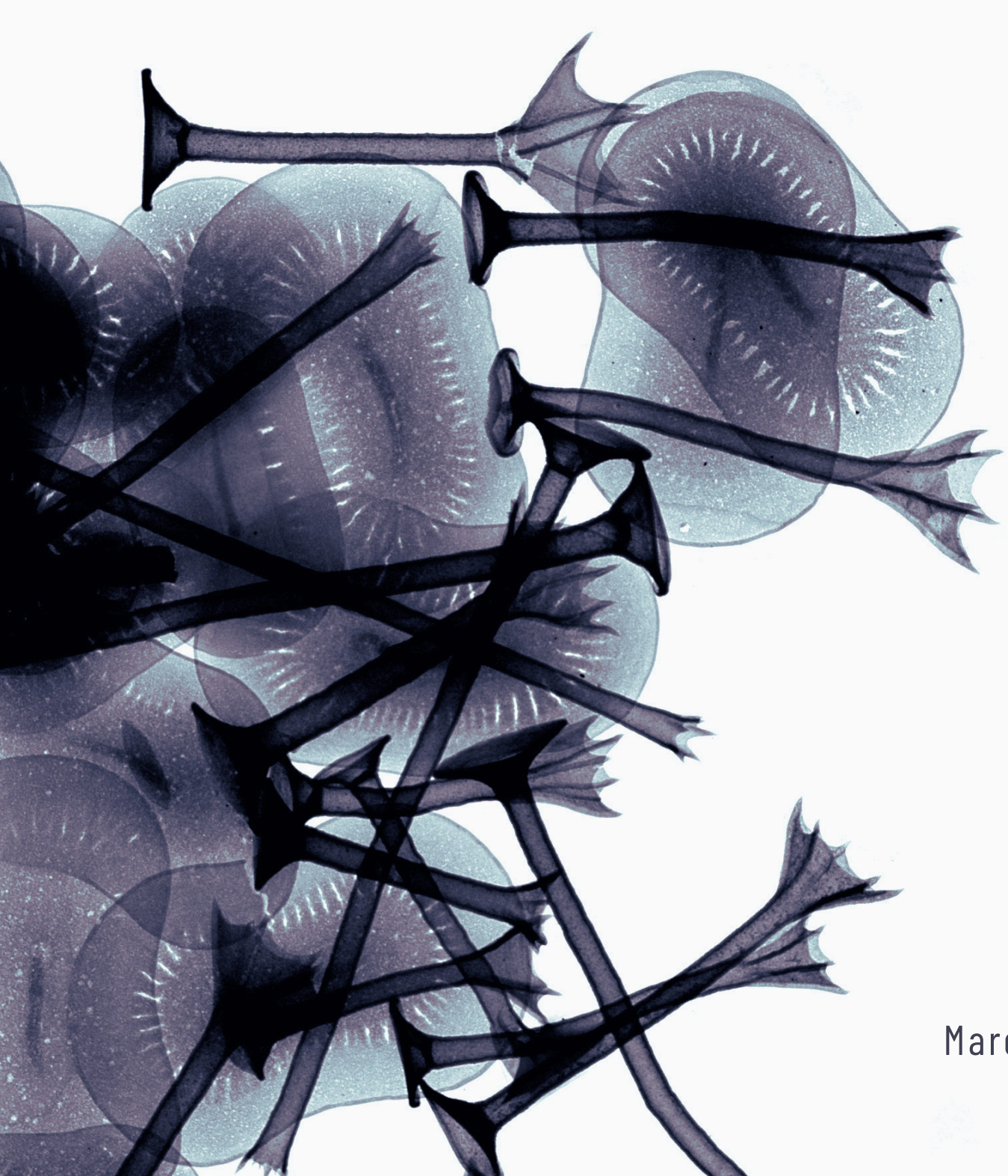
AMOEBA DISCOVERY



Issue 1

March 2025

Cover picture: Skeletal elements of Acanthocystis nichollsi (Haptista, Centroplasthelida): transmission electron microscopy, digital color. Micrograph by Vasily Zlatogursky.

WHAT'S INSIDE

Who is behind the *Magazine*

Bio

My fascination with the natural world began in early childhood. I pursued my biology studies at the University of Novi Sad (Serbia), where I earned my PhD in 2020, specializing in the taxonomy of testate amoebae from the Dinaric Alps. A Swiss government scholarship later brought me to the University of Neuchâtel, where I conducted postdoctoral research on the diversity of testate amoebae in the Jura Mountains. The next chapter of my academic journey led me to China, where I studied testate amoebae in East and Southeast Asia. Immersed in biodiverse ecosystems of the tropical and subtropical regions, I became captivated by the geographical distribution of rare and exotic species. In China, I founded and taught the world's first university course dedicated exclusively to testate amoebae, alongside several other biodiversity-related courses. Although I'm not currently affiliated with a university, my work as an independent researcher remains ongoing. Through this magazine, I aim to bring the microscopic world into focus for a broader audience—unveiling the incredible life forms that exist just beyond the reach of the naked eye.

Dr. Stefan Luketa

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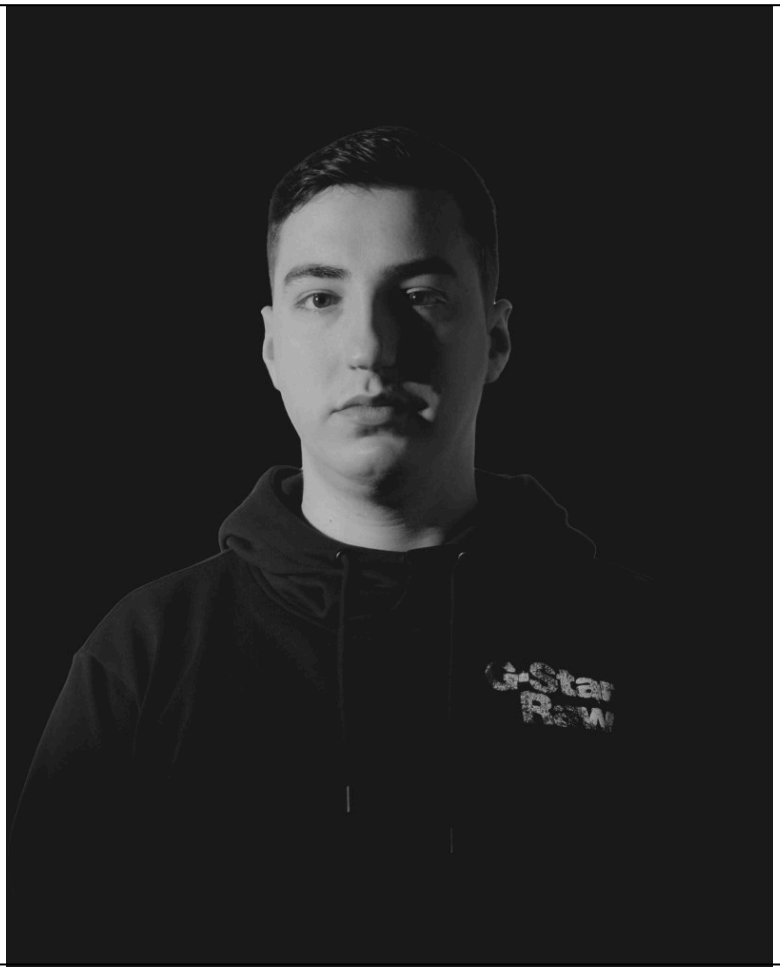
From the Editor



The 21st century appears to be the age of science, a time when microscopy is flourishing with professional researchers studying microscopic organisms, many of whom are based at universities. In today's competitive academic environment, many of these researchers are primarily focused on publishing papers aimed at their peers in the scientific community. Meanwhile, the availability of affordable and accessible microscopic equipment has led to the growth of a vibrant community of amateurs and enthusiasts who are captivated by observing life at the microscopic level. However, there are few opportunities for these two groups—professional and amateur microscopists—to connect, largely due to the lack of platforms that facilitate their interaction. Inspired by the need for such a space, I came up with the idea of creating *Amoeba Discovery*, a free online magazine dedicated to everyone who shares a passion for life on the microscopic scale.

Without you, Amoeba Discovery is just an idea—together, we make it a reality

True slime mold *Trichia crateriformis*.
Photo by Alison Pollack, check her profile:
https://www.inaturalist.org/people/alison_pollack

The primary focus of the magazine is on protists and tiny metazoans, but we will also explore topics such as the history of microscopy, various microscopic methods and techniques, and the intriguing structures of fungi, plants, and animals at the microscopic level. These subjects will be examined through three key types of content: in-depth articles, interviews with experts, and reviews of the latest publications in the field.

Do you have captivating illustrations of the microscopic world you'd like to share through *Amoeba Discovery*? Or perhaps you'd like to contribute an article or share your story in an interview? I'd love to hear your ideas and suggestions for future issues of our quarterly magazine. Together, we can help fulfill its mission: to connect professional researchers with amateur microscopists and nature lovers eager to explore the fascinating mysteries of the microscopic world.

Finally, I encourage you to follow *Amoeba Discovery* on social media to stay updated on the latest content. And if you enjoy the magazine's concept and content, please help spread the word to your friends and colleagues so we can connect with all the lovers of the microscopic world around the globe. Without you, *Amoeba Discovery* is just an idea—together, we make it a reality.

Stefan Luketa

Editor-in-Chief

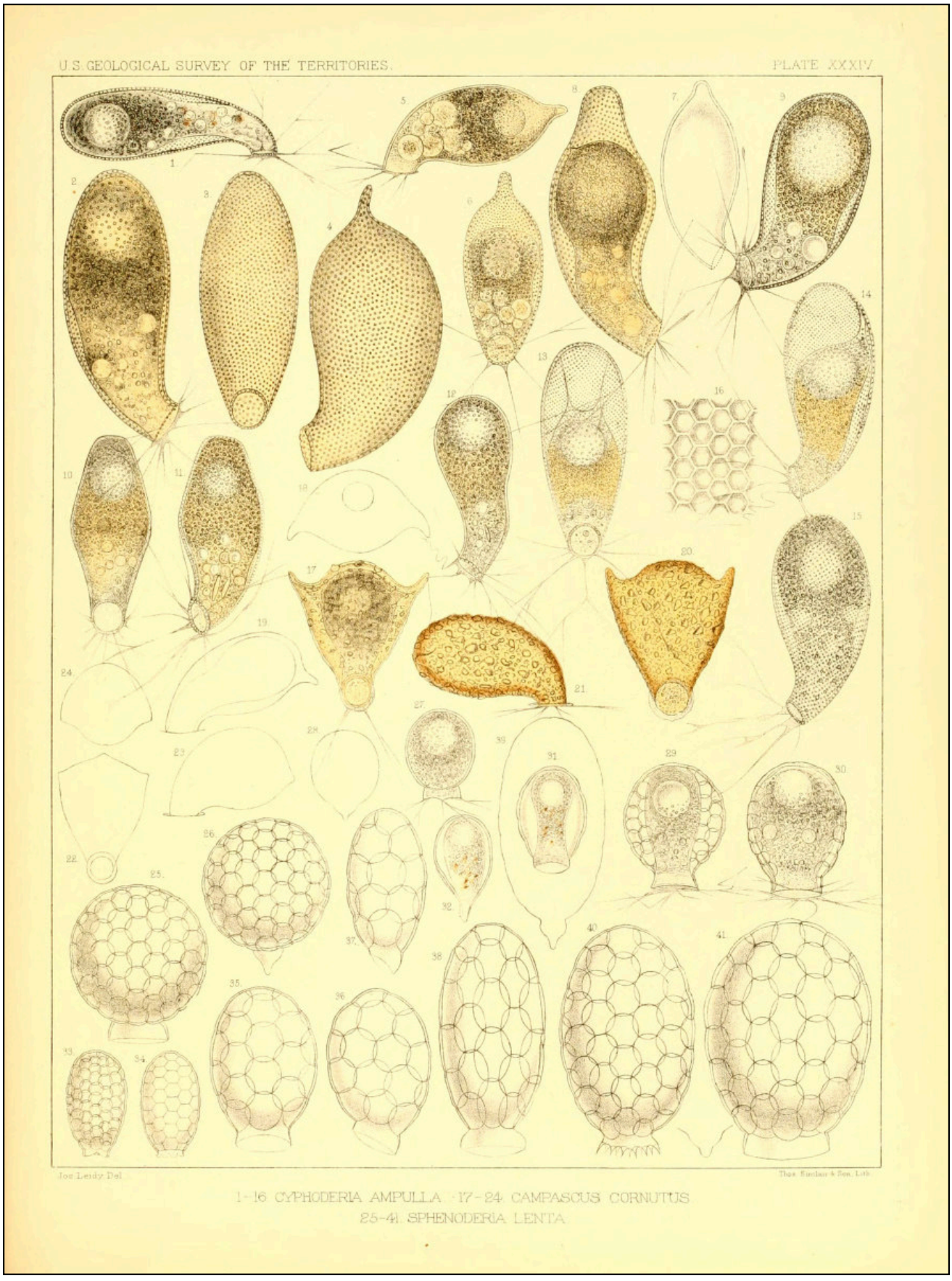
Testate Amoebae

A Hidden World Awaiting Exploration

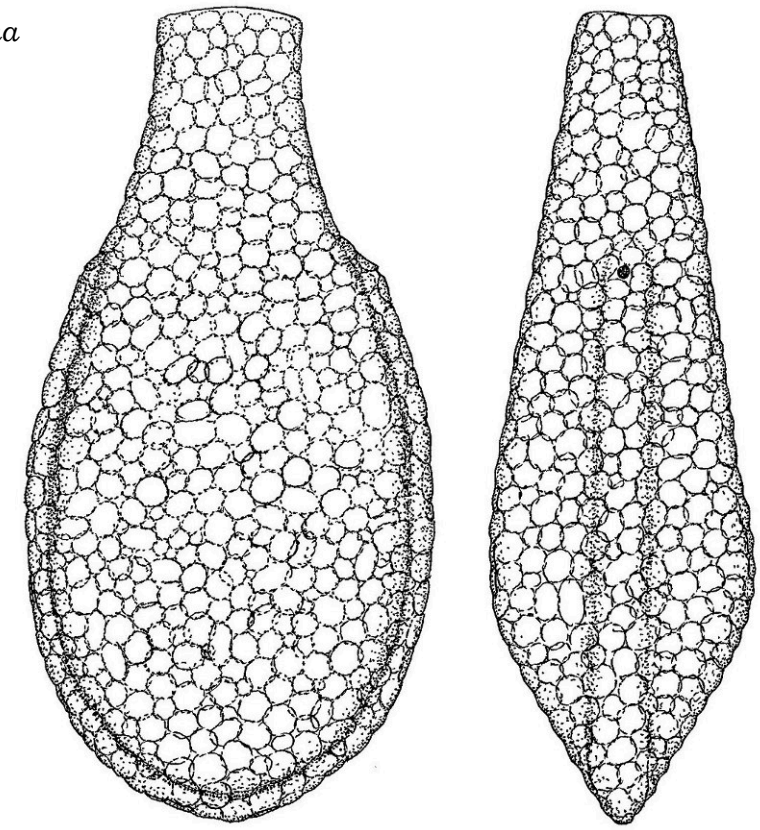
By Dr. Stefan Luketa

Testate amoebae are fascinating microscopic organisms with protective shells that play a key role in nutrient cycling across various ecosystems. Their relatively large size and slow movement make them especially appealing to microscopy amateurs and enthusiasts, offering a unique opportunity to explore biodiversity on a microscopic scale. Many species remain undiscovered in local habitats, making testate amoebae an exciting area of study. By documenting their observations, enthusiasts contribute to citizen science, expand biogeographic knowledge, and strengthen connections within the scientific community. Exploring testate amoebae allows us to appreciate the hidden wonders of biodiversity and recognize our role in uncovering them.

Drawings of various testate amoebae from *Fresh-Water Rhizopods of North America* by Joseph Leidy (1879)


U.S. GEOLOGICAL SURVEY OF THE TERRITORIES.
PLATE XXXIV
Joe Leidy Del.
Thos. Sinclair & Son, Lith.
1-16 CYPHODERIA AMPULLA. 17-24 CAMPASCUS CORNUTUS.
25-41 SPHENODERIA LENTA.

Drawings of *Planocarina marginata* from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



Introduction

In the concealed corners of our planet's ecosystems—spanning from verdant wetlands to the dim recesses of shadowy forests—a captivating microcosm teems with life. Testate amoebae, these often-overlooked unicellular organisms, play an unexpectedly vital role in sustaining the intricate balance of nature. Their abundance and diversity, coupled with their unique shell structures, make them an ideal model for taxonomy and evolutionary studies, leading to a surge in research over the past two decades. As we grapple with the pressing challenges of biodiversity loss and climate change, understanding these microscopic marvels becomes increasingly crucial.

In this article, we invite you to journey into the world of testate amoebae—discovering their intricate structures, remarkable lifestyles, and compelling reasons to further explore these hidden wonders. Prepare to unveil the secrets of this microworld, where nature's delicate balance is maintained by some of its smallest inhabitants.

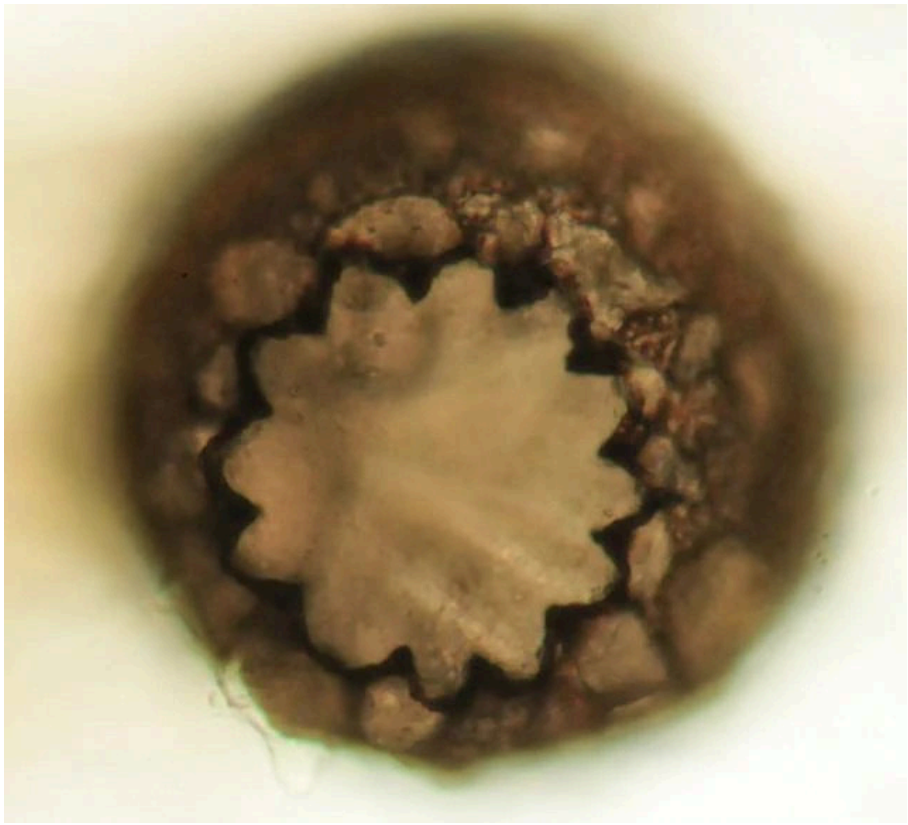
Shell of *Netzelia corona* with three horns. Photo by Ferry Siemensma, www.arcella.nl

What do testate amoebae look like?

Testate amoebae are a captivating group of single-celled microorganisms that showcase remarkable morphological diversity, highlighting their adaptive strategies across various environments. These protists, classified within the broader category of amoeboid organisms, exhibit an array of forms and sizes, yet all share a defining characteristic: they are enveloped in a protective shell, or test. The construction of this shell varies significantly, utilizing materials such as silica, calcium carbonate, or organic substances. This diversity in composition not only serves to shield the amoebae from predation and environmental stressors but also reflects their evolutionary adaptations to specific habitats.

> THE CONSTRUCTION OF TESTATE AMOEBA SHELLS VARIES SIGNIFICANTLY, UTILIZING MATERIALS SUCH AS SILICA, CALCIUM CARBONATE, OR ORGANIC SUBSTANCES

Aperture of *Netzelia corona* with 12 denticulate lobes. Photo by Ferry Siemensma, www.arcella.nl



Some species of testate amoebae present an intriguing puzzle for researchers, primarily described through their empty shells, leaving much of their biology enshrouded in mystery. Many of these organisms feature agglutinated shells formed from quartz grains, known as xenosomes. However, the robust and opaque nature of these shells poses significant challenges for studying their cellular structures. The thick walls obstruct light and hinder transmission electron microscopy, complicating our efforts to examine the intricate details of their cytoplasm.

Surrounding their cells is a thin, elastic plasma membrane that plays a crucial role in maintaining the amoeba's life processes. This semi-permeable membrane regulates the exchange of substances, ensuring homeostasis while facilitating nutrient uptake and waste removal. Additionally, it allows the amoeba to change shape and form pseudopodia, which are essential for locomotion and feeding.

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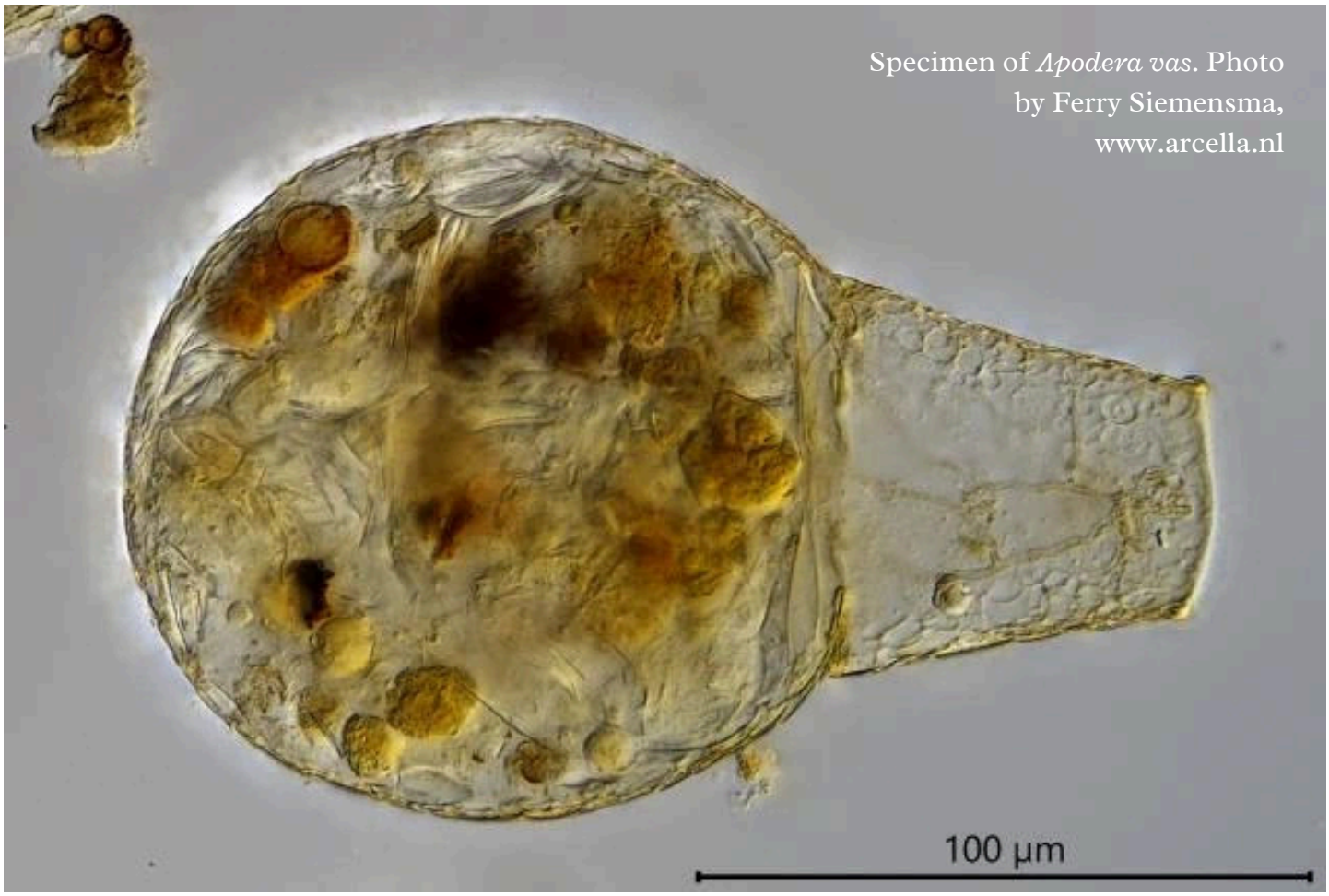


Specimen of *Apodera vas*. Photo by Ferry Siemensma, www.arcella.nl

At the core of these organisms lies the protoplasm, comprising both the cytoplasm and the nucleus. The cytoplasm is typically organized into two distinct zones. The anterior zone consists of a granular matrix filled with food and digestive vacuoles, numerous mitochondria for energy production, and peripheral vesicles containing organic cement. In contrast, the posterior zone features a denser matrix housing the nucleus, surrounded by a compact mass of granular endoplasmic reticulum and abundant ribosomes. This region also contains one or more Golgi complexes and several contractile vacuoles, strategically positioned near the nucleus and adjacent to the cell membrane.

Contractile vacuole *is a specialized organelle found in freshwater testate amoebae that helps regulate water balance by expelling excess water from the cell.*

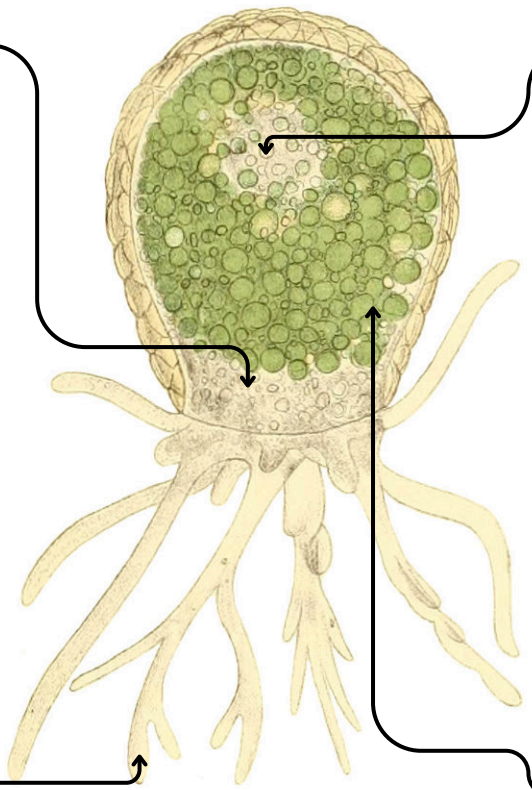
General Structure of Testate Amoebae

Shell

This protective structure can be composed of various materials, including sand grains, organic matter, or even the remains of other microorganisms. It offers protection from predators and harsh environmental conditions. The shape and structure of the shell vary among species, making it a key feature for identification and classification.

Cytoplasm

It is the gel-like substance that fills the interior of the cell, surrounding the nucleus and containing organelles. The cytoplasm facilitates the movement of materials within the cell, ensuring that essential processes such as metabolism and waste elimination occur efficiently.



Nucleus

It is a vital component that contains the genetic material necessary for reproduction and metabolic processes. The nucleus is usually singular, though some species have more than one. The arrangement of the nucleus can also aid in species identification, as it often displays distinctive features, such as the number of nucleoli.

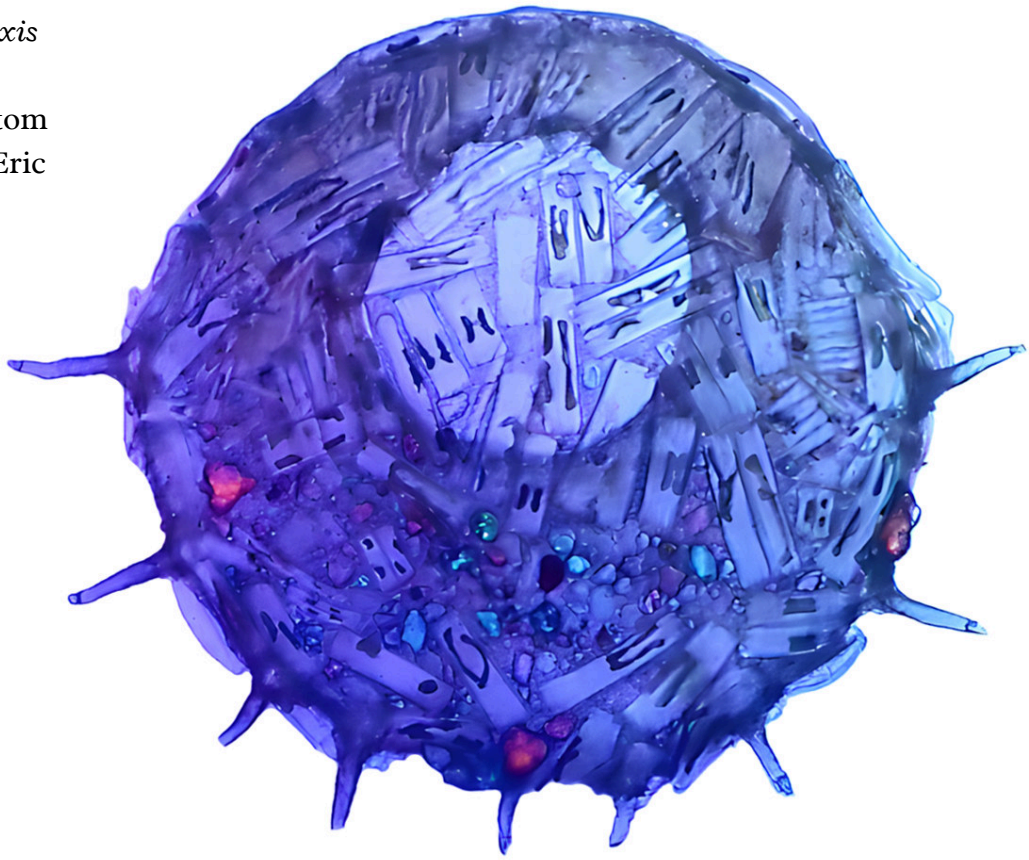
Pseudopodia

Pseudopodia are temporary, extension-like structures formed by the amoeba's flexible cytoplasm. They are crucial for locomotion, feeding, and interaction with the environment. Testate amoebae typically use pseudopodia to move in a crawling manner, extending and retracting them in a process known as amoeboid movement.

Symbionts

Testate amoebae sometimes host a variety of symbiotic organisms within their cytoplasm, including bacteria and algae. The symbiotic algae provide the amoeba with nutrients through photosynthesis, while, in turn, benefiting from the protection and stable environment offered by the amoeba's cell.

Drawing source: Leidy J. 1879. Fresh-water Rhizopods of North America. Report of the United States Geological Survey of the Territories. 12: 1-324.



Shell of *Centropyxis discoides* with incorporated diatom shells. Photo by Eric Farang.

Shells with incorporated diatoms

Some testate amoebae are master architects, crafting their shells entirely from organic materials that they secrete themselves. Others, however, take a more resourceful approach, scavenging their surroundings for building materials. They carefully embed environmental particles into a layer of organic matter, reinforcing their protective homes. While the choice of materials largely depends on what is available in their habitat, these tiny engineers are not random in their selections—they actively seek out materials that best suit their needs.

Among the many options, some amoebae show a distinct preference for diatom shells. Diatoms, microscopic algae encased in intricately designed silica frustules, offer an exceptional building material —strong, lightweight, and beautifully ornamented. When a testate amoeba encounters a suitable diatom, it does not simply absorb it into its structure. Instead, it carefully manipulates the frustule using its pseudopodia, positioning it with precision. With the help of sticky organic secretions, the amoeba secures the new addition, sometimes even reshaping its own shell to accommodate the piece perfectly.

A stunning example of this phenomenon was discovered by Eric Farang, who found a particularly beautiful specimen of *Centropyxis discoides*. This species is relatively large, with an average size of around 250 µm, and it has a notably flattened shell compared to other *Centropyxis* species. Typically, the shell is made up of granular organic material, interspersed with a few flat sand grains, although it is rarely fully covered. However, the specimen documented by Farang is an exception—completely covered with diatom frustules.

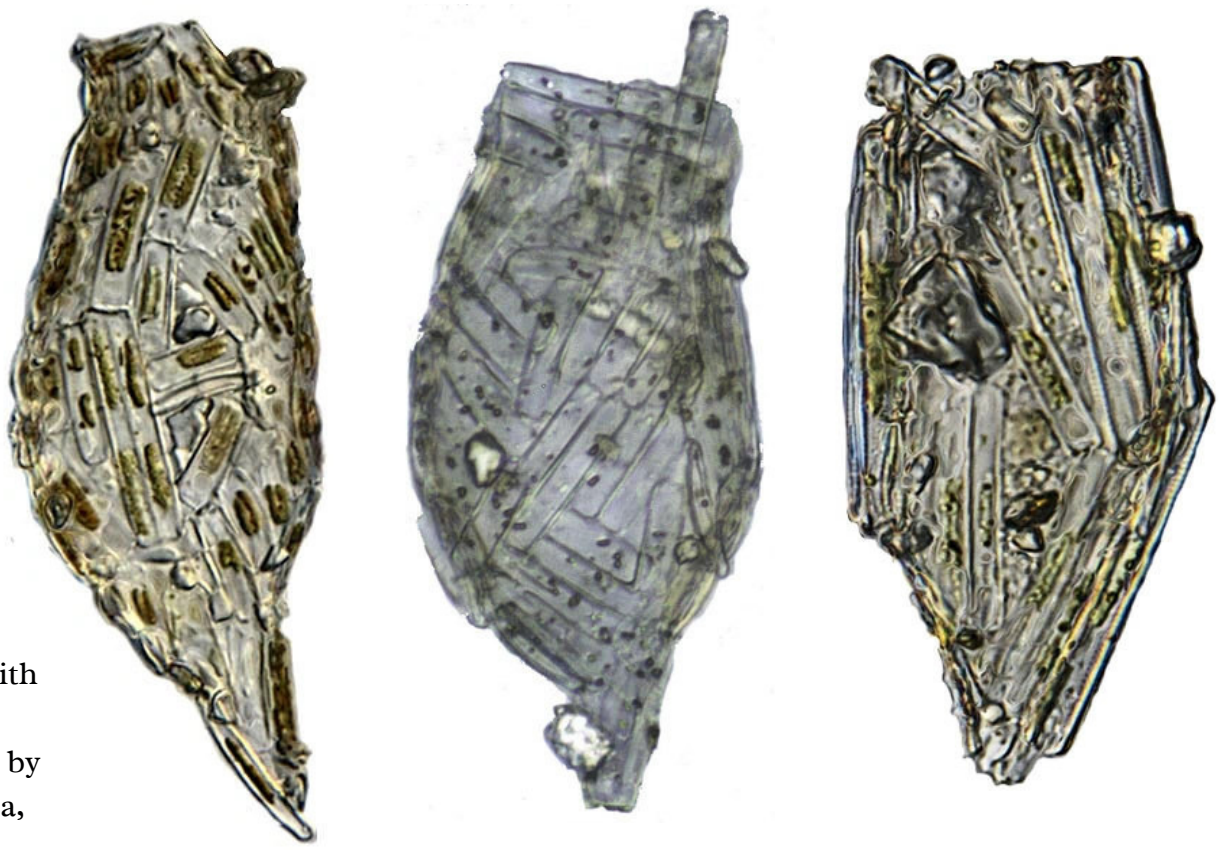
One fascinating example comes from Ferry Siemensma, who meticulously documented numerous *Difflugia bacillifera* specimens. These amoebae exhibit remarkable variations in the design of their shells, with the outlines often obscured by a diverse array of diatom frustules integrated into their structure. In some cases, the shell appears to be entirely composed of diatoms, creating a striking mosaic of microscopic algae. The siliceous materials that make up these shells are equally diverse: some frustules are nearly as long as the body of the shell itself, while others are much smaller, measuring around 10 µm in length. Additionally, spherical siliceous cysts from chrysomonad flagellates are also incorporated, further enriching the shell's structure.

Shells of *Difflugia bacillifera* with incorporated diatoms. Photos by Ferry Siemensma, www.arcella.nl

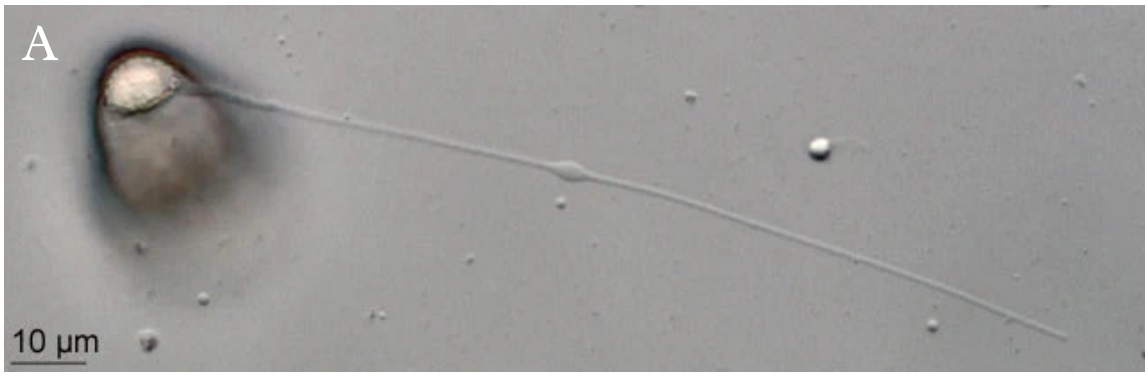
Siemensma's research extends to *Cylindrifflugia bacillariarum*, where he observed similarly diverse shells. It is believed that the foundational structure of these shells consists of small shell plates, with diatoms serving a reinforcing role. The organic cement that binds these materials together is especially intriguing. In some instances, it forms a patterned structure composed of small rings, while in others, it appears as delicate strands interspersed between the particles, contributing to the overall stability and cohesion of the shell.

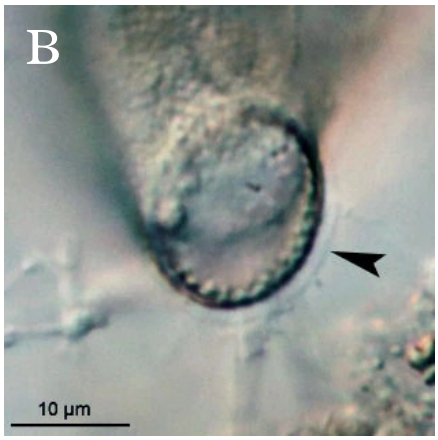
But why do testate amoebae incorporate diatom frustules into their shells? The true purpose of this behavior remains a mystery. Do these frustules offer a practical advantage—perhaps a stronger yet lighter alternative to sand particles? Or is there something more at play? Some researchers speculate that by incorporating diatom shells, amoebae may be engaging in a form of mimicry, disguising themselves to blend seamlessly into their environment and evade predators.

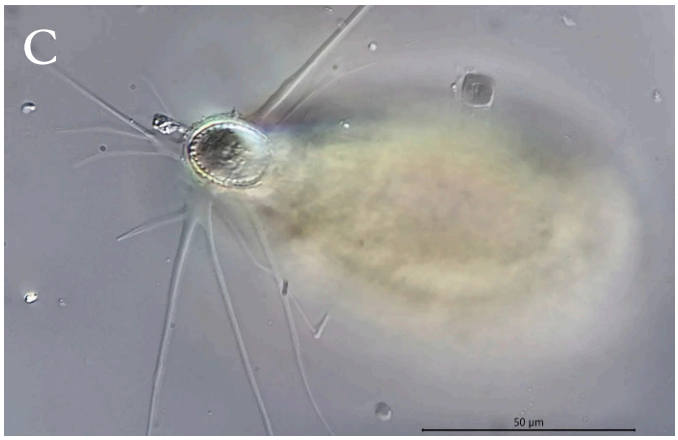
This remarkable adaptation showcases the unexpected complexity of microscopic life, where survival often depends not just on individual resilience, but on the ability to shape and integrate with the surrounding world in astonishingly innovative ways.



Shells of *Cylindrifflugia bacillariarum* with incorporated diatoms. Photos by Ferry Siemensma, www.arcella.nl





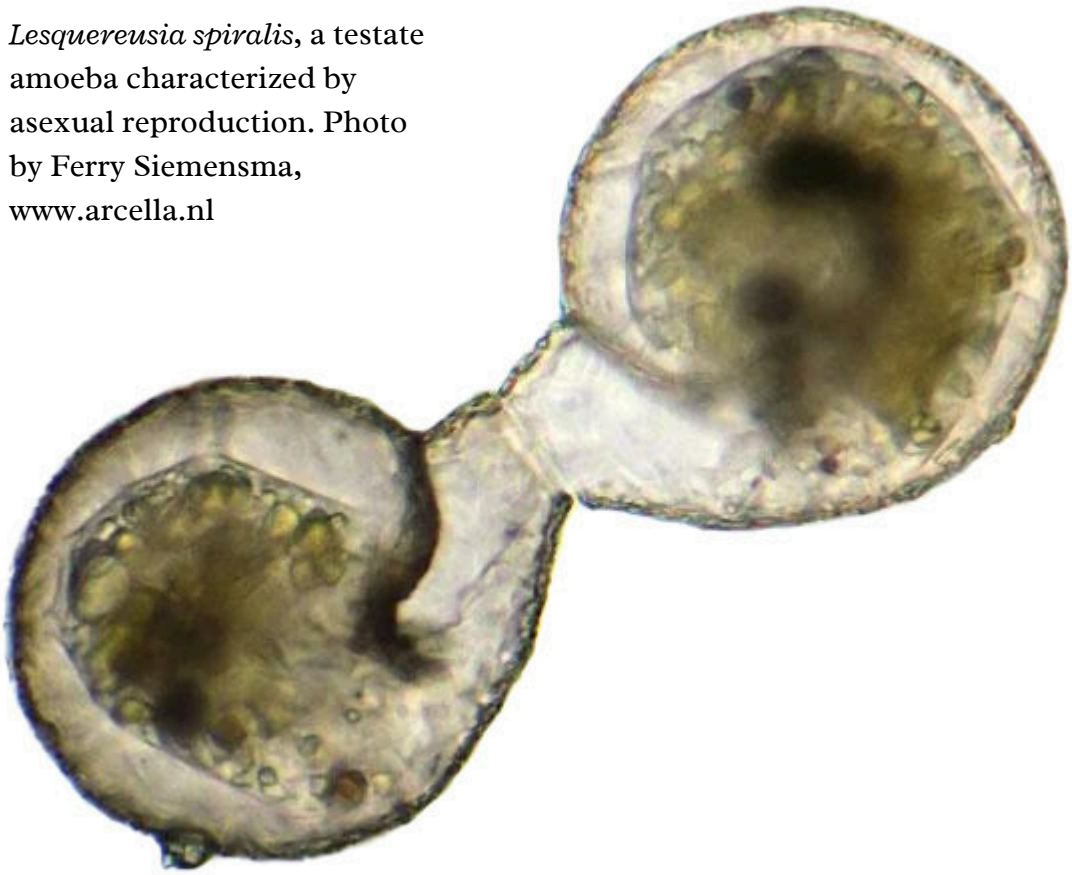


Pseudopodia: (A) *Cyphoderia laevis* – specimen with a very long filopodium; (B) *Cyphoderia ampulla* – specimen with an aperture surrounded by a presumed organic veil; (C) *Cyphoderia ampulla* – specimen with several filopodia. Photos by Ferry Siemensma, www.arcella.nl

Lifestyle of testate amoebae

The mode of movement in testate amoebae is nothing short of fascinating. By extending their pseudopodia, these microscopic organisms navigate their surroundings with remarkable agility. This ability to form temporary projections enables them to crawl along surfaces, effectively capturing food particles such as bacteria, algae, and organic debris. In this role, testate amoebae are vital players in the microbial food web, influencing population dynamics and significantly contributing to nutrient cycling across diverse ecosystems. Their feeding behavior illustrates a fundamental ecological function, aiding in the regulation of organic matter and supporting the overall health of their habitats.

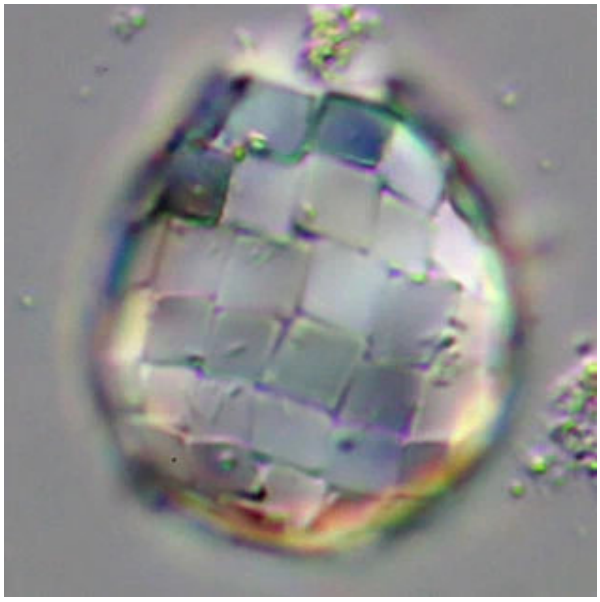
> BY EXTENDING THEIR PSEUDOPODIA, TESTATE AMOEBAE NAVIGATE THEIR SURROUNDINGS WITH REMARKABLE AGILITY

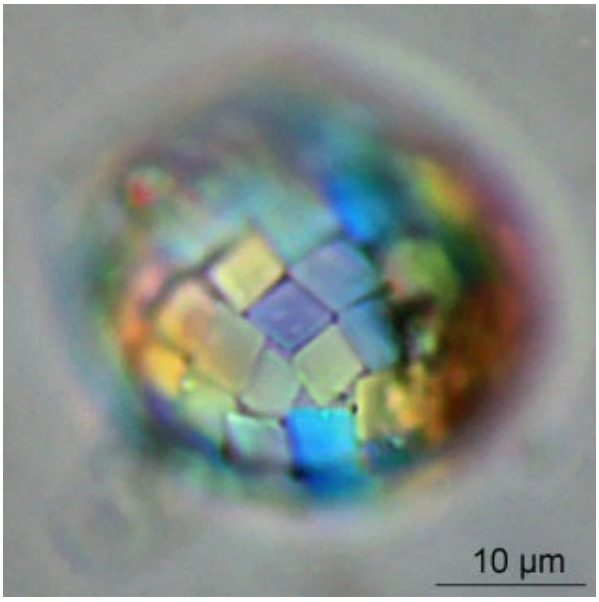


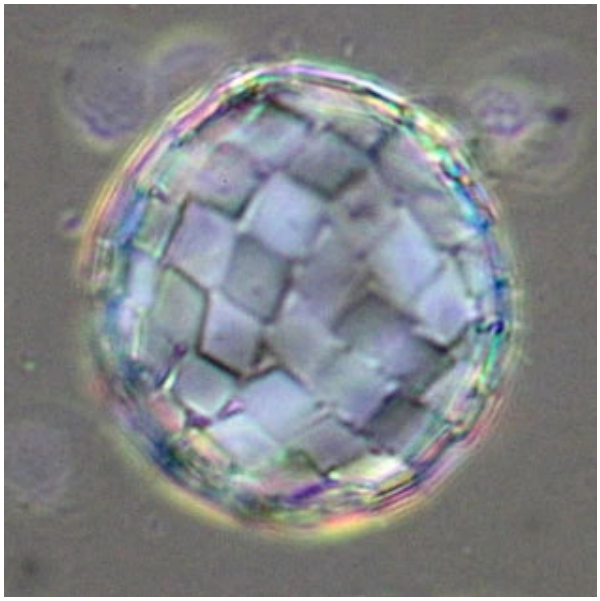
Lesquereusia spiralis, a testate amoeba characterized by asexual reproduction. Photo by Ferry Siemensma, www.arcella.nl

Reproduction in testate amoebae primarily occurs through binary fission, a process that ensures the replication of the parent cell. During this method, an identical daughter cell is formed, with subsequent fission typically occurring via a closed form of mitosis known as orthomitosis. This efficient reproductive strategy allows for rapid population growth, with doubling times ranging from two to twelve days depending on species and environmental conditions. Interestingly, testate amoebae often reproduce more quickly in laboratory settings than in their natural habitats, where environmental stresses can impact their life cycles.

For many years, it was widely believed that testate amoebae exclusively reproduced asexually. However, evidence for sexual reproduction within this group remained elusive until the late 19th and early 20th centuries. It wasn't until the pioneering studies of Valkanov in the 1960s that the complexity of reproductive strategies in testate amoebae began to emerge. Valkanov's research identified four distinct types of copulation, suggesting that sexual reproduction may indeed play a significant role in the life cycles of these organisms.







Paraquadrula irregularis, a testate amoeba characterized by sexual reproduction. Photos by Ferry Siemensma, www.arcella.nl

The capacity for both asexual and sexual reproduction reflects the remarkable adaptability of testate amoebae. In stable environments, asexual reproduction can dominate, facilitating swift population expansion. Conversely, in fluctuating or stressful conditions, the potential for sexual reproduction offers a strategic advantage by fostering genetic diversity. Such adaptability is crucial for the survival of these microorganisms as they thrive in various ecological niches.

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THE CAPACITY FOR BOTH ASEXUAL AND SEXUAL REPRODUCTION REFLECTS THE REMARKABLE ADAPTABILITY OF TESTATE AMOEBAE

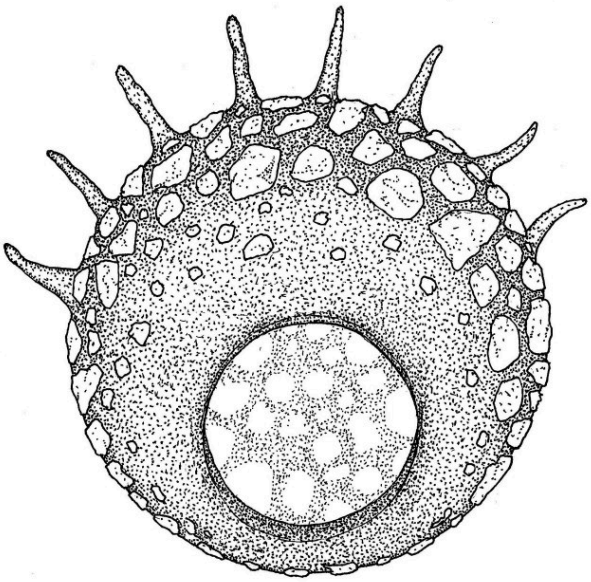
Mosses, a typical habitat of testate amoebae. Photo by Stefan Luketa.

Habitats testate amoebae inhabit

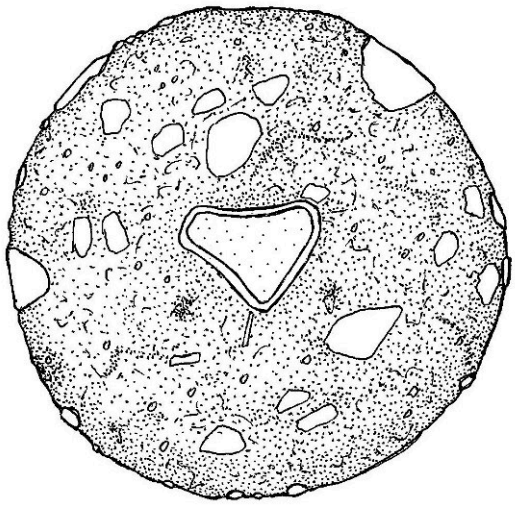
Globally distributed from tropical regions to the polar extremes, testate amoebae inhabit a wide variety of environments, including terrestrial, freshwater, brackish, and marine ecosystems. Their presence in these diverse habitats speaks to their remarkable adaptability and resilience. However, in terrestrial settings such as forest mosses, soils, and leaf litter, testate amoebae face a critical limitation: they require a thin film of moisture to survive. This delicate layer of water is essential for their movement across surfaces like moss and decaying plant matter, enabling their unique locomotion through pseudopodia. Without this vital moisture, their ability to move—and, consequently, to feed—becomes severely

> BY EXTENDING THEIR PSEUDOPODIA, TESTATE AMOEBAE NAVIGATE THEIR SURROUNDINGS WITH REMARKABLE AGILITY

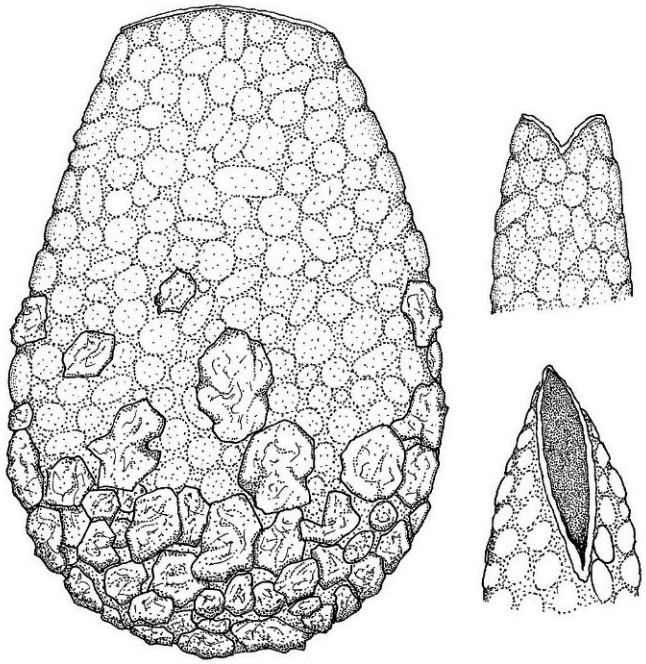
Drawings of common testate amoeba species from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



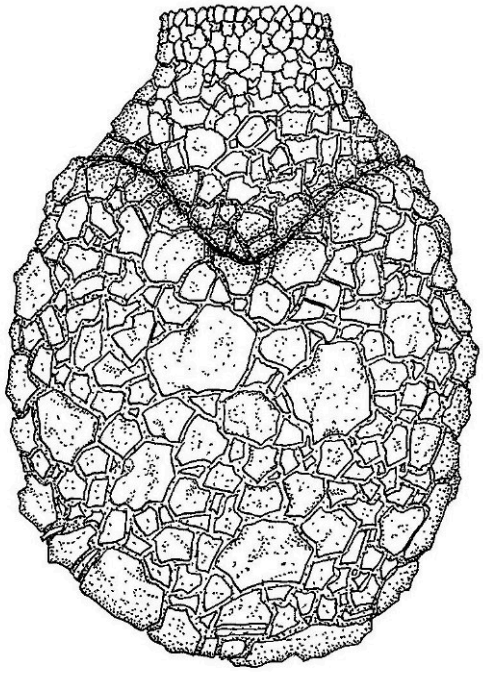
Centropyxis aculeata



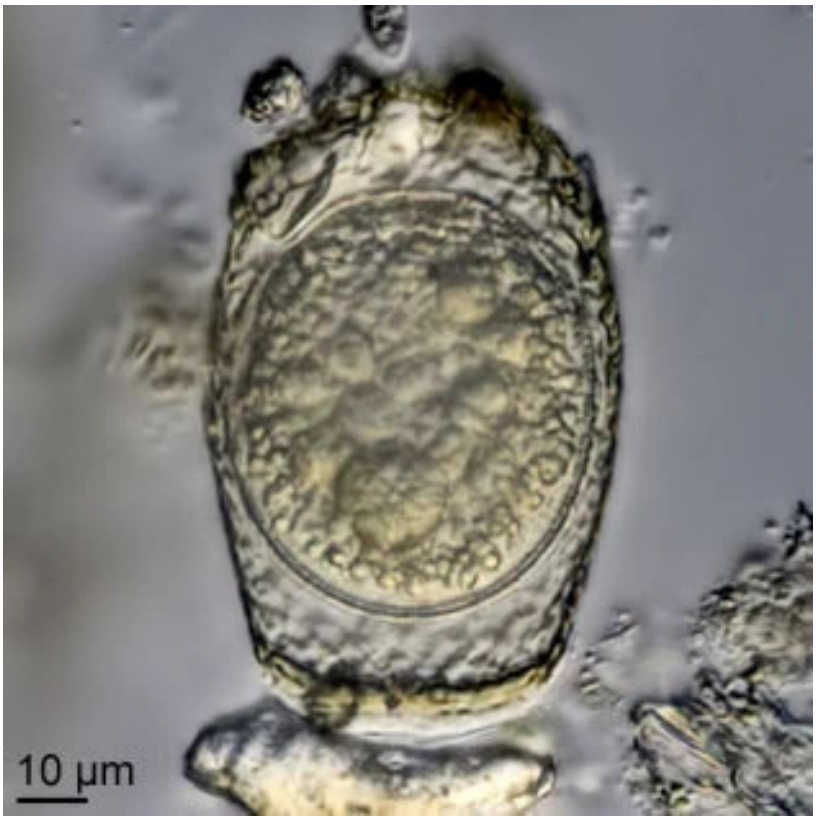
Trigonopyxis arcula



Heleopera petricola



Zivkovicia compressa



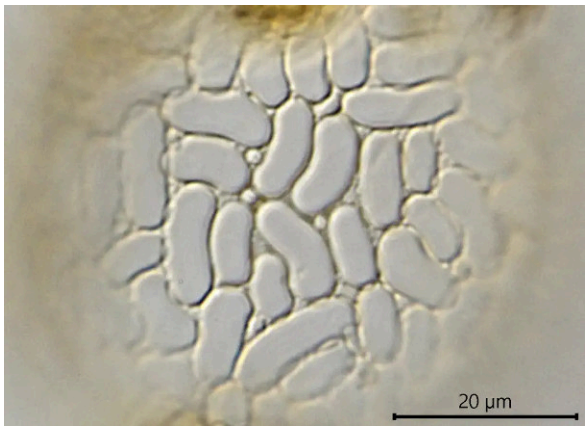
Shell of *Heleopera petricola* with a cyst inside. Photo by Ferry Siemensma, www.arcella.nl

Shell of *Padaungiella lageniformis* with a cyst inside. Photo by Ferry Siemensma, www.arcella.nl

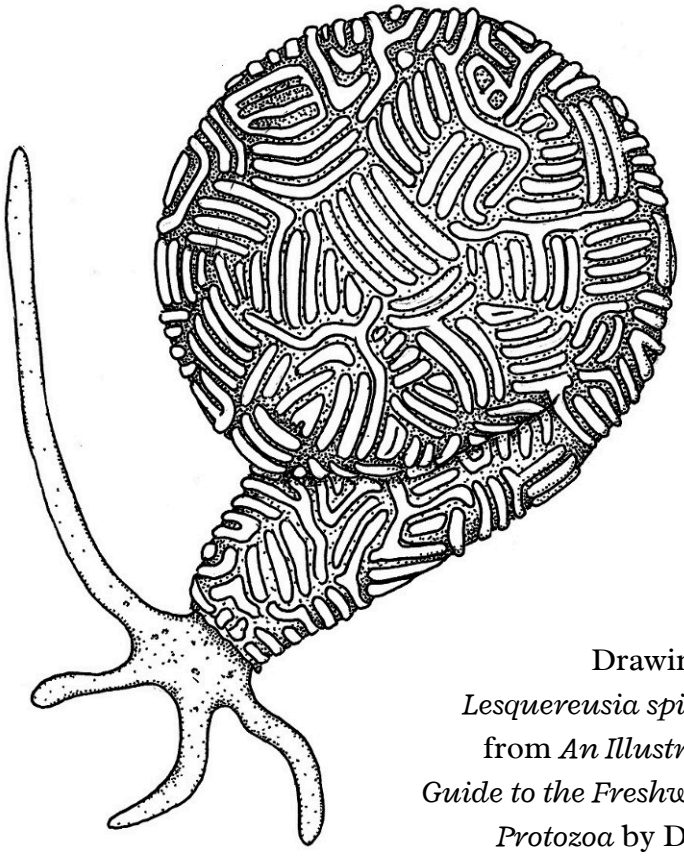
restricted. This dependence on water not only constrains their movements but also limits their capacity to colonize completely dry environments, highlighting a significant evolutionary factor in their ecological distribution.

Some species of testate amoebae can enter a dormant state in response to unfavorable environmental conditions, allowing them to survive until more suitable circumstances arise. In challenging environments—such as during droughts, extreme temperatures, food scarcity, or anaerobic conditions—these organisms form resistant cysts, serving as a vital survival mechanism.

Typically generated within the amoeba's shell, these cysts are encased in a thick organic membrane that provides robust protection. During the encystment process, amoebae undergo significant physiological changes. They reduce the volume of their cytoplasm and the number of organelles, entering a dormant state that can last for several months. This remarkable adaptation enables them to withstand harsh conditions until the environment becomes more conducive to life.



Detail of the shell wall of *Lesquereusia epistomium*. Photo by Ferry Siemensma, www.arcella.nl



Drawing of *Lesquereusia spiralis* from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

Why study testate amoebae?

Testate amoebae captivate microscopy enthusiasts with their impressive size and distinctive features. Generally larger than most flagellates and ciliates, these organisms offer a significant advantage for observation. Their deliberate, unhurried movements invite close examination, allowing amateur microscopists to explore their intricate behaviors and diverse structures in remarkable detail. This accessibility positions testate amoebae as ideal subjects for those eager to delve into the peculiar and often overlooked lifestyles of microbial life.

These remarkable organisms inhabit a variety of environments, from serene ponds and lush mosses to the rich soil beneath our feet. Each of these ecosystems harbors its own unique array of testate amoeba species, many of which remain

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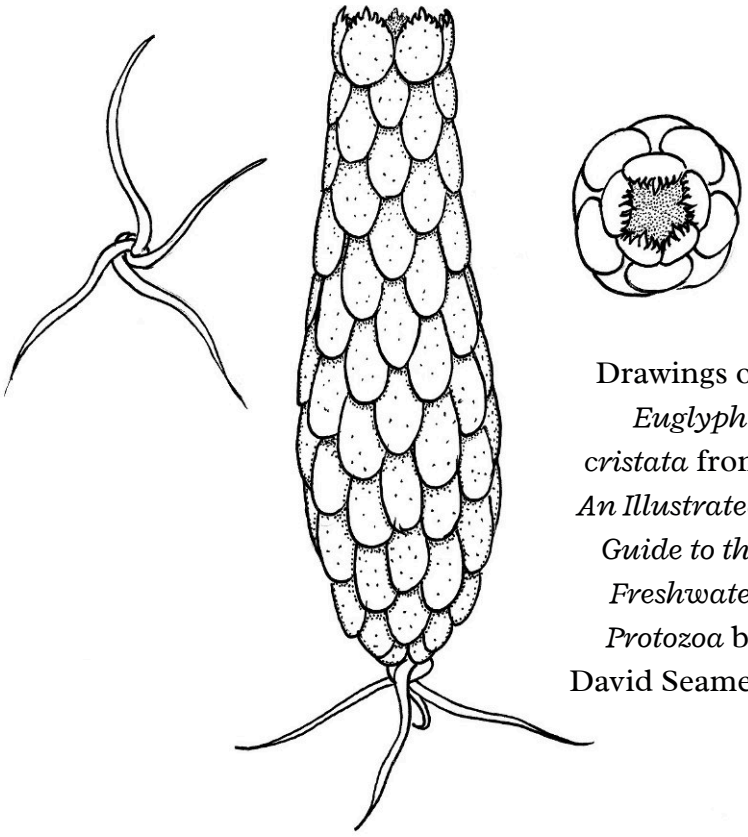
GENERALLY LARGER THAN MOST FLAGELLATES AND CILIATES, TESTATE AMOEBAE OFFER A SIGNIFICANT ADVANTAGE FOR OBSERVATION

Photo of *Euglypha cristata*. Photo by Ferry Siemensma, www.arcella.nl

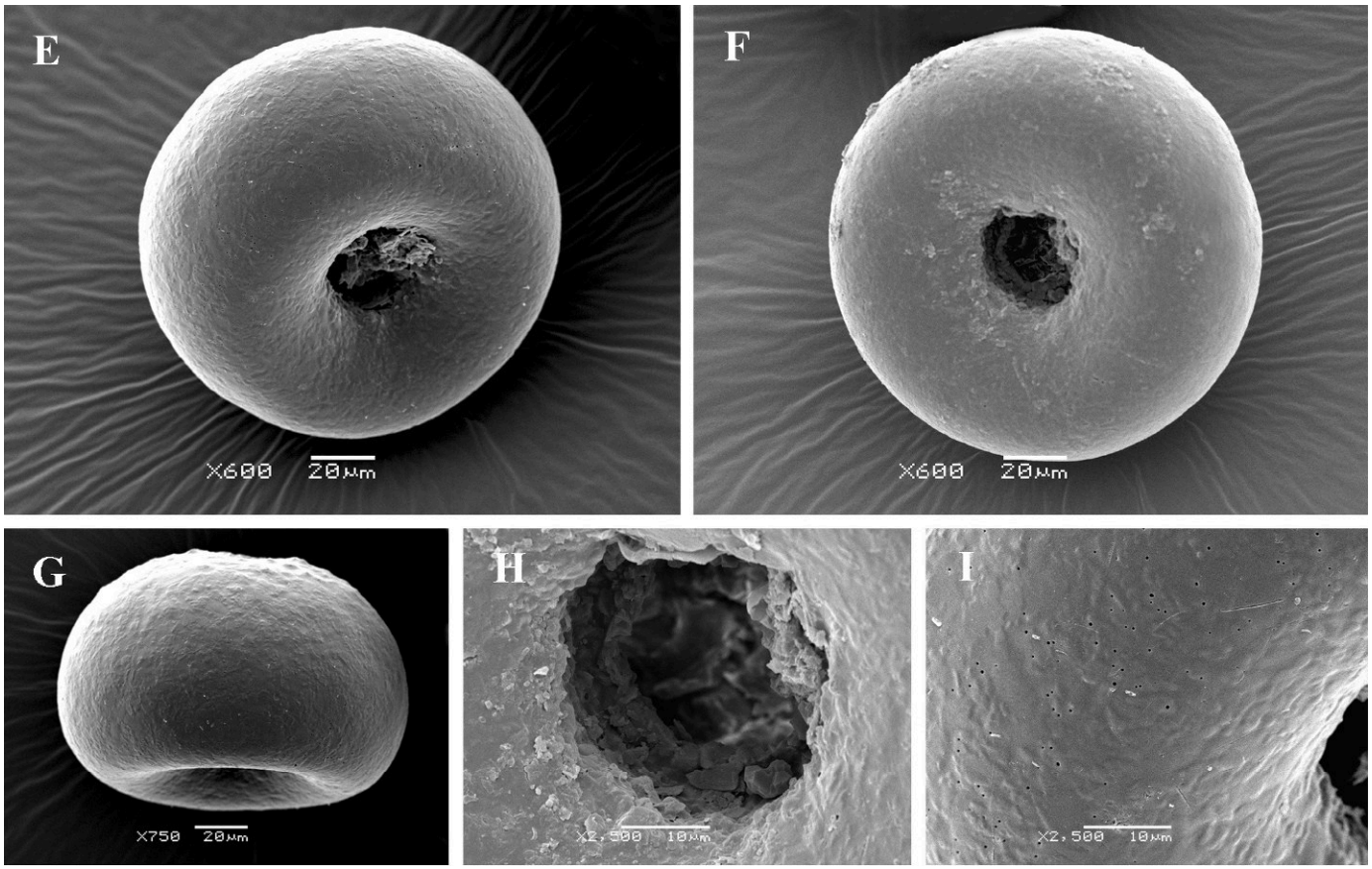


Drawings of *Euglypha cristata* from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

undescribed. This presents an exciting opportunity for both seasoned researchers and enthusiastic amateurs alike. By exploring these local habitats, individuals can contribute to citizen science initiatives that deepen our understanding of ecological dynamics and the myriad forms of life that share our planet.

