisting of subcellular analogs to a cornea, lens, iris, and retina[1,8,9]. These planktonic cells are uncultivated and rarely encountered in environmental samples, obscuring the function and evolutionary origin of the ocelloid. By using isolated-organelle genomics, single-cell genomics, TEM, and FIB-SEM tomography, we show that ocelloids are built from pre-existing organelles, including a cornea-like layer made of mitochondria and a retinal body made of anastomosing plastids. We found that the retinal body forms the central core of a network of peridinin-type plastids, which in dinoflagellates and their relatives originated through an ancient endosymbiosis with a red alga[2]. As such, the ocelloid is a chimeric structure, incorporating organelles with different endosymbiotic histories. The anatomical complexity of single-celled organisms may be limited by the components available for differentiation, but the ocelloid shows that pre-existing organelles can be assembled into a structure so complex that it was initially mistaken for a multicellular eye[3]. Although mitochondria and plastids are acknowledged chiefly for their metabolic roles, they can also serve as building blocks for greater structural complexity in the absence of multicellularity.

Many organisms can orient to light. In single-celled algae, such as *Chlamydomonas* and many dinoflagellates, an "eyespot" directs photons onto photoreceptors on the flagellum, allowing the cell to respond to the intensity and direction of light[4, 5]. A vastly more complex structure is found in warnowiid dinoflagellates: the eye-like ocelloid. Ocelloids consist of subcellular components resembling a lens, a cornea, iris-like rings and a pigmented cup called the retinal body[6-9], which together so resemble the camera-type eyes of some animals that they have been speculated to be homologous[10] (Figures 1 and 2). The first description of a warnowiid was dismissed as a cell that had scavenged the eye from a jellyfish[3]. Ultrastructural studies of the ocelloid subsequently suggested that the retinal body might be derived from a plastid, in that it contains thylakoid-like membranes during cell division[4,8,9].

The ocelloid is among the most complex subcellular structures known, but its function and evolutionary relationship to other organelles remains unclear. This poor state of knowledge can be attributed to the fact that warnowiid cells are uncultivated and rarely encountered in environmental samples, with as few as two cells reported from the plankton per year for some species[11]. Modern single-cell genomics approaches, however, provide opportunities to study uncultivated eukaryotes at the molecular level, including rare species[12,13,14]. In an attempt to learn more about the cell biology of ocelloids, we employed a single-cell genomics approach on three different genera of warnowiids: *Erythropsidinium agile*, *Warnowia* sp., and *Nematodinium* sp. We also investigated ultrastructure details and the phylogenetic origin of the retinal body in *Nematodinium* sp. by using focused ion beam scanning electron microscopy (FIB-SEM) and a novel, single-organelle genomics approach.

Plastid and mitochondrial features of the ocelloid

Thylakoid-like structures have been reported only once before in the retinal body[6], so we examined the ultrastructure of the ocelloid in *Nematodinium* sp. and *Erythropsidinium agile* using single-cell transmission electron microscopy (TEM). During interphase, the retinal body contains highly ordered waveform membranes (Figure 2), which are perpendicular to the plane expected for thylakoids in a chloroplast. However, we confirmed that near the end of interphase, the waveform membranes dedifferentiated into a plastid-like arrangement made of double-stacked thylakoid-like structures (Supplementary Figure 2). Thus, the thylakoids and waveform membranes represent two modes of the same membrane system. Moreover, we

found that the retinal body of *Nematodinium* sp. fluoresces strongly under 505 nm light—the primary wavelength for chlorophyll excitation (Supplementary Figure 1e). In *Nematodinium*, we also found mitochondria in the ocelloid; where they formed a cornea-like layer overlying the lens (Figure 1c, Supplementary Figure 3)[1].

