# How exaptations facilitated photosensory evolution: Seeing the light by accident

*Gregory S. Gavelis[1)2)*], Patrick J. Keeling[2)] and Brian S. Leander[2)]*

**Exaptations are adaptations that have undergone a major change in function. By recruiting genes from sources originally unrelated to vision, exaptation has allowed for sudden and critical photosensory innovations, such as lenses, photopigments, and photoreceptors. Here we review new or neglected findings, with an emphasis on unicellular eukaryotes (protists), to illustrate how exaptation has shaped photoreception across the tree of life. Protist phylogeny attests to multiple origins of photoreception, as well as the extreme creativity of evolution. By appropriating genes and even entire organelles from foreign organisms via lateral gene transfer and endosymbiosis, protists have cobbled photoreceptors and eyespots from a diverse set of ingredients. While refinement through natural selection is paramount, exaptation helps illustrate how novelties arise in the first place, and is now shedding light on the origins of photoreception itself.**

**Keywords:**
co-option; cryptochrome; lens; neofunctionalization; plastid; rhodopsin; stigma

## Introduction

Light provides most of the energy in the biosphere, and also information relevant to all organisms living in the photic zone, as well as dark ecosystems illuminated by bioluminescence [1, 2]. The selective advantages of exploiting this information have resulted in a great diversity of photoreceptive systems (Fig. 1). Eyes (or eyespots) in animals and some protists are extraordinarily complex, and how this complexity evolved has been a longstanding question [3]. It is clear that visual systems have become superbly suited to their tasks through the gradual refinement of pre-existing features such as photoreceptors, photopigments, and lenses. But how did these features acquire photosensory roles in the first place?

Gould and Vrba coined the term "exaptation" to describe traits that became used for different functions than those for which they had originally evolved [4]. This concept is useful to explain the evolution of some important features. For instance, the feathers of *Archaeopteryx* were originally adapted for warmth, but through exaptation, they became reconfigured for flight. Thus, exaptations are adaptations that have undergone a major change in function.

In eye evolution, the force of exaptation became apparent when researchers disentangled the phylogenetic history of individual "crystallin" proteins that form the lens. Studies ranging from birds to mollusks found that lens crystallins had evolved numerous times independently, and were recruited from disparate proteins involved in functions such as glycolysis and stress responses [5]. These discoveries also helped to illustrate the molecular mechanisms underpinning exaptation. The first common mechanism is "co-option" (also called "gene sharing") where a single gene gains a new function by being expressed in two different spatial or temporal contexts [6]. A prime example is found in the lens of birds and reptiles, where "delta crystallin" is produced in high amounts, while the same gene product is found at lower concentrations in other tissues, where it functions as arginosuccinate lyase (probably its ancestral function) [7]. The second common mechanism of exaptation is "neofunctionalization" in which a gene is duplicated, with the copy acquiring a new and independent function.

Since the elucidation of lens crystallins, the conceptual usefulness of exaptation has broadened further, and is now a consideration in even more ancient features of

DOI 10.1002/bies.201600266

[1)] School of Life Sciences, Arizona State University, Phoenix, AZ, USA
[2)] Departments of Botany and Zoology, Canadian Institute for Advanced Research, University of British Columbia, Vancouver, Canada

***Corresponding author**:
Gregory S. Gavelis
E-mail: zoark0@gmail.com

photoreception [8]. This includes the Pax-6 master control genes that initiate eye development [9], as well as diverse phototransduction pathways in animals [10], and even the origins of opsins in animals [11]. Here we expand on this theme, mainly drawing on discoveries in microbial eukaryotes (protists), such as phytoplankton and unicellular fungi. Photoreception in several protists has originated not just through co-option and neofunctionalization, but through the acquisition of genes from other organisms via "lateral gene transfer." In some cases, foreign organelles have been transferred laterally through endosymbiosis, which, in turn, have been recruited for photosensory roles (e.g. as eyespots). In the first section, we review how exaptation has produced several photoreceptor proteins across different eukaryotic groups. In the second section, we discuss how exaptation has repurposed entire organelles for visual functions, namely chloroplasts and mitochondria.

## Eukaryotes evolved photoreceptors multiple times from light-driven enzymes

Photoreceptors are the proteins and associated pigments necessary to detect light, making them the foundation of vision (the term "photoreceptor" is also used to refer to photosensory cells such as rods and cones, but we use it here strictly in reference to photosensory proteins). A few decades ago, it was conceivable that photoreception had evolved only once in the history of life [12], but it is now clear that photoreceptors have evolved multiple times in eukaryotes alone – sometimes more recently than expected [11, 13]. The usefulness of these receptors is evident in the observation that true phototaxis (i.e. tracking light in three dimensions), evolved at least 10 times independently in eukaryotes (Fig. 2), with two apparent origins in animals and eight in protists [13, 14]. Photoreception must have predated phototaxis and enabled this diversification. While the number of times photoreception itself evolved is still uncertain, its origins are clearly manifold, based on the diversity of photoreceptor proteins across eukaryotes.

One of the newest debates – and the one most pertinent to vision in humans – is whether the opsins of animals were exapted from melatonin receptors [11, 15, 16]. Despite the intriguing possibility that a hormone receptor gained the ability sense light, we will not discuss this here, as opsin evolution has been thoroughly reviewed elsewhere [10, 17]. Microbial photoreceptors, meanwhile, are rarely included in reviews of eye evolution, yet they have much to teach us about photosensory evolution.

### Microbial rhodopsins display functional plasticity that spans metabolic and sensory roles

Like opsins, microbial rhodopsins have seven transmembrane helices and bind to retinal – a derivative of vitamin A. These ancient photoreceptors have a scattered distribution across all three domains of life [18]. Their phylogeny differs greatly from that of their "host" organisms, indicating a history of rampant lateral gene transfer [19]. Rhodopsins are particularly amenable to lateral gene transfer due to their self-contained organization – the retinal-binding region and its effector (a transmembrane channel or pump) are on the same protein [18]. Therefore, their light-driven mechanisms are likely to survive lateral gene transfer, while delivery to new hosts with new cellular contexts creates opportunities for exaptation. The phylogenetic distribution of microbial rhodopsins suggests several transitions from metabolic roles (as light-driven pumps) to photosensory molecules (as light-gated ion channels), and vice versa [18]. Some major protist groups rely on sensory rhodopsins for phototaxis, such as green algae (e.g. *Chlamydomonas* and *Volvox*), cryptophytes [20–22] and probably dinoflagellates [22–24]. Other protists evidently use rhodopsins for metabolic functions, such as diatoms, which are thought to use these light driven pumps to take up dissolved iron across the plasma membrane (which is the main growth-limiting mineral in much of the open ocean) [25]. In the predatory dinoflagellate *Oxyrrhis marina*, bacteriorhodopsins are clustered around vesicles in the cytoplasm, perhaps to acidify digestive vacuoles via light-driven proton pumping [26], while bacteriorhodopsins in the dinoflagellate *Pyrocystis lunula* are co-expressed with genes involved in a circadian clock [27]. Even the giant viruses of algae have been found to encode rhodopsins (predicted as sensory proton channels), which they could potentially use to manipulate host behavior [28]. With the manifold functions of microbial rhodopsins coming to light, the emerging picture is one of promiscuous lateral gene transfer and neofunctionalization.

