Evidence for the acquisition of a proteorhodopsin-like rhodopsin by a chrysophyte-infecting giant virus

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Running Head: Proteorhodopsin encoded by chrysophyte virus

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Abstract

Chrysophytes are widespread nanoflagellate protists in aquatic ecosystems with diverse trophic roles as primary producers and bacterivores. While molecular evidence suggests that chrysophytes are commonly infected by giant viruses, no previous isolates have been reported. Here, we describe the first isolated chrysophyte-infecting virus, Chrysophyceae Clade H virus SA1 (ChrysoHV). ChrysoHV and its mixotrophic host were isolated from surface waters in the tropical North Pacific. The ChrysoHV capsid (290 ± 40 nm diameter) is associated with a loose, sac-like membrane that extends its effective diameter (720 ± 120 nm), and presents a long, thin tail extending 1,200 (± 240) nm in length. This morphology has not been previously observed in other virus isolates or environmental surveys. The sequenced, assembled genome is 1.19 Mbp, and phylogenetic analysis places ChrysoHV as the third cultivated member of the *Aliimimivirinae* subfamily in the *Mimiviridae* family of giant viruses. ChrysoHV is the first cultivated member of *Aliimimivirinae* to encode a rhodopsin. Moreover, ChrysoHV encodes three rhodopsins total, two heliorhodopsins and one proteorhodopsin. No other virus-derived rhodopsin has been found in the proteorhodopsin clade, and the loss of retinal binding hints at a light-independent function. ChrysoHV also encodes nine genes with closest known homologs from marine cyanobacteria, mostly related to nutrient uptake. This suggests that lateral gene transfer occurs between abundant marine bacteria and giant viruses, supporting the conceptual model that genes are horizontally transferred between ingested prey and infectious virions within predatory protists.

Importance

Chrysophytes are abundant eukaryotic phytoplankton with diverse trophic strategies ranging from photosynthesis to phagotrophy, and have been studied as models of mixotrophy among marine protists. However, no chrysophyte-infecting viruses had been cultivated, leaving a major gap in our understanding of viral diversity. This study reports the functional and genomic characterization of the first isolated chrysophyte-infecting virus, the giant virus ChrysoHV. The virion morphology is unusual, with a loosely associated outer membrane and a long thin tail. The viral genome encodes three rhodopsins, one of which is affiliated with a bacterial proteorhodopsin lineage not previously identified in viruses. ChrysoHV also encodes genes with closest homologs in marine cyanobacteria, suggesting that phagocytic protists may provide a conduit for horizontal gene transfer from bacterial prey to viral pathogens. This isolate offers a useful model for exploring giant virus genomics and the function of a new viral rhodopsin.

Main text

Introduction

Chrysophytes are single-celled or colonial protists in the class Chrysophyceae, and they are globally abundant in freshwater and marine ecosystems (1, 2). Chrysophytes play multiple ecosystem roles as primary producers and bacterivores, and include phago-mixotrophic forms that perform photosynthesis and ingest prey, strictly heterotrophic phagotrophs, and potentially non-phagotrophic phototrophs (3). In subtropical ocean gyre ecosystems, which span approximately a quarter of the global surface ocean (4), chrysophytes play an important role as predators of marine cyanobacteria (5, 6), influencing the community structure at the base of the food web. Isolates from Chrysophyceae Clade H have been shown to be voracious consumers of

70 the cyanobacterium *Prochlorococcus* (7), the dominant primary producer in subtropical gyres
71 and most abundant phototroph in the ocean (8). Additionally, single cell amplified genomes of
72 Chrysophyceae Clade H have been shown to encode endogenous viral elements from the
73 *Nucleocytoviricota* phylum (9), referred to as giant viruses (10). Genetic marker surveys of
74 protist and giant virus distributions from the *Tara* Oceans global dataset also indicate that
75 chrysophytes commonly co-occur with the giant virus family *Mimiviridae* (11), and further
76 RNA-seq studies revealed that chrysophytes are in fact native hosts of mimivirids (12).
77 However, chrysophyte-infecting giant virus isolates have not been previously described. In this
78 study we detail the isolation and initial characterization of a giant virus that infects a Clade H
79 chrysophyte, ChrysoHV.

80 Giant viruses encode vast genomic repertoires that include numerous homologs of
81 cellular genes (13). These cellular homologs may augment or compensate for host functions
82 compromised during infection (14–16), or interfere with host defenses (17). Among the most
83 commonly found viral homologs of cellular genes are microbial rhodopsins (18–20), which are
84 photoreceptive proteins that may aid in the generation of ion gradients or intracellular signaling
85 (21, 22). Viral rhodopsin homologs have been experimentally confirmed to convert light energy
86 into electrochemical potential across multiple clades: viral rhodopsin groups I and II (14, 23, 24),
87 channelrhodopsin (15), and heliorhodopsin (17). Furthermore, these viral rhodopsin homologs
88 are globally distributed across aquatic habitats, with their relative abundance decreasing over
89 depth with the attenuation of sunlight (14, 25).

90 Although giant viruses are diverse and globally abundant (11, 19, 20), a relatively small
91 fraction of their known diversity is represented by cultivated isolates (26). Likewise, giant virus
92 rhodopsin homologs are widespread and potentially functional, but only a few rhodopsin-

93 encoding giant viruses have been isolated and remain in culture with their native host (16, 17,
94 27). Cultivated isolates are important for establishing virus-host linkages, and furthermore, can
95 reveal novel gene lineages that may go undetected in environmental datasets and clarify the
96 evolutionary history of highly divergent sequences (16). Through the isolation and
97 characterization of ChrysoHV, we identify a previously undescribed lineage of viral rhodopsin
98 that appears to derive from bacterial proteorhodopsin.
99

100 **Results & Discussion**

101 *Host range, infection dynamics, and morphology*

102 We tested the host range of ChrysoHV against seven protist isolates from Station
103 ALOHA, spanning three classes: three Chrysophyceae, three Dictyochophyceae, and one
104 Bolidophyceae representative. This resulted in no lysis for six of the seven isolates (Fig. 1B)
105 (Supplementary Fig. S1). Only the representative of chrysophyte Clade H, lysed when
106 challenged with ChrysoHV (Fig. 1). The apparent burst size, *i.e.*, estimated number of virions
107 produced per cell, was 24 ± 2 ([mean ± SD]) (Fig. 1A). The virion particles produced from the
108 infection of chrysophyte strain UHM3501 displayed the notable morphological features of an
109 icosahedral capsid (290 ± 40 nm diameter [mean ± SD]) associated with a loose membrane (720
110 ± 120 nm), and a long tail (1,200 ± 240 nm length) terminating in tail fibers (210 ± 700 nm
111 length) (Fig. 2A-C).
112

