JMBE Journal of Microbiology & Biology Education

The Diverse World of Protists—an Ideal Community with which to Introduce Microscopy in the Microbiology Teaching Laboratory

Julia Van Etten,[a] Ramaydalis Keddis,[b] Jessica Lisa,[b,c] and Ines Rauschenbach[b]
[a]Graduate Program in Ecology and Evolution, Rutgers University, New Brunswick, New Jersey, USA
[b]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, New Jersey, USA
[c]Department of Biology, Georgian Court University, Lakewood, New Jersey, USA

KEYWORDS microscopy, community science, discovery-based learning, observation, protists

INTRODUCTION

Although the scientific community and its students are typically more acquainted with eukaryotes as plants, animals, and fungi, a majority of eukaryotic diversity is made up of kingdom-level groupings of largely unicellular and microscopic organisms called *protists*. Protists were the first eukaryotes, originating from the unique primary endosymbiotic event in which an archaeal proto-eukaryote engulfed an α-proteobacterium that ultimately became the mitochondrion, revolutionizing physiology, altering the trajectory of life, and ultimately creating the biodiversity found in nature today (1–3). Protists provide insights into major evolutionary transitions, play environmentally and economically important roles in nutrient cycling and public health, and represent a physiologically diverse array of lifestyle strategies, from photosynthesis to parasitism to extremophily and beyond (4–10). Furthermore, protists occupy diverse niches, are typically larger and more charismatic than prokaryotes, and are ubiquitous in soil and aquatic environments, making them easy to collect, observe, and study. However, most microbiology courses neglect to include protists in their curricula, or they are seldom discussed in depth, despite being a topic of active research and discovery (11–15).

In the manuscript, we present a hands-on, inquiry-based laboratory module that can be implemented across many ability levels of microbiology laboratory courses. This module serves as an introductory or refresher microscopy lesson that allows students to explore the world of protists and their significance to microbiology. Topics such as ubiquity, the "rare biosphere," single-cell genomics, cell physiology and behavior (e.g., taxis), endosymbiosis, and the origins of multicellularity can be incorporated into the discussion. This activity also presents an opportunity to use the campus or local surroundings as a field site and allows students to gain experience as naturalists, a relevant endeavor due to the increase in field-based molecular analyses (e.g., references 16 and 17), the prominence of community science (previously termed "citizen science") to inform formal research studies (18, 19), and the need to continue traditional microscopy to complement these methods in the discovery of novel species (20, 21).

Address correspondence to Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, New Jersey, USA. E-mail: inesrau@sebs.rutgers.edu.
The authors declare no conflict of interest.
Received: 11 June 2021, Accepted: 11 December 2021, Published: 31 January 2022

PROCEDURE

Intended student audience

This lab module has been added to our 200-level Introduction to Microbiology course (taken by students with one semester of biology and chemistry), 300-level General Microbiology course (taken by mixed science majors), and 400-level Applied Microbiology course (taken by microbiology majors). This module can be used for both face-to-face and remote/online learning.

Laboratory timeline and microscopic observations

As a prelab activity, students review the microscope parts and their functions and watch a video of how to prepare a wet mount (22) (https://youtu.be/_nezlOWxNrY). At the beginning of the lab, background information on protists is introduced with a minilecture (Appendices 1, 2). Students then either use their own samples or use lab-provided samples to observe and identify protists. We offer both options to increase student engagement. Instructors review how to prepare the samples and use the microscope, after which students work independently with the microscopes to find appropriate organisms. The time for this exercise is flexible, depending on the length of the laboratory session. It is recommended to plan at least 1.5 to 2+ h (Appendix 3). Instructors work individually with students throughout the period to take or draw pictures of the organisms observed through the microscopes (Appendix 4). The pictures and descriptions are added to the students' laboratory notebooks, and the grading rubric provided in Appendix 5 is applied for scoring.

Extension of laboratory exercise

(i) iNaturalist. We incorporate iNaturalist (https://www.inaturalist.org), jointly facilitated by the California Academy of Sciences and the National Geographic Society, which allows students to serve in an official capacity as community scientists and contribute to active projects. Users around the world contribute photographs of and/or sounds from organisms they observe in a particular location. This is a great resource for beginner naturalists because users can upload a finding, and other users can help identify the organism. User-recorded observations link to the location in which they are found, creating a record of sightings in a geographical area (https://www.inaturalist.org/projects/passion-puddle-rutgers-univ-in-new-brunswick-nj-usa), and can also be linked to preestablished biodiversity projects that contribute data to official research projects (e.g., the "Passion Puddle Project," https://www.inaturalist.org/projects/the-passion-puddle-project). For this lab exercise, students were able to use their own or instructor-created user accounts (Appendix 6). By reporting the location of the observation or associated project upon upload, all entries are easy to identify by teaching assistants and professors for grading purposes and assistance with identifications.