Observing testate amoebae reveals the complex interactions within microbial communities. These microorganisms do not exist in isolation; they play integral roles in the ecosystems they inhabit, engaging in intricate relationships with bacteria, algae, and other microorganisms. Through these interactions, testate amoebae facilitate nutrient cycling and energy flow, underscoring the delicate balance that sustains life at the microscopic scale. By studying these interactions, we gain a richer appreciation for the intricacies of ecosystem functioning and the vital roles even the smallest organisms play in maintaining ecological harmony.

The artistry of testate amoebae is equally striking. Each organism's shell, crafted from a variety of materials, showcases a stunning fusion of form and function. The unique shapes and intricate designs of these microscopic



Scanning electron micrographs of *Cyclopyxis puteus*.
From: Todorov and Bankov (2019), doi: 10.3897/ab.e38685

structures not only invite scientific inquiry but also inspire photography and artistic expression, bridging the realms of science and art. This intersection can engage a broader audience, encouraging them to marvel at the hidden wonders of the microbial world.

Despite their prevalence, many regions—including significant areas of Europe—remain underexplored in terms of testate amoeba diversity. This gap presents a golden opportunity for researchers and hobbyists to document species diversity and distribution, contributing invaluable data to biogeographical and ecological studies. With just a light microscope, anyone can embark on this journey, making the exploration of these fascinating microorganisms accessible to a wide audience.

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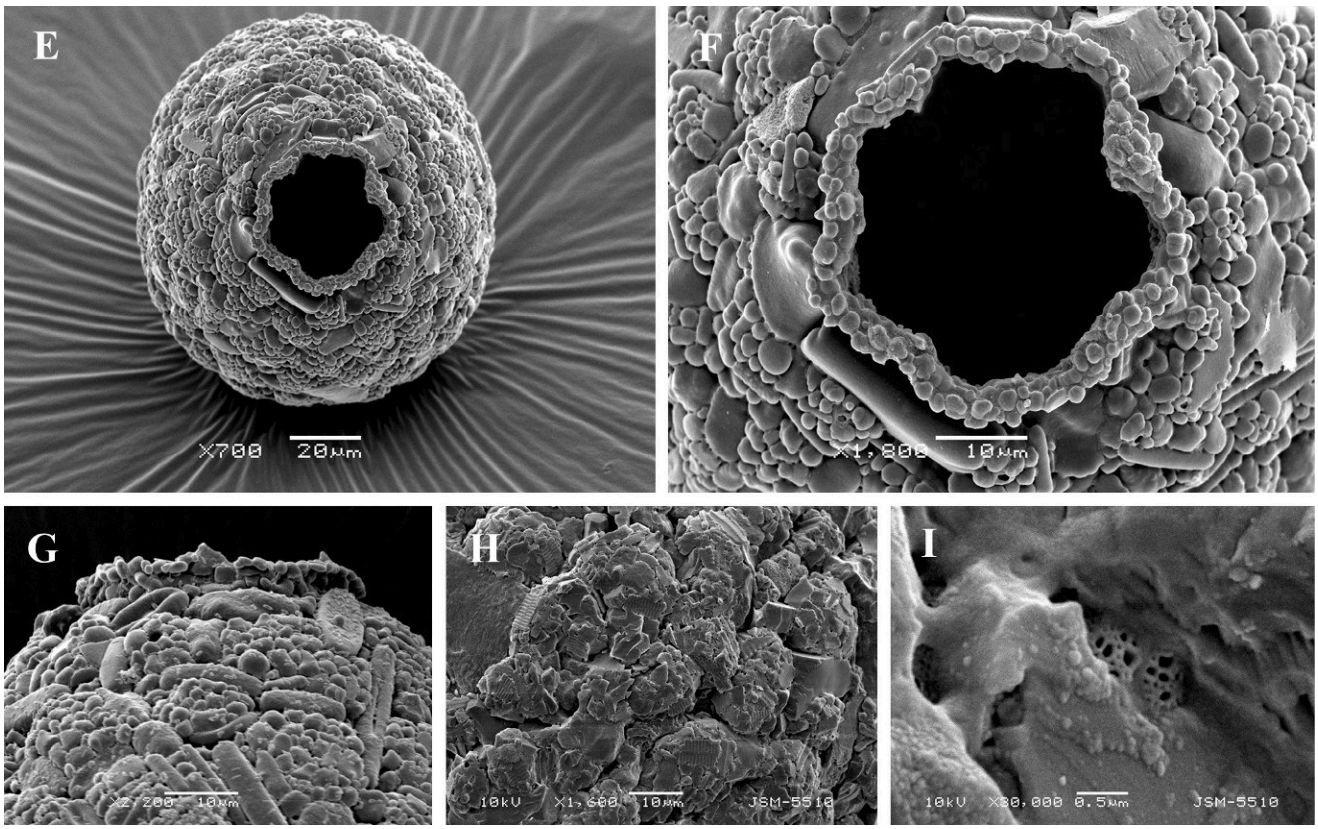
DESPITE THEIR PREVALENCE, MANY REGIONS—INCLUDING SIGNIFICANT AREAS OF EUROPE—REMAIN UNDEREXPLORED IN TERMS OF TESTATE AMOEBA DIVERSITY



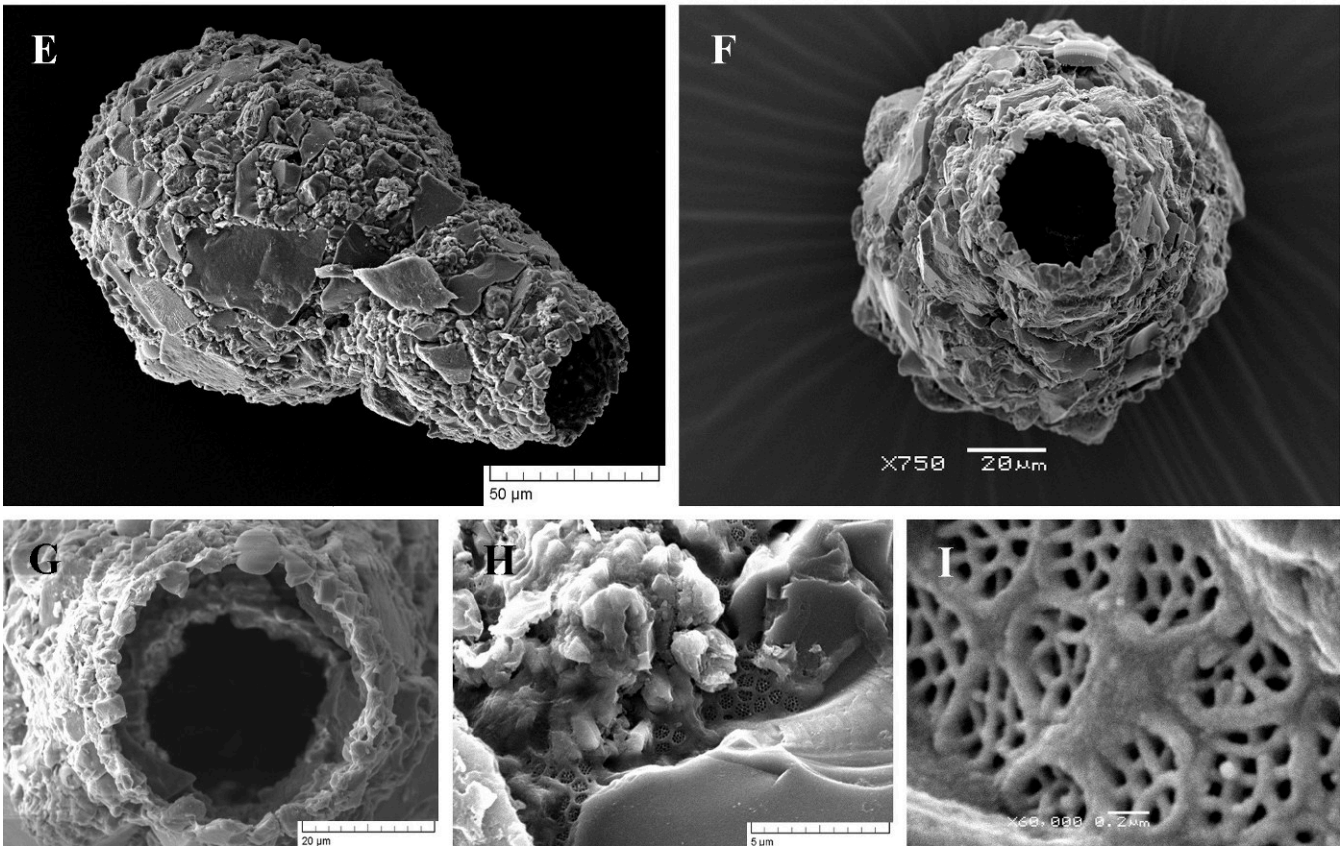
Scanning electron micrographs of *Netzelia tuberculata*.
From: Todorov and Bankov (2019), doi: 10.3897/ab.e38685

How to study testate amoebae?

Embarkng on the journey of discovering testate amoebae opens a window into a fascinating yet often overlooked aspect of our natural world. Contrary to the belief that advanced laboratory equipment is necessary, you can begin your exploration with just a good-quality light microscope capable of at least 400x magnification. An oil immersion lens can significantly enhance your ability to observe the intricate details of these remarkable microorganisms.

Your first step involves gathering samples from diverse habitats—ponds, marshes, or patches of moist soil. Using a pipette, carefully collect water from various depths or scoop sediment from the bottom, taking care not to disturb the organisms too much. Gently mixing your sample in water can help release the amoebae from their

> CONTRARY TO THE BELIEF THAT ADVANCED LABORATORY EQUIPMENT IS NECESSARY, YOU CAN BEGIN YOUR EXPLORATION WITH JUST A GOOD-QUALITY LIGHT MICROSCOPE CAPABLE OF AT LEAST 400X MAGNIFICATION

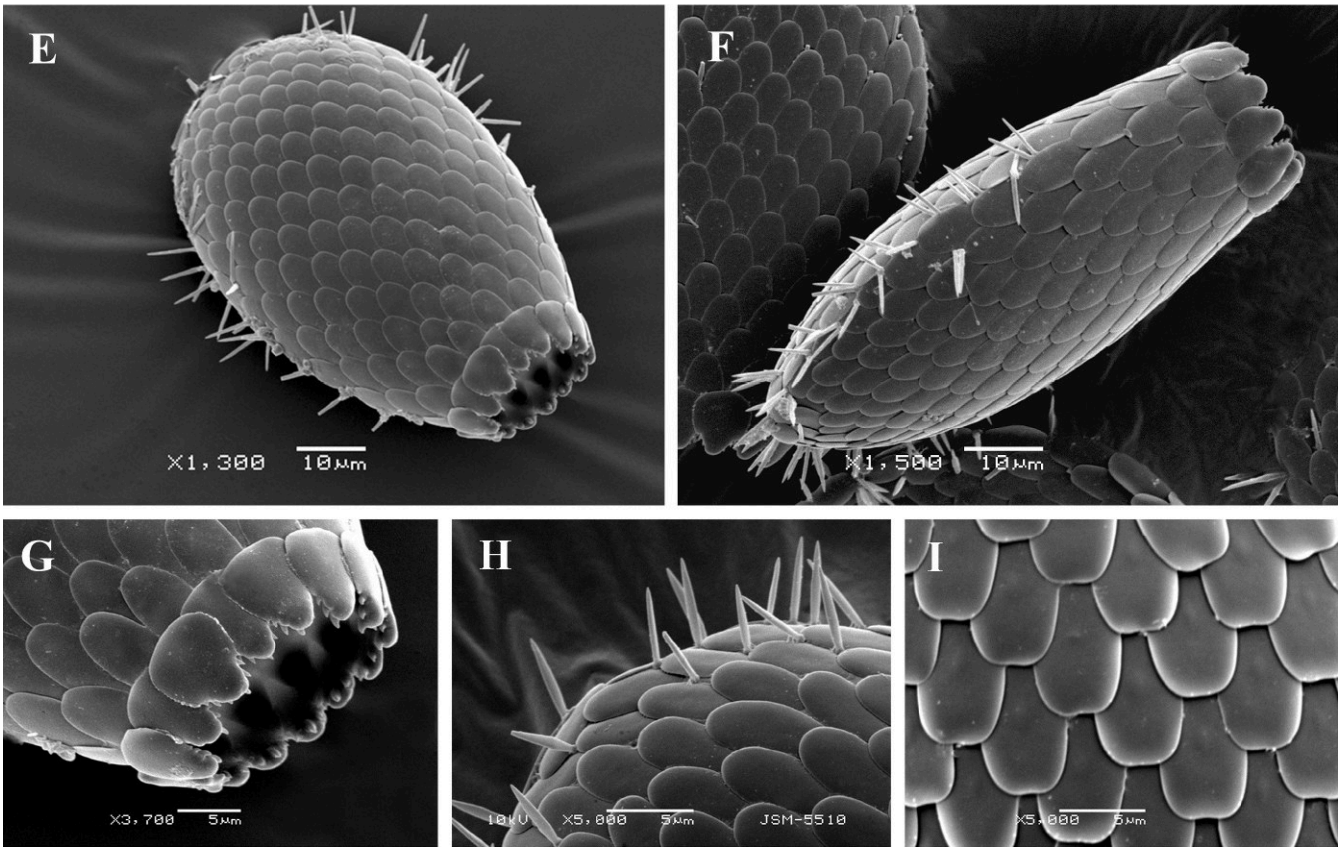


Scanning electron micrographs of *Lagenodifflugia vas*.
From: Todorov and Bankov (2019), doi: 10.3897/ab.e38685

substrate. Once you have prepared your sample, place a drop on a microscope slide, cover it with a slip, and start your observations. Patience is key; take the time to observe the myriad shapes and movements.

As you delve deeper, pay close attention to the tests, or protective shells, of these amoebae. You may find an array of intricate patterns and textures that vary dramatically between species. Some tests are ornate, composed of silica and resembling delicate glass sculptures, while others may appear more simplistic and organic. With higher magnification, you can appreciate the fine details that contribute to their unique forms. These microorganisms are not only captivating because of their varied shapes —from spherical to elongated or asymmetrical—but also due to their fascinating behaviors. Witnessing them glide smoothly across the slide, extending and retracting their pseudopodia, showcases their interaction with the environment as they feed on bacteria and algae.

Having a reliable field guide or reference material on testate amoebae is invaluable for identifying the various species you may encounter during your explorations. These resources provide detailed

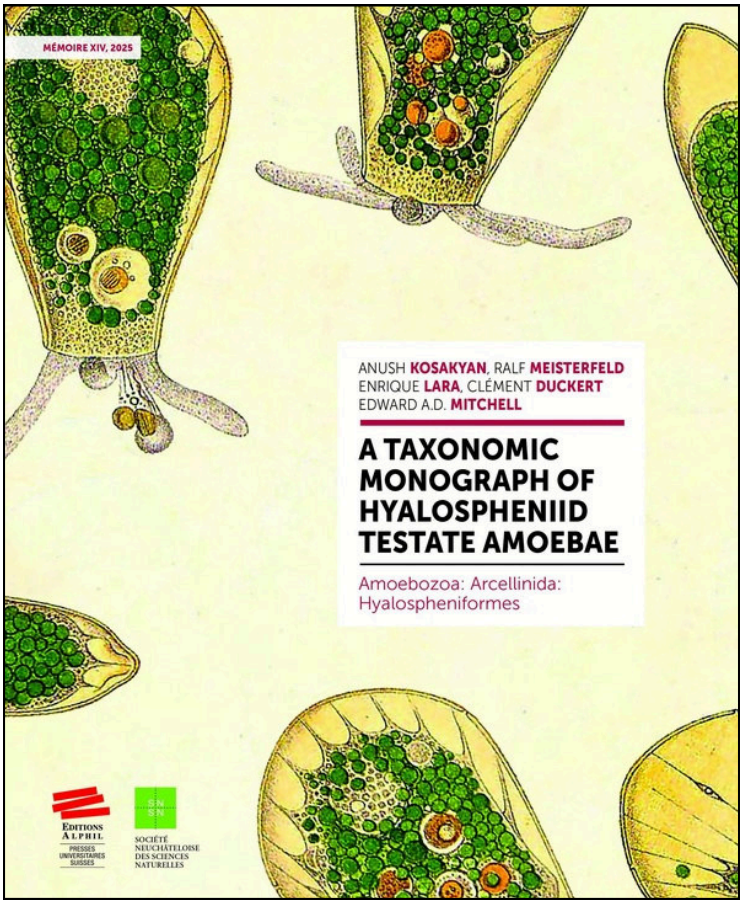


Scanning electron micrographs of *Euglypha compressa*.
From: Todorov and Bankov (2019), doi: 10.3897/ab.e38685

descriptions, illustrations, and key characteristics that can help you distinguish between the diverse forms of these fascinating microorganisms. Whether you choose a printed guide or an online resource, having access to accurate information enhances your observational skills and deepens your understanding of these organisms. As you become more familiar with their unique features, you'll find joy in recognizing different species and uncovering the rich diversity within this microscopic world. Each identification adds another layer to your journey, transforming each encounter into a meaningful discovery.

Documenting your observations is vital. Keep a notebook or digital record to log species identified, their characteristics, and any intriguing behaviors you encounter. Engaging with the community of fellow enthusiasts can enrich your exploration. Sharing your findings on social media or joining online forums dedicated to microscopy and environmental science allows for meaningful discussions and valuable feedback. These platforms foster a sense of collaboration and shared discovery, enhancing both your knowledge and that of the wider community.

Recently published monograph on hyalospheniid testate amoebae by an international team of researchers



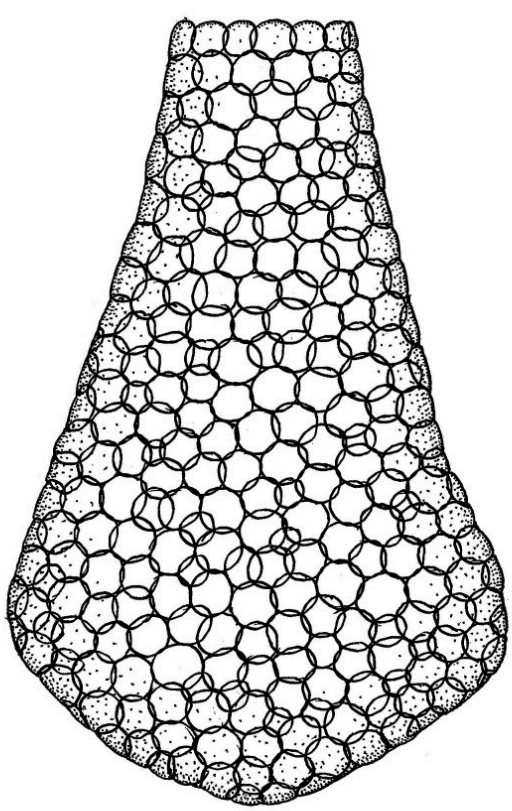
Neglected testate amoebae from Australia

Microscope enthusiasts can truly collect very significant data regarding the diversity and distribution of testate amoebae, and here I will present such a case.

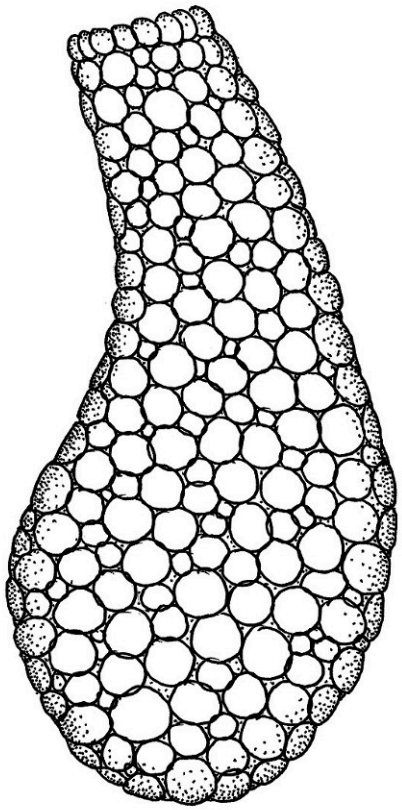
In February 2025, an international team of researchers published a monograph presenting the most comprehensive analysis of diversity and geographic distribution of hyalospheniid testate amoebae. These amoebae have long intrigued researchers with their unpredictable geographic distribution across the planet. The monograph was based on an enormous amount of data scattered accross many papers and monographs, but one crucial piece of the puzzle was largely missing—Australia. Why was some important data from this continent not included? The answer lies in the unusual story of one microscopy enthusiast and his privately published book.

> MICROSCOPE ENTHUSIASTS CAN TRULY COLLECT VERY SIGNIFICANT DATA REGARDING THE DIVERSITY AND DISTRIBUTION OF TESTATE AMOEBAE

Drawings of two rare *Argynnia* species from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



Argynnia triangulata

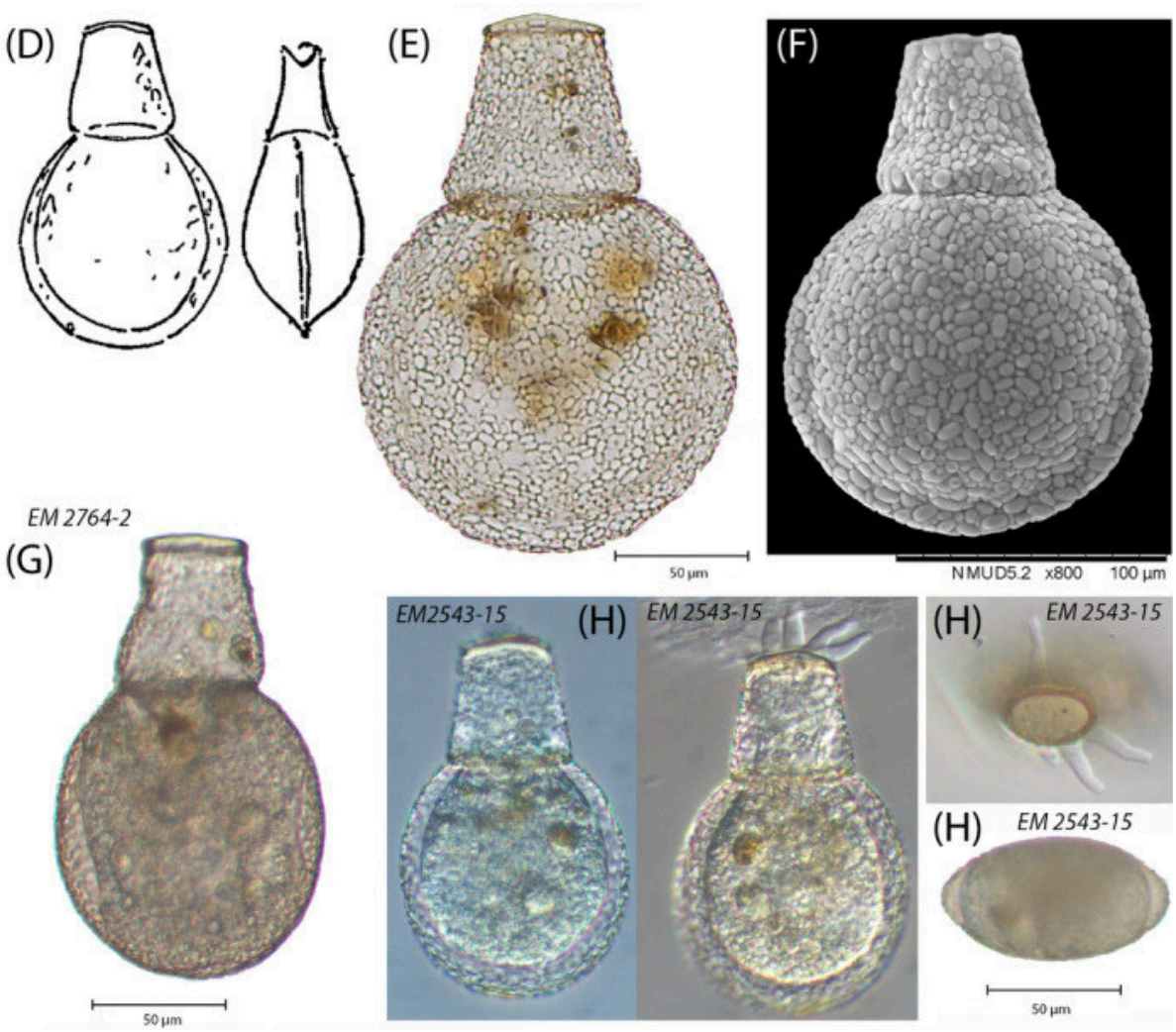


Argynnia repanda

David Seamer is not a typical scientist. He does not hold a professorial title, does not work at a prestigious research institution, and has not published papers in leading scientific journals. Yet, his work over the past decades could change the way we understand the microscopic world of Australia's freshwater ecosystems. David spent 20 years researching southeastern Australia and Tasmania, traveling in his converted school bus—which served as both his home and his laboratory. Equipped with a light microscope and boundless curiosity, he collected samples from lakes, rivers, ponds, swamps, and streams, carefully examined them, and created detailed illustrations.

Among the many microscopic organisms he documented, his findings on testate amoebae from the group

DAVID SEAMER SPENT 20 YEARS RESEARCHING SOUTHEASTERN AUSTRALIA AND TASMANIA, TRAVELING IN HIS CONVERTED SCHOOL BUS—WHICH SERVED AS BOTH HIS HOME AND HIS LABORATORY

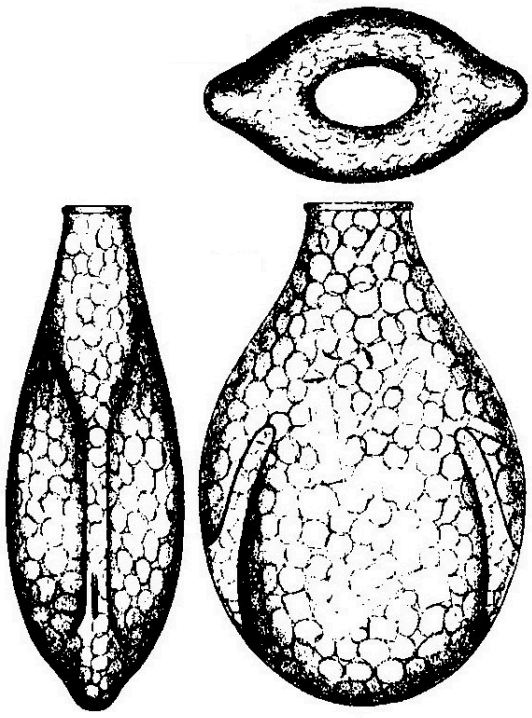


Apodera angatakere, a rare testate amoeba from New Zealand, superficially described and ignored for 92 years. From: Duckert et al. (2021), doi: 10.1111/jeu.12867

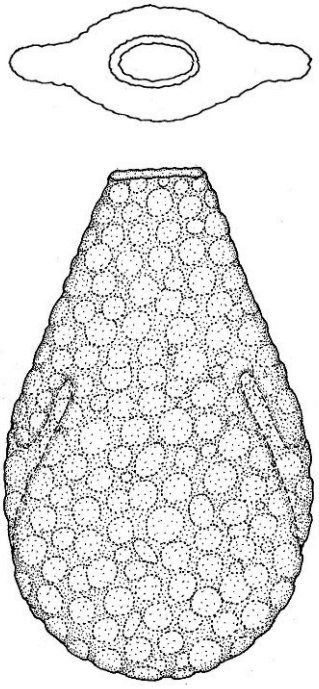
Hyalospheniformes are particularly noteworthy. The issue is that all of this data was published in a privately printed book, overlooked by most testate amoeba researchers. So, without access to his work, the scientific community was unable to include Australian data in the global biogeographic analysis.

Testate amoebae from the Hyalospheniformes group offer important insights into the evolution and biogeographic patterns of microscopic organisms. Their diversity and geographic distribution can reveal how species spread and adapt to different ecosystems. Since Australia is an ecologically unique continent, data from its landscapes could significantly contribute to our understanding of the global distribution patterns of these organisms.

Hyalospheniid testate amoebae, with their unmistakable shell shape and structure, have long been considered excellent models for testing biogeographic hypotheses—even before the advent of molecular methods. Among them, the genus *Cornutheca* stands out. Its species, notable for their large size and distinctive shell morphology, are highly recognizable, making it unlikely that they would have been overlooked by researchers. In current scientific literature, the prevailing narrative holds that species of the genus *Cornutheca* have been found in only two regions: Eastern North America and Eastern Asia. This limited distribution seemed well established—until an unexpected discovery challenged the dominant view, though it has largely remained overlooked in academic circles. In his book, David Seamer documented the presence of *Cornutheca saccifera* in the Strathbogie Ranges of southeastern Australia. This remarkable finding marks the first recorded occurrence of a *Cornutheca* species in the Southern Hemisphere, raising new questions about the true range and dispersal history of these testate amoebae.

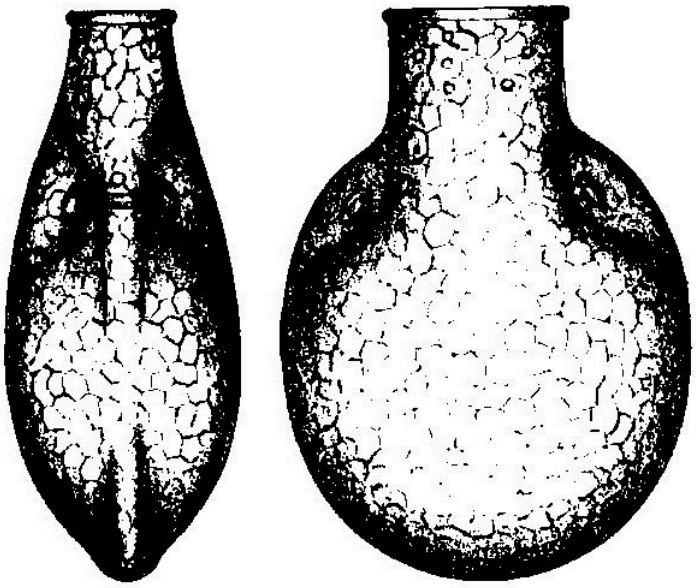


Drawings of *Cornutheca saccifera* published with the first description of the species. From: Wailes (1913), doi: 10.1111/j.1096-3642.1913.tb01776.x

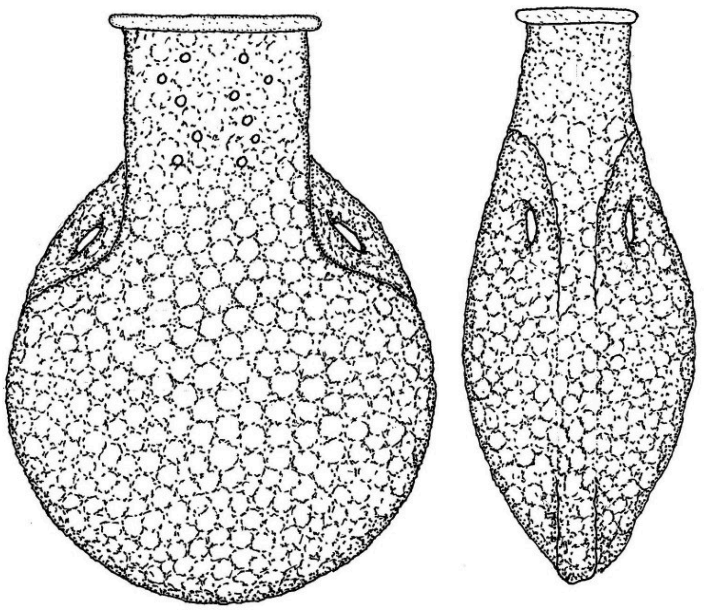


Drawing of *Cornutheca saccifera* from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

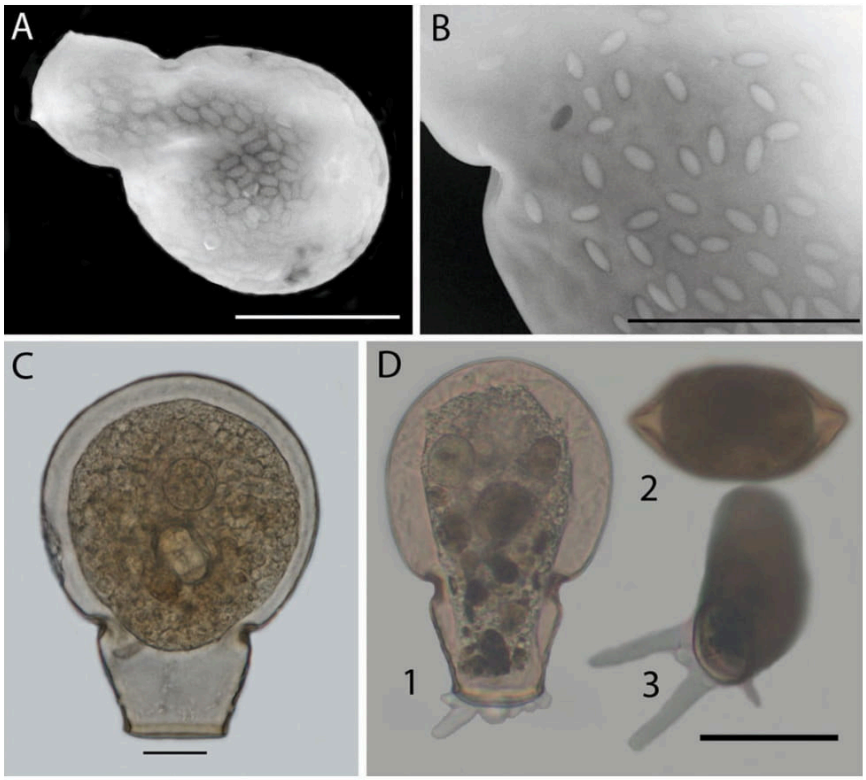
A uniquely specialized species with distinct morphological traits, *Certesella murrayi* was long thought to be confined to South America. This species is highly distinctive and difficult to misidentify, as it stands apart from the other three species in the genus *Certesella*. Its most defining features include a short, hollow keel at the base of the neck and a sharply differentiated neck that contrasts with the rest of the shell, rather than gradually tapering toward the aperture. Named after James Murray, who first collected the moss samples where it was discovered, the species was initially described by George Wailes in 1913 based on specimens from Brazil and Chile. Decades later, in 1980, Maria Cristina Vucetich reported its presence in Argentina, further reinforcing the belief that *Certesella murrayi* might be an endemic organism restricted to the continent. This assumption persisted until an unexpected discovery in January 2006, when David Seamer identified the species in Jacky's Marsh, Tasmania. This finding challenged previous notions of its distribution, suggesting instead that *Certesella murrayi* is not a South American endemic but rather a circumaustral species—one with a broader range spanning the Southern Hemisphere.



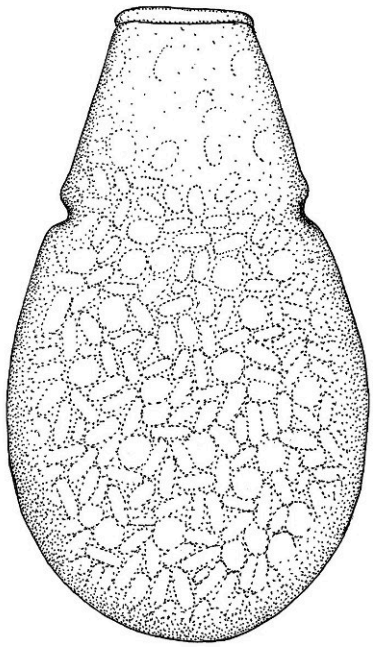
Drawings of *Certesella murrayi* published with the first description of the species. From: Wailes (1913), doi: 10.1111/j.1096-3642.1913.tb01776.x



Drawings of *Certesella murrayi* from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

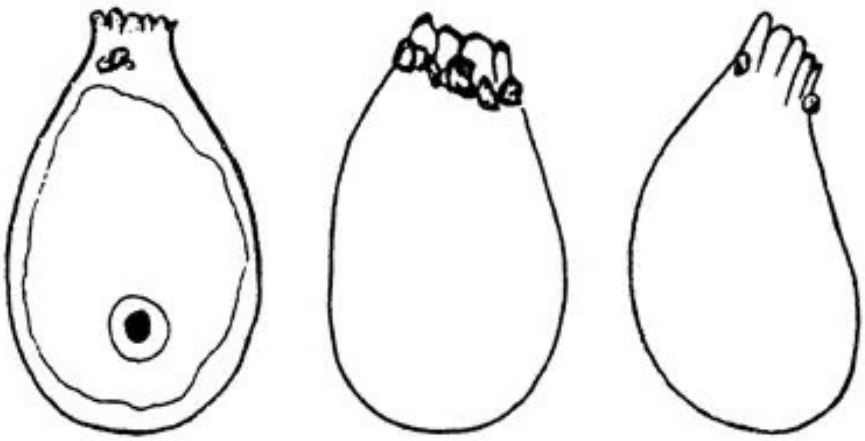


Micrographs of *Alocodera cockayni* from New Zealand. From: Kosakyan et al. (2025), doi: 10.33055/ALPHIL.00614

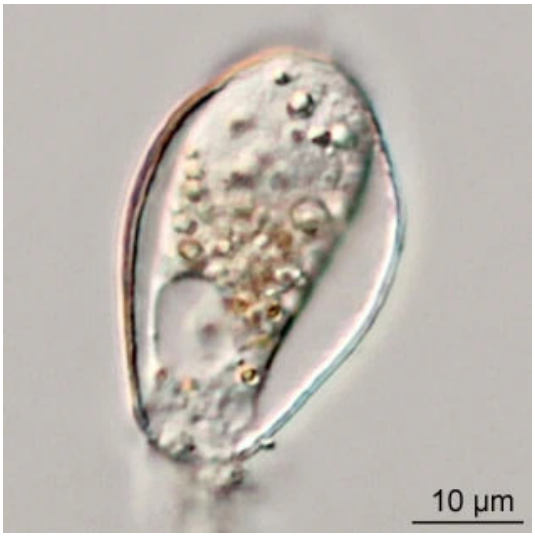


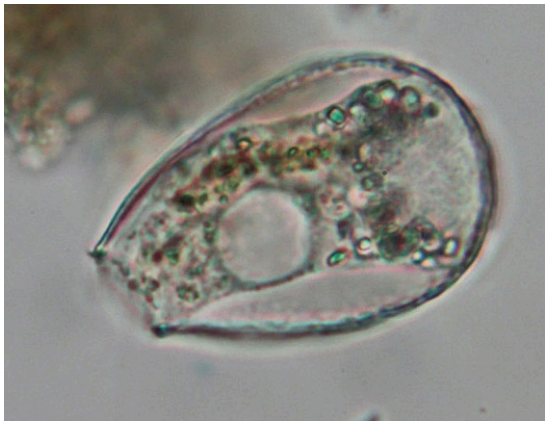
Drawing of *Alocodera cockayni* from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

So far, only a single species from the genus *Alocodera* has been recognized—*A. cockayni*, a circumaustral endemic. However, this opinion may be an oversimplification. What appears to be a single species could, in fact, be a complex of multiple closely related species, grouped together due to the unclear boundaries that separate them.The strongest evidence for this hypothesis lies in an intriguing pattern: specimens of *A. cockayni* seem to fall into two distinct size groups. Some individuals have shells that never exceed 100 micrometers, while others possess significantly larger shells, measuring over 120 micrometers. Strikingly, no specimens have been found with shell lengths between these two ranges—suggesting the presence of at least two separate species rather than a continuous variation within one. A particularly notable example comes from April 2006, when David Seamer recorded a specimen in Bogong National Alpine Park, Victoria. This individual had a shell length of 125 micrometers, placing it firmly within the larger group. Its discovery adds further weight to the idea that *A. cockayni* may not be a single species, but rather a hidden mosaic of biodiversity waiting to be fully understood.



Drawings of *Feuerbornia lobophora*. From: Jung W (1942) Süd chilenische Thekamöben (Aus dem südchilenischen Küstengebiet, Beitrag 10). Arch. Protistenkd. 95: 253-356.





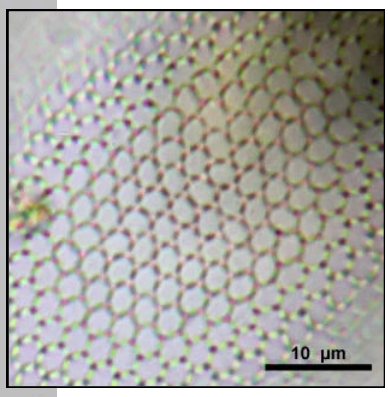
Photos of *Cyphoderia laevis*, a species similar in shape to *Feuerbornia lobophora*. Photo by Ferry Siemensma, www.arcella.nl

Among the many discoveries made by David Seamer, one stands out as particularly fascinating—a mysterious, unidentified species of testate amoeba from the enigmatic genus *Feuerbornia*. This genus, shrouded in mystery, was formally described in 1942 based on samples collected from soil mosses deep in the rainforests of Chile. Since then, only a single species has been known to science: *Feuerbornia lobophora*, an amoeba whose overall appearance closely resembles species from the genus *Cyphoderia*.

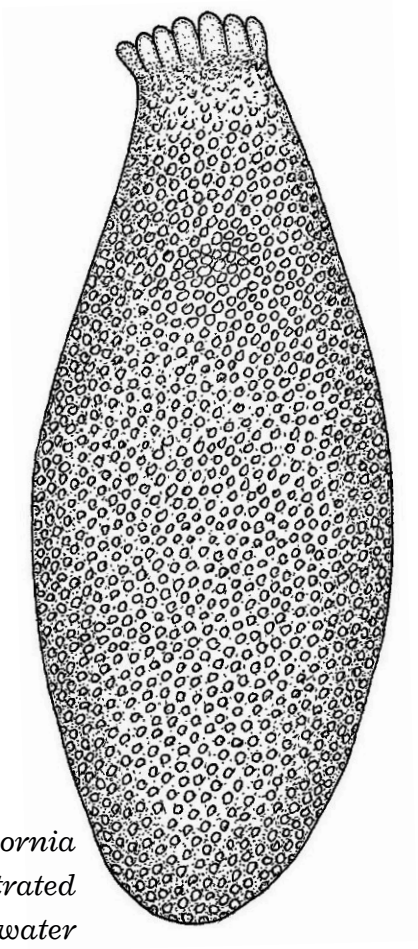
What makes *F. lobophora* so intriguing is its elusiveness. Since its initial description, no researcher has ever encountered it again. As a result, almost nothing is known about its biology or structure—no one has had the opportunity to study it using modern scientific methods.

The only defining feature that sets it apart is the presence of "thickened lobes" around its aperture, a trait that makes it unmistakable yet frustratingly difficult to find in nature. And then came David's discovery. In the remote alpine landscapes of southeastern Australia, he stumbled upon a testate amoeba that unquestionably belonged to the genus *Feuerbornia*, yet it did not quite match the description of *F. lobophora*.

Photo of *Cyphoderia* sp. Photo by Ferry Siemensma, www.arcella.nl



Cyphoderia ampulla, detail of the shell wall. Photo by Ferry Siemensma, www.arcella.nl

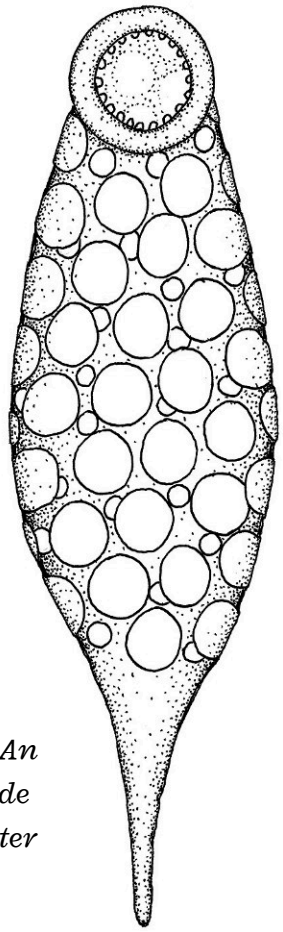


Drawing of *Feuerbornia* sp. from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

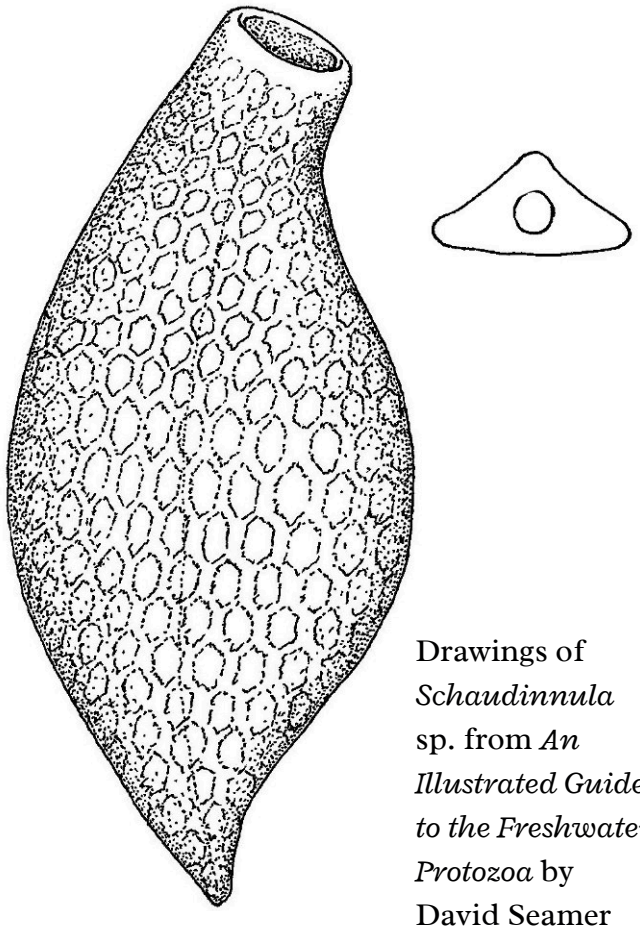
The differences were striking. The specimen he recorded measured just 45 micrometers in shell length—significantly smaller than *F. lobophora*, whose shells range between 74 and 85 micrometers. Moreover, its overall shape was more elongated, lacking the foreign bodies that typically form a collar-like structure at the base of *F. lobophora*'s apertural lobes.

All evidence points to an exciting conclusion: this is an entirely new species, one that has remained unnoticed by science until now. But to confirm its existence, more research is needed. Falls Creek Alpine Park in Victoria, the very place where David made his discovery, holds the key to unlocking this mystery. A dedicated expedition must return to sample the *Sphagnum* mosses that serve as its habitat, to observe this elusive amoeba once more, and to finally study it using modern scientific techniques. Only then can a formal description be published, giving this species its rightful name and place in the scientific record.

This is more than just the identification of a new species



Drawing of *Playfairina caudata* from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

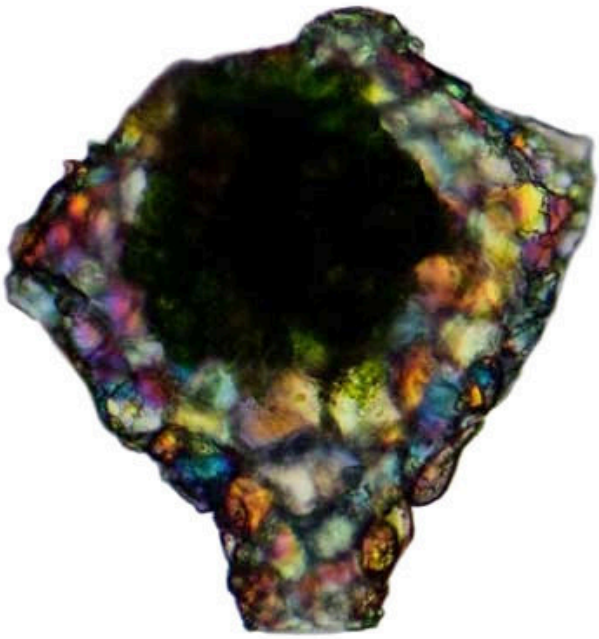


Drawings of *Schaudinnula* sp. from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

—it is the unraveling of one of the greatest mysteries among testate amoebae. For decades, the genus *Feuerbornia* has remained a ghost in the world of protistology, evading researchers at every turn. Now, for the first time, the veil may finally be lifted, revealing a hidden chapter in the evolutionary story of these enigmatic microorganisms.

This story raises an important question: how much valuable data remains unnoticed because it is collected by independent researchers outside academic circles? Seamer's work is proof that an individual, driven by curiosity, can contribute to science just as much as researchers employed in laboratories. Future publications on testate amoebae will likely include David's data, revealing even more unknown secrets of these microscopic creatures. Until then, his story remains an example of how science does not belong solely to universities and institutes—but to anyone willing to view the world through a microscope and ask the questions to which we still have no answers.

I hope this article on testate amoebae has sparked your curiosity and inspired you to explore the hidden world of these extraordinary organisms. The microscopic realm is full of wonders waiting to be discovered, and your observations could make a valuable contribution to our understanding of biodiversity on a microscopic scale.



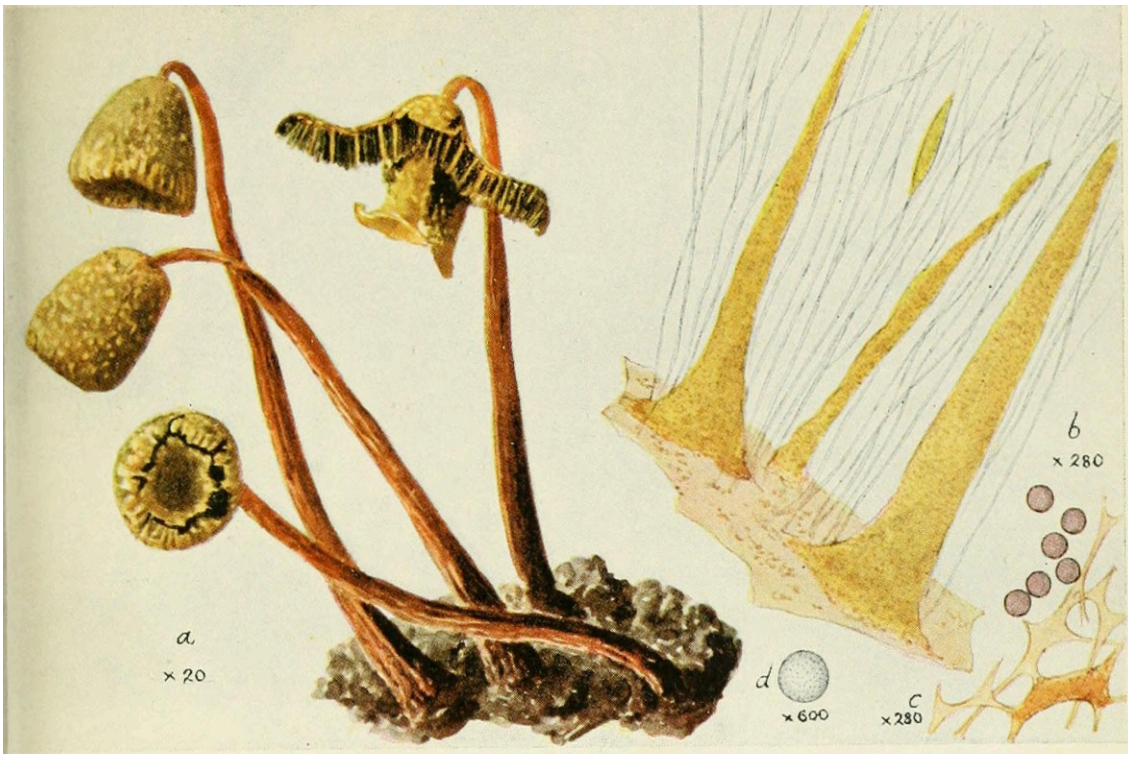
Difflugia nodosa, one of the largest testate amoeba species, with a shell length of up to half a millimeter. Photos by Ferry Siemensma, www.arcella.nl

Gulielma Lister In the Footsteps of Slime Molds

By Dr. Stefan Luketa

Gulielma Lister was a pioneering figure in the study of slime molds—enigmatic organisms that had long confounded scientists. With unparalleled dedication, she transformed these curious creatures into subjects of serious scientific inquiry. Through meticulous field observations, rigorous taxonomic studies, and stunning watercolor illustrations, Lister revealed slime molds as complex organisms that challenge traditional boundaries between plants, animals, and fungi. Her work not only redefined our understanding of these fascinating life forms but also opened a window into the deeper mysteries of life itself. Today, slime molds continue to intrigue researchers and nature lovers alike, thanks to Lister's groundbreaking contributions that continue to inspire exploration in the natural world.

Portrait of Gulielma Lister, September 1926. Photo credit: William George. Copyright: Essex Field Club Archive



Drawings of *Physarella oblonga* from *A Monograph of the Mycetozoa* by Gulielma Lister

Introduction

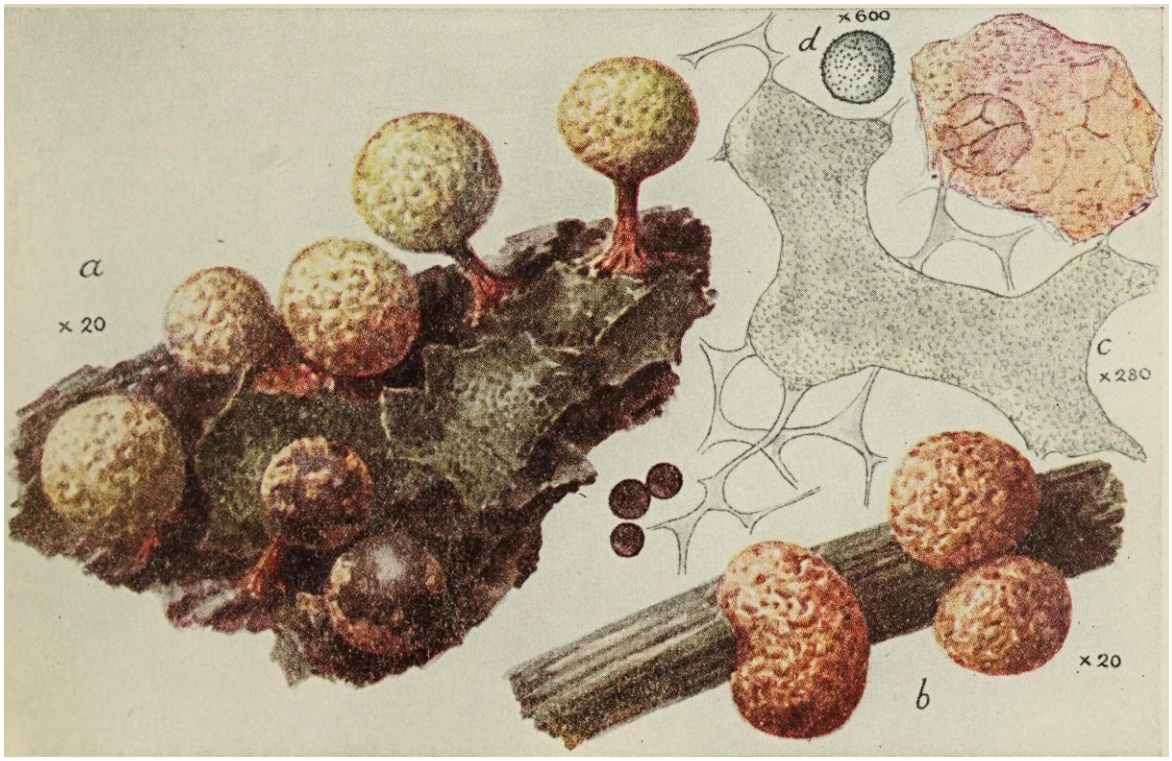
At first glance, slime molds may seem like little more than an oddity of the natural world—unassuming, translucent blobs that ooze and pulsate across damp forest floors. But to the few scientists who have ventured into their strange, elusive world, slime molds are a source of endless fascination, offering clues to the very nature of life itself. Among these scientists was Gulielma Lister, a trailblazer whose work in the early 20th century fundamentally changed our understanding of these remarkable organisms. Yet, despite her groundbreaking contributions, Lister remains one of the most underappreciated figures in the history of mycology. Though she never sought fame or formal academic recognition, Lister's research on slime molds was revolutionary. Her precise field notes, detailed taxonomic descriptions, and exquisite watercolor illustrations brought clarity to the previously misunderstood world of Mycetozoa. Through her lens, slime molds were no longer just curiosities of the forest; they became a window into the complexities of evolution, behavior, and life cycles—complexities that blurred the boundaries between plants, animals, and fungi.

Gulielma Lister at her home, Sycamore House, Leytonstone, circa 1930

Early life and education

Gulielma Lister was born on October 28, 1860, at Sycamore House in Leytonstone, East London, into a family deeply immersed in intellectual and artistic pursuits. The Lister family, known for its Quaker heritage, placed a strong emphasis on education, personal integrity, and public service—values that profoundly shaped Gulielma's upbringing. As the daughter of Arthur Lister, a wine merchant with a passion for natural history, and Susanna Tindall, a trained artist, Gulielma's early life was marked by a unique fusion of science and art—two realms that would later merge seamlessly in her scientific work.

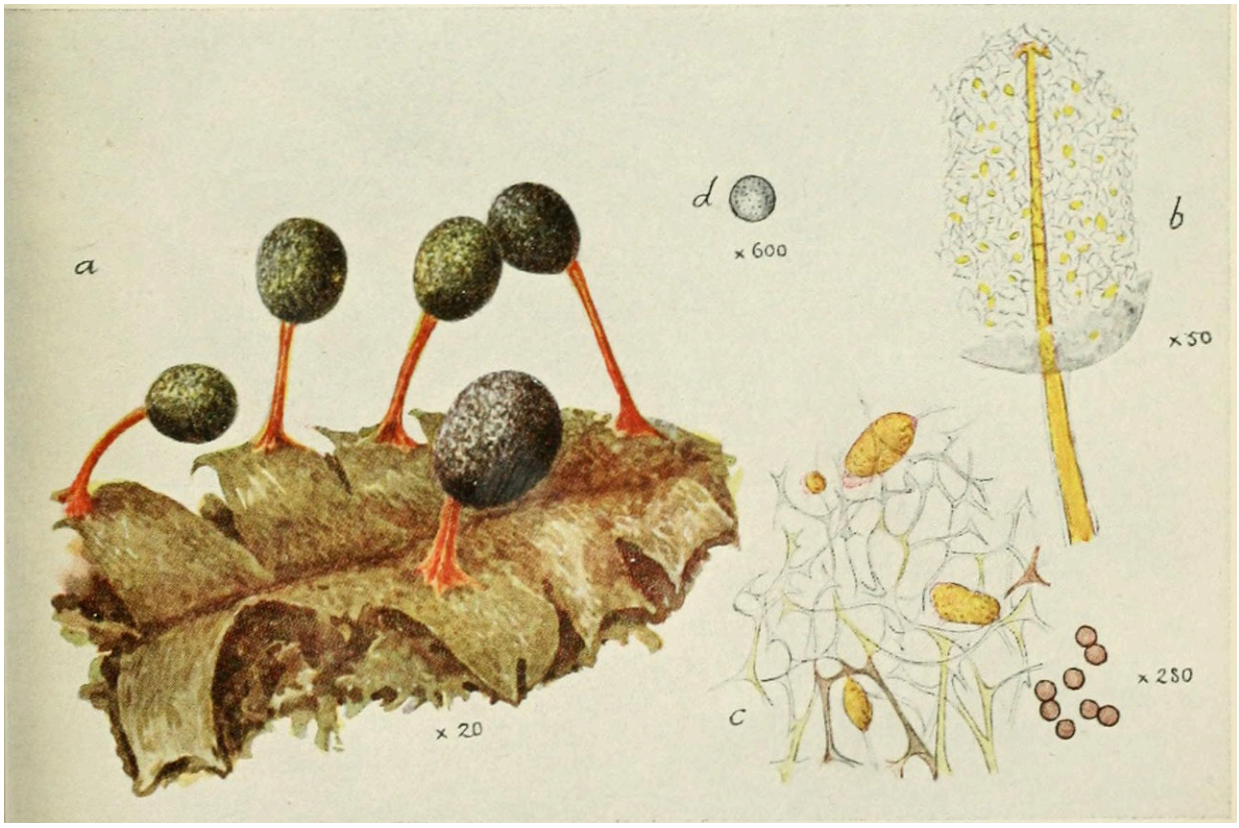
Quakers *are people who belong to the Religious Society of Friends, a Christian movement founded in 17th-century England that emphasizes direct experience with God, simplicity, equality, and pacifism.*



Drawings of *Physarum citrinellum* from *A Monograph of the Mycetozoa* by Gulielma Lister

From an early age, Lister was immersed in the world of natural history. Her father, Arthur Lister, was a self-taught botanist and mycologist whose fascination with fungi and the natural world became a defining feature of his life. Although he made his living as a wine merchant, his true passion lay in the study of Mycetozoa, the group of organisms we now call slime molds. Gulielma accompanied him on many of his field excursions, where she learned firsthand about plant life and fungi. These early explorations planted the seeds for what would become her lifelong fascination with mycology, particularly the study of slime molds. While her formal education was somewhat unconventional for the time, Lister's home environment served as an extraordinary classroom. Her mother, Susanna, was a trained artist, and her artistic sensibilities profoundly influenced Gulielma's

***Arthur Lister** (1830–1908) was a British botanist and mycologist best known for his research on slime molds. His work, including "A Monograph of the Mycetozoa" (1894), contributed significantly to the biology and diversity of these organisms.*



Drawings of *Physarum penetrale* from *A Monograph of the Mycetozoa* by Gulielma Lister

approach to science. The precision and attention to detail required in scientific illustration became one of Lister's signature strengths. Her ability to translate what she observed in the natural world into exquisite watercolor paintings was not only a personal passion but also a vital tool in her scientific work. Many of her illustrations—some of which are preserved in the collections of the Natural History Museum and Kew Gardens—showcase her talent for capturing the fine details of the organisms she studied, whether it was the intricate structure of slime molds or the delicate textures of various fungi.