### A novel photoreceptor was produced by gene fusion

A new photoreceptor protein has originated relatively recently in the blastomycotes: fungi with flagellated zoospores that are attracted to green light. By sequencing the genome of *Blastocladiella emersonii*, and through subsequent experimentation, Avelar et al. identified the molecular basis for this positive phototaxis: a surprising gene fusion of a microbial rhodopsin to a guanalyl cyclase catalytic domain [14]. The product is a photosensory protein that transmits its signal to cytosolic G proteins, rather than to membrane-bound channels as in microbial rhodopsins. Inhibition studies indicate that the protein is necessary for phototaxis [14].

Phototaxis specifically tuned to green light has also been observed in *Ulkenia*, a member of the Thraustochytrida: a group of protists that has independently evolved a fungus-like saprotrophic lifestyle [29]. Amon and French found this attraction to target the bioluminescence of the bacterium *Vibrio fischeri*, on which *Ulkenia* zoospores feed. The concept that cells could use phototaxis to track bioluminescent prey is intriguing, though the photoreceptors in this group await identification.

### Cryptochromes originated from DNA repair enzymes

A blue-light response is found in a diverse family of proteins: the cryptochromes and photolyases. Each protein forms a

complex with a flavonoid pigment that becomes chemically altered by exposure to blue light [30]. In photolyases, light-activated flavins restore specific bonds in damaged nucleotides, thereby repairing UV damage to DNA. Photolyases are widespread throughout archaea, bacteria, and eukaryotes, while cryptochromes have a more scattered distribution [31]. Phylogenetic studies indicate that cryptochromes arose independently in animals and plants (and probably several other groups) by gene duplication from photolyases, followed by loss of their original function in DNA repair [32]. This neofunctionalization allowed cryptochromes to take on roles regulating blue light-dependent transcription in plants and algae and to entrain circadian rhythms in animals [33–35].

Cryptochromes have no known visual roles, though the ciliated larvae of sponges appear to employ them in phototaxis [36]. Phototaxis has only been investigated in a few sponge taxa, so this innovation might be specific to demosponges [37, 38]. Alternately, cryptochromes could have lent the ancestral form of photoreception in animals, only to be replaced in eumetazoans by the evolution of opsins. Many eumetazoans still upregulate cryptochromes in their primary or pineal eyes for circadian entrainment [39]; and this might be a relic of a more ancient visual role. Testing these hypotheses will require an exploration of cryptochrome activity in deep-branching animals and their larvae. This would provide a glimpse into the early evolution of phototactic processes in animals [31].

Cryptochromes perform roles beyond phototaxis and circadian clocks; they are necessary for magnetoreception in butterflies and *Drosophila* [40] and possibly birds [41, 42]. The mechanism by which cryptochromes detect magnetic fields is currently debated, but in insects, magnetotaxis requires photoactivation by blue light. Upon activation, the flavin chromophore becomes photoreduced by electron transfers, potentially producing radical pairs that orient in a magnetic field [31]. While the particulars of this mechanism are unclear, the centrality of cryptochromes in insect phototaxis has been demonstrated [40]. Thus, cryptochromes illustrate the meandering path of evolution, as they originated from DNA-repair enzymes, were then neofunctionalized as photoreceptors, and lastly, in insects, underwent a third round of exaptation, as makeshift compasses in the brain.

## How chloroplasts and mitochondria contributed to photosensory structures in microbes

On the size spectrum between photoreceptor proteins and multicellular eyes is a realm of photoreceptive organelles, as found in several groups of protists. These generally consist of a photoreceptor combined with a shading structure (or "eyespot") to shield light from one side and thereby ensure the photoreceptor is only monitoring light from one direction. Most protist eyespots are plastid-derived. This is illustrated by the model organism *Chlamydomonas*, a flagellated green alga: its red/orange eyespot is found in the chloroplast, and is formed from several lipid droplets pigmented with carotenoids [43, 44] (carotenoids are the principle shading pigment of protist eyespots – not to be confused with photoreceptor pigments).

In green algae, both plastids and carotenoid biosynthesis genes [45] were derived from the "primary endosymbiosis" between an early eukaryote and a cyanobacterium – which is now the plastid in green algae, red algae, and glaucophytes [46]. Since then, photosynthesis has spread through several "secondary endosymbiotic" events, where plastids of red and green algae have been acquired by other groups, such as kelps, dinoflagellates, cryptophytes, and haptophytes (Fig. 3). Many of these diverse algal lineages have independently retailored photosynthetic plastids into eyespots [47, 48]. Some algal groups have eyespots in the cytoplasm rather than the plastid (e.g. euglenids and eustigmatophytes), but might still rely on symbiotically derived carotenoids to pigment the eyespot [45].

Plastid endosymbioses can also serve as vehicles for genes that can eventually be neofunctionalized in the same way that carotenoid pigments have. Euglenids perform phototaxis with light-activated adenylyl cyclase [49]. Based on its high sequence similarity to a protein in cyanobacteria, Jekely suggested that this unusual photoreceptor spread to euglenids via lateral gene transfer from their green-alga derived plastids. He also proposed a similar scenario for the origin of a photoreceptive flavoprotein in heterokonts, but in this case from a red alga [13].