113 *Genomic characterization*

114 The ChrysoHV genome was assembled as a putatively linear DNA sequence of 1.19 Mbp
115 encoding 1,138 predicted genes with a G+C content of 23.42% (Fig. 2B). The encoded genes

116 have diverse putative functions, including the ribosomal proteins eL40 and S27a, making it the
117 second isolated eukaryote-infecting virus known to encode ribosomal proteins (21), and the first
118 to encode S27a (Supplementary Table S1). ChrysoHV also encodes electron transport proteins
119 such as cytochrome b5, widespread in prokaryotes, eukaryotes, and giant viruses (Supplementary
120 Table S1) (28), along with succinate dehydrogenase flavoprotein and iron-sulfur subunits
121 (Supplementary Table S1). Additionally, the virus encodes seven ABC transporters including
122 one specific to phosphate transport (XVM28869.1) and two AmtB ammonium transporters. Of
123 these ammonium transporters, one (XVM28992.1) has 55% amino acid sequence identity (aaID)
124 to *Prochlorococcus* and *Synechococcus* homologs (Supplementary Table S1). Likewise,
125 ChrysoHV encodes three porins with 44-66% aaID to *Prochlorococcus* iron and carbohydrate
126 porins (XVM28452.1, XVM28569.1, XVM28570.1), two urea ABC transporter substrate-
127 binding proteins with 81-90% aaID to a *Prochlorococcus* homolog (XVM29005.1,
128 XVM29006.1), a semiSWEET sugar transporter with 44% aaID to a *Prochlorococcus* homolog
129 (XVM28124.1), a sulfotransferase family protein with 55% aaID to a *Prochlorococcus* homolog
130 (XVM28964.1), and a N-acetylmuramoyl-L-alanine amidase for cell wall remodeling with 39%
131 aaID to a *Prochlorococcus* homolog (XVM29061.1) (Supplementary Table S1). The presence of
132 genes whose closest known relatives are *Prochlorococcus* homologs suggests extensive
133 horizontal gene transfer from cyanobacterial prey of the chrysophyte to a giant virus that infects
134 it.

135 The ChrysoHV genome also includes a region of putatively light-sensitive proteins,
136 encompassing a proteorhodopsin-like protein surrounded by two photolyases and followed by a
137 heliorhodopsin (Fig. 2D, Fig. 3C, Supplementary Table S1). The positioning and shared

138 orientation of the photolyases and heliorhodopsin around the proteorhodopsin-like protein
139 implies that they may be part of the same operon.
140
141 *Sequence characterization of a distinct lineage of proteorhodopsin*
142 Phylogenetic analysis of ChrysoHV's rhodopsin placed it within the proteorhodopsin
143 clade (Fig. 3A). The sequence alignment of transmembrane domain helix-III (TM3) indicates
144 that the ChrysoHV rhodopsin is spectrally tuned to blue light (glutamine, Q; Fig. 3A), which is
145 the dominant spectral tuning residue in proteorhodopsins from Station ALOHA, the isolation site
146 of ChrysoHV (29). The two most closely related rhodopsin sequences were recovered from
147 metagenomes from the Black Sea at 50 m depth (SAMN27960419) and the North Pacific at 5 m
148 depth in the 0.22-3μm size fraction (SAMEA2623059). These rhodopsins may also absorb blue
149 light (Fig. 3A) and share a poly-N (asparagine) tract at the start of the rhodopsin coding
150 sequence.
151 ChrysoHV does not encode β-carotene, a precursor to retinal, the light-absorbing
152 chromophore in proteorhodopsin, and the salt bridge between K102 and E228 most likely
153 inhibits retinal binding. This loss of retinal binding, if associated with a change in rhodopsin
154 function, could explain the high rate of sequence divergence of ChrysoHV's proteorhodopsin
155 compared to the rest of the proteorhodopsin clade (Fig. 3A). Understanding how the rhodopsin
156 may function without retinal binding could be important to interpreting the function of viral
157 rhodopsins detected below the photic zone (30, 31). Further investigation is needed to confirm
158 whether the ChrysoHV proteorhodopsin can confer other physiological benefits in the absence of
159 light absorption, similar to the *Psychroflexus torquis* proteorhodopsin ptqPR, which acts as a
160 phospholipid scramblase to preserve membrane potential, fluidity, and integrity (32).

161 ChrysoHV also lacks the conserved DTE proton pumping motif found in most
162 proteorhodopsins, and instead, possesses an ETL motif (Fig. 3A), which has been detected in
163 proteorhodopsins from deep water at Station ALOHA (Fig. 3A) (29). A comparison of the
164 predicted structure of the conserved domain of the ChrysoHV rhodopsin against existing
165 functional characterizations revealed that it was most similar (35.9% aaID) to the uncultured
166 gamma-proteobacterium clone HOT 75m4 rhodopsin (Fig. 3B). This rhodopsin was confirmed to
167 pump protons (H^+) (33), and, like ChrysoHV, was collected from Station ALOHA (33). These
168 results suggest that ChrysoHV may have acquired the proteorhodopsin from a marine bacterium,
169 which, like *Prochlorococcus*, may be prey for chrysophyte Clade H.
170
171 *Comparison to photoreceptive proteins across Nucleocytoviricota*
172 ChrysoHV is the third cultivated isolate in the *Aliimimivirinae* subfamily of *Mimiviridae*
173 (Fig. 2E); other known isolates include CroV BV-PW1 (34), infecting a heterotrophic
174 nanoflagellate, and CV-XW01 (35), infecting a green alga. In this subfamily, only two other
175 representatives, cPacV-1605 and GVMAG-M-3300023174-43, encode rhodopsins, which belong
176 to viral rhodopsin group II (Fig. 2A). However, 40 other *Aliimimivirinae* representatives encode
177 at least one photolyase homolog (Fig. 2E), either the (6-4) photolyase or DASH-type
178 cryptochrome. Only ChrysoHV and cPacV-1605 encode multiple rhodopsin and photolyase
179 types, including two heliorhodopsins (Fig. 3A). However, cPacV-1605 encodes a viral rhodopsin
180 (group II) and ChrysoHV encodes a proteorhodopsin (Fig. 3A). Furthermore, instead of being
181 nested within a photoreceptive gene neighborhood, the position of the viral rhodopsin in cPacV-
182 1605 (locus tag: CPAV1605_207, GenBank ID: VVU94485.1) is further away from the
183 photolyases (locus tag: CPAV1605_133, CPAV1605_136; GenBank ID: VVU94411.1,