Single-cell and single-organelle genomics show a plastid origin of the retinal body

In order to further investigate the possible plastid origin of the retinal body, we first examined transcriptomes from isolated cells of *Erythropsidinium agile* and *Warnowia* sp., both of which lack photosynthetic plastids. From polyadenylated cDNA libraries, we found that these heterotrophic genera expressed multiple photosynthesis-related genes, including light-harvesting proteins (Supplementary Table 1). In addition, *Warnowia* sp. expressed three transcripts corresponding to the chloroplast-soluble peridinin-chlorophyll-binding protein, which is distinctive for dinoflagellate peridinin-type plastids[14]. These proteins are likely present in the retinal body because it is the only pigmented structure apparent in light micrographs of *Erythropsidinium agile* and *Warnowia* sp. (Supplementary Figures 1a-b).

The provenance of the retinal body is, however, complicated by the complex evolution of plastids in dinoflagellates[15]. While the ancestral peridinin-type plastid of dinoflagellates was initially acquired from a red alga, several dinoflagellates have since replaced this plastid with those from either a haptophyte, a cryptophyte, a diatom, or a green alga, and several non-photosynthetic lineages have been found to possess relict plastids[2,15,16]. In order to investigate the phylogenetic origin of the retinal body more directly, we characterized genes from isolated organelles. Single cells of *Nematodinium* sp. were micro-dissected, and their individual retinal bodies were isolated (Figure 1). The individual retinal bodies from five cells were pooled, lysed, and their DNA was amplified with phi29 polymerase through multiple displacement amplification. As a control for contamination from remnants of the host cell cytoplasm during microdissection (e.g., from cryptic peridinin plastids clinging to the retinal bodies), we also pooled five intact *Nematodinium* sp. cells and subjected them to the same procedures for DNA amplification and sequencing. From sequence databases derived from both samples, we identified genes that are encoded in the plastid of other dinoflagellates. Overall, six plastid genes were identified from isolated retinal bodies, PsaB, PsbA, PsbB, PsbD, PetB, PetD, spanning photosystems I and II. These genes grouped strongly with the peridinin-containing plastids of dinoflagellates in individual and concatenated phylogenetic analysis (Figure 3, Supplementary

Figures 6-7), and collectively, plastid-encoded genes represented 13% of all reads. By contrast, the proportion of plastid/nuclear DNA in the whole-cell amplification was <0.0001%. The representation of plastid DNA in the retinal body was, therefore, over 1,600 fold higher than in whole cells (Figure 1).

Reconstructing organelle networks with FIB-SEM tomography

Although the genomic data suggests that the retinal body is a derived plastid, there is another potential source of plastid DNA within the cell. Our isolates of *Nematodinium* contained small brown-pigmented bodies with double-stacked thylakoids typical of peridinin-type plastids. The presence of these plastids in addition to the retinal body raises the possibility that *Nematodinium* has two different morphotypes of peridinin plastids within the same cell. However, the physical relationship between these plastid types was unclear because the retinal body does not appear connected to the more canonical plastids under TEM; moreover, the retinal body appears differentiated throughout the cell cycle and produces daughter retinal bodies through binary fission[6,8].

To investigate the physical connections between the different components of the ocelloid and surrounding structures, such as peridinin-type plastids, we performed FIB-SEM tomography on a single isolated cell of *Nematodinium* sp. The three-dimensional reconstructions of our FIB-SEM data demonstrated that the outer membrane of the retinal body is fused to a network of adjacent plastids, forming a membranous web throughout the cell (Figure 4e). Complex plastid networks such as this have been described in other algal lineages (e.g., euglenophytes and green algae), but they are difficult to infer from individual TEM sections and were accordingly overlooked in previous ultrastructural studies of *Nematodinium*[20].