### Some protists "steal" eyespots from their prey

Early stages in the symbiotic acquisition of an eyespot are reflected in *Hatena arenicola*, a relative of cryptophytes. Okamoto and Inouye described this heterotrophic genus from marine sand, as well as its peculiar interaction with a species of *Nephroselmis*, the flagellated green alga that is its preferred prey [50, 51]. Following engulfment, *Nephroselmis* is not digested, but grows tenfold to fill most of *Hatena*'s cytoplasm, with considerable modification to both itself and its host. Specifically, *Hatena* resorbs its feeding apparatus (evidently becoming a phototroph) and this region becomes occupied by the symbiont. Remarkably, the symbiont enlarges and aligns its eyespot with the most anterior part of *Hatena*'s plasma membrane. When *Hatena* divides, only one daughter cell inherits the symbiont (and its eyespot), while the other assembles a feeding apparatus, evidently resuming heterotrophy. Okamoto and Inouye interpreted this as a transitional phase between an opportunistic and an obligate endosymbiosis. The modification of the eyespot suggests that *Hatena* is phototactic during the symbiosis, raising the intriguing possibility that *Hatena* relies on photoreceptors housed in its endosymbiont communicating with host machinery for motility.

An even more transient acquisition is found in the ciliate *Strombidium oculatum*. It grazes on algae but delays digestion of their plastids, presumably to harvest their photosynthate. McManus et al. observed a strain of *S. oculatum* feeding on *Ulva* zoospores in its tidepool habitat [52]. In addition to sequestering their plastids, *S. oculatum* collected eyespots "stolen" from its prey and assembled them into a larger eyespot at the anterior end of the cell. Both *Strombidium* and

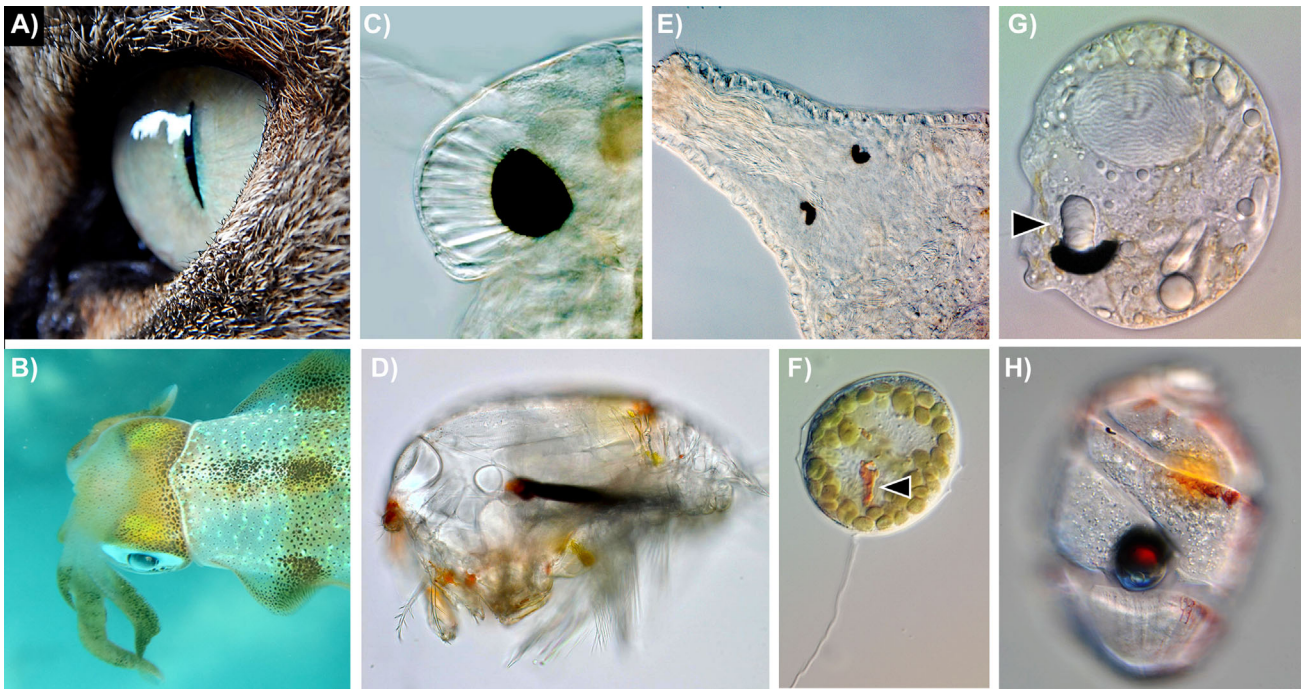


**Figure 1.** Examples of eyes and eyespots. Vertebrates (**A**: a housecat) and cephalopods (**B**: a firefly squid) have converged on similar camera-type eyes for high acuity vision. In comparison, arthropod eyes have much lower resolution, with most taxa possessing multi-lensed "compound" eyes (**C**: *Evadne*, a waterflea). Other arthropods have more bizarre arrangements, such as **D**: *Coryceaus*, a copepod in which each eye resembles a telescope, with two lenses aligned serially before the retina. The smallest lensed eyes among animals are found in rhabdocoel flatworms (**E**: *Ceratopera axi*), which form tiny lenses within mitochondria. Beyond animals, many single cells use eyelike structures for directional phototaxis, such as dinoflagellates, some of which have chloroplast-derived "eyespots" (**F**: *Kryptoperidinium*, arrow denotes eyespot). One group, the warnowiid dinoflagellates, has surprisingly complex "ocelloids" that resemble camera-type eyes, and are comprised of both chloroplasts and mitochondria. **G**: Side view of the ocelloid of *Nematodinium*, denoted by arrow. **H**: The frontal view of the ocelloid in *Warnowia*, where some red eyeshine is visible. Photographs by Emma Gavelis (A), Jonas Gavelis (B), Niels Van Steenkiste (E), Franz Niedl (H), and GSG (C, D, F, and G).

*Hatena* demonstrate the benefits of retaining the eyespots of their prey or symbionts (i.e., for phototaxis), rather than metabolizing these oil-rich droplets. The same is true for the several phytoplankton lineages that have lost photosynthesis but retain pigmented eyespots, some of which are formed from relict plastids [53, 54].

