14. Beier, C., Hovhannisyan, A., Weiser, S., Kung, J., Lee, S., Lee, D.Y., Huie, P., Dalal, R., Palanker, D., and Sher, A. (2017). Deafferented adult rod bipolar cells create new synapses with photoreceptors to restore vision. J. Neurosci. 37, 4635–4644.

15. Care, R.A., Anastassov, I.A., Kastner, D.B., Kuo, Y.-M., Della Santina, L., and Dunn, F.A. (2020). Mature retina compensates functionally for partial loss of rod photoreceptors. Cell Rep. 31, 107730.

16. Scalabrino, M.L., Thapa, M., Chew, L.A., Zhang, E., Xu, J., Sampath, A.P., Chen, J., and Field, G.D. (2022). Robust cone-mediated signaling persists late into rod photoreceptor degeneration. eLife 11, e80271.

17. Murphy, G.J., and Rieke, F. (2008). Signals and noise in an inhibitory interneuron diverge to control activity in nearby retinal ganglion cells. Nat. Neurosci. 11, 318–326.

18. Doroudchi, M.M., Greenberg, K.P., Liu, J., Silka, K.A., Boyden, E.S., Lockridge, J.A., Arman, A.C., Janani, R., Boye, S.E., Boye, S.L., et al. (2011). Virally delivered channelrhodopsin-2 safely and effectively restores visual function in multiple mouse models of blindness. Mol. Ther. 19, 1220–1229.

19. Macé, E., Caplette, R., Marre, O., Sengupta, A., Chaffiol, A., Barbe, P., Desrosiers, M., Bamberg, E., Sahel, J.-A., Picaud, S., et al. (2015). Targeting channelrhodopsin-2 to ON-bipolar cells with vitreally administered AAV Restores ON and OFF visual responses in blind mice. Mol. Ther. 23, 7–16.

20. Kralik, J., van Wyk, M., Stocker, N., and Kleinlogel, S. (2022). Bipolar cell targeted optogenetic gene therapy restores parallel retinal signaling and high-level vision in the degenerated retina. Commun. Biol. 5, 1116.

Eukaryotic evolution: Deep phylogeny does not imply morphological novelty

Brian S. Leander[1,2]
[1]Department of Zoology, University of British Columbia, Vancouver, BC 1Z4 V6T, Canada
[2]Department of Botany, University of British Columbia, Vancouver, BC 1Z4 V6T, Canada
Correspondence: bleander@mail.ubc.ca
https://doi.org/10.1016/j.cub.2022.12.016

Eukaryotic diversity is often depicted as a molecular phylogenetic tree consisting of a few supergroups that originated over a billion years ago. A new study reveals an ancient group of tiny phagotrophic flagellates that reinforces inferences about early evolutionary history.

Microbial organisms have dominated the planet in both abundance and phylogenetic diversity for over three billion years, yet the novel traits of many of these lineages remain poorly understood or completely unknown. It is well known, however, that some microbes are more complex than others at both morphological and genetic levels, reflecting diverse modes of nutrition, ecological roles, and evolutionary histories. For instance, the origin of eukaryotes about a billion years ago involved several key innovations that increased cellular complexity, including the emergence of a cytoskeletal system that facilitates locomotion, reproduction, and the ingestion of bacterial prey cells through a process called 'phagotrophy'[1]. Although several lineages of modern phagotrophic eukaryotes have retained their appetite for bacteria, some phagotrophic eukaryotes eat other microbial eukaryotes instead. Phagotrophic modes of nutrition are inferred to reflect the early evolutionary history of eukaryotes, which ultimately set the stage for the astounding array of biological novelty found within the known supergroups, such as the independent origins of multicellularity, parasitism, secreted cell coverings, intracellular armor, extrusomes, and photosynthesis via endosymbiosis. A new study by Tikhonenkov et al.[2] sheds additional light onto the earliest stages of eukaryotic evolution through the cultivation and characterization of several new strains of tiny phagotrophic flagellates (< 10 μm) that not only have retained many ancestral traits, but collectively form a new group of eukaryotes, called the Provora.

The first known member of the Provora, *Ancoracysta twista*, was described in 2017 by Janouškovec et al.[3] and, at the time, was considered an orphan lineage within the tree of eukaryotes, because it did not nest within any of the known supergroups[4]. Advances in phylogenomics and phylotranscriptomics have led to the discoveries of several other orphan lineages of tiny, morphologically streamlined phagotrophic flagellates[4–8]. The newly reported Provora now contains seven species that were collected from diverse and distant geographical locations, including the Caribbean Sea, Black Sea, Red Sea, Pacific Ocean, and Atlantic Ocean. The global distribution of this new group is bolstered by re-analyses of previously published environmental rDNA sequence surveys of eukaryotic diversity[2]. These analyses also suggest that members of the Provora might be numerically rare in specific locations despite their cosmopolitan distributions, making single-cell isolation and cultivation efforts particularly important.