Despite the limitations placed on women's education during the Victorian era, Lister had access to a rich intellectual environment that nurtured her scientific curiosity. She attended Bedford College for

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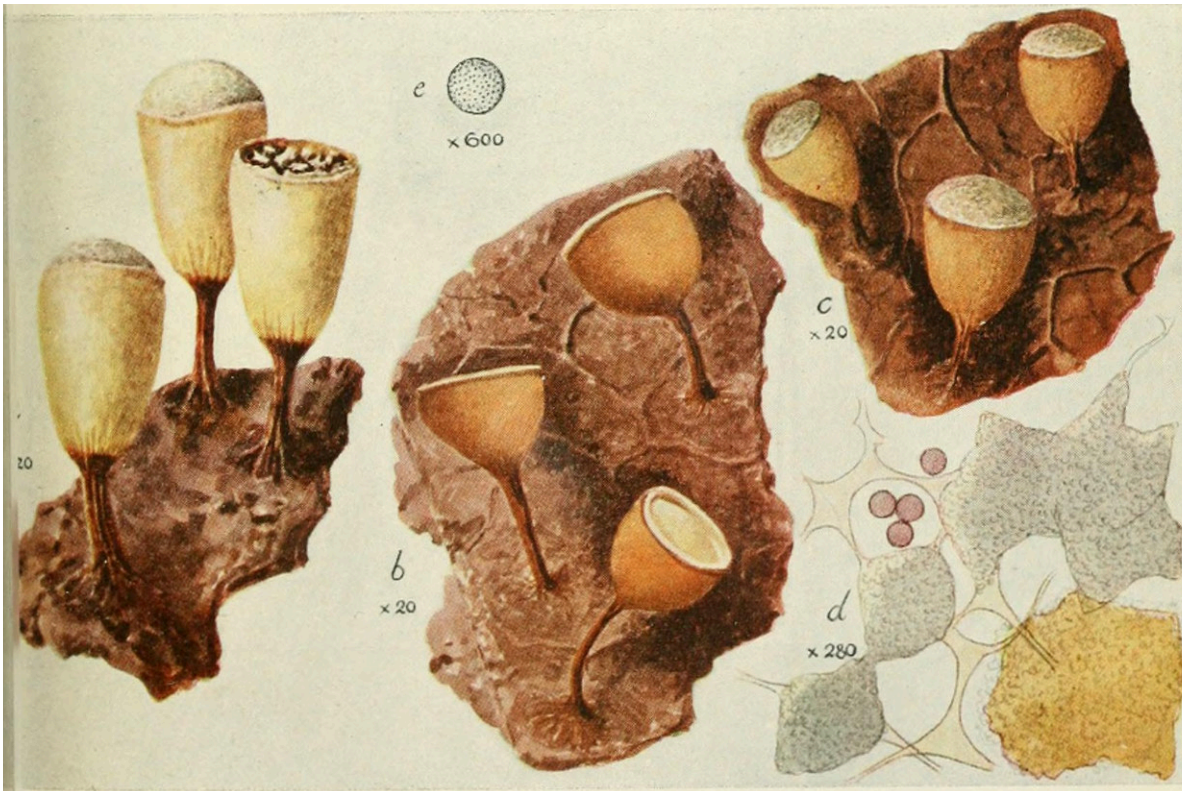
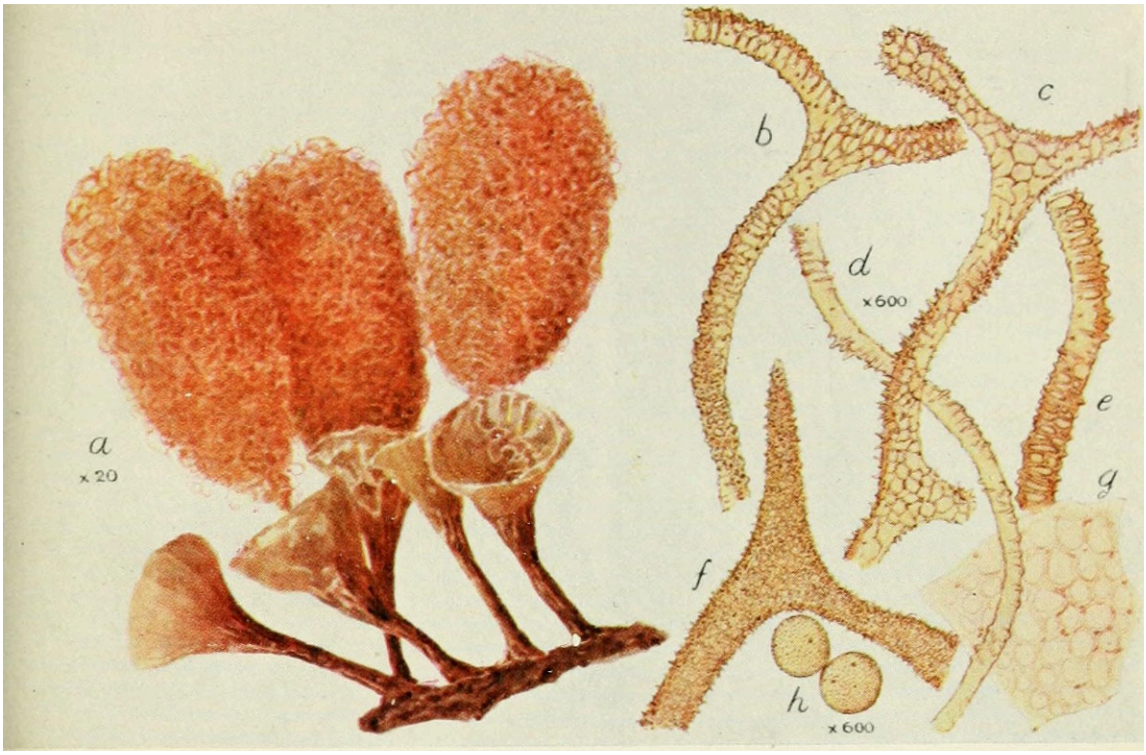
DESPITE THE LIMITATIONS PLACED ON WOMEN'S EDUCATION DURING THE VICTORIAN ERA, LISTER HAD ACCESS TO A RICH INTELLECTUAL ENVIRONMENT THAT NURTURED HER SCIENTIFIC CURIOSITY



Drawings of *Craterium minutum* from *A Monograph of the Mycetozoa* by Gulielma Lister

Women in London for one year, where she received formal instruction in systematic and structural botany. Although her time at Bedford College was brief, it provided Lister with a solid grounding in the basics of botany, particularly plant morphology and classification. However, it was her informal education at home—guided by her father's extensive library of botanical texts and field notes—that had the greatest impact on her intellectual development. Lister's early education was not confined to books. The Lister family home, filled with specimens, botanical prints, and journals from Arthur's many mycological studies, was a sanctuary of learning. Gulielma learned how to carefully observe, classify, and record her findings, all while engaging in stimulating discussions about the natural world with her father and other members of the Quaker community. These early lessons in fieldwork, taxonomy, and observation laid the foundation for her future career in science. It was in this environment of intellectual rigor and curiosity that Lister's love for mycology —and her fascination with slime molds—began to take root.



Drawings of *Arcyria ferruginea* from *A Monograph of the Mycetozoa* by Gulielma Lister

The Lister Family Legacy in Mycology

Gulielma was largely self-taught in many aspects of her education, as, apart from a one-year stay at Bedford College for Women, she received most of her instruction at home. Her days often began in the laboratory or out in the field, observing microscopic organisms in their natural habitats. Gulielma was a key figure in the development of the science of plasmodial slime molds, and her passion for this field was deeply rooted in the pioneering work of her father, Arthur Lister. A botanist and mycologist, he laid the foundations for the study of Mycetozoa with his work "A Monograph of the Mycetozoa", published in 1894, which became an indispensable reference in the field. Gulielma was not just an observer; she was an active participant in this scientific endeavor, working alongside her father as both his field and laboratory assistant. Together, they studied and recorded their observations, often working at home—first at Sycamore House in Leytonstone, and later at their coastal home, Highcliff, in Lyme Regis.

Her close collaboration with her father was not purely professional. Like him,

Drawings of *Badhamia nitens* from *A Monograph of the Mycetozoa* by Gulielma Lister

Gulielma was deeply captivated by the world of slime molds—these unusual organisms that were the focus of their research. Over the years of working with him, her expertise in the field grew, and her contributions became increasingly significant. In addition to assisting with data collection for "A Monograph of the Mycetozoa", Gulielma played a crucial role in the preparation of the "Guide to the British Mycetozoa" (1895), which became one of the most important references for identifying British slime molds.

After her father's death in 1908, Gulielma did not simply continue his work; she took it in new directions. She built upon the foundational principles of his research, adding new information in a second, expanded edition of the "Guide". Her contribution to this edition was particularly evident in the artistic illustrations: she included

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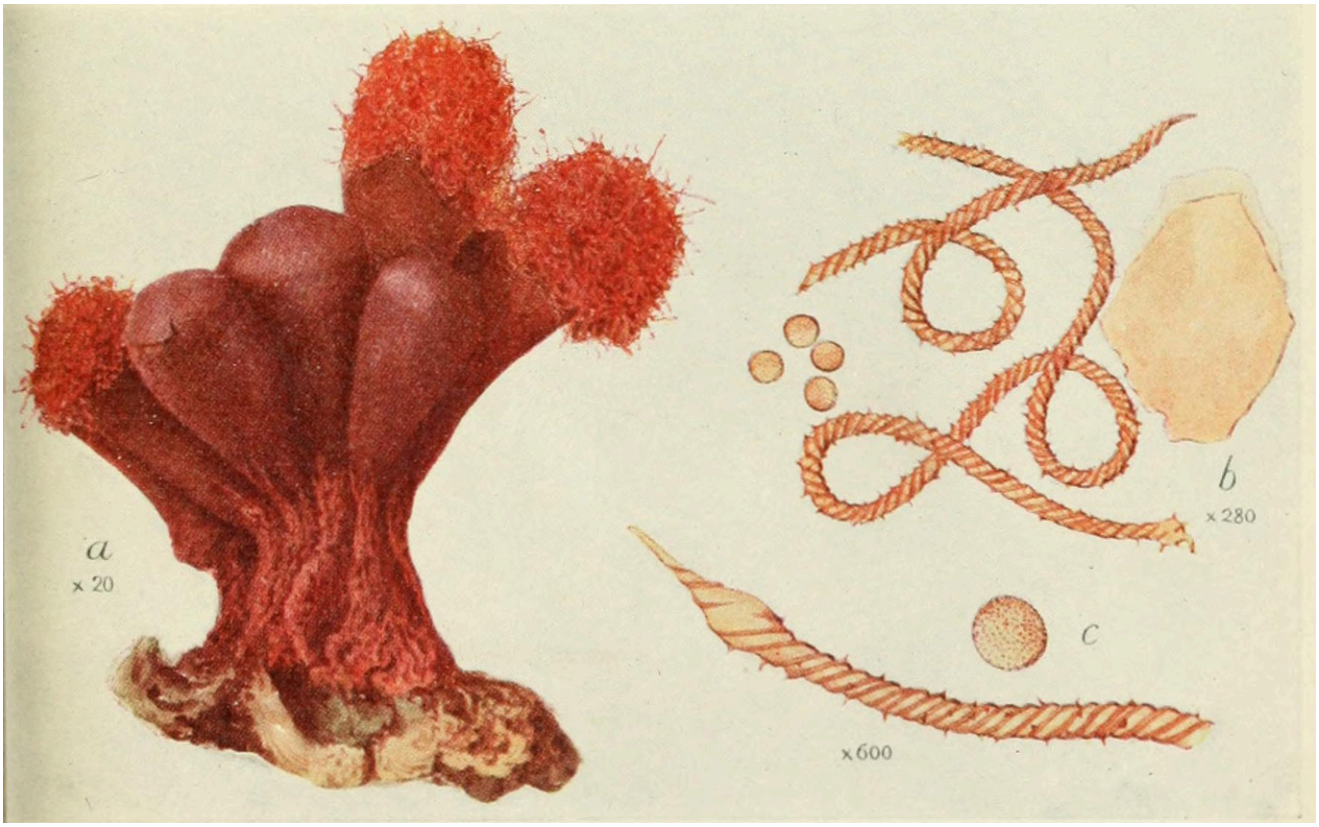
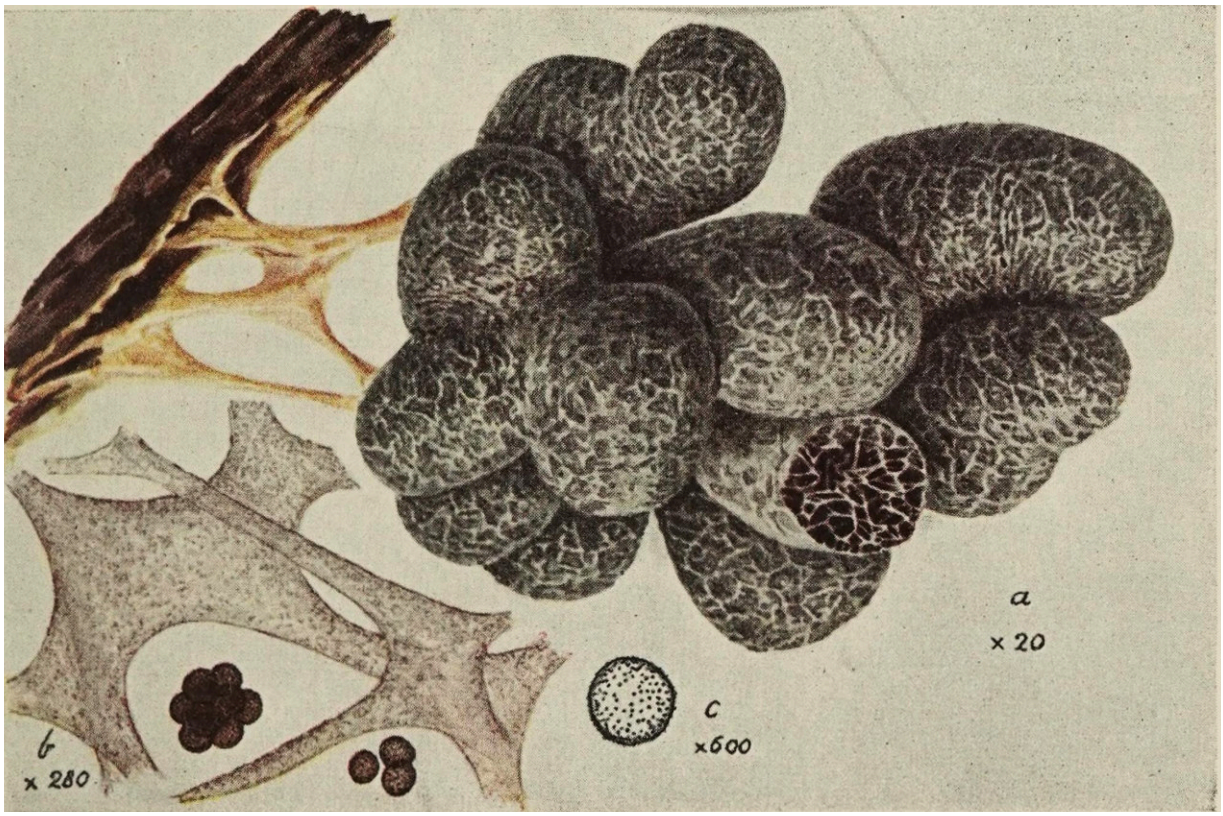


Drawings of *Hemitrichia vesparium* from *A Monograph of the Mycetozoa* by Gulielma Lister

precise, hand-painted watercolors of slime molds, which were not only of artistic value but also served as invaluable visual tools for the scientific community. These illustrations helped future researchers better understand and identify these microscopic organisms.

Although her scientific work was widely recognized, Gulielma was not formally affiliated with major institutions like the British Museum or the Royal Botanic Gardens at Kew. Most of her research career was spent as an independent scholar, focused on discovery and exploration rather than institutional titles. Her work was driven not by a desire for academic recognition but by a deep love for nature and a passionate investigative instinct.

Nevertheless, this did not diminish her influence. She collaborated with some of the most significant collections in the United Kingdom and France, including the British Museum, Kew Gardens, and the Natural History Museum in Paris. These collaborations helped her work gain international recognition.



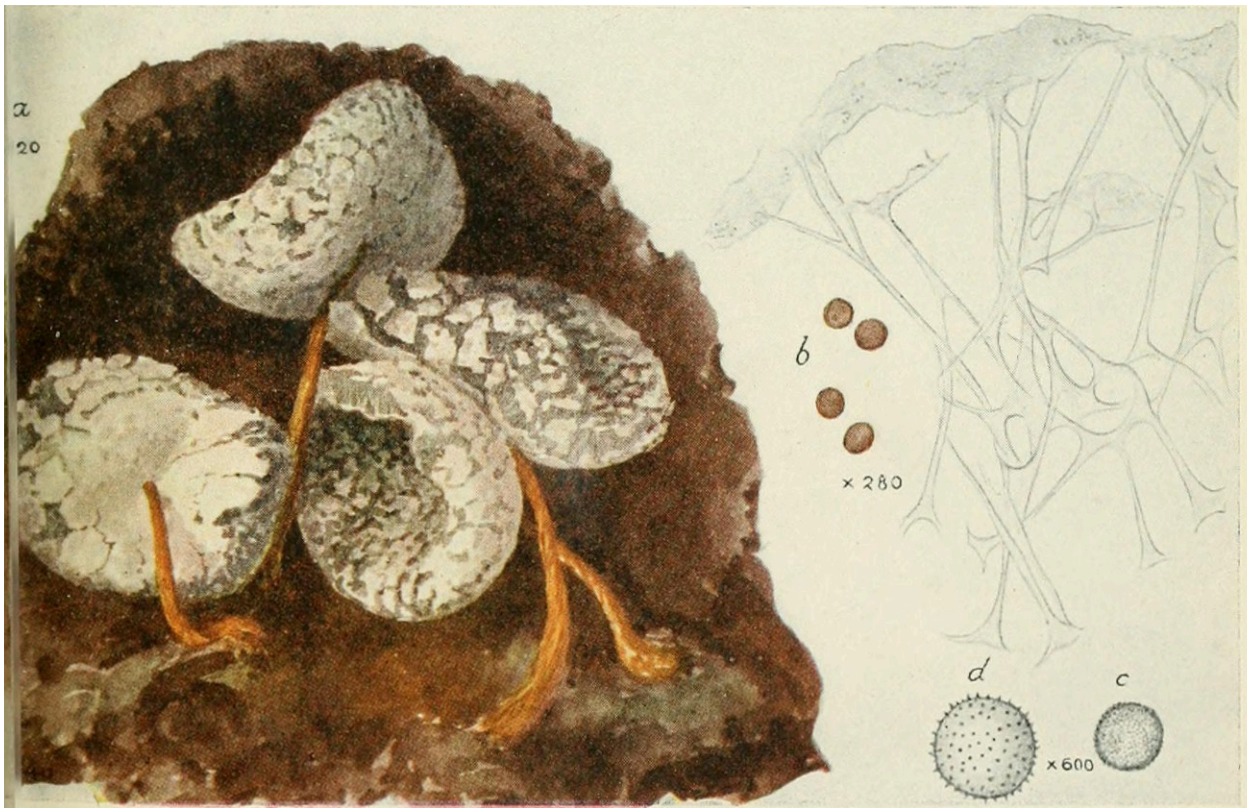
Drawings of *Badhamia utricularis* from *A Monograph of the Mycetozoa* by Gulielma Lister

The Study of Slime Molds

The organisms that so fascinated Lister—slime molds—are among the most peculiar of all living things. Mycetozoa are neither true fungi nor protozoa, but are often described as a separate group that exhibits characteristics of both. They exist in two distinct stages: a mobile, amoeba-like plasmodium that moves and feeds, and a stationary, spore-producing fruiting body. Their unique life cycle, combining traits of both protozoa and fungi, makes them a subject of particular intrigue and complexity. For much of the 19th and early 20th centuries, their classification and behavior remained shrouded in mystery.

Lister's studies brought clarity to many aspects of Mycetozoa. She developed detailed methods for observing and classifying different species,

> SLIME MOLDS EXIST IN TWO DISTINCT STAGES: A MOBILE, AMOEBA-LIKE PLASMODIUM THAT MOVES AND FEEDS, AND A STATIONARY, SPORE-PRODUCING FRUITING BODY



Drawings of *Trichamphora pezizoidea* from *A Monograph of the Mycetozoa* by Gulielma Lister

significantly advancing the taxonomy and understanding of slime molds. Her pioneering work included careful field studies, as well as detailed illustrations that captured the various forms of slime molds in vivid color. These watercolors, alongside her meticulous field notes, became invaluable resources for both researchers and amateurs alike.

One of her most important contributions was her revision of the taxonomy of Mycetozoa, particularly the Myxogastria, the largest and most diverse group within slime molds. Lister's work helped identify and classify many new species, while her detailed descriptions provided a deeper understanding of their complex life cycles and ecological roles. At a time when many mycological studies were constrained by limited technology and resources, Lister's keen observations and meticulous data recording set her apart as a highly skilled and careful scientist.

Myxogastrids *(Myxogastria) differ from other groups of slime molds primarily in their plasmodial stage, where they form a large, multinucleate mass (plasmodium) that moves and engulfs food.*

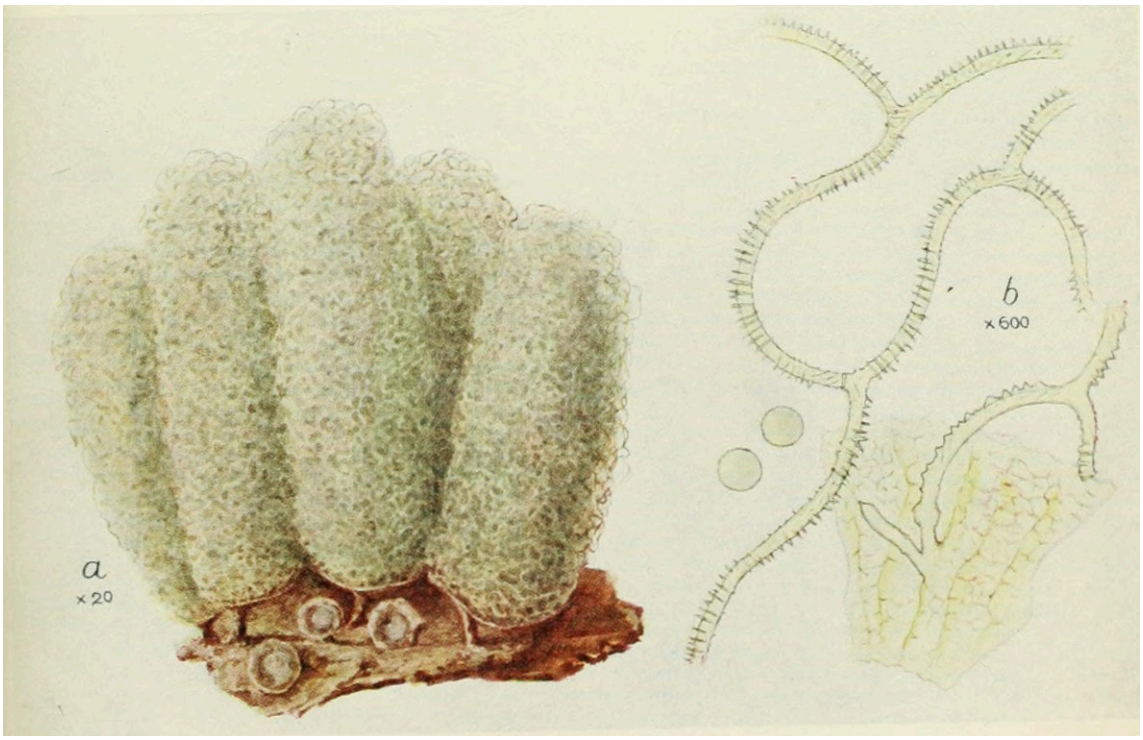
Group photograph of the *British Mycological Society*, at the Haslemere Fungus Foray, 25-30 September 1905

A Pioneer in Academic Societies

The name of Lister resonates through the annals of mycology, not only for her groundbreaking research but also for her steadfast commitment to nurturing a community of naturalists and advancing the science of fungi. As a founding member of the British Mycological Society in 1903, Lister played an instrumental role in shaping the early years of this influential organization. Her involvement went beyond membership; she was twice elected president, first in 1912 and again in 1932, and was named an honorary member in 1924—a recognition of her unparalleled contributions to the field.

Lister's leadership extended far beyond the British Mycological Society. She was a trailblazer for women in science, becoming the first

> AS A FOUNDING MEMBER OF THE BRITISH MYCOLOGICAL SOCIETY IN 1903, LISTER PLAYED AN INSTRUMENTAL ROLE IN SHAPING THE EARLY YEARS OF THIS INFLUENTIAL ORGANIZATION

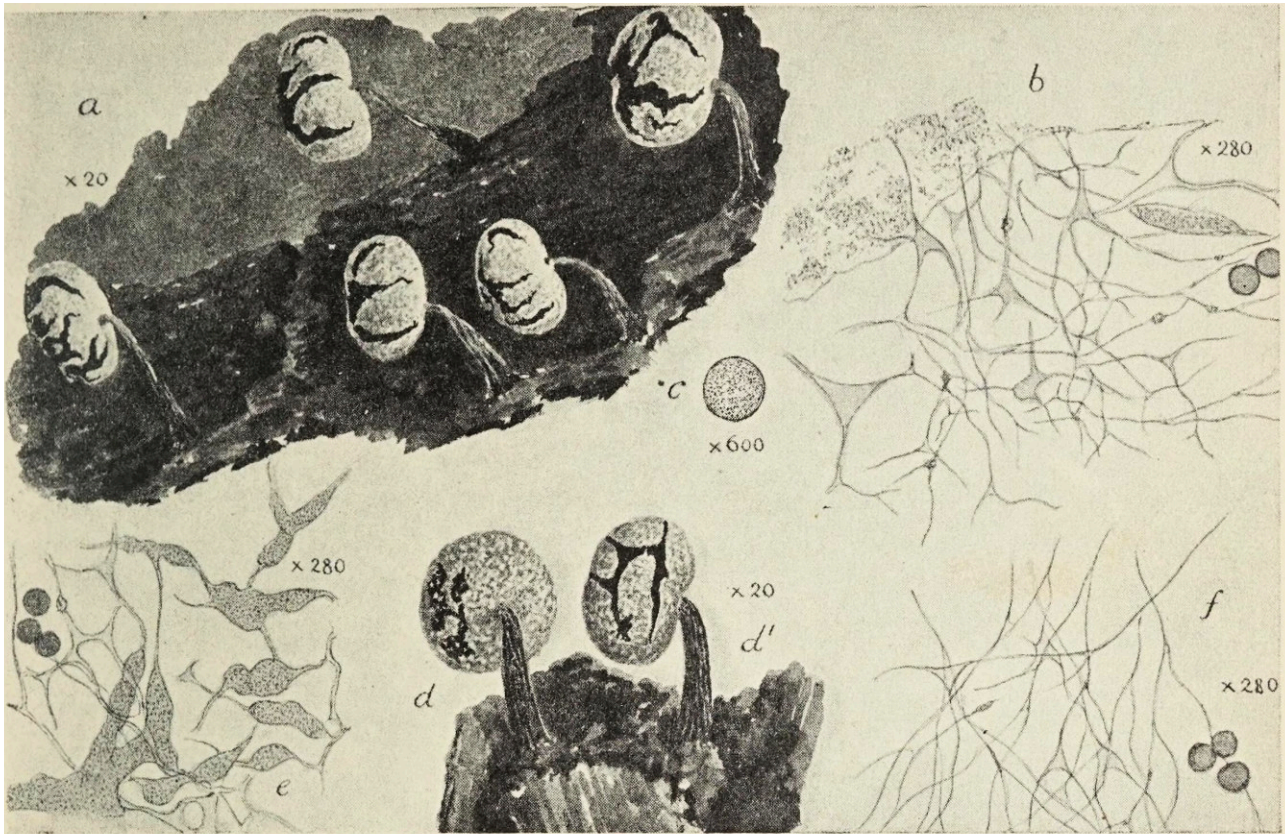


Drawings of *Arcyria glauca* from *A Monograph of the Mycetozoa* by Gulielma Lister

female president of the Essex Field Club. In this role, she brought her passion for the natural world into the field, leading excursions and fostering an environment where aspiring naturalists could learn through hands-on experience. Her mentorship, which inspired many young botanists and mycologists, was grounded in a deep love for nature's intricacies and a belief in the importance of community-driven scientific inquiry.

Her standing in the scientific community was further cemented with her election as one of the first female fellows of the Linnean Society of London, an honor that marked a milestone for women in science. Throughout her involvement, she served on the Society's council and remained an active participant in its affairs until her death. Lister's legacy is not merely one of academic achievement, but of a profound, lifelong dedication to advancing science and empowering future generations of naturalists—particularly women—who would follow in her footsteps.

*The **Linnean Society of London** is one of the world's oldest active biological societies, founded in 1788 and named after the Swedish botanist Carl Linnaeus, the father of modern taxonomy. It is renowned for hosting the first public presentation of Charles Darwin and Alfred Russel Wallace's theory of natural selection in 1858.*



Drawings of *Physarum nutans* from *A Monograph of the Mycetozoa* by Gulielma Lister

Global Correspondence

Lister's dedication to advancing the frontiers of scientific knowledge transcended the borders of Britain. Her intellectual curiosity knew no bounds, and she cultivated an extensive network of correspondence with mycologists around the world. From Europe to North America and as far afield as Japan, her insights and research findings were shared and respected by leading experts in the field. In one remarkable instance, her contributions to the study of slime molds earned her a rare token of appreciation: the Emperor of Japan himself sent Lister a pair of exquisite enamel vases as a gesture of gratitude for her assistance with his own research.

Beyond the exchange of ideas, Lister also cultivated close professional ties with fellow

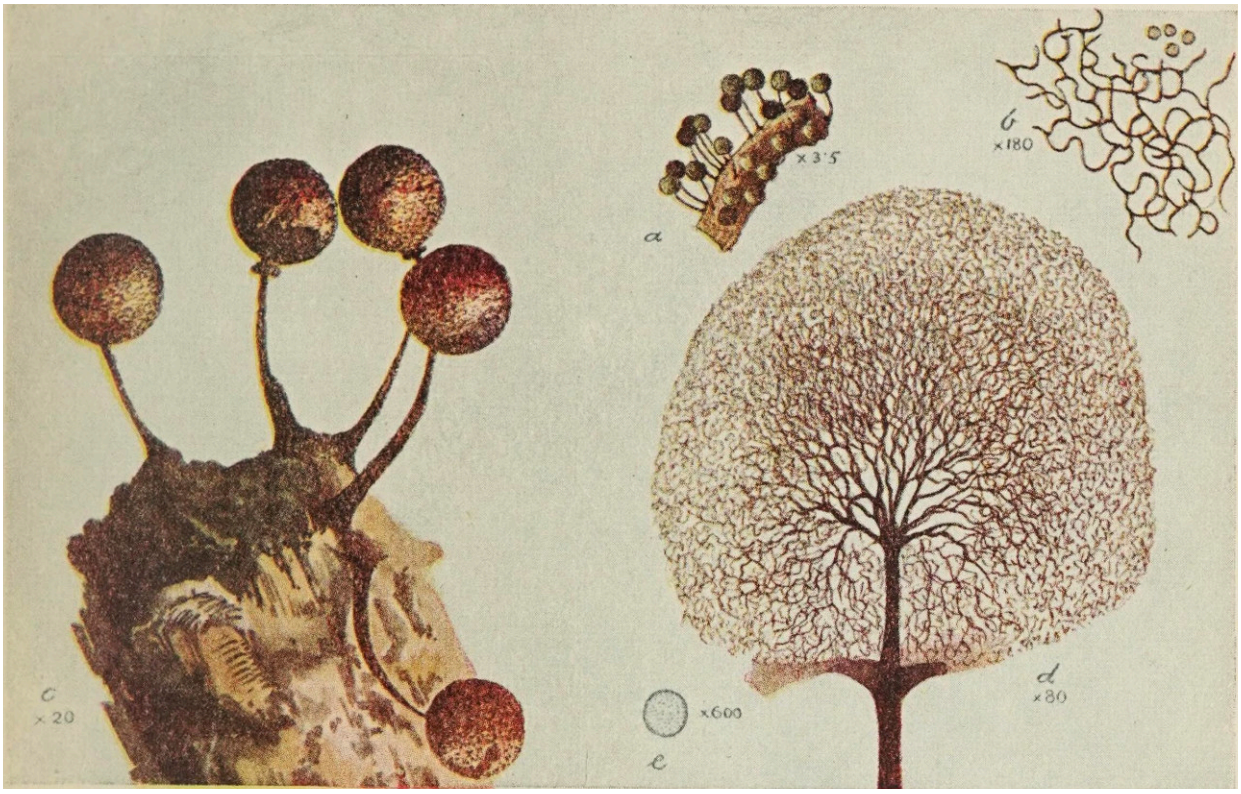
> THE EMPEROR OF JAPAN HIMSELF SENT LISTER A PAIR OF EXQUISITE ENAMEL VASES AS A GESTURE OF GRATITUDE FOR HER ASSISTANCE WITH HIS OWN RESEARCH

Alice Hibbert-Ware
(1869-1944)

Józef Tomasz Rostafiński
(1850-1928)

naturalist Alice Hibbert-Ware. Together, they embarked on extensive travels throughout Europe and New Zealand, combining their shared passion for fungi and ornithology. These journeys were not mere leisure; they were integral to Lister's work. The diverse ecosystems they explored offered invaluable opportunities to study fungi and slime molds in their natural habitats. Lister's intellectual pursuit of Myxogastria, in particular, took her across Europe, where she made it a point to stay abreast of the latest developments in the field. Her commitment to scholarship was so profound that she even learned Polish to read the works of Józef Tomasz Rostafiński, a pioneering researcher in slime molds.

Lister's global contributions also extended to Ireland, where she played a significant role in the Royal Irish Academy's Clare Island Survey. Her research on slime molds in Ireland further solidified her reputation as one of the foremost authorities on the subject. Through her tireless fieldwork and collaborations with international researchers, Lister's legacy grew not only within the scientific communities of Britain but also across the world, making her a key figure in the global study of mycology.



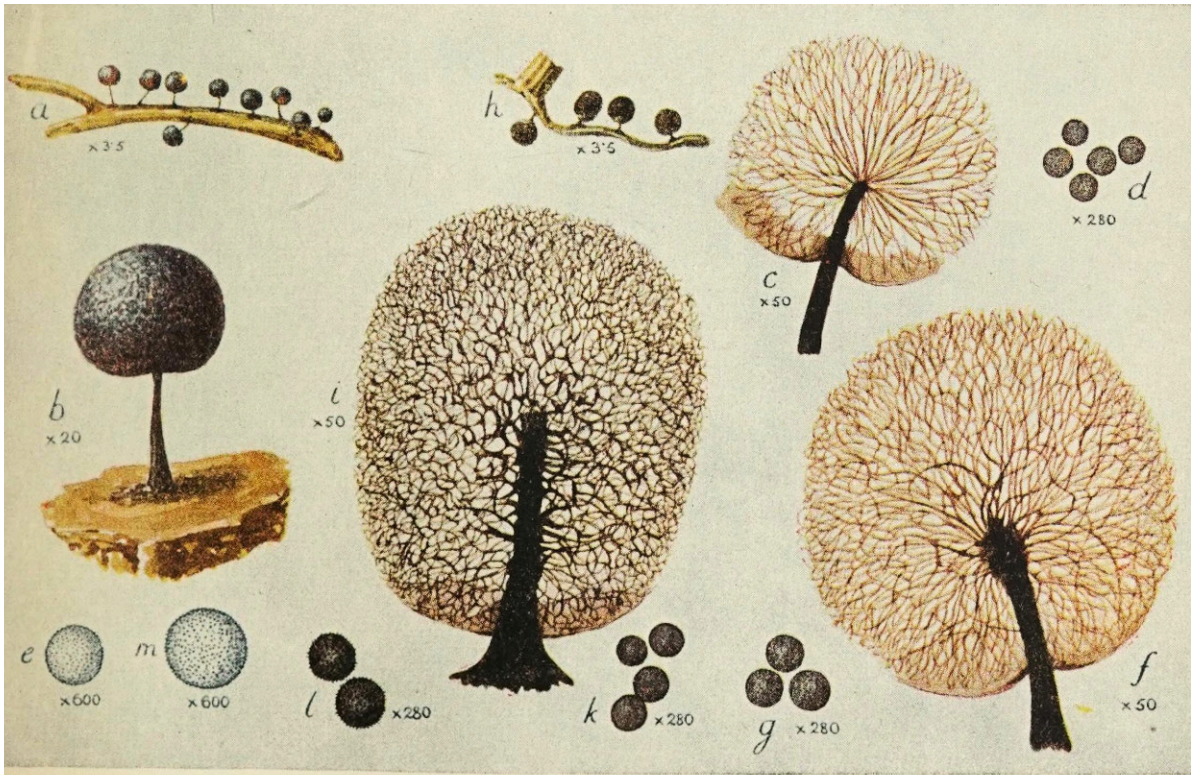
Drawings of *Lamproderma arcyrionema* from *A Monograph of the Mycetozoa* by Gulielma Lister

Legacy and Recognition

Gulielma Lister passed away on May 18, 1949, at the age of 88, following a stroke. She died in the same house where she was born, in Leytonstone—an appropriate conclusion to a life so deeply intertwined with the land and its natural wonders. Lister's legacy, however, endures far beyond her passing, preserved in the collections she helped shape and in the field of mycology that she profoundly influenced.

Her extensive botanical and mycological collections are now housed in some of the world's most esteemed institutions, including the Natural History Museum in London, Kew Gardens, and the Stratford Museum. The research notebooks she meticulously compiled, documenting the work she and her father carried out on historical collections, were bequeathed to the British Mycological Society, where

> LISTER'S EXTENSIVE BOTANICAL AND MYCOLOGICAL COLLECTIONS ARE NOW HOUSED IN SOME OF THE WORLD'S MOST ESTEEMED INSTITUTIONS



Drawings of *Lamproderma violaceum* from *A Monograph of the Mycetozoa* by Gulielma Lister

they continue to serve as an invaluable resource for contemporary researchers. In addition, her detailed scientific illustrations—executed with the same precision as her written work—remain preserved in the museum's archives, further solidifying her dual legacy as both a scientist and an artist.

While she never sought the spotlight, Lister's contributions to the study of slime molds are undeniable. Her tireless dedication to scientific inquiry, her passion for the natural world, and her unwavering commitment to the pursuit of knowledge have left an indelible mark on the history of science. In the complex and often overlooked realm of slime molds, Lister's name is synonymous with meticulous observation, intellectual rigor, and a relentless drive to uncover the mysteries of nature—qualities that continue to inspire mycologists, botanists, and naturalists around the world.

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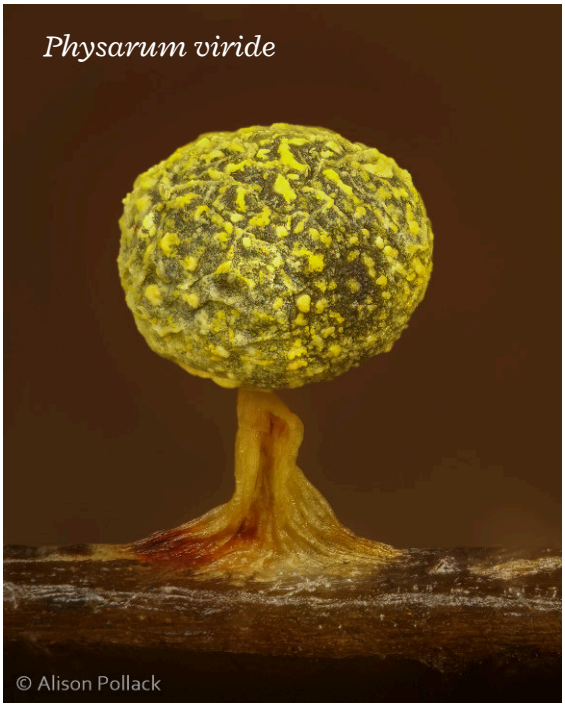
LISTER'S DETAILED SCIENTIFIC ILLUSTRATIONS—EXECUTED WITH THE SAME PRECISION AS HER WRITTEN WORK—REMAIN PRESERVED IN THE MUSEUM'S ARCHIVES



At the dawn of the 20th century, Gulielma Lister introduced the scientific community to the intricate beauty of slime molds through her detailed illustrations. At the time, photographic technology was not advanced enough to accurately capture the delicate forms and vivid colors of their fruiting bodies, which typically measure only a few millimeters in size. Today, thanks to cutting-edge photographic equipment capable of revealing these miniature wonders in stunning detail, a growing community of photographers has dedicated itself to documenting and celebrating these fascinating organisms. In many ways, Lister's work laid the foundation for this modern movement, inspiring a new generation of enthusiasts more than a century after her drawings first brought slime molds into the spotlight.

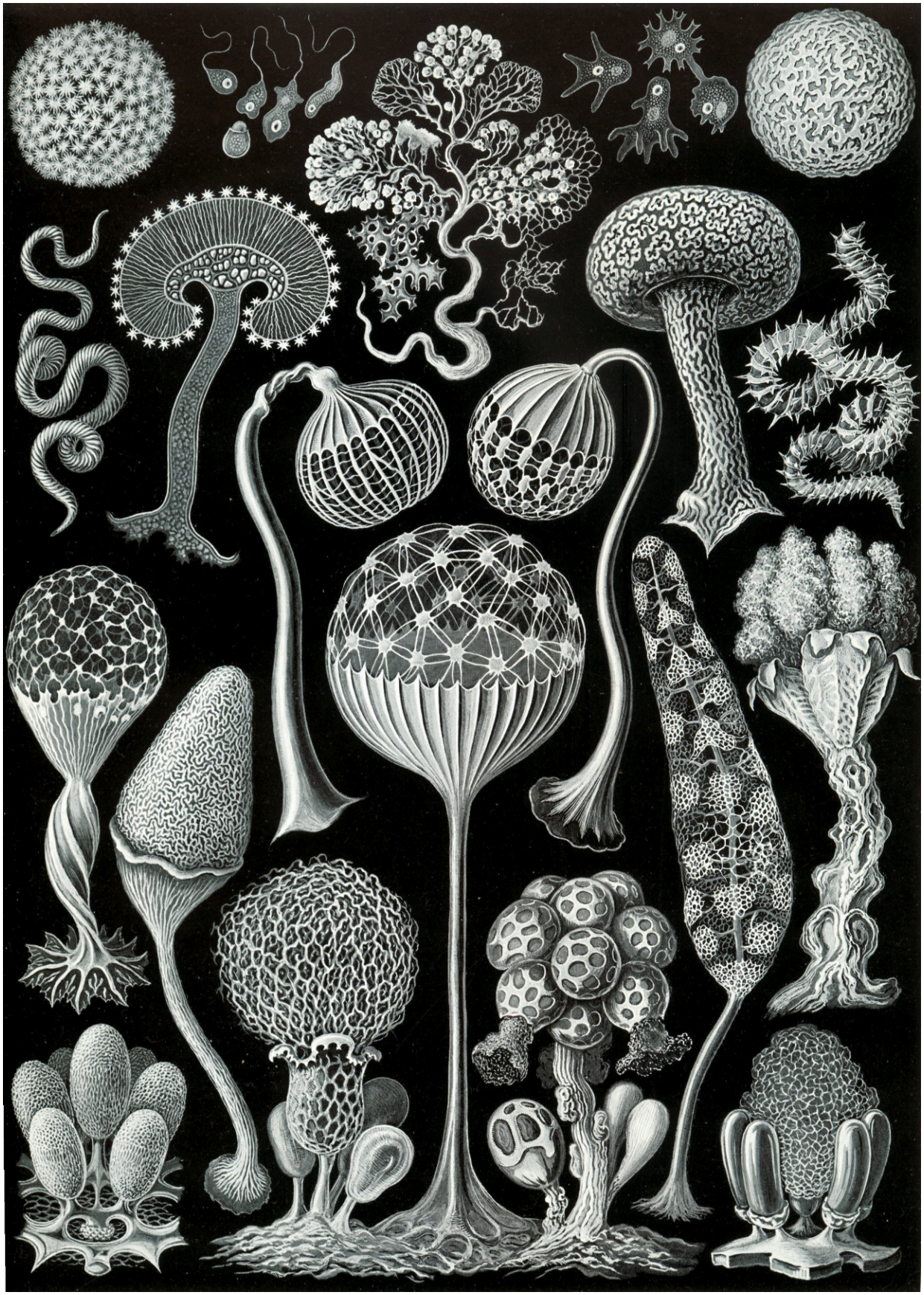
One of the most acclaimed photographers specializing in slime molds is Alison Pollack, whose stunning photos of these fascinating organisms can be explored at https://uk.inaturalist.org/people/alison_pollack.

From Blob to The Beauty
Fruiting Bodies of Plasmodial Slime Molds

By Dr. Stefan Luketa

Plasmodial slime molds are truly remarkable creatures, captivating in their ability to transform in ways that seem almost magical. They start their journey as a plasmodium—a strange, shapeless mass, made up of many nuclei but no fixed form. In this early stage, they aren't concerned with appearances. Instead, they focus all their energy on feeding and growing, spreading out in search of nutrients, almost like a living, pulsing network. But here's where it gets even more intriguing: when the conditions are just right—when the environment signals it's time—the plasmodium undergoes a dramatic change. It transforms into something entirely different: a complex, stunning fruiting body. This is where the slime mold's story takes a new turn.

Drawings of various plasmodial slime molds from *Kunstformen der Natur* by Ernst Haeckel (1904)

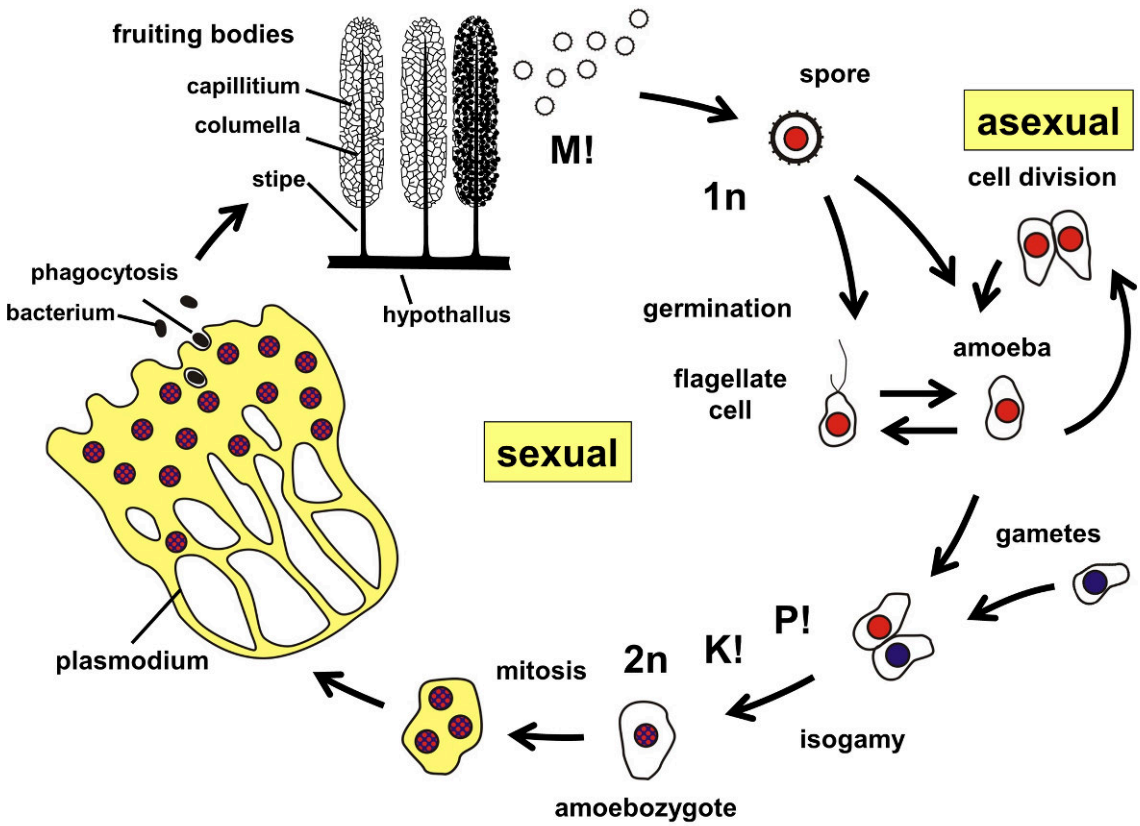


Fruiting bodies of *Trichia* sp. Photo by Alison Pollack, https://www.inaturalist.org/photos/462791474

Introduction

Imagine walking through a damp forest, the air thick with moisture and the ground covered in decaying leaves and rotting wood. If you look closely, you might spot a curious organism—an almost mystical creature—moving silently across the forest floor. This is a plasmodial slime mold, a living marvel that thrives in the moist, organic matter of its environment. Its life cycle is anything but ordinary, unfolding in a series of intricate stages that often seem to defy the rules of nature.

Fruiting bodies, the reproductive structures of slime molds, typically form on decaying organic material, such as leaves or wood, which provide a rich environment for feeding and development. As the plasmodium withdraws from its feeding grounds, it begins to form these fruiting bodies, which can vary widely in appearance. Some are small and round, while others are long and spindly, and each can have its own unique structure. The process is a testament to the resilience and adaptability of slime molds, whose remarkable life cycle continues to intrigue scientists and nature lovers alike.



Life cycle of *Stemonitis* sp. Picture source: Photo source: https://commons.wikimedia.org, CC BY-SA 3.0 license.

Fructification of the Plasmodium

One of the most fascinating moments in this cycle is the process of fructification, when the slime mold, having spent time feeding and growing, undergoes a transformation. The plasmodium, which is the vegetative phase of the slime mold, suddenly begins to prepare for reproduction. What triggers this shift is still something of a mystery. Some scientists believe that changes in environmental conditions, like shifts in humidity, temperature, or even a period of starvation, might signal to the plasmodium that it's time to reproduce. However, once the process of fructification begins, there is no turning back. Any interruption at this stage can result in malformed or deformed fruiting bodies, a clear sign of just how delicate this transformation is.

Fructification typically begins when a mature plasmodium—an amoeba-like, multinucleate

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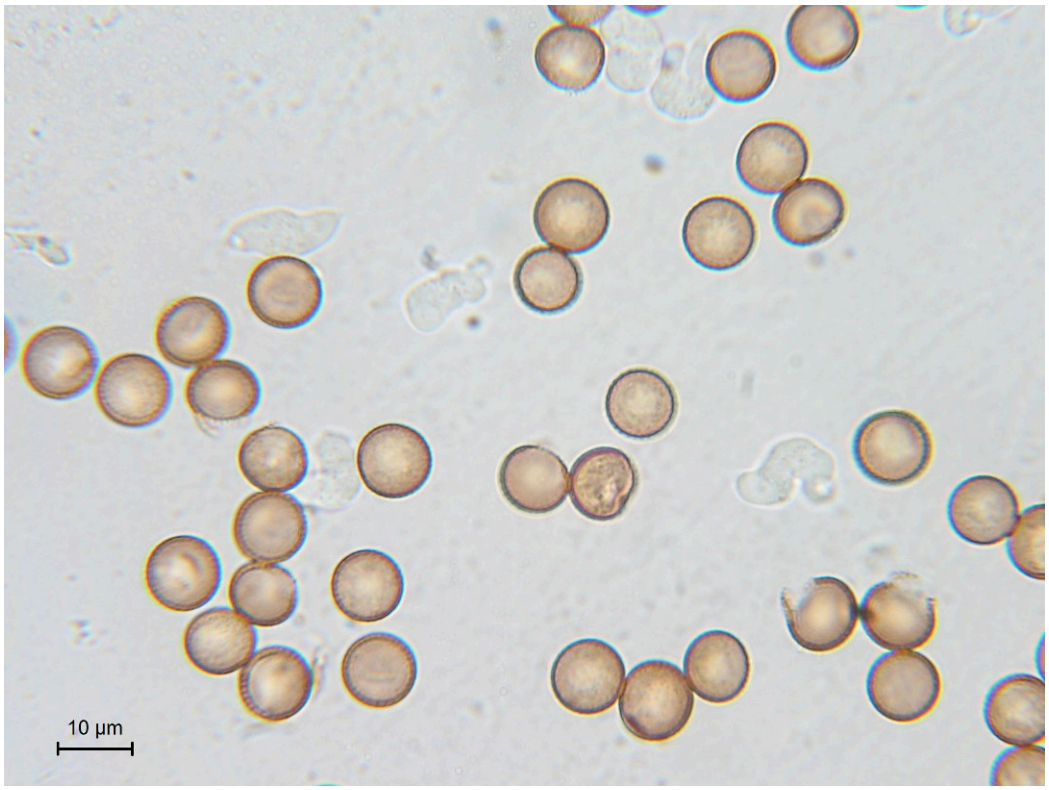
FRUCTIFICATION TYPICALLY BEGINS WHEN A MATURE PLASMODIUM—AN AMOEBA-LIKE, MULTINUCLEATE MASS OF PROTOPLASM — ABANDONS ITS FEEDING BEHAVIORS



Budded myxoflagellates of *Symphytocarpus flaccidu*s and opened spores. Photo source: https://commons.wikimedia.org, CC BY-SA 3.0 license.

mass of protoplasm — abandons its feeding behaviors. Under optimal conditions, the plasmodium will stop foraging for food and begin to exhibit phototaxis, specifically a positive response to light. This behavior is a crucial part of the process, as the plasmodium moves toward a dry, well-lit area, often in search of conditions that will allow for the efficient dispersal of spores. The attraction to light may be seen as an evolutionary adaptation that maximizes spore distribution in environments favorable for the next generation.

The plasmodium, having completed its movement toward the light, then begins the formation of fruiting bodies. These fruiting bodies are where spore production occurs, and they come in a variety of shapes and sizes, depending on the species. In some cases, these structures can be quite small, measuring less than a millimeter, while in extreme cases, they can grow to massive proportions, up to one square meter in size and weighing as much as 20 kilograms, as seen in species like *Brefeldia maxima*.

> IN SOME CASES, FRUITING BODIES CAN BE QUITE SMALL, MEASURING LESS THAN A MILLIMETER, WHILE IN EXTREME CASES, THEY CAN GROW TO MASSIVE PROPORTIONS, UP TO ONE SQUARE METER IN SIZE AND WEIGHING AS MUCH AS 20 KILOGRAMS

Fruiting bodies of *Alwisia bombarda*. Photo by Gim Siew Tan, https://www.inaturalist.org/photos/258015468

Structure of the Fruiting Bodies

The fruiting bodies of plasmodial slime molds exhibit a fascinating variety of shapes and sizes, which can change depending on the species and environmental conditions. These structures typically form in areas rich in organic matter, such as decaying wood, fallen leaves, or moist substrates found in forests and woodlands. In the early stages of development, fruiting bodies appear as tiny, translucent droplets that gradually grow and change shape as they mature. There are several distinct types of fruiting bodies, the most common of which are sporangium, aethalium, and plasmodiocarp.

Sporangia are typically smaller and more rigid than aethalia, with a clearly defined outer coat that protects the spores until they are ready to be

> IN THE EARLY STAGES OF DEVELOPMENT, FRUITING BODIES APPEAR AS TINY, TRANSLUCENT DROPLETS THAT GRADUALLY GROW AND CHANGE SHAPE AS THEY MATURE

Sporangia of *Cribraria* sp. Photo by Alison Pollack, https://www.inaturalist.org/observations/260873147

released. In contrast, aethalia develop into larger, fleshy masses that can resemble succulent or gelatinous formations. These masses change over time, growing into larger structures that may vary in color—from light yellow to dark red, purple, or even black—depending on the slime mold species. Plasmodiocarp is often more slender and branched, with segmented parts along the stem or branches, where spores are formed.

Despite the differences in appearance, all of these fruiting body types share one common feature: their ability to grow rapidly and produce spores in large quantities, ensuring successful reproduction and species dispersal. Interestingly, plasmodial slime molds do not always form fruiting bodies. This development typically occurs only after the organism reaches a certain stage in its life cycle, when environmental conditions are favorable for reproduction.

Aethalium of *Fuligo septica*. Photo by Karen Andrea Boehme, https://www.inaturalist.org/photos/477598989

Aethalium

Aethalium typically appears as a large, fleshy, and succulent mass, with its shape and size varying depending on the species. It is commonly found in round or oval forms, though branched structures can also occur. Due to its considerable size, aethalium is often one of the most striking sights in nature, particularly in humid forests or on decaying tree branches. The size of an aethalium can range from just a few millimeters to several centimeters in diameter. Its color can vary from yellow and orange to red and even black. The color of an aethalium often reflects its stage of maturity, with younger aethalia being bright yellow or orange, while darker, black aethalia indicate that the spores have matured and are ready for release.

When viewed under a microscope, aethalium consists of a protoplasm rich in various organelles and components that support its growth and function. Unlike some other fruiting bodies of Myxogastria, aethalium lacks a distinct division into internal layers. The protoplasm is homogeneous, containing numerous nuclei, as aethalium originates from the plasmodium, which is a multinucleate structure. Instead of forming separate cells, these nuclei exist within one large, multinucleate mass. This organization allows the aethalium to grow rapidly and efficiently, as resources in the

Aethalium of *Reticularia lycoperdon*. Photo by Anita Bubak, https://www.inaturalist.org/photos/451332268

protoplasm can be evenly distributed among the nuclei. Although aethalium develops from the plasmodium and not all of its components are fully differentiated, partial differentiation of cells does occur during its growth. This enables the organism to function effectively, particularly in terms of spore production.

Aethalium develops from plasmodium through several distinct stages. Initially, the plasmodium takes on an amoeboid shape, moving through the soil or decaying organic material while feeding on microscopic organisms and microbial particles. As the plasmodium reaches the appropriate stage of development, a process of differentiation is triggered. At this point, the plasmodium withdraws from its feeding area and begins to form the fruiting body. The plasmodium condenses into a large mass of protoplasm, which then expands and gradually transforms into the fleshy, succulent mass known as aethalium. This transformation marks the shift from the vegetative phase to the reproductive phase of the slime mold's life cycle.

As aethalium grows, the protoplasm stabilizes and organizes into a homogeneous mass, which then continues to develop and mature. During this phase, spores begin to form within the protoplasm of the aethalium. As the spores mature, the aethalium undergoes a transformation, typically transitioning into a darker color. The mass can turn black, signaling that

Aethalium of *Tubifera ferruginosa*. Photo by Alison Pollack, https://www.inaturalist.org/photos/444037669

the spores have fully developed and the fruiting body is now ready to release them into the surrounding environment. These spores are microscopic and highly resistant to adverse conditions, such as drought and extreme temperatures. Thanks to their exceptional resilience, the spores can endure for extended periods, remaining dormant until they encounter a moist, nutrient-rich environment that is conducive to germination.

When the spores reach maturity, the aethalium opens or breaks apart, releasing the spores into the surrounding environment. These spores are dispersed through various means, such as wind, water, or by coming into contact with animals or insects. As the spores travel, they seek out suitable conditions for growth. Once they encounter an environment that provides the necessary moisture and nutrients, they germinate, giving rise to a new plasmodium. This marks the beginning of a new life cycle for the slime mold, continuing the process of growth, development, and eventual fructification.

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THANKS TO THEIR EXCEPTIONAL RESILIENCE, THE SPORES CAN ENDURE FOR EXTENDED PERIODS, REMAINING DORMANT UNTIL THEY ENCOUNTER A MOIST, NUTRIENT-RICH ENVIRONMENT THAT IS CONDUCIVE TO GERMINATION

Plasmodiocarps of *Hemitrichia serpula*. Photo by Maria Vasilyeva, https://www.inaturalist.org/photos/466858441

Plasmodiocarp

Plasmodiocarp is a fruiting body that forms from the plasmodium when the slime mold is ready to reproduce. Unlike the larger, fleshy aethalia, plasmodiocarp is smaller and more linear in shape, with a distinct structure and method of organizing spores. While aethalia are typically large, fleshy masses of protoplasm in which spores develop within a single, unified mass, plasmodiocarp has a more segmented form. This segmented structure allows the spores to be arranged along the stem or axis of the fruiting body, making the process of spore release more efficient and organized. The shape and organization of plasmodiocarp thus support a different reproductive strategy, one that facilitates the spread and dispersal of spores in a more controlled manner.

Plasmodiocarp begins to form when the plasmodium, a multinucleate protoplasm that

> UNLIKE THE LARGER, FLESHY AETHALIA, PLASMODIOCARP IS SMALLER AND MORE LINEAR IN SHAPE, WITH A DISTINCT STRUCTURE AND METHOD OF ORGANIZING SPORES

Plasmodiocarps of *Physarum aeneum*. Photo by Katja Schulz, https://www.inaturalist.org/photos/341408333

has previously migrated and fed on microorganisms in its environment, reaches reproductive maturity. At this stage, the plasmodium withdraws its resources from the feeding site and begins to differentiate, giving rise to a fruiting body that develops along a substrate such as wooden branches, decaying leaves, or organic matter. The stem or axis on which the plasmodiocarp forms may be very thin and microscopic, but its unique structure allows for more efficient distribution of spores compared to other fruiting bodies. This arrangement helps optimize the release and dispersal of spores, ensuring better chances for the slime mold's survival and reproduction.

Within the stem of the plasmodiocarp, spores form along segments that run the length of the structure. As the plasmodiocarp develops, it creates specialized regions rich in nutrients, providing the ideal conditions for spores to form. These spores are highly resilient, capable of withstanding drought and

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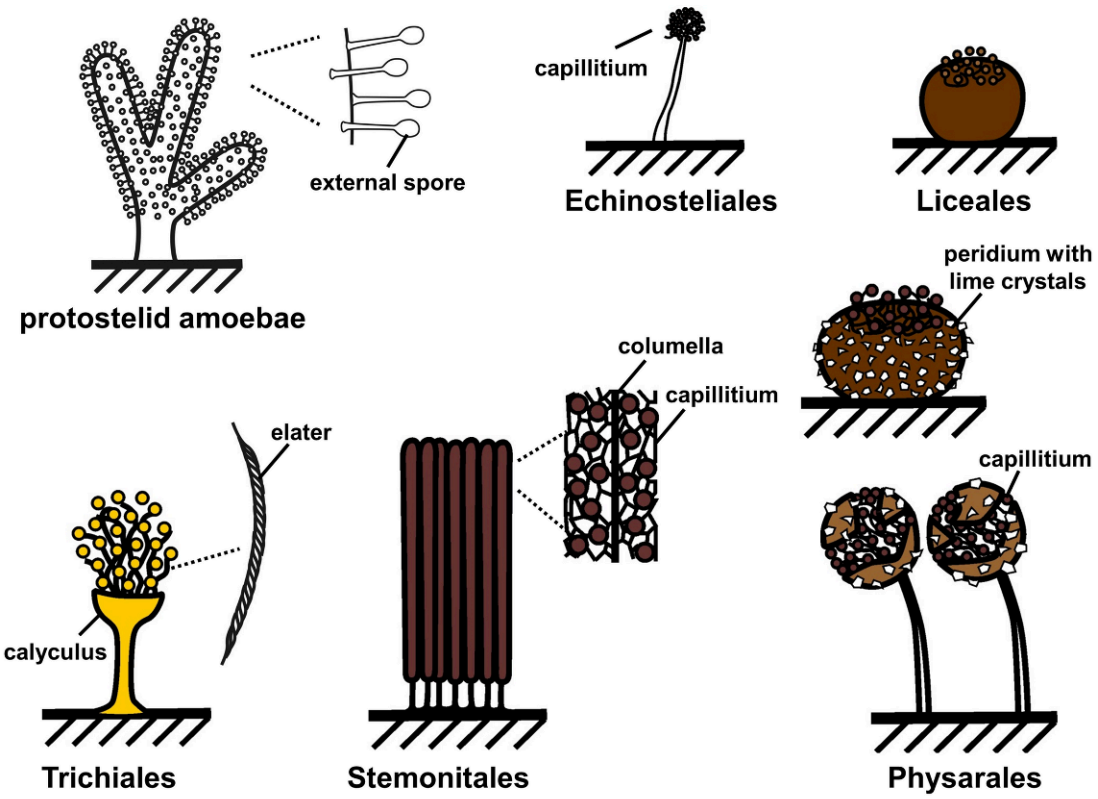
Plasmodiocarps of *Physarum cinereum*. Photo by Urmas Tartes, https://www.inaturalist.org/photos/412314317

temperature fluctuations. As the spores mature, the plasmodiocarp opens or breaks apart, releasing the spores into the surrounding environment. The spores then spread through various means, including water, wind, or by hitching rides on animals and insects. This dispersal allows the spores to travel across new ecosystems, facilitating the expansion of the organism and the continuation of its life cycle.

Compared to other fruiting structures like sporangia, which are often smaller and more tightly protected, plasmodiocarp is more complex in both its form and the way it releases spores. While spores in sporangia develop within solid capsules that open upon maturity, plasmodiocarp features a segmented structure that facilitates more efficient spore distribution. This segmented design allows spores to be arranged along the stem or axis, enabling a more organized release. Additionally, plasmodiocarp is typically larger and more durable than sporangia, allowing it to survive under favorable conditions for longer periods. As a result, it can release spores gradually over an extended time, improving the chances of successful dispersal and colonization.



Sporangia types in the protostelids and in the myxogastrid groups (Echinosteliales, Liceales, Trichiales, Stemonitales, Physarales). Picture source: https://commons.wikimedia.org, CC BY-SA 3.0 license.

