

PIENAAR LAB MANUAL

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Welcome

I am very happy that you have joined the [Pienaar Lab](#) in the University of FIU's Biological Sciences Department. I hope you'll learn a lot about evolution and the multidisciplinary tools we use to study it. Your time here will involve developing new skills (coding, data analysis, molecular and other lab skills, writing, giving talks, field work, critical thinking etc.), making new friends, and hopefully having a great deal of fun throughout the whole process.

This lab manual was inspired by several others, and borrows heavily from them (e.g. [this one](#) , [this one](#) and [this one](#)). It's also a work in progress. If you have ideas about things to add, or what to clarify, talk to me (Jason, the PI). This lab manual is licensed under a [Creative Commons Attribution - NonCommercial 4.0 International License](#).

When you join the lab, you're expected to read this manual.

Expectations and Responsibilities

Everyone

We are aiming for an engaging, collaborative, hostility free, intellectually challenging, and rewarding lab environment. This does not happen without everyone putting some effort in. Here is a minimum set of things all lab members are expected to do:

Big, overall picture

- Work on what you are passionate about, pursue your question relentlessly, but do so with integrity.
- Work carefully - time spent on careful planning, experimental design, forethought, and documenting your activities is invaluable. It is also ethical and how science works – always keep in mind that you or someone else has to be able to repeat your experiment, this is only possible if you carefully document everything you do. Never forget that you yourself are in the process of becoming a scientist.
- Become okay with mistakes and failures and learn how to turn them into positive learning experiences. We all make mistakes, we all have experiments that fail, we all have things we can improve on. Improvement will only happen if you are honest about them with yourself, other lab members and any collaborators you are working with. This also means learning to accept constructive criticism and to act on it - it is how we grow.
- Make sure you are aware of what academic misconduct is. Science is about pursuing the truth, not feeding our egos. It is never okay to plagiarize, omit data, make up data, or fudge results in order to get a paper published, a thesis finished or a grant proposal done.

- Support your fellow lab mates, help them out when they need it (even if you are not on the specific project), let them vent when they need to. The benefits of this will quickly become apparent when you need help on your own project or someone to listen to your current frustrations. At the same time, don't exploit your lab mates and expect them to spend time on parts of your project unfairly. If you find that your lab mates are spending significant amounts of time and effort on your project, and vice versa, come and speak to me so that we can determine an equitable solution (co-authorship for example).
- Respect your fellow lab mates. Respect both their strengths and weaknesses and respect diversity. Any discrimination based on race, sex, religion, culture, sexual orientation, nationality or age are never acceptable and are grounds for dismissal from the lab, regardless of how productive you are.
- If you are struggling with a scientific problem or personal problem, feel free to talk to me about it – I would like to help. It is definitely okay to go through hard patches (we all do, and often we end up better scientists because of it).
- Socialize – although not required, socializing with your fellow lab mates and graduate students / postdocs outside of work time will do wonders for building what could become lasting collaborations and friendships. Often the best ideas are born out of time spent together outside of the lab.

Small, day to day picture

- If you are sick, stay home and take care of yourself. Reschedule meetings, and ask if you need any help (with critical experiments etc. during that time).
- You are not expected to come in after hours on weekends, or holiday (or any specific times) but you are expected to get your work done. This may require learning some time management skills, and especially dealing with procrastination.
- Show up for lab meetings, one on one meetings, and any other commitments you made. Also respect the fact that others have very busy schedules. If you want feedback on something, give the person enough time to get you feedback. Do not leave grants, papers, conference applications etc. to the last minute – give your collaborators and advisors enough time to provide meaningful feedback. This is both respectful, and in your own best interest towards successfully completing your project / degree etc.
- Keep any shared workspaces clean, neat and organized and ready for the next user. This is part of being respectful, and also part of maintaining lab safety and productivity. If you see anything out of place, clean it up, even if you didn't do it. This is the best way to get repeat offenders to clean up after themselves, especially if the mess is part of their ongoing experiment. If you do need to maintain some organized chaos over shared work spaces, make sure you communicate this to all lab members, and place sticky notes explaining why the "mess" needs to stand for a while. Also, make sure everyone is okay with this.
- Adhere to the lab dress code and safety rules (closed shoes, long pants) – we do work with chemicals and equipment and must abide by these rules (feel free to keep a set of the required clothes in the lab).

Principle investigator

All of the above as well as:

- Support you (academically, emotionally and towards funding yourself and your project)
- Give feedback in a timely manner
- Be available in person (through regular scheduled meetings, email, unplanned meetings etc.)
- Make lab direction and functioning transparent to all members
- Support your career development (promoting your work, introducing you to collaborators, writing recommendation letters, helping you attend conferences as finances permit, helping you prepare for the next step – grad school, post doc, faculty, industry)

Post docs

All of the above as well as:

- Develop your own, independent lines of research
- Help train and mentor students in the lab (both undergraduate and graduate)
- Present your work internally and externally
- Apply for your own grants and participate in lab grant writing (this is part of professional development)
- Apply for jobs (when you are ready, but no later than the 4th year as a postdoc)
- Challenge me (Jason) when I'm wrong, and share your unique expertise with the lab.

Graduate Students

All of the above as well as:

- Develop your dissertation research. For an MS, you should have at least one substantial experiment or set of experiments that answers a big picture question that you are developing. For a PhD, you should have at least three substantial experiments geared towards your big picture questions. Much of your work has to be done independently, but that does not mean in a vacuum. Others in the lab are there to help if you need it, especially when you are just starting out.
- Help mentor undergraduate student in the laboratory (and yes they can help you collect your data)
- Present your work internally and externally
- Apply for small grants (learn how to find them, and how to research them to find one that fits with what you are trying to do).
- Make sure you understand and meet all departmental and college deadlines.
- Prioritize your research time. Teaching is important, but ultimately it is your research that gets you to the next step of your career.

Undergraduate students

All of the above as well as:

- Work towards your own research project (in collaboration with the PI, post docs or graduate students)
- Help new students adjust to the lab by answering any questions you know the answers to
- Develop your weekly research schedule
- Attend all lab meetings (when you can) and be prepared to present your research at the lab meetings
- Make sure you perform safety training before doing any actual lab work – ask if you need it or are unsure whether you need it for a specific task
- If you are earning course credit, make sure you are putting enough hours in (1 credit hour = 3 hours per week in the lab at least)

General policies

Hours

Being in the lab is a good way of learning from others, helping others, building camaraderie, having fast and easy access to resources (and people) you need, and being relatively free from distractions at home (e.g., your bed or Netflix). That said, hours in academia are more flexible than other jobs -- but you should still treat it as a real job (40 hours/week) and show up to the lab. My primary concern is that you get your work done, so if you find that you are more productive at home (lab-mates can be chatty sometimes), feel free to work at home occasionally. If you have no meetings, no participants, and no other obligations that day, it might be a good day to work at home – but you can't do this all the time, and I expect to see everyone in the lab on a regular basis (but see Noise Policy).