184 VVU94414.1) and heliorhodopsins (locus tag: CPAV1605_533, CPAV1605_1309; GenBank ID:
185 VVU95557.1, VVU94808.1) (36). ChrysoHV is the only cultivated giant virus to encode the
186 proteorhodopsin lineage of rhodopsin along with two heliorhodopsins (Fig 2E, Fig. 3A).
187
188 *Global ocean distribution of ChrysoHV phylotypes and proteorhodopsins*
189 Analysis of the distribution of ChrysoHV phylotypes (≥ 90% PolB aaID) from the *Tara*
190 Oceans dataset (11) revealed their prevalence in subtropical surface ocean sites in the Pacific and
191 Indian Ocean as well as the Mediterranean Sea (Fig. 4A). Notably, at station 132, north of the
192 Hawaiian archipelago, ChrysoHV phylotypes were detected in both the mixed layer and
193 mesopelagic zone, suggesting connectivity between surface and deep ocean virus populations,
194 which has previously been observed in the tropical North Pacific (25). The abundance of the
195 ChrysoHV proteorhodopsin-like protein (≥ 80% PolB aaID) was also highest in the subtropical
196 surface ocean (Fig. 4B), but detected at fewer overall sites than the ChrysoHV PolB.
197 Additionally, the ChrysoHV proteorhodopsin relative abundance was highest at station 132,
198 indicating that these proteorhodopsin sequences may be derived from a ChrysoHV phylotype.
199
200 **Conclusion**
201 In the marine environment, diverse microorganisms from proteobacteria to microalgae
202 utilize rhodopsins to supplement their energetic needs (29, 37). SAR11 bacteria, of which
203 *Pelagibacter* is an abundant subclade, account for 25% of all plankton (38), and are a likely prey
204 source for mixotrophic algae such as Clade H chrysophytes. The discovery of a proteorhodopsin-
205 like protein encoded by a giant virus supports the conceptual model that lateral gene transfer is
206 driven by the co-mingling of infectious virions and ingested prey within the same host cell (39).

207 The presence of nine genes whose closest known relatives are *Prochlorococcus* homologs further
208 suggests that prey-virus HGT has played an important role in the evolution of ChrysoHV. By
209 leveraging naturally co-occurring and experimentally accessible virus-host pairs, this study lays
210 the groundwork for future investigations into how the virocell environment of predatory protists
211 influences gene acquisition in giant viruses. As the only cultivated isolate known to encode one
212 microbial rhodopsin and two heliorhodopsins, this system provides the opportunity for novel
213 insights into the role of rhodopsins in giant virus infection strategies.
214

215 **Materials and Methods**

216 *Isolation and cultivation*

217 The five mixotrophic protists used in this study include one chrysophyte (UHM3501),
218 one bolidophyte (UHM3600), and three dictyochophytes (UHM3020, UHM3053, UHM3071),
219 all of which were isolated from the epipelagic zone (5–100m) of Station ALOHA through serial
220 dilutions of whole seawater samples enriched with *Prochlorococcus* (7). The mixotrophs were
221 cultivated at 24°C at approximately 200–220 µmol photons m^{-2} s^{-1} irradiance to mimic the high
222 light conditions at station ALOHA (40). The unialgal cultures were grown on K medium without
223 dissolved, inorganic nitrogen sources ($NaNO_3$ and NH_4Cl). Cultures were instead amended with
224 cyanobacteria prey (*Prochlorococcus* MIT9301) for their nitrogen source (7, 41). In addition,
225 two heterotrophic chrysophytes (UHM3530 and UHM3560) were used in this study. These
226 strains were isolated from seawater collected from the mixed layer (5–50 m) at Station ALOHA
227 in 2022 and 2024 through serial dilutions of seawater samples. The heterotrophs were cultivated
228 on filtered seawater amended with sterile quinoa grains to stimulate growth of ambient bacteria
229 as their food source.

230 The Chrysophyceae Clade H virus SA1 (ChrysoHV) was isolated by challenging the
231 chrysophyte isolate UHM3500 with unfiltered seawater collected from 25 m depth at Station
232 ALOHA on 04 May 2019. The lytic agent detected in the challenge was rendered clonal by serial
233 dilutions to extinction (42). This is the first chrysophyte-infecting virus isolated from Station
234 ALOHA, thus the strain designation SA1. In addition to the host used for isolation, the virus was
235 found to infect a related chrysophyte strain UHM3501 (Supplementary Fig. S1). Both strains are
236 members of Chrysophyceae Clade H, but differ in their grazing kinetics and 18S rRNA gene
237 sequences (7). The virus was maintained on both chrysophytes through serial, monthly
238 challenges of exponential phase culture with lysate from the preceding challenge until
239 chrysophyte strain UHM3500 was lost. Approximately three to four days post-infection (DPI),
240 infected cultures were stored at 4°C to serve as lysate for the next round of infection.
241
242 *Host range tests and flow cytometry*
243 We challenged isolates of Ochrophyta protists, both mixotrophs and heterotrophs, with
244 raw ChrysoHV lysate, then tracked the population dynamics of the protists and viruses over time
245 using flow cytometry. The mixotrophic algal protists were tracked every two days up to ten DPI,
246 and the heterotrophic protists were tracked every day up to five DPI. The experimental design
247 included triplicate, 25 mL protist cultures for both negative controls and infection treatments. To
248 prepare the infection treatment, 500 µL of unfiltered lysate (3-6 × 10^5 virus-like particles mL^{-1}
249 final concentration) was added to exponential or early stationary phase protist cultures. The
250 samples were fixed with ice-cold glutaraldehyde at 0.5% final concentration, incubated for 15
251 min at 4°C, and then flash frozen in liquid nitrogen (43).