Sporangium

Sporangia are reproductive bodies that resemble small caps or spheres. These structures form as a result of changes in the plasmodium, a multinucleate layer of protoplasm that develops during the vegetative phase of slime molds. When the plasmodium reaches a certain stage of growth and maturity, the initiation of a process occurs that leads to the formation of sporangia.

Sporangia typically have a distinctive outer layer known as the peridium. This outer layer can vary in texture, often being soft and elastic, but in certain cases, it becomes tough and resistant to external conditions. Inside the sporangium, one of the most notable features is the capillitium—a network of thread-like structures that crisscross the interior. These threads play a crucial role in supporting and protecting the spores, which are produced in large numbers during the process of fructification.

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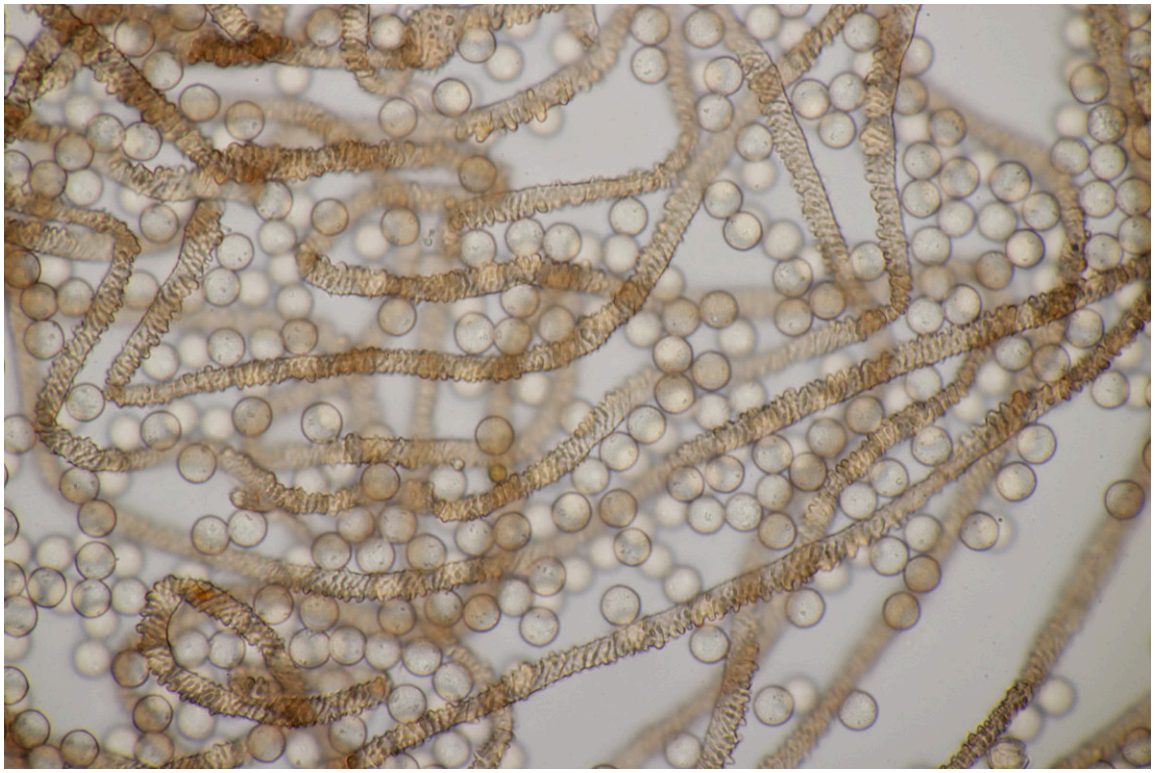
> INSIDE THE SPORANGIUM, ONE OF THE MOST NOTABLE FEATURES IS THE CAPILLITIUM—A NETWORK OF THREAD-LIKE STRUCTURES THAT CRISSCROSS THE INTERIOR

The stalk of the fruiting body of *Arcyria marginata* is composed of cells, covered with tender slime cover. Magnification ×400. Photo source: https://commons.wikimedia.org, CC BY 4.0 license.

The capillitium not only helps preserve the integrity of the fruiting body but also aids in the dispersal of spores, ensuring the continuation of the species. While nearly all plasmodial slime molds feature capillitium, there are a few exceptions. For example, in some species of the genus *Echinostelium*, the capillitium may be absent or drastically reduced, highlighting the diversity within this fascinating group of organisms.

The spores of plasmodial slime molds are remarkably resilient, able to withstand harsh environmental conditions. This durability enables them to survive prolonged periods of drought, extreme temperatures, and other unfavorable factors, which makes them ideal for long-distance dispersal. When the fruiting body dries out, the spores are released into the surrounding environment, primed to enter the next stage of the slime mold's life cycle. Ready to endure the challenges of their new surroundings, these spores can remain dormant until conditions improve, ensuring the continued survival and spread of the slime mold species.



Capillitium and spores of *Arcyria stipata*. Photo by Björn Sothmann, https://www.inaturalist.org/photos/103750448

Once the fruiting bodies have matured and dried, the spores are released into the surrounding environment, ready to begin their journey to new locations. The primary means of spore dispersal is through the wind, which can carry the tiny, lightweight spores over vast distances. However, small animals such as woodlice, mites, and beetles also contribute significantly to the dispersal process. These creatures may pick up spores by coming into contact with the fruiting bodies, or they might ingest them and later excrete the spores in new areas. Running water can also aid in spore dispersal, though it plays a less prominent role compared to wind and animal vectors. Through these various methods, the spores are spread across different habitats, ensuring the slime mold's continued survival and potential for colonization.

The dispersal process is crucial for the continuation of the slime mold's life cycle. By spreading spores over a wide area, slime molds enhance the chances that their offspring will find new environments rich in resources. This widespread dispersal increases the likelihood of successful colonization in diverse habitats, ensuring the species' survival. Moreover, the ability to disperse over long distances provides a critical advantage, allowing the slime mold to endure even when local conditions become unfavorable for growth. In this way, dispersal plays a key role in both the resilience and adaptability of slime molds.

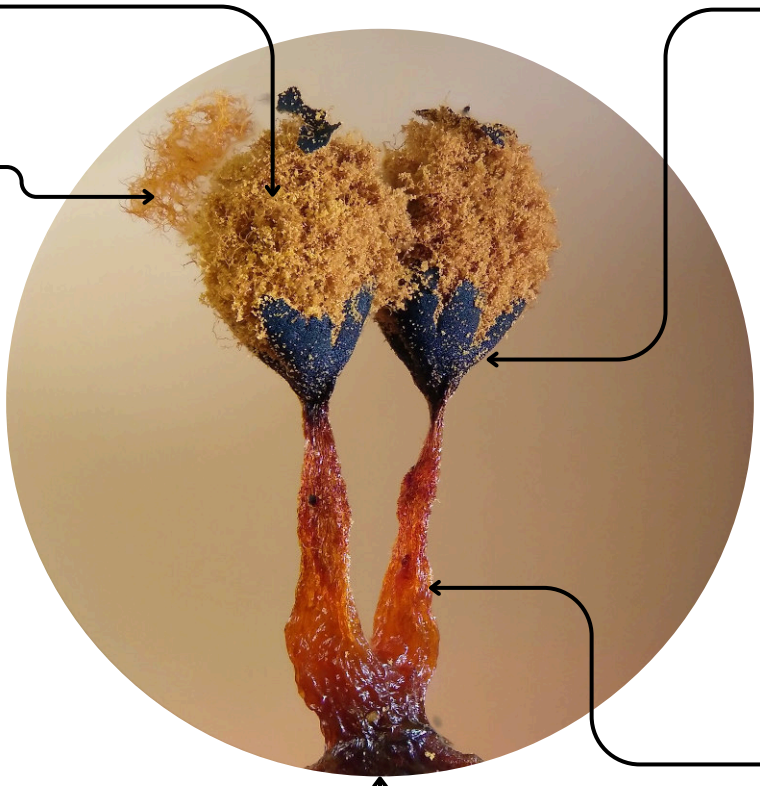
Sporangium of Plasmodial Slime Molds

Spores

They are microscopic structures surrounded by a hard, protective layer, which allows them to survive under unfavorable conditions such as drought, low temperatures, and other extremes. When the fruiting body matures, the spores are released and dispersed into the environment, often with the help of wind, water, or animals that carry them. Spores can remain dormant for extended periods until they encounter favorable conditions for germination.

Capillitium

It is a mass of sterile fibers within a fruiting body interspersed among spores. Capillitium helps release spores into the air, allowing them to travel long distances before settling in new environments.



Peridium

It is the layer on the surface of the fruiting body. This protective layer helps preserve the spores during their development, shielding them from negative external factors and allowing them to survive under unfavorable conditions until the moment of dispersal.

Hypothallus

It is a structure typically composed of a thin, often inconspicuous layer of protoplasm that lies beneath the fruiting body and anchors it to the substrate. The hypothallus enables the growth and development of the fruiting body, as well as its stability before the spores are released.

Stalk

This structure connects the hypothallus to the head of the fruiting body. The stalk allows the fruiting body to be raised above the substrate, increasing the chances of spore dispersal. In some species, the stalk is short and inconspicuous, while in others it is long and clearly visible. In certain cases, the stalk may be fragile or flexible, while in other species it can be firm and woody.

Photo source: https://commons.wikimedia.org

Sporangia of *Colloderma oculatum*. Photo by Alison Pollack, https://www.inaturalist.org/photos/470777378

Environmental Factors

Although the exact triggers for fructification in slime molds remain not fully understood, researchers have identified several environmental factors that appear to influence the process. Changes in humidity, temperature, pH, and even periods of starvation are believed to signal the plasmodium to transition into the reproductive phase. However, these factors do not trigger fructification in a uniform way across all species. While some species may require a specific combination of conditions, others may respond to just one or two environmental cues. This variability suggests that different slime mold species have evolved unique mechanisms for detecting and responding to environmental changes.

Sporangia of *Diachea leucopodia*. Photo by Alison Pollack, https://www.inaturalist.org/photos/266126452

Sporangia of *Physarum oblatum*. Photo by Alison Pollack, https://www.inaturalist.org/photos/246413103

For example, certain species of slime molds only initiate fructification during dry periods when the surrounding environment becomes inhospitable for active plasmodium growth. In such cases, the slime mold shifts to reproduction as a survival strategy, ensuring the continuation of the species. The process of fruit body formation begins when the plasmodium ceases its search for food, begins moving toward areas with light, and eventually forms the fruiting bodies that will release spores. This shift from a vegetative to a reproductive state is irreversible. If disruptions occur during this transition, it may result in malformed or deformed fruit bodies, indicating that the plasmodium has not successfully completed its transformation.

Fructification in plasmodial slime molds is a remarkable process that ensures the survival and dispersal of these fascinating organisms. Though much about the triggers for fruit body formation remains unknown, it is clear that changes in environmental conditions such as light, temperature, and moisture are integral to the transition from the vegetative state to reproduction. The wide variety of fruit body forms, ranging from simple sporangia to complex aethalia and plasmodiocarps, showcases the adaptability and complexity of these organisms. With their unique spore dispersal mechanisms and resilient reproductive structures, plasmodial slime molds continue to intrigue scientists and provide insight into the wonders of nature's adaptability.

Sporangia of *Metatrichia vesparia*. Photo by Stu Pickell, https://www.inaturalist.org/photos/416659769

Heliozoa Hunter
ZLATOGURSKY

Microscopic organisms known as heliozoans may seem elusive, but for Dr. Vasily Zlatogursky, a leading expert in their biology and diversity, they reveal a fascinating world full of surprises. In this interview, he unveils the secrets of this enigmatic group of protists, explaining how they live, hunt, and thrive in their microscopic ecosystems.

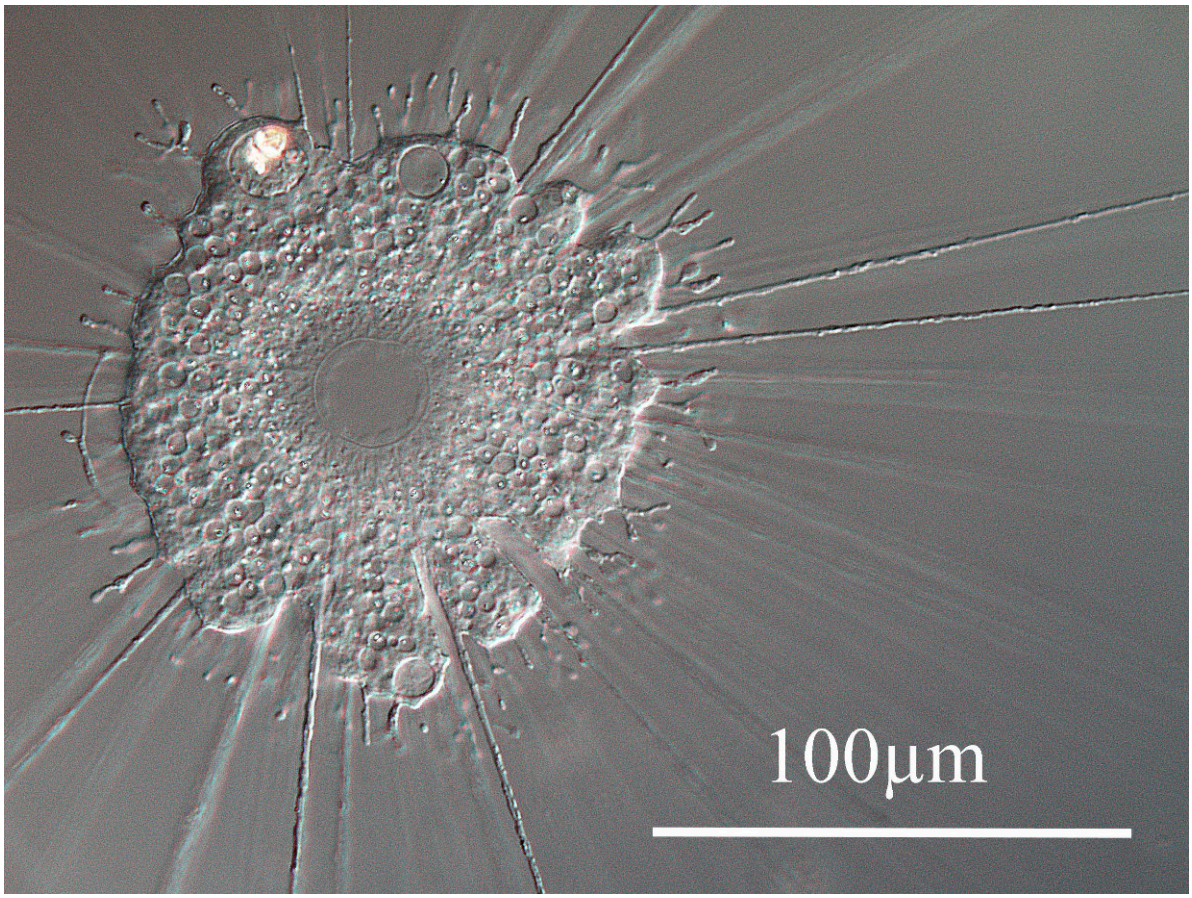
Interviewed by
Dr. Stefan Luketa

In the world of microscopic organisms, few creatures capture the imagination quite like heliozoans. These tiny protists, named for their sun-like appearance, extend delicate cytoplasmic rays from their bodies—structures that not only give them an otherworldly beauty but also serve a crucial role in how they interact with their environment.

Thanks to advancements in microscopy and molecular biology, researchers are now uncovering new species and gaining deeper insights into the evolutionary history of these enigmatic organisms. Leading the charge in heliozoa research is Dr. Vasily Zlatogursky, a scientist whose work has been instrumental in revealing the hidden world of these fascinating protists. Through meticulous microscopic observations and cutting-edge DNA sequencing techniques, Dr. Zlatogursky continues to shed light on their biology, helping us understand not only heliozoa themselves but also the broader evolutionary web of life they are part of.

In this interview, Vasily takes us into the fascinating world of heliozoa, sharing what we know about them today and how our understanding of their diversity has evolved over the past decades. We discuss how these organisms adapt to different environmental conditions, the role they play in ecosystems, and the modern methods used to study them.

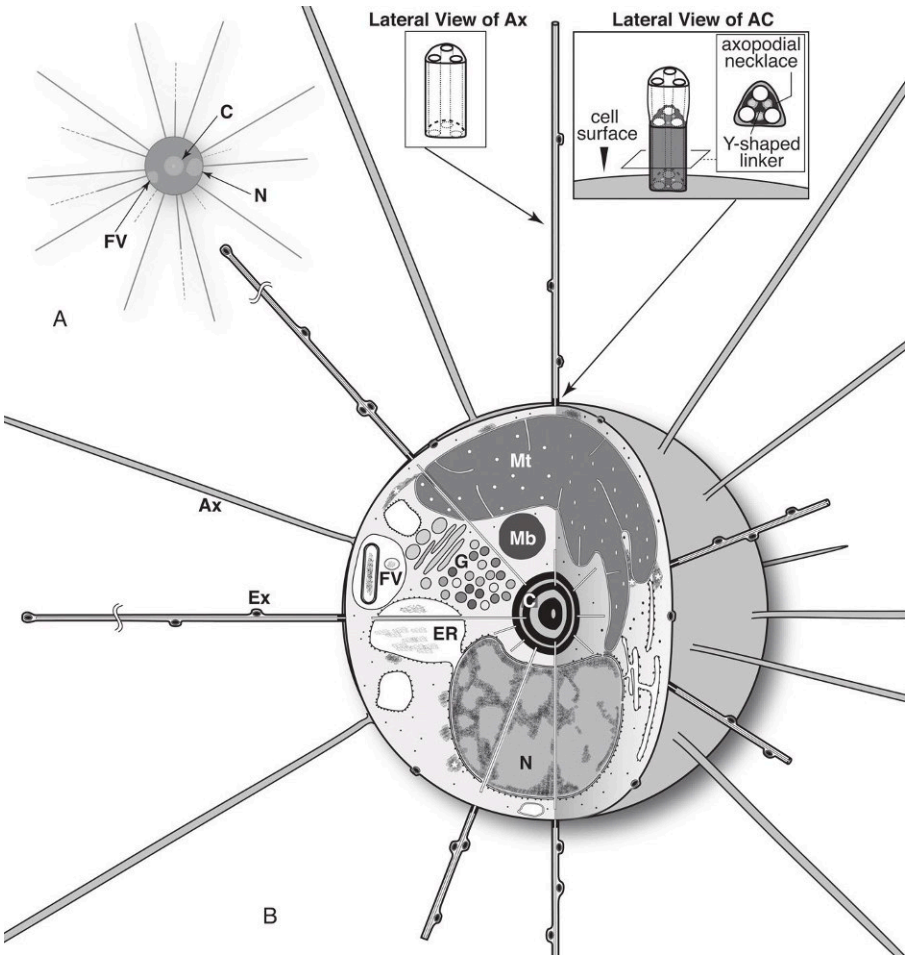
Vasily Zlatogursky at the Protist-2016 conference. Photo by Pavel Flegontov.



Actinophrys cf. *taurianini* (Stramenopiles: Actinophryida); differential interference contrast. Photo by Vasily Zlatogursky.

To begin our conversation, could you provide us with a modern and accessible explanation of the term "heliozoans"?

Unfortunately, there is no universally accepted definition for "heliozoan" and a lot of different, barely similar, and distantly related organisms were designated as such. The name refers to sun-like shape, and the term was coined by Ernst Haeckel in his famous book "Generelle Morphologie der Organismen" (1866). The group included an *Actinosphaerium*—a giant, multinucleated amoeboid cell with axopodia, which we now know belongs to the Stramenopiles. Strangely, *Actinophrys*—a close relative of *Actinosphaerium*—was not classified as a heliozoan but was instead placed in Monera (roughly equivalent to what we now call prokaryotes), as Haeckel did not believe it had a nucleus. In 1874, Haeckel's students Richard Hertwig and Edmund Lesser redefined Heliozoa as amoeboids with



Schematic cell drawings of microhelid *Microheliella maris*. A. Schematic cell image under the light microscope. B. Schematic diagram of cell ultrastructure and organelle orientation. N, nucleus; Mt, mitochondrion; Mb.microbody; G, Golgi apparatus; Ax, axopodium; AC, axopodial collar; Ex, extrusome; white asterisks, dense foci. From: Yabuki et al. (2012), doi: 10.1016/j.protis.2011.10.001

ray-like pseudopodia and without a central capsule (internal skeleton). This was their argument in a debate with Richard Greeff—another of Haeckel's students—who classified these organisms as "freshwater radiolaria". Today, we understand that such organisms have evolved multiple times in the history of eukaryotes. I would say that the prime examples of "heliozoans" are actinophryids in Stramenopiles, centrohelids in Haptista, and microhelids in Cryptista—eukaryotes completely devoid of cilia or flagella, maintaining their cell shape primarily through microtubules, a rare trait among eukaryotes. Interestingly, the only other cells that rely primarily on microtubules for their shape are animal neurons, which makes heliozoans a promising laboratory model.

HELIOZOA ARE COMPLETELY DEVOID OF CILIA OR FLAGELLA, MAINTAINING THEIR CELL SHAPE PRIMARILY THROUGH MICROTUBULES, A RARE TRAIT AMONG EUKARYOTES

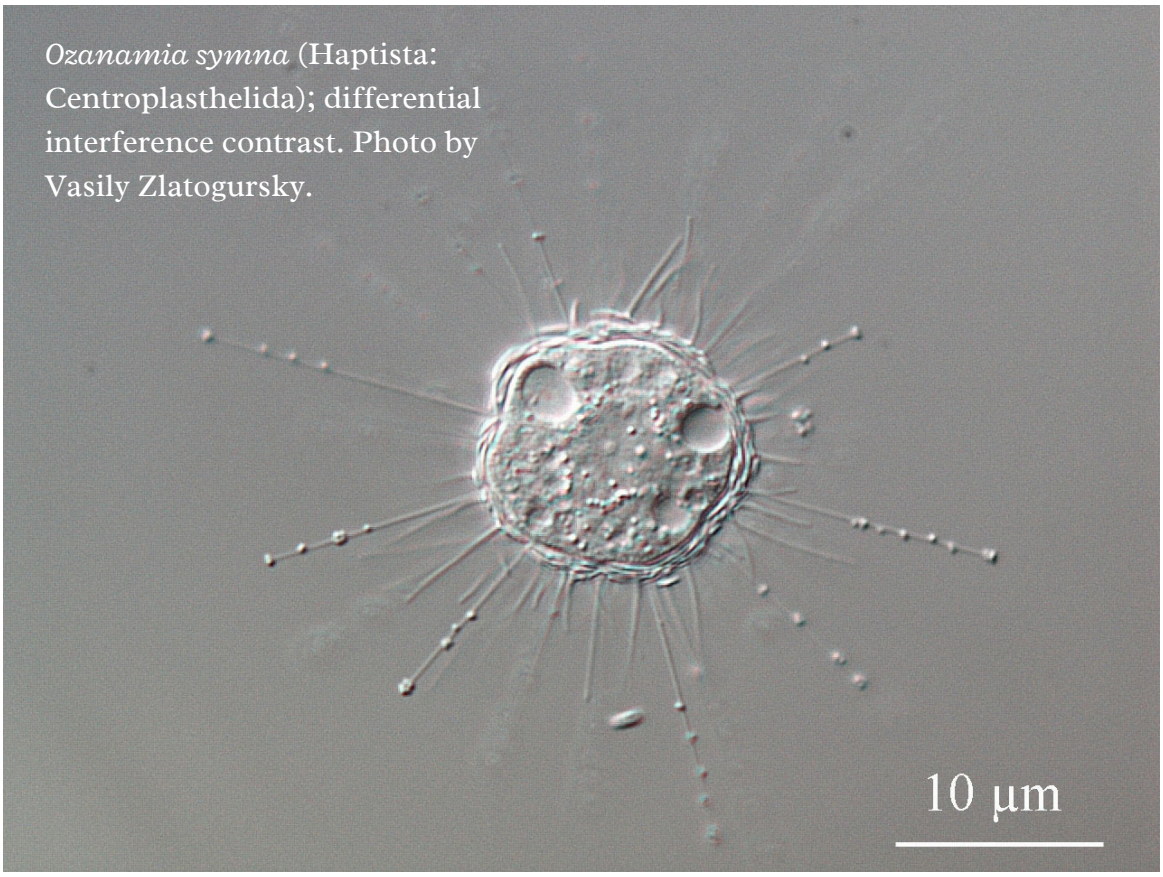
How many species of heliozoans have been identified so far?

As for the number of described species, this depends on the definition discussed above and many other factors. I maintain an up-to-date checklist of valid centrohelid species, which currently includes 170 names, though there are likely many more waiting to be described. Other lineages contain far fewer species, typically fewer than a dozen, but this is most likely due to the lack of dedicated studies.

An up-to-date checklist of valid centrohelid species, compiled by Dr. Vasily Zlatogursky, can be downloaded via the link:

https://docs.google.com/document/d/1YlZoA3UBLOuYCIbacpDIXJjBcSZaAYzug2RDaz7Q3Mg/edit?tab=t.0

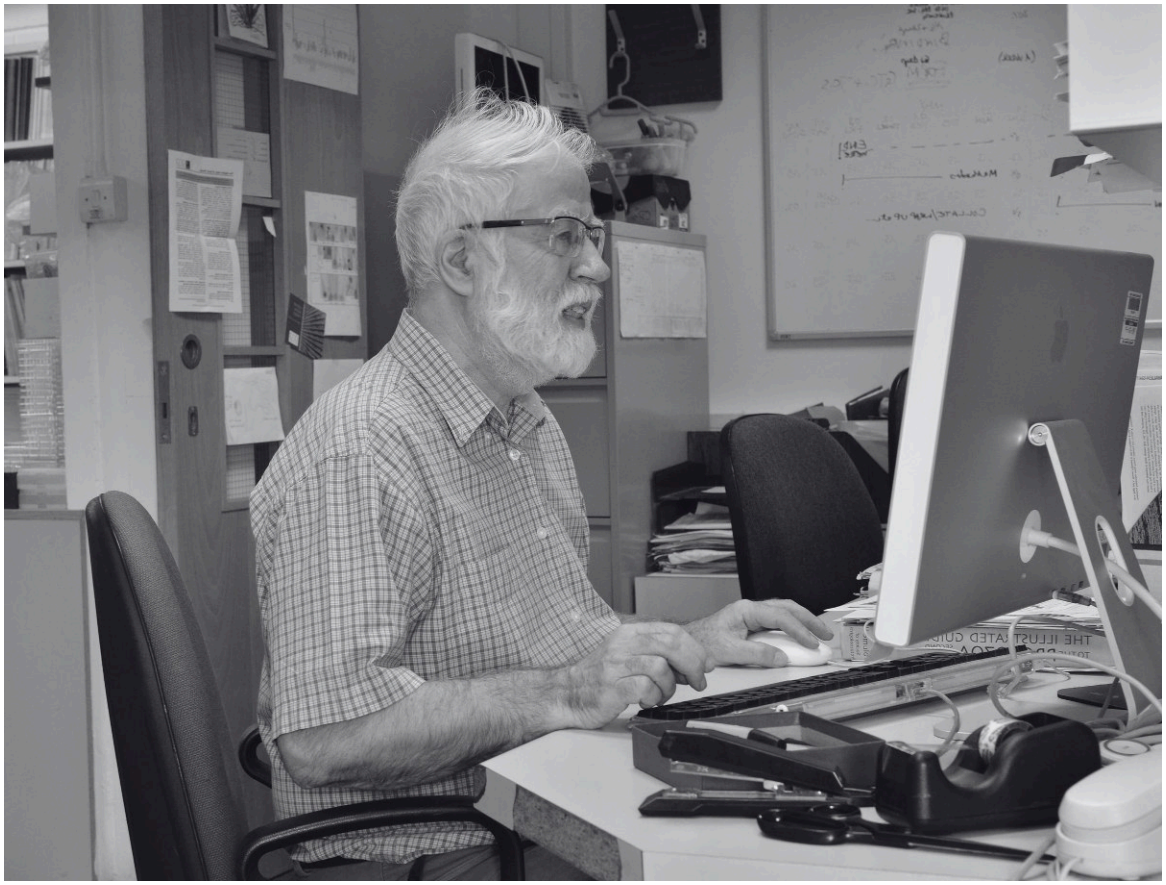
Skeletal elements of *Acanthocystis nichollsi* (Haptista, Centroplasthelida); transmission electron microscopy. Micrograph by Vasily Zlatogursky.



Ozanamia symna (Haptista: Centroplasthelida); differential interference contrast. Photo by Vasily Zlatogursky.

What sparked your interest in researching heliozoans, and what aspects of these fascinating organisms drew you to them?

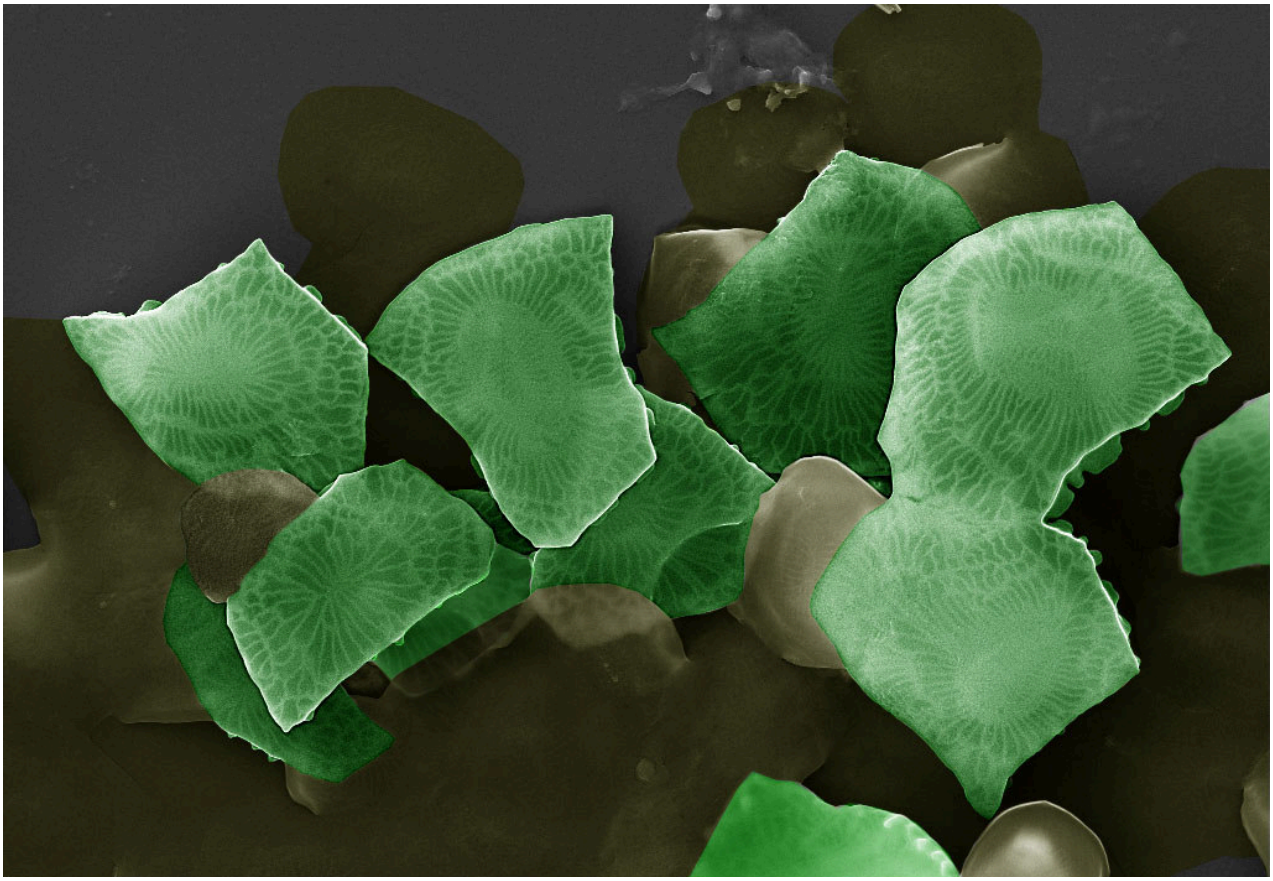
I always wanted to study insects because I was fascinated by the beauty of their shapes and colors. However, when I entered university, I was also eager to join the Department of Invertebrate Zoology because I liked the team of people working there. Heliozoans became a perfect compromise—just as beautiful as insects, but something I could study in my favorite department. Since then, my passion has been to find, discover, and describe these stunning organisms and to share their beauty with the world by publishing and showcasing their portraits, sometimes depicting complex and beautiful shapes nobody could see.



Thomas Cavalier-Smith (1941—2021)

At the start of your career, was there a particular researcher in the field of heliozoans whose work you admired or who influenced your approach to research?

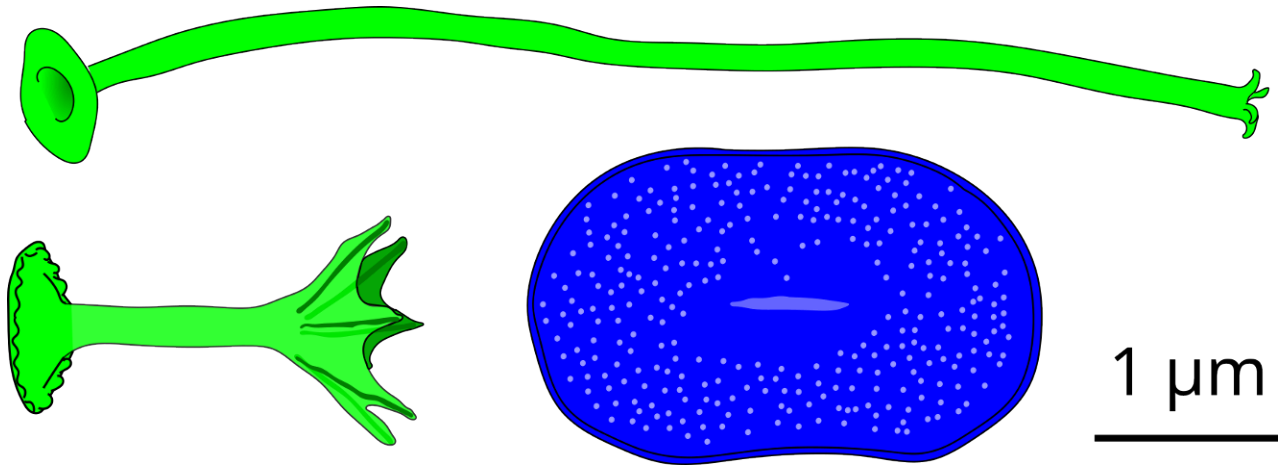
My scientific crush has always been Professor Thomas Cavalier-Smith. I was drawn to his brilliant mind, surprising and sometimes controversial ideas, his deep knowledge of protist diversity, and the speed with which he accepted and interpreted new information. I consider myself lucky to have had the opportunity to discuss some of his and my own ideas with him at conferences, and I will always regret that we never published together as co-authors. That was the plan at some point, but it never materialized due to various obstacles.



Cyst scales of *Raphidiophrys heterophryioidea* (Haptista: Centroplasthelida); scanning electron microscopy, digital colors. Micrograph by Vasily Zlatogursky.

Your research primarily focuses on centrohelids, the largest group of heliozoans. What distinguishes this group from other heliozoans, and what makes them particularly interesting to study?

Yes, centrohelids are my favorite eukaryotic lineage. Apart from their remarkable beauty, I find them fascinating from a biological perspective. Their cells are covered with siliceous scales, which come in a variety of highly complex shapes. Many people are familiar with the siliceous shells (frustules) of diatoms, but imagine a single cell covered with thousands of nano-sized "diatom shells"—that would be a typical centrohelid. Nobody knows the exact function of these scales. Some researchers believe they serve as a defense against predators, while others suggest they help the cell adhere to the substrate. However, neither hypothesis fully explains their intricate structure. My view is that this can be explained by neutral evolution—the increase in complexity that is not driven



Spine scales (green) and plate scales (blue) of *Acanthocystis crispus* (Haptista: Centroplasthelida). Schematic drawings by Vasily Zlatogursky.

by selective pressure, but rather by random fluctuations, positive feedback loops, and, who knows, perhaps even the fundamental laws of chemistry and physics. After all, these scales are essentially giant crystals, and crystals are naturally capable of self-assembly, forming complex shapes. At the same time, we cannot rule out the possibility that there is some selective advantage to the shape of the scales that we have not yet identified. Centrohelids are truly wizards of biomineralized scale formation, and the same species can alter its morphology, switching between different scale shapes. For example, they can form special types of scale morphologies for different purposes, such as forming a wall in a dormant stage or building an adhesive stalk. So, the shape might actually play a yet unrecognized role, which I find to be an intriguing mystery!

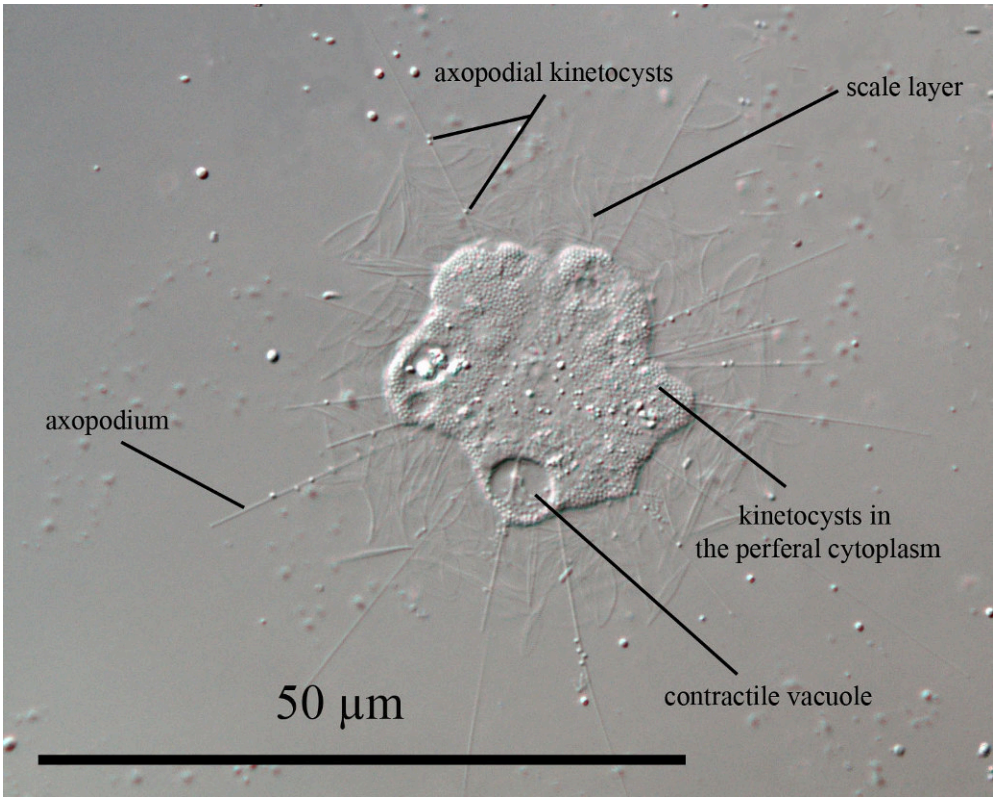
> THE SHAPE OF CENTROHELID SCALES MIGHT ACTUALLY PLAY SOME YET UNRECOGNIZED ROLE, WHICH I FIND TO BE AN INTRIGUING MYSTERY!

Concentrating samples in the laboratory. Photo by Vasily Zlatogursky.

Where are heliozoans typically found, and what methods do you use to collect them for your research?

You can find them literally everywhere—in freshwater, brackish water, marine habitats, and soil. Some can even be found in extreme environments, such as highly acidic habitats. I have rarely encountered a water sample that did not contain any heliozoans; if you look carefully enough, they are always present. However, due to their small size and lack of active movement, they are often overlooked. There is no point in searching fixed samples, as a cell with retracted axopodia is very hard to distinguish from tiny spherical non-living particles.

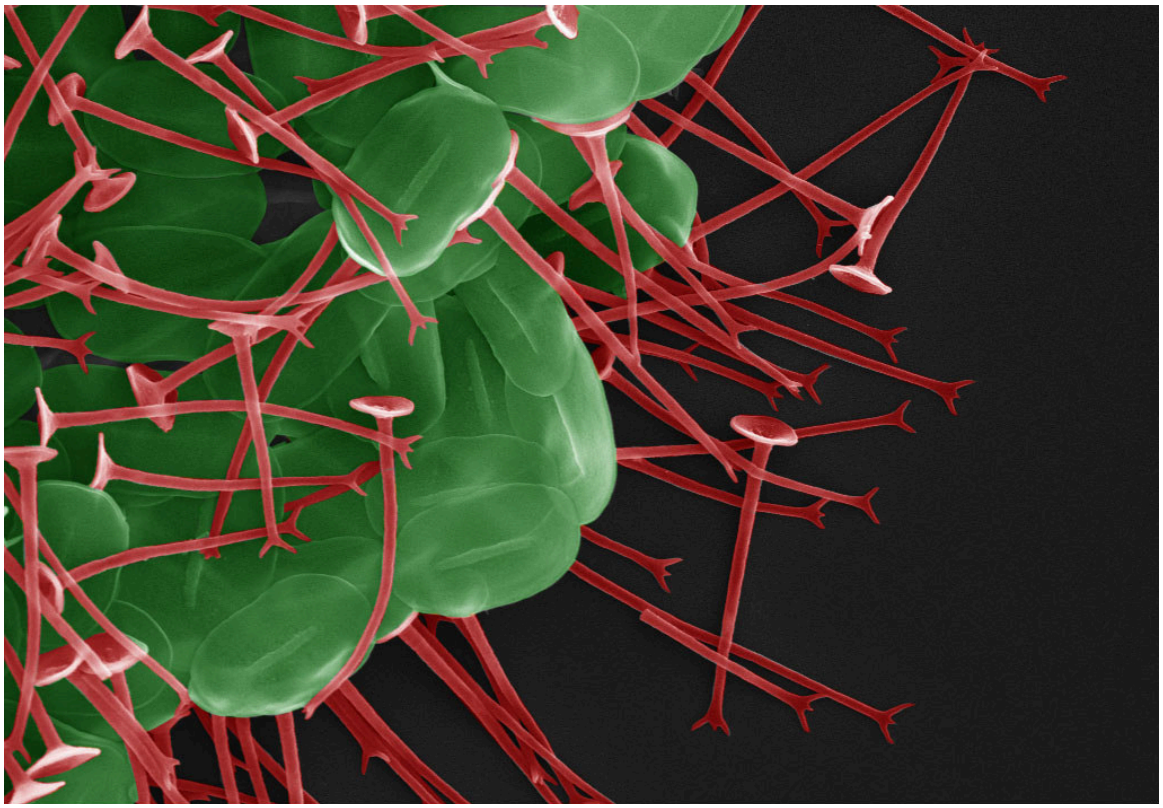
To find heliozoans, you essentially need a fresh water-containing sample. However, heliozoans are top predators on the microscopic scale, so their abundance is rarely high. Think of the number of lions compared to zebras in the savanna. To increase your chances of finding them, you either need to concentrate the sample—by collecting it with a plankton net (although this can often damage fragile cells)—or retain it on a filter membrane, or incubate the sample with nutrients to allow potential prey organisms to multiply.



Raphidocystis ambigua (Haptista: Centroplasthelida); differential interference contrast. Note contractile vacuole, which helps cells to survive in freshwater environment. Photo by Vasily Zlatogursky.

What is the relationship between heliozoans and the salinity of the water they inhabit? Are they sensitive to changes in salinity?

Heliozoans can be found in a broad variety of salinities and in freshwater. Kirill Mikrjukov, a prominent researcher on heliozoans, had an extravagant theory: that in centrohelids, the same species inhabit both marine and freshwater habitats, and that centrohelids are therefore globally distributed, not restricted by any salinity barriers. I have always been skeptical about this idea and thought that it could be explained by the tendency to "lump" many sometimes very dissimilar findings under one and the same species name, which was very typical of Mikrjukov. But recently, in our metabarcoding study led by Elena Gerasimova, we found that there are indeed centrohelids with a very broad salinity tolerance—the same species can thrive in almost freshwater conditions and at salinities as high as twice the oceanic level. This indicates that Mikrjukov may have been right, at least about some centrohelid lineages, but of course, future studies—especially those involving the isolation of actual cells rather than just DNA—are necessary.

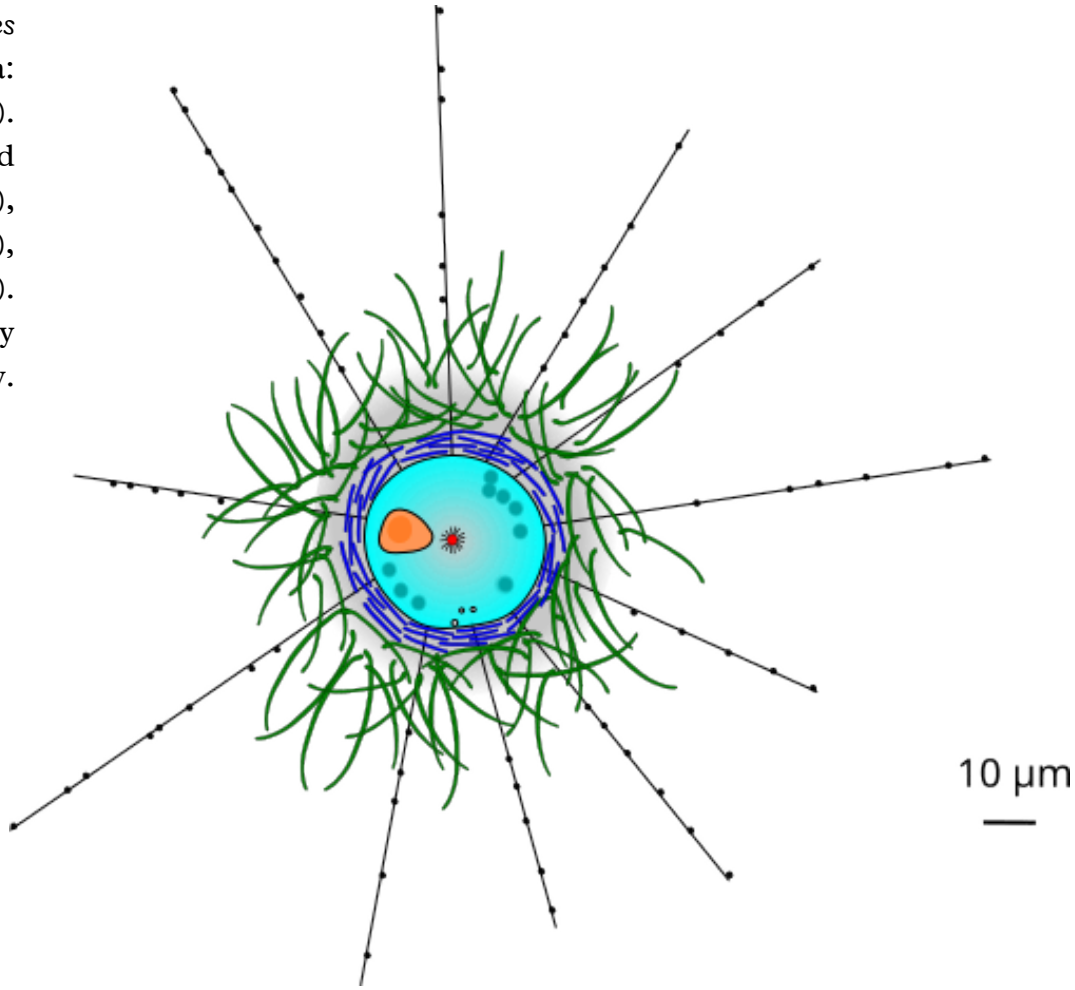


Acanthocystis crescenta (Haptista: Centroplasthelida); scanning electron microscopy, digital colors. Plate scales shown in green, spine scales—in red. Micrograph by Vasily Zlatogursky.

Are the skeletal elements of centrohelids species-specific, and how do they contribute to the classification and taxonomy of these organisms?

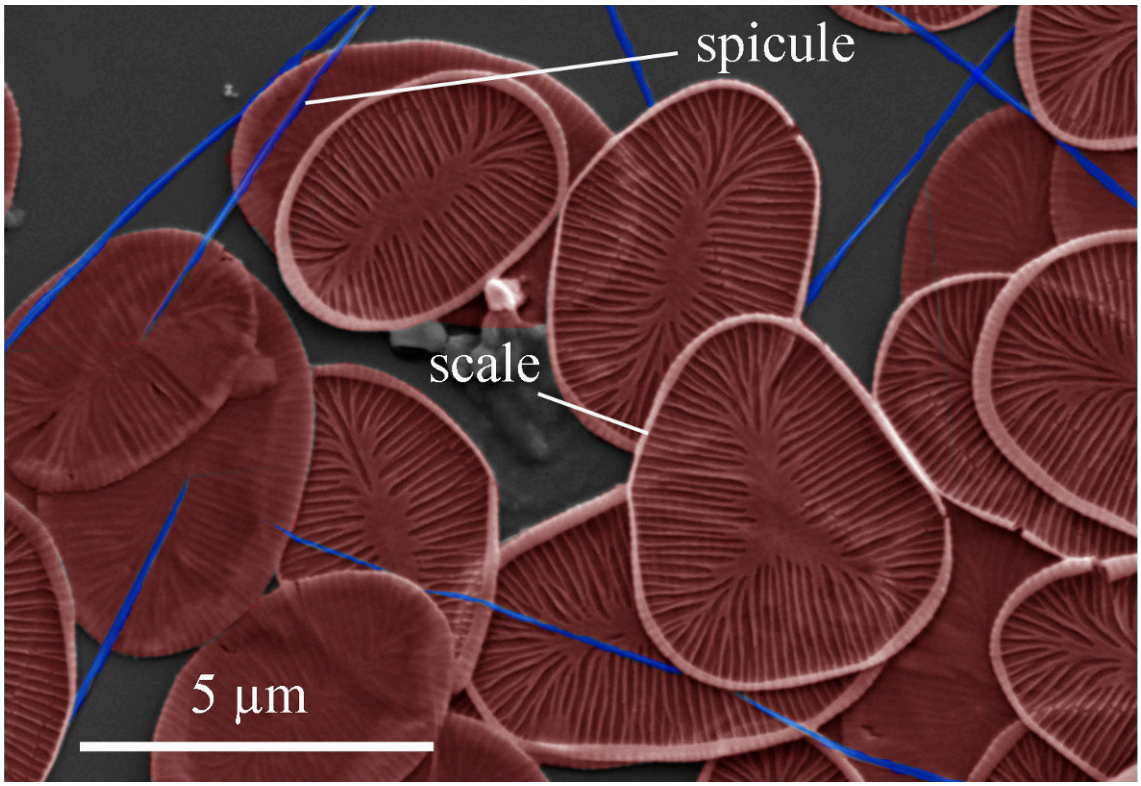
Yes, this is a great question, and I have thought about it a lot. Species identification and description in centrohelids are based mostly on scale morphology, which makes a lot of sense because these are the most character-rich structures. This might be one of the reasons why many more centrohelid species have been described compared to other heliozoans. But centrohelids are agamous organisms; no sexual process has been described for this lineage, and

Agamous organisms *are organisms that reproduce without the involvement of sexual processes or the fusion of gametes (reproductive cells).*



Ricksol blepharistes (Haptista: Centroplasthelida). Spine scales (green) and plate scales (blue), nucleus (orange), centrosome (red). Schematic drawing by Vasily Zlatogursky.

the biological species concept is barely applicable. This raises the question of where to draw the line between species: how different must the scales be to deserve a separate species name? For now, the accumulating molecular data adds a new layer to the problem, as we also need to decide on a sequence similarity threshold—where to draw the species line. And what we badly need is parallel data, where the same strains are studied both morphologically and using molecular methods. As I mentioned above, there are taxonomists—"lumpers"—who tend to unify many similar forms under a single species name. I am the opposite—a "splitter"—and I try to carefully distinguish between morphological features as long as they are consistently observed. In one study, I was able to compare sequences within and between species of the genus *Acanthocystis*, which I recognize morphologically. To my surprise, despite my tendency to "split" species, there was a lot of genetic diversity and heterogeneity within a morphological "species", suggesting the presence of cryptic species not recognized morphologically. This supports the idea that "splitting" is the right approach for improving taxonomy.

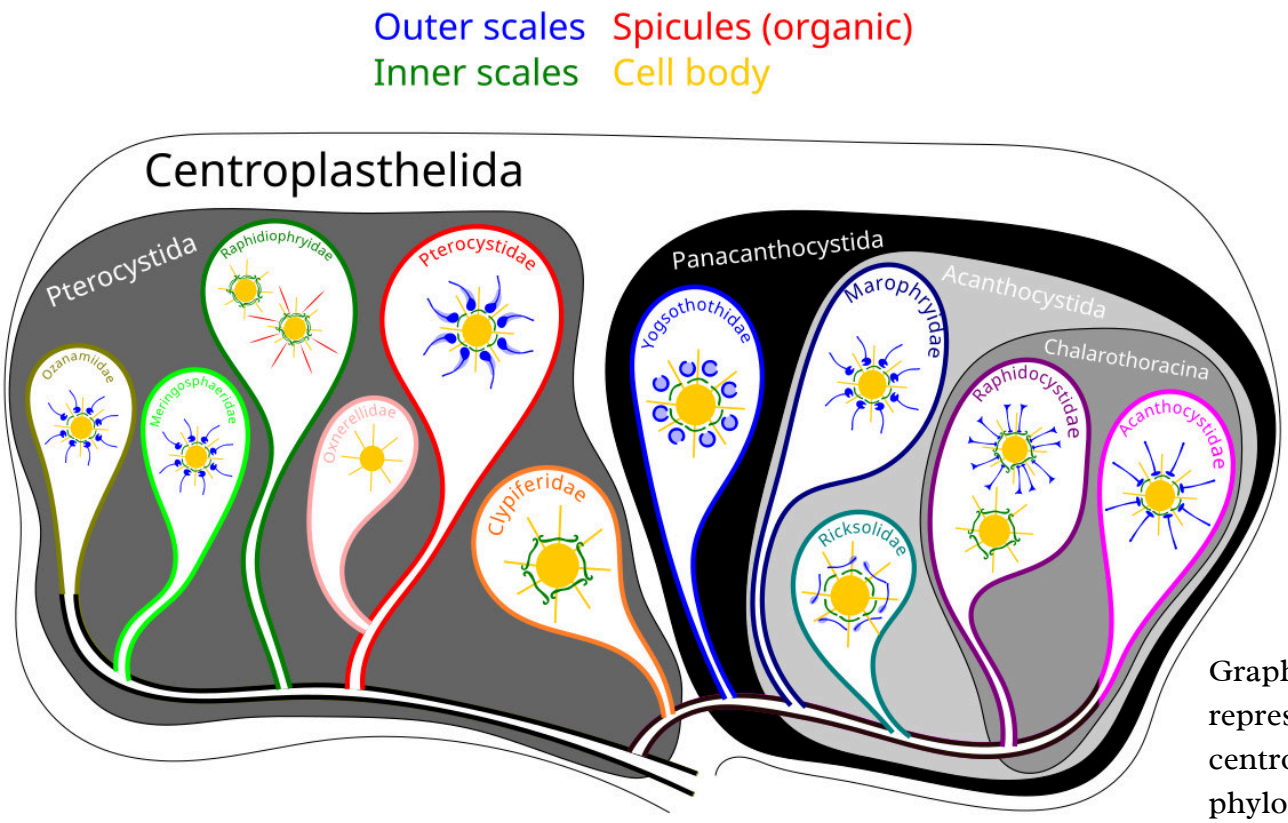


Raphidiophrys heterophryoidea (Haptista: Centroplasthelida); scanning electron microscopy, digital colors. Micrograph by Vasily Zlatogursky.

Based on the molecular sequences you've obtained, you proposed an interpretation of the evolution of cell coverings. Could you share your insights on how the skeletal elements on the surface of centrohelid cells evolved?

Actually, if you want an honest answer, we still don't know how centrohelid scales evolved. Back in 2007, when Thomas Cavalier-Smith and Sophie von der Heyden published the first molecular phylogenetic analysis of scale evolution in centrohelids, the picture seemed nice, logical, and comprehensive. The scale morphology was being mapped onto the tree topology quite nicely. Sometimes, I feel that I've spent my whole career gradually disrupting this nice initial picture. The more data we obtained, the less logical the picture became.

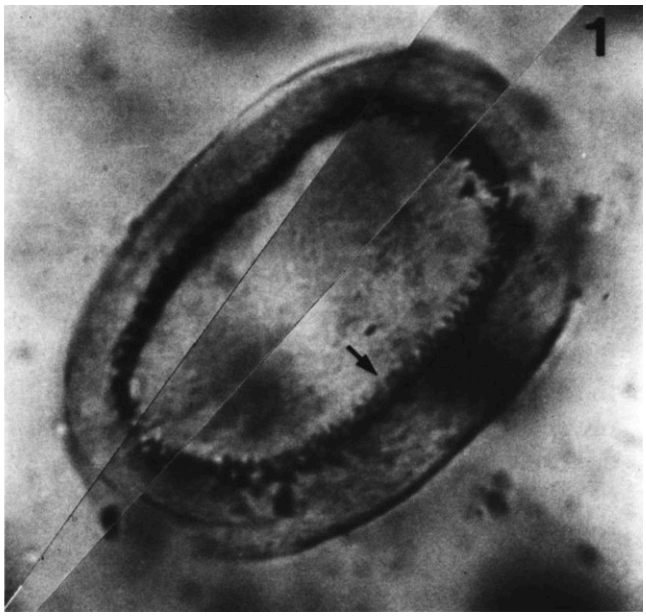
Tree topology *refers to the branching pattern of a phylogenetic tree that illustrates the evolutionary relationships between different species or groups. It shows how species or lineages are related through common ancestors, with each branch representing a lineage and each node representing a common ancestor.*

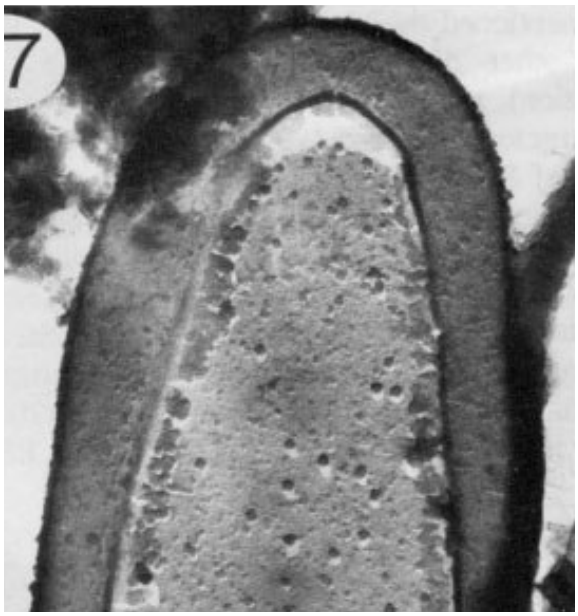


Graphical representation of centrohelid phylogeny. Modified from Gerasimova et al. (2023), doi: 10.1111/jeu.12992

Multiple parallel emergences of similar characters, secondary loss of characters in derived clades, and surprising relationships between dissimilar morphotypes—we discovered many such examples. Partly, I think this might be due to the low resolution power of the small subunit ribosomal RNA gene, which is mainly used to reconstruct centrohelid phylogeny. The deepest branches of the tree just lack support. A study with phylogenomics (including hundreds of genes, not just one) is necessary for a representative set of centrohelid species to improve tree resolution. But I would be surprised if some of the conflicts between morphology and phylogenetic relationships didn't persist in a well-resolved tree: evolution doesn't owe us any logic or nice patterns and can take peculiar and counterintuitive paths. Future studies will show...

Low resolution power *refers to the limited ability of a particular dataset, method, or analysis to clearly distinguish or resolve the evolutionary relationships between different species or groups.*



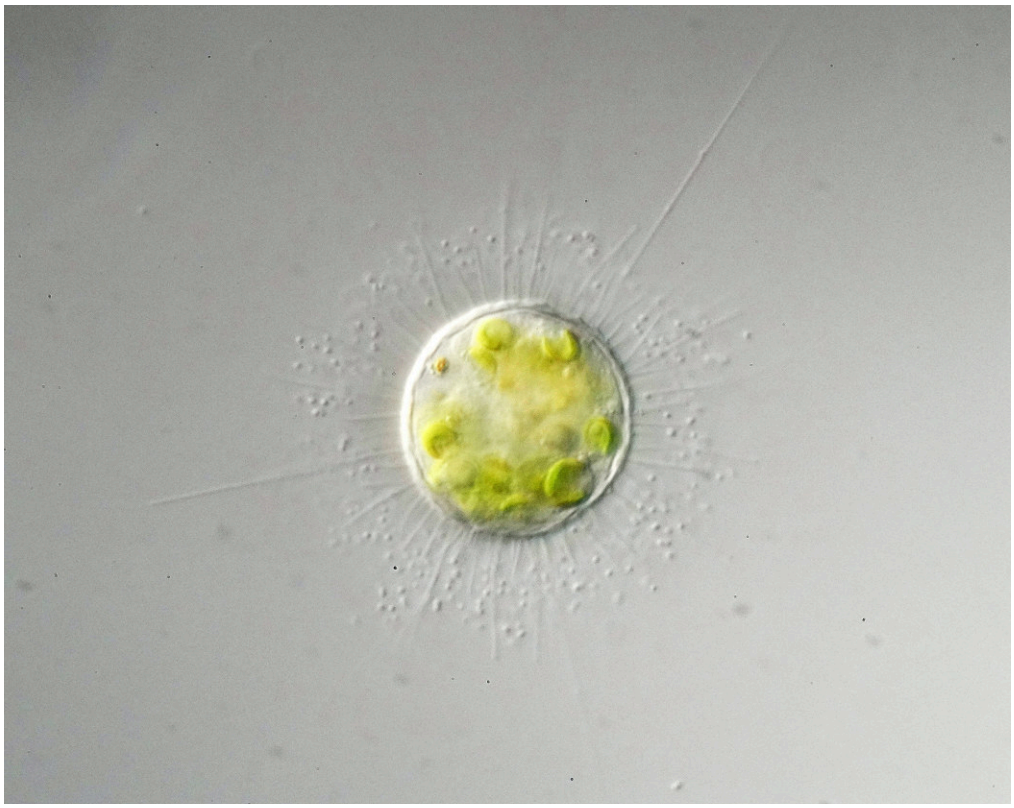


Left: Cambrian microfossil known as *Bicorniculum* from Allison and Hilgert (1986), doi: 10.1017/S0022336000022538; *Right:* Plate scales of *Raphidocystis marginata* (Haptista: Centroplasthelida) from Nicholls and Düerrschmidt (1985), doi: 10.1139/z85-288

Given that most heliozoans have skeletal elements, it's likely that some of them are preserved as fossils. What can we learn from the fossil record about ancient heliozoans, and what discoveries have been made so far?

Yes, absolutely a fair question, and strangely, the fossil record for centrohelids is almost entirely absent. As estimated by molecular clock analysis, centrohelids must be one of the most ancient eukaryotic lineages, having split from a common ancestor with haptophytes well before the Cambrian explosion, as early as 1600–1900 million years ago. At the same time, the unambiguous findings of fossil centrohelid scales I am aware of are almost modern—dating from less than a million years ago. There are many much older scale nannofossils. When I look at some of them,

***Molecular clock** is a method used in molecular biology and genetics to estimate the time of divergence between two species or groups of organisms based on the rate at which genetic mutations accumulate over time.*



Acanthocystis cf. *penardi* (Haptista: Centroplasthelida); differential interference contrast. Note multiple *Chlorella* symbionts. Photo by Vasily Zlatogursky.

they are reminiscent of centrohelid scales, even though they are not usually recognized as such. For example, scale microfossils from the early Cambrian, known as *Bicorniculum*, are very similar to extant *Raphidocystis*, but these are not particularly complex scales, and one can find similar shapes in extant chrysophytes. This is hard to know for sure. I believe that the centrohelid fossil record does exist, but it has not been studied enough.

We need more collaboration between micropaleontologists and protistologists, like myself. If anyone working on microfossils is reading this, feel free to contact me—I would love to chat with someone about the potential of finding fossil centrohelids. They can be searched for both in actual rocks and, in fact, in the literature as well. Many may have already been described, but simply not identified as centrohelids.