Tomographic reconstructions also confirmed a close association between mitochondria and the lens of the ocelloid. The mitochondria surrounding the lens were interconnected and formed a sheet-like “cornea” layer that was consistent with TEM data. The corneal layer surrounded all regions of the lens except for a few minor perforations and the side facing the retinal body (Figure 4e). Like the connection of the retinal body to the plastid network, the corneal mitochondria appear to form a continuous network with mitochondria in the nearby cytoplasm. The ocelloid, therefore, represents an intriguing mixture of components with endogenous and endosymbiotic origins; the lens composed of stacked vesicles formed

endogenously in the endomembrane system, and the retinal body and cornea layer are plastids and mitochondria, respectively (Figure 4)[15, 20, 21].

Prior to this study, there was little evidence for homology between the ocelloid and other structures found in dinoflagellates[4]. Based on its resemblance to camera-type eyes, a relationship was even suggested between the ocelloid and the eyes of some animals[10]. To the contrary, our findings indicate that the ocelloid is a conglomerate of several membrane-bound organelles, including endomembrane vesicles, mitochondria and plastids. The ocelloid is likely homologous to the much simpler eyespots found in several other lineages of dinoflagellates (Supplementary Figure 4), most of which share features in common with the peridinin plastid[4,18,19]. Peridinin plastids stem from an ancient red alga that was incorporated by the common ancestor of all myzozoans (dinoflagellates, chromerids, and apicomplexans), many of which (including all apicomplexans) subsequently lost photosynthesis and reduced their plastids to cryptic, morphologically simple structures[2]. While morphological reduction is a common trend among endosymbiotic organelles, the ocelloid in warnowiids demonstrates that increased complexity can also arise.

A hypothesis for the function of ocelloids

In order to understand the function of the ocelloid, a basic knowledge of the life history of warnowiid dinoflagellates is required. Understanding warnowiid behavior is a very difficult problem, however, because their cells are rarely encountered, have never been cultivated and degrade rapidly when removed from the plankton[11]. Nevertheless, we observed one important detail of warnowiid life history using TEM of individual cells isolated directly from the ocean. Specifically, we found that the food vacuoles in *Nematodinium* contained trichocysts (Supplementary Figure 5), which are defensive extrusive organelles found in dinoflagellates[26]. These data suggest that *Nematodinium* feeds on other dinoflagellates, so one hypothesis is that the ocelloid is involved in the detection of other dinoflagellates as prey. Some dinoflagellates are capable of bioluminescence[27], but most transmit polarized light through their distinctively large nucleus of permanently condensed chromosomes[28]. An intriguing possibility is that the ocelloid sensing polarized light, and by extension preferred prey. Testing such a specific phototactic behaviour will be challenging until warnowiids are brought into culture. Nevertheless, the genomic and detailed ultrastructural data presented here have resolved the

basic components of its origins, and demonstrate how evolutionary plasticity of mitochondria and plastids can generate an extreme level of subcellular complexity that ostensibly supports predatory lifestyles within marine planktonic communities.

METHODS

Collection

From 2005 to 2009, *Erythropsidinium agile* and *Warnowia* sp. were collected from the marine water column in the vicinity of Shimoda, Japan. On an inverted light microscope, cells of *Erythropsidinium agile* were identified based on the presence of an ocelloid and a piston organelle (Supplementary Figure 1b, Supplementary Video 1). Cells of *Warnowia* sp. were recognized as ocelloid-bearing cells encircled three or more times by a helical groove (Supplementary Figure 1a, Supplementary Video 2). In the summer of 2012 and 2013, *Nematodinium* sp. was collected from surface water in Bamfield Inlet, Bamfield, BC, Canada with a 20 micron plankton net. Cells of *Nematodinium* sp. were identified based on the presence of an ocelloid and nematocysts (Supplementary Figure 2c). Uncultivated *Nematodinium* sp. cells containing putative prey organisms (visible as pigmented vacuoles) were chosen for transmission electron microscopy, so that their feeding habits could be inferred from intracellular remnants (Supplementary Figure 5).

cDNA Libraries from Warnowia sp. and Erythropsidinium agile

Total RNA was extracted from two cells of *Erythropsidinium agile*, and four cells of *Warnowia* sp. Polyadenlyated transcripts were reverse transcribed and cloned into competent *E. coli* cells **(from ? kit)**. We obtained 1,152 and 2,658 clones from *Erythropsidinium agile* and *Warnowia* sp., respectively. Of these, 752 and 1,180 had cDNA inserts, and were Sanger sequenced (Supplementary Table 1).