## Rare dinoflagellates sculpted organelles into eyelike "ocelloids"

The most famous eyespot was described by Hertwig in 1884 from a single planktonic cell isolated from the Mediterranean Sea [55]. The cell bore an eyelike structure, or "ocelloid," with a lens, iris-like rings, and pigmented retinal body that were similar to the camera-type eyes of animals, but at a subcellular scale. Hertwig's description was met with harsh criticism from the zoologist Karl Vogt, who argued that the cell had scavenged the eye from a cnidarian [56]. Hertwig was ultimately vindicated: the cell is now known to be a warnowiid dinoflagellate, specifically *Erythropsidinium*, which is a heterotrophic relative of many plastid-bearing groups [57, 58]. Moreover, its eyespot is clearly a sub-cellular structure [59]. Early ultrastructural studies suggested that the pigmented component of the ocelloid might be a modified plastid. Namely, this "retinal body" divides by binary fission shortly before the rest of the cell and has internal waveform membranes that take on a thylakoid-like appearance during division [59]. Using high resolution microscopy and single cell genomics, it was recently demonstrated that the retinal body is a peridinin-type plastid [53]. This suggests that the retinal body is homologous to the plastid-derived eyespots found in some other dinoflagellates [53, 54, 60]. Moreover, the lens is surrounded by a cornea-like layer comprised of mitochondria, and even a reflective crystalline layer reminiscent of the tapetum lucidum in many nocturnal animals [53, 61]. Compared to other eyespots and even some eyes in animals – the ocelloid is extremely complex.

Francis calculated that the lens dimensions of the ocelloid in *Nematodinium* were sufficient to focus light on the retinal body [62]. But contrary to frequent speculation [63], warnowiids probably cannot "see" images, simply as a matter of scale. The diameter of the retinal surface is only slightly larger than a light wave's amplitude (~2 μm), and as such, could not fit an image of much more than one "pixel." This would seem to limit the ocelloid to a mere directional photodetector. But why would warnowiids invest in a lens, iris-like structures, and retinal body, to achieve what other algae accomplish with far simpler eyespots? The answer may lie in a landmark treatise on optics in algae, by Foster and Smyth, in which they

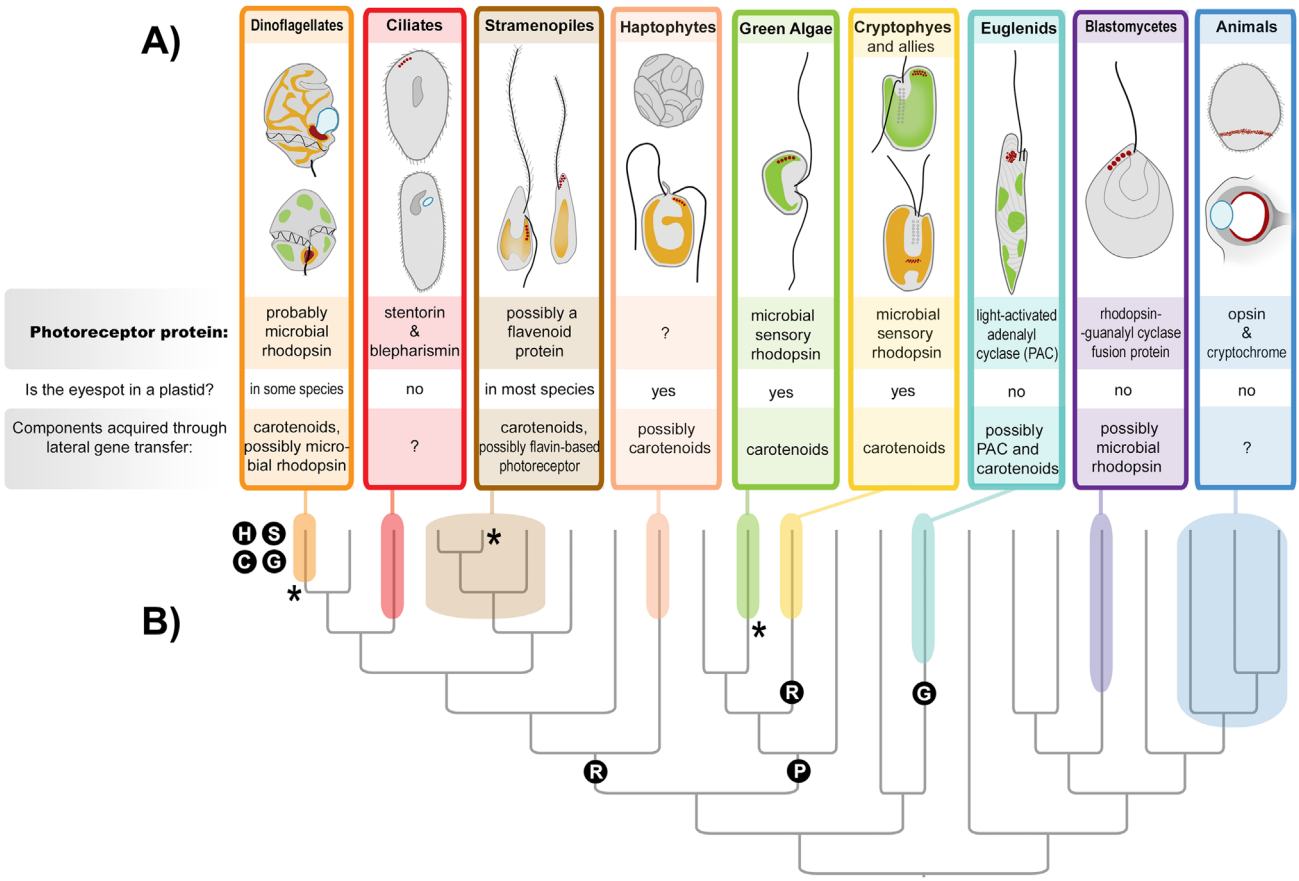


**Figure 2.** Diversity of photoreceptors in eukaryotes. **A**: Eight major eukaryotic groups are capable of phototaxis. Basic details of these systems are summarized from ref. [13] and citations therein. **B**: Phototactic groups (highlighted branches) are shown within a phylogenetic context (tree based on ref. [80]). Proteorhodopsin genes were acquired from bacteria multiple times (asterisks), though they are not necessarily used in phototaxis. Photoreception in algae was influenced by multiple acquisitions of photosynthesis (shown as black circles), via other eukaryotes such as haptophytes (H), stramenopiles (S), cryptophytes (C), green algae (G), red algae (R), as well as the initial "primary plastid" (P) from a cyanobacterium [46].

figured that eyespots could, in principle, track qualities beyond the mere intensity of light, such as color and polarization [64]. Stacked, waveform membranes in the retinal body have invited comparison to a polarization-filter [65], and it was recently discovered that *Nematodinium* hunts other dinoflagellates [53], which have chromosomes that circularly polarize light [66]. These fragments of evidence suggest that the ocelloid facilitates the tracking of prey via polarotaxis. The lens, in this scenario, would serve simply to concentrate light on the photoreceptors.