(ii) Remote learning. The lab module is easily adaptable to hybrid or online learning. The larger size of protists and other eukaryotic cells allows students to utilize inexpensive home, foldable, or wooden microscopes to observe the samples. Foldable microscopes are available online and can be mailed to students nationally and internationally. For remote learning, instructors used a synchronous online meeting time to introduce the topic and materials. Students were then given time to obtain samples and work with their microscopes (Appendix 7). For asynchronous online learning, instructors record the minilecture and instructions on how to prepare the samples, and students then emailed or uploaded their findings to their respective online learning program.

Safety issues

Our laboratory adheres to the standards set forth by the ASM Guidelines for Biosafety in Teaching Laboratories (23) and Rutgers University (24). Students in the laboratory are required to wear personal protective equipment at all times, which includes a lab coat, lab glasses/goggles, and gloves. Lab benches and microscopes are disinfected before and after sampling. The water samples are autoclaved after use and disposed of according to policy.

CONCLUSION

Over the past few years, we have completely transformed the introductory microscopy lab module in our microbiology courses. We first changed our instruction from having students look at a wax pencil-drawn X to observing prepared fungal and diatom slides. Both were appropriate activities aligned with one of our course goals. While students learned the different parts of the microscope, applied differences between bright-field and phase-contrast microscopy to various samples, and had time to practice using the equipment, the activities did not elicit much enthusiasm among students to discover unknown microbes and take images of the specimens. We were looking for alternative and more exciting ways to introduce students to the microscope and found exactly what was needed to engage our students. This new module has changed the instructional methods from a guided, rigid framework of teaching about the microscope to independent exploration by students. The enthusiasm of students with various skill levels about discovering protists from our campus pond has translated into stunning images and contributed to ongoing biodiversity projects on campus.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.7 MB.

ACKNOWLEDGMENTS

The materials used in this lab module were purchased with funds received from the School of Environmental and Biological Sciences Campus as Classroom seed fund. The iPads used in our teaching lab were purchased with grant support from SEBS Instructional Computing Funds. We thank the Rutgers Teaching Excellence Network (Rutgers TEN) for funding this publication NSF IHR/DUE IUSE (#2013315). We also thank Debashish Bhattacharya and the Bhattacharya lab for funding the initial exploration of Passion Puddle that led to the creation of this laboratory exercise and for their ongoing contributions to the field of protist biology.

We thank our teaching laboratory technician, Jean Katz, and our teaching assistants for supporting our efforts in providing excellent undergraduate education.

REFERENCES

1. Eme L, Spang A, Lombard J, Stairs CW, Ettema TJG. 2017. Archaea and the origin of eukaryotes. Nat Rev Microbiol 15:711–723. https://doi.org/10.1038/nrmicro.2017.133.
2. Roger AJ, Muñoz-Gómez SA, Kamikawa R. 2017. The origin and diversification of mitochondria. Curr Biol 27:R1177–R1192. https://doi.org/10.1016/j.cub.2017.09.015.
3. Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, Takaki Y, Takano Y, Uematsu K, Ikuta T, Ito M, Matsui Y, Miyazaki M, Murata K, Saito Y, Sakai S, Song C, Tasumi E, Yamanaka Y,