However, there are several reasons beyond rarity that would explain why it is difficult to find tiny phagotrophic flagellates with global distributions, the most important of which are that sampling tiny fast-moving cells from a specific spot within a vast continuous ocean is essentially random and our overall knowledge of marine microbial diversity is

still exceedingly limited. The simple fact that members of the Provora have been captured repeatedly suggests that they are probably not rare. We now know, for instance, that diplonemids are among the most common and widespread groups of planktonic phagotrophic flagellates in the world's oceans despite initial assumptions that they were also relatively rare[9,10]. In order to explain the rarity of the provoran species in environmental rDNA sequence surveys, Tikhonenkov et al.[2] compared these tiny phagotrophic flagellates with apex animal predators by stating "as eukaryovorous predators their rarity relative to other microbes is not surprising and does not indicate a lack of ecological impact any more than a lion's rarity compared to wildebeest does". However, lions are rare because there is not enough energy moving through the food web to support a large population of large apex predators. This is unlikely to be true for tiny marine phagotrophs like provoran species, because they occur at the lowest trophic levels in aquatic food webs by eating the most abundant energy source on the planet outside of photosynthesis: other free-living microbes. Moreover, tiny marine phagotrophs are then eaten by organisms occurring at higher levels in the food web, such as meiofauna and zooplankton, which are well-known to be abundant and ubiquitous components of aquatic communities[11].

Although diverse at molecular and metabolic levels, what is most remarkable about many microbes is how stable they have been at the morphological level for hundreds of millions of years. From the perspective of gross morphology, the species within the Provora are nearly identical to each other and to hundreds of other species within several different supergroups of eukaryotes (Figure 1). This display of apparent morphostasis over vast phylogenetic distances provides compelling evidence for reconstructing the ancient phagotrophic ancestors of eukaryotes as a whole. In fact, the degree of morphostasis in some of these tiny phagotrophic flagellates with deep phylogenetic positions includes fine details associated with the overall organization of their relatively complex microtubular cytoskeletons[12].

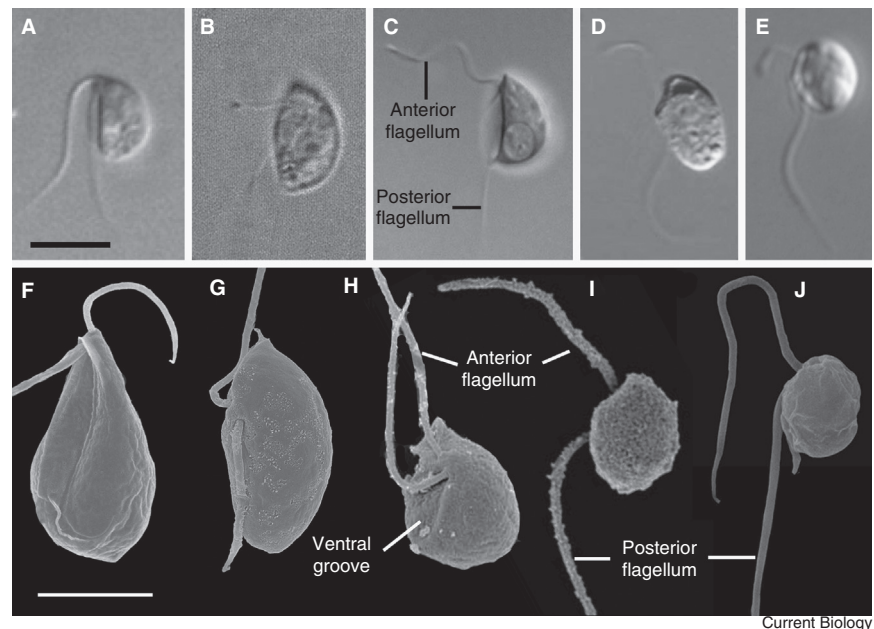The reoccurring morphotype of these tiny bacterivorous and eukaryovorous