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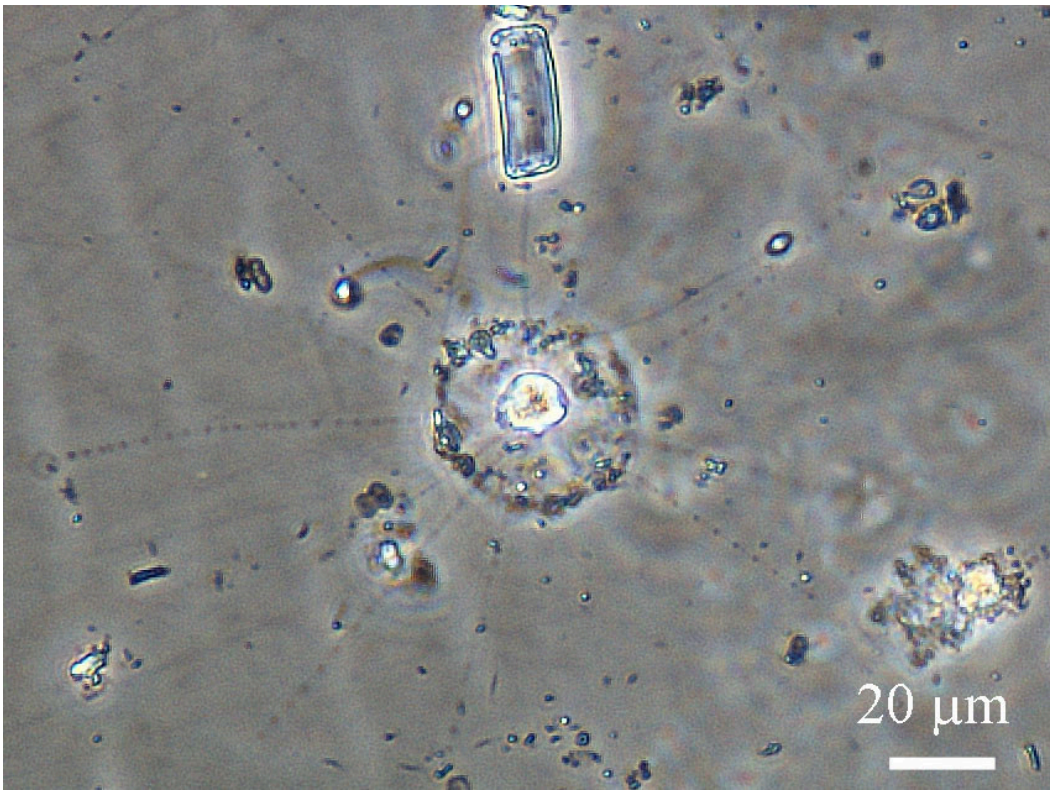
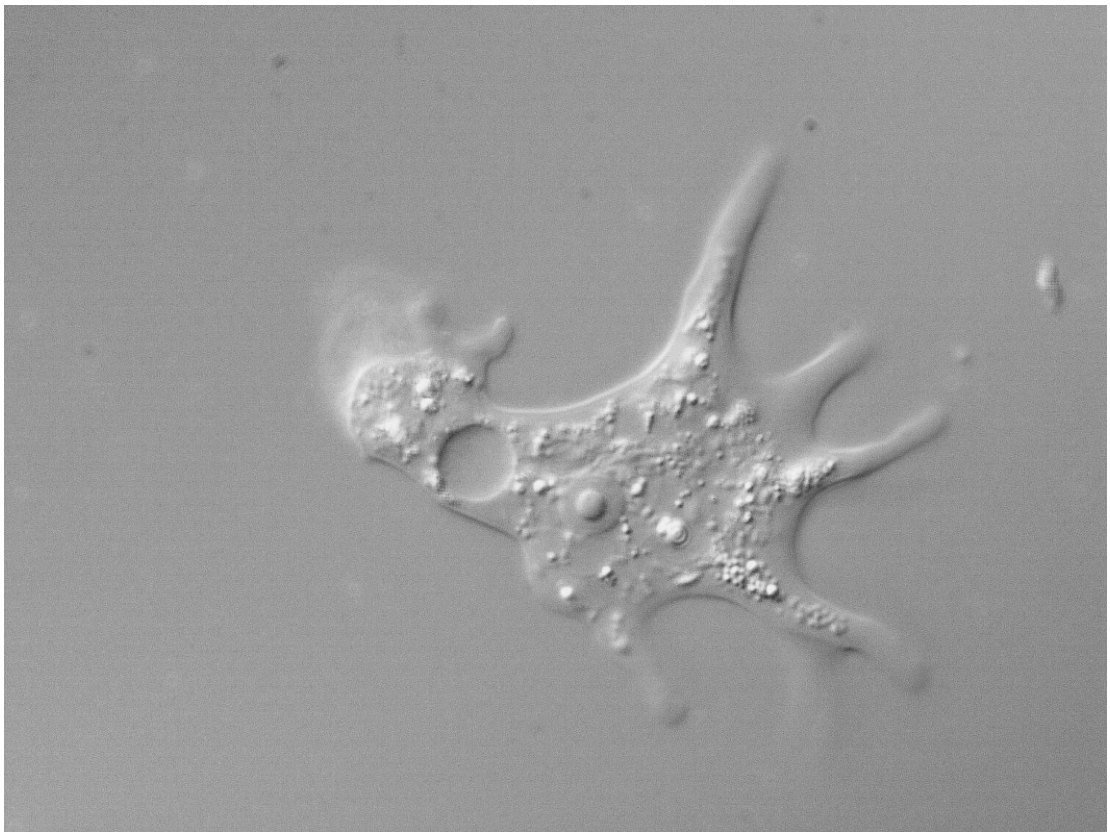


An undetermined centrohelid without scales, but with mucous sheath instead; phase contrast. Photo by Vasily Zlatogursky.

Species identification and taxonomy of centrohelids are typically based on the shape and structure of their scales. However, there are also species without scales. What characteristics can be used to distinguish scale-less centrohelid species from one another?

Unfortunately, at the current state of knowledge, none have been identified. This is problematic not only for naked species but also for forms with purely organic skeleton elements, known as spicules. These forms were once recognized as members of a single genus, *Heterophrys*, but now we see that such forms are scattered throughout the phylogenetic tree and may be entirely unrelated. The naked lineage, *Oxnerella*, is currently the only one identified, and the same goes for the mucous-coated *Chlamydaster*. However, it may only be a matter of time before other similar, unrelated forms are discovered for these two as well. For such forms, molecular methods currently remain the only viable solution for identification.



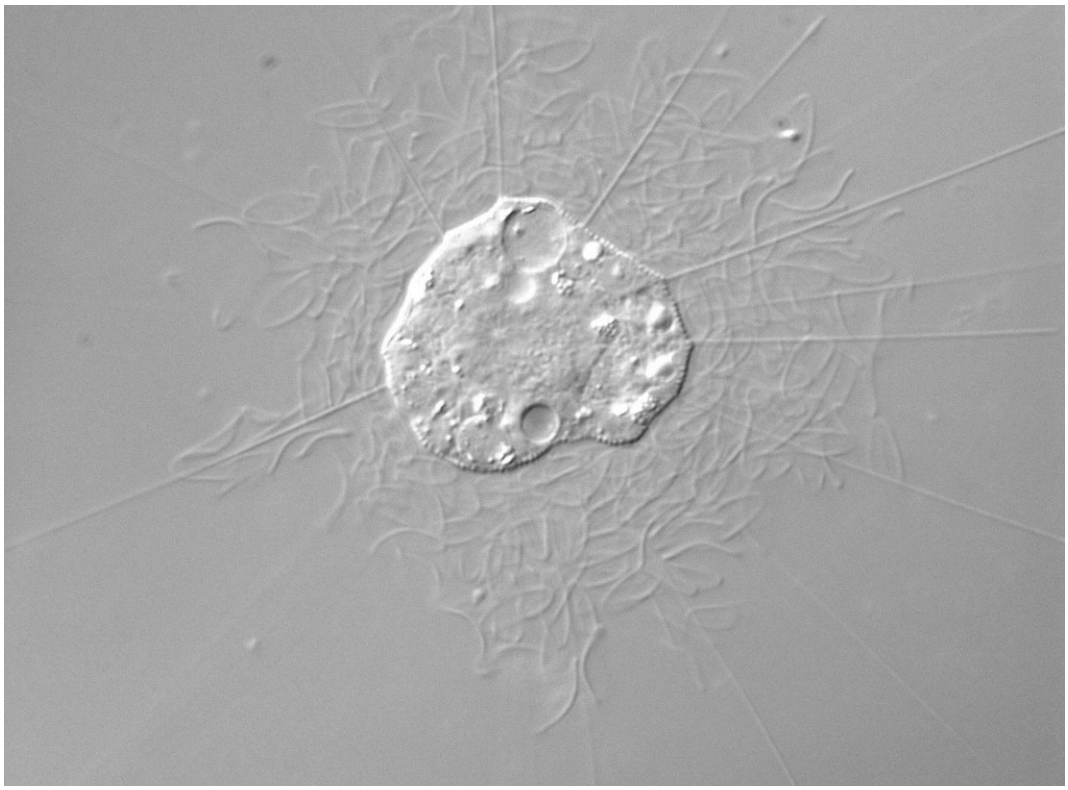
Korotnevella sp. (Amoebozoa: Dactylopodida); differential interference contrast. Photo by Vasily Zlatogursky.

Besides heliozoans, you have also studied naked amoebae, such as those from the genus *Korotnevella*. Species in this genus are known for their cell surfaces covered with complex, boat-like scales, which are believed to be species-specific and help with clear species identification. However, your research has shown different findings. Could you elaborate on your findings and how they challenge this conventional view?

The situation with *Korotnevella* was similar to what was described above for centrohelids: each morphological species was genetically heterogeneous, potentially representing a number of cryptic species. But just as with centrohelids, in dactylopodids the diversity is far from fully revealed, and generalizations are difficult due to the so-called "taxonomic impediment"—the known species are just the tip of the iceberg of undescribed diversity, which makes any final conclusions premature.

Cryptic species *refers to a group of organisms that are morphologically similar or identical but belong to different species, often distinguishable only through genetic differences. These species are typically identified through molecular analysis rather than traditional taxonomy.*

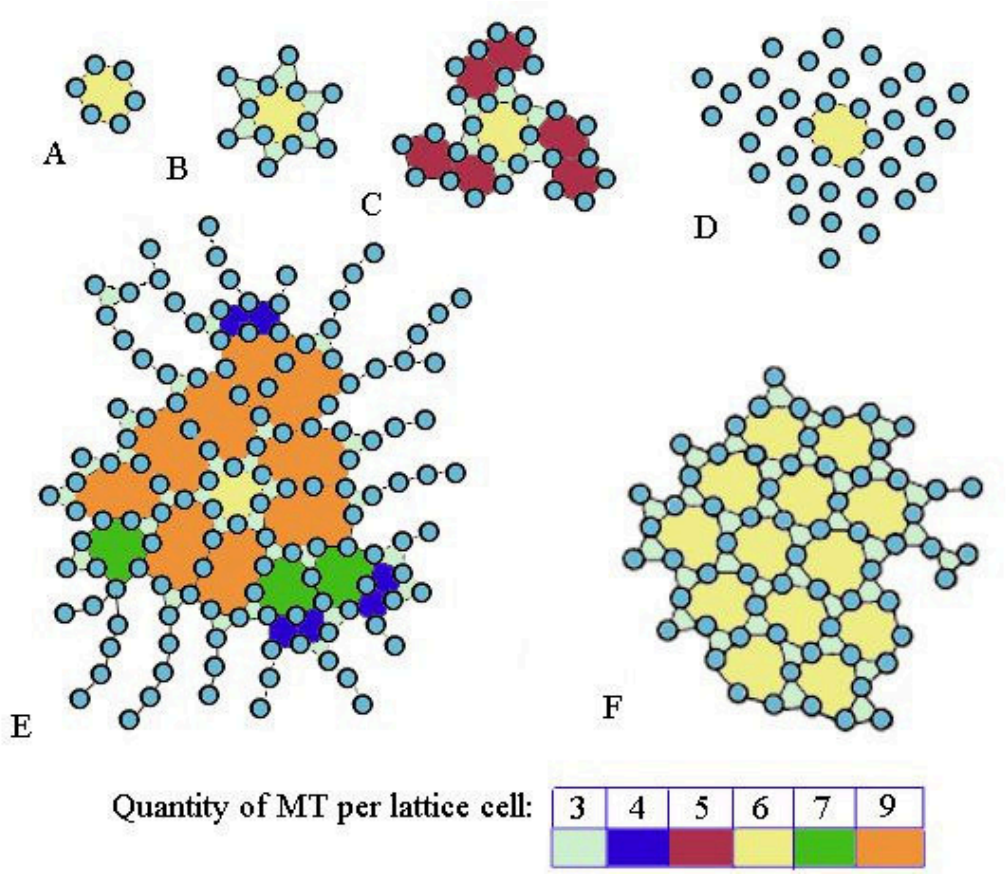
Raphidocystis symmetrica (Haptista: Centroplasthelida); differential interference contrast. Note axopdia. Photo by Vasily Zlatogursky.



One of the most distinctive features of centrohelids, visible under a light microscope, is the presence of axopodia. Could you describe the structure of these cell extensions and their function in the organism?

Axopodia are straight, non-branching pseudopodia, supported by a bundle of microtubules. For historical reasons, the bundle is referred to by the same term as the 9+2 structure of the eukaryotic cilium/flagellum—the axoneme. However, it does not contain doublets, dynein arms, etc. Instead, the microtubules are connected by rigid protein linkers, which provide stiffness to the entire structure. The number of linkers per microtubule is called valence—analogous to the valence of atoms in chemistry.

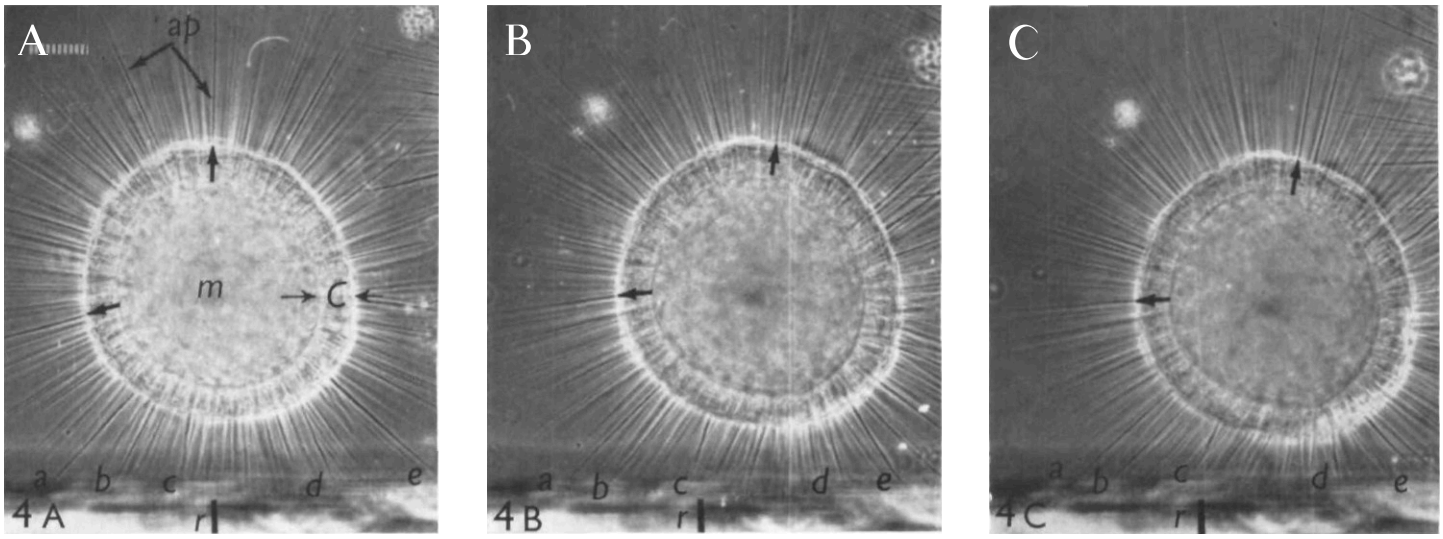
***Axoneme** is the central structure of a flagellum/cilium, consisting of a core arrangement of microtubules that extend from the base to the tip. It typically features a "9+2" microtubule arrangement, where nine pairs of microtubules form a ring around two central microtubules.*



Variations of the axonemal patterns in centrohelids, as visible on a cross-section, note microtubules and protein linkers. Schematic drawings by Vasily Zlatogursky.

The combination of microtubules of different valence can create various patterns in the arrangement of the tubules, which are especially evident in the cross-section of the axopodium. Interestingly, Lewis Tilney—one of the pioneers of eukaryotic cytoskeleton studies—predicted the theoretical possibility of some of these patterns, which were later discovered in real axopodia. Axopodia are not unique to heliozoans; another group that typically possesses them is the radiolaria. What is less known is that some ciliates and apicomplexans can also form axopodia. Centrohelids and other heliozoans typically use axopodia for prey capture; their axopodia are equipped with extrusomes—"stinging" organelles—that help kill and/or entrap the prey.

Cytoskeleton *is a complex network of protein filaments and tubules inside the cell that provides structural support, shape, and mechanical strength. It also plays a crucial role in intracellular transport and cell division.*



Record of the locomotioof *Actinosphaerium eichhorni*, at the following time intervals: A) 0 sec: B) 160 sec: C) 240 sec. From: Watters (1968), doi: 10.1242/jcs.3.2.231

Do heliozoans move actively, or are they primarily carried by water currents?

Typically, heliozoans are substratum-associated, but some can be temporarily or constantly planktonic. In the water column, they are passively floating. As for substratum-associated forms, they can attach with axopodia or use special stalks, remaining motionless or employing an active "walking" movement with the help of axopodia. In actinophryids, this walking is very slow, and as the British biologist John Kitching graphically said, "watching *Actinophrys* through the microscope is

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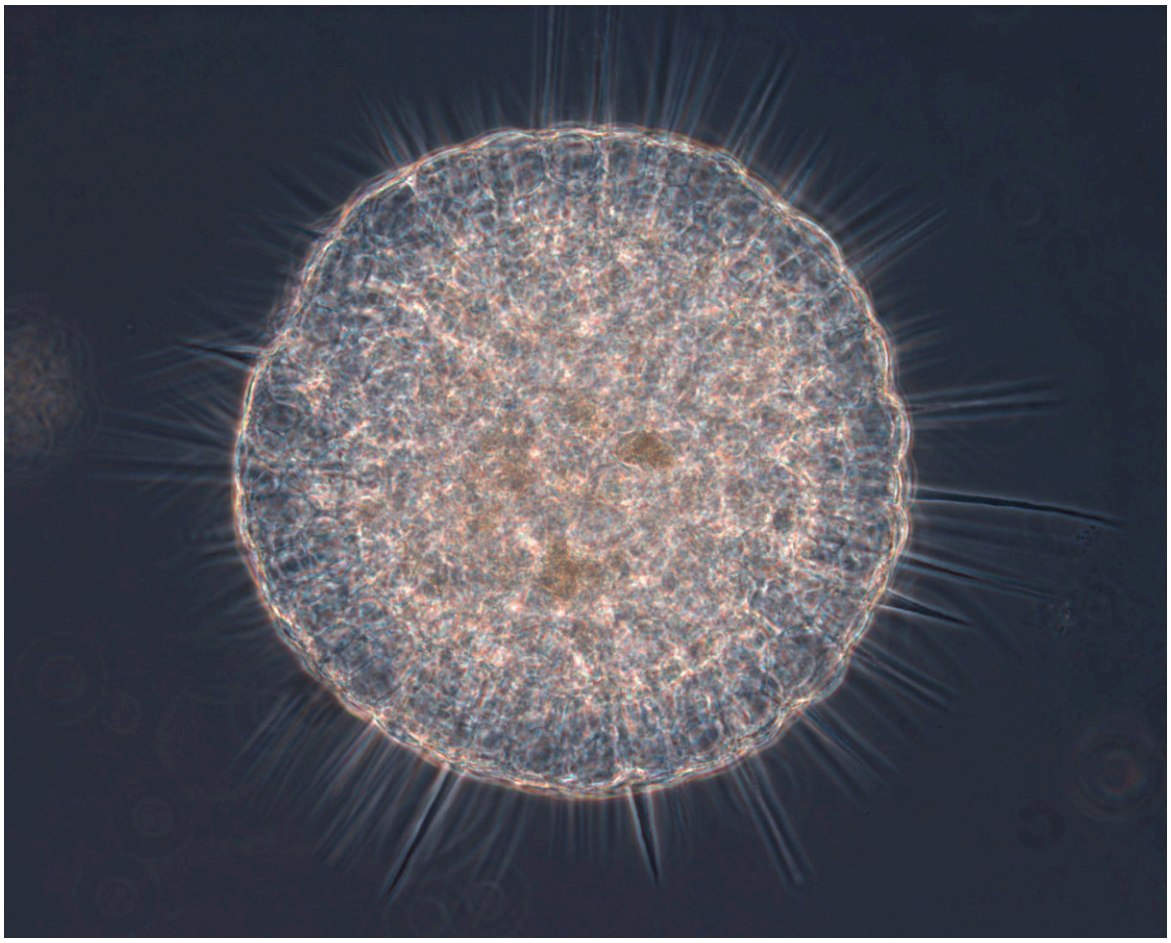
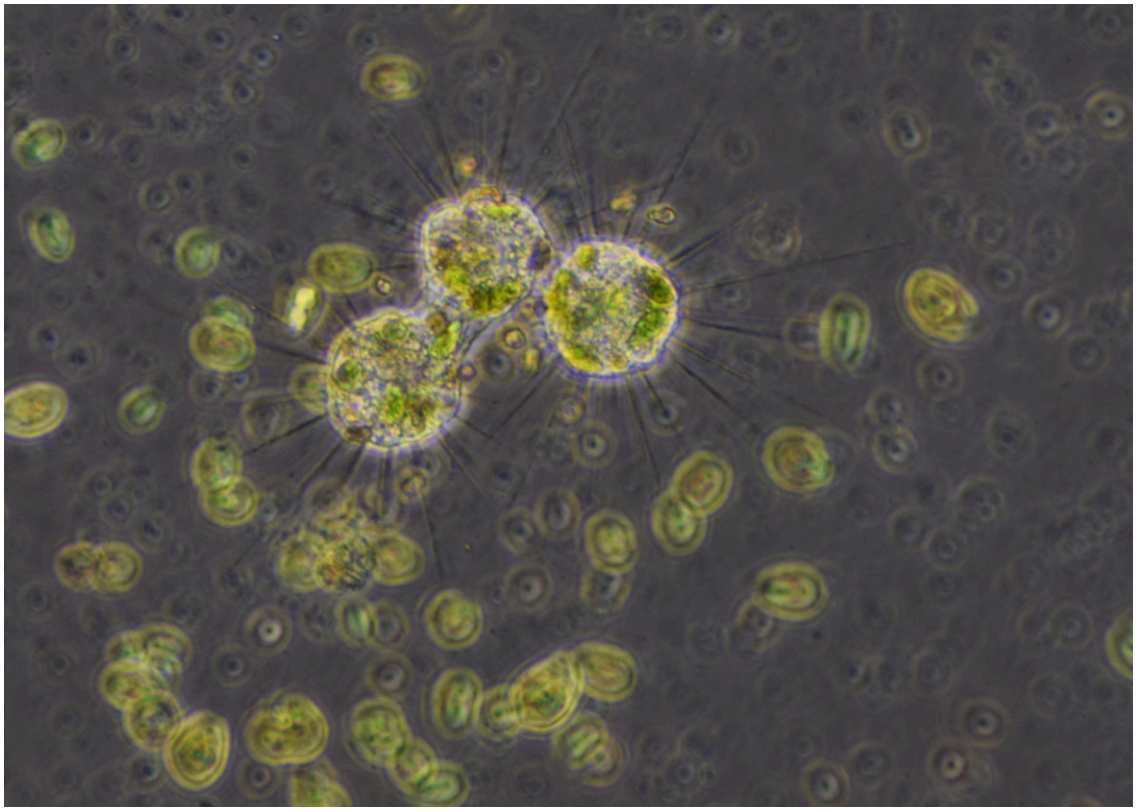


Actinosphaerium sp. (Stramenpiles, Actinophryida) phase contrast. Photo by Vasily Zlatogursky.

about as exciting as watching the minute hand of a clock". Centrohelids, such as *Acanthocystis*, can be quite fast, and their movement is easy to observe. In actinophryids, the movement mechanism is better studied and is based on the contraction of the axopodia. As these are attached to the substratum, the cell slides or rolls thanks to axopodial contraction. There are contractile tubules that run parallel to the axoneme and contract in an ATP-independent fashion, and the axoneme (which, I should remind you, is a bundle of microtubules) breaks into smaller blocks and is transported to the cytoplasm. The movement of centrohelids is less understood. *Acanthocystis* can not only contract but also actively bend its axopodia, yet we still have no idea what the molecular mechanism of this movement is.

ATP (Adenosine Triphosphate) *is a molecule that carries energy within cells. It is often referred to as the "energy currency" of the cell because it provides the necessary energy for a variety of cellular processes, such as protein synthesis and cell division.*



Actinophrys sol (Stramenopiles: Actinophryidae) feeding on *Chlorogonium* sp. (Archaeplastida: Chlorophyta); phase contrast. Note multiple algal cells entrapped in a steaky mucous around the cells of heliozoans. Photo by Vasily Zlatogursky.

What do heliozoans feed on, and what methods do they use to capture their food?

Heliozoans are primarily predators of other eukaryotes and can usually feed on a variety of prey. Surprisingly, they can sometimes feed on organisms that are considerably larger than themselves. Actinophryids achieve this by forming large peripheral vacuoles adjacent to the cell and by feeding collectively, where many individual cells form a common giant food vacuole. During this collective feeding process, the nuclei can divide, so after sharing a meal, they may find that more individuals are leaving the

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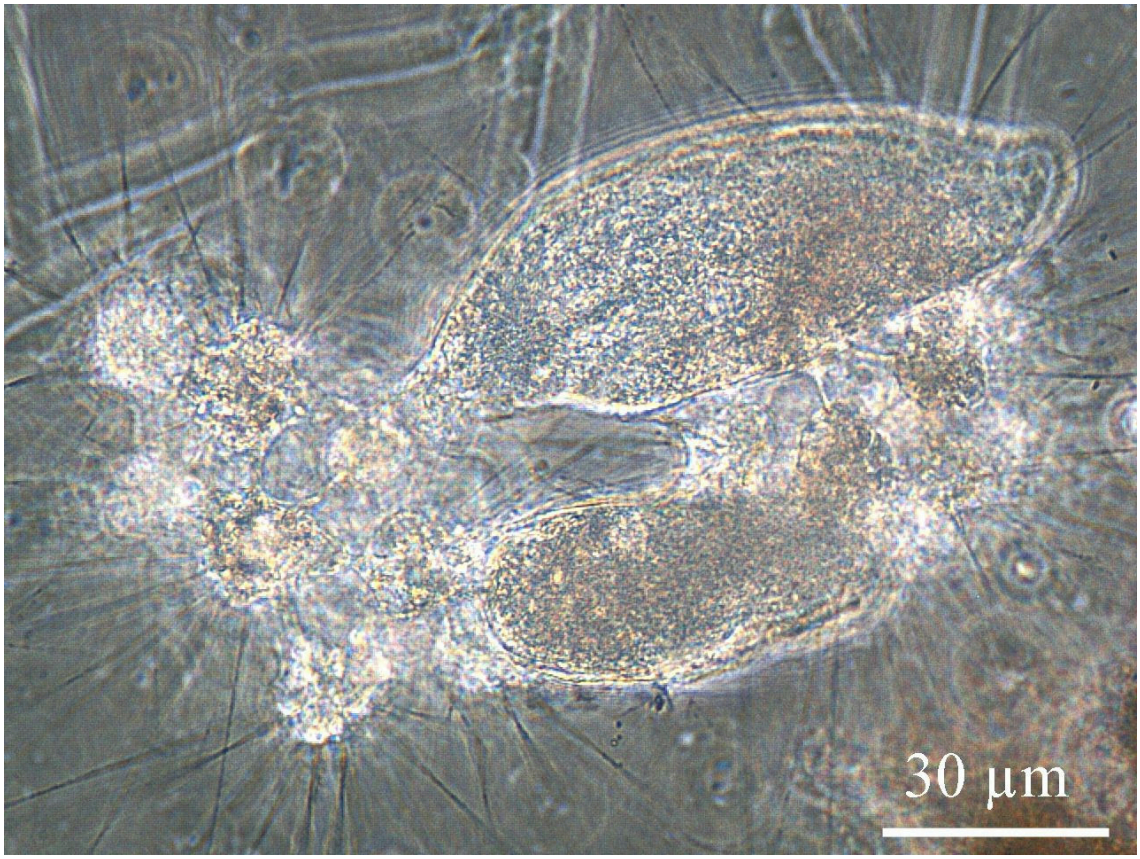
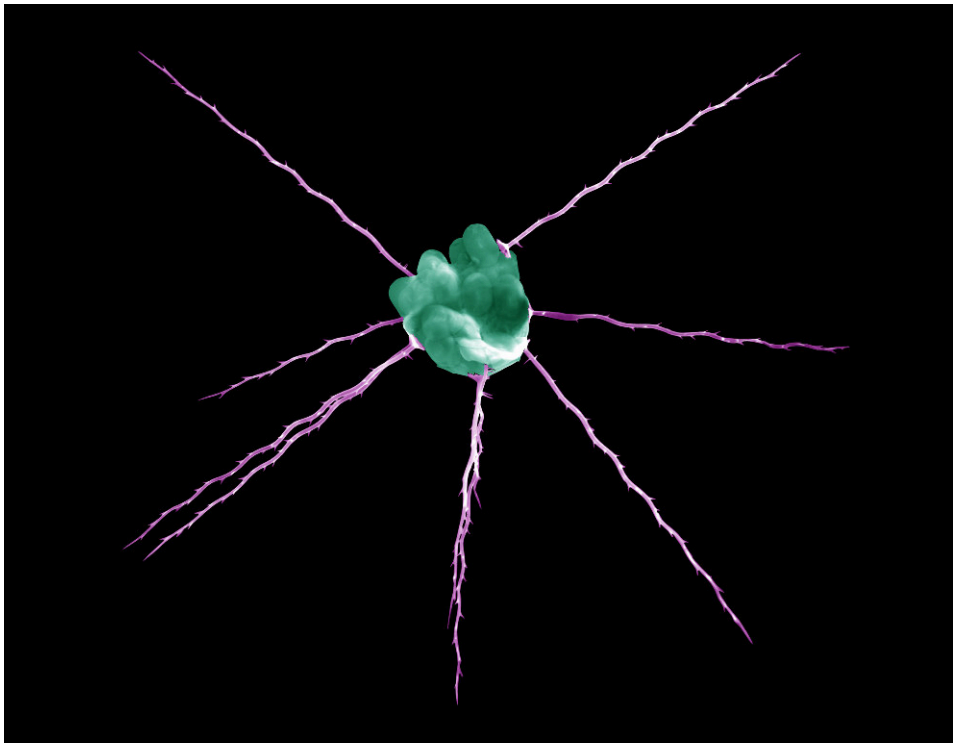


A net-like colony of *Raphidiophrys* sp. (Haptista: Centroplasthelida) is capable of catching ciliates that are much larger than the individual members of the colony. Photo by Vasily Zlatogursky.

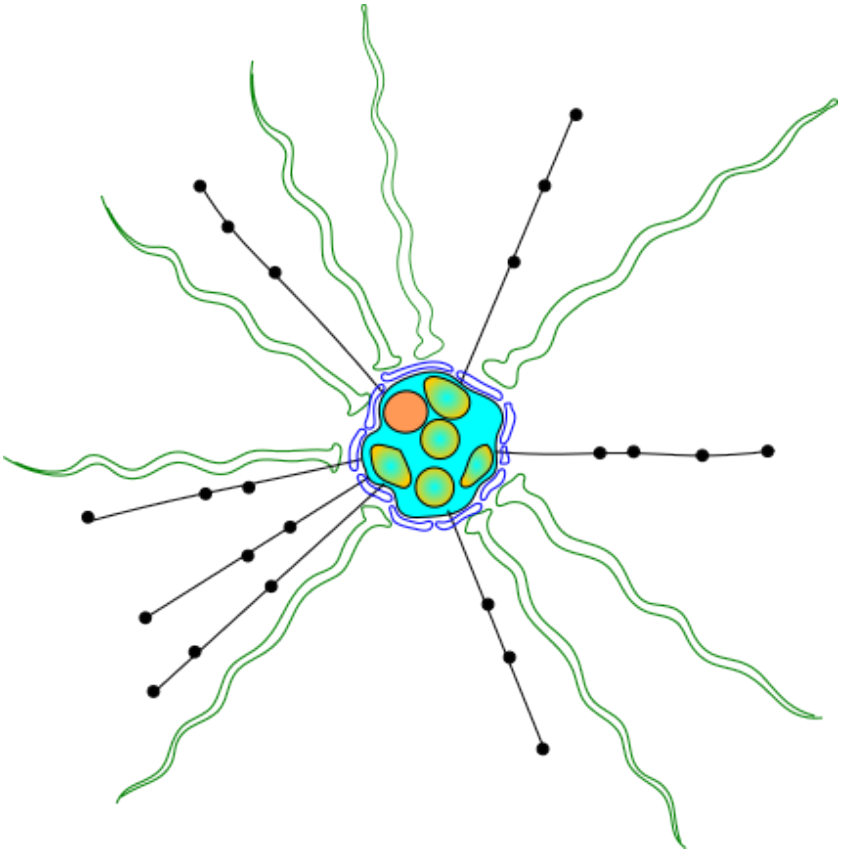
table than there were when they started eating. Using such strategies, actinophryids can sometimes consume small animals, such as rotifers and gastrotrichs, which is impressive because they are just single cells, while rotifers are multicellular, just like us. Centrohelids can also feed on organisms larger than themselves, but they achieve this by teaming up in advance to form large net-like colonies connected by cytoplasmic bridges. This is observed, for example, in the genus *Raphidiophrys*. The prey is typically entrapped using axopodia, which bear stinging extrusomes, but actinophryids are sometimes more "creative," setting up sticky traps for actively swimming cells, much like spiders do on land, or modifying the behavior of ciliates, apparently causing their cilia to beat in reverse. As a result, a ciliate trying to escape ends up moving directly toward the danger. But sometimes prey manages to escape. For example, *Chlamydomonas* can shed its flagella when entrapped by a heliozoan, much like lizards can sacrifice their tails. This is what the centrin star in the flagellum transitional zone of green algae is actually for!



Meringosphaera mediterranea (Haptista: Centroplasthelida); scanning electron microscopy, digital colors; plate scales shown in green, spine scales—in magneta. Photo by Vasily Zlatogursky.

You have studied the fascinating phenomenon of kleptoplastidic symbiosis in marine centrohelids. Could you explain what this phenomenon is, how it works, and its significance in the biology of these organisms?

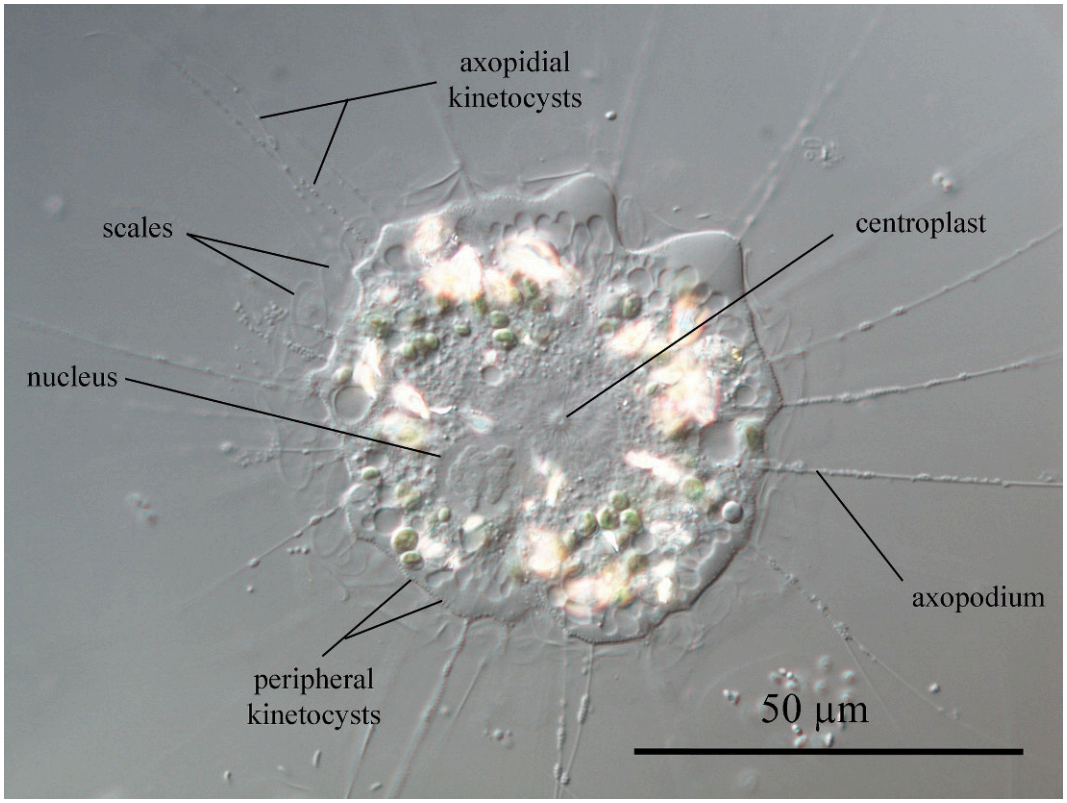
Yes, this is an amazing story, one that is still unfolding, and I am sure there are more discoveries to come soon. But, as I often have to say in this interview, we still don't actually know how it works and are just starting to scratch the surface of the topic. In short, there was an enigmatic genus of eukaryotes called *Meringosphaera*, which was described by algologists more than a hundred years ago. People debated whether it was a chrysophyte, a xanthophyte, or something else. It was only protistologists working on heliozoans, such as Monika Dürrschmidt and Naja Vørs, who noted the drastic similarity of its scales to those of centrohelids. But their remarks remained mostly unnoted, and *Meringosphaera* stayed within the realm of algae. However, when I was working in Fabien Burki's laboratory in Sweden—by the way, I'm really happy to rejoin it now—I managed to observe living cells, not fixed or dead ones, which is what algologists often deal with. And I saw that this was indeed a heliozoan; I observed prominent axopodia with extrusomes! The molecular data confirmed this conclusion.



Meringosphaera mediterranea (Haptista: Centroplasthelida); line drawing. Note chromatophores in the cytoplasm. Schematic drawing by Vasily Zlatogursky.

But the question remained: why are they green, and what are the photosynthetic bodies in its cytoplasm that had been deceiving researchers for so long? The subsequent work, led by Megan Sørensen in Fabien's lab, revealed plastid genome sequences from some unknown dictyochophytes in single-amplified genomes, which I generated. However, surprisingly, there was no signal for nuclear genes related to dictyochophytes. This led us to hypothesize that *Meringosphaera* steals plastids from some dictyochophyte algae, but this seems to be something more than just kleptoplasty. Heliozoans seem to have genes acquired from algae that help maintain the plastids alive. But again, these results are very preliminary. There are many species of *Meringosphaera* that we are just beginning to distinguish from one another. Apparently, the origins of their plastids can also differ. These cells turn out to be an important part of ocean plankton; they are found in nearly every marine environment and can be very abundant. It is hard to believe that we completely missed and misunderstood such a huge component of the system until recently!

***Kleptoplasty** is a phenomenon in which an organism temporarily "steals" and retains chloroplasts from algae or other photosynthetic organisms for its own use. These stolen chloroplasts, known as kleptoplasts, continue to perform photosynthesis inside the host organism, providing it with energy.*

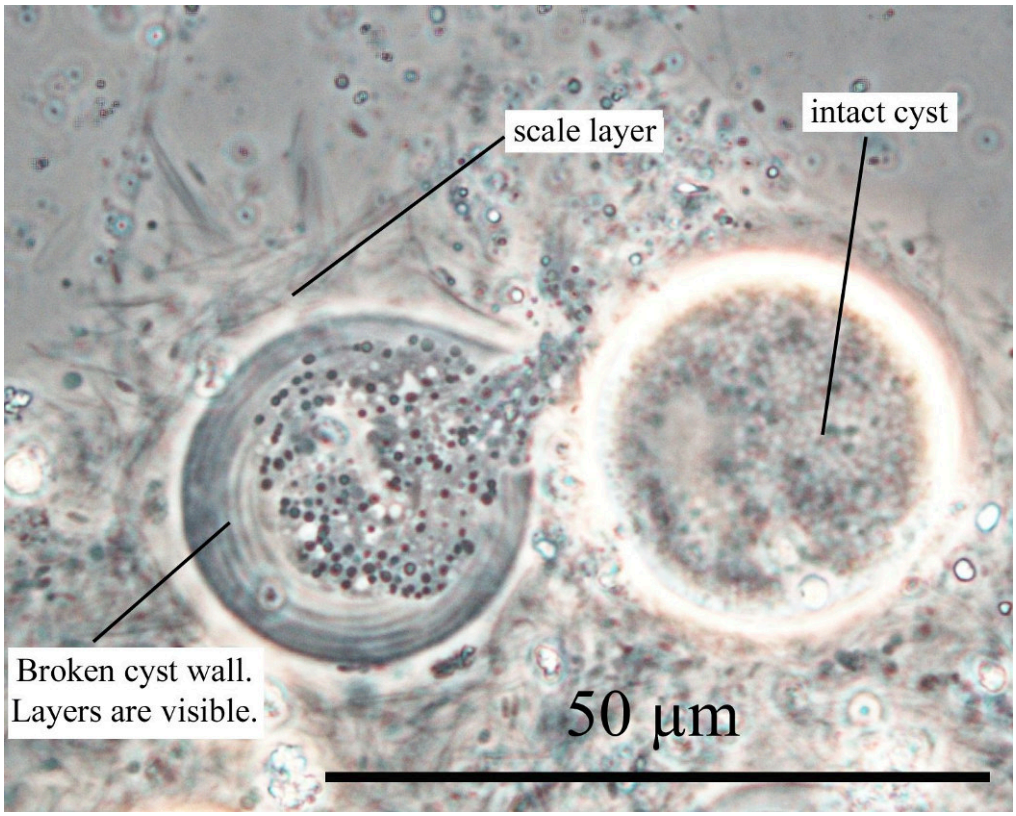


Raphidiophrys intermedia (Haptista: Centroplasthelida); differential interference contrast. Note centrosome (so called "centroplast"). Photo by Vasily Zlatogursky.

Throughout your career, you have studied the ultrastructure of heliozoan cells. One of the characteristic organelles in centrohelids is the centroplast. Could you tell as its structure and explain its role in the cell?

Following Cavalier-Smith, I am avoiding the term *centroplast* because it introduces unnecessary terminological complexity. I would prefer to call it the centrosome or the interphase microtubule-organizing center, which emphasizes that it is functionally similar to, and likely homologous with, what we also have in our cells. The mitotic (cell nucleus division) images of centrohelids and animals are very similar! But yes, the centrosome in centrohelids is organized in a quite special way, taking the form of an electron-dense disc sandwiched between two less dense hemispheres. This structure nucleates microtubules, which form the bundles of axonemes that then extend into axopodia. It occupies so much space in the center of the cell that there is no room for the nucleus, and it is pushed to a peripheral position and partly penetrated by axonemes. Centrohelids lost the ability to build a eukaryotic flagellum, but this centrosome might be a remnant of the flagellar basal body.

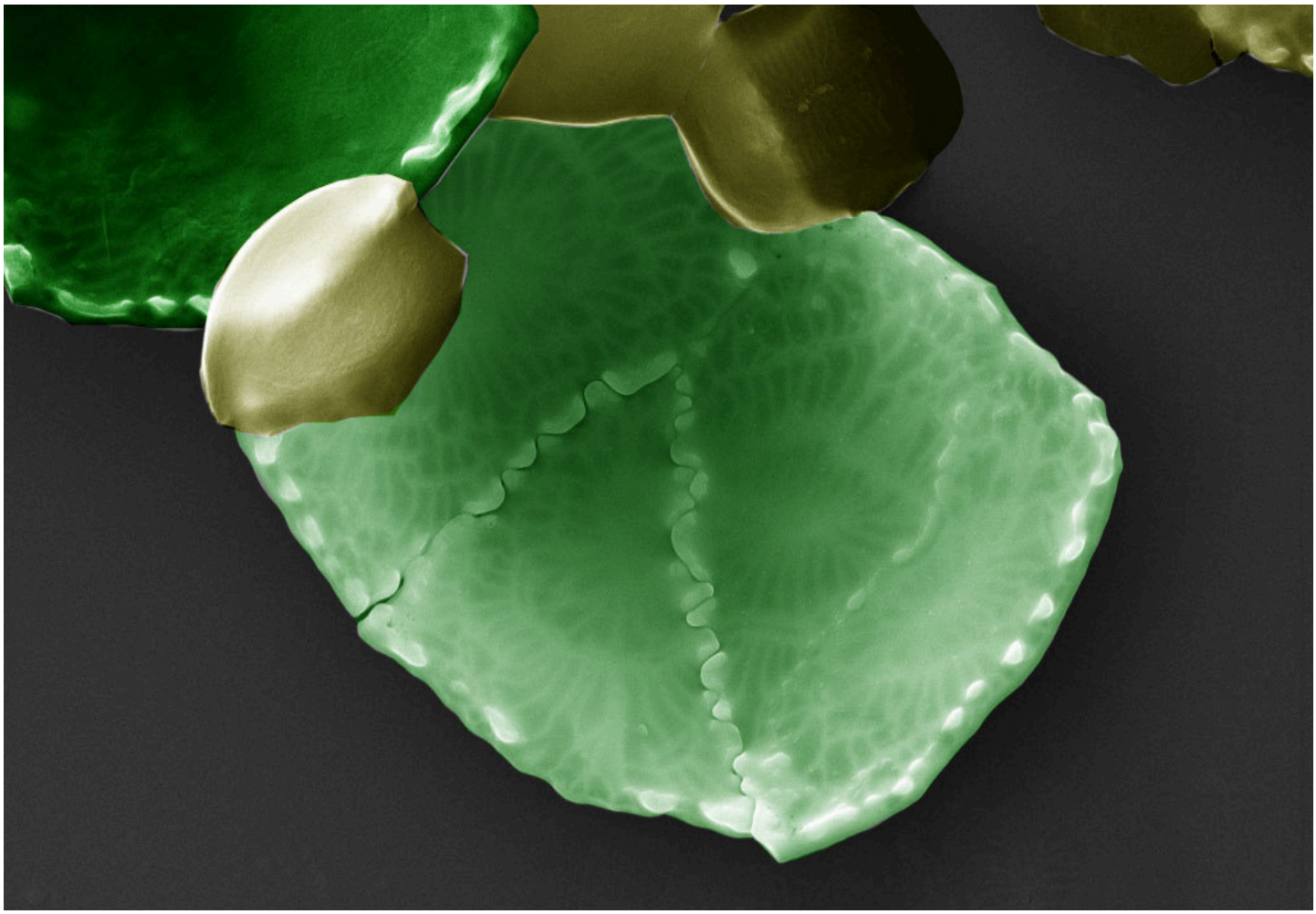
Cysts of *Raphidocystis ambigua* (Haptista: Centroplasthelida); phase contrast. Photo by Vasily Zlatogursky.



While much attention has been given to the scales on the cell surface, cyst scales have received far less focus. In your study on this topic, one of the most intriguing discoveries was the unique, puzzle-like arrangement of cyst scales. Could you tell us more about these cyst scales and their biological role?

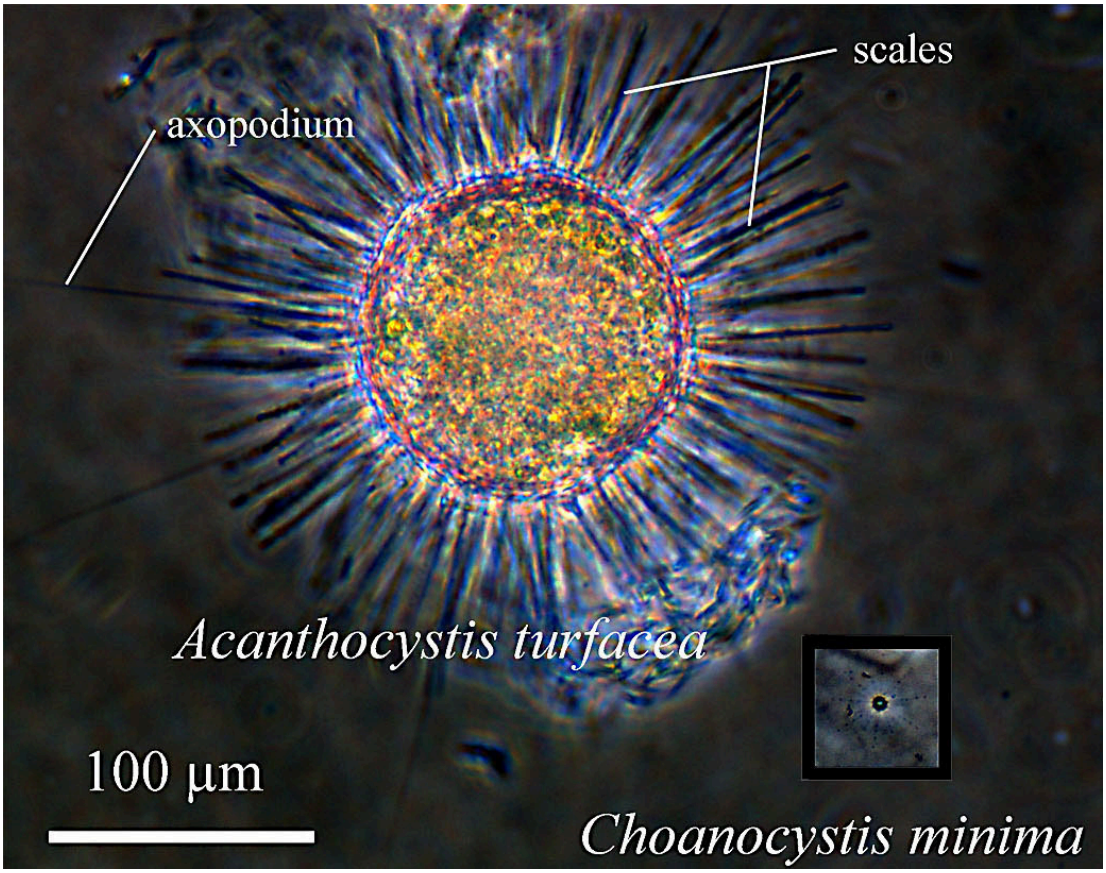
Yes, this is the work of my student Daria Drachko, and I am really proud of her results and conclusions about centrohelid cysts. The cysts are dormant stages that help the organism survive harsh conditions. The scales serve to protect the cell inside, forming multiple layers on the surface. On one hand, the bigger the cell wall, the better—more layers, thicker walls—more protection, right? But the problem is that, at some point, the heliozoan needs to exit the cyst and resume active life. We have found that the

THE CYSTS ARE DORMANT STAGES THAT HELP THE ORGANISM SURVIVE HARSH CONDITIONS. THE CYST SCALES SERVE TO PROTECT THE CELL INSIDE, FORMING MULTIPLE LAYERS ON THE SURFACE.



Cyst scales of *Raphidiophrys heterophryioidea* (Haptista: Centroplasthelida); scanning electron microscopy, digital colors. Note the mosaic connection of scales. Micrograph by Vasily Zlatogursky.

centrohelid *Raphidiophrys elongata* builds a cyst wall made of scales tightly cemented together, and when it exits the cyst, it breaks a hole in the cyst wall and squeezes out. That is why, for this species, the cyst wall needs to be kept relatively thin. But in the other related species, *Raphidiophrys heterophryoidea*, the cyst wall is made of scales forming a mosaic layer—the scales fit together like jigsaw puzzle pieces. Such a cyst wall doesn't need to be broken—it just disassembles easily into separate scales. And that is why such a "demountable" wall can be made much thicker, which is the case for *R. heterophryoidea*. After exiting the cyst, this species is for some time covered with cyst scales (a.k.a. puzzle pieces), giving it a really bizarre look. So, it could be considered another species if you don't know what is actually going on.



Acanthocystis sp. and in insert *Ozanamia curvata* (=*Choanocystis minima*) (Haptista: Centroplasthelida), both shown to scale; phase contrast. Photos by Vasily Zlatogursky.

During your undergraduate thesis research, you discovered three new species, including the smallest heliozoan species known at the time. Could you share more about this discovery and the process behind identifying these new species?

This was the moment in my work when I felt really weird. The cells of *Choanocystis minima*—the smallest centrohelid described so far—are not only really tiny, but they also attach to the substratum very tightly. I didn't have this species in culture and was studying individual cells found in the samples. If you touched the cell with a glass capillary, it would get damaged right away, so I used my own eyelash instead to carefully and gently make the cell detach from the

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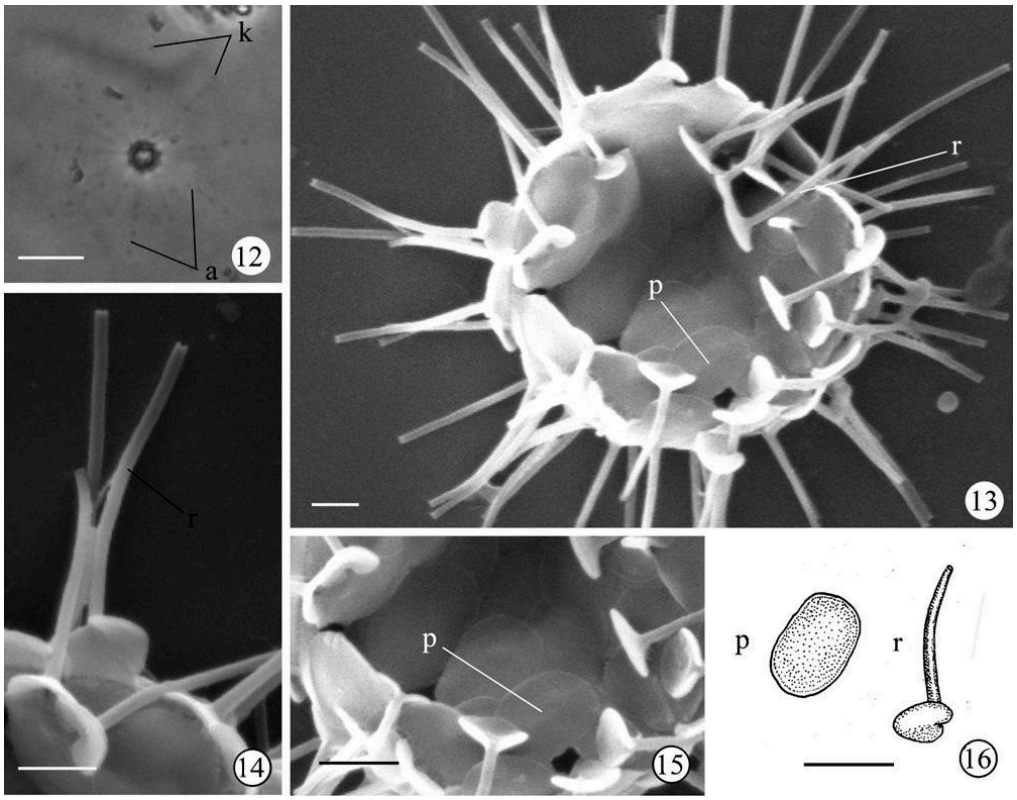
THE CELLS OF CHOANOCYSTIS MINIMA—THE SMALLEST CENTROHELID DESCRIBED SO FAR—ARE NOT ONLY REALLY TINY, BUT THEY ALSO ATTACH TO THE SUBSTRATUM VERY TIGHTLY



Ozanamia curvata (=*Choanocystis minima*) live (12) and in the scanning electron microscope (13–16). 12. General view of the cell. 13. General view of the scale layer. 14. Radial scales with heart-shaped base. 15. Plate scales and basal parts of radial scales. 16. Line drawing of radialand plate scale. a – axopodia, k – kinetocyst, p – plate scale, r – radial scale. Scale bars 10 micrometers (Fig. 12) and 1 micrometer (Figs 13–16). From: Zlatogursky (2010), doi: 10.1016/j.ejop.2010.01.003

substratum without damaging it. I spent hours and days doing this and felt really strange. But this work paid off, because this species was not only revealed to be new but also a size record-breaker! Unfortunately, I didn't obtain molecular data for it during my undergraduate research, and I've never seen it since. I would really be interested in reisolating it now and studying it in more detail.

Actually, recently, it turned out that I made a mistake: *Choanocystis minima* is synonymous with *Ozanamia curvata*—a species described earlier by Cavalier-Smith and von der Heyden. As an undergraduate, I overlooked the similarity between these two forms, as Kenneth Nicholls recently rightly pointed out. This is an important correction, but it does not disprove the size record; Cavalier-Smith and von der Heyden did not provide reliable size measurements in their description.

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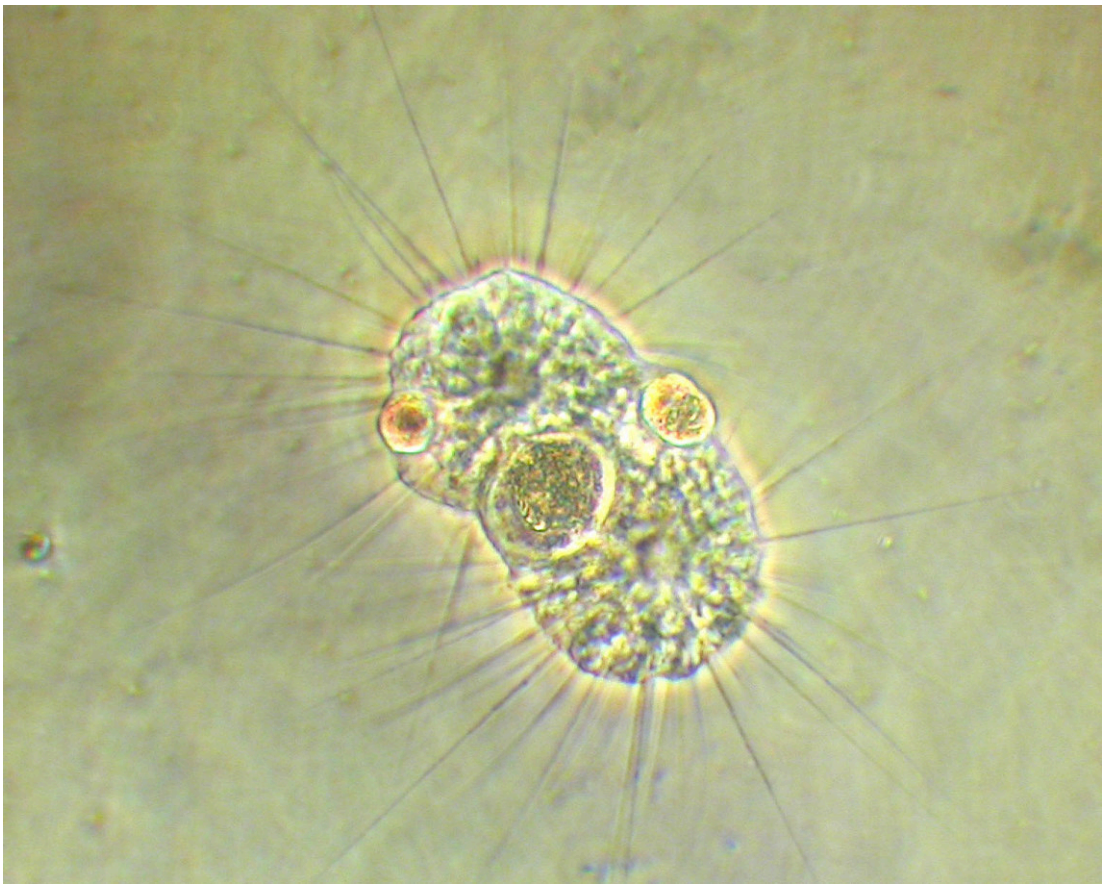
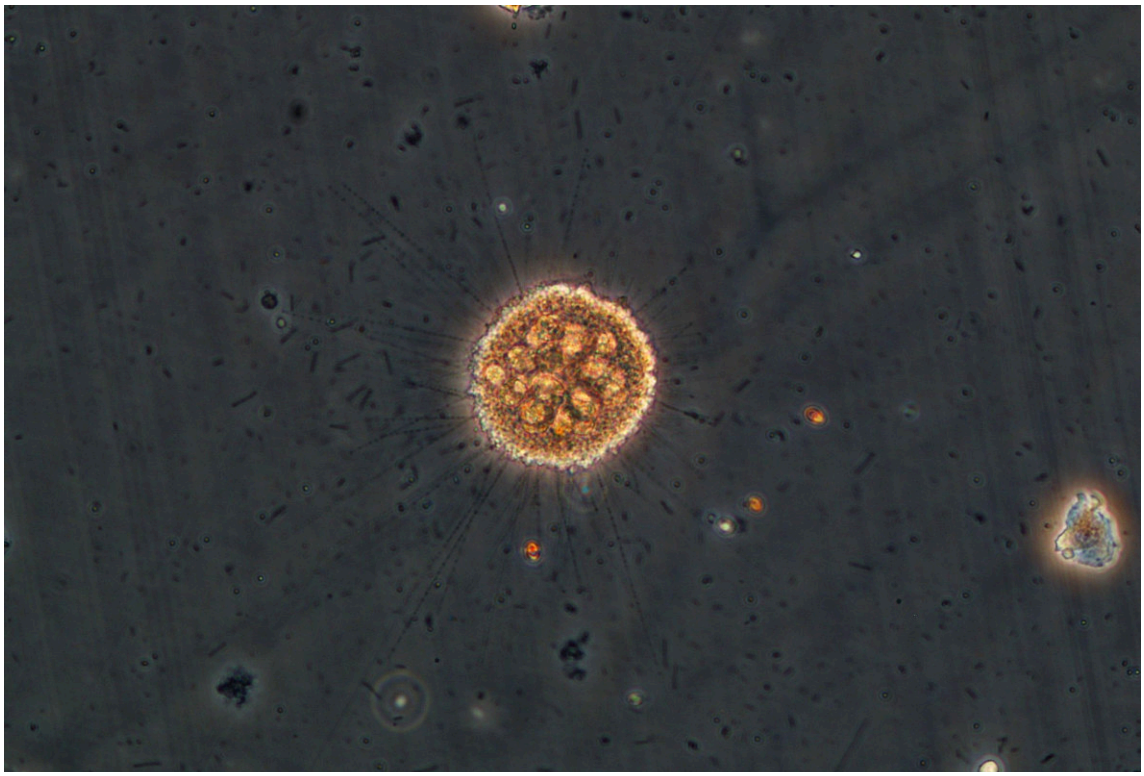
RECENTLY, IT TURNED OUT THAT I MADE A MISTAKE: CHOANOCYSTIS MINIMA IS SYNONYMOUS WITH OZANAMIA CURVATA—A SPECIES DESCRIBED EARLIER BY CAVALIER-SMITH AND VON DER HEYDEN



Two cells of *Actinophrys* sp. (Stramenopiles: Actinophryida) share a common food vacuole; phase contrast. Photo by Vasily Zlatogursky.

Most amateur microscopists only have access to light microscopy. Are there any specific light microscopy techniques that can significantly improve the accuracy of heliozoan identification?

This is definitely a problem because, in the end, we don't want centrohelids to be neglected by ecologists and amateur microscopists, who should also be able to recognize them—not just by experts who search for them specifically. Of course, it would not be possible for them to repeat all the complex work we do with electron microscopes and sequencing if they just want to identify the organism they see. I think there is a huge need for an identification guide that allows species to be determined without having a cutting-edge laboratory. This is something I am aiming for, but first, we need to describe lots of species and have a more or less adequate picture of the true diversity. For now, unfortunately, it is sometimes easier to find a new centrohelid species than to find a known one again.

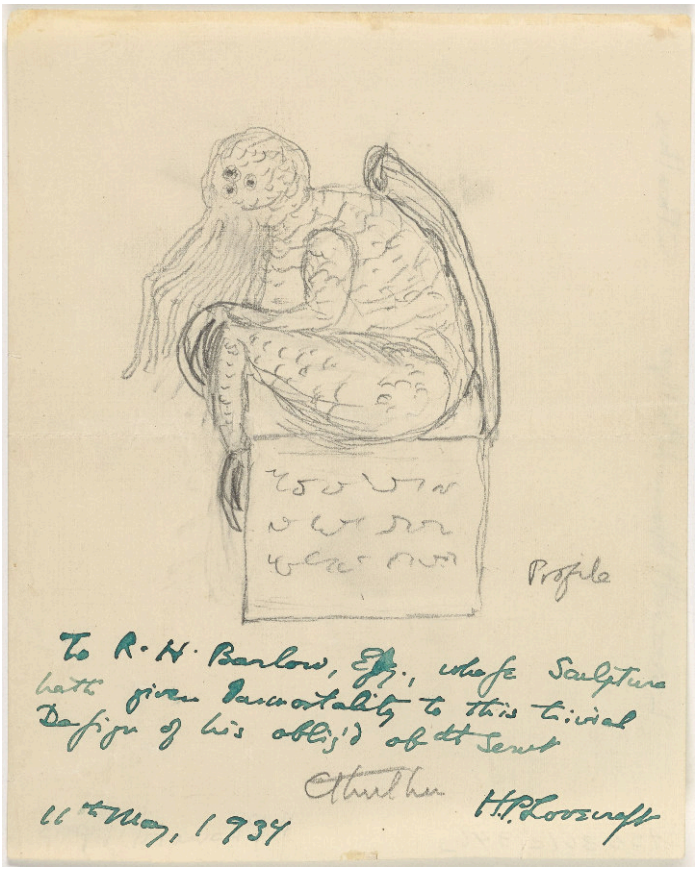


Colony of *Yogsothoth cartheri* (Haptista: Centroplasthelida); phase contrast. Photo by Vasily Zlatogursky.

