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Evolution: No end in sight for novel incredible (heterotrophic) protists

Julius Lukeš[1,*], Ivan Čepička[2], and Martin Kolísko[1]
[1]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, and Faculty of Science, University of South Bohemia, 37005 České Budějovice (Budweis), Czech Republic
[2]Department of Zoology, Faculty of Sciences, Charles University, 12800 Prague, Czech Republic
*Correspondence: jula@paru.cas.cz
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The microbial eukaryotes known as protists are of immense importance for our understanding of eukaryotic biology. Although it is often difficult to convince funding bodies to sponsor research projects aimed at finding new protist lineages, such discoveries usually provide new and fundamental insights into cell and evolutionary biology, and ecology.

Probably more scientists study sparrows than all the free-living microbial eukaryotes (protists) combined. This is quite unfortunate, not because the former are unworthy, but because the latter not only contribute substantially to planetary health, but they also represent the majority of functional and evolutionary eukaryotic diversity on Earth[1]. This fact usually comes as a surprise to people studying macroscopic eukaryotes, yet the diversity of protists is bound to grow even further, as implied by the fact that 50% of eukaryotic genes expressed in the ocean do not have any match in public databases and/or lack any reliable phylogenetic affiliation[2]. Studies like the one on *Meteora sporadica* by Eglit, Shiratori *et al.*[3], published in this issue of *Current Biology*, superimpose intriguing protists over the unassigned sequences. This peculiar heterotrophic protist has a giant mitochondrial genome, unusual morphology and ultrastructure, and moves by use of bizarre 'swinging arms'. Surprisingly, however, sequences of its nuclear genes revealed that it belongs to the obscure and species-poor supergroup (kingdom) Hemimastigophora, with which it otherwise does not seem to have anything in common. First described by German protistologists in 2002[4], *Meteora* attracted attention mostly because of its unique rowing movement and by a failure to find its taxonomic home; it has remained in the *incertae sedis* category[5] until now.

Although the study by Eglit, Shiratori *et al.*[3] is exciting, it is not surprising that yet another free-living heterotroph is not closely related to any other known microbial eukaryote — a recurrent theme in the field! Indeed, completely unknown protists and/or those that have been described only morphologically seem to represent the biggest source of untapped eukaryotic diversity. Thanks to Eglit, Shiratori *et al.*[3], *Meteora* is no longer enigmatic, as it has now joined a disparate band that brings together deep-branching lineages, most of which have been discovered only recently, such as Picozoa, *Rhodelphis*, *Anaeramoeba*, *Barthelona*, *Microheliella*, and Provora[6–11]. Some of these organisms have been known for a very long time but remained mysterious until recently (for example, the morphologically