Figure 1. Phagotrophic flagellates.
Light and scanning electron micrographs of tiny phagotrophic flagellates representing different supergroups of eukaryotes that originated over a billion years ago. (A) Light micrograph of *Andalucia godoyi* (Supergroup = Discoba; reproduced from Lara et al.[15]). (B) Light micrograph of *Colpodella edax* (Supergroup = Alveolata; reproduced from Leander et al.[16]). (C) Light micrograph of *Cantina marsupialis* (Supergroup = Stramenopiles; reproduced from Yubuki et al.[17]). (D) Light micrograph of *Rhodelphis limneticus* (Supergroup = Archaeplastida; reproduced from Gawryluk et al.[14]). (E) Light micrograph of *Nibbleromonas arcticus* (Supergroup = Provora; reproduced from Tikhonenkov et al.[2]). (F) Scanning electron micrograph of *Kipferlia bialata* (Supergroup = Metamonada; reproduced from Yubuki et al.[18]). (G) Scanning electron micrograph of *Colpodella edax* (Supergroup = Alveolata; reproduced from Leander et al.[16]). (H) Scanning electron micrograph of *Rictus lutensis* (Supergroup = Stramenopiles; reproduced from Yubuki et al.[19]). (I) Scanning electron micrograph of *Rhodelphis limneticus* (Supergroup = Archaeplastida; reproduced from Gawryluk et al.[14]). (J) Scanning electron micrograph of *Nibbleromonas kosolapovi* (Supergroup = Provora; reproduced from Tikhonenkov et al.[2]). (A–E) Images at same scale, bar = 3 μm; (F–J) Images at same scale, bar = 3 μm. Panels A–C and G reprinted with permission from John Wiley and Sons. Panels D, E, I, and J reprinted with permission from Springer Nature © 2019; 2022.

phagotrophs consists of a rounded cell less than 10 μm in diameter with two flagella and a ventral groove or 'cytostome', which functions as a 'mouth'. The anterior flagellum is used mainly for swimming, and the posterior flagellum tends to sit in the ventral groove to facilitate feeding. If the bacterial or eukaryotic prey cells are also tiny (<10 μm), then they are usually consumed by whole-cell phagocytosis; if the prey cells are relatively large (>10 μm), then other feeding behaviours are used to access pieces of the prey cell instead, such as 'nibbling' and vampire-like cytoplasmic sucking called 'myzocytosis'. Ultimately, these tiny phagotrophs take on a morphotype that is well-adapted to arguably the most stable environment on earth: a vast ocean brimming with bacteria and other minuscule free-living eukaryotes. This

abiotic continuity combined with the relatively low competition for incredibly abundant resources appears to have produced several lineages of tiny phagotrophs that retain similar morphology but are phylogenetically divergent.

In summary, these tiny phagotrophs, which are sometimes only 3 μm in diameter, fall far outside our realm of normal experiences and sit very close to the wall of minimum complexity required for a fully functional eukaryotic cell. This highly streamlined morphology means that distinguishing representatives of different lineages from one another at the morphological level involves analyses of fine ultrastructural details, mostly associated with the microtubular flagellar apparatus, mitochondrial cristae, and extrusive organelles[3,12,13]. Therefore, most novelty in tiny phagotrophic lineages

like the newly reported Provora is not at the behavioural or morphological level, but is associated with the composition and organization of their nuclear and mitochondrial genomes[2,3,14]. A report of a new group of tiny phagotrophic flagellates is exciting not because it shows morphological novelty, but because it shows morphostasis over a vast amount of time and has a compelling phylogenetic position and genetic information that connects this ancestral morphotype to larger, more complicated and sometimes more familiar groups of eukaryotes, like animals, fungi, plants, kelps, dinoflagellates, apicomplexan parasites, and red algae. So far, the Provora only contains tiny phagotrophic flagellates, but perhaps new lineages with more complex morphologies and behaviours will someday be discovered and shown to be more derived members of this new group of eukaryotes. Nonetheless, there is little doubt that if you could travel back in time and observe the most recent common ancestor of most, if not all, modern eukaryotes using a light or scanning electron microscope, it would look very similar to the tiny phagotrophic flagellates described in Tikhonenkov *et al.*[2] and illustrated in Figure 1.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Leander, B.S. (2020). Predatory protists. Curr. Biol. *30*, R510–R516.

2. Tikhonenkov, D.V., Mikhailov, K.V., Gawryluk, R.M.R., Belyaev, A.O., Mathur, V., Karpov, S.A., Zagumyonnyi, D.G., Borodina, A.S., Prokina, K.I., Mylnikov, A.P., *et al.* (2022). Microbial predators form a new supergroup of eukaryotes. Nature *612*, 714–719.

3. Janouškovec, J., Tikhonenkov, D.V., Burki, F., Howe, A.T., Rohwer, F.L., Mylnikov, A.P., and Keeling, P.J. (2017). A new lineage of eukaryotes illuminates early mitochondrial genome reduction. Curr. Biol. *27*, 3717–3724.

4. Keeling, P.J., and Burki, F. (2019). Progress towards the tree of eukaryotes. Curr. Biol. *29*, R808–R817.

5. Lax, G., Eglit, Y., Eme, L., Bertrand, E.M., Roger, A.J., and Simpson, A.G.B. (2018). Hemimastigophora is a novel supra-kingdom-level lineage of eukaryotes. Nature *564*, 410–414.

6. Shalchian-Tabrizi, K., Eikrem, W., Klaveness, D., Vaulot, D., Minge, M.A., Le Gall, F., Romari, K., Throndsen, J., Botnen, A., *et al.* (2006). Telonemia, a new protist phylum with affinity to

chromist lineages. Proc. Biol. Sci. *273*, 1833–1842.