Together with your colleagues, you discovered specific colonial centrohelids and created the genus *Yogsothoth*. Could you describe the structure of these heliozoans and what sets them apart from other centrohelids?

This finding was made by me and my student Yegor Shishkin-Skarð, and it is one of the coolest centrohelid species I have ever seen. It forms colonies of many, sometimes up to 30 cells, but this is not unusual, as it has also been observed in other centrohelids. What is unusual is, as often in my studies, the scales. Each cell is covered with plate scales, but the whole colony itself is covered with really unusual scales, which do not resemble the scales of any other centrohelids. These scales are boat-shaped or helmet-shaped, with a big cavity inside—a totally novel scale type. What I find very interesting is that these cavity-scales do not belong to any of the individual cells. This huge, very thick layer of scales is what the cells form together cooperatively and use as a colonial, not individual, structure. This puts *Yogsothoth* on a surprisingly advanced level of multicellularity with novel shared anatomical features, as Polish researcher Łukasz Lamża recently called it.

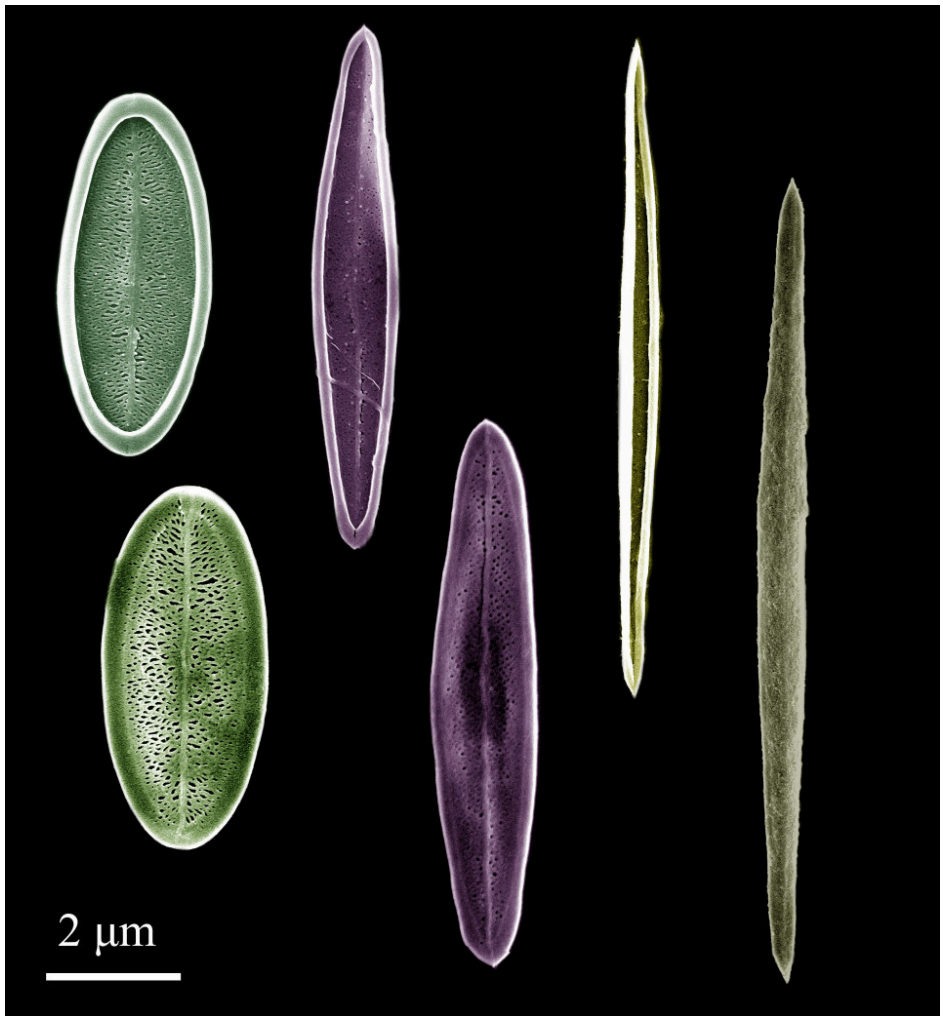
H. P. Lovecraft (1890—1937), the creator of the Cthulhu Mythos



A sketch of Cthulhu drawn by Lovecraft

Could you share the story behind the fascinating name of the genus *Yogsothoth*?

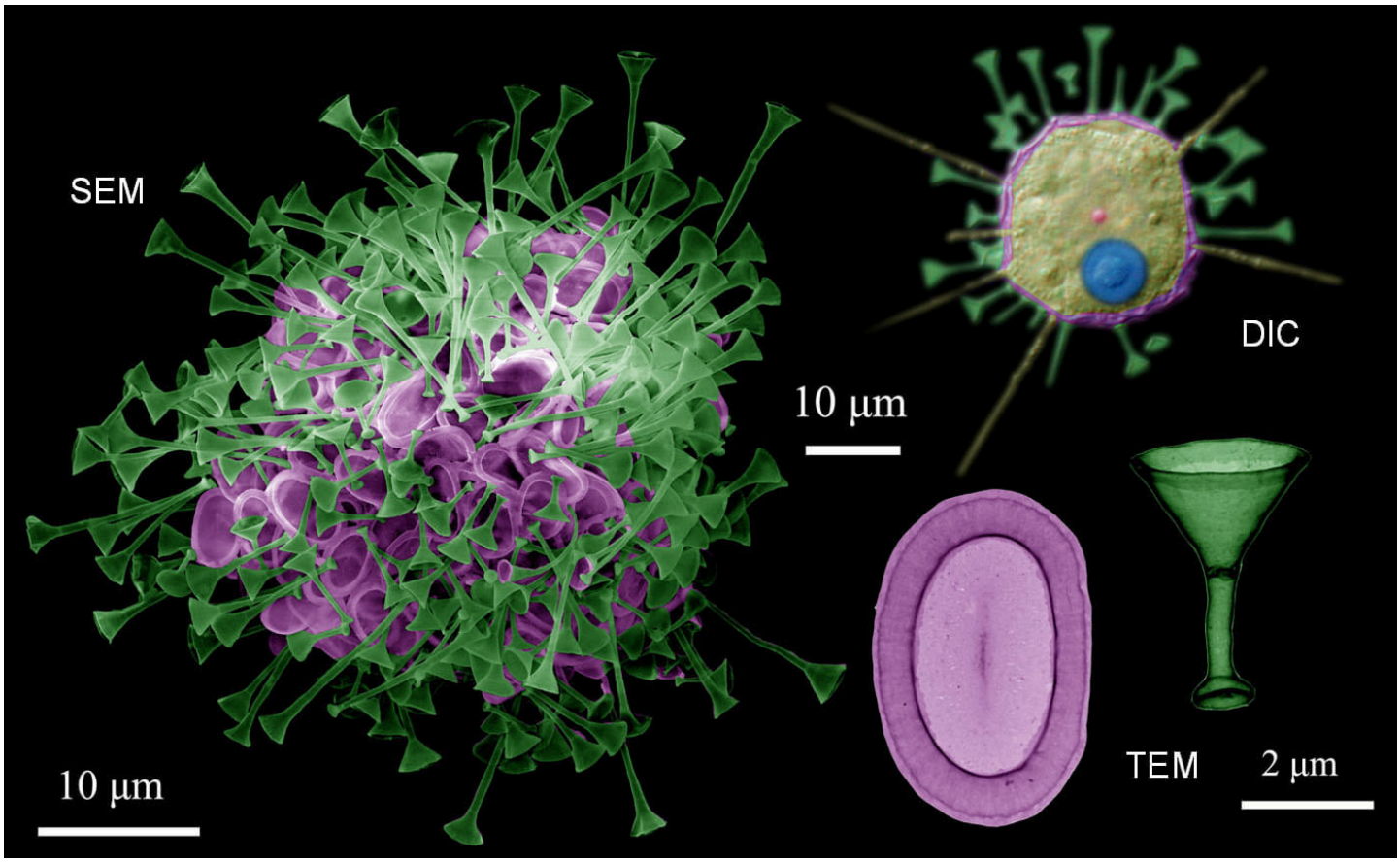
The name refers to Yog-Sothoth, a creature from Howard Phillips Lovecraft's book, part of the fictional Cthulhu Mythos universe. Daria and Yegor are big fans of Lovecraft, not me, by the way! But I agreed with them that the description of Yog-Sothoth by Lovecraft (as a conglomeration of glowing spheres) matches the heliozoan morphology quite well, so I didn't object. It is not the first protist named after a Lovecraft character, by the way. There is a flagellate from a termite's gut named *Cthulhu*.



Scales of *Raphidocystis ambigua* (Haptista: Centroplasthelida); scanning electron microscopy, digital colors. Note the superficial similarity to diatom shells! Micrographs by Alisa Mironchikova.

Can you tell us a bit about the publications you currently have in preparation? Should we expect descriptions of new heliozoan species to be released soon?

I am preparing a paper on centrohelid phylogenomics. I have my own genomic and transcriptomic data, a lot of data from colleagues and collaborators, and data from public sources. Currently, I am working on the analysis of all these sources together to create the first comprehensive, well-sampled phylogenomic tree of centrohelids. Hopefully, it will help answer important questions about their evolution that I mentioned in the beginning. Additionally, increasing taxon sampling may help place centrohelids more accurately within the whole family tree of eukaryotes. In the latest studies, their position changes from one analysis to another, but currently, researchers rely on data from just 4–5 species. I am working on improving this considerably! And… yes, new species descriptions will be included in this paper as well—stay tuned.



Raphidocystis glabra (Haptista: Centroplasthelida). Spine scales in green, plate scales in purple, nucleus in blue, cytoplasm in yellow and centrosome in red; digital colors. Micrographs by Vasily Zlatogursky.

Are you active on social media? Do you think there are enough resources on protistology available on these platforms to help introduce the fascinating world of protists to a wider audience?

Many protistologists are actively engaged on social media platforms like X and BlueSky. These microblogging sites are particularly effective for rapidly sharing newly published research papers, conference updates, and other relevant information. I am very active on these platforms, where I post research updates and share content from fellow protistologists. On BlueSky, there is even a protist starter pack available (https://bsky.app/starter-pack/oliverio.bsky.social/3l6zuxosq6q2x), along with the #protistsonsky hashtag for easy discovery of related content.

@zlatogursky.bsky.social

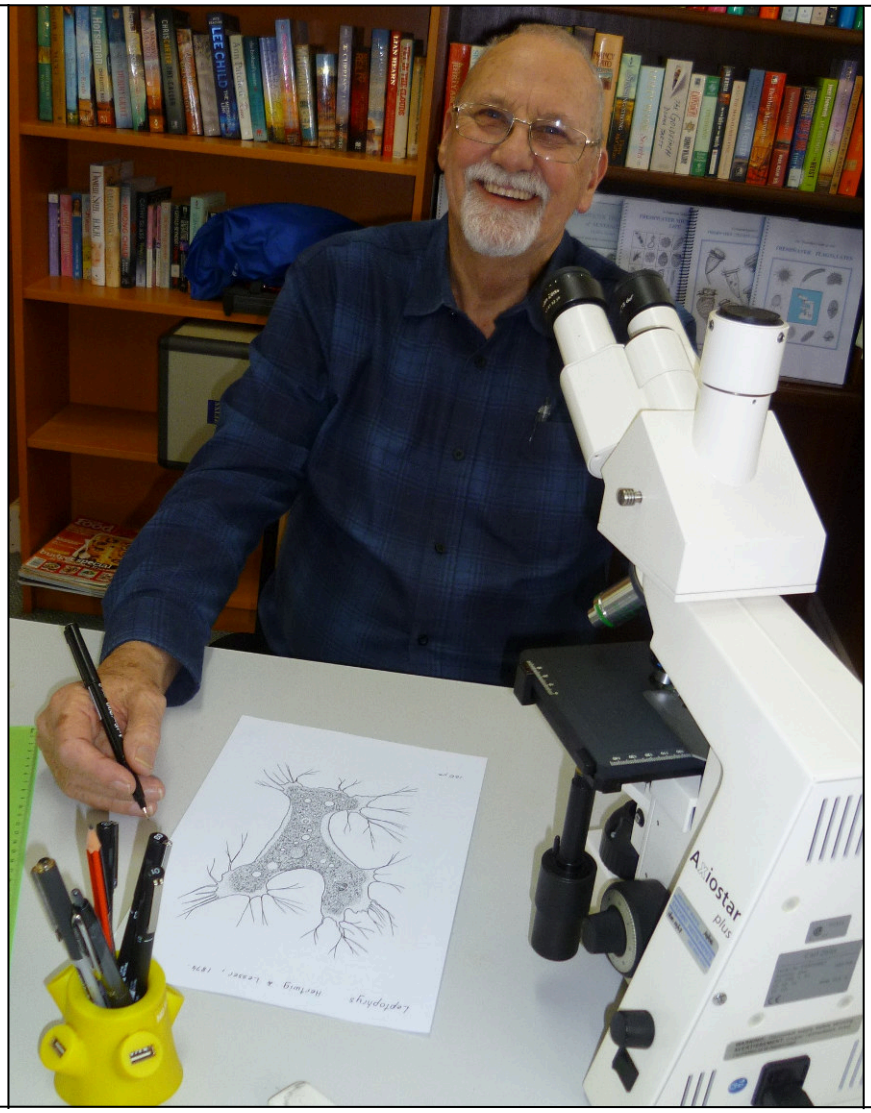
@zlatogursky

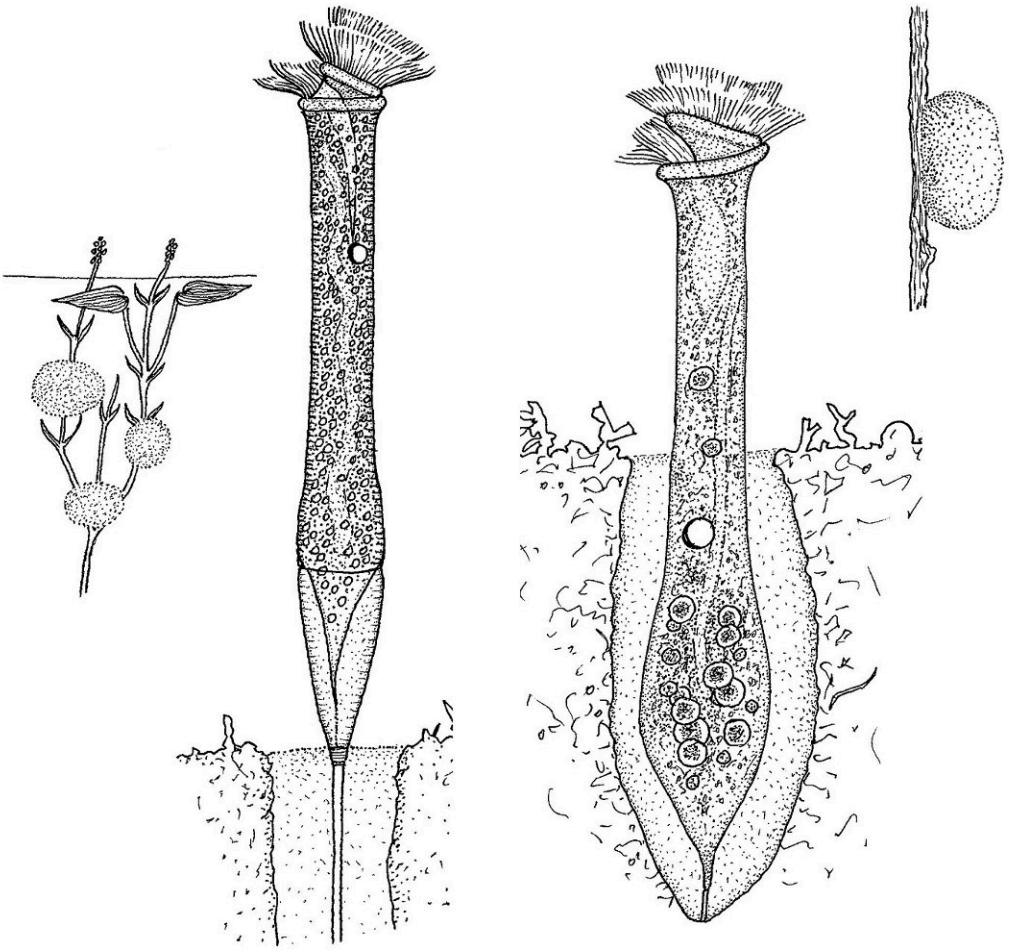
DAVID SEAMER

The Bus Expedition into the World of Microscopic Life

David Seamer is a passionate researcher who spent two decades living and working in a converted school bus, which served as both his home and laboratory. Traveling across Australia, including Tasmania, he delved into the hidden world of microscopic life, conducting research and creating detailed drawings.

Interviewed by
Dr. Stefan Luketa





Drawings of *Ophrydium* sp. from *An Illustrated Guide to the Freshwater Free-living Peritrichs* by David Seamer

Australia, a vast land of untamed wilderness and breathtaking natural beauty, conceals secrets that remain largely hidden from the world. While the continent's diverse landscapes—ranging from lush rainforests to arid deserts—are renowned for their unique ecosystems and rich biodiversity, it is the microscopic world, thriving in the smallest corners, that still holds many mysteries. Despite extensive research on larger organisms, the microscopic organisms within Australia's freshwater ecosystems remain largely unexplored.

Enter David Seamer—a man whose passion for the unseen world of microscopic organisms has fueled an extraordinary journey. Unlike most researchers, David isn't anchored to a university or academic institution. A self-taught scientist with an adventurous spirit, his unconventional career began in 1993 when he converted an old school bus into both his mobile home and laboratory. From that humble vehicle, David ventured into some of

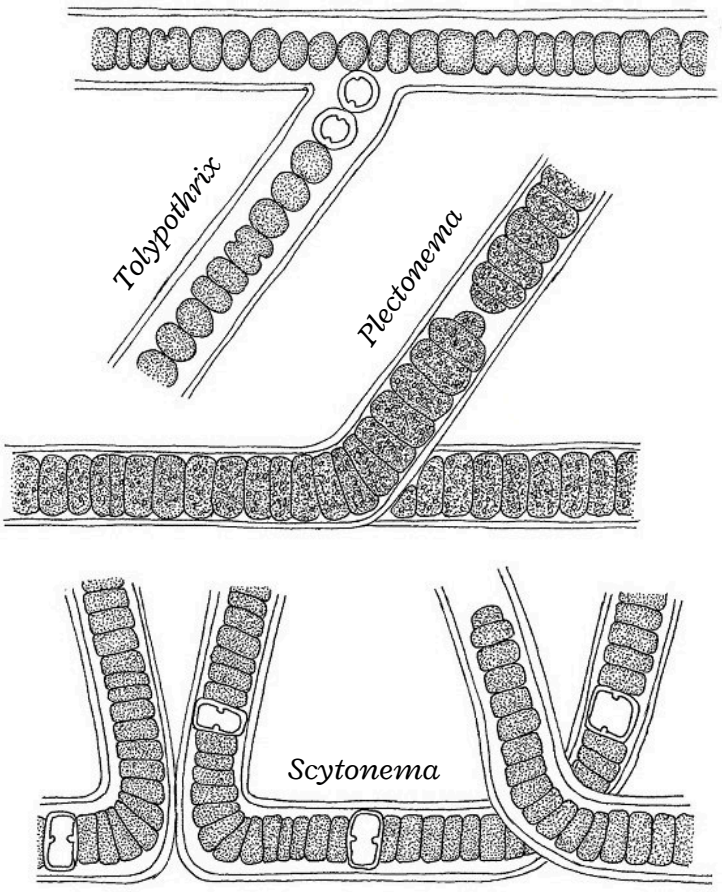
Australia's most remote and isolated regions, driven by an insatiable curiosity that would ultimately uncover the hidden life forms thriving in the freshwater ecosystems of Australia and Tasmania.

Traveling in his bus for 20 years, David's research has not only contributed to the scientific understanding of microscopic organisms but has also brought their beauty and complexity to the forefront through his breathtaking illustrations. His drawings, which capture the elegance and mystery of these tiny life forms, offer a glimpse into a world that most of us will never see—a world that David has come to know as both a scientist and an artist.

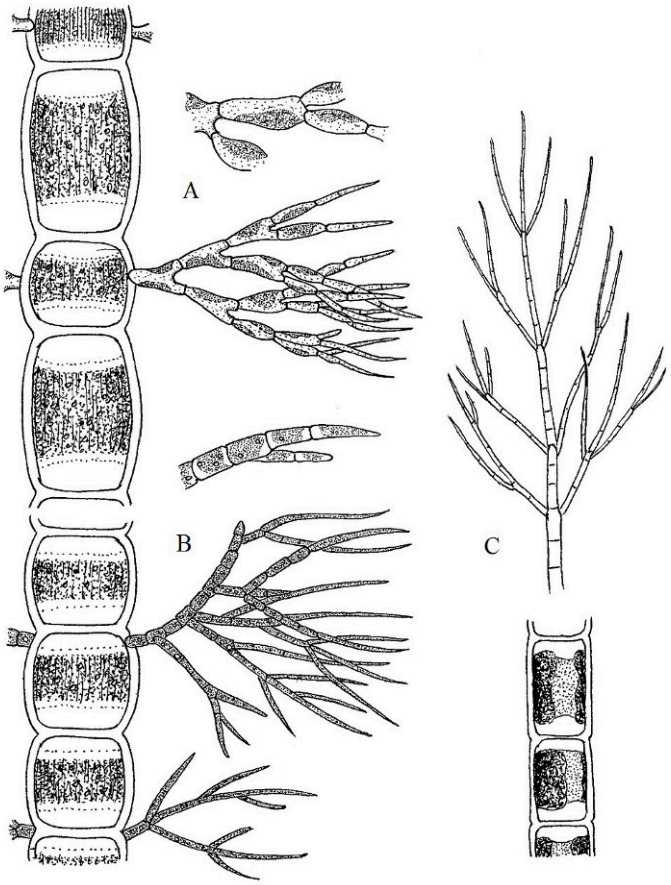
Each stroke of his pencil tells a story of discovery, bringing to life organisms that exist on the very fringes of our understanding.

Now settled in the quiet town of Wangaratta, David continues his work—writing, studying, and uncovering new insights into the microscopic life that thrives in Australia.

Cyanobacteria



Filamentous green algae



Drawings from *A Beginners Guide to Freshwater Microscopic Life* by David Seamer

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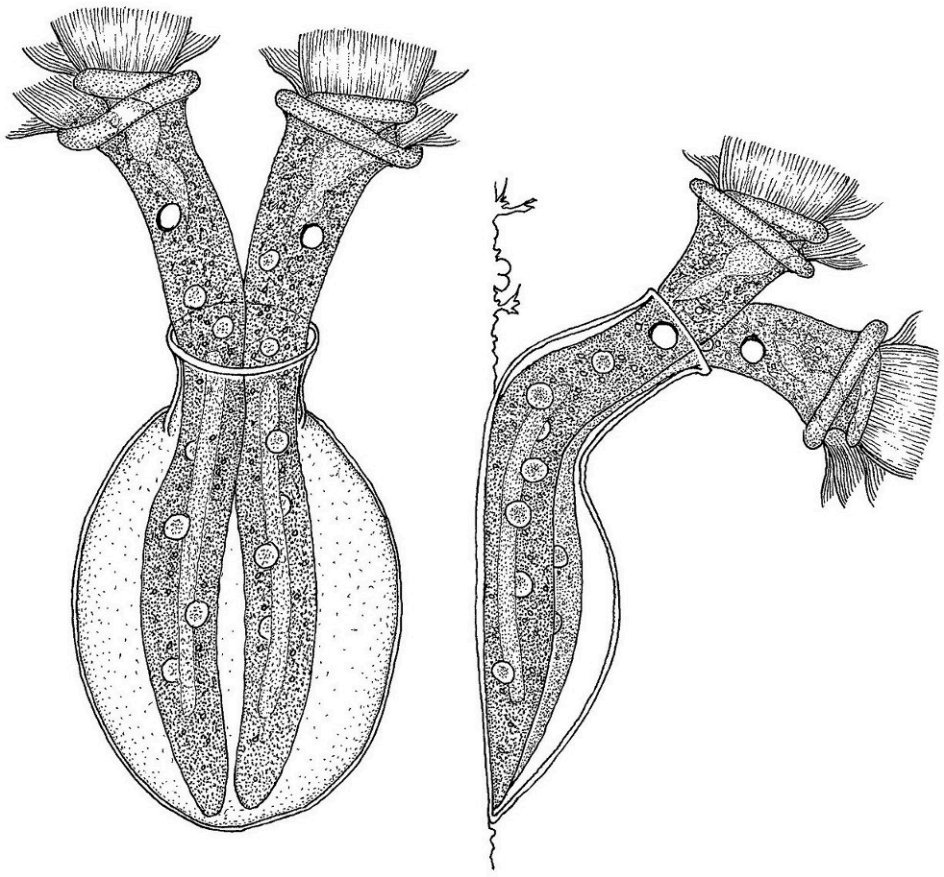
Drawing of *Platycola* sp. from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

DAVID HAS PUBLISHED SEVEN BOOKS, EACH A TESTAMENT TO HIS DECADES-LONG PASSION FOR DOCUMENTING MICROSCOPIC LIFE



He has published seven books, each a testament to his decades-long passion for documenting these creatures, with hundreds of his illustrations gracing their pages.

In this interview, we embark on a journey through David's world—a world of microscopic discovery. He will take us behind the scenes of his research, recounting the challenges he has faced over the years, the techniques he uses to "hunt" microscopic organisms, and how he transforms his observations into works of art. Along the way, we'll learn about some of his most exciting discoveries and explore the contents of his books, offering a rare glimpse into the extraordinary diversity of life thriving in the hidden corners of Australia.

Te Awamutu College, New Zealand

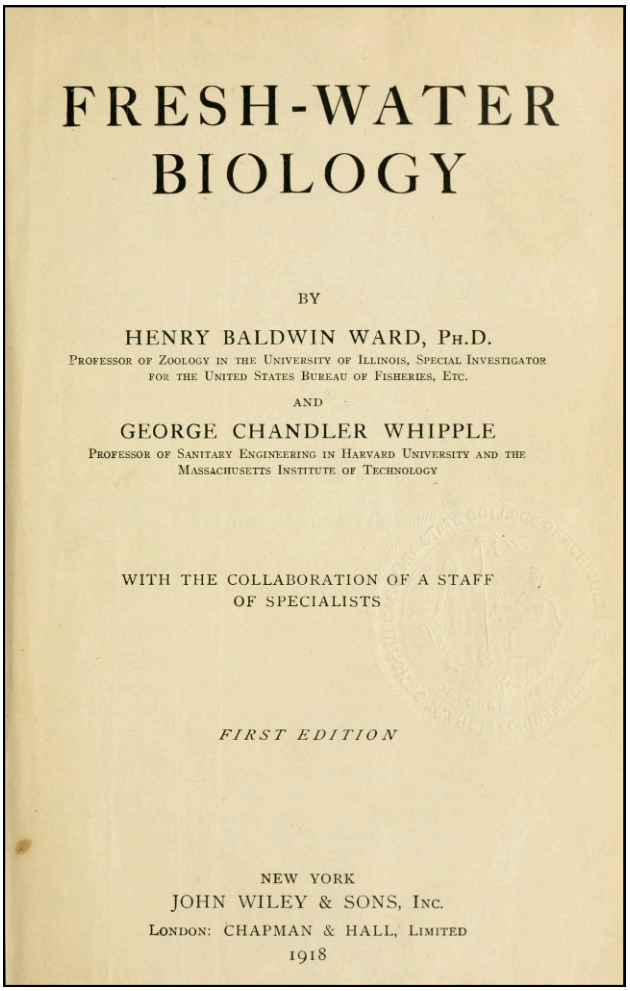
David at the age of 19

Born in New Zealand, could you share some insights about your childhood there and how it may have influenced your later work?

I was the youngest of four children and afforded a great deal of freedom. I had always had a fascination with nature and all living things, and being blessed with the ability to draw, I spent many hours studying and drawing things I had collected, such as flowers and insects. My mother often complained that my room was more like a zoo than a bedroom. I had several old aquariums in which I kept butterfly larvae, small lizards, water beetles, etc. At age 13, I started a biology club in which every available child in the town was encourage to cycle off to bush-covered hills to collect insects, along with whatever else we could find, take them home, and attempt to identify them. I guess this was the catalyst for what turned out to be a life of exploring the micro-world.

What first sparked your interest in studying microscopic life, and how did that passion evolve over time?

During a double period of biology in high school, we were studying *The Cell* using amoeba that the teacher had collected from a nearby wetland. We were asked to draw an amoeba and its relevant features, such as the nucleus, contractile vacuole, etc., when suddenly some strange shape swam by. I quickly caught up and drew it. It was then that I noticed other peculiar shapes, drew them, and by the end of the period, I had several pages of drawn weird things. I asked the teacher what these were, and he pointed me to a copy of Ward and Whipple's *Freshwater Biology*. When I saw drawings of not only what I had recorded but also the range of microscopic life, I was hooked. I saved up my lawn-mowing pocket money and bought my first microscope.



FRESH-WATER BIOLOGY

BY

HENRY BALDWIN WARD, PH.D.

PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF ILLINOIS, SPECIAL INVESTIGATOR FOR THE UNITED STATES BUREAU OF FISHERIES, ETC.

AND

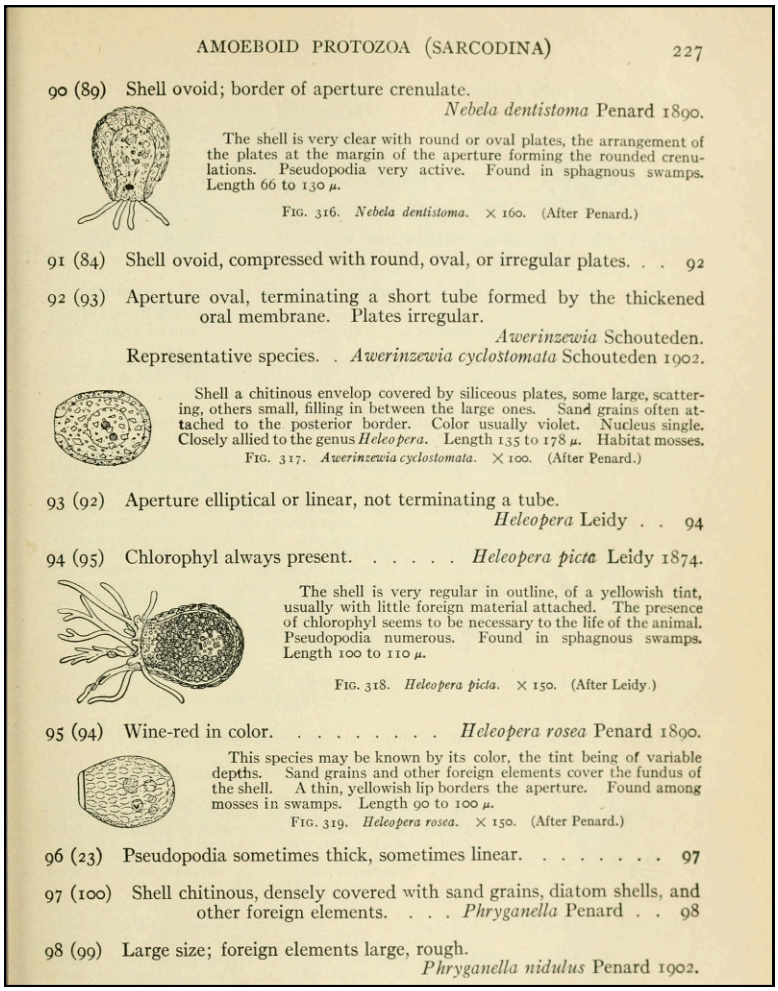
GEORGE CHANDLER WHIPPLE

PROFESSOR OF SANITARY ENGINEERING IN HARVARD UNIVERSITY AND THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

WITH THE COLLABORATION OF A STAFF OF SPECIALISTS

FIRST EDITION

NEW YORK
JOHN WILEY & SONS, INC.
LONDON: CHAPMAN & HALL, LIMITED
1918



90 (89) Shell ovoid; border of aperture crenulate.
Nebela dentistoma Penard 1890.

The shell is very clear with round or oval plates, the arrangement of the plates at the margin of the aperture forming the rounded crenulations. Pseudopodia very active. Found in sphagnous swamps. Length 66 to 130 μ.

FIG. 316. *Nebela dentistoma*. × 160. (After Penard.)

91 (84) Shell ovoid, compressed with round, oval, or irregular plates. . . 92

92 (93) Aperture oval, terminating a short tube formed by the thickened oral membrane. Plates irregular.
Awerinzewia Schouteden.
Representative species. . *Awerinzewia cyclostomata* Schouteden 1902.

Shell a chitinous envelop covered by siliceous plates, some large, scattering, others small, filling in between the large ones. Sand grains often attached to the posterior border. Color usually violet. Nucleus single. Closely allied to the genus *Heleopera*. Length 135 to 178 μ. Habitat mosses.

FIG. 317. *Awerinzewia cyclostomata*. × 100. (After Penard.)

93 (92) Aperture elliptical or linear, not terminating a tube.
Heleopera Leidy . . 94

94 (95) Chlorophyl always present. . . . . *Heleopera picta* Leidy 1874.

The shell is very regular in outline, of a yellowish tint, usually with little foreign material attached. The presence of chlorophyl seems to be necessary to the life of the animal. Pseudopodia numerous. Found in sphagnous swamps. Length 100 to 110 μ.

FIG. 318. *Heleopera picta*. × 150. (After Leidy.)

95 (94) Wine-red in color. . . . . *Heleopera rosea* Penard 1890.

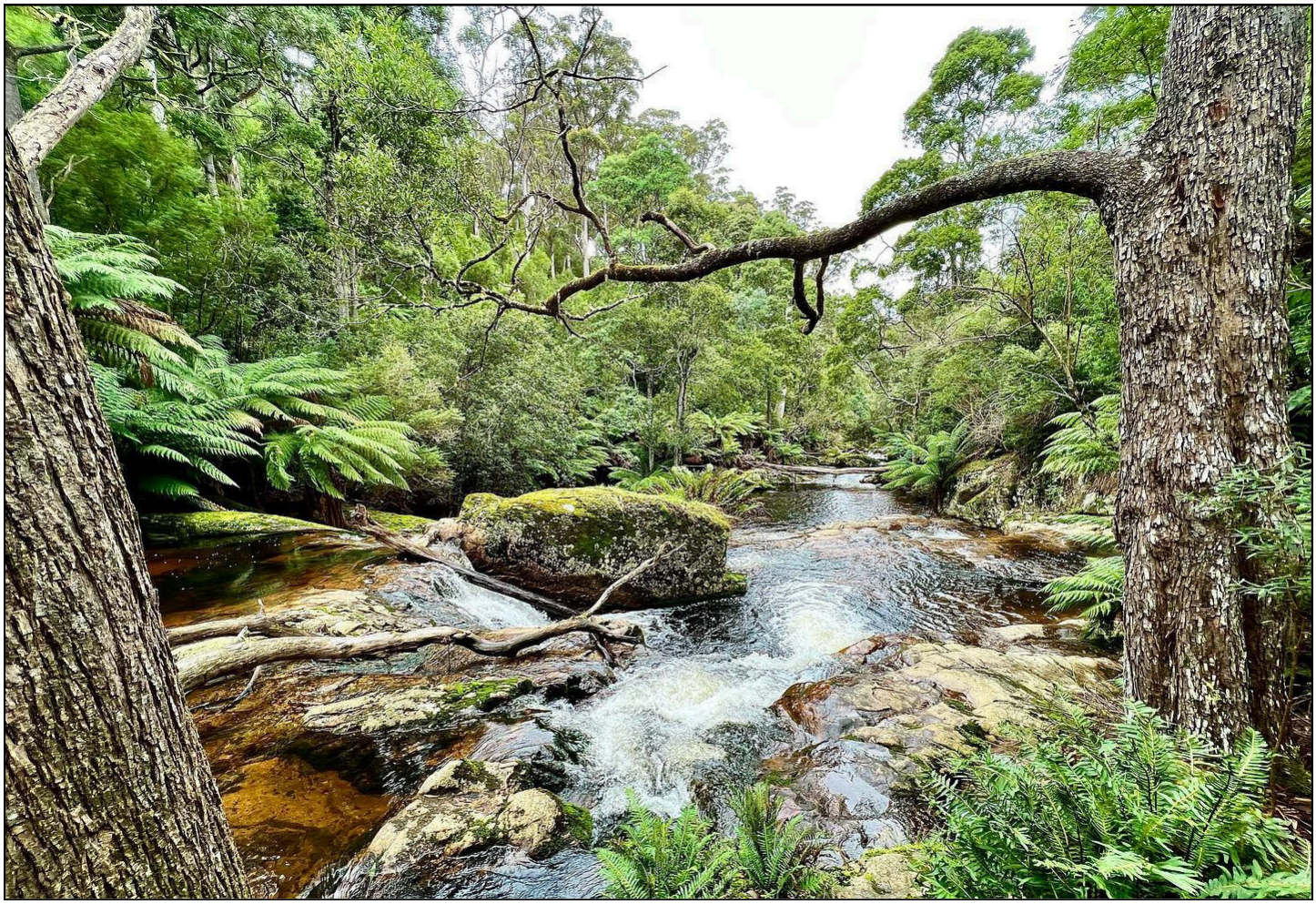
This species may be known by its color, the tint being of variable depths. Sand grains and other foreign elements cover the fundus of the shell. A thin, yellowish lip borders the aperture. Found among mosses in swamps. Length 90 to 100 μ.

FIG. 319. *Heleopera rosea*. × 150. (After Penard.)

96 (23) Pseudopodia sometimes thick, sometimes linear. 97

97 (100) Shell chitinous, densely covered with sand grains, diatom shells, and other foreign elements. . . . *Phryganella* Penard . . 98

98 (99) Large size; foreign elements large, rough.
Phryganella nidulus Penard 1902.



Typical Tasmanian mountain creek

After moving from New Zealand to Australia, did you begin collecting samples and studying microscopic organisms there?

When I was twenty, I moved from New Zealand to Australia. I took my microscope with me and, whenever I could, sampled the local aquatic environments. I continued to do so, and over the years, I have slowly built up a considerable collection of recordings.

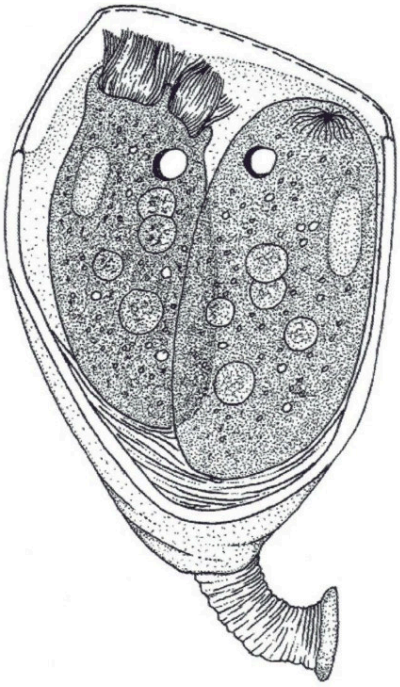
In 1984, you moved to Tasmania, an island with unique ecosystems situated off the southern coast of Australia. How did this relocation open up new opportunities for researching the island's specific ecosystems, particularly in the field of microscopic life?

Tasmania gave me the opportunity to sample new environments, such as alpine ponds, *Sphagnum* bogs, and relatively virgin wetlands, which only added to my collection. A chance meeting with Professor Peter Tyler from Hobart University on an intercity flight gave further incentive to broaden my algal studies of desmids and diatoms, two groups that, to date, I had only previously glanced at.

Cradle Mountain Lake, Tasmania

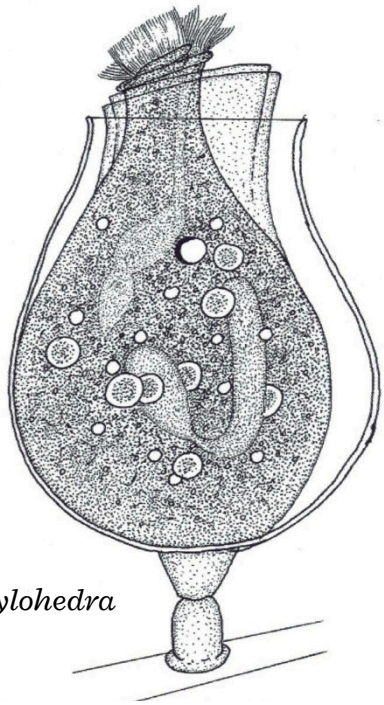
In 1993, you made the unusual decision to lead a highly adventurous life in Tasmania for the next seven years. Could you share more about what this period was like and how it influenced both your personal and professional journey?

With the breakdown of my marriage in 1993, I had some serious decisions to make about my life's direction. I decided that life was too short to waste, so I chose to do the thing I loved. I bought an old school bus and converted it into a mobile home/laboratory in which I traveled around Tasmania, collecting, drawing, and cataloguing much of that state's algae and protozoa.

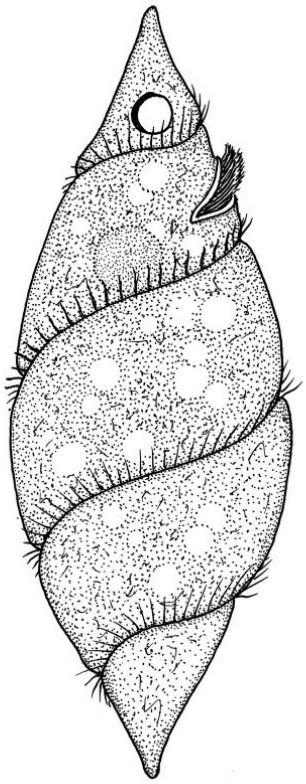


Cyclodonta

The representatives of three genera of ciliates from Tasmania.



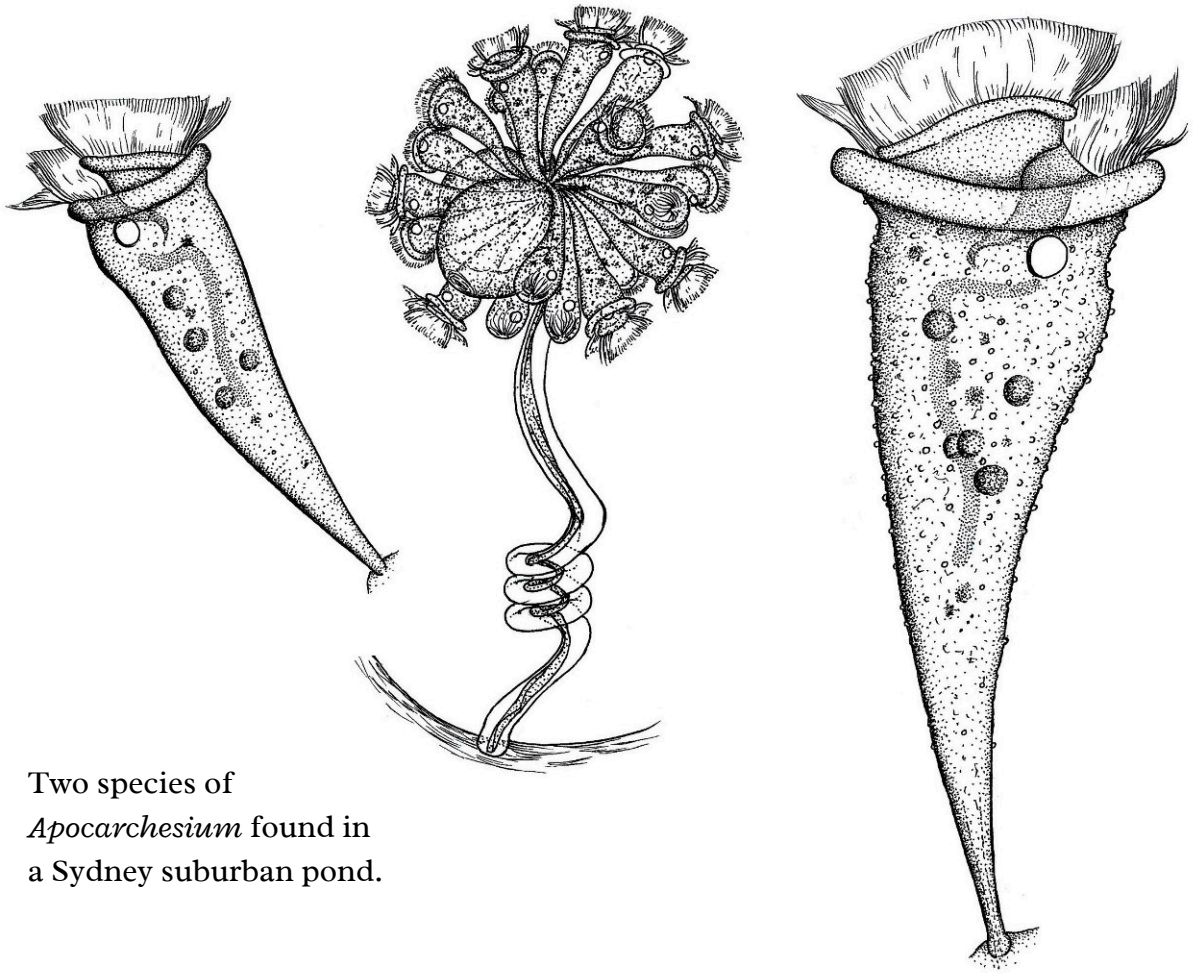
Stylohedra



Mycterothrix

During your time in Tasmania, what are some of the most fascinating microscopic species you discovered?

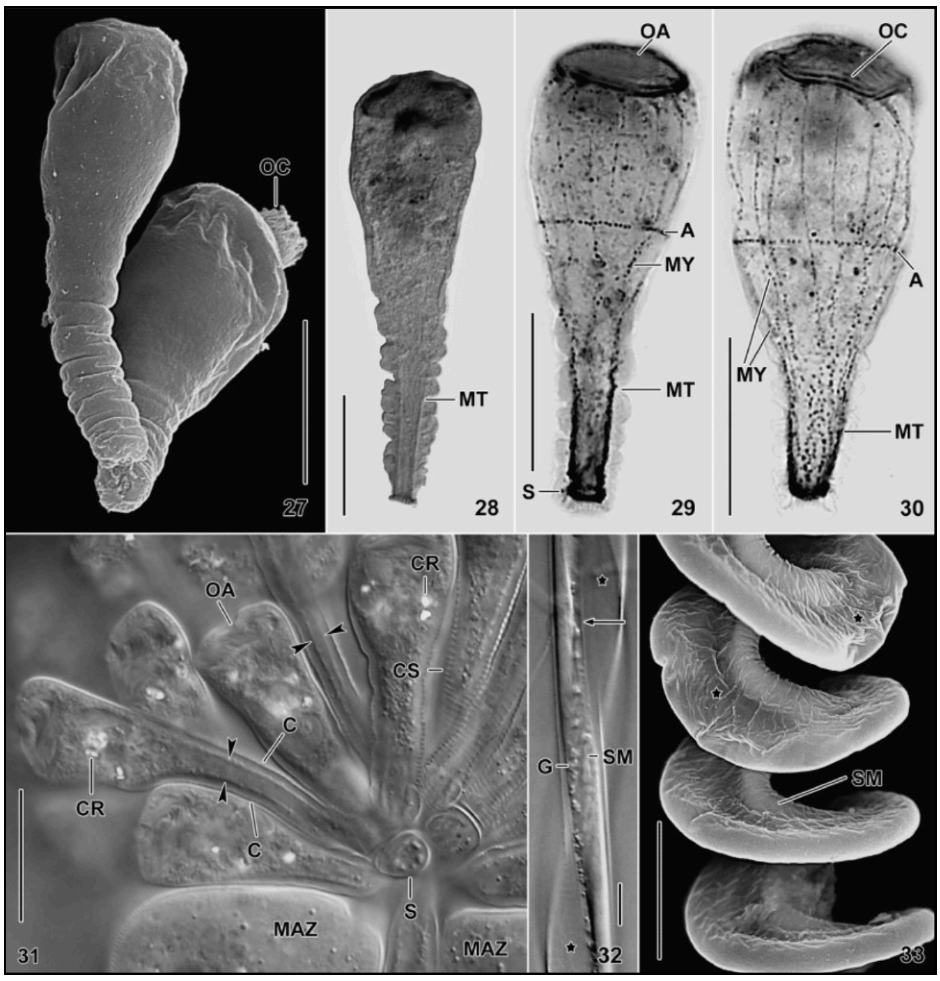
During this time, I found and recorded some unusual ciliates that I had never encountered before and, in some cases, since. Among these were *Cyclodonta*, an uncommon loricated peritrich found in Goulds Lagoon in suburban Hobart; *Stylohedra*, another uncommon loricated peritrich, was recorded from Chain of Lagoons, a coastal pond at St. Helens on Tasmania's east coast; as well as *Mycterothrix*, a quite rare ciliate that was found in nearby Jocks Lagoon.



Two species of *Apocarchesium* found in a Sydney suburban pond.

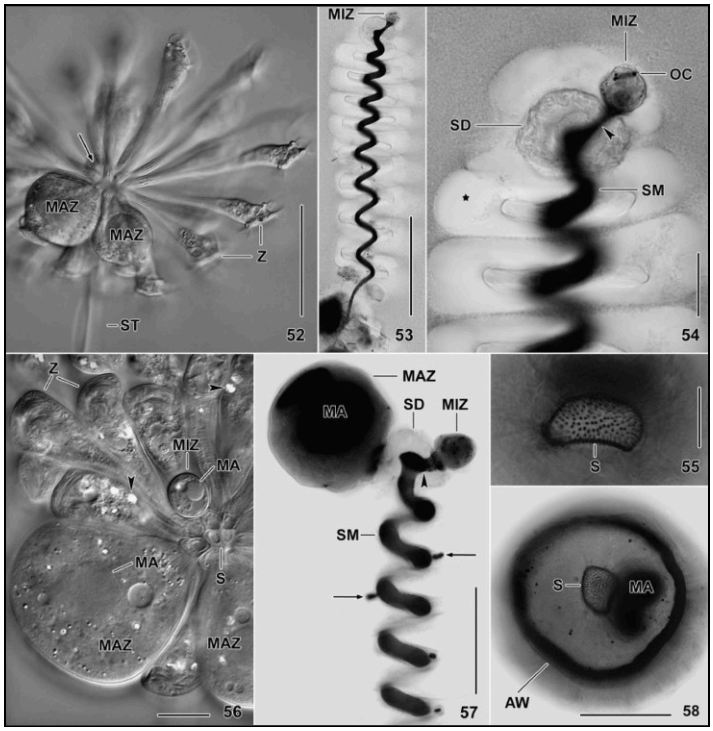
After seven years of intensive research on microscopic organisms in Tasmania, you continued your research journey in South-East Australia, where you encountered vastly different habitats. What were some of the most surprising or exciting species you discovered in that region?

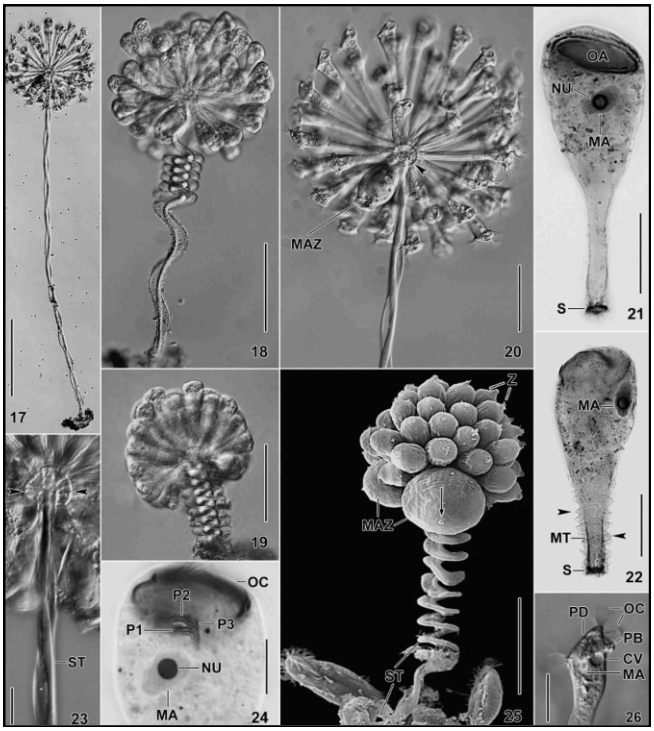
In a pond in Sydney in 2002, I found two different species of a peritrich that just did not key out to any published genus. I did extensive drawings and took photographs of it. In 2009, two Chinese biologists (Ji & Kusuoka) found it in Japan and described and named it *Apocarchesium rosettum*. In 2010, Norf & Foissner found a new species, *Apocarchesium arndti*, in Austria, and since then, both species have been recorded from various parts of the world. However, the two species I found in Sydney are quite different and remain unnamed.



Apocarchesium arndti

Norf H., Foissner W. 2010. A new flagship peritrich (Ciliophora, Peritrichida) from the river Rhine, Germany: *Apocarchesium arndti* n. sp. Journal of Eukaryotic Microbiology. 57(3): 250-264.





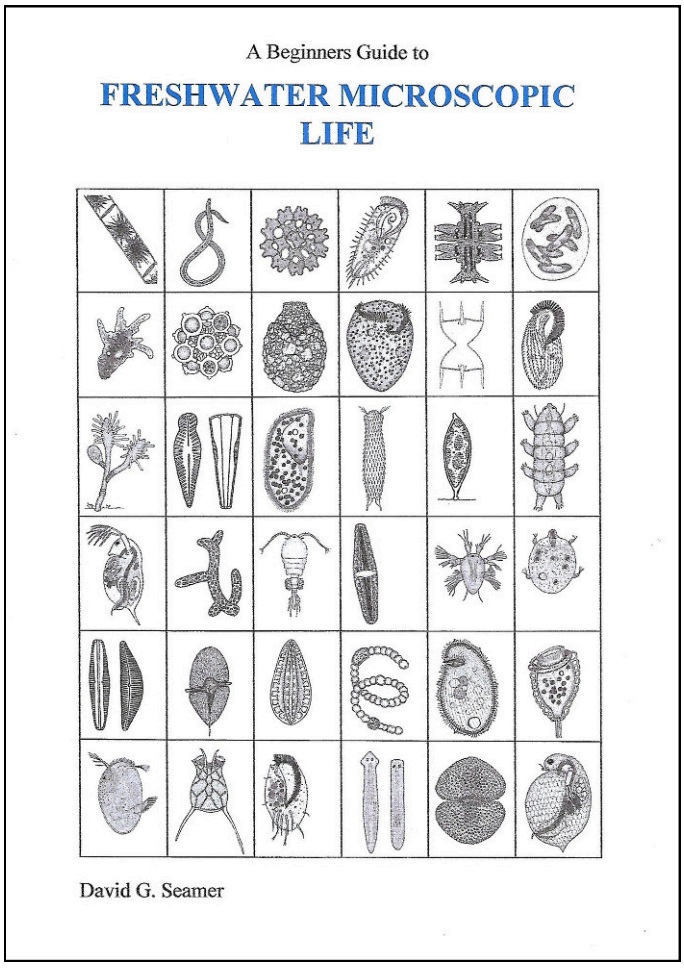
Reedy Creek, Victoria, south-east Australia

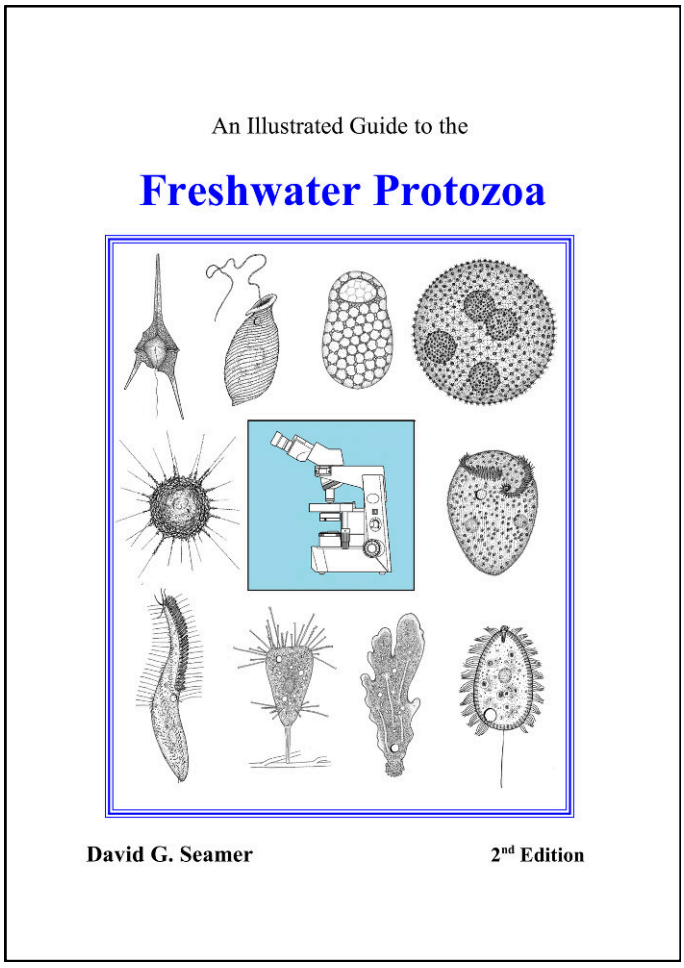
Out of all the areas you researched in South-East Australia, which one did you find to be the most fascinating, and why?

Oh, wow, what a question! I can't say that there is any one area that fascinates me more than any other. Each environment can be unique, with its own mixture of common and sometimes not-so-common species, and at each location, it is like going on a safari, for one never knows what one will discover. Sometimes, even the most mundane-looking pond can be a treasure trove of exciting organisms.

In 2013, you decided to conclude your 20-year adventure by settling in a rural town in Victoria. Was this decision motivated by a desire to synthesize the knowledge and experiences you gained from your extensive research, or was there another reason behind it?

There were two main reasons for my decision to cease my exploring adventure. One was the need to take stock and seriously look at what I was going to do with the vast accumulation of knowledge I had gathered over the previous thirty or so years. It was then, at a suggestion from a friend, that I decided to use my drawings and associated information to create a series of guides to help others identify what they had found with their microscopes. The second reason for settling down was that my dear old, normally reliable bus was starting to give up her mobility, and keeping her in a safe and viable condition was getting too expensive.



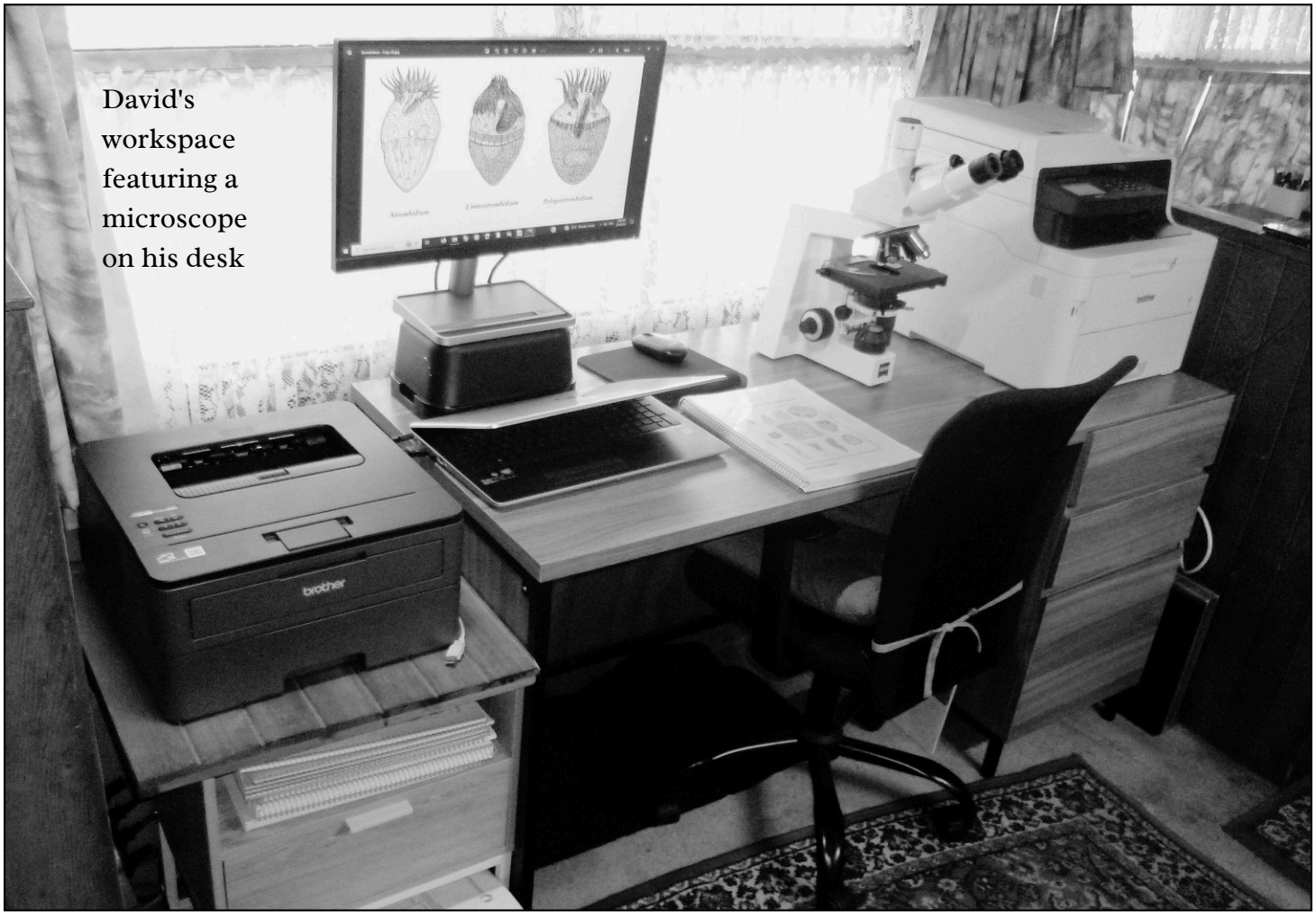


What advice would you give to beginners who are purchasing their first microscope? What key features or details should they focus on to get the most out of their experience, especially when studying microscopic life?

Stick to well-known brands and buy the best you can afford. While the microscope body and mechanics are important, the quality of its lenses is what will determine the quality of the experience. Don't be misled by offers that give you huge magnifications. The 100x oil immersion objective lens provides the highest magnification in most microscopes. Anything above that is just marketing.

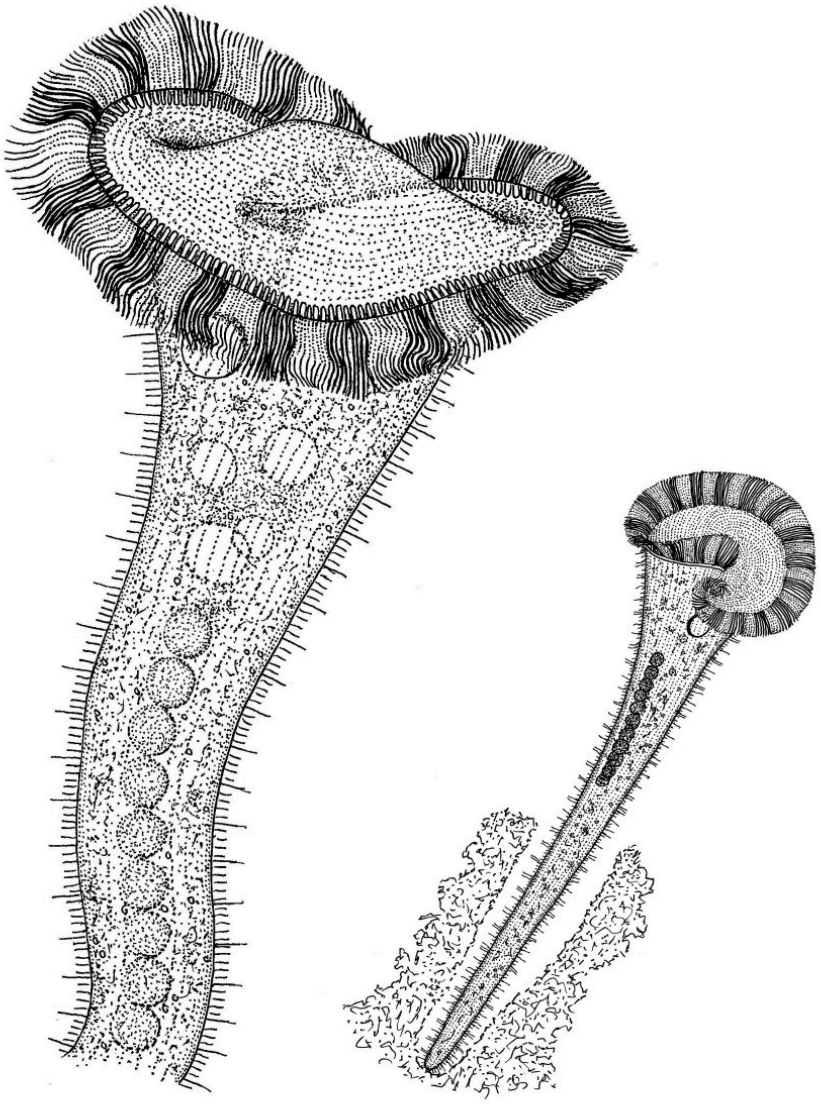
Do you think keeping a notebook with notes and sketches of observed microscopic organisms is a useful practice for beginners? How important is this for tracking progress and improving observational skills?