These considerations will remain speculative until basic aspects of warnowiid life history are uncovered: a difficult undertaking given that warnowiid cells are rare, fragile, and resistant to culture. Two further warnowiid genera (*Warnowia* and *Erythropsidinium*) were recently found to eat copepod eggs [67]; thus warnowiids are not strictly predators of other dinoflagellates. Remarkably, Gomez noticed that – during pauses in flagellar swimming – *Erythropsidinium* pivots its ocelloid independently of the cell body, akin to a vertebrate eye rotating in its socket [67]. Perhaps this motion assists in scanning the environment. Much about the ocelloid remains uncertain, such as what spectral signal the cell is "looking" for, and how it transmits this information into useful behavior.

Hayakawa et al. purportedly localized bacteriorhodopsin mRNAs to the retinal body in *Erythropsidinium* [68]. But these results should be taken with some caution, since rhodopsin was found to be nuclear-encoded in most if not all other eukaryotic studies (making it unlikely to reside in the retinal body plastome). Nevertheless, rhodopsin is the most likely phototactic pigment in dinoflagellates, based on other experimental studies [24, 64]. In order to understand the ocelloid, a logical next step would be to determine in which membranes rhodopsin (if present) is embedded, ideally through immunolocalization and electron microscopy. This could help elucidate ocelloid function. For instance, if rhodopsin lies inside the retinal body, this would substantiate the idea that its convoluted membranes serve to increase surface area for rhodopsin molecules, i.e. the same way that ciliary and microvillar photoreceptor cells in animals maximize photosensitivity. An alternate, undiscussed scenario is that rhodopsin is embedded in the plasma membrane. This is the case in other algae with channelrhodopsins (e.g. green algae and cryptophytes). In warnowiids, an invagination of the plasma membrane reaches into the center of the ocelloid, forming an "ocellar chamber" between the lens and the retinal

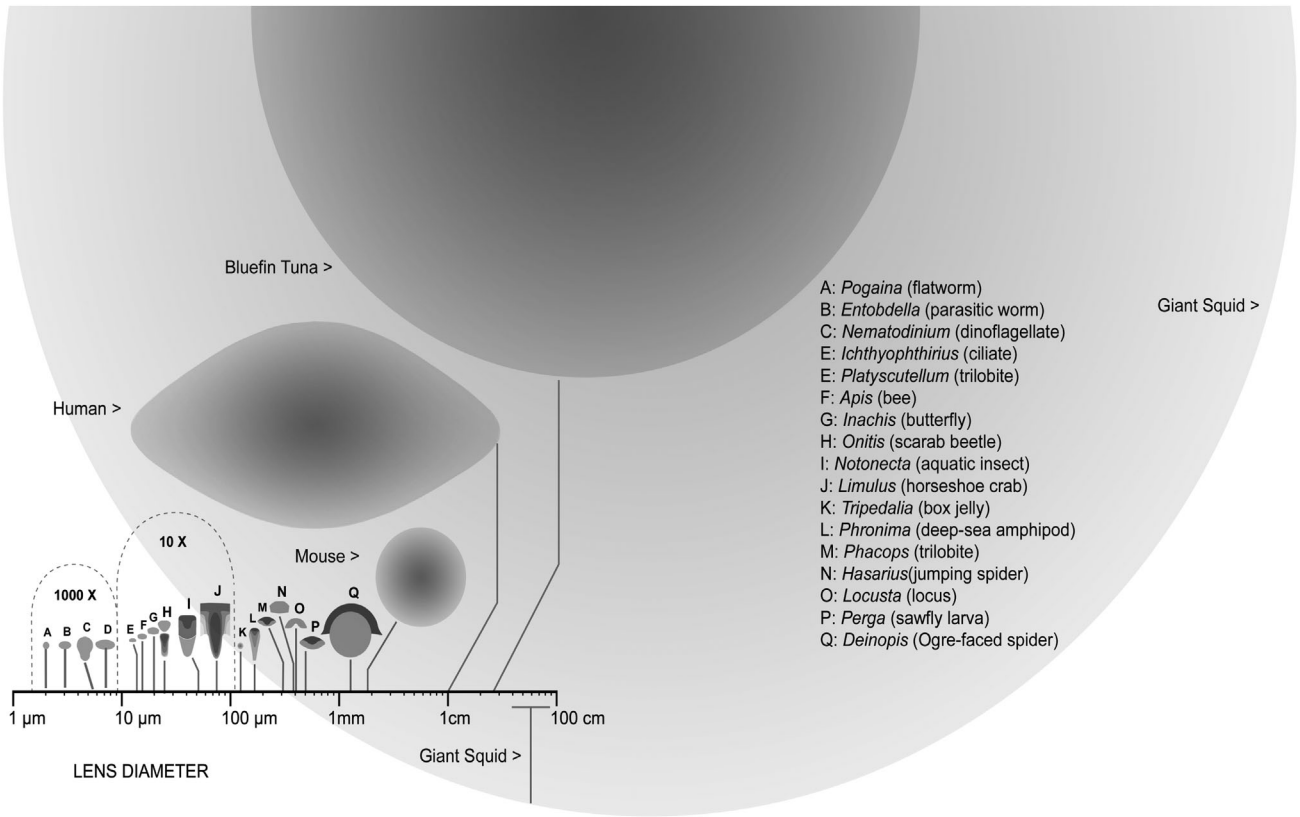


**Figure 3.** The size spectrum of nature's lenses. A great diversity of lenses exist across eukaryotes, many of which are too small for spatial resolution, and might simply serve to concentrate light. Lenses are drawn to logarithmic scale.

body [69], which could house plasma-membrane bound rhodopsins. However, this scenario would place the retinal body beneath them, meaning that it could not filter incoming light (as in the polarotaxis hypothesis). Clearly, there is no shortage of hypotheses about ocelloid function, only a lack of warnowiid cells to study. This should be remedied by collecting them in the tropical pacific waters where they are most abundant [68].