Fluorescence and DIC Microscopy

Red epifluorescence of the *Nematodinium* sp. retinal body was excited with a 505 nm Argon laser on a Zeiss Axioplan inverted microscope (Supplementary Figure 1a). Differential interference contrast (DIC) observations of *Nematodinium* sp., *Warnowia* sp., and *Erythropsidinium agile*, were performed using the same microscope (Supplementary Figure 1).

Single-Cell Transmission Electron Microscopy of Uncultivated *Nematodinium* sp.

Each isolated cell of *Nematodinium* sp. was micropipetted onto a poly-L-lysine coated slide. Cells were fixed with 2% glutaraldehyde in filtered seawater for 30 min on ice. After two washes in filtered seawater, cells were post-fixed in 1% OsO_4 for 30 min. Cells were dehydrated through a graded series of ethanol (50%, 70%, 85%, 90%, 95%, 100%, 100%), infiltrated with acetone-resin mixtures (acetone, 2:1, 1:1, 1:2, Epon 812 resin), and embedded in Epon 812 resin. Polymerization at 60° C produced a resin-embedded cell affixed to the glass slide. Using a power drill, resin was shaved to a 1 mm^3 block, which was removed from the glass slide with a fine razor. The block, containing a single cell, was super glued to a resin stub in the desired orientation for sectioning. Thin sections were produced with a diamond knife, post-stained with uranyl acetate and lead citrate and viewed under a Hitachi H7600 Transmission Electron Microscope.

Isolation of the Retinal Bodies of *Nematodinium* sp.

In preparation for single-organelle genomics, five cells of *Nematodinium* sp. with no visible prey contents were selected in order to minimize the chances of genetic contamination. Each cell of *Nematodinium* sp. was micropipetted onto a slide in a droplet of TE buffer and affixed to a patch of Poly-L-Lysine. Cells were lysed with nuclease-free water. The nucleus and other cell contents were gently dislodged with rinses of TE buffer, leaving the retinal body behind for manual isolation (Figure 1d). Five different retinal bodies were isolated and pooled onto a new, sterile slide, and washed three times with TE buffer.

Single-Organelle Genomics of *Nematodinium* sp.

In order to test for the presence of a plastid genome in the retinal body, we performed genomic amplification using phiX 29 polymerase (Repli-G mini kit, Qiagen) on five individually isolated retinal bodies that were then pooled together. We performed a control reaction by amplifying a pool of five whole cells of *Nematodinium* sp. using the same procedures as for the retinal bodies. The whole cell amplification provided a measure of overall plastid DNA concentration, against which the retinal body plastid DNA concentration could be compared. In order to minimize amplification bias, each reaction was divided into four aliquots, run in parallel, and pooled after the 15 hr amplification period. Paired end sequencing on an Illumina MiSeq yielded 9,798 reads from the retinal bodies, versus 501,338 reads from whole cells. From these

reads, plastid genes were assembled using the de novo assembly program Ray, which fragmented the reads into a variety of hash sizes ("kmers"), then assembled them. We found the assembly from 53 bp kmers to be optimal, recovering six partial plastid genes (Figure 1d,e). In order to estimate the concentration of plastid reads in the whole cell vs. isolated retinal body amplifications, we counted plastid reads in Bowtie [**Ref**], a read mapping program, then divided them by the total number of reads in each amplification to measure plastid gene concentration. (Figure 1d,e).