I have personally kept notes and sketches all my life. I find that sometimes going back over them may bring relevance to a new observation. Time, date, place, and a short description of the sampling site, as well as pH, are also important details to note.



David's workspace featuring a microscope on his desk

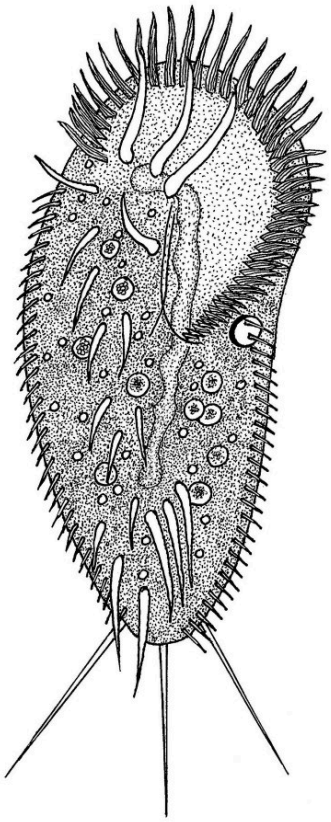
> WHILE THE MICROSCOPE BODY AND MECHANICS ARE IMPORTANT, THE QUALITY OF ITS LENSES IS WHAT WILL DETERMINE THE QUALITY OF THE EXPERIENCE



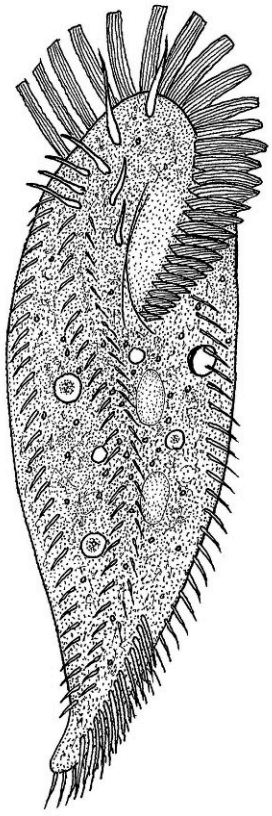
> THE 100X OIL IMMERSION OBJECTIVE LENS PROVIDES THE HIGHEST MAGNIFICATION IN MOST MICROSCOPES. ANYTHING ABOVE THAT IS JUST MARKETING.

Drawings of *Stentor introversus* from *An Illustrated Guide to the Freshwater Ciliate Stentor* by David Seamer

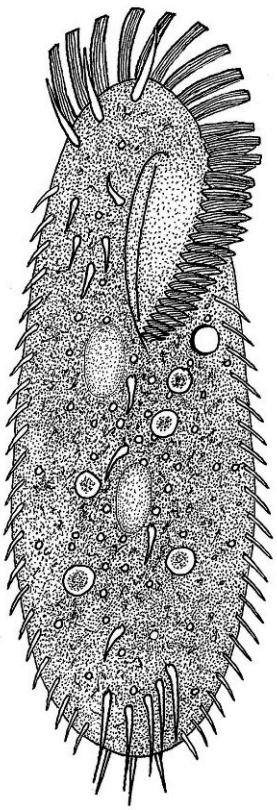
Drawings of ciliates from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



Stylonychia (Metastylonychia) nodulinucleata



Uroleptus musculus



Oxytricha fallax

Many beginners wonder if drawing is an outdated technique for recording the structures and shapes of microscopic organisms, given the advances in digital photography. Do you believe that photographs can fully replace drawings, or is there still value in sketching for scientific observation?

While micro-photography is an exciting development and amazing imagery is being accomplished, especially with Differential Interference Contrast (DIC) and photograph stacking, the proponents usually focus on the mechanics of photography and the results more than on their subject. When one draws an organism, one must actually look at and examine the subject, and so I believe one gains a better understanding of its physical nature. While it may take some time to set up your equipment, pressing a camera's shutter release takes a fraction of a second, whereas drawing your subject takes much more time, effort, and commitment. Some of my drawings take more than an hour to complete, but once done, I can honestly say, 'I truly know you'.

David is holding the plankton net

> FOR COLLECTING FROM OPEN WATER OR THE PELAGIC REGION, I USE A 30-MICRON PLANKTON NET, AND I FIND THIS TO BE AN ESSENTIAL PIECE OF EQUIPMENT

How do you capture microscopic organisms that inhabit the pelagic zone, or the open water of aquatic ecosystems? What methods or techniques do you find most effective for collecting these organisms?

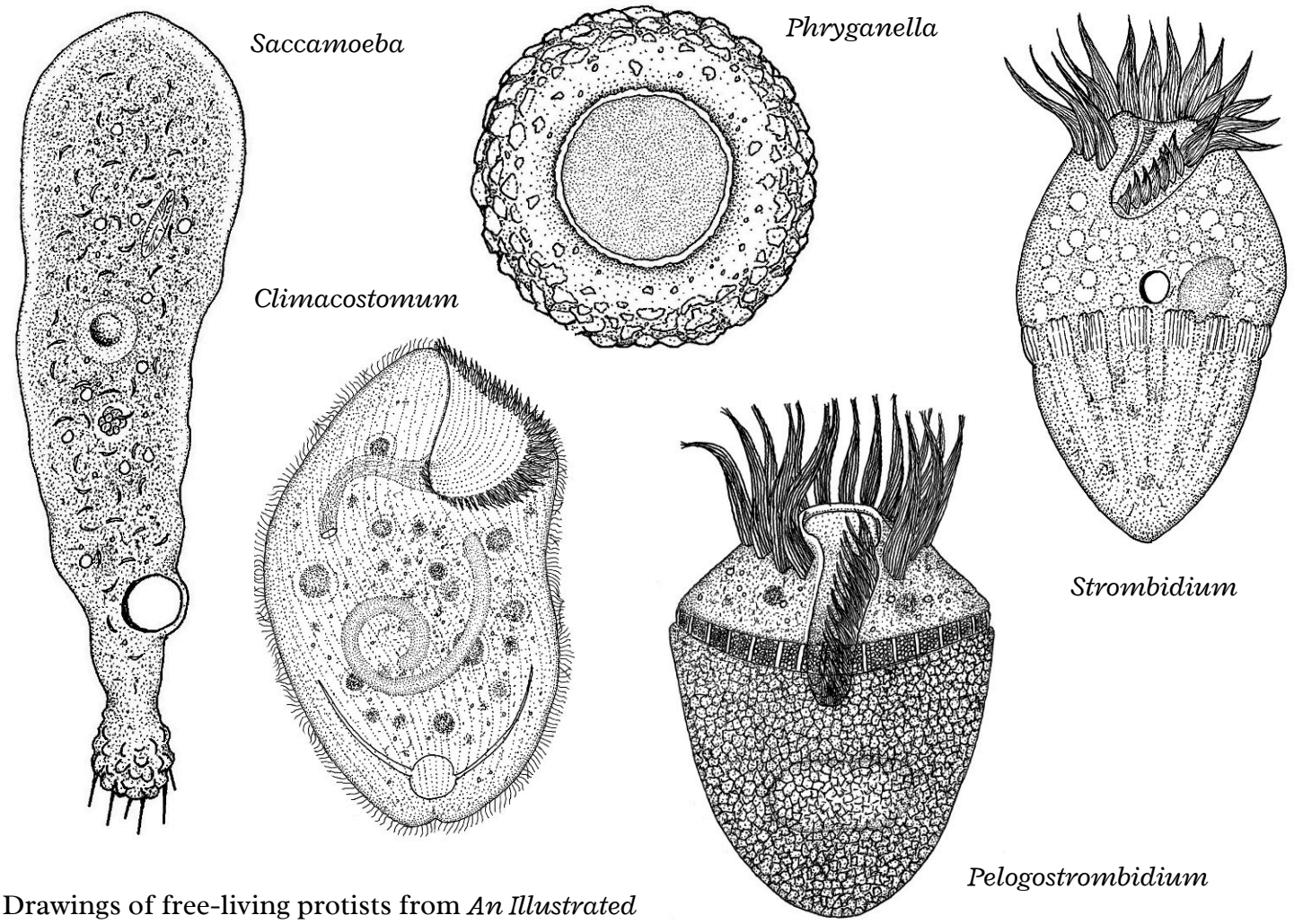
For collecting from open water or the pelagic region, I use a 30-micron plankton net, and I find this to be an essential piece of equipment. The plankton net can also be used to carefully drag across the marginal plants that grow in the shallow areas along the edges of ponds, but you should keep in mind the possible damage caused by sticks or rocks.

What techniques do you use to capture microscopic organisms that live on the bottom of aquatic ecosystems, such as in sediments or on submerged surfaces? How do these methods differ from those used for pelagic organisms?

For collecting samples from the detritus at the bottom of a pond or from the surface of solid submerged objects such as logs or rocks, I regularly use a cheap kitchen basting pipette. This collects a concentrated amount from a small area, whereas a plankton net collects a concentrated sample from a large volume of water.

When collecting samples, would you recommend beginners observe live microscopic organisms right away, or should they focus on preserving them for later study? What are the pros and cons of each approach?

While many ciliates will live in 'captivity' for quite a long time, others will die shortly after collecting, so immediate examination is essential if you want to study these species. Also, things such as natural shape are important for identification, and since some protists can either explode and disappear or be distorted with preservation, live examination is important. The way a ciliate moves may also be essential in defining a genus, as is the composition of the cilia of the cytostome and other defining features, which are best seen in live organisms. Diatoms are best when cleaned of chlorophyll, and they can be permanently mounted. With testate amoebae, while the original occupant may be dead and gone, their shells can be made into a permanent slide and examined well into the future.

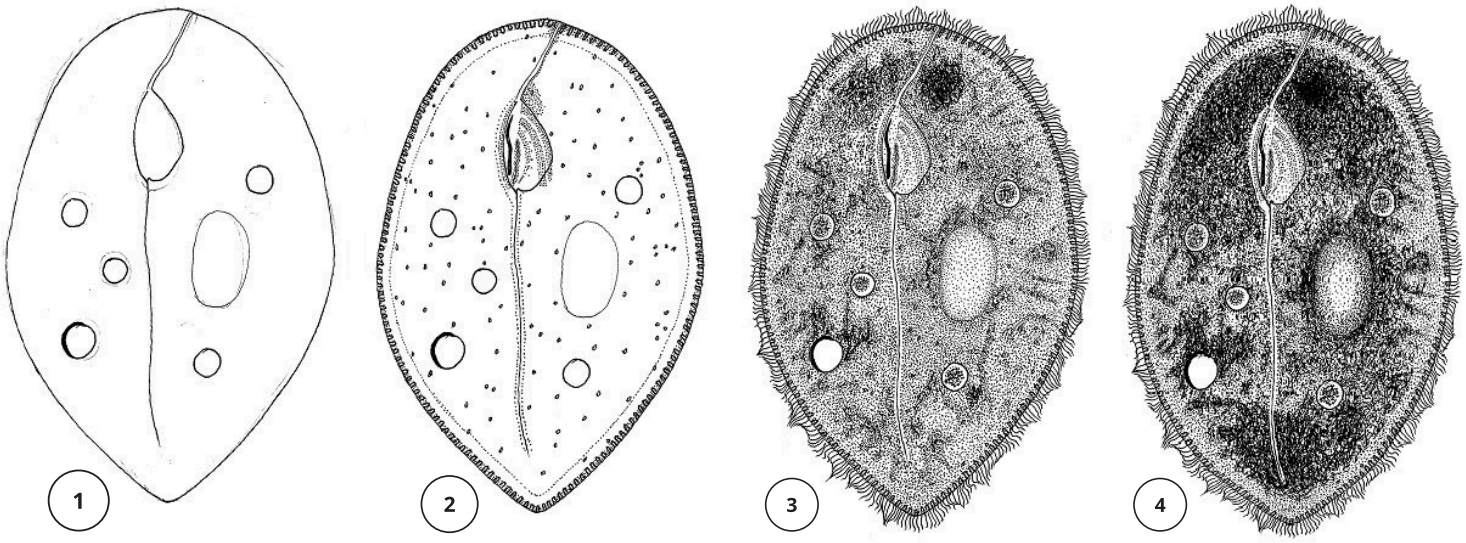


Drawings of free-living protists from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

Having illustrated many guides throughout your career, could you walk us through the techniques you use to create such detailed and accurate drawings of microscopic organisms? Are there specific tools or methods you rely on for precision?

I use phase contrast to illuminate my subject and do not use a camera lucida, but draw directly from life. Accurate dimensions are essential, so I use an eyepiece micrometer to establish the length and width, and, if possible, depth. Firstly, I draw the shape and relative features such as the cytostome, nucleus, food, and contractile vacuoles in pencil. Then, when satisfied with their correctness, I repeat with a fine felt-tipped pen. I then start 'filling in' the cytoplasm, remembering that it is not all one uniform density. I use a dot method, working from the outside in. Because the cytostome or mouth can be so important for accurate identification, I try to make this as detailed as possible.

Steps in drawing a ciliate by David Seamer



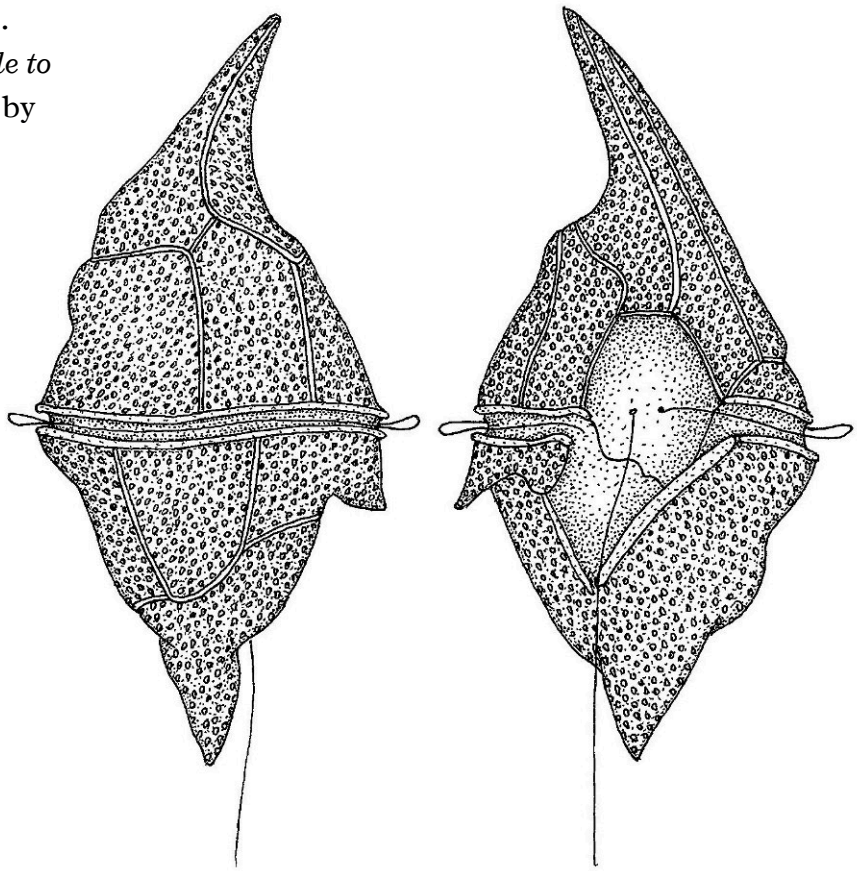
I first draw the general shape using accurate measurements and all the obvious features. It is important to draw only what you see and not what you think should be there. If you don't see it—don't draw it.

Then, details like granules and the peripheral trichocysts that lay just below the cell's pellicle. Fine detail should be given to the cytostome ciliation, which is important for identification.

Next come the food vacuoles, the nucleus or nuclei, if more than one. The cytoplasm is rarely uniform and often has rougher patches where, perhaps, pieces of past meals are still evident.

Finally, take a good look at the subject and try to identify what is distinctive about this particular genus. In this case, Frontonia has very dark patches at both ends, so I emulate them.

Drawing of *Ceratium* sp. from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



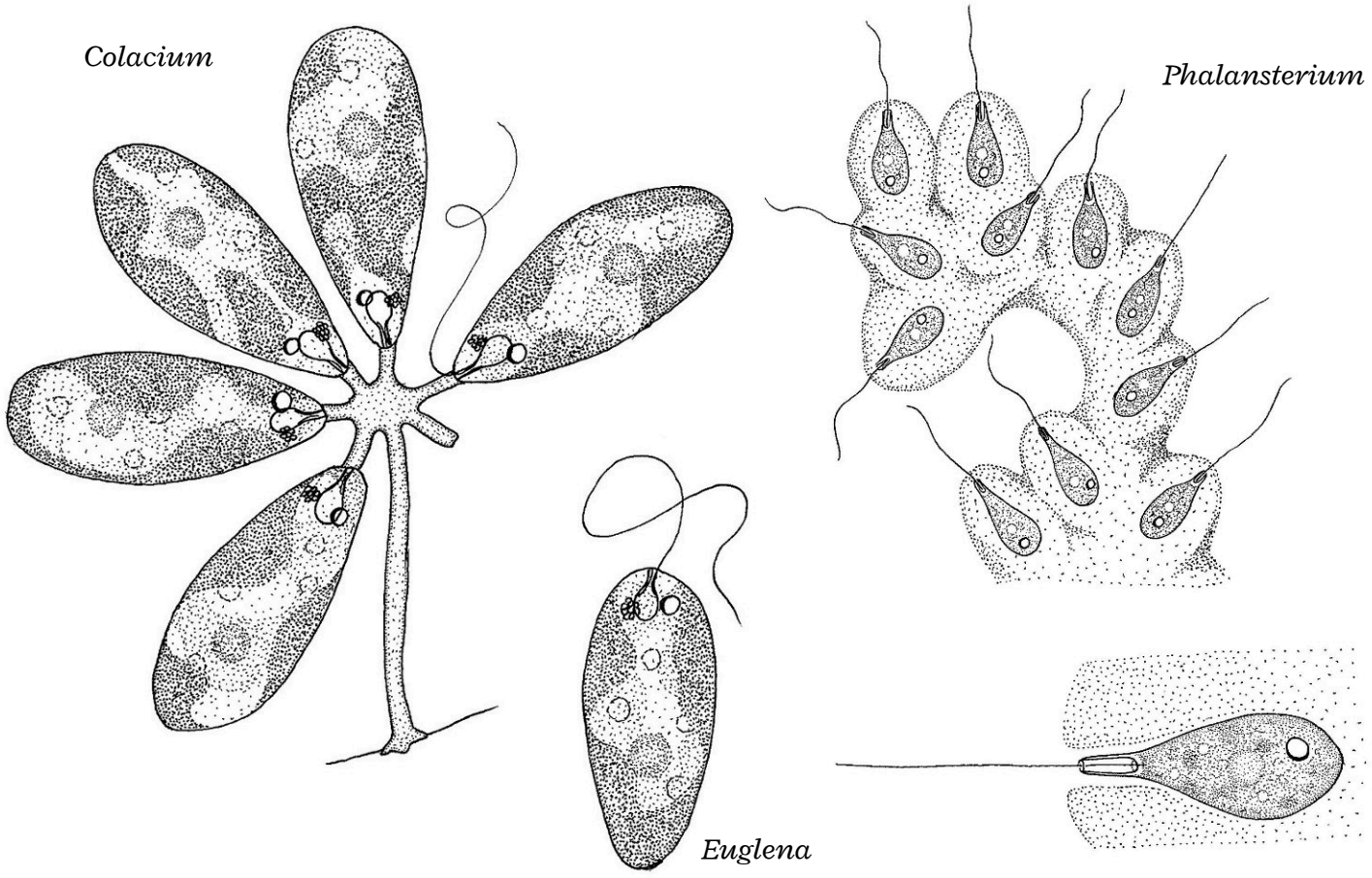
What are the most challenging aspects of illustrating microscopic organisms, especially considering their intricate structures and small size? How do you overcome these difficulties to achieve accuracy and detail in your drawings?

In two words: patience and observation. One must spend time carefully observing the organism, always looking for key indicators. The number and position of the nucleus or nuclei, if more than one, are important. In the case of ciliates, the location and type of cytostome or mouth, as well as the position and number of contractile vacuoles, are key factors. Their colour, if any, and the absence or presence of trichocysts just below the pellicle are also important.

With testate amoebae, not only the shape and size but also the composition of their shells or tests should be determined. If unfamiliar with the genus, try to find a live amoeba, as the nature of its pseudopodia and the way they move—whether flowing or eruptive—will help determine its taxonomy. With flagellates, the number and position of flagella, as well as whether they are solitary or colonial, are the obvious keys. Movement and color are also important.

You are the author of several books on freshwater microscopic life. What would you say is the biggest challenge you face when creating these books, particularly in balancing scientific accuracy with making the content accessible to a wider audience?

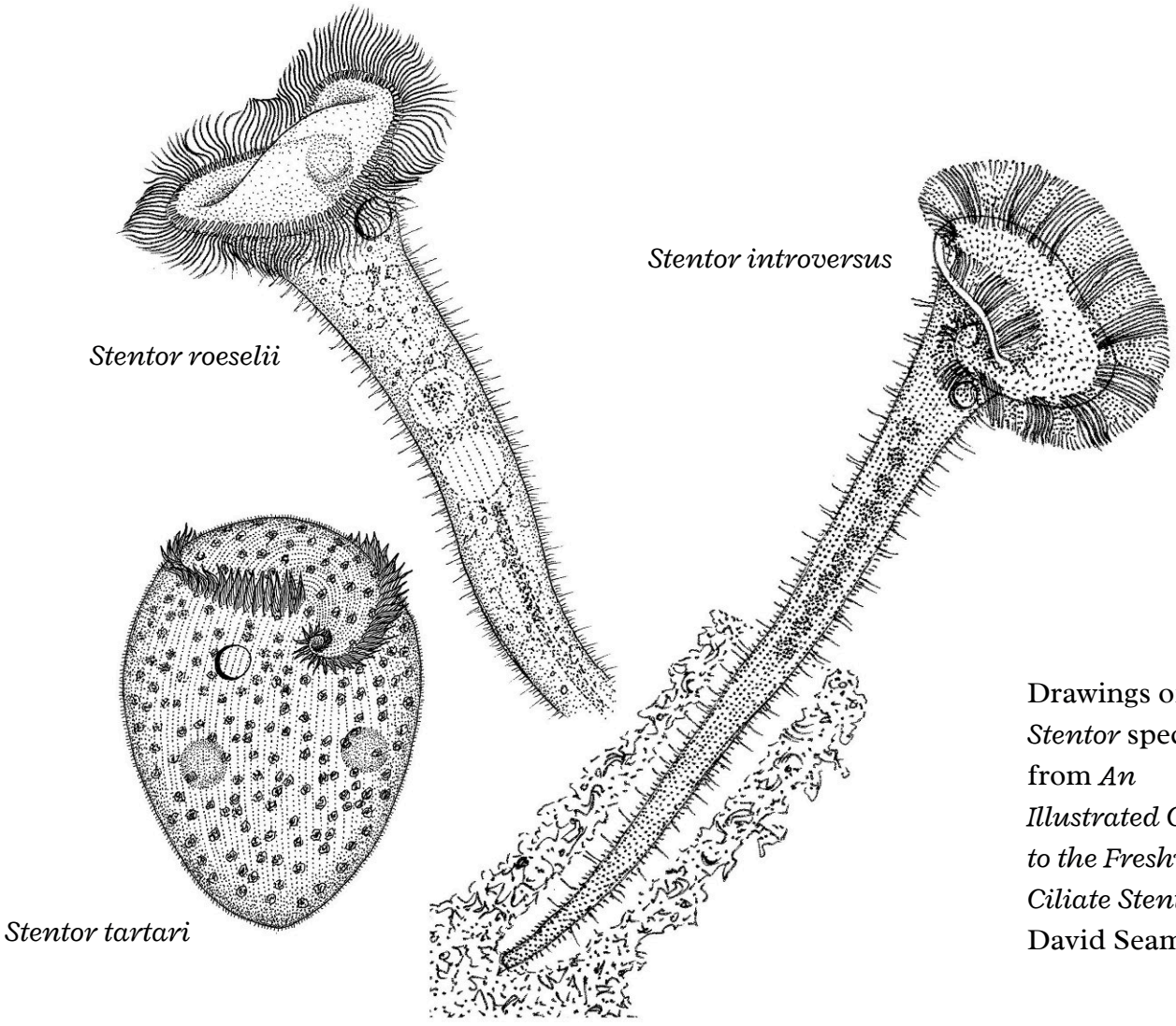
When I first started studying protozoa, the lack of appropriate reference and resource material was my biggest challenge. The books that were available to a high school student in the 1960s were either so simple that they were useless or written by scientists for scientists, and so were equally useless. When I decided to write my guides, I was determined, while being as scientifically accurate as possible, to make them user-friendly not only for amateurs, enthusiasts, students, and teachers, but also for professionals who had only a passing knowledge of the subject. Another big and constant challenge is trying to keep up with the latest changes in taxonomy, as organisms are constantly being assessed and sometimes reassigned to differing genera or allocated an entirely new name.



Drawings of flagellates from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

How many species are currently recognized within the genus *Stentor,* and what key characteristics can be used to distinguish them from one another? Are there specific features that you would recommend beginners focus on to accurately identify these ciliates?

There are currently 19 recognized *Stentor* species. There are three main identification indicators, and one of these is the shape and number of nuclear material. It can be singular, vermiform, or take the form of a long sausage, nodular, or a slightly separated row of beads, or moniliform, appearing as a closely joined string of beads. Another important indicator is the color of the pigment granules. *Stentor* can be blue, blue-green, pink, red, yellow-brown, violet, amethyst, purple, or totally lacking in color. They can also be green due to the presence of the symbiont algae *Zoochlorella*, which is another indicator of species.

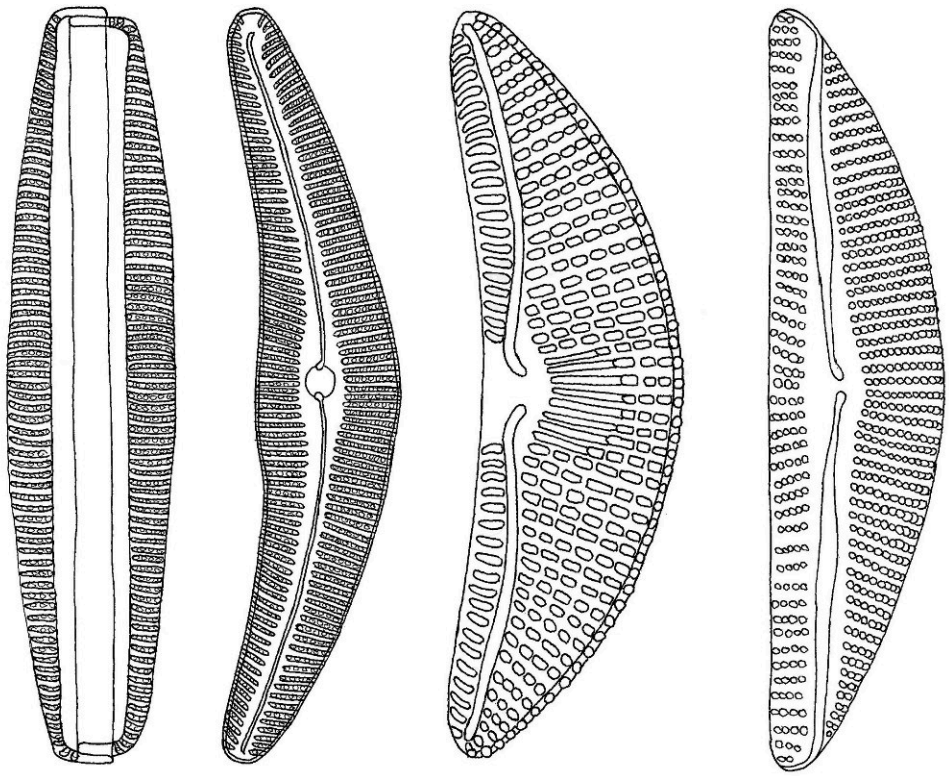


Drawings of *Stentor* species from *An Illustrated Guide to the Freshwater Ciliate Stentor* by David Seamer

In the numerous samples you collected from freshwater ecosystems in Australia, you encountered diatoms. Over the years, you created detailed drawings of many diatom species and eventually compiled them into a special book to help beginners with their identification. For precise species-level identification of diatoms, is an advanced light microscope necessary, or are there other methods that can be just as effective?

Firstly, let me say that I am not an expert on diatoms and only wrote this simple beginner's guide because of numerous requests to do so. One does not need a special microscope or accessories to ID diatoms. Diatoms are classified by their shape and size, and the two main types are centric and pennate. Centric diatoms are round and bilaterally symmetric, while pennate diatoms are oblong and exhibit lateral symmetry. The sides of the frustules (girdle) have a very different appearance from the top (valve), so they may need to be turned to aid identification. Identification also relies on comparing the intricate arrangements and patterns of the holes and slits on the surface of their shell or frustule. This is best done when the contents of the diatom are removed, and there are several ways of doing this, such as using organic solvents or an active agent, like hydrogen peroxide.

Drawings of diatoms from *Beginner Guide to the Freshwater Diatoms* by David Seamer



Cymbella *Amphora* *Encyonema*

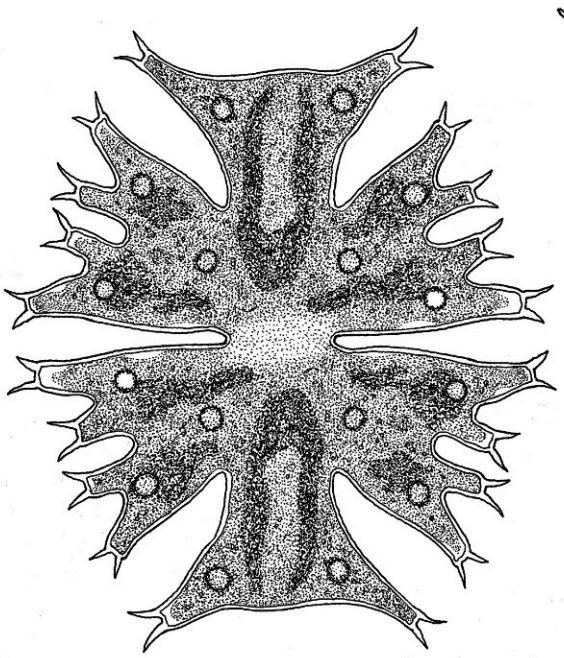
Desmids are a common and species-rich group of algae to which you dedicated a book. These green algae are often easier to identify than diatoms, especially with the excellent illustrations in your book. Why do you think beginners should pay particular attention to desmids, and what makes them an interesting group to study?

There are almost 40 genera, with approximately 6,000 species, all with something in common. They consist of two identical semi-cells that perfectly mirror each other, some of which are best described as extraordinary and beautiful. They may be found as individuals, as a filament of joined cells, or as a colony within a mucilaginous envelope.

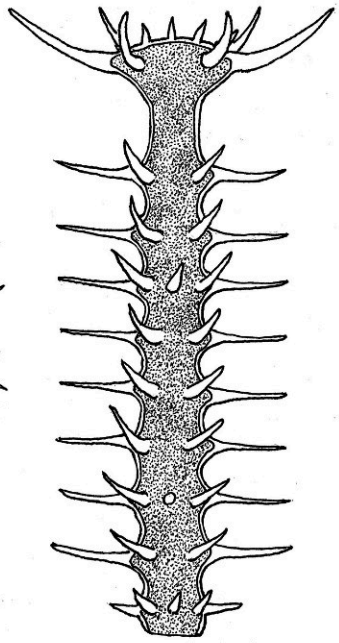
If someone wanted to collect a wide variety of desmid species, what types of habitats would you recommend searching in? Are there specific environmental conditions that are ideal for finding these algae?

Desmids can be found in freshwater habitats around the world, including ponds, rivers, and lakes, but they prefer wetlands with low nutrient levels, so fens, bogs, and mires are good places to look for desmids.

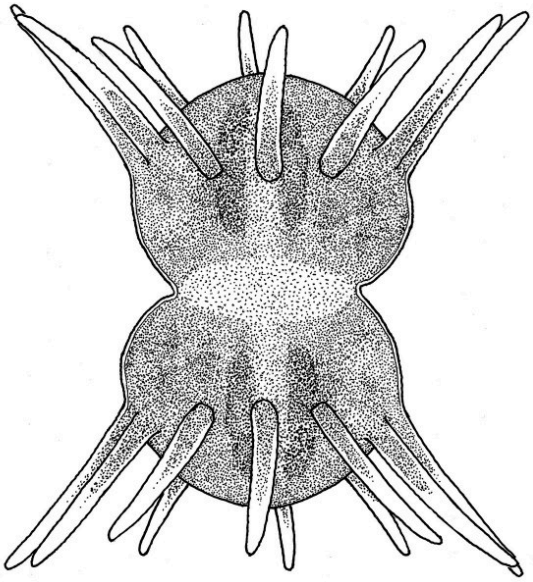
Drawings of desmids from *Beginner Guide to the Desmids* by David Seamer



Micrasterias



Triplocerus

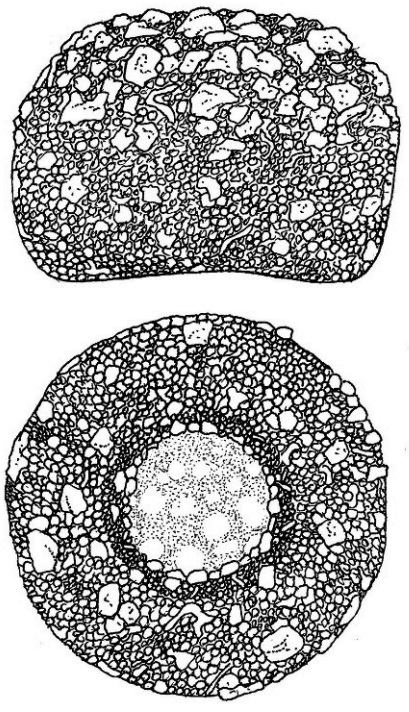


Staurastrum

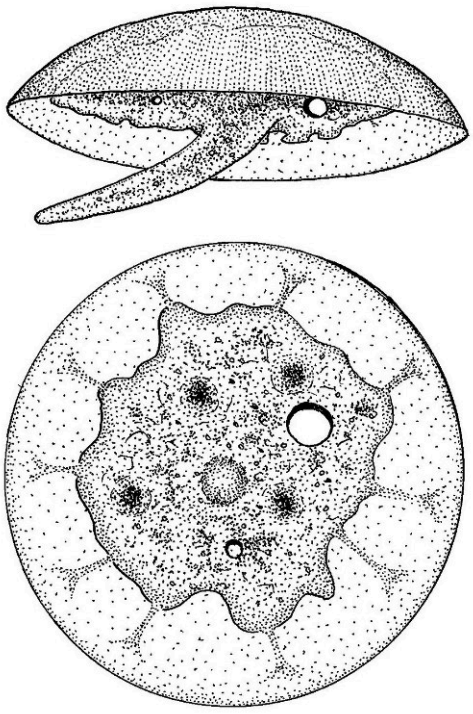
One of your most well-known books is "An Illustrated Guide to the Freshwater Protozoa", in which you gave special attention to amoeboid protists. During your research, did you come across any rare species of amoebae that particularly caught your attention, and what made them stand out?

During my studies in Tasmania and southeast mainland Australia, I found and recorded a variety of amoebae, both naked and testate, from various geographical locations, including alpine and lowland *Sphagnum* bogs, riverside wetlands, and inner-city garden ponds. Most were relatively common, but some were quite unique. I found the *Sphagnum* bogs particularly interesting, as they revealed a huge diversity of cosmopolitan genera, like *Cyclopyxis*, *Lesquereusia*, and many species of *Arcella* and *Galeripora*, as well as many testate amoebae endemic to the Southern Hemisphere, such as *Apodera*, *Alocodera*, and *Certesella*. There were also a number of more exotic species, such as *Playfairina*, *Schaudinnula*, and I found what may be a new species of *Paramphitrema*.

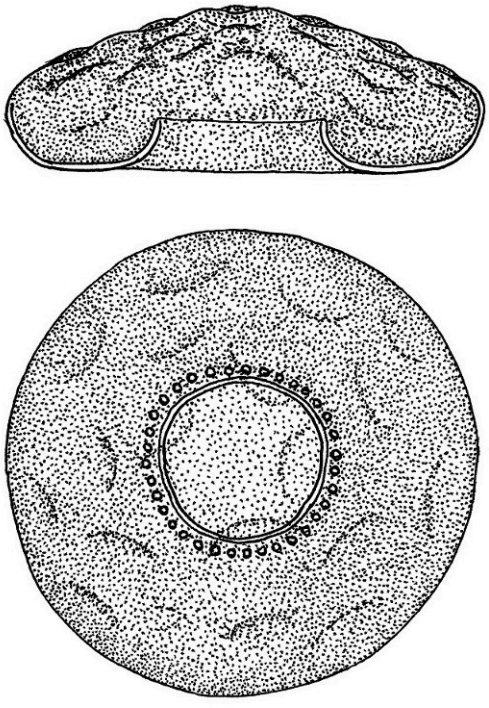
Drawings of testate amoebae from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



Cyclopyxis



Microchlamys



Galeripora

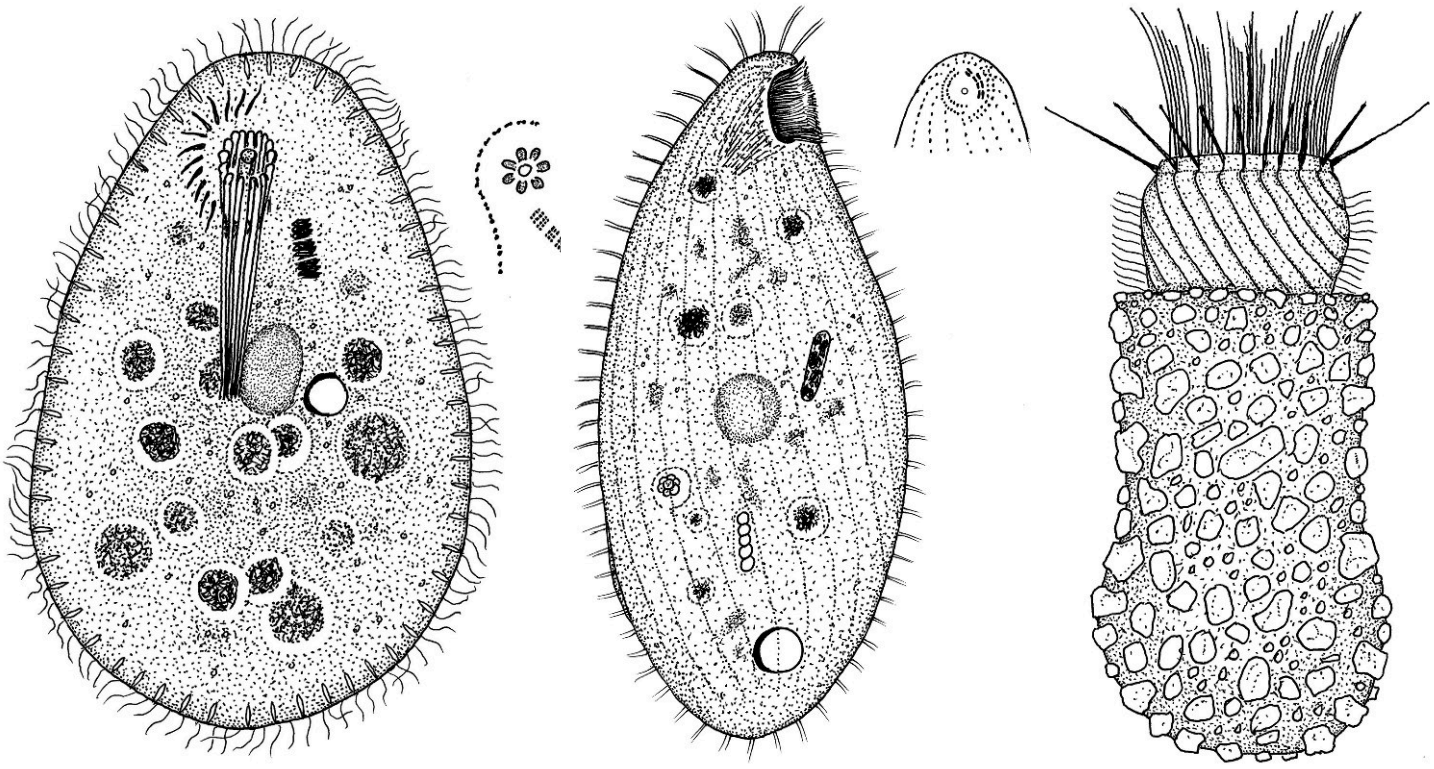
Do you think beginners should prioritize species identification when observing microscopic organisms, or should they focus more on understanding the behavior and interactions of these organisms?

I personally think identification is more important, because if you know the name of something but don't have sufficient time or means to observe its behavior, you can always research it. However, if you know how it behaves, that won't necessarily help you discover its identity.

Do you have a favorite group of microscopic organisms that you find particularly fascinating or rewarding to study? What is it about them that captures your interest?

If I am honest, I would have to say that ciliates in general, but especially the peritrichs, are what I find fascinating. What I find particularly interesting is the enormous diversity and the subtle changes that separate the different genera. However, the entire range of protists is enough to fill a lifetime of wondrous study.

Drawings of ciliates from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



Naxella

Platyophrya

Codonella

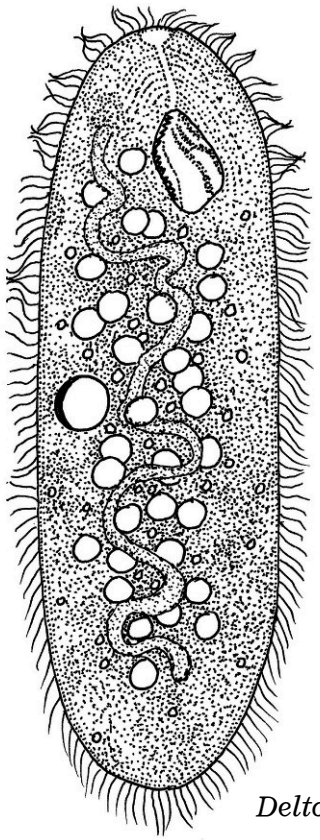
Which group of microscopic organisms do you find the most challenging to observe and illustrate, and what makes them particularly difficult to capture accurately?

Possibly the Hymenostomata, because many of them are small but move frequently and very quickly, making continuous observation difficult. Also the hypotrichs, because the difference between genera is based on the pattern of the cirri and so one must be extremely accurate in ones drawings. Naked amoeba are the hardest to identify because of their constant shape changing.

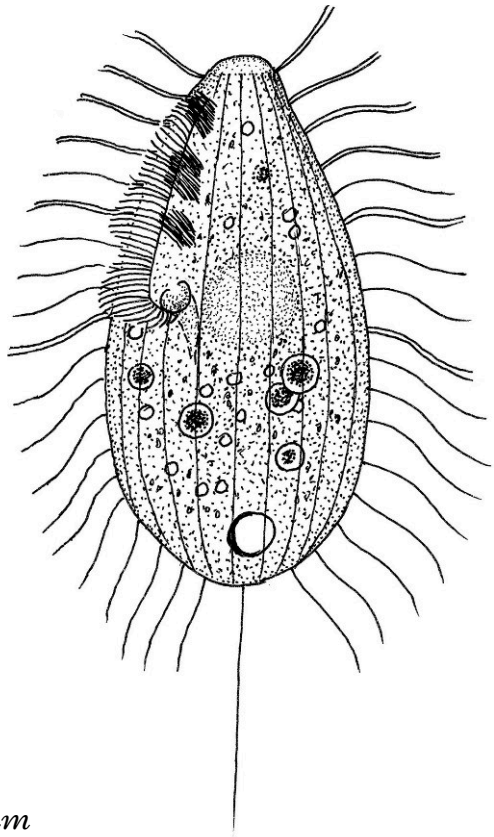
Was there a particular book or author that inspired you when you decided to write your first book on microscopic life? How did their work influence your approach to writing and research?

Initially, there was *Freshwater Biology* by Ward and Whipple, and the two-volume set by Colin R. Curds and colleagues. More recently, my inspiration has come from the amazing works of the esteemed, late, and sadly missed Prof. Dr. Wilhelm Foissner. The accuracy and detail of the illustrations in all these works reaffirmed my resolve to do the same.

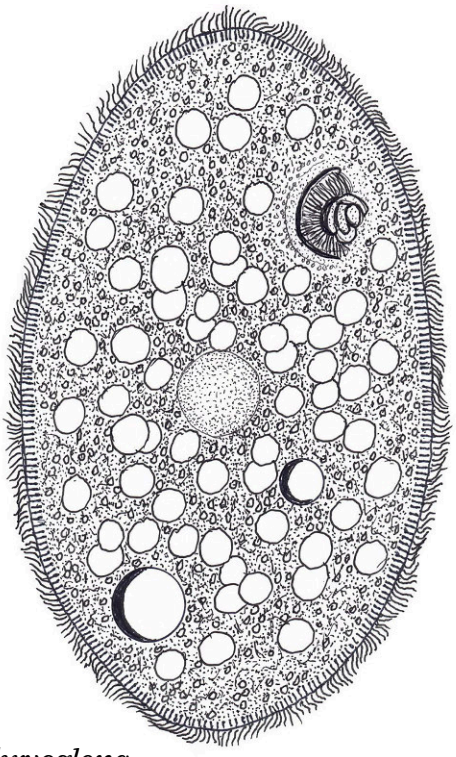
Drawings of Hymenostomata from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer



Deltopylum



Uronema



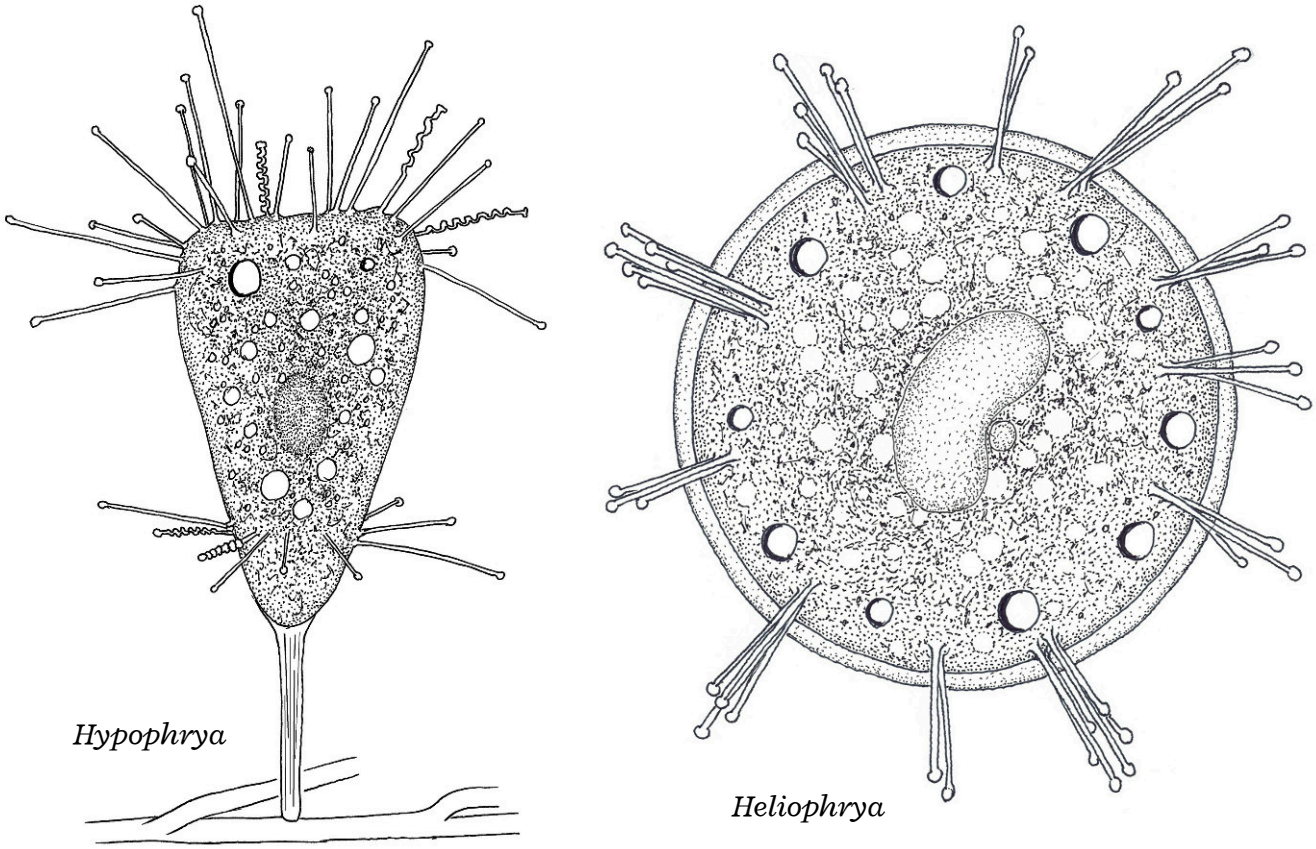
Ophryoglena

You dedicated one of your books to the freshwater free-living peritrichs, a fascinating group of ciliates. The cover features illustrations of nine very different ciliates — what is it about these organisms that makes them particularly captivating to you?

As I have already said, what I find fascinating about the order Peritrichida, is the enormous diversity and the subtle changes that separate the different genera. One cannot help but be amazed by the multiplicity of design created by these unicellular organisms.

The genus *Vorticella* is one of the most well-known in the peritrich group. Did you encounter these ciliates often during your research.

As you say, *Vorticella* is one of the most well-known peritrichs and is found in almost every sample, with perhaps the exception of fast-flowing water. However, there are many vorticellids that look like *Vorticella* but are not, and careful study of their stalk, for example, is needed to confirm identification. Also, colonial peritrichs, such as *Carchesium* and *Zoothamnium*, when first establishing a new colony as a single individual, may be easily misidentified as *Vorticella*.



Drawings of suctorians from *An Illustrated Guide to the Freshwater Protozoa* by David Seamer

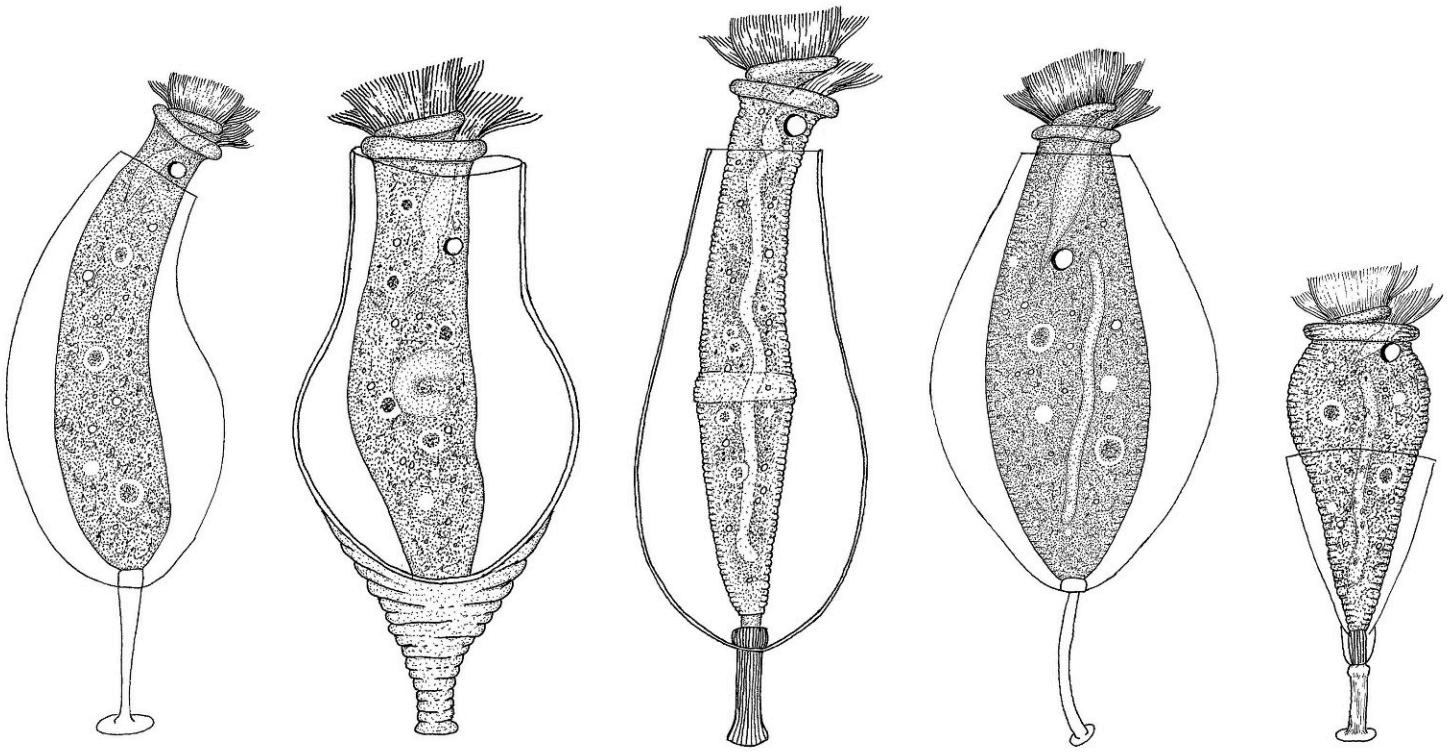
Peritrichs from the genus Cothurnia were among the first microscopic organisms observed by Antoine van Leeuwenhoek when he invented his microscope in the 17th century. These ciliates clearly had a profound impact on your work, as you dedicated an entire book to them. What was it about *Cothurnia* that inspired such a deep focus in your research?

While many of the loricated peritrichs have had multiple guides and species reviews, *Cothurnia* has not. Possibly because of the subtleness of species, this genus has been largely overlooked, and having found a number of their species myself, I thought they deserved to be better represented.

What features make the genus *Cothurnia* unique, and what sets it apart from other similar ciliates?

Each of the loricated peritrichs has a unique feature that defines its genus. *Cothurnia* is no different and, like all peritrichs, has its own set of identification indicators. Their tests are anchored to the substrate via a stalk, as with *Pyxicolar* and *Pseudothuricola*, but they have no operculum.

Drawings of *Cothurnia* species from *An Illustrated Guide to the Freshwater Peritrich Cothurnia* by David Seamer



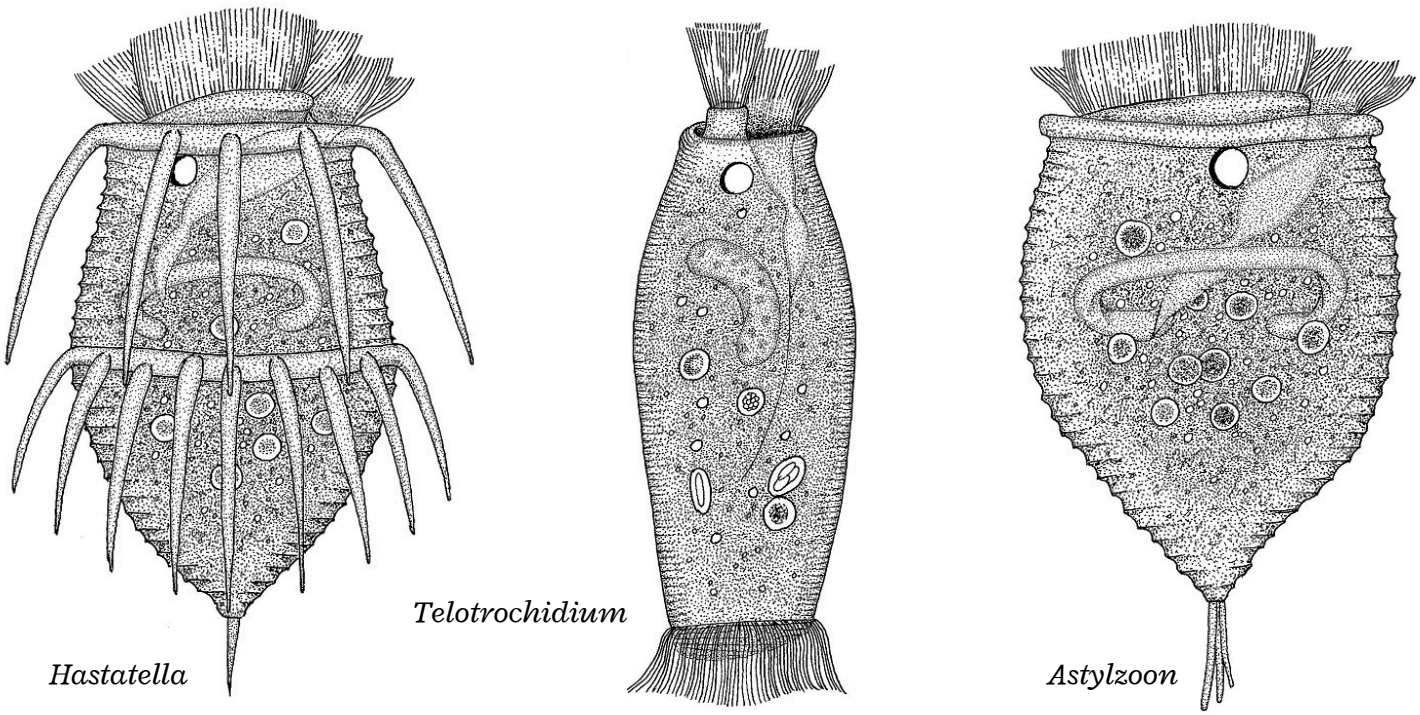
C. recurvata *C. bavarica* *C. imberbis* *C. clausiens* *C. acuta*

Can you tell us about the lesser-known group of peritrichs that lack a contractile stalk—those species that actively move through the aquatic environment? What makes their behavior and characteristics so unique?

Whilst most peritrichs are sessile, that is to say they are anchored to a particular spot, there area small number that are not and are found swimming in the water column. These motile genera include *Astylozoon*, *Hastatella*, *Opisthonecta*, and *Telotrochidium*.

Ciliates are known for being active and fast-moving organisms. How do you manage to observe the tiny details within their cells and accurately transfer them onto a drawing?

Ciliates, by their very nature and structure, can be active and fast-moving, and therefore some species are difficult to observe in detail. However, with the application of patience and various inhibitors, fine details can be recorded. Methylcellulose or Protoslo (Protoslow) can be added to the microscope slide, which will slow ciliates down while maintaining their shape and movement. A little cotton wool added to the slide will also inhibit ciliate movement.



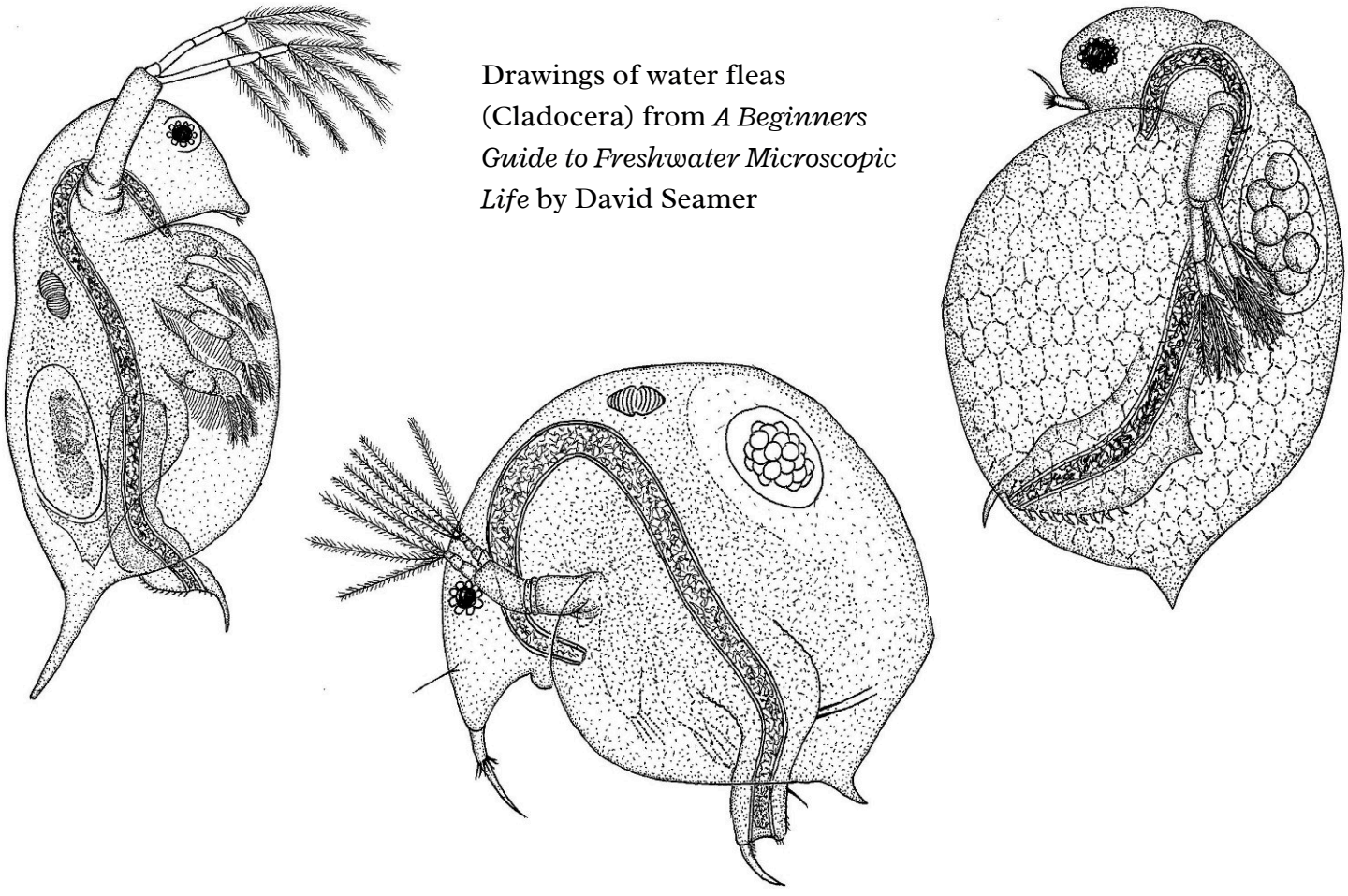
Drawings of peritrichs that lack a contractile stalk from *An Illustrated Guide to the Freshwater Free-living Peritrichs* by David Seamer

Your comprehensive book, *A Beginner's Guide to Freshwater Microscopic Life*, is an invaluable resource for beginners, as it covers all groups of microscopic organisms found in freshwater ecosystems. You devoted special attention to invertebrate groups such as cladocera, copepoda, rotifera, and gastrotricha. Which of these groups do you find most fascinating to study, and why?

To be honest, I don't particularly find multicellular invertebrates that interesting, but my readers are bound to come across them as they explore the micro-world, so I have included them. It is always better to have a more comprehensive knowledge base than a lesser one.

Finally, what are you currently working on? Are there any new books you're planning to undertake in the near future?

I am currently working on a new book, *Pond Life Beyond the Naked Eye*, which is a guide positioned between *A Beginner's Guide to Freshwater Microscopic Life* and *An Illustrated Guide to the Freshwater Protozoa*, which I hope to complete by the year's end.



Drawings of water fleas (Cladocera) from *A Beginners Guide to Freshwater Microscopic Life* by David Seamer

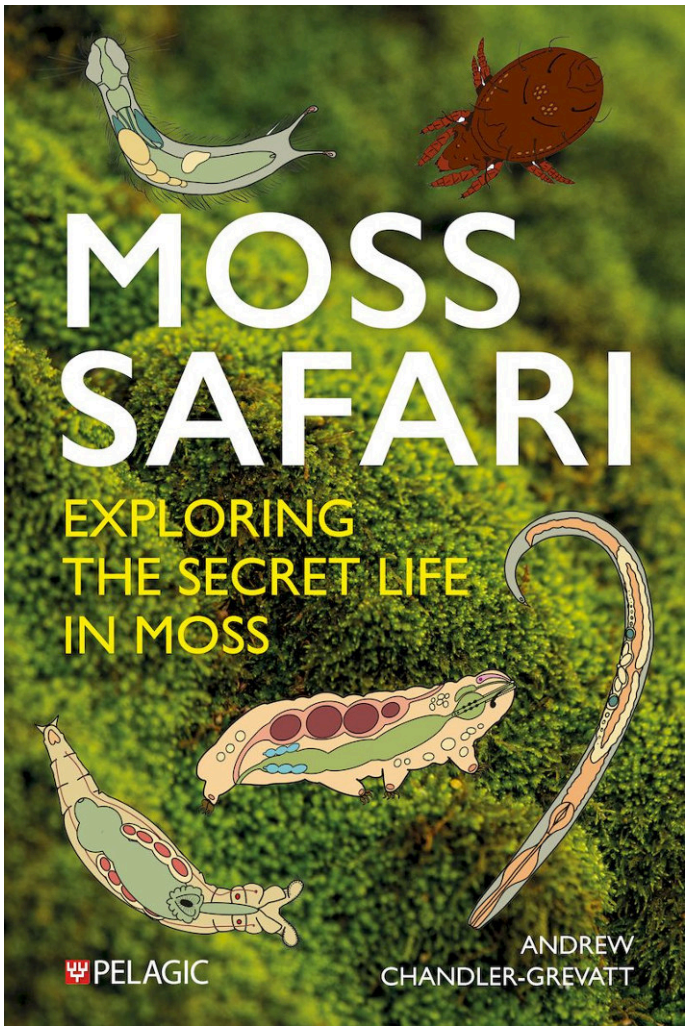
CHANDLER-GREVATT

Inspiring Young Explorers Through Moss Safari

Dr. Andrew Chandler-Grevatt created Moss Safari, a unique project that inspires young minds to explore the microscopic wonders of moss. In this interview, he shares the inspiration behind the idea, the importance of fostering scientific curiosity in children, and the current state of microscopy popularization.