### Lenses abound in eyes too small for spatial vision

Organellar lenses are found in other groups as well. A refractile "watchglass organelle" is present in some ciliates, such as *Ichthyophthirius* [70] – a common pathogen of fish – as well as in *Ophryoglena*, which loses phototaxis if its lens is removed by microdissection [71]. Several animals also possess subcellular lenses. Acoelomorph flatworms and the larvae of monogenean flatworms (Fig. 1E) form tiny crystalline lenses within mitochondria overlying the retina [72, 73] (Fig. 1E). These crystals measure as few as two microns in diameter, making them – to the best of our knowledge – the smallest lenses in a eukaryote (Fig. 3A). Due to the optical constraints that we mentioned in warnowiids, all of these lenses are too small to form images. Nevertheless, small lenses are widespread in nature (Fig. 3), including among the larval stages of several invertebrate phyla. These animals have simple lensed eyes with neither the optics nor innervation to interpret spatial images [74]. Thus, it seems that lenses generally did not initially evolve for spatial resolution, but simply to concentrate light on the photoreceptors and thereby increase sensitivity.

## Conclusions and outlook

In sum, exaptation allowed not only for the evolution of lenses, but also eyespots and photoreceptor proteins – the latter being necessary for any form of photoreception at all. It seems that blind lineages can acquire "vision" in one of two ways: (i) evolving photoreceptors through exaptation (which is how the very first photoreceptor protein must have evolved) or (ii) appropriating a photoreceptor gene from another lineage. Protists in particular seem to have benefited from lateral gene transfer (This is not surprising, given that any foreign gene inserted into the nuclear genome of a unicellular organism will be inherited by one of its offspring. By contrast, animals and plants will only pass on genes inserted directly into eggs, sperm or other germline cells.). In practice so far, photoreceptors that have undergone lateral gene transfer in nature have also proven amenable to directed gene transfer in the lab.

In the past decade, microbial photoreceptors have been harnessed for use in optogenetics: a new field that uses light-gated ion channels to control processes in living tissues [20, 21, 75]. By implanting transgenic photoreceptors, membrane potential can be controlled from a distance, using light alone. For instance, bio-robots can be made to swim using light-

stimulated muscle tissues, the reactivity of which stems from Channelrhodopsin-2 (ChR2) [76]. While optogenetics began with the use of ChR2 from *Chlamydomonas*, it is employing new protist photoreceptors at an increasing rate, from channelrhodopsins in cryptophytes to the rhodopsin-fusion protein in *Blastocladiella* [21, 77, 78]. Algal photoreceptors also have therapeutic potential. As of this writing, the first human trial has commenced in the effort to restore monochromatic vision using ChR2 from green algae [79]. Thus, exploring the evolution of photoreceptor proteins does more than foster an appreciation of nature's creativity: it illuminates the path to technologies that may lay ahead.

### Acknowledgments

We would like to thank our three reviewers (including Todd Oakley) for their valuable input, as well as Emma Gavelis, Jonas Gavelis, Niels Van Steenkiste, and Franz Neidl for contributing vivid imagery of living specimens, and Cally Gavelis (Fig. 1A) for posing. This work was supported by grants from the National Science and Engineering Research Council of Canada to BSL (2014-05258) and PJK (227301).