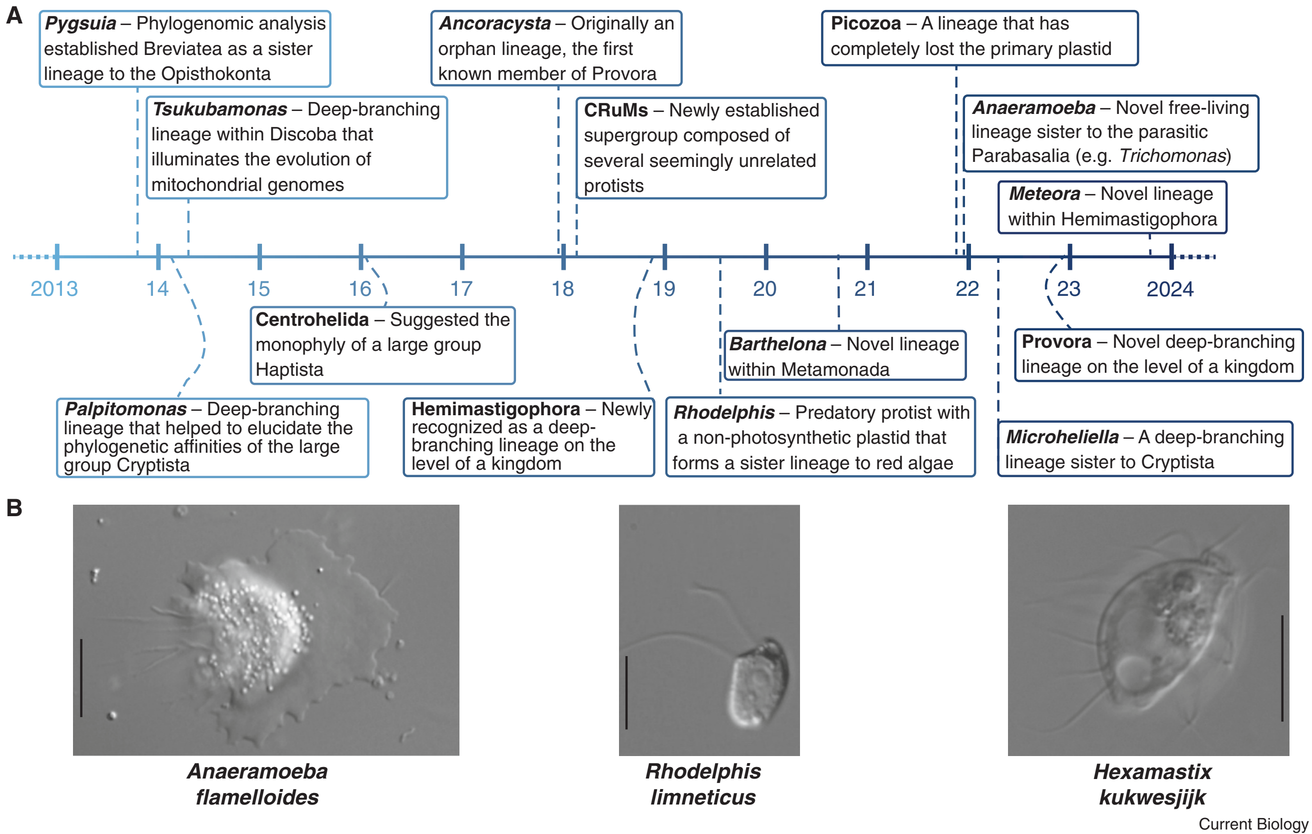

Figure 1. Novel important lineages of protists.
(A) Timeline of studies published within the last decade that presented (phylo)genomic data from protists that form major lineages in the eukaryotic tree. (B) Light micrographs of three examples of important novel protistan lineages. Scale bars denote 10 μm. *Rhodelphis limneticus* image reproduced from Gawryluk *et al.*[6], with permission from Springer Nature. *Hexamastix kukwesjijk* image reproduced from Lax *et al.*[20], with permission from Springer Nature. Photo of *Anaeramoeba flamelloides* in a laboratory culture by Ivan Čepička.

conspicuous hemimastigophoran *Spironema multiciliatum* was described in 1893[12]), whereas others are fresh additions to the known protist diversity. More than three centuries since the discovery of 'animalcules' by van Leeuwenhoek, protists are nowadays the only eukaryotes where the description of a new species may simultaneously mean the discovery of a new phylum, kingdom, or even supergroup.

Entirely 'novel' lineages are not the only kind of major discoveries happening in the protist field, since explorations of poorly studied lineages, as a rule, generate fascinating biological insights as well. For example, studies of the closest microbial relatives of animals, including choanoflagellates, ichthyosporeans, and filastereans, enlightened our understanding of the evolution of multicellularity[13]. Work on the molecular biology of the flagellate *Blastocrithidia nonstop*, which parasitizes the intestine of true bugs, has not only shown that it uses an alternative genetic code with all three stop codons reassigned but also identified novel mechanisms behind its functioning[14]. Another example, the heterotrophic flagellate *Monocercomonoides exilis*, which lives in the chinchilla intestine, has turned out to be the only currently known mitochondrion-lacking eukaryote, with the 'fingerprints' of its lost organelle removed from its nuclear genome[15].

Each of these studies offered a fresh look into the acquisitions, losses, and evolution of organelles, cellular (ultra)structure, and expansions and contractions of gene families, etc. Although these might sound like questions of interest to only a narrow group of protist aficionados, they have frequently had far-reaching implications. Microbial eukaryotes redefined our views of numerous fundamental processes in biology, such as the emergence of multicellularity, the evolution of plastids and photosynthesis, mitochondrial metabolism and functions, the evolution of parasitism, the stability of organelles, and horizontal gene transfer[13,15–17]. Protists often serve as inspiration for cell and molecular biology, rewriting textbook chapters, as novel biochemical and cellular processes are often first discovered in microbial eukaryotes. Parasitic trypanosomes are particularly prominent in this respect[18], most likely due to the fact that, as serious human pathogens, they have been studied far better than other protists. And since some protistan lineages might have lived on this planet for a billion-plus years, remaining in some kind of morphological and genetic stasis, they also allow us to model characteristics of the last eukaryotic common ancestor[19].

However, these new or poorly understood lineages that have been the source of important discoveries are hard to come by for unexpected reasons. Foremost among these, exploratory research, seemingly without testable hypotheses, is not favored by most funding bodies, and the question of whether a new lineage of eukaryotes might be found is not always generally fundable. Many environments that are likely to hold novel eukaryotic microbes, such as deep-sea mud, are notoriously hard and expensive to sample, not to mention the even bigger challenge of obtaining living cells from such locations. On the other hand, the world of protists is so underexplored that it is not folly to try discovering a new eukaryotic supergroup on a beach behind your house (actually, some organisms mentioned above were obtained almost exactly this way). Regardless of how they are found, this is when the most time-consuming part begins, namely bringing the studied organism into culture, as overcoming this exceedingly difficult bottleneck is essential for satisfactory in-depth characterization. Counterintuitively, many interesting protist lineages were discovered as unexpected constituents of laboratory cultures established for entirely different reasons, but the discoveries themselves were by no means random, *amat victoria curam*. Very recently, culture-free methods like single-cell genomics and transcriptomics have become available that partially bypass at least some of these hurdles[7].