252 The samples were stored at -80°C up to four days, then thawed at room temperature and
253 processed within the same day on a CytoFlex-S (Beckman Coulter Life Sciences, Indianapolis,
254 IN, USA) in the Biological Electron Microscope Facility (BEMF) at the University of Hawai‘i at
255 Mānoa. The samples were split into two fractions, with 200 µL allocated towards quantifying
256 cell abundances and loaded directly onto a 96-well plate, and 5 µL allocated toward quantifying
257 virus abundances. The latter fractions were diluted into 195 µL solutions of TE (pH 8.0) with
258 2.5X SYBR Green I nucleic acid stain (Invitrogen, Thermo Fisher Scientific, Waltham, MA,
259 USA) and incubated at 80°C for 20 min in darkness (44), then loaded onto a 96-well plate. The
260 cell counts were run on auto-record for one minute at a flow rate of 20 µL min^{-1} for the algae and
261 30 µL min^{-1} for the heterotrophs. The mixotroph populations were distinguished through
262 chlorophyll fluorescence and 488 nm, 90° side scatter (SSC), whereas the heterotrophs were
263 detected using SYBR Green fluorescence and 488 nm, 90° SSC (45). The viral-like particle
264 (VLP) population was identified through SYBR Green fluorescence and 405 nm, 90° violet side
265 scatter (VSSC), enhancing marine virus detection (46). The samples were run on auto-record for
266 one minute at a flow rate of 10 µL min^{-1}. Both algae and virus populations were delineated
267 through a custom Python script using the following packages: fcsparser (v0.2.8), pandas (v2.2.2),
268 matplotlib (v3.7.1), while the FlowJo (v.10.10.0) software was used to delineate the
269 heterotrophic protist population. Apparent burst size was determined by dividing the maximum
270 concentration of VLPs post-infection by the inferred number of lysed cells, calculated as the
271 difference between the cell concentration on the day of infection minus the minimum
272 concentration post-infection.
273
274 *Electron microscopy*

275 Transmission electron microscope (TEM) micrographs of viral particles were prepared
276 from raw, unfixed lysate and negatively stained with 2% uranyl acetate for 45 sec on 200-mesh
277 copper grids with carbon-stabilized formvar support that had been rendered hydrophilic by glow
278 discharge (47). Images were captured with a Hitachi HT7700 transmission electron microscope
279 (Tokyo, Japan) at the Biological Electron Microscope Facility (BEMF) at the University of
280 Hawai‘i at Mānoa. Particle dimensions were measured with ImageJ from Fiji (v2.16.0/1.54p)
281 (48), including: capsid diameter (n = 38), envelope (n = 34), tail (n = 17), and tail fibers (n = 6).
282
283 *DNA extraction, sequencing, assembly, and annotation*
284 To prepare sufficient viral biomass for DNA sequencing, DNA was extracted from 610
285 mL of lysate (6.4×10^6 VLP/mL), after allowing the infection of chrysophyte Clade H sp.
286 UHM3501 to clear four DPI. The unfiltered lysate was concentrated and pooled through serial
287 centrifugations (6X) in a 100 kDA Centricon Plus centrifugal filter (MilliporeSigma, Burlington,
288 MA, USA) at 3,500 × *g* at 20°C for 15 minutes. Approximately 200 ng of DNA was extracted
289 from the concentrate using the ZymoBiomics Quick-DNA High Molecular Weight MagBead Kit
290 (Zymo Research, Irvine, CA, USA), flash frozen in liquid nitrogen, stored at -20°C, then shipped
291 on dry ice to the US Department of Energy Joint Genome Institute (JGI). There, the DNA was
292 sequenced using PacBio Multiplexed 6–10 kb Low Input parameters.
293 A PacBio Low Input (DNA) library was constructed and sequenced using the PacBio
294 SEQUELIIe platform (Pacific Biosciences, Menlo Park, CA USA), which generated 3,267,906
295 reads totaling 31,033,080,767 bp. Reads were deduplicated using pbmarkdup (v1.0.3) (49).
296 BBDuk (v38.99) (50) was used to remove reads that contained PacBio control sequences and
297 PCR adapters, and IceCreamFinder (v38.99) (51) was used to remove reads without SMRTbell

298 sequences. The final filtered fastq contained 3,257,268 reads totaling 30,921,351,264 bp with
299 mean read length of 9,493 bp.
300 The filtered reads (31 Gb) were assembled using Flye (v2.9) (52) with the "--meta --
301 pacbio-hifi" argument. From this initial assembly, viral contigs were identified and grouped
302 through a low-complexity binning workflow, utilizing CONCOCT (v1.1.0) (53), MaxBin
303 (v2.2.7) (54), MetaBAT2 (v2.15-5) (55), and SemiBin2 (v1.5.1) (56). For each binning strategy,
304 the viral bin was identified by assessing the presence of giant virus marker genes (10) with
305 GVClass (v1.0) (57), and the MetaBAT2-generated viral bin was used in downstream analysis.
306 Subsequently, circular consensus sequencing reads were recruited against these identified viral
307 contigs, and a 100 Mb subsample of these reads was assembled using Flye without the "--meta"
308 argument (v2.9) (52). This iterative assembly resulted in one high-quality viral contig, which was
309 polished using two rounds of Racon (v1.6.0) (58) to ensure accuracy.
310 Gene calling was performed with GVClass (v1.0) (57), and the 1.196 Mb viral genome
311 had an estimated 93.77% coding density. Gene annotation was performed with KofamScan
312 (v1.3.0) (59) and eggnog mapper (v2.1.12) (60) with annotations of genes of interests confirmed
313 with BLASTp (61). tRNAs were annotated with tRNAscan-SE (v2.0.12) (62).
314
315 *Phylogenetic analysis*
316 Phylogenetic analysis of ChrysoHV with IQ-TREE (v2.2.6) (63) was based on a
317 concatenated alignment of seven core proteins: SFII, RNAPL, PolB, TFIIB, TopoII, A32, and
318 VLTF3 (10). Query sequences were retrieved from the GVMAGs (20) and GenBank Assembly
319 (64) databases, and quality filtered to contain at least four out of seven core proteins determined
320 through hmmsearch (v3.3.2, E-value $\leq 1 \times 10^{-10}$) (65). Additionally, the query sequences were

filtered to remove genomes with more than 10 copies of any core protein and more than four core proteins with multiple copies. The remaining 990 query sequences were aligned with MAFFT (v7.520) (66) and trimmed with trimAl (-gt 0.1; v1.4.1) (67), then concatenated with a custom python script (68). The trimmed, concatenated alignment was used for phylogenetic analysis using the mixture model LG+I+F+G4 and ultrafast bootstrap supports (option: -bb 1000) (v2.2.6) (63). The tree was annotated with iTOL (v6) (69).