The authors have declared no conflict of interest

## References

1. **Ellis EA, Oakley TH**. 2016. High rates of species accumulation in animals with bioluminescent courtship displays. *Curr Biol* **26**: 1916–21.
2. **Thomas KN, Robison BH, Johnsen S**. 2017. Two eyes for two purposes: in situ evidence for asymmetric vision in the cockeyed squids *Histioteuthis heteropsis* and *Stigmatoteuthis dofleini*. *Philos Trans R Soc London B Biol Sci* **372**: 1–9.
3. **Oakley TH, Pankey MS**. 2008. Opening the "black box": the genetic and biochemical basis of eye evolution. *Evol Educ Outreach* **1**: 390–402.
4. **Gould SJ, Vrba ES**. 1982. Exaptation—a missing term in the science of form. *Paleobiology* **8**: 4–15.
5. **de Jong WW, Hendriks W, Mulders JWM, Bloemendal H**. 1989. Evolution of eye lens crystallins: the stress connection. *Trends Biochem Sci* **14**: 365–8.
6. **Piatigorsky J, Wistow GJ**. 1989. Enzyme/crystallins: gene sharing as an evolutionary strategy. *Cell* **57**: 197–9.
7. **Piatigorsky J, O'Brien WE, Norman BL, Kalumuck K**, et al. 1988. Gene sharing by delta-crystallin and argininosuccinate lyase. *Proc Natl Acad Sci USA* **85**: 3479–83.
8. **Plachetzki DC, Oakley TH**. 2007. Key transitions during the evolution of animal phototransduction: novelty, "tree-thinking," co-option, and co-duplication. *Integr Comp Biol* **47**: 759–69.
9. **Nilsson D-E**. 1996. Eye ancestry: old genes for new eyes. *Curr Biol* **6**: 39–42.
10. **Oakley TH, Speiser DI**. 2015. How complexity originates: the evolution of animal eyes. *Annu Rev Ecol Evol Syst* **46**: 237–60.
11. **Feuda R, Hamilton SC, McInerney JO, Pisani D**. 2012. Metazoan opsin evolution reveals a simple route to animal vision. *Proc Natl Acad Sci USA* **109**: 18868–72.
12. **Eakin RM**. 1965. Evolution of photoreceptors. *Cold Spring Harb Symp Quant Biol* **30**: 363–70.
13. **Jekely G**. 2009. Evolution of phototaxis. *Philos Trans R Soc B Biol Sci* **364**: 2795–808.
14. **Avelar GM, Schumacher RI, Zaini PA, Leonard G**, et al. 2014. A rhodopsin-guanylyl cyclase gene fusion functions in visual perception in a fungus. *Curr Biol* **24**: 1234–40.
15. **Fredriksson R, Shioth HB**. 2005. The repertoire of G-protein-coupled receptors in fully sequenced genomes. *Mol Pharmacol* **67**: 1414–25.
16. **Mackin KA, Roy RA, Theobald DL**. 2014. An empirical test of convergent evolution in rhodopsins. *Mol Biol Evol* **31**: 85–95.
17. **Spudich JL, Yang C-S, Jung K-H, Spudich EN**. 2000. Retinylidene proteins: structures and functions from Archaea to humans. *Annu Rev Cell Dev Biol* **16**: 365–92.
18. **Sharma AK, Spudich JL, Doolittle WF**. 2006. Microbial rhodopsins: functional versatility and genetic mobility. *Trends Microbiol* **14**: 463–9.
19. **Ugalde JA, Podell S, Narasingarao P, Allen EE**. 2011. Xenorhodopsins, an enigmatic new class of microbial rhodopsins horizontally transferred between archaea and bacteria. *Biol Direct* **6**: 52.
20. **Govorunova EG, Sineshchekov OA, Spudich JL**. 2016. Structurally distinct cation channelrhodopsins from cryptophyte algae. *Biophys J* **110**: 2302–4.
21. **Govorunova EG, Sineshchekov OA, Janz R, Liu X**, et al. 2015. Natural light-gated anion channels: a family of microbial rhodopsins for advanced optogenetics. *Science* **349**: 647–50.
22. **Sineshchekov OA, Govorunova EG, Jung K-H, Zauner S**, et al. 2005. Rhodopsin-mediated photoreception in cryptophyte flagellates. *Biophys J* **89**: 4310–9.
23. **Sineshchekov OA, Jung K-H, Spudich JL**. 2002. Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci USA* **99**: 8689–94.
24. **Hartz AJ, Sherr BF, Sherr EB**. 2011. Photoresponse in the heterotrophic marine dinoflagellate *Oxyrrhis marina*. *J Eukaryot Microbiol* **58**: 171–7.
25. **Marchetti A, Schruth DM, Durkin CA, Parker MS**, et al. 2012. Comparative metatranscriptomics identifies molecular bases for the physiological responses of phytoplankton to varying iron availability. *Proc Natl Acad Sci USA* **109**: E317–25.
26. **Slamovits CH, Okamoto N, Burri L, James ER**, et al. 2011. A bacterial proteorhodopsin proton pump in marine eukaryotes. *Nat Commun* **2**: 183.
27. **Okamoto OK, Hastings JW**. 2003. Novel dinoflagellate clock-related genes identified through microarray analysis. *J Phycol* **39**: 519–26.
28. **Yutin N, Koonin EV**. 2012. Proteorhodopsin genes in giant viruses. *Biol Direct* **7**: 34.
29. **Amon JP, French KH**. 2004. Photoresponses of the marine protist *Ulkenia* sp. zoospores to ambient, artificial and bioluminescent light. *Mycologia* **96**: 463–9.
30. **Sancar A**. 2003. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chem Rev* **103**: 2203–38.
31. **Chaves I, Pokorny R, Byrdin M, Hoang N**, et al. 2011. The cryptochromes: blue light photoreceptors in plants and animals. *Annu Rev Plant Biol* **62**: 335–64.
32. **Selby CP, Sancar A**. 2006. A cryptochrome/photolyase class of enzymes with single-stranded DNA-specific photolyase activity. *Proc Natl Acad Sci USA* **103**: 17696–700.
33. **Facella P, Lopez L, Chiappetta A, Bitonti MB**, et al. 2006. CRY-DASH gene expression is under the control of the circadian clock machinery in tomato. *FEBS Lett* **580**: 4618–24.
34. **Brunelle SA, Hazard ES, Sotka EE, Van Dolah FM**. 2007. Characterization of a dinoflagellate cryptochrome blue-light photoreceptor with a possible role in circadian control of the cell cycle. *J Phycol* **43**: 509–18.
35. **Stanewsky R, Kaneko M, Emery P, Beretta B**, et al. 1998. The cry(b) mutation Identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell* **95**: 681–92.
36. **Leys S, Cronin T, Degnan B, Marshall J**. 2002. Spectral sensitivity in a sponge larva. *J Comp Physiol A Sensory Neural Behav Physiol* **188**: 199–202.
37. **Rivera AS, Ozturk N, Fahey B, Plachetzki DC**, et al. 2012. Blue-light-receptive cryptochrome is expressed in a sponge eye lacking neurons and opsin. *J Exp Biol* **215**: 1278–86.
38. **Müller WEG, Wang X, Schröder HC, Korzhev M**, et al. 2010. A cryptochrome-based photosensory system in the siliceous sponge *Suberites domuncula* (Demospongiae). *FEBS J* **277**: 1182–201.
39. **Sancar A**. 2000. Cryptochrome: the second photoactive pigment in the eye and its role in circadian photoreception. *Annu Rev Biochem* **69**: 31–67.
40. **Gegear RJ, Foley LE, Casselman A, Reppert SM**. 2010. Animal cryptochromes mediate magnetoreception by an unconventional photochemical mechanism. *Nature* **463**: 804–7.
41. **Maeda K, Henbest KB, Cintolesi F, Kuprov I**, et al. 2008. Chemical compass model of avian magnetoreception. *Nature* **453**: 387–90.
42. **Rodgers CT, Hore PJ**. 2009. Chemical magnetoreception in birds: the radical pair mechanism. *Proc Natl Acad Sci USA* **106**: 353–60.
43. **Satoh M, Hori T, Tsujimoto K, Sasa T**. 1995. Isolation of eyespots of green algae and analyses of pigments. *Bot Mar* **38**: 467–74.
44. **Schmidt M, Gessner G, Luff M, Heiland I**, et al. 2006. Proteomic analysis of the eyespot of *Chlamydomonas reinhardtii* provides novel insights into its components and tactic movements. *Plant Cell Online* **18**: 1908–30.