Given the difficulties mentioned above, it might come as a surprise how often fundamentally novel organisms are being discovered, and one has to cheerfully conclude that there is still a lot left to be explored. Indeed, the rate of discovery of new lineages does not seem to be slowing down, but rather the opposite is true (Figure 1). The question we should ask is: Where next? Are there nodes on the tree of eukaryotes where major discoveries are to be anticipated, whether in their diversity, functional, or evolutionary aspects? Although there is no clear answer to these questions (since undiscovered eukaryotic diversity still seems to be infinite), there are some candidate positions of burning interest. For example, unicellular relatives of multicellular groups and free-living relatives of parasites and symbionts are of immense interest as they may enlighten us on the evolutionary changes associated with a particular lifestyle. Last but not least, there are ecologically important lineages known almost exclusively from environmental surveys, with very little or no information available about their biology. It is almost guaranteed (actually the word 'almost' may even be omitted) that these protists evolved cellular features and processes highly diverged from textbook eukaryotes.

Another seemingly unanswerable question is what representatives of these as yet undiscovered eukaryotic supergroups look like and where they live. Combining information from the recent principal findings, we predict that many of these exciting unicells are likely small (even for protists), rare in their environment (hence the name *Meteora sporadica*: "after the Sporades Basin, where the species was sporadically found"[4]), and almost completely missing from environmental sequence libraries. Although not abundant, they may be widespread or even cosmopolitan — *Meteora* has been found in Greece, Croatia, and Japan, both in shallow and deep-sea waters. Indeed, most of these elusive protists are likely to be marine and particle-associated (that is, they live in sediments, the stinkier the better). Hence, digging in the mud for a new eukaryotic supergroup might be a good start.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Sexual selection: When crickets go quiet

Allen J. Moore
Department of Entomology, University of Georgia, Athens, GA 30602, USA
Correspondence: ajmoore@uga.edu
https://doi.org/10.1016/j.cub.2023.12.006

Mutations that change male cricket song should be at a disadvantage because the song is used by females to choose amongst males. A new study caught evolution in action and showed that females may have flexible preferences, and new songs may even be preferred so that the mutations spread.

One of the most common experiences of nature is hearing cricket song at dusk. These are males calling out to females, with the function of attracting them for opportunities to mate. Once a female arrives, males use a courtship song to induce mounting and sperm transfer. While male song is common, its evolution is complicated. Females are not passive recipients of male song and actively choose between attractive and less attractive signals. Such communication systems require both an active signaler and an active receiver to function, and thus evolution is expected to act on both simultaneously. How then do new signals evolve? New variants of existing songs are likely to fare poorly because rare variants are easily lost through drift or through selection against signals that have a poor match with receivers and thus attract few mates. Such counter-selection is especially expected for sexual signals, which are thought to be coadapted between the (typically) male signaler and the preference of the female, either through genetic coupling or genetic covariances arising from linkage disequilibrium[1]. Signals that are too quiet, the wrong pitch, or the wrong tempo would seem unlikely to spread in a population in which preferences exist. A new study by Zhang *et al.*[2] in this issue of *Current Biology* documents the very rapid evolution of a novel courtship signal in a Hawaiian population of field crickets (*Teleogryllus oceanicus*), arising from a wing mutation that appeared less than a decade ago.

The Hawaiian Islands seem to provide more than their fair share of windows into evolution. On the Big Island of Hawaii, a wing mutation arose in 2017 resulting in "small-wing" compared to the wild-type "long-wing" males in *T. oceanicus*. Happily, researchers have been monitoring *T. oceanicus* populations for several decades[3] and so had the unique opportunity to catch the origin and to be able to study the spread of this mutation through the population in real time. Wings are critical to the production of cricket calls because calling songs are created by the crickets rubbing together specialized structures on their forewings, and variation in calls amongst species reflects the variation in these structures. Indeed, the typical carrier frequency (pitch) and amplitude (loudness) of the calls of small-wing males differ from those of the wild type by being both higher-pitched and quieter. Consistent with this being a relatively new trait in the population, the calls of small-wing males had much more variable frequencies and amplitude than those of the wild-type long-wing males. Six years is not much time for selection to have refined the small-wing trait. However, communication reflects coevolution of both signal and reception and so the question is how female preferences covary, if at all, with this new trait. After all, crickets sing to attract and influence the mating of females and, like other crickets, *T. oceanicus* females have distinct preferences for the calls they find attractive[4].

Unexpectedly, Zhang *et al.*[2] found that *T. oceanicus* females displayed broad preferences and, rather than suffering a mating disadvantage by not matching the characteristics preferred by females, small-wing males suffered no disadvantage. Although initial female responses were faster with long-wing males, there was no difference in mounting rate or spermatophore transfer. Even more surprisingly, although the sample size is small and therefore this result is still preliminary, some small-wing males never sang at all but still achieved an equally successful mating rate. It appears that small-wing males may even have some advantages in short-range interactions with females.

The big question that Zhang *et al.*[2] address is why are female preferences so permissive toward small-wing males? This isn't the case for all mutants. In other populations, a mutation resulting in flat-wing males has arisen. These males do not

