Quick guide

# Provora

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***What is Provora?*** Provora is a group of unicellular eukaryotes comprised of microbial predators, many of which eat their prey by 'nibbling' them to death. This group remained undiscovered until 2022, despite being one of the ancient branches in the tree of life, on par with major eukaryotic supergroups (Figure 1). Under the microscope, provorans can be easily mistaken for many other predatory unicellular organisms — they are micron-sized, fast-swimming, rounded cells with two flagella. Their deep placement in the eukaryotic phylogeny dates the emergence of Provora to over one and a half billion years ago.

***What are eukaryotic supergroups?*** Advances in genomic sequencing and molecular phylogenetics since the beginning of the 21st century have revealed ancient relationships between superficially dissimilar organisms, many of which had not previously been considered to be close kin. This field has transformed our view of eukaryotic phylogeny and the tree of eukaryotes, resulting in one with a small number of ancient lineages that each encompass a large amount of biodiversity. These new assemblages were termed supergroups. The supergroup concept of eukaryotic phylogeny replaced the traditional 'kingdom' of Protozoa with around ten supergroups that encompass almost the entire known diversity of eukaryotes, plus a few groups that remain stubbornly mysterious. It also showed that most eukaryotic diversity is made up of microbial protists, and that multicellular eukaryotes evolved many times from different protist ancestors. The eukaryotes most familiar to us, animals, fungi, and plants, are now important but subsidiary branches in a phylogenetic tree of unicellular organisms. Provorans form their own new supergroup, and at the genomic level are roughly as diverse as animals and fungi combined.

***If the genetic diversity of provorans is so great, why do they all look the same?*** Diversity, like beauty, can be in the eye of the beholder. Although it is true that provorans are all small predators, when viewed at a more detailed level they are quite diverse. Indeed, provorans consist of two ancient sub-groups, Nebulidia and Nibbleridia, which differ greatly in their ultrastructure, feeding habits, and genomes. They share only 20–25% of estimated gene families, consistent with their high level of genetic diversity. Both nebulids and nibblerids feed on other unicellular eukaryotes using a ventral groove (a type of cellular mouth) and extrusomes (a cellular harpoon) to attack their prey. They fire this harpoon at their prey, tethering themselves to the victim and allowing the predator to devour the cell. In the case of nibblerids, the prey may exceed the predator in size: their ventral groove is armoured with subcellular microtubules and tooth-like protrusions that give them the ability to pinch off pieces of the larger prey cell. This is a unique and previously unseen mechanism of microbial feeding. Size is often a limiting factor for predator–prey interactions, yet the biting behavior displayed by provorans shows that interactions in which the game is larger than the hunter should be taken into account when modeling microbial food webs and matter and energy flows in aquatic ecosystems.

***How many species of Provora are there?*** Currently, only seven species of Provora are known from cultured strains. These are split into four genera — *Nibbleromonas*, *Ubysseya*, *Nebulomonas*, and *Ancoracysta*. The two latter genera fall under the Nebulidia lineage, and the two former are within Nibbleridia. This is not very many species, but looking at environmental sequence data (rRNA gene sequences that were obtained from natural samples of uncultivated diversity) shows that there are other provorans yet to be discovered. These probably belong to different, as yet unknown, genera and families, and what their structure and behaviours are like remains to be seen.

***How did they get their names?*** The name Provora is a syllabic abbreviation of the English words 'PROtists', 'deVOuring', and 'voRAcious', reflecting their predatory lifestyle. The names *Nibbleromonas* and *Nebulomonas* originate from the Latin 'monas' (unicellular organism) and the English 'nibbler' and the Latin 'nebulosus' (misty), respectively. *Nibbleromonas* alludes to the 'nibbling' feeding behaviour of these flagellates, whereas *Nebulomonas* reflects the enigmatic status of the genus, whose evolutionary position has remained unclear since the discovery of its first known representative, *Ancoracysta twista*. *Ancoracysta* is named after the bizarre stinging organelles of this organism, the ancoracysts, whose shape resembles that of an anchor. And, finally, the name *Ubysseya* comes from UBC (University of British Columbia) and 'The Ubyssey', the university's official student newspaper. *Ubysseya* was found in marine waters near Vancouver using a UBC research vessel.

***Where do we find them?*** It is noteworthy that the Provora were not isolated from exotic habitats or through particularly adventurous fieldwork. Instead, they were found in ordinary sea water, sediments, and coral samples. These samples were collected around the world, including the Red, Black, and Caribbean Seas, and the Pacific and Arctic Oceans. High-throughput environmental sequencing shows that provorans live almost everywhere in marine environments, are less common in brackish or fresh waters, and are not found in soil.