Yamaguchi T, Kamagata Y, Tamaki H, Takai K. 2020. Isolation of an archaeon at the prokaryote–eukaryote interface. Nature 577:519–525. https://doi.org/10.1038/s41586-019-1916-6.
4. Knoll AH. 2011. The multiple origins of complex multicellularity. Annu Rev Earth Planet Sci 39:217–239. https://doi.org/10.1146/annurev.earth.031208.100209.
5. Baldauf S, Romeralo M, Carr M. 2013. The evolutionary origin of animals and fungi, p 73–106. *In* Trueba G, Montúfar C (ed), Evolution from the Galapagos. Springer, New York, NY.
6. Sebé-Pedrós A, Degnan BM, Ruiz-Trillo I. 2017. The origin of Metazoa: a unicellular perspective. Nat Rev Genet 18:498–512. https://doi.org/10.1038/nrg.2017.21.
7. Cheng S, Xian W, Fu Y, Marin B, Keller J, Wu T, Sun W, Li X, Xu Y, Zhang Y, Wittek S, Reder T, Günther G, Gontcharov A, Wang S, Li L, Liu X, Wang J, Yang H, Xu X, Delaux P-M, Melkonian B, Wong GK-S, Melkonian M. 2019. Genomes of subaerial Zygnematophyceae provide insights into land plant evolution. Cell 179:1057–1067.e14. https://doi.org/10.1016/j.cell.2019.10.019.
8. Dawson D. 2005. Foodborne protozoan parasites. Int J Food Microbiol 103:207–227. https://doi.org/10.1016/j.ijfoodmicro.2004.12.032.
9. Grattan LM, Holobaugh S, Morris JG. 2016. Harmful algal blooms and public health. Harmful Algae 57:2–8. https://doi.org/10.1016/j.hal.2016.05.003.
10. Schwelm A, Badstöber J, Bulman S, Desoignies N, Etemadi M, Falloon RE, Gachon CMM, Legreve A, Lukeš J, Merz U, Nenarokova A, Strittmatter M, Sullivan BK, Neuhauser S. 2018. Not in your usual top 10: protists that infect plants and algae. Mol Plant Pathol 19:1029–1044. https://doi.org/10.1111/mpp.12580.
11. Caron DA, Worden AZ, Countway PD, Demir E, Heidelberg KB. 2009. Protists are microbes too: a perspective. ISME J 3:4–12. https://doi.org/10.1038/ismej.2008.101.
12. Ohtsuka S, Suzaki T, Horiguchi T, Suzuki N, Not F (ed). 2015. Marine protists. Springer Japan, Tokyo, Japan.
13. Sibbald SJ, Archibald JM. 2017. More protist genomes needed. Nat Ecol Evol 1:e0145. https://doi.org/10.1038/s41559-017-0145.
14. Burki F, Roger AJ, Brown MW, Simpson AGB. 2020. The new tree of eukaryotes. Trends Ecol Evol 35:43–55. https://doi.org/10.1016/j.tree.2019.08.008.
15. Van Etten J, Bhattacharya D. 2020. Horizontal gene transfer in eukaryotes: not if, but how much? Trends Genet 36:915–925. https://doi.org/10.1016/j.tig.2020.08.006.
16. Leggett RM, Clark MD. 2017. A world of opportunities with Nanopore sequencing. J Exp Bot 68:5419–5429. https://doi.org/10.1093/jxb/erx289.
17. Watsa M, Erkenswick GA, Pomerantz A, Prost S. 2019. Genomics in the jungle: using portable sequencing as a teaching tool in field courses. bioRxiv. https://doi.org/10.1101/581728.
18. Bonney R, Shirk JL, Phillips TB, Wiggins A, Ballard HL, Miller-Rushing AJ, Parrish JK. 2014. Next steps for citizen science. Science 343:1436–1437. https://doi.org/10.1126/science.1251554.
19. Simoniello C, Jencks J, Lauro FM, Loftis JD, Weslawski JM, Deja K, Forrest DR, Gossett S, Jeffries TC, Jensen RM, Kobara S, Nolan L, Ostrowski M, Pounds D, Roseman G, Basco O, Gosselin S, Reed A, Wills P, Wyatt D. 2019. Citizen-science for the future: advisory case studies from around the globe. Front Mar Sci 6:225. https://doi.org/10.3389/fmars.2019.00225.
20. Keeling PJ, Burki F. 2019. Progress towards the tree of eukaryotes. Curr Biol 29:R808–R817. https://doi.org/10.1016/j.cub.2019.07.031.
21. Van Etten J. 2020. Red algal extremophiles: novel genes and paradigms. Micros Today 28:28–35. https://doi.org/10.1017/S1551929520001534.
22. Keddis R, Rauschenbach I. 2020. Microbiology laboratory exercises. Rutgers University, New Brunswick, NJ. https://doi.org/10.7282/t3-fzc0-a870.
23. ASM. 2019. Guidelines for biosafety in teaching laboratories. https://asm.org/getattachment/3c1eb38c-84d7-472f-aa9b-5d695985df21/2019-Biosafety-Guidelines.pdf. Accessed February 25, 2021.
24. Rutgers REHS. 2019. Rutgers University biosafety guidelines for teaching laboratories. https://ipo.rutgers.edu/sites/default/files/RU%20Biosafety%20Guide.pdf. Accessed February 25, 2021.