45. **Frommolt R, Werner S, Paulsen H, Goss R**, et al. 2008. Ancient recruitment by chromists of green algal genes encoding enzymes for carotenoid biosynthesis. *Mol Biol Evol* **25**: 2653–67.
46. **Keeling PJ**. 2013. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annu Rev Plant Biol* **64**: 583–607.
47. **Dodge JD**. 1983. The functional and phylogenetic significance of dinoflagellate eyespots. *Biosystems* **16**: 259–67.
48. **Dodge JD, Crawford RM**. 1969. Observations on the fine structure of the eyespot and associated organelles in the dinoflagellate *Glenodinium foliaceum*. *J Cell Sci* **5**: 479–93.
49. **Iseki M, Matsunaga S, Murakami A, Ohno K**, et al. 2002. A blue-light-activated adenylyl cyclase mediates photoavoidance in *Euglena gracilis*. *Nature* **415**: 1047–51.
50. **Okamoto N, Inouye I**. 2006. *Hatena arenicola* gen. et sp. nov., a katablepharid undergoing probable plastid acquisition. *Protist* **157**: 401–19.
51. **Okamoto N, Inouye I**. 2005. A secondary symbiosis in progress? *Science* **310**: 287.
52. **McManus GB, Zhang H, Lin S**. 2004. Marine planktonic ciliates that prey on macroalgae and enslave their chloroplasts. *Limnol Oceanogr* **49**: 308–13.
53. **Gavelis G, Hayakawa S, White R, III, Gojobori T**, et al. 2015. Eye-like ocelloids are built from different endosymbiotically acquired components. *Nature* **523**: 204–7.
54. **Hehenberger E, Imanian B, Burki F, Keeling PJ**. 2014. Evidence for the retention of two evolutionary distinct plastids in dinoflagellates with diatom endosymbionts. *Genome Biol Evol* **6**: 2321–34.
55. **Hertwig R**. 1884. Erythropsis agilis, eine neue Protozoe. *Jahrb Z Anat Entw* **10**: 204–12.
56. **Kofoid CA, Swezy O**. 1921. The free-living unarmored Dinoflagellata. Berkeley: Memoirs of the University of California. p. 1–562.
57. **Gavelis GS, White RA, Suttle CA, Keeling PJ**, et al. 2015. Single-cell transcriptomics using spliced leader PCR: evidence for multiple losses of photosynthesis in polykrikoid dinoflagellates. *BMC Genomics* **16**: 528.
58. **Hoppenrath M, Bachvaroff TR, Handy SM, Delwiche CF**, et al. 2009. Molecular phylogeny of ocelloid-bearing dinoflagellates (Warnowiaceae) as inferred from SSU and LSU rDNA sequences. *BMC Evol Biol* **9**: 116.
59. **Greuet C**. 1977. Structural and ultrastructural evolution of ocelloid of Erythropsidinium-pavillardi-Kofoid-and-Swezy (dinoflagellate Warnowiidae, Lindemann) during division and palintomic divisions. *Protistologica* **13**: 127–43.
60. **Moestrup Ø, Hansen G, Daugbjerg N**. 2008. Studies on woloszynskioid dinoflagellates III: on the ultrastructure and phylogeny of *Borghiella dodgei* gen. et sp. nov., a cold-water species from Lake Tovel, N. Italy, and on *B. tenuissima* comb. nov. (syn. *Woloszynskia tenuissima*). *Phycologia* **47**: 54–78.
61. **Greuet C**. 1968. Organisation ultrastructurale de l'ocelle de deux peridiniens Warnowiidae, *Erythropsis pavillardi* Kofoid et Swezy et Warnowia pulchra Schiller. *Ed du Cent Natl la Rech Sci* **4**: 209–30.
62. **Francis D**. 1967. On the eyespot of the dinoflagellate, Nematodinium. *J Exp Biol* **47**: 495–501.
63. **Colley NJ, Nilsson D-E**. 2016. Photoreception in phytoplankton. *Integr Comp Biol* **56**: 764–75.
64. **Foster K, Smyth R**. 1980. Light antennas in phototactic algae. *Microbiol Rev* **44**: 572–630.
65. **Greuet C**. 1982. Photorécepteurs et phototaxie des flagellés et des stades unicellulaires d'organismes inférieurs. *Ann Biol* **21**: 98–141.
66. **Liu J, Kattawar GW**. 2013. Detection of dinoflagellates by the light scattering properties of the chiral structure of their chromosomes. *J Quant Spectrosc Radiat Transf* **131**: 24–33.
67. **Gómez F**. 2017. The function of the ocelloid and piston in the dinoflagellate *Erythropsidinium* (Gymnodiniales, Dinophyceae). *J Phycol* in press https://doi.org/10.1111/jpy.12525
68. **Hayakawa S, Takaku Y, Hwang JS, Horiguchi T**, et al. 2015. Function and evolutionary origin of unicellular camera-type eye structure. *PLoS ONE* **10**: e0118415.
69. **Dodge JD, Greuet C**. 1987. Dinoflagellate ultrastructure and complex organelles. In Taylor FJR ed; *The Biology of Dinoflagellates*. Oxford: Blackwell Publishing Inc. p. 92–142.
70. **Lynn DH, Frombach S, Ewing MS, Kocan KM**. 1991. The organelle of Lieberkuhn as a synapomorphy for the Ophryoglenina (Ciliophora: hymenostomatida). *Trans Am Microsc Soc* **110**: 1–11.
71. **Kuhlmann H-W**. 1998. Do phototactic ciliates make use of directional antennas to track the direction of light? *Eur J Protistol* **34**: 244–53.
72. **Sopott-Ehlers B**. 1992. Photoreceptors with mitochondrial lenses in *Pogaina suecica* (Plathelminthes, Rhabdocoela). *Zoomorphology* **112**: 11–5.
73. **Rohde K, Watson NA, Chisholm LA**. 1999. Ultrastructure of the eyes of the larva of *Neoheterocotyle rhinobatidis* (Platyhelminthes, Monopisthocotylea), and phylogenetic implications. *Int J Parasitol* **29**: 511–9.
74. **Randel N, Jékely G**. 2016. Phototaxis and the origin of visual eyes. *Philos Trans R Soc B Biol Sci* **371**: 20150042.
75. **Hegemann P**. 2008. Algal sensory photoreceptors. *Annu Rev Plant Biol* **59**: 167–89.
76. **Park S-J, Gazzola M, Park KS, Park S**, et al. 2016. Phototactic guidance of a tissue-engineered soft-robotic ray. *Science* **353**: 158–62.
77. **Scheib U, Stehfest K, Gee CE, Korschen HG**, et al. 2015. The rhodopsin-guanylyl cyclase of the aquatic fungus *Blastocladiella emersonii* enables fast optical control of cGMP signaling. *Sci Signal* **8**: rs8.
78. **Wietek J, Broser M, Krause B, Hegemann P**. 2016. Identification of a natural green light absorbing chloride conducting channelrhodopsin from *Proteomonas sulcata*. *J Biol* **291**: 4121–7.
79. 2016. RST-001 Phase I/II Trial for Retinitis Pigmentosa – Full Text View – ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT02556736
80. **Burki F, Kaplan M, Tikhonenkov DV, Zlatogursky V**, et al. 2016. Untangling the early diversification of eukaryotes: a phylogenomic study of the evolutionary origins of Centrohelida, Haptophyta and Cryptista. *Proc R Soc B Biol Sci* **283**: 20152802.