For graduate students, I understand having to be away for classes and TA-ing, but show up to the lab on a regular basis when you don't have those obligations (but see Noise Policy for more details).

To encourage lab interaction, try to be in most weekdays during 'peak' hours (assuming no other obligations) – e.g., between 11am and 4pm. This is not a hard rule, you can work at home occasionally (see Noise Policy for more details), and I understand other obligations. But keep it in mind.

Noise Policy

I love that lab members get along and want to spend time with one another. This is a critical aspect of a productive, friendly, and positive lab environment. But I also realize that you are all very busy and want to have a place to focus and work quietly.

Motivated by the concerns of some lab members, and in conversation with them, we have devised a set of policies so that you can all work effectively. These policies do not preclude socializing at specific, agreed-upon times (e.g., lunch, happy hours); in fact, we encourage you to! These policies also do not preclude meeting with one another to discuss research, classes, life, etc; again, we encourage you to! But keep these policies in mind:

1. General quiet time: Quiet time is between 9am and 5pm in the lab. Please respect other people's needs to work quietly in the lab during those times by lowering your voice and generally keeping noise to a minimum (i.e. unless unilaterally agreed upon, please don't play music aloud). If you do need to talk, do it quietly and/or set up a meeting in a room with closed doors.
2. Headphone rule: If someone is wearing headphones, respect their need for quiet. Do not tap them on the shoulder to talk. Do not talk loudly next to them. Exception: if there is a fire alarm or other emergency and they are not aware, do alert them for their own safety.
3. Flexible work locations: Feel free to work from home, a library, an unused classroom, or anywhere else when Policies 1 & 2 aren't enough, or you just need a day of privacy. With respect to working from home: no need to alert Jason. It's nice having people around to help each other and for us to talk in person, so do not work from home *all* the time, but do so when you need to.

PI Office Hours

In addition to weekly meetings (see below), and occasionally dropping by the lab, you can find Jason in his office (SEC 2312). His door is almost always open (except when it's not); if it is, feel free to ask for a chat. If the door is closed, assume that Jason is either gone, in a meeting in his office, or has deadlines to meet and does not want to be disturbed – please send a message (text or email) rather than knocking in that case.

Meetings

Weekly Lab Meetings

Weekly lab meetings (~1.5 hours each) are meant to be a forum for trainees to present project ideas and/or data to get feedback from the rest of the group. Projects at any level of completion (or even not yet started!) can benefit from being presented. These lab meetings can also be

used to talk about methods, statistical analyses, new papers, and career development. For paper discussions, everyone must come to the lab meeting having read the paper and prepared with comments and questions to contribute. Some weeks we may explore a particular issue and have people read different papers – in that case, come to a lab meeting having read your paper and be prepared to summarize it for the group. Each week a different lab member (grad students and postdocs) will lead the lab meeting by choosing a paper to read, or presenting their research for feedback.

Each trainee (RA, student, post-doc) is expected to present at least once every semester. These meetings are informal, and you can do what you wish with your slot – just be prepared to contribute something substantive. Lab members are also expected to attend every meeting (obviously, illnesses, doctor appointments, family issues, etc. are a valid reason for missing a meeting). Undergraduate students are encouraged to attend as often as possible (assuming it fits in their course schedule).

Occasionally, we may have joint lab meetings with other faculty in the department – these may be combined with our weekly lab meeting or an additional meeting. We will also use lab meetings (or ad-hoc scheduled meetings) to prepare for conference presentations and give people feedback on job talks or other external presentations.

Individual Meetings

At the beginning of each semester, we will set a schedule for weekly meetings. Each full-time lab member (RAs, graduate students, post-docs) will have a 30 minute slot set aside to meet with Jason, if needed. If scheduling conflicts arise (e.g., because of travel), we can try to reschedule for another day that week. If there is nothing to discuss, feel free to cancel the meeting or just drop by for a brief chat.

Jason will meet with undergraduate students according to need; postdocs and graduate students should meet with their undergraduate mentee on a regular basis.

Deadlines

One way of maintaining sanity in the academic work is to be as organized as possible. This is essential because disorganization doesn't just hurt you, it hurts your collaborators and people whose help you need. When it comes to deadlines, tell your collaborators as soon as you know when a deadline is, and make sure they are aware of it the closer it gets. Don't be afraid to bug them about it (yes, bug Jason as well).

Give Jason at least one week's (but preferably many more weeks) notice to do something with a hard deadline that doesn't require a lot of time (e.g., reading/commenting on conference abstracts, filling out paperwork, etc).

Give Jason *at least* two weeks' notice (preferably more) to do something with a hard deadline that requires a moderate amount of time (e.g., a letter of recommendation).

If you want feedback on research and teaching statements, or other work that requires multiple back-and-forth interactions between you and Jason before a hard deadline, give him as much time as you can; at the very least three weeks.

For manuscript submissions and revisions (i.e., which either have no deadline at all or only a weak deadline), send drafts to Jason as soon as you have them, and bug him to give you feedback if he hasn't responded in two weeks – papers are important!

Presentations

Learning to present your research is important. Very few people will read your papers carefully (sad, but true) but you can reach a lot of people at conference talks and posters. Also, if you plan on staying in academia, getting a postdoc position and getting a faculty position both significantly depend on your ability to present your data. Even if you want to leave academia, presentations are likely to be an important part of your job. Additionally, every time you present your work, you are representing not just yourself but the entire lab.

It is therefore highly encouraged that you seek out opportunities to present your research, whether it is at departmental talk series and events, to other labs (within or outside of UA), at conferences, or to the general public. If you are going to give a presentation (a poster or a talk), be prepared to give a practice presentation to the lab at least one week ahead of time (two weeks or more are advisable for conference presentations, and *many* weeks ahead of time are advisable for job talks, which require much refining). Practice talks will help you feel comfortable with your presentation, and will also allow you to get feedback from the lab and implement those changes well in advance of your real presentation.

Templates for posters will be available, and you can use those as much or as little as you'd like. Some general rules for posters should be followed: minimize text as much as possible (if you wrote a paragraph, you're doing it wrong), make figures and text large and easy to see at a distance, label your axes, and make sure different colors are easily distinguishable. Other than that, go with your own style.