Interviewed by
Dr. Stefan Luketa

New book *Moss Safari* by Andrew Chandler-Grevatt



In a world where digital screens often dominate children's attention, fostering curiosity about the natural world can be a challenge. But what if science could become an adventure—one that leads young explorers into the hidden wonders of nature? This is exactly what Dr. Andrew Chandler-Grevatt aims to achieve with *Moss Safari*, an engaging program that introduces children to the fascinating world of microscopy through moss and the tiny life forms it harbors.

Through hands-on exploration, kids get the chance to peer into a world invisible to the naked eye, discovering tardigrades, protozoa, and other microscopic creatures living in moss. More than just a science lesson, *Moss Safari* is an invitation to observe, question, and develop a lifelong love for discovery.

In this interview, Andrew shares the inspiration behind the project, the importance of introducing microscopy to children, and how looking at a drop of water through a microscope can change the way we see the world.

Andrew observes moss-squeezes under the microscope

What sparked your initial interest in microscopy and science? Was there a specific moment or experience that particularly influenced your path into this field?

I had a little plastic microscope as a boy—probably around seven years old—given to me one Christmas by my parents. I remember looking at prepared slides of insect parts and plant structures, and I still have those sets today. I used to examine dead insects, particularly spider fangs, flies, and fleas from my pet dog and cats. I also had a couple of kids' books about microscopy and have always been fascinated by hidden worlds—the deep sea, distant planets, and creatures we can only see through a microscope.

“

I HAD A LITTLE PLASTIC MICROSCOPE AS A BOY—PROBABLY AROUND SEVEN YEARS OLD—GIVEN TO ME ONE CHRISTMAS BY MY PARENTS

Andrew photographs mosses in the forest

Did you have a role model or someone in your childhood who influenced your decision to pursue biology? If so, how did they shape your passion for the field?

My grandmother had a rural upbringing and could name most plants, insects, and birds. She had hefty natural history books on her shelf, and when I opened them, pressed flowers would often fall out. My dad was a keen gardener and freshwater fisherman, always showing me interesting animals, insects, and plants. My mum learned bird identification from my dad, and it became a shared experience—we still watch garden birds together and sometimes visit the wetlands to observe from a hide.

“

MY GRANDMOTHER HAD A RURAL UPBRINGING AND COULD NAME MOST PLANTS, INSECTS, AND BIRDS

Wet mosses after rain usually contain a lot of active microscopic organisms

My passion for biology feels innate—I've always been curious about living things, whether it's our pets, garden wildlife, or creatures in rock pools. I loved science at primary school, particularly watching tadpoles develop into frogs. At secondary school, my fascination grew thanks to teachers like Mr. James, who showed me stomata on a leaf under a microscope, and Mrs. Shephard, who encouraged my interest in biology, animal welfare, and the environment. I originally wanted to be a vet but didn't get the grades, so I pursued a degree in biological sciences, followed by a master's in crop production in a changing environment, coinciding with the first IPCC report.

As a teacher, I try to help my students see the world through a biological or ecological lens —understanding that everything is connected. In Moss Safari, for example, we see how a diatom in a moss cushion is linked to the sun, gas exchange, and the global nutrient cycle.

IN MOSS SAFARI WE SEE HOW A DIATOM IN A MOSS CUSHION IS LINKED TO THE SUN, GAS EXCHANGE, AND THE GLOBAL NUTRIENT CYCLE

It is not necessary to take a large amount of moss; a small tuft is sufficient

Could you share the story behind the creation of Moss Safari? What first sparked your passion for exploring the microscopic world of mosses, and how did that lead to the founding of the project?

It all started with a colleague, Professor Jonathan Bacon, a neuroscientist at the University of Sussex. We bonded over a passion for science education and worked on research projects about children's understanding of neurons and the nervous system. Alongside this, Jonathan introduced me to a lab activity where undergraduates examined life in moss simply by squeezing it and observing the water under a microscope. His favourite organism was the tardigrade, and he even ran this as an outreach activity with a local school.

I tried the moss squeeze method at home and was instantly hooked by the tiny life forms in the moss that had fallen from my roof. I started observing, recording, and researching these organisms. When I shared my discoveries with colleagues and trainee science teachers, they loved it—but many felt unsure about doing the activity themselves. That's when I developed Moss Safari. Inspired by traditional safaris, I chose five common multicellular organisms—the "Microscopic Big Five"—and created simple guide sheets to make the activity accessible to everyone, from children to adults.

Two of the fifteen Duralab microscopes donated by VITTA Education to use for Moss Safari outreach

Tell us more about Moss Safari. What are the main goals of the project, and what do you hope to achieve through it?

At its simplest, Moss Safari is an interactive event where I squeeze soaked moss, place a few drops of water on a microscope slide, and project the microscopic world onto a big screen. I introduce the audience to the moss habitat and the Big Five: mites, nematodes, rotifers, tardigrades, and gastrotrichs. We explore the slide together, stopping to examine any creatures we find, and I share their stories—their life cycles, adaptations, and significance in the local and global ecosystem.

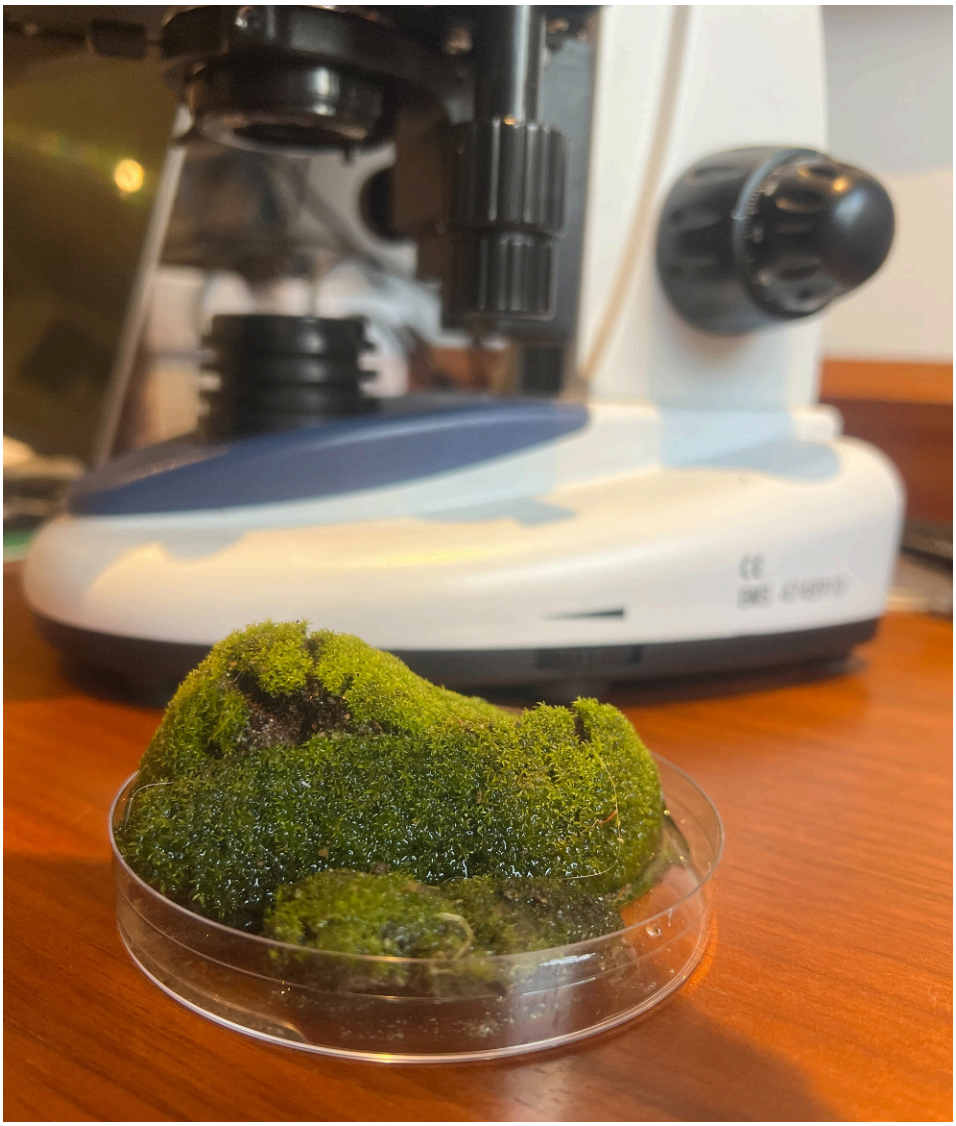
Over time, the goals of Moss Safari have evolved. I now focus on three key aims:
(a) *Connecting people with nature* – encouraging awareness and appreciation of moss and its hidden life.
(b) *Promoting microscopy* – making it accessible and helping people feel confident using a microscope.
(c) *Increasing participation in STEM* – inspiring both children and adults, particularly those who haven't had the opportunity to explore microscopy before.

During in-person big screen events, Andrew projects the microscope's view onto a large screen, guiding the audience through a live safari

Could you explain the concept of "microscopes as science capital"? How does this idea relate to making science more accessible and engaging for a wider audience?

When people encounter a microscope, they react in two ways: either with excitement and curiosity or with hesitation, unsure how to use it. Science Capital—a concept introduced by Professor Louise Archer at UCL—describes the science-related experiences and knowledge that shape a person's engagement with science.

Some children arrive at school with high Science Capital because they have family members who discuss or engage in science, have prior knowledge, or have done hands-on activities like using a microscope or telescope. Others have low Science Capital and may feel disconnected from science. My belief is that a positive



All you need for a moss safari is a shallow plastic container with a small amount of water, a clump of moss, a pipette, a slide, a cover glass, and a microscope

experience with a microscope early on can change that—helping children feel capable, engaged, and more likely to pursue science-related paths. I really believe microscopes are a huge part of Science Capital—if you've had a good experience using one as a kid, you're more likely to feel at home in science later on. This hands-on experience builds confidence and opens doors to curiosity-driven exploration. The more familiar and comfortable children are with scientific tools, the more likely they are to see science as something they can participate in, rather than something distant or inaccessible.

> THE MORE FAMILIAR AND COMFORTABLE CHILDREN ARE WITH SCIENTIFIC TOOLS, THE MORE LIKELY THEY ARE TO SEE SCIENCE AS SOMETHING THEY CAN PARTICIPATE IN

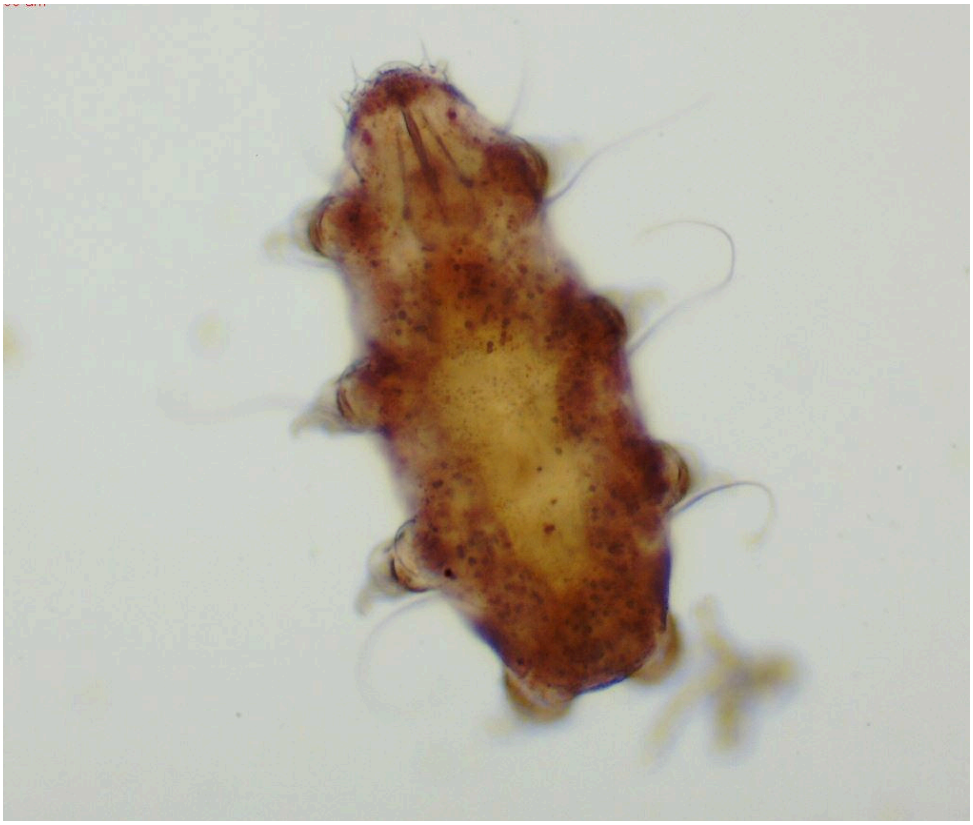
Live Moss Safari, an interactive workshop

Could you describe what your workshops at science festivals are like? What activities do children seem to enjoy the most, and are there any that they find particularly challenging?

I run three types of Moss Safari events:
(a) *Online Live Moss Safari* – a 50-minute virtual journey where participants watch a live feed of the microscopic world, with storytelling and discussions about the creatures we find.
(b) *In-Person Big Screen Events* – I project the microscope's view onto a large screen, guiding the audience through a live safari. The real-time reactions—wows, oohs, and ahs—are fantastic.
(c) *Hands-On Festival Stands* – at events like *New Scientist Live*, visitors use pipettes to collect water from moss, place it on a slide, and discover what they've caught.

One challenge is ensuring children have a good first experience with a microscope. I collaborate with VITTA Education, using their durable microscopes, and focus on simple, hands-on techniques to make microscopy accessible.

Water bears (tardigrades) capture kids' imaginations the most because they are so resilient, so cute, and can survive in space

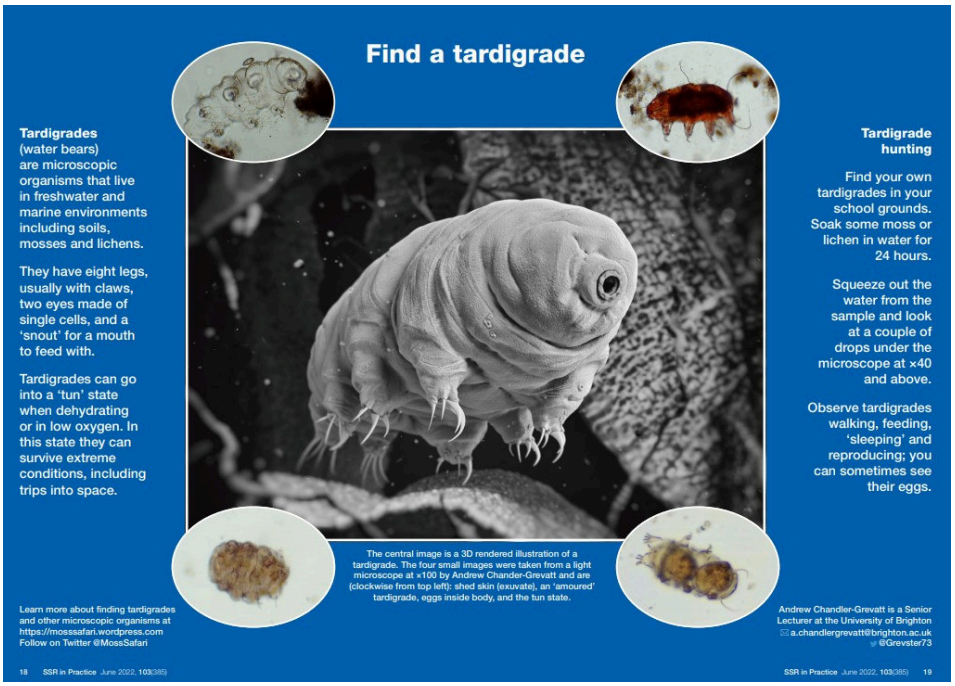


When children first explore microscopy, what aspects of microscopic organisms tend to fascinate them the most? Are there certain discoveries or features that seem to captivate their curiosity?

In my experience, it is seeing something moving that creates the most interest. Seeing a wiggling nematode, a feeding rotifer or a whizzing ciliate will create various reactions from 'yuck – what is that?' to 'wow – what was that?' Children like to be disgusted and surprised; it's an emotional response that causes curiosity.

Tardigrades capture kids' imagination the most, because they are so resilient, so cute and can survive in space. It fuels their imagination of creatures unknown to them that have 'superpowers' to survive the harshest of environments.

Having someone to tell the stories of the organism, their significance to our lives adds to the curiosity. For example, the bdelloid rotifers are all female and so we are curious how they have existed so long without males. Nematodes have won three Nobel Prizes based on the work scientists have done to understand them. This leads to lots of questions.



Moss Safari had a centre-fold feature in *School Science Review in Practice*, June 2022, encouraging teachers to *Find a Tardigrade*

How can microscopy inspire scientific curiosity in children? What key lessons or insights can they gain from observing microorganisms under a microscope, and how does this shape their understanding of the natural world?

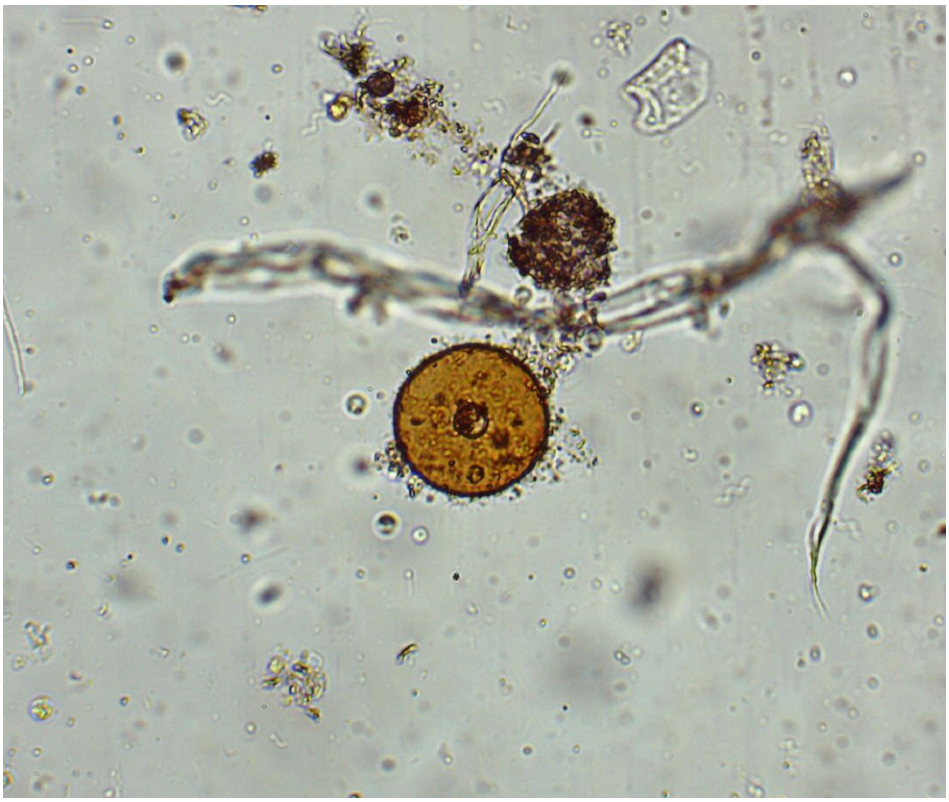
Looking through a microscope takes you into another world. It's unknown, so there is a sense of discovery. I often emphasise that you are the first human to see that individual organism, so they get a sense of privilege. This often leads to a sense of stewardship, wanting to protect the animals and the moss.

It reveals that there is more to the world than we can actually see. It shows that ants have faces, sugar is made up of tiny crystal cubes and in the case of moss, there is literally a complete ecosystem that is invisible to the naked eye.

It also extends their understanding of the natural world to beyond just themselves. I did a session on 'What is Life?' using Moss Safari and showed that humans are not typical organisms, most organisms are likely to be single celled and photosynthetic.

Finally, I think it is about connection. When they have seen what lives in moss, they are more likely to see moss in the world around them. Even in the cities there is moss, and this makes a connection with nature on their doorstep.

Andrew's favourite single-celled organisms are shelled amoebae from the genus *Arcella*



Do you have any favorite organisms to observe under the microscope? What is it about them that fascinates you the most?

I have been researching the microscopic Big Five for my book on Moss Safari and for each chapter, I became more and more fascinated by these organisms. Not just their adaptations and ability to survive such harsh conditions, but also that there is so much still unknown. Most of the discoveries about these animals are being made right now or at least within the last decade. The gaps in knowledge fascinate me. So I ended up falling in love with each organism for different reasons as I came to get to know them better.

However, if you want me to state my favourite. It must be the moss piglet, the tardigrade. Their diversity, their resilience, their ability to adapt fascinates me. I have already started writing about them for a future book. And I also have a favourite single celled organism. It's *Arcella*, the golden testate amoeba. To me they are like finding lucky pennies on a Moss Safari, I always stop and take a photo of them. I like trying to see what is inside their shell and when I see an active one, well I can watch it for ages.

You can follow Andy Chandler-Grevatt on BlueSky for news about Moss Safari

With your presence on various social media platforms, how do you think the rise of these platforms in the past two decades has influenced the way science is communicated to children? What role do they play in making scientific education more accessible and engaging?

Interestingly, for me, social media does not reach children. There are a lot of restrictions and most children are unable to access social media until they are in their teens. I haven't got into TikTok or Instagram as much as I should. However, Twitter/X in the past has connected me with other enthusiasts, Facebook groups have really helped me make connections with other amateurs and educators and more recently BlueSky has helped me connect with moss enthusiasts and other educators.

There are science teachers and school science technicians from the UK and all over the world who are obsessed with Moss Safari and use it in their schools. They are my main contacts about Moss Safari, often sharing their finds, asking questions about what they have found. I have two active hashtags #mosssafariID and #iseemoss.

Andrew at New Scientist Live 2024, an event that takes place at London ExCeL each year and attracts tens of thousands of visitors daily

What are some of the biggest challenges you face when educating children about microscopic organisms and microscopy? How do you address or overcome these challenges in your workshops to keep them engaging and informative?

Although microscopes are getting more affordable, they do present a cost for parents. However, a microscope is a comparative price to a computer game or console, so for many parents this is affordable.

Microscopy can be very complicated, but it doesn't have to be. Moss Safari uses only the most basic techniques. Adjusting the focus, the light source and the diaphragm. There are no stains, filters or special techniques involved. There are some brilliant videos out there that use expensive microscopes and sophisticated techniques to capture the images. I want to show case what is accessible, I want other people to be able to easily repeat what they see me doing. I hope then, once they get confidence, that participants will go on to more advanced approaches.

Andrew at The Association for Science Education Conference 2025, a great annual event to meet science educators nationally and internationally

What do school teachers identify as the main barriers to incorporating Moss Safari into their teaching?

Time. Moss Safari takes time and although it is curriculum linked, it does not always provide the organism you need on demand. For example, *Euglena* and *Paramecium* are listed in the National curriculum and exist in moss, but to observe them, you are better off getting a culture. The current National Curriculum in England is content heavy and doesn't allow for more open-ended practical. However, there are lots of opportunities in after school clubs, open evenings, science fayres, science weeks and so on which a lot of schools use Moss Safari for.

Schools face several challenges, the cost of a class set, ensuring that microscopes are maintained, having enough working microscopes to use and having teachers who after confident in using microscopes. Poor quality, unmaintained microscopes will give students a poor early experience that may put them off.

Moss Safari has collaborated with Vitta Education/Edulab to produce Moss Safari Kits for schools

In today's digital age, how do you strike a balance between traditional teaching methods and the use of new technologies when educating children?

Generally, teachers are very skilled at developing their teaching practice to include the latest technologies. That is technology has made microscopy more accessible. If you can afford a microscope and a camera for it, you are set up to record, research and share what you find. However, smart phones are fantastic for getting pictures from the eyepiece to record what you are seeing live. This is great in school situations (if they are allowed phones!).

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GENERALLY, TEACHERS ARE VERY SKILLED AT DEVELOPING THEIR TEACHING PRACTICE TO INCLUDE THE LATEST TECHNOLOGIES

Andrew organizes Hands-On Festival Stands at events like New Scientist Live, where visitors use pipettes to collect water from moss, place it on a slide, and discover what they've caught

What have been some of the most challenging moments you've faced in developing Moss Safari?

In live Moss Safari's – it's getting my laptop to connect to a school or other location's projector is always the most challenging half hour of any session! That's my main headache, but I've got a new laptop that is compatible with most connectors.

Then it is making sure I get to show off at least a couple of the live Big Five on the Moss Safari. As it's random, it doesn't always work. If we don't find a tardigrade, people leave disappointed!

The biggest challenge remains making it financially viable, at the moment I do this in my spare time and would love to make it work without having to take lots of money from schools. My target audience are often those least able to afford it, so I am still trying to find smarter ways of generating income to support activities.

In the next issue of *Amoeba Discovery*, we will discuss the book *Moss Safari*, which will be published on 27 May 2025. Picture is AI generated.

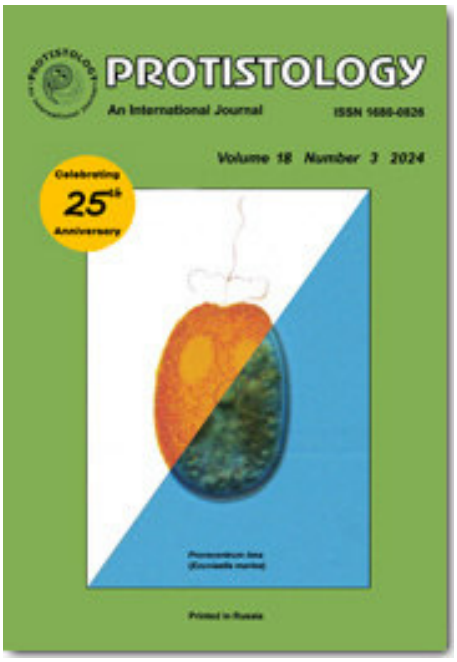
What's next for Moss Safari? Are there any exciting plans for expansion?

I'm excited about the upcoming publication of my book on the Microscopic Big Five, which I've been working on for a couple of years. I'm also developing outreach activities in Brighton and Sussex, particularly for young people from disadvantaged backgrounds, providing them with hands-on experience using microscopes.

Funding remains a challenge. Most of my work is voluntary, with some income from school visits and events. I'm seeking funding for a nationwide assessment of school microscopes to better support science education. I'm also always on the lookout for collaborations to bring the hidden life of moss to new audiences.

Ultimately, my goal is simple: to share the wonder of the microscopic world and make it accessible to as many people as possible.

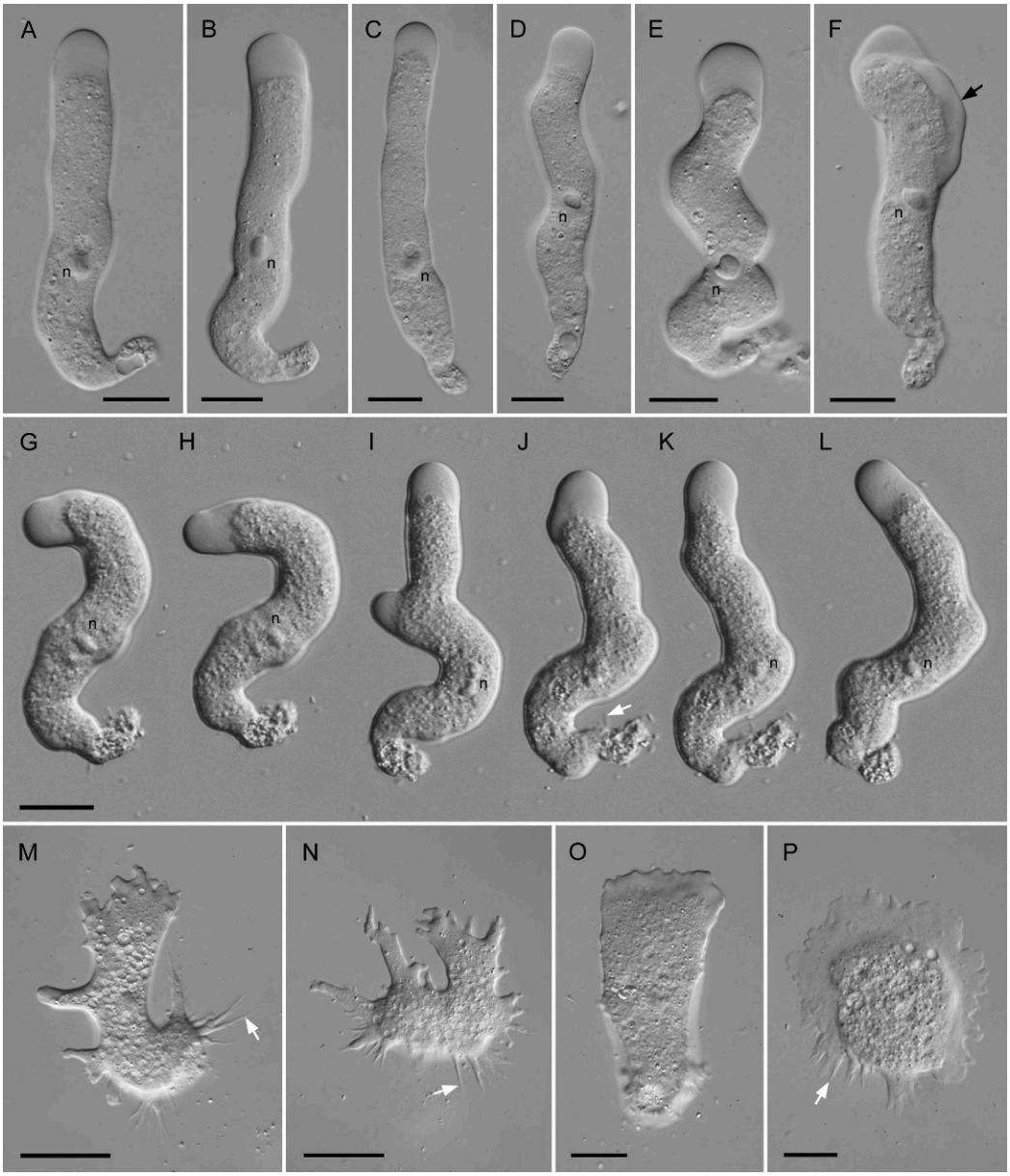
Reinvestigated Endemic Amoeba from the Island in the Largest European Lake



Kulishkin NS, Smirnov AV (2024) New data on *Leptomyxa neglecta* (Amoebozoa, Tubulinea, Leptomyxida). Protistology, 18: 40-51. DOI: 10.21685/1680-0826-2024-18-1-4

Lake Ladoga, the largest freshwater lake in Europe, has a fascinating history that stretches back thousands of years. Located in the northwestern part of Russia, its story is intertwined with the ancient Baltic Ice Lake, a massive body of water that existed nearly 10,000 years ago. At that time, Lake Ladoga was part of a much larger freshwater system, playing a crucial role in the early formation of the Baltic Sea. Initially, it was connected to the sea through a small strait, which allowed water to flow between the two. But as time passed, the region's geography began to shift due to natural changes. Slowly but surely, Lake Ladoga became more isolated from the Baltic Sea. By the Middle Ages, the lakehad fully separated, forming a distinct ecosystem that was entirely freshwater. This shift was pivotal in shaping the unique biodiversity that exists in Lake Ladoga today.

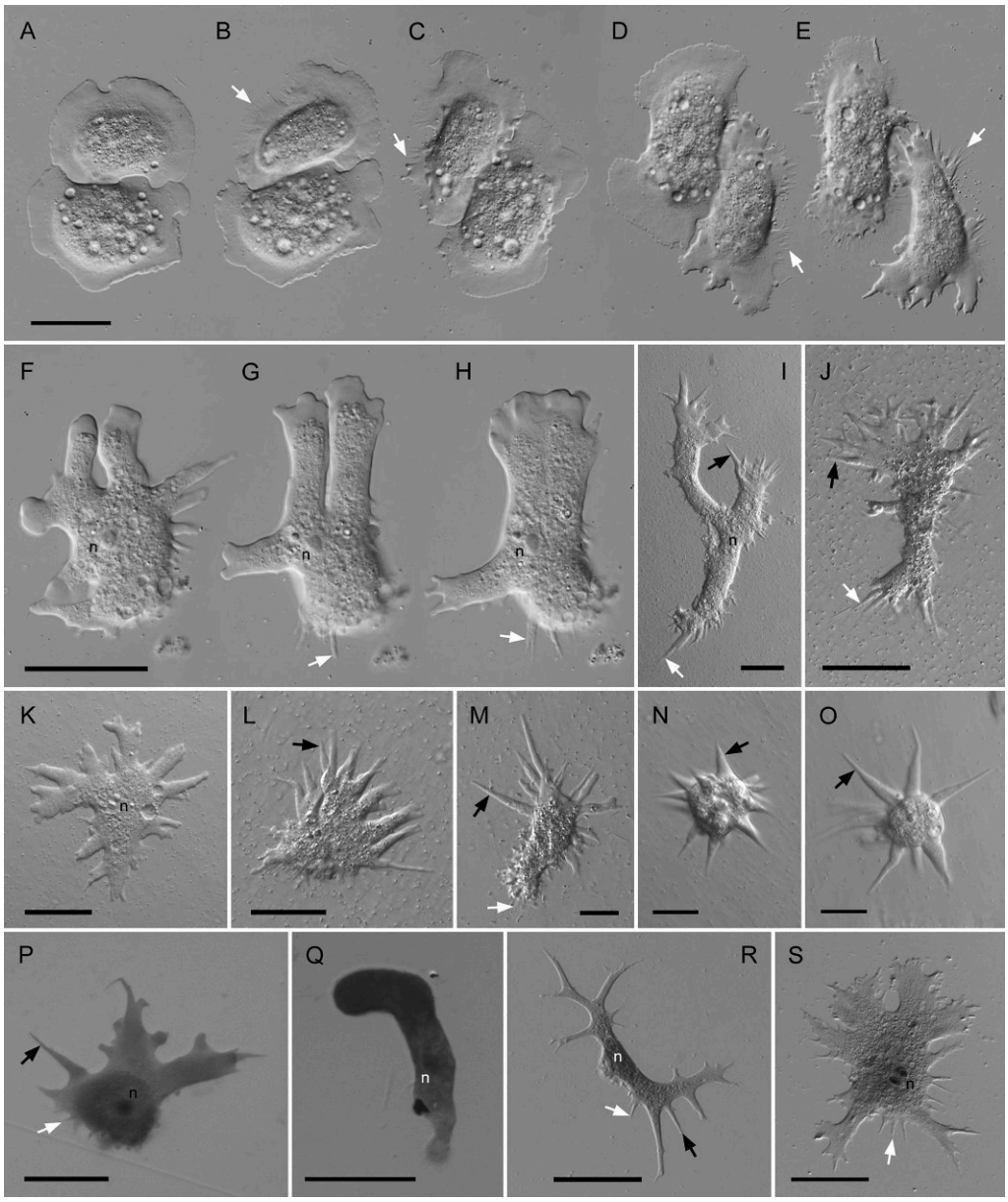
In the early 1990s, Alexey Smirnov embarked on a study of the amoebae living in Lake Leshevoe, situated on Valamo Island in the heart of Lake Ladoga. During his research,



Light micrographs of moving *Leptomyxa neglecta*. From: Kulishkin and Smirnov (2024), doi: 10.21685/1680-0826-2024-18-1-4

he discovered an intriguing species, which he initially believed belonged to the genus Rhizamoeba. Over the following years, Smirnov returned to the island several times, carefully collecting samples to better understand the structure and behavior of this mysterious amoeba. His persistence paid off, and by 2009, he had gathered enough data to officially name the species Rhizamoeba neglecta.

The early descriptions of this species were rudimentary, consisting only of linear drawings. Later, Smirnov and his colleague Nikita Kulishkin used phase-contrast microphotographs taken with a film camera to illustrate the amoeba. However, the equipment they used was not of the highest quality, so they decided to conduct a new

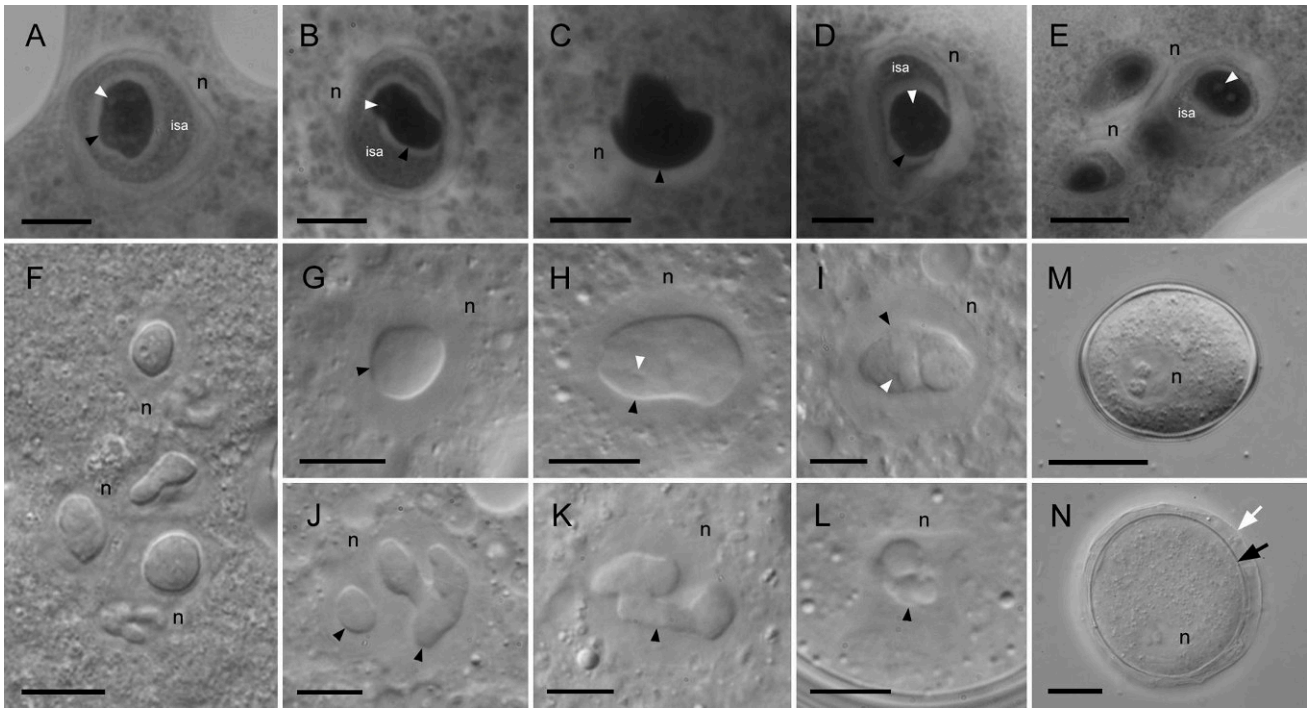


Light micrographs of *Leptomyxa neglecta*. From: Kulishkin and Smirnov (2024), doi: 10.21685/1680-0826-2024-18-1-4

study using state-of-the-art microscopic technology. Their goal was not only to refine their understanding of the amoeba's structure and behavior but also to investigate whether the species could be found in other lakes on Valamo Island.

In June 2019, they traveled back to Valamo Island and collected sediment samples from Lake Nikonovskoe, which was just a kilometer away from Lake Leshevoe, where the species had originally been discovered. Their research confirmed that the amoeba was found only in these two lakes, making it endemic to the island.

The results of their study were published in April 2024, accompanied by high-quality micrographs that provided a clearer picture of the species.



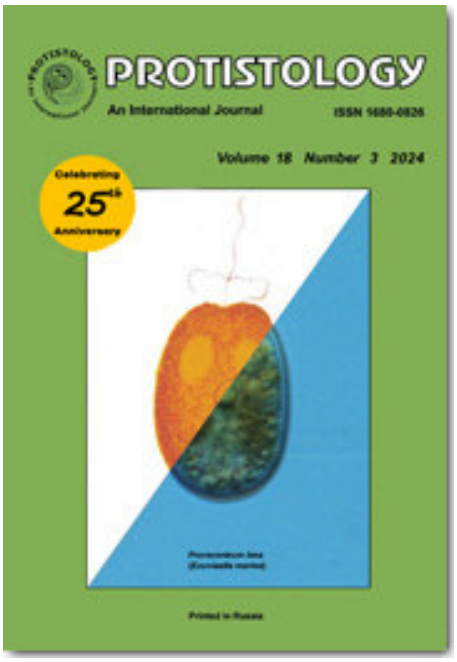
Light micrographs of the nuclei of *Leptomyxa neglecta* in the permanent stained preparations. From: Kulishkin and Smirnov (2024), doi: 10.21685/1680-0826-2024-18-1-4

Among the fascinating observations they made was the fusion of two pseudopodia. Initially, the amoeba formed two short pseudopodia that were closely spaced and oriented in the same direction. As time passed, these pseudopodia extended forward, causing the contact area to grow. Eventually, the space between the two pseudopodia was filled with hyaloplasm, followed by granuloplasm, and the anterior end of the cell transformed into a hyaline lobe.

For the first time, the researchers also described cysts in Leptomyxa neglecta. These cysts, which were rare, were only observed in older cultures that had been maintained for six months or more. The cysts varied in structure, with both spherical single-walled and double-walled forms present. Inside the cysts, the nuclei were crumpled, containing nucleoli made up of 10 to 12 closely packed granules of varying sizes. This discovery added another layer to the understanding of this unique species, further solidifying its fascinating place in the ecosystem of Lake Ladoga.

Review by Dr. Stefan Luketa

A Forgotten Amoeboflagellate Rediscovered 140 Years Later

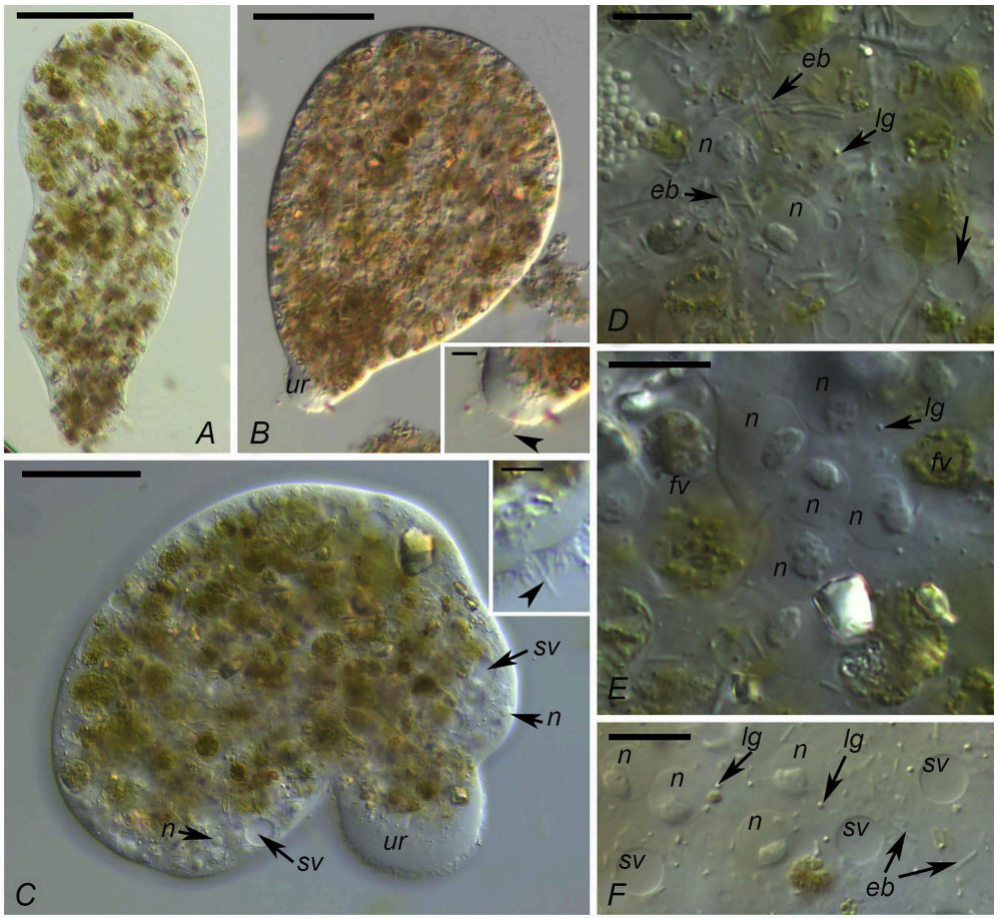


Chistyakova L, Goodkov A, Frolov A (2024) Rediscovery and redescription of *Pelomyxa quarta* (Gruber, 1884) comb. nov. (Archamoebae, Pelobiontida): another pelomyxa rescued from oblivion. Protistology, 18: 232-239. DOI: 10.21685/1680-0826-2024-18-3-5

A few days ago, scientists published an exciting discovery in the icy waters of the Arctic Ocean: *Katarium polorum*, a new cold-loving amoeba that's turning heads in the world of microbiology. Discovered by Marcel Dominik Solbach, this tiny organism is only about 15 micrometers in diameter, but it's packed with some pretty impressive survival strategies for living in one of the most extreme environments on Earth.

What makes *Katarium polorum* stand out? For starters, the organism is encased in an organic shell, with a small round opening. From this opening, a threadlike extension of cytoplasm often pushes out, forming delicate, branching pseudopodia. These slender extensions are used to explore the surrounding environment—almost like tiny feelers on a quest for food or new territory. Interestingly, these amoebae don't seem to be ones for settling down. Instead of attaching to surfaces like many other microorganisms, they float freely in their surroundings, continuously extending and retracting their pseudopodia, as if navigating an ever-changing landscape.

The primary diet of *Katarium polorum* seems to be diatoms, though it has also been seen feasting on bacteria. But it's not just the way it moves or feeds that's captivating scientists—it's also how it



Pelomyxa quarta, light microscopy (DIC). A–C – Pelomyxae during locomotion, inserts – uroid; D–F – details of the cell structure. Abbreviations: ur – uroid, sv – structural vacuoles, fv – food vacuoles, n – nucleus, lg – lipid globules, eb – prokaryotic endocytobionts, arrowheads – flagella. Scale bars: A–C – 50 µm; D–F –10 µm, inserts – 5 µm. From: Chistyakova et al. (2024), doi: 10.21685/1680-0826-2024-18-3-5

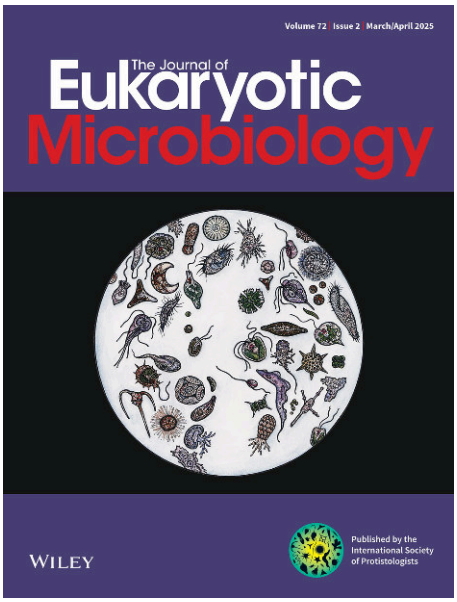
behaves as it ages. In older cultures, the amoeba begins to clump together, sometimes forming clusters of several or even dozens of cells. Among these groupings, researchers have found enormous, multinucleate cells—known as "monstrosities"—offering a fascinating look at the amoeba's biological complexity. These giant cells seem to represent a key piece of the puzzle when it comes to understanding how this organism thrives in the frigid waters of the Arctic and Antarctic Oceans.

Another intriguing feature of *Katarium polorum* becomes visible in these older cultures: a distinct layer of reflective granules or crystals that form a horizontal band within the cell's cytoplasm. Despite these changes, the amoeba remains active, continuing to feed, move, and divide. It's as if it is constantly adapting to its harsh surroundings, constantly evolving to survive the coldest places on the planet.

The discovery of *Katarium polorum* opens new doors in our understanding of life at extreme temperatures, shedding light on how microscopic organisms can not only survive but thrive in conditions that would be unthinkable for most life forms.

Review by Dr. Stefan Luketa

New Testate Amoeba Discovered in the Polar Oceans



Solbach MD, Bonkowski M, Dumack K (2024) *Katarium polorum* n. sp., n. g., a novel thecofilosean amoeba (Cercozoa, Rhizaria) from the polar oceans. Journal of Eukaryotic Microbiology, e13071. DOI: 10.1111/jeu.13071

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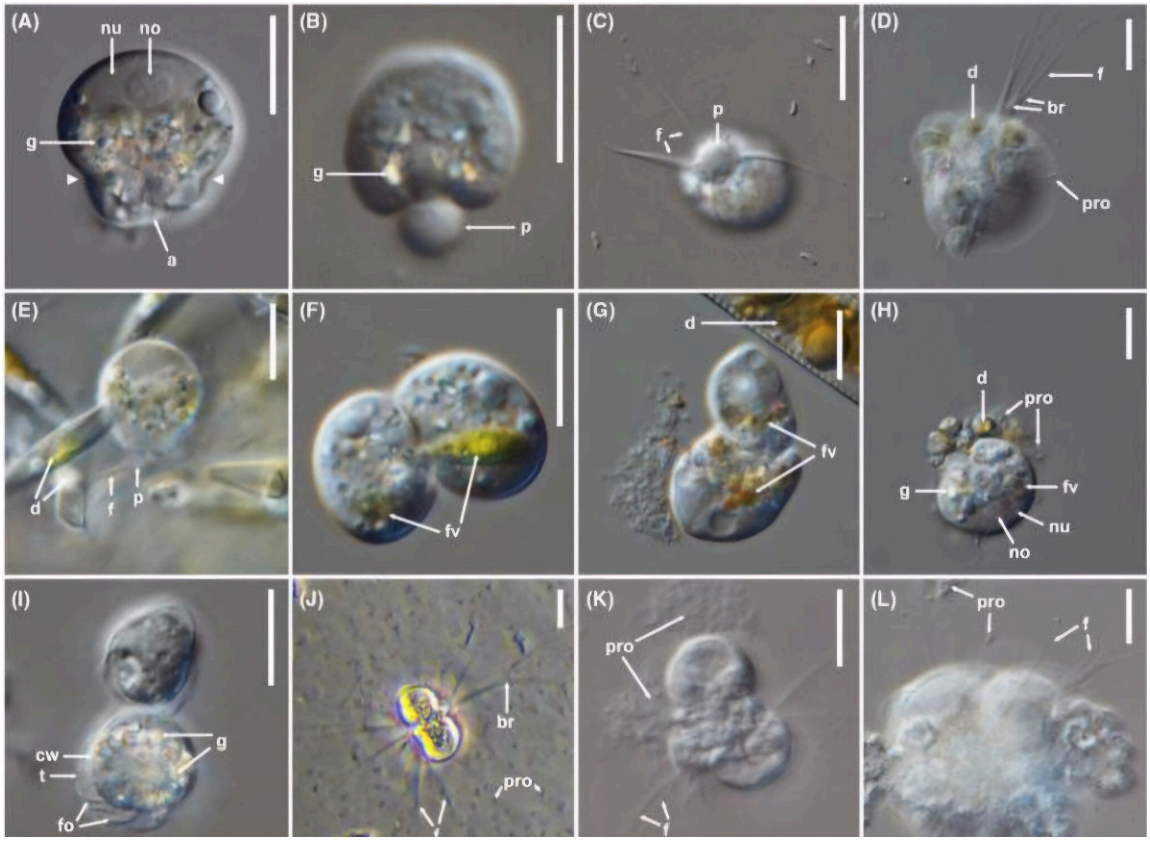

Cellular features of *Katarium polorum* investigated by light microscopy. Scale bars: 10 micrometers. From: Solbach et al. (2024), doi: 10.1111/jeu.13071

behaves as it ages. In older cultures, the amoeba begins to clump together, sometimes forming clusters of several or even dozens of cells. Among these groupings, researchers have found enormous, multinucleate cells—known as "monstrosities"—offering a fascinating look at the amoeba's biological complexity. These giant cells seem to represent a key piece of the puzzle when it comes to understanding how this organism thrives in the frigid waters of the Arctic and Antarctic Oceans.

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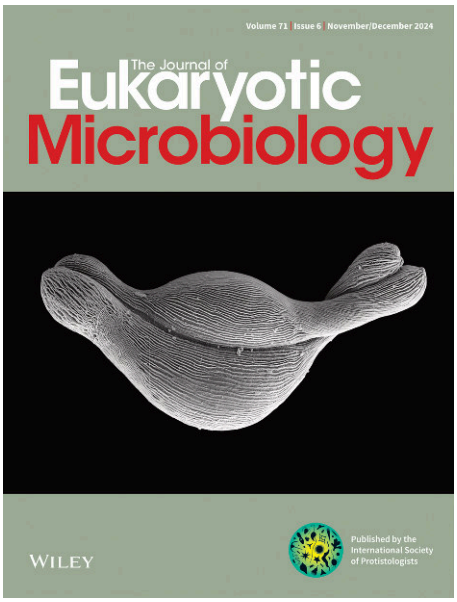
Review by Dr. Stefan Luketa

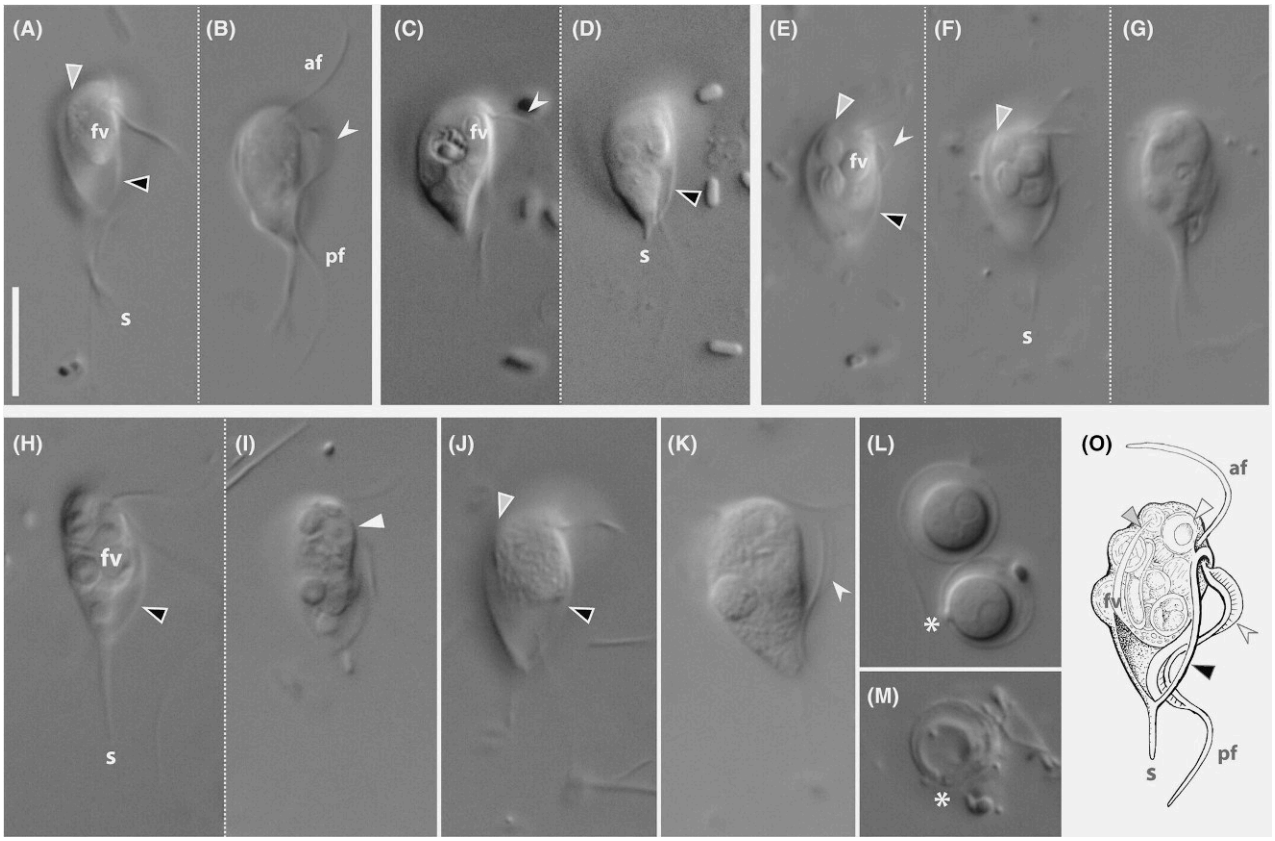
New flagellates, named skoliomonads, discovered in oxygen-free habitats



Eglit Y, Williams SK, Roger AJ, Simpson AGB (2024) Characterization of *Skoliomonas* gen. nov., a haloalkaliphilic anaerobe related to barthelonids (Metamonada). Journal of Eukaryotic Microbiology, e13048. DOI: 10.1111/jeu.13048

Adapting to environments with limited or no oxygen is one of the most remarkable evolutionary achievements, particularly when observed in single-celled organisms such as metamonads. These organisms are especially intriguing due to their extensive biological modifications. Many metamonads either possess modified mitochondria or lackthem altogether, which distinguishes them from most other eukaryotes. Instead of relying on oxygen-dependent energy production, these organisms have evolved alternative metabolic pathways that enable them to survive in some of the most extreme environments on Earth.

In November 2024, scientists introduced a novel genus of metamonads, *Skoliomonas*, which offers fresh insights into life in oxygen-deprived habitats. Skoliomonads are characterized by asymmetric morphology, with a rounded anterior and a sharply pointed posterior that extends into a long spike, often nearly as long as the organism's entire body. This distinctive tail is a prominent feature, and the organism’s dorsal surface rises into a pronounced hump, while the ventral side is flattened and features a groove

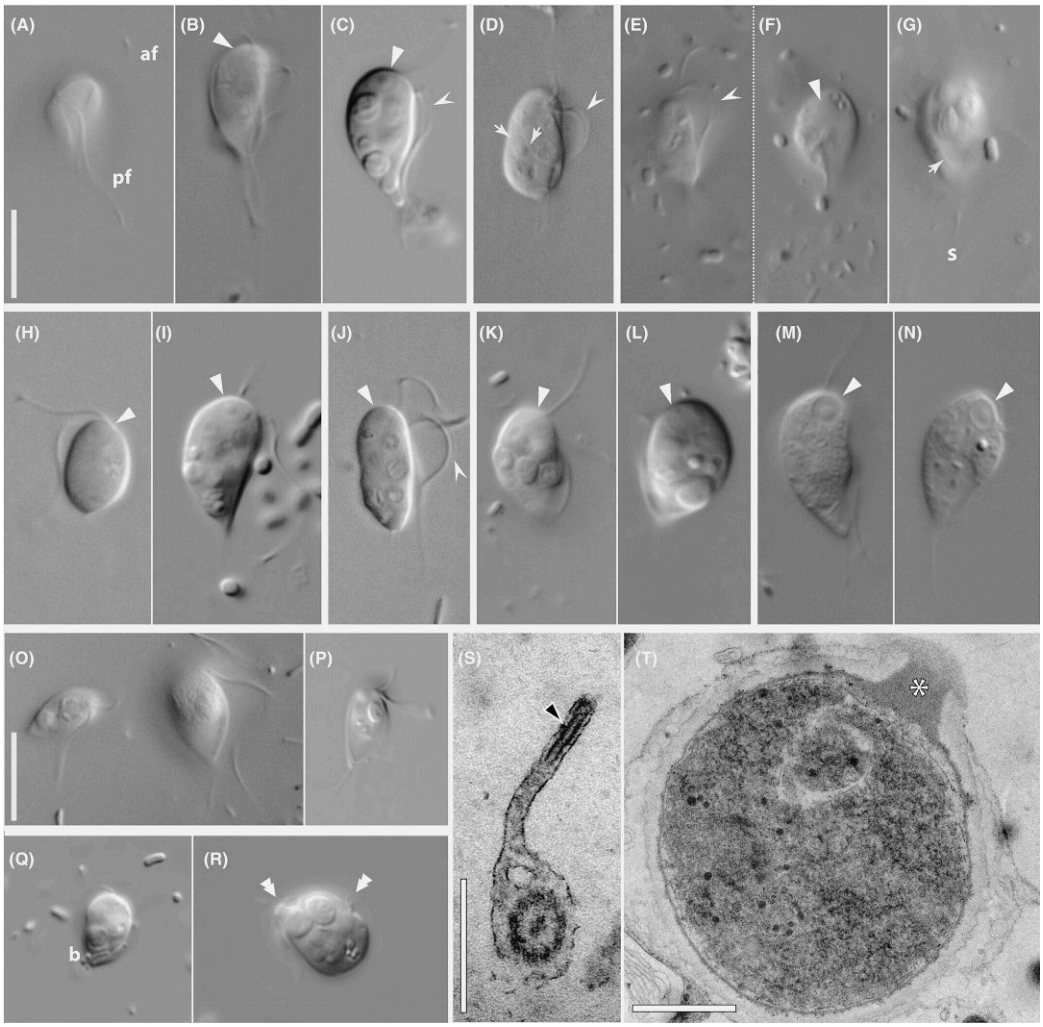


Differential interference contrast (DIC) images showing the general morphology of *Skoliomonas litria* (A-M) and general diagram of a skoliomonad cell (O). Scale bar is 10 micrometers for all micrographs. From: Eglit et al. (2024), doi: 10.1111/jeu.13048

along its right edge. At the terminus of this groove lies a scythe-shaped cytopharynx, a specialized feeding structure essential for nutrient acquisition.

As skoliomonads move through their environment, they utilize the cytopharynx to capture bacteria, which are subsequently digested in vacuoles located along the dorsal side of the cell. These vacuoles enlarge and round out as digestion progresses, providing clear evidence of the organism's feeding process. For locomotion, skoliomonads rely on two flagella: one flagellum sweeps in a large arc, generating a powerful yet fluid movement.

The feeding mechanism of skoliomonads is highly specialized and adapted to their environment. The ventral groove plays a crucial role in capturing and processing food. Although skoliomonads have not been observed filtering water currents under experimental conditions, they are capable of anchoring themselves to surfaces using their sharp posterior spike before detaching and resuming locomotion.



Additional light (A-R) and transmission electron (S-T) microscopy images for the four *Skoliomonas* sp. isolates. Scale bars: 10 micrometers in A and O; 500 nanometers in S; 1 micrometer in T. (A–R) are at the same scale. Eglit et al. (2024), doi: 10.1111/jeu.13048

Under unfavorable conditions, skoliomonads can form cysts that provide structural protection and enhance the organism's survival in extreme environments. These cysts have a double wall and a protruding plug. Within the cyst, the nucleus is positioned near the anterior, and the nucleolus—particularly prominent in certain isolates—is eccentrically located. On the left side of the cell, digestive vacuoles distort the organism's shape as they extend across the dorsal side during feeding.

The discovery of skoliomonads significantly deepens our understanding of how life adapts to extreme, oxygen-deprived environments. Although these organisms possess a relatively simple structure, they reveal a complex realm of biological specialization. Their distinctive morphology, feeding mechanisms, and capacity to form protective cysts underscore the extraordinary adaptability of life, even in some of Earth's most inhospitable ecosystems.

Review by Dr. Stefan Luketa

Quotes

THOMAS CAVALIER-SMITH (1941-2021)

From: https://doi.org/10.1098/rstb.2015.0476

Multicellularity evolves in two ways. Naked cells, as in animals and slime moulds, evolve glue to stick together. Walled cells modify wall biogenesis to inhibit the final split that normally makes separate unicells, so daughters remain joined.

Many amoebae have become multicellular, but only temporarily for spore dispersal, not feeding.

Sponges are related to other animals and choanoflagellates are the closest protozoan relatives of animals.

Among extant animals, only sponges could have evolved directly from protozoa without changing feeding mode.

I contend that it was not the presence of potential glue molecules, but the rare ability of choanoflagellate cells to stick together yet still feed as before that made stem choanoflagellates our ancestors.

Amoeba Discovery

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