Molecular Phylogenetic Analyses

The six plastid genes, photosystem I P700 apoprotein A2 (PsaB), photosystem II protein D1 (PsbA), photosystem II CP47 protein (PsbB), photosystem II protein D1 (PsbD), cytochrome b6 (Petb), and cytochrome b6/f complex subunit 4 (PetD) were translated, and their amino acids aligned with a representative set of eukaryotes in Muscle [**Ref**], with fast-evolving and ambiguously aligned regions removed in Gblocks 0.91b [**Ref**]. Accession numbers are listed in Supplementary Figures 6 and 7. The concatenated alignment of 1,618 amino acids is available upon request. The amino acid substitution model (Protein GTR gamma) was estimated using the Models package in Mega 6.0.5 [**Ref**]. A maximum likelihood phylogeny was run with 500 bootstraps in RAxML. A second, Bayesian analysis was run for 10,000 generations in MrBayes 3.2 [**Ref**], using the high-heating setting of (nchains = 4), to account for rapid evolution of dinoflagellate plastids. These maximum likelihood analyses were run both for the mutliprotein dataset, and also for each protein gene individually (Supplementary Figures 6 and 7). A dinoflagellate phylogeny was estimated using 18S and 28S ribosomal rDNA sequences, concatenated as 2,331 nucleotide alignment, across 36 dinoflagellate taxa including published sequences from *Nematodinium* sp., *Warnowia* sp. and *Erythropsidinium agile*.

Focused Ion Beam Scanning Electron Microscopy (FIB-SEM)

Cells of *Nematodinium* sp. were individually transferred into a droplet of filtered seawater. Cells were frozen immediately to minimize fixation artefacts, using a Leica EM HPM 100 high-pressure freezer (Leica, Wetzlar, Germany). Freeze substitution was subsequently used to remove the aqueous content of the cells and replace it an acetone solution containing 5% water, 1% osmium tetroxide and 0.1% uranyl acetate, at -80 °C for 48 hours, -20 °C for 6

hours, then graded back to 4 °C over 13 hours. The prepared samples were washed twice in 100% acetone. Two cells were recovered by micropipette. Each cell was placed on a separate Thermonox (Fahlenbach, Germany) coverslip, where it adhered to a patch of poly-L-lysine. In preparation for FIB-SEM, cells were infiltrated with a 1:1 mix of acetone and Embed 812 resin for two hours, then 100% resin overnight. A second Thermonox coverslip was applied, sandwiching each cell in a thin layer of resin between the coverslips. Resin was polymerized at 65 °C for 24 hours. The top coverslip was then removed with a razor blade to expose the resin face overlying the cell.

A single cell was imaged by a FEI Helios NanoLab 650 dual beam FIB-SEM. The ion beam milled through the cell in 20 nm increments, yielding 190 image slices. Slices were aligned as a z-stack in Amira 5.5. Features of interest, including mitochondria and chloroplasts, were semi-automatically segmented: that is, manually traced in approximately one of every three slices, before automatic interpolation filled in the volumes between the slices. Images that did not pass quality screening because of fluctuations in microscope beam power and autofocus were not directly segmented, but were interpolated from segmentation on neighbouring images, per manufacturer's instructions. Surfaces of the mitochondria, chloroplasts, and vesicles were generated, smoothed and colorized to produce a 3-dimensional model of the components that form the ocelloid.

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Figure Legends

Figure 1: **Genomics and structure of organelles in the ocelloid.** a. Illustration of *Nematodinium* sp. showing the basic components of the ocelloid with their putative organellar origins. b. Transmission electron micrograph of the ocelloid of *Erythropsidinium agile*, including the lens (l), crystalline layer (c), and retinal body (R). c. Transmission electron micrograph of the ocelloid of *Nematodinium* sp., depicting the edge of the lens (l) where it is overlain by a cornea like layer of mitochondria (asterisks). d. Genomic reads amplified from five whole cells of *Nematodinium* sp. e. Genomic reads amplified from five retinal bodies after they were micro-dissected from individual cells of *Nematodinium* sp.