Jason is also happy to share slides from some of his talks if you would like to use a similar style. You'll get a lot of feedback on your talks in any case, but other people's slides might be helpful to you as you are setting up your talk. As with posters, feel free to go with your own style as long as it is polished and clear.

Recommendation Letters

Letters of recommendation are extremely important for getting new positions and grants. You can count on Jason to write you a letter if you have been in the lab at least one semester (it's

hard to really know someone if they have only been around for a few months). Exceptions can be made if students or postdocs are applying for fellowships shortly after starting in the lab.

If you need a letter, notify Jason as soon as possible with the deadline (see Deadlines for guidance), your CV, and any relevant instructions for the content of the letter. If the letter is for a grant, also include your specific aims. If the letter is for a faculty position, also include your research and teaching statements. In some cases (especially if short notice is given), you may also be asked to submit a draft of a letter, which will be modified based on Jason's experience with you, made more glamorous (people are much too humble about themselves!), and edited to add anything you left out that Jason thinks is important. This will ensure that the letter contains all the information you need, and that it is submitted on time.

Code of conduct

Essential policies

All lab members are expected to abide by FIU's policies on discrimination and harassment which can, and must be read [here](#)

We will not tolerate any form of discrimination or harassment and we are committed to making a safe, friendly and accepting environment for lab members and visitors alike. If you notice any recurrent discriminatory behavior, please tell the PI immediately. If the PI is the one being discriminatory please inform the dept. chair.

Photos and videos

We respect the privacy and comfort of lab members by only taking photos or video recordings of them with their explicit knowledge and consent. This is especially important in situations where a lab member would otherwise not be aware of you taking a photo and therefore cannot object if they do not want you to – e.g., if they are wearing one of our VR headsets or are being scanned. To avoid ambiguity about when a lab member is vs is not aware of photos being taken, we ask that everyone obtain consent from lab members before taking photos or videos, and obtain consent again before posting any images on social media. This is done to respect others' privacy and acknowledge that people have varying degrees of comfort related to being photographed and especially with having those photographs shared on social media.

The goal of this is to foster an environment where everyone feels safe to be who they are, take risks, and have fun, without worry or self-consciousness. If someone wants to be photographed doing something fun or silly in lab events, and consents to be photographed, by all means go ahead! Just please respect the privacy of those who do not want that.

Scientific integrity

The lab is committed to ensuring research integrity, and we take a hard line on research misconduct. We will not tolerate fabrication, falsification, or plagiarism.

Think about the goal of science and why you are here: you're here to arrive at the truth, to get as close as we can to facts about our study systems. Not only is research misconduct doing you a disservice, it's also a disservice to the field. And it risks your entire career. It is never right and never worth it. Don't do it.

Reproducible Research

If you gave someone else your raw data, they should be able to reproduce your results exactly. This is critical, because if they can't reproduce your results, it suggests that one (or both) of you has made errors in the analysis, and the results can't be trusted. Reproducible research is an essential part of science, and an expectation for all projects in the lab. Even worse is when you cannot reproduce your own results.

For results to be reproducible, the analysis pipeline must be organized and well documented. To meet these goals, you should take extensive notes on *each step* of your analysis pipeline. This means writing down how you did things every step of the way (and the *order* that you did things), from any pre-processing of the data, to running models, to statistical tests. It's also worth mentioning that you should take detailed notes on your experimental design as well. Additionally, any code you develop should also be clearly annotated. We all know what it's like to sit down, quickly write a bunch of code to run an analysis without taking time to put comments on it, and then having no idea what we did a few months down the road. Comment your code so that every step is understandable by an outsider. Finally, it is highly encouraged that you use some form of version control to keep track of changes in your code, manuscripts, etc.

Reproducibility is related to replicability, which refers to whether your results can be obtained again with a *different* data set. That is, if someone ran your study again (with a different group of participants), do they get the same results? If someone ran a conceptually similar study, do they get the same results? Science grows and builds on replicable results – one-off findings don't mean anything. Our goal is to produce research that is both reproducible and replicable.

Authorship

Like other labs, we will follow the APA guidelines with respect to authorship:

"Authorship credit should reflect the individual's contribution to the study. An author is considered anyone involved with initial research design, data collection and analysis, manuscript drafting, and final approval. However, the following do not necessarily qualify for authorship: providing funding or resources, mentorship, or contributing research but not helping with the publication itself. The primary

author assumes responsibility for the publication, making sure that the data are accurate, that all deserving authors have been credited, that all authors have given their approval to the final draft; and handles responses to inquiries after the manuscript is published."

At the start of a new project, the student or postdoc taking on the lead role can expect to be first author (talk to Jason about it if you aren't sure). Jason will typically be the last author, unless the project is primarily under the guidance of another PI and Jason is involved as a secondary PI – then Jason will be second to last and the main PI will be last. Students and postdocs who help over the course of the project may be added to the author list depending on their contribution, and their placement will be discussed with all parties involved in the paper. If a student or post-doc takes on a project but subsequently hands it off to another student or postdoc, they will most likely lose first-authorship to that student or post-doc, unless co-first-authorship is appropriate. All of these issues will be discussed openly, and you should feel free to bring them up if you are not sure of your authorship status or want to challenge it.

Old projects

If a student or postdoc collects a dataset but does not completely analyze it or write it up within two years after the end of data collection, Jason will re-assign the project (if appropriate) to another person to expedite publication. If a student or postdoc voluntarily relinquishes their rights to the project prior to the 2-year window, Jason will also re-assign the project to another individual. This policy is here to prevent data (especially expensive data) from remaining unpublished, but is also meant to give priority to the person who collected the data initially.

Lab resources

Management Systems for Personnel, Inventory & Data

Synology NAS drive, google drive and box

Still need to set this up

Lab website: <https://jpienaar.wixsite.com/my-site/guides>

Data management

The following is used as a standard template for grant submissions

DATA MANAGEMENT PLAN (@UA) needs to updated for FIU

1. Data and Materials Produced

This project will generate field-collected, species abundance data, Oxford Nanopore Technologies DNA sequencing, Illumina DNA sequencing, Illumina RNA sequencing, phylogenetic inferences, images of

biological specimens, measurements and analyses of experimental tardigrade / moss interactions, bioinformatic workflows and scripts, R code for implementing phylogenetic comparative method extensions.