Gene phylogeny of microbial rhodopsins with a heliorhodopsin outgroup were built with IQ-TREE (v2.2.6) (63) using model LG+F+R10 and ultrafast bootstrap supports (option: -bb 1000) and annotated with iTOL (v6) (69). Representatives of rhodopsin homologs from cultivated virus isolates include: EhV-202 (heliorhodopsin) (17), PgV-12T and -16T (viral rhodopsin I) (27), and FloV-SA2 (viral rhodopsin II) (16). Additionally, the gene phylogeny includes synthetic construct: vPyACR_21921 (channelrhodopsin) (15), and rhodopsin homologs from single amplified genomes (SAGs) and metagenome assembled genomes (MAGS): ChoanoV1 (viral group II) (14), cPacV-1605 (viral group II) (70), and OLPV 1 and 2 (viral groups I & II) (36). Query rhodopsin sequences were retrieved from NCBI and the supplementary materials of Olson *et al*. (2018), which included proteorhodopsin sequences recovered from metagenomic sequences at Station ALOHA (29). The alignment was built with MAFFT (L-INS-i; v7.520) (66) and trimmed with trimAl (-gt 0.1; v1.4.1) (67).

The phylogeny of near-complete 18S rRNA genes from select algal isolates were built with IQ-TREE (v2.2.6) (63) using GTR+I+G model with ultrafast bootstrap support (option: -bb 1000) and annotated with iTOL (v6) (69). The nucleotide sequences were retrieved from NCBI, aligned with MAFFT (L-INS-i; v7.520) (66) and trimmed with trimAl (-gt 0.1; v1.4.1) (67).

Gene distribution in the global ocean

Comparisons to *Nucleocytoviricota* PolB amino acid sequences from the *Tara* Oceans dataset (11) were performed using BLASTp (v2.13.0, E-value $\leq 1 \times 10^{-120}$) (61), and sequences with 90% or greater aaID were plotted using a custom R script with the following packages: maps (v3.4.2), mapdata (v2.3.1), and ggplot2 (v.3.5.1). The relative abundance of the PolB genes were normalized to the total number of *Nucleocytoviricota* PolBs (11). Likewise, ChysoHV proteorhodopsin-like protein sequences from the *Tara* Oceans dataset with a minimum of 80% aaID were identified through Ocean Gene Atlas (71, 72). The relative abundance of ChysoHV proteorhodopsin-like protein sequences were normalized to total metagenomic reads in the given sample (71, 72).

Protein structure predictions

The conserved domain of the ChrysoHV rhodopsin structure was predicted using ColabFold (v1.5.5) (73) and compared against the AlphaFold Protein Structure Databases–SwissProt (74) and CATH (v4.4) (75) using Foldseek (76). The conserved domain of the ChrysoHV rhodopsin and HOT 75m4 Gamma-proteobacterium proteorhodopsin structures were rendered in ChimeraX (v1.8.dev202403010247) (77).

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Author Contributions

GFS, KFE, and PB developed, designed, and acquired funding support for the project. CRS isolated the virus and algae strains. CW and QL assisted with the collection of virus host-range data. FS provided sequencing support and supervised the bioinformatics analyses. KL assisted

with the virus genome assembly. JT assisted with gene phylogeny workflows. CRS and PB imaged the virus. PB wrote the manuscript with input from all authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

ChrysoHV sequencing data is available through the NCBI sequence read archive SRR34002084, and the genome assembly has been deposited to NCBI GenBank under accession ID PV764857. The mixotrophic protists used in the study, GenBank IDs: MZ611734.1 (Chrysophyceae Clade H sp. UHM3500), MZ611720.1 (Chrysophyceae Clade H sp. UHM3501), MN615710.1 (*Florenciella* sp. UHM3020), MZ611733.1 (Dictyochophyceae clade X sp. UHM3020), MZ611705.1 (*Rhizochromulina* sp. UHM3071), MZ611730.1 (*Triparma* sp. UHM3600), are available through NCMA. The virus and heterotrophic protists used in this study, GenBank IDs: PV717318.1 (*Paraphysomonas* sp. UHM3560) and PV717319.1 (*Spumella* sp. UHM3530), are available through the Marine Viral Ecology Laboratories at the University of Hawai‘i at Mānoa upon request to KFE and GFS. Additional data and scripts on gene alignments, phylogenies, genome annotation, protein structural predictions, host range challenges, and biogeography are available on the Dryad repository: http://datadryad.org/share/8duyiXJ3riDLgLbseVaWvUBnbOPOM4SkBw9WNBaRYA4.