Figure 2: **Ultrastructure of the retinal body in *Nematodinium* sp.** A composite of 12 electron micrographs depicting a glancing section through the retinal body and pigmented ring or "iris" of *Nematodinium* sp. Scale bar = 1 µm. Crystalline layer (c). Lipid droplets (l). Pigmented ring (p) Waveform membranes (W).

Figure 3: **Phylogeny of retinal-body encoded proteins.** Six partial plastid genes from the retinal body of the ocelloid in *Nematodinium* sp. were amplified. Photosystem I P700 apoprotein A2 (PsaB), photosystem II protein D1 (PsbA), photosystem II CP47 protein (PsbB), photosystem II protein D1 (PsbD), cytochrome b6 (Petb), and cytochrome b6/f complex subunit 4 (PetD) were translated and concatenated for a 1,618 amino acid alignment. The tree was inferred by analyzing the 42-taxon alignment using maximum likelihood (ML). Statistical support for the branches was evaluated using 500 ML bootstrap replicates and Bayesian posterior probabilities. Support values are shown for all branches within the Myzozoa, a clade comprised of dinoflagellates, chromerids, and apicomplexans. Groups bearing plastids of red algal ancestry are highlighted in red or pink. Other plastids and cyanobacteria are shown in grey.

Figure 4: Three-dimensional reconstruction of the ocelloid of *Nematodinium* sp. using FIB-SEM tomography. a. Stack of a halved cell, showing the nucleus and ocelloid (box). b. FIB-SEM slice of the ocelloid, depicting lens, mitochondria (blue) and retinal body (red). c. Translucent FIB-SEM stack of the region surrounding the ocelloid, including lens (yellow) and full plastid network

(red). d. Reconstructions of the ocelloid and its component parts, including the mitochondrial "cornea" layer, vesicular lens, and retinal body.

Supplementary Table 1. **Plastid-associated proteins expressed by heterotrophic warnowiids.** From *Erythropsidinium agile* and *Warnowia* sp., 752 and 1,180 polyadenylated cDNAs were sequenced, respectively. Transcripts predicted to function in the photosystem are shown, annotated via BlastX.

	Annotation	Clone Count	Top BlastX Hit	Error
Erythropsidinium agile	Light harvesting protein	2	*Symbiodinium* sp. C3	2^{-67} 8^{-23}
Warnowia sp.	Light harvesting protein	3	*Symbiodinium* sp. C3	1^{-48} 1^{-37} 4^{-37}
	Chloroplast ferredoxin	2	*Alexandrium fundyense*	7^{-29} 1^{-17}
	Chloroplast soluble peridinin-chlorophyll-a-binding protein precursor	3	*Symbiodinium microadriaticum*	1^{-31} 1^{-25} 1^{-21}

Supplementary Figure Legends

Supplementary Figure 1: **Light micrographs (LM) of warnowiids used in this study.** a. Still frame LM from a video of *Warnowia* sp. b. LM of *Erythropsidinium agile*. c. LM of *Nematodinium* sp. with a nematocyst (arrowhead). d. LM of the ventral side of *Nematodinium* sp. showing red pigmentation of the retinal body. e. Epifluorescence image of the same cell and angle, showing red fluorescence of the retinal body excited by 505 nm light. f. LM of *Nematodinium* sp. showing a bright spot of reflectivity (i.e., "eyeshine") (arrowhead) in the ocelloid. Scale bars = 10 µm.

Supplementary Figure 2: **Transmission electron micrographs (TEM) of thylakoid membranes in *Nematodinium* sp.** a. TEM showing a small, peripheral plastid in *Nematodinium* sp. with typical thylakoids (t) resembling peridinin plastids in other dinoflagellates. b. TEM showing thylakoids in the iris region of the ocelloid. c. TEM showing thylakoids in the iris positioned beside waveform membranes (w) of the retinal body, during interphase. d. TEM showing a retinal body towards the end of interphase, in which the waveform membranes dedifferentiate and are continuous with the typical thylakoids.