2. Standards, Formats and Metadata

All researchers will be required to maintain laboratory notebooks in order to document experimental design, processes and characterizations of samples. Field-collected specimens will be labeled with unique IDs and detailed field notebooks kept by all research personnel, and voucher Tardigrade specimens will be stored in the lab until such time as they can be placed in the appropriate museum or herbarium. Details of the research proposal products will be made available via text, tables, plots and images in peer-reviewed journal articles. Results of the research analyses will be made available in spreadsheet tables and will be saved in standard formats such as CSV or XLSX. Images will be saved in standard formats such as JPEG, TIFF, or PNG. Manuscripts will appear in PDF format and all include all text, calculations, tables and figures. Raw DNA and RNA sequence data will be saved in the format it is received from the specific sequencing technology. The standard for Illumina sequencing is .fastq files. The standard for Oxford Nanopore Technologies is HD5 and .fastq files. Up to 500Gb of raw data is expected per organism sequenced (including DNA and RNA). Final pipelines will be archived github and results will comprise summary statistics stored as .txt/.csv files. Figures and diagrams will also be saved as .eps prior to finalization at JPEG, TIFF, or PNG formats. Code, including computational models and bioinformatic workflows and scripts, will be saved as standard .txt files and disseminated through dryad (datadryad.org) and through PI Pienaar's github repository. The R code for PCM extensions will be built into the mvSLOUCH R package available on the CRAN repository.

3. Roles and Responsibilities

PI Pienaar will take responsibility for the management and sharing of the research data. At least 2 copies of data will be maintained, e.g., main computer and backup to PI Pienaar's lab server, and backup server. Digital laboratory notebooks, analyses and writing will be maintained on researcher computers and backed up to a collaborative FIU (UA) Box directory. UA Box is free to all members of the UA community and their collaborators with no restrictions on size or storage. PI Pienaar will maintain access to all backup copies as a contingency plan for personnel departure. Adherence to the DMP will be checked quarterly.

4. Dissemination Methods

Prior to submitting papers for journal review data will be released according to the dissemination practices described below. Data will be archived at the time of release via public repositories. All digital images of voucher specimens will be archived on the iDigBio repository maintained by NSF. Computational models, bioinformatic scripts and workflows used during this project will be made freely available on PI Pienaar's github site. Results obtained during this project will be disseminated through publications submitted to: (a) the bioRxiv preprint server (biorxiv.org); and (b) open access scientific journals. Each field-collected sample will be registered with the National Center for Biotechnology Institute (NCBI) as a BioSample and each sequencing project will be registered with NCBI as a BioProject at the beginning of this research. Assembled genome sequences will be deposited with NCBI and disseminated through the NCBI ftp site (ftp://ftp.ncbi.nlm.nih.gov/genomes/). Raw .fastq sequence reads will be deposited with the NCBI Sequence Read Archive (SRA) and disseminated through the NCBI ftp site. Digitized images will be deposited with the National Resource for Digitized Collections (iDigBio.org). Phylogenetic matrices and inferred trees will be deposited with the Open Tree of Life project with sufficient metadata to replicate the analyses and evaluate the phylogenetic hypotheses.

5. Policies for Data Sharing and Public Access

All data and code will be made publicly available either at or prior to submitting peer-reviewed publications for journal review (Years 1-5). Data will be contributed to the NCBI public databases (described below). In addition, data will be submitted to supplementary materials sections of peer-reviewed journals. Data will be maintained in machine readable formats and accompanied by detailed metadata to enable open re-use of the data. Code for phylogenetic comparative analyses and

agent based modeling will be distributed under the GNU General Public License version 3 (GPLv3). The distributions will include a user manual as a README file and example vignettes to facilitate ease of use.

6. Archiving, Storage and Preservation

Sequencing of genomes and transcriptomes will result in raw data that can be shared via the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA; <https://www.ncbi.nlm.nih.gov/sra>) that is freely accessible. Annotated genomes will be made available via the NCBI Nucleotide database (<https://www.ncbi.nlm.nih.gov/nucleotide/>) which is freely accessible. Collaborator Fierst has developed a tutorial for submitting both raw sequence reads and annotated whole genome sequences to NCBI (<https://github.com/BamaComputationalBiology/Applied-Bioinformatics/blob/master/CFG-NCBI-Submission.md>). The NCBI and Dryad are trusted repositories for data archiving. In addition, data will be archived on archival institutional repository servers at UA.

Equipment (@UA)

A list of the labs equipment can be found here

The following is used as a standard template for grant submissions, note that a lot of the molecular equipment is actually situated in Dr Fierst's lab across the passageway as we have pooled many of our resources

LABORATORY:

Pienaar has approximately 1500 square feet of laboratory space (a one-bay 750 square foot lab dedicated to microscopy and organismal culturing, and a one-bay 750 square foot lab equipped with state of the art molecular biology equipment). These two spaces are adjacent to each other and situated directly between collaborator Fierst's computational biology lab and collaborator Ciesla's pharmacology lab, and on the same floor as collaborator Chtarbanova's and Fierst's molecular biology labs, all within the state-of-the-art (built 2011) Science and Engineering Complex at the University of Alabama.

CLINICAL:

No clinical facilities are to be used for this grant.

ANIMAL:

Animal facilities are available, but none will be used for this project.

COMPUTATIONAL:

The Pienaar lab has one MacPro workstation (12 core, 64GB RAM), four IMac workstations with 8GB RAM, and a PC workstation with similar specs running windows 10, dedicated to digital microscopy. Each computer is linked to multi-terabyte external hard drives for backup, and genomic data will be stored on project-specific RAID drives (120 TB total), as well as on UA servers (see below). All computers are also backed up on off-site hard drives and via cloud backup with CrashPlan PRO. Computers are loaded with various software including Mathematica, Microsoft Office, Filemaker Pro, Adobe Acrobat, Photoshop and Illustrator, R, Geneious 10 bioinformatics package, and diverse open-source bioinformatics, population genetics and phylogenetic tree building analysis software (latest versions of ASTRAL and BEAST). The UA Office for Information Technology will be responsible for long-term archiving of all data on the UA enterprise-class Compellent SAN platform with daily replication/replay to a secure Tier 2 disaster recovery facility in Atlanta. This platform offers 3 tiers of performance utilizing solid-state, 15,000 rpm, and 7,200 rpm disk drives, and also includes robotic tape storage capability for archiving of data for long-term retention. UA also offers unlimited storage

The Pienaar lab also has access to the rest of the UAHPC system (described below) and can run jobs on these machines using up to 48 cores at once and lasting up to one week. The lab also has access to the Alabama Supercomputer Authority's servers.