7. Brown, M.W., Heiss, A.A., Kamikawa, R., Inagaki, Y., Yabuki, A., Tice, A.K., Shiratori, T., Ishida, K.-I., and Hashimoto, T. (2018). Phylogenomics places orphan protistan lineages in a novel eukaryotic super-group. Gen. Biol. Evol. *10*, 427–433.

8. Yabuki, A., Kamikawa, R., Ishikawa, S., Kolisko, M., Kim, E., Tanabe, A.S., Kume, K., Ishida, K.-I., and Inagki, Y. (2014). *Palpitomonas bilix* represents a basal cryptist lineage: Insight into the character evolution in Cryptista. Sci. Rep. *4*, 4641.

9. Flegontova, O., Flegontov, P., Malviya, S., Audic, S., Wincker, P., De Vargas, C., Bowler, C., Lukeš, J., and Horák, A. (2016). Extreme diversity of diplonemid eukaryotes in the ocean. Curr. Biol. *26*, 3060–3065.

10. Gawryluk, R.M.R., Del Campo, J., Okamoto, N., Strassert, J.F.H., Lukeš, J., Richards, T.A., Worden, A.Z., Santoro, A.E., and Keeling, P.J. (2016). Morphological identification and single-cell genomics of marine diplonemids. Curr. Biol. *26*, 3053–3059.

11. Schratzberger, M., and Ingels, J. (2018). Meiofauna matters: The roles of meiofauna in benthic ecosystems. J. Exp. Mar. Biol. Ecol. *502*, 12–25.

12. Yubuki, N., and Leander, B.S. (2013). Evolution of microtubule organizing centers across the tree of eukaryotes. Plant J. *75*, 230–244.

13. Tikhonenkov, D.V., Strassert, J.F., Janouskovec, J., Mylnikov, A.P., Aleshin, V.V., Burki, F., and Keeling, P.J. (2020). Predatory colponemids are the sister group to all other alveolates. Mol. Phylogenet. Evol. *149*, 106839.

14. Gawryluk, R.M.R., Tikhonenkov, D.V., Hehenberger, E., Husnik, F., Mylnikov, A.P., and Keeling, P.J. (2019). Non-photosynthetic predators are sister to red algae. Nature *572*, 240–243.

15. Lara, E., Chatzinotas, A., and Simpson, A.G.B. (2006). *Andalucia* (n. gen.)—the deepest branch within jakobids (Jakobida; Excavata), based on morphological and molecular study of a new flagellate from soil. J. Eukaryot. Microbiol. *53*, 112–120.

16. Leander, B.S., Kuvardina, O.N., Aleshin, V.V., Mylnikov, A.P., and Keeling, P.J. (2003). Molecular phylogeny and surface morphology of *Colpodella edax* (Alveolata): Insights into the phagotrophic ancestry of apicomplexans. J. Eukaryot. Microbiol. *50*, 334–340.

17. Yubuki, N., Pánek, T., Yabuki, A., Cepicka, I., Takishitac, K., Inagakid, Y., and Leander, B.S. (2015). Morphological identities of two different marine stramenopile environmental sequence clades: *Bicosoeca kenaiensis* (Hilliard 1971) and *Cantina marsupialis* (Larsen and Patterson, 1990) gen. nov., comb. nov. J. Eukaryot. Microbiol. *62*, 532–542.

18. Yubuki, N., Simpson, A.G.B., and Leander, B.S. (2013). Comprehensive ultrastructure of *Kipferlia bialata* provides evidence for character evolution within the Fornicata (Excavata). Protist *164*, 423–439.

19. Yubuki, N., Leander, B.S., and Silberman, J.D. (2010). Ultrastructure and molecular phylogenetic position of a novel phagotrophic stramenopile from low oxygen environments: *Rictus lutensis* gen. et sp. nov. (Bicosoecida, incertae sedis). Protist *161*, 264–278.

Plant physiology: The to-and-fro of hormone signals to respond to drought

Mingyuan Zhu[1] and Philip N. Benfey[1,2,*]
[1]Department of Biology, Duke University, Durham, NC 27708, USA
[2]Howard Hughes Medical Institute, Duke University, Durham, NC 27708, USA
*Correspondence: philip.benfey@duke.edu
https://doi.org/10.1016/j.cub.2022.12.007

Xerobranching, a temporary suppression of root branching when water is limiting, is controlled by the plant hormone abscisic acid (ABA). A recently published study reveals how root branching is dynamically controlled by redistribution in opposite directions of ABA and auxin.

Climate change has led to significant increases in drought severity in many agricultural regions[1]. We, as humans,

have developed various practices to deal with drought, including selective breeding and efficient irrigation. Plants have also