Figures

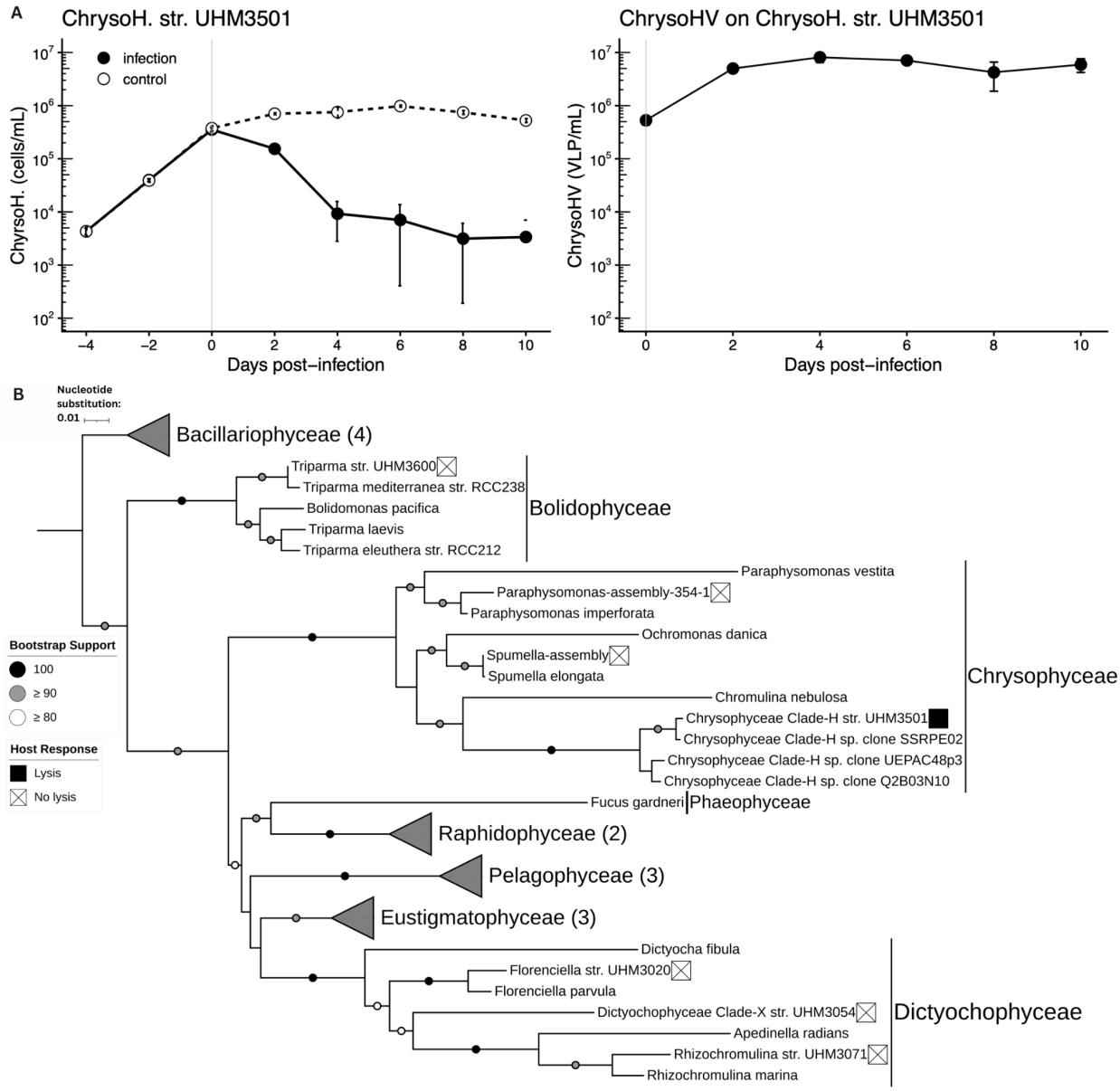


Figure 1. **ChrysoHV infection dynamics and host range tests.** (A) The left panel shows a time series of Chrysophyceae Clade H sp. UHM3501 abundance (cells/mL) in response to the addition of raw ChrysoHV lysate. The control treatment is shown in the black dashed line with white-fill points, and the infection treatment shown in the solid black line with black-fill points. The right panel shows the abundance of ChrysoHV (VLP/mL) during the same time series. Days pre- and post-infection are displayed on the x-axis. Error bars represent the standard deviation of the triplicate counts. (B) Maximum Likelihood (ML) 18S rRNA phylogeny (GTR+I+R model)

420 of the Ochrophyta phylum with isolates challenged in bold. The white or black boxes by the
421 bolded isolate names indicate whether the protist culture did not lyse (white with “X”) or lysed
422 and produced virions (black) in response to the ChrysoHV challenge. The node symbols indicate
423 the bootstrap values equal to or greater than 80% (white), 90% (gray) or 100% (black). There is
424 no node symbol for branches with less than 80% support.
425