OFFICE:

Pienaar has a private office for consultation (~ 250 square feet) on the same floor as his laboratory, his graduate students and postdocs are provided with private booths within shared graduate student and postdoctoral office spaces.

MAJOR EQUIPMENT:

Pienaar Lab: The Pienaar laboratory is equipped with state-of-the-art infrastructure for molecular lab work, including a fume hood and a fully integrated biological safety cabinet that provides a sterile work environment for DNA and RNA isolation. For specimen storage, the lab is equipped with a 23 cu-ft -86°C ultracold freezer, three 20 cu-ft -20°C freezers, and two 49 cu-ft 4°C refrigerator. For PCR and other temperature sensitive applications the lab houses two BioRad C1000 touch cyler thermocyclers. Other molecular equipment includes a Nanopure UV/UF water purification system, liquid nitrogen dewars, frozen tissue homogenizer, a Qubit 4 fluorometer for DNA and RNA quantification, Agilent magnetic capture plate, two microcentrifuge heating blocks, incubator with tube rotisserie, shaking incubator, water bath, multiple sets of pipettes dedicated for particular applications, including automatic and multi-channel pipettors, vortexers, hot plate/spinner, digital pH meter, and assorted beakers, flasks, graduated cylinders, tubes, plates, etc. For centrifugation, the lab has two microcentrifuges, 4 mini-spinners, and a versatile refrigerated Eppendorf 5810 centrifuge capable of spinning plates, microcentrifuge tubes, or conical tubes. For DNA/RNA electrophoresis, the lab contains power supplies, various sized gel boxes and a networked BioDoc-it gel visualizer and documentation system, a single unit containing a UV transilluminator, dark cabinet, digital camera, and windows PC. The lab also has access to an Agilent Bioanalyzer, a Covaris sonicator, and a microplate reader as shared departmental molecular facilities

The smaller, one bay Pienaar's laboratory is furthermore equipped with four 80x and one 120X magnification dissecting stereo microscopes each connected to high resolution recording equipment, and three high magnification (1000x) compound microscopes, also connected to recording equipment, as well as two large temperature, light and humidity controlled growth chambers for organismal culturing.

Both labs are equipped with two large sinks and standard safety equipment including 4 eye-washes and emergency showers. Safety equipment including spill kits, protective eye-wear, various types of protective gloves, and laboratory coats and first aid kits are all kept in standard areas of the lab for easy access. Areas containing hazardous or flammable materials are properly labeled and stored in fireproof cabinets, key protocols for proper equipment use are attached to equipment, and a library of MSDSs, equipment and chemical documentation, and lab operating procedures is available. All personnel are trained annually in laboratory safety procedures through UA's Environmental Health and Safety training program.

OTHER RESOURCES:

As dictated by the NSF Grant Proposal Guide (Chapter II.C.2.i), organizational resources necessary for this project committed by The University of Alabama are described here.

Chemical analysis resources:

The University of Alabama chemistry facility is located adjacent to the Biological Sciences building to the Science and Engineering Complex and faculty and students have free access to it, provided they are trained. This facility also has a full-time employee designed solely to help faculty and students process samples.

Computational resources:

UAHPC (formerly RC2) is a 84 node (1400 core) cluster featuring Dell PowerEdge M610s and M620s with ~21.8 Teraflops theoretical sustained performance. 18 nodes contain two Intel Hexa Core Nehalem Xeon X5650 processors, 48GB of SDRAM, and the 59 newest nodes contain two Intel Octa Core E5-2650 or E5-2640v2 processors and 64GB of RAM per node. 3 nodes contain two Intel Quad Core Nehalem Xeon X5550 processors, 64 GB of SDRAM and 1 node contains two Intel Hexa Core Nehalem Xeon X5650 processors, and 48GB of SDRAM. There are three high memory nodes. These compute nodes are controlled by a Dell PowerEdge M830 master node containing two 10-core processors, and 3TB of 15,000 RPM SAS6 Hard Drive capacity for sharing applications and home directories across the cluster. In addition, two dedicated storage nodes allow for efficient handling of data between compute nodes and the data storage devices. The storage nodes are

connected via PERC H700 or H810 controllers to a total of approximately 100 TB of storage in five Dell PowerVault MD1200s, plus another 20TB of internal disks in the second storage node. The storage nodes have 10G connectivity to the internet.

All nodes are connected internally within their Dell M1000e chassis by Infiniband 4x QDR at a throughput of 40 Gbit/s, and all the chassis are interconnected through a pair of external Infiniband switches (2:1 oversubscribed). Storage is shared between nodes using NFS on IPoIB.

- 2x Penguin IceBreakers storage chains running ZFS mounted through NFS for a total of 560TB usable capacity. This storage is used as an active project repository on both clusters.
- A 720TB disk-based backup environment. The backup environment will serve the above-mentioned storage devices, except for the scratch space provisioned on both clusters.

Graduate student support: Graduate students at the University of Alabama have shared office facilities and access to necessary support including small grant funding opportunities and partial travel support. Partial or full 12 month GTA support is also available, and priority for these GTA lines is generally given to graduate students of new faculty.

Herbarium resources: The UA and UNA (University of North Alabama) herbaria collectively contain ~ 6000 described moss specimens that are in the process of being digitized. These facilities are available to my lab, and provide an invaluable resource to aid in moss identification. Specimens collected for this proposal will be archived in the UA Herbarium.

Microscopy resources: The University of Alabama Biological Sciences Optical Analysis Facility has a Hitachi H7650 TEM, a Nikon C2 Confocal Microscope and a Hitachi SU3500 Variable Pressure SEM. In addition, the lab has numerous light microscopes, microtome sectioning equipment, facilities for specimen preparation, support and ancillary equipment for the electron microscopes and histology equipment to support our more technical microscopic needs.

Workshop hosting resources

UA is a large, public institute with numerous state-of-the-art workshop hosting facilities and resources. The workshops planned for this proposal, are small and focused by design given the complex nature of the phylogenetic comparative methods. They will therefore be held in one of the UA Biological Sciences department undergraduate training laboratories over the interim period between summer classes.