***If they live everywhere, why did it take so long to discover this group?*** The Provora have existed for probably over a billion years and they are distributed in common habitats across the globe — in other words they have been living right under our noses. So, why did it take so long to find them? The answer is likely a combination of factors. First, their small size, unremarkable appearance, and relatively low abundance makes them easy to overlook, and required meticulous cultivation practices and microscopy, followed by comparative analyses of the sequencing data to recognize what they were. Small predatory unicellular organisms are

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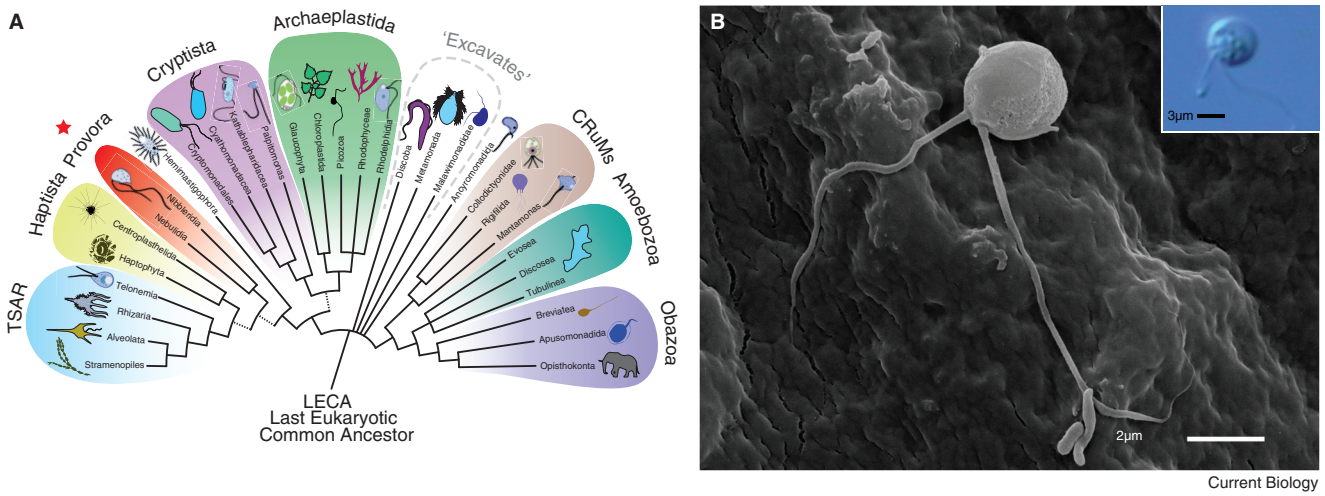


**Figure 1. Provora and their place among eukaryotes.**
(A) The eukaryote tree of life and phylogenetic placement of Provora denoted by star symbol. Tree topology is based on a consensus of recent phylogenomic studies; unresolved branching order among lineages is shown as multifurcation; branches with uncertain support across reconstructed phylogenies are indicated with dashed lines. Currently, around ten supergroups of eukaryotes are recognized, with several additional groups without a clear position in the tree. Provora form their own supergroup diverging somewhere around the base of the supergroups Haptista and TSAR, but in some trees even appearing sister to Hemimastigophora — another enigmatic group of early diverging predatory protists. Eukaryote silhouettes modified from Galindo *et al*. (2022). Amoeba proteus icon modified from Gareth Monger (CC BY 3.0). Elephant icon modified from Jan A. Venter, Herbert H. T. Prins, David A. Balfour and Rob Slotow (vectorized by T. Michael Keesey) (CC BY 3.0). (B) Cell morphology of the provoran genus *Nibbleromonas* visualized by scanning electron microscopy and differential interference contrast light microscopy (inset). Roundish cells with two heterodynamic flagella inserted into separate flagellar pockets and tapering tips (acronemes). Electron microscopy image used with permission by Artem Belyaev.

inherently rare in nature, just like large predators are rarer than their prey. But unlike large animals, they are easily overlooked and to the non-expert eye 'all look the same'. Molecular sequence surveys should have sidestepped this problem, but here their relative abundance and the lack of taxonomic affiliation were the issue: provoran sequences were actually sampled in the past, but were discarded from analyses because they could not be classified or fell short of read-depth cut offs commonly employed to exclude artifacts and contaminants. Therefore, without the data from cultured strains there was no way to see that these sequences were actually real. These technical explanations are likely correct, but they also obscure the depth of this problem, which is as though we carried out decades of research on the African savannah using tools all biased to overlooking 'cats'.

### *What can we learn from them?*

Provorans are a good example of how desperately underexplored the microbial world is. The vast majority of biodiversity is microbial, but microbial life is so small, unfamiliar, and difficult to understand intuitively that it is most often simply ignored when talking about global biodiversity and ecosystems (for example, they are rarely or never even mentioned in policy discussions about biodiversity loss). However, microbes, including microbial eukaryotes, form the foundation and backbone of all food webs, and the organisms we do often discuss are all reliant on this microbial foundation for survival. And if microbial diversity is understudied, the *most* understudied part of that diversity is microbial predators. Like animal predators, such as wolves and lions, microbial predators are numerically rare but play an outsized role in their ecosystems as they regulate the populations of their prey. The discovery of Provora as a new eukaryotic supergroup also emphasizes more generally that our knowledge about the tree of life is incomplete. Even while we debate how to preserve biodiversity across the globe, we still lack fundamental knowledge about the extent and identity of that diversity, mostly in the microbial realm. It is almost certain that additional undiscovered microbial lineages remain to be found, and each of these has the potential to fill in major gaps in our understanding of major transitions in evolution, and how life interacts today.

### *Where can I find out more?*

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### DECLARATION OF INTERESTS

The authors declare no competing interests.

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