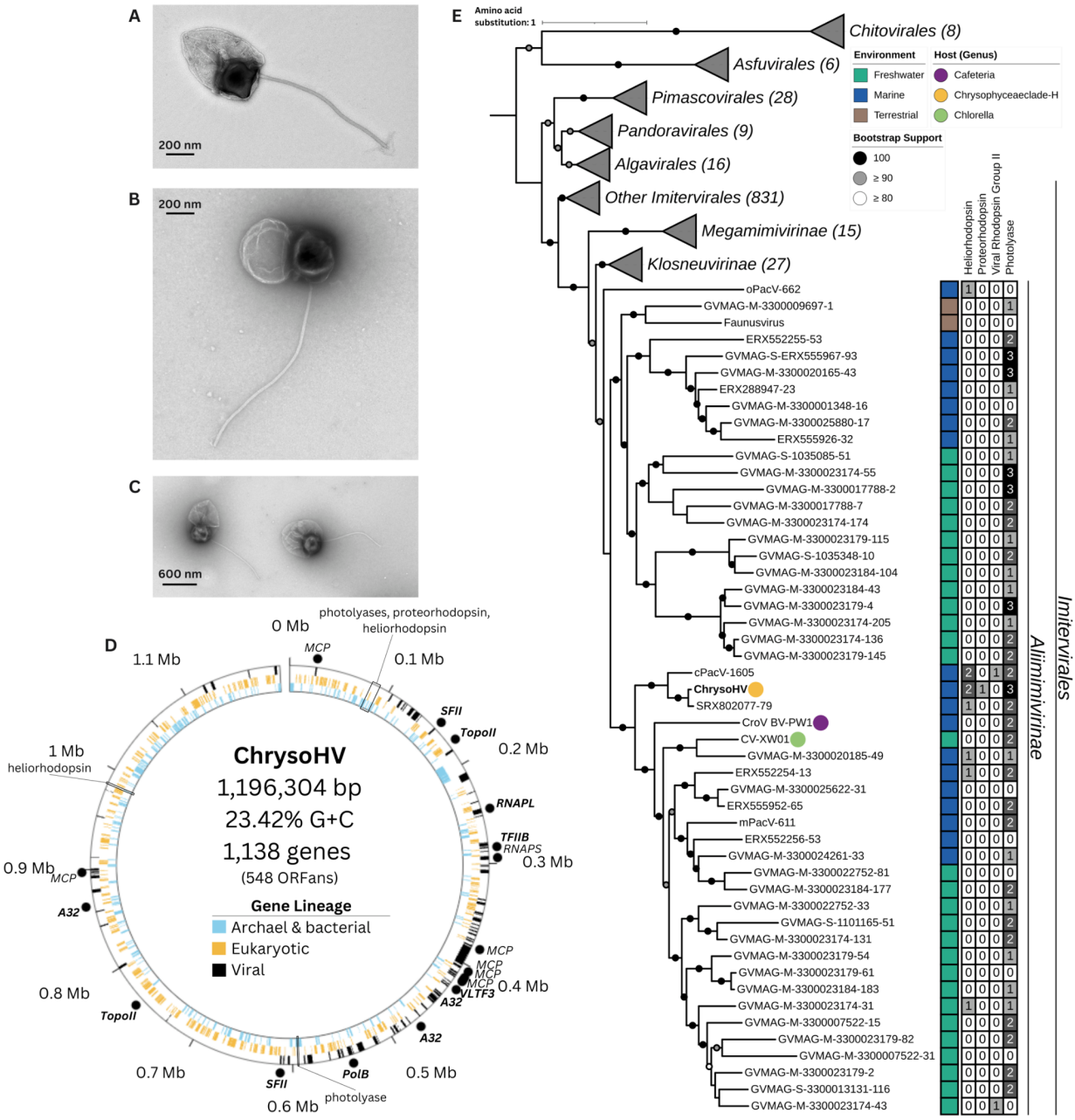


Figure 2. **ChrysoHV ultrastructure, genome, and phylogenetic analysis.** (A) Transmission electron microscopy (TEM) micrograph of ChrysoHV propagated on UHM3501. (B, C) TEM micrograph of negatively stained ChrysoHV propagated on UHM3500. (D) Genome plot of ChrysoHV with track placement and color showing gene lineage annotated according to domain, inner to outer tracks showing: archaeal and bacterial (blue), eukaryotic (yellow), and viral

432 (black) lineages. Phylogenetically informative giant virus marker genes are represented as black
433 dots on the outside track and show gene name. The genes used in the *Nucleocytoviricota*
434 phylogenetic analysis are bolded. (E) Maximum Likelihood (ML) phylogeny of the
435 *Nucleocytoviricota* phylum inferred from a concatenated alignment of amino acid sequences of
436 marker genes (SFII, RNAPL, PolB, TFIIB, TopoII, A32, VLTF3) using model LG+I+F+G4 with
437 ultrafast bootstrap supports (-bb 1000) shown by node symbol color– white (≥ 80%), gray (≥
438 90%), and black (100%) with no node symbol for supports < 80%. The tree is rooted with
439 *Chitovirales* as the outgroup, and the scale bar represents one amino acid substitution per site.
440 The frequency of microbial rhodopsins and photolyases present in *Aliimimivirinae*
441 representatives are annotated in the grayscale matrix, and the environment the representative was
442 collected from is shown in jade (freshwater), blue (marine), and brown (terrestrial).
443