Lab protocols

Field work

Bryophytes and associated meiofauna

Before field trip, gather the following in the field trip backpack

- Collection envelopes
- Sample description sheets
- Stapler (to staple description to envelope)
- Charged Canon EOS Rebel T23 camera (or cellphone for pictures)
- Something to write with
- A GPS (we use free GPS apps on our smart phones, e.g. GPS locator)
- Small spade or sharp implement to scrape tough moss samples off substrate
- Permission to sample in the specific area (if required)

Collecting samples

- At the field site, collect enough moss to “stuff” the collection envelope (about 1 hand full, unless it is a small moss, don't take more than 25% of the plant so that it can reestablish itself, remember they are very slow growers), if present, make sure to get moss with sporophytes (helps with identification),
- Document sample site with descriptions on sample sheet,
- Take pictures as follows (in this order at all times)
 - Staple filled-out description sheet to collection envelope with sample in it.
 - Take legible photograph of description
 - For each moss sample collected, take one “zoomed out” picture to capture the habitat, and one “close up” picture to capture the moss phenotype in its natural habitat (take the time to get well-focused pictures, these become essential for identifying the moss later).

Processing samples in the lab

- Remove data card from camera, place in data slot in back of Mac desktop
 - Open “NO NAME” disk image
 - Drag the set of images and description picture associated with a sample into their own folders created with the same name as the collection no. on the description sheet (data management TALLY)
 - Place moss-containing envelopes in “unprocessed box” and allow them to dry for at least 24 hours.
- Divide each Bryophyte sample into two portions
 - One stays in the collection envelope as a source of food for tardigrades and for culturing experiments
 - The other is used to extract meiofauna, to identify the moss, and upon further drying, will become the herbarium specimen (to be placed in description sheet packet when folded)

Long term storage of samples for later analysis

Lukasz Michalczyk (Polish collaborator) recommends the following protocol

1. Air dry the sample (if moist)
2. Place in labeled brown envelope
3. Vacuum seal envelope in plastic
4. Store at -20 in Freezer (each envelope in own bag to facilitate ease of use later)

Extracting Meiofauna from Bryophytes

We primarily use the Baerman pan methods developed by P Davison

(http://tardigradehunters.weebly.com/uploads/2/5/9/3/25930056/baermann_pan_instructions2014breif.pdf).

1. Set up pans and fill with DI / Rain / Spring water till water level is barely touching nylon mesh
2. Place one ply tissue on top of mesh, make sure tissue is surrounded by water on all sides (i.e. don't allow the water to flow out the pan by capillary motion)

3. Place a number of “pinches” of Bryophyte samples on top of the tissue
 - a. If Moss, use 5 grams
 - b. If bark etc, use enough material to try cover square of tissue
4. Cover the pan with a lid (prevents evaporation), and label lid with unique Id number for sample.
5. Allow Meiofauna to migrate into lower pan for 12-48 hours
6. Pre-wet 35 Micron sieve with DI water
7. Pour Baerman pan water through sieve, rinse with DI water
8. For the last rinse, use Rain water to wash out sieve into a petri dish
9. Label petri dish lid and lower half side with unique ID

Data entry

Taxon Identification

DNA Barcoding

Overview: 1) collect related tardigrade species, 2) extract genomic DNA, 3) PCR with barcoding primers, 4) check products on gel with 100bp ladder, 5) purify with PCR purification kit, 6) elute into 50ul ddh20, 7) ship for sequencing

Genomic DNA extraction

Invitrogen PureLink Genomic DNA mini kit

DNA extraction protocol (borrowed from Dr Stanislava Chtarbanova @ UA Tuscaloosa for single drosophila flies)

1. *Squishing buffer (SB)*
 10mM TRIS-Cl pH 8.2
 1 mM EDTA
 25 mM NaCl
 200ug/ml Proteinase K (enzyme diluted fresh each time from frozen stock)
2. Place X tardigrades in a 0.5ml tube (PCR tube), mash for 5-10s with a pipette tip containing 50ul of SB without expelling the liquid (sufficient liquid escapes from the tip during squishing)
3. Incubate at 25-37°C (or room temp) for 20-30 minutes
4. Inactivate Proteinase K by heating to 95°C for 1-2 min

DNA extraction protocol:

(Modified protocol generated from Casquet et al. 2012)

1. Put N tardigrades in distilled water for a day. Then transfer them to a 1.5ml Eppendorf tube (**Note: a single individual works for this protocol in principle, but DNA concentration is lower**).

2. Put the open lid tube under the fume hood until all the water evaporates.
3. Add 40µl 10% Chelex 100 resin solution to the tube (Kaczmarek et al. 2019) with the addition of 2.7 µl of 200ng/µl Proteinase K.
4. Incubate the tube at 55°C for 5hr with occasional vortexing under 500 RPM (Kaczmarek et al. 2019), and 2-3 times centrifuge (8000 G for 1-2mins).
5. Inactivate the proteinase K by incubating at 95°C for 8-10 minutes (according to Dr. Chtarbanova).
6. Add 20µl of MQ or distilled water to the tube and centrifuge for 2 mins at 8000 G. Extract 40µl of DNA (to the level of remaining Chelex beads at the bottom), and transfer it to the new Eppendorf tube (Kaczmarek et al. 2019).

Note: Tardigrades can preserve in 90% ethanol or keep frozen at -80°C in a drop of water for later analysis.

DNA extraction from moss for PCR (worked for Caleb Turberville, good quality DNA as per nanodrop)

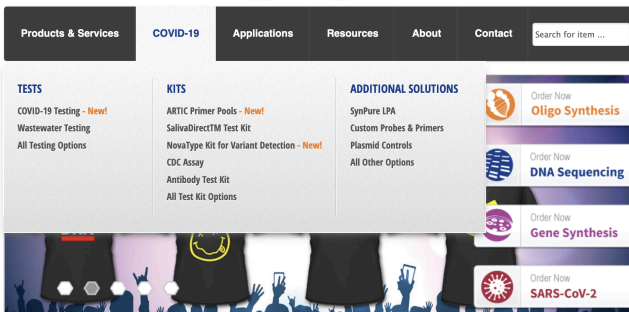
1. Grind protonemal plant material in liquid nitrogen with sterilized mortar and pestle.
2. Prewarm (microwave for 25 seconds) CTAB and N-lauroylsarcosine.
3. Add per gram fresh weight: 3 mL CTAB buffer, 300 µL N-lauroylsarcosine buffer, 60 µL Proteinase K stock, and (optional) 120 µL β-Mercaptoethanol.
4. Incubate at 60°C for 1 h in a shaking water bath. Cool to room temperature.
5. Add 1 vol of sterile 5 M ammonium acetate, mix, and incubate for 5 min.
6. Centrifuge for 10 min at 15,000 g at room temperature.
7. Precipitate the DNA from the supernatant with 1 vol of isopropanol at -20°C for 30 min.
8. Centrifuge for 30 min at 12,000 g at 4°C.
9. Wash pellet with 70% ethanol, dry, and redissolve in 3 mL TE buffer.
10. Add 100 µL of RNase stock. Incubate at 37°C for 15 min.
11. Increase the vol to 5 mL with TE. Perform 2 extractions with 1 vol of chloroform-isoamylalcohol (24:1) (optional just use 1 volume phenol:chloroform).
12. Precipitate the DNA from the aqueous phase with isopropanol. Centrifuge as above.
13. Wash pellet with 70% ethanol, dry, and redissolve in 500 µL TE buffer.