Supplementary Figure 3: **Transmission electron micrographs (TEM) of the cornea-like layer of mitochondria in the ocelloid of *Nematodinium* sp.** a: Low-mag TEM of the ocelloid, with rectangles delimiting the areas of higher magnification shown in images b, c and d. b, c, d: High magnifications of structures bordering the lens (L). Asterisks = mitochondria. P = pigmented ring. R = retinal body.

Supplementary Figure 4: **Dinoflagellate eyespot types within a phylogenetic context.** Diagrams of whole cells and eyespots are shown for all dinoflagellates for which both ultrastructural descriptions and 18S and 28S ribosomal rDNA sequences have been published. Eyespot diagrams highlight plastid-like structures (crimson), as well as mitochondria (dark blue), lens-like vesicles (light blue), lipid droplets (red dots), and crystalline layers (grey dashes). The phylogenetic tree was inferred from a 2,331-nucleotide alignment of concatenated 18S and 28S ribosomal rDNA sequences across 36 genera; statistical support was evaluated with 500 bootstraps using maximum likelihood and 10,000 generations of Bayesian analysis. Bootstrap

values above 60% are shown. For some taxa, 18S and 28S ribosomal sequences were concatenated from different species within the genus. Only the genus is shown for these taxa.

Supplementary Figure 5: **Light micrographs (LM) and transmission electron micrographs (TEM) showing food vacuoles in *Nematodinium* sp.** a. Differential interference contrast LM showing a cell with prey (P) visible as green tinted food vacuole. b. Differential interference contrast LM showing a cell in which the condensed dinoflagellate-type nuclei are visible as birefrigent chromosomes (n) in both the predator and prey. c. Differential interference contrast LM of a *Nematodinium* sp. cell containing digested prey (arrowhead) and co-occuring with potential prey, a smaller dinoflagellate. d. TEM showing a food vacuole inclusion consisting of a bolus of discharged trichocysts. e. Longitudinal TEM section of discharged dinoflagellate-type trichocysts showing their characteristic striation pattern.

Supplementary Figure 6: **Individual ribosomal gene and photosystem protein gene trees.** For c. and d., the photosystem genes for *Nematodinium* sp. were amplified from the retinal body of the ocelloid. Support values for all phylogenies were calculated from 100 bootstraps using Maximum Likelihood analysis. a. 18S ribosomal rDNA gene phylogeny derived from a 1,717 bp alignment across 33 dinoflagellate taxa. b. 28S ribosomal rDNA gene phylogeny derived from a 970 bp alignment across 43 dinoflagellate taxa. For both a. and b., warnowiids are highlighted in yellow and *Nematodinium* sp. is highlighted in black. c. Photosystem I P700 apoprotein A2 (PsaB) protein phylogeny derived from a 508 AA alignment across 42 photosynthetic taxa. d. Photosystem II protein D1 (PsbA) protein phylogeny derived from a 360 AA alignment across 39 photosynthetic taxa. For c. and d., dinoflagellates are shaded in grey, and *Nematodinium* sp. is highlighted in black.

Supplementary Figure 7: **Individual photosystem protein trees.** All the photosystem genes from *Nematodinium* sp. were amplified from the retinal body of the ocelloid. Support values for all phylogenies were calculated from 100 bootstraps using Maximum Likelihood analysis. a. Photosystem II CP47 (PsbB) protein phylogeny derived from a 504 AA alignment across 38 photosynthetic taxa. b. Photosystem II protein D1 (PsbD) phylogeny derived from an 342 AA alignment across 42 photosynthetic taxa. c. Cytochrome b6 (Petb) protein phylogeny derived from an 216 AA alignment across 32 photosynthetic taxa. d. Cytochrome b6/f complex subunit

457 4 (PetD) protein phylogeny derived from an 161 AA alignment across 31 photosynthetic taxa.

458 Dinoflagellates are shaded in grey, and *Nematodinium* sp. is highlighted in black.