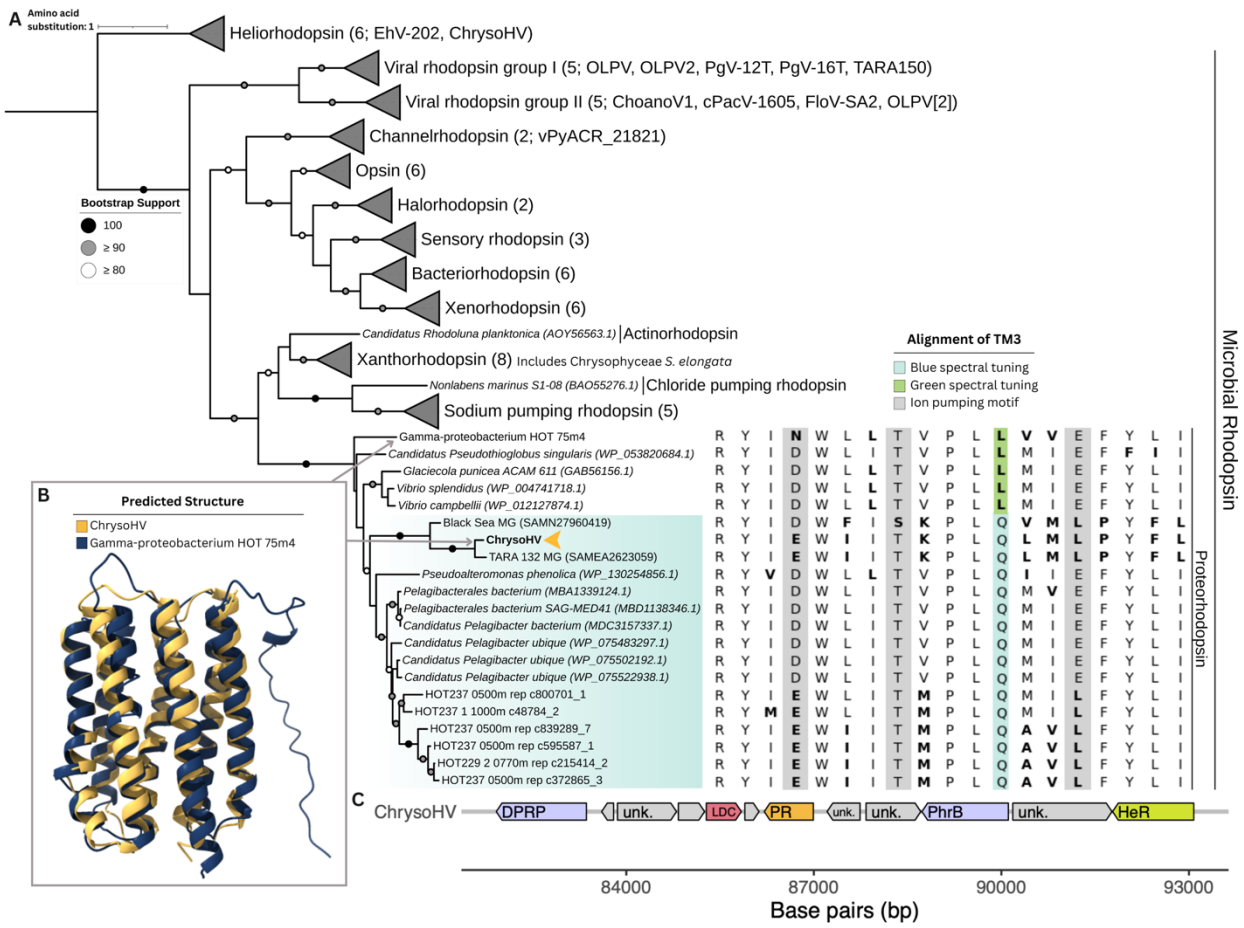
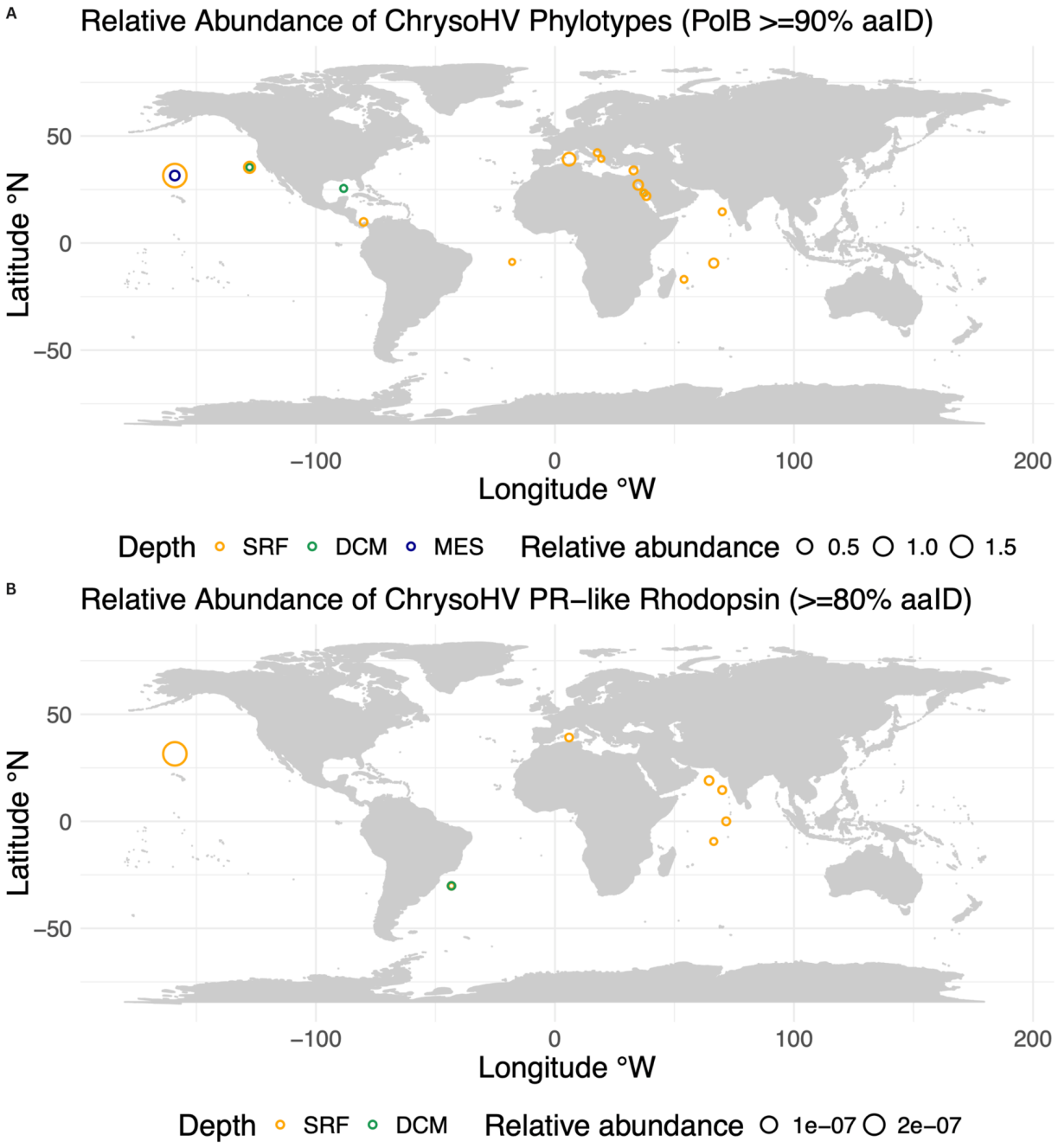


Figure 3. **Rhodopsin gene phylogeny, predicted structure, and gene neighborhood.** (A) Microbial rhodopsin ML phylogeny with heliorhodopsin as the outgroup, built with model LG+F+R10. Select representatives of viral rhodopsin homologs and putative chrysophyte rhodopsins are referred to in the parenthesis of their corresponding collapsed clade. The proteorhodopsin (PR) clade is annotated with the alignment of transmembrane helix 3 (TM3) outlining the ion-pumping motif (gray) and the spectral tuning amino acid (green or blue). Residues that differ from the most common residue at that locus are in bold (B). The predicted structure of ChrysoHV's proteorhodopsin-like protein's conserved domain (yellow) and the HOT 75m4 Gamma-proteobacterium proteorhodopsin (blue). (C) Gene neighborhood of photoreceptive proteins in ChrysoHV, showing the proteorhodopsin (locus tag: ChrysoHV_0101;

GenBank ID: XVM28022.1) next to the enzyme lysine decarboxylase (locus tag: ChrysoHV_0099, GenBank ID: XVM28020.1), between two photolyases (locus tag: ChrysoHV_0095, ChyrsoHV_0104; GenBank ID: XVM28016.1, XVM28025.1), and one heliorhodopsin (locus tag: ChrysoHV_0106, GenBank ID: XVM28027.1).



461 **Figure 4**. **Distribution and relative abundance of ChrysoHV PolB and PR**
462 **(proteorhodopsin)**. (A) Global distribution of phylotypes with ≥ 90% PolB aaID to ChrysoHV
463 from the *Tara* Oceans dataset. Size of the circle indicates the relative abundance of the summed
464 phylotypes at each given sample site to total *Nucleocytoviricota* PolB sequences per site. (B)
465 Global distribution of homologous PR with ≥ 80% aaID to the ChrysoHV proteorhodopsins with
466 size of the circle indicating relative abundance normalized to total metagenomic reads per site.
467 Sample depth is shown through color: surface (SRF: 3-7 m, orange), deep chlorophyll maximum
468 (DCM: 7-200 m, green), and mesopelagic (MES: 200-1,000 m, purple).
469

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