Barcode Sequencing

We have been using Eurofins genomics

1. Log into Eurofins genomics (username: jpienaar, pwd: tardigrade)
2. Choose Order now DNA sequencing 2nd from top on the right (see figure below)

3. Choose tube sequencing - submit tube order
4. Choose number of samples to be submitted
5. choose standard and simpleseq tubes
6. Add your primers to the list on the left if they are not there already (or choose premix if using the PCR Barcoding primers)



Culturing Protocols

Bryophytes

We use a small scale modification of methods used to culture the model moss *Physcomitrella patens*. We maintain both liquid subcultures (that need attention every 2 weeks) and solid subcultures (that need subculturing once a month).

You will need: Petri dishes, Ehrlenmeyer flasks, space in the growth chamber, Calcium nitrate, magnesium sulfate, potassium nitrate, potassium phosphate dibasic, sucrose, DI water, various 1L bottles, a balance, an autoclave, large pipetting devices (10 ml)

Protocol

1. Make the following stock solutions for Knop's growth medium (each in 1L DI H₂O) and sterilize each by autoclaving on the liquid cycle (note we can also just buy premade Knops).
 - 25g/l KH₂PO₄
 - 25g/l KCL
 - 25g/l MgSO₄x7H₂O
 - 100g/l Ca(NO₃)₂
2. Make 1L liquid Knop medium, take 10ml of each stock solution, add 12.5mg FeSO₄x7H₂O, fill bottle up to 1L with DI H₂O (i.e. add ~960ml), and adjust the Ph to 5.8 with KOH or HCL.
3. To make solid media, repeat step 2 but add 1.2 % w/v Agar and sterilize by autoclaving, then pour into petri dishes before it solidifies. These petri dishes can be stored at room temperature for up to 4 weeks in a sealed bag, but it is best to make fresh medium for the monthly sub-culturing.

4. Start by rinsing your recently sampled plant multiple times under the DI water tap till it is clean of all debris – make sure it is only one type of moss by IDing it under the microscope (separate different moss / liverwort species from each other). To start the culture, add 1% w/v sucrose to 30 ml of liquid Knop medium in 100ml sterile flask, and inoculate it with 2-3 small, individual moss plants. Disrupt the plants for 60s at 19000rpm with the tissue homogenizer (the sucrose promotes fast growth). Place these samples in the light cycle growth chamber. After 2 weeks, inoculate 200ml Knop medium in a 500ml flask with 100mg/l dry weight of your initial cultures (this larger flask should NOT contain sucrose). These should be homogenized weekly, and sub-cultured with 100mg/l dry weight every 2 weeks. They serve as back up cultures. To determine the dry weight of a given sample, take 3 10ml samples and dry them for 2 hours at 105c, and weight them (average weight gives you dry weight per 10ml, so multiply by 100 to get dry weight for 1l, then work backwards to figure out how much liquid to take for inoculation).

Tardigrades

Following Stec et al (2016), we try to culture Tardigrades in 2% Agar plates in KCM solution

KCM solution (1000 ml)

1000ml diH₂O

31.33 ul 1M KCl

24 ul 1M CaCl₂

10.7 ul 1M MgSO₄

2% Agar – measure 2 grams Agar powder, pour into flask with 100ml KCM solution, microwave for ~ 2 minutes (stopping every 30s to swirl flask), When cool enough to touch, but still in liquid form, pour into 12 or so small petri dishes, label petri dishes with medium and date - place in the fridge for later use (store for up to a month with lids on, and parafilm wrap)

GENOMICS RESOURCES

Assembly software

Canu

[Canu](#) is a fork of the [Celera Assembler](#) designed for high-noise single-molecule sequencing (such as the PacBio RSII or Oxford Nanopore MinION).

Lot of useful downloads and tutorials here: <https://canu.readthedocs.io/en/latest/>

PERSONNEL

Post Docs

Jesualdo Fuentes-Gonzalez (has key, ends August 31, 2021)

PhD students

Ana Perez (Fall, 2022)

Justin Rosario (Fall, 2022)

Jessica Gonzalez (Spring, 2023)

MS Students

Undergraduates / OPS

Hannan Shaban (Senior)

Kevin Murillo (OPS)

Alexis Mas (OPS)

Past members

Adam Trautwig (post doc, now at Emory)

Sogol Momeni (Started Fall 2019) (Lukasz Ciesla took over main advisor role)

Caleb Turberville (January 2019, graduated Fall 2021)

Jacob Loeffelholz (January 2020, graduated Fall 2021)

Winifred (Elise) Warren (Sophomore)

Chase Mahler (Senior)

Regan (sophomore)

Connor Russel

Blakely (Greer) Norris (Sophomore)

Mehdi Noudali

Morphological descriptions of species commonly encountered

Ramazzotti & Maucci 1983:

Suborder Echiniscoidea has a length/width ratio of 2/1 to 3/1 and length/thickness ratio of about 3/1.

Echiniscus, Pseudechiniscus, and Mopsechiniscus: reddish/orange coloring to free granulations, green color in Echiniscus viridis group.

Echiniscus: small lipid bodies (red/orange spherical cells) free inside the body cavity liquid.

Depth of color in Echiniscus does not change during cryptobiosis.

Echiniscus chemical reaction, including an intense blue coloring when in contact with sulfuric acid, are those of carotene.

Echiniscus never completely lose their color.

In a population of Echiniscus of the blumi group, a coloration is darker in shady environments and lighter, even yellow, in very sunny environments.

Echiniscidae are always lacking the median cephalic cirrus.

All the body appendages (spines and filaments) vary greatly, not only in size, but sometimes also in their presence or absence, even in a single population (e.g. in *Echiniscus testudo*).

Page 37 and 38 for changing and unchanging characters for single species.

All adult Echiniscidae four rounded claws, larvae have two single claws often with spurs.

In many Echiniscidae the posterior legs have a dentate collar. Larvae have incomplete dentate collars.

The armored Echiniscidae feed exclusively on chlorophyll cells in moss.

In *Echiniscus*, there are only two longitudinal dorsal muscles.

Echiniscidae eyes are red (occasionally black).

Cyst is observable in *Echiniscus* although it's rare in Echiniscidea.

No one has succeeded in culturing armor (Echiniscidae) such as *Echiniscus* because of more environmental requirements which are unknown.

Family Echiniscidae have dorsal armor, legs with 4 claws (e.g. *Echiniscus*).

Always observe the tardigrade in a fresh state, inside a drop of water before making a permanent preparation (since mounting media makes the eyespots disappear and dissolves).

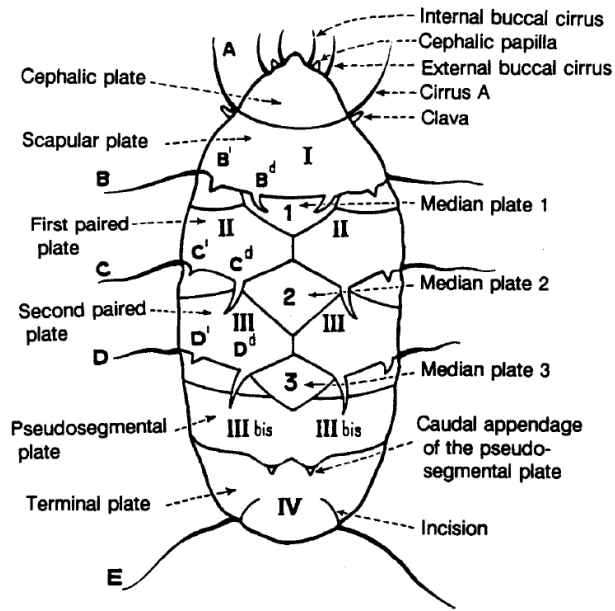


Fig. 7 - Arrangement of the plates and the appendages in a *Pseudechiniscus* (schematic).

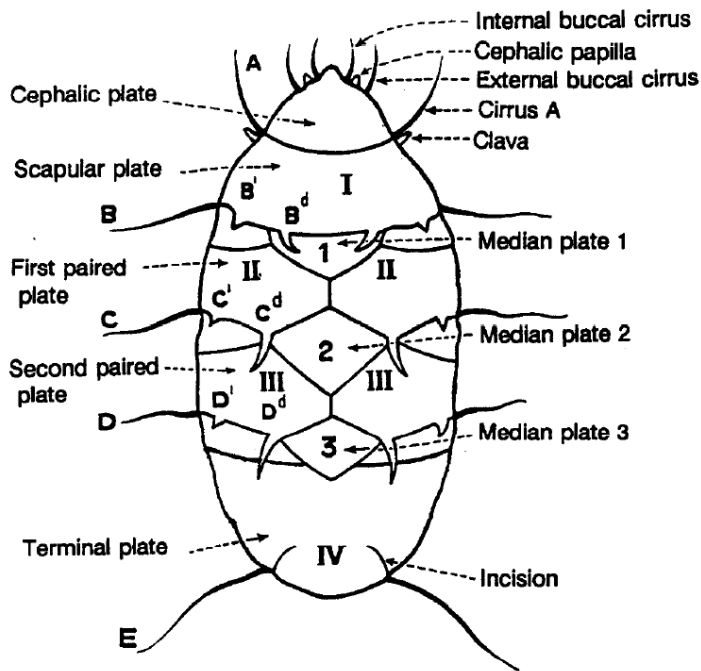


Fig. 5 - Plan of the arrangement of the plates and appendages in an *Echiniscus*.

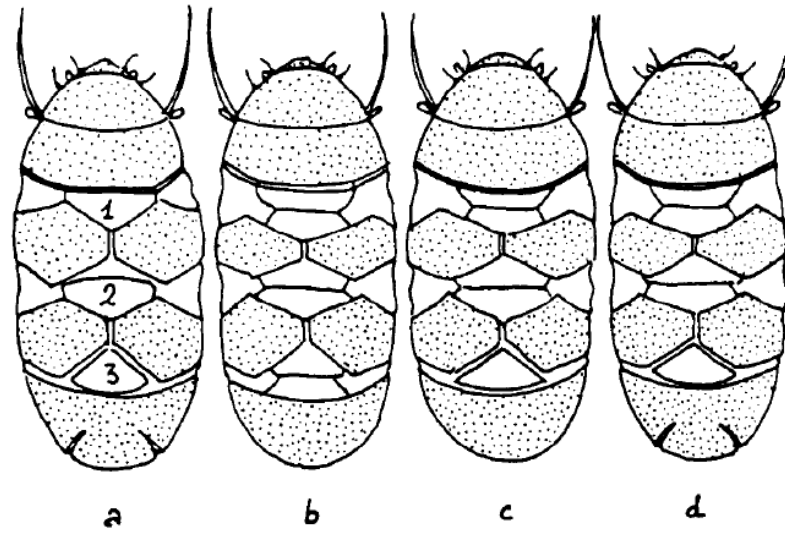


Fig. 6 - Arrangement of the plates in the genera: a, *Echiniscus*; b, *Bryochoerus*; c, *Bryodelphax*; d, *Hypechiniscus*.

Fontoura & Morais 2011:

Genus *Pseudechiniscus* with 37 species is very homogeneous.

2 main groups: *Pseudechiniscus suillus* & *Pseudeschiniscus victor*.

P. suillus has 28 species characterized by the absence of trunk cirri and *P. victor* group characterized by trunk cirri.

FIELD DATA COLLECTION SCRATCHPAD

The aim is to turn our phones into efficient, easy to use data recorders for moss / lichen samples that geotags and time stamps pictures, records field notes about habitat and substrate, takes light and moisture readings.

Essentials (<http://brunalab.org/apps/>)

Lanyard, waterproof case, lenses for macro pics, selfie stick for tops of branches pics

CitiSci.org

Data sheet entries (modeled on bryophyte portal)

Family

Species Name

Observer name

Collection number (date+number eg. 21_01_01_12) was the 12th species collected on the first of January 2021.

Date Collected

Locality_Description

Latitude

Longitude

Altitude

Habitat

Substrate

Associated_tardigrades

Associated_rotifers

Associated_nematodes

LAB MEETINGS

LAB MEETING NOTES: February 2, 2024

Notetaker: Jesualdo A. Fuentes-González

OLD BUSINESS

Jess

Assembling committee: 3 members confirmed (Jason Pienaar, Janna Fierst, Jessica Lee), others pending.

Justin

Reaching out to committee members to schedule quals, one member still uncertain.

Ana

Reaching out to committee members to schedule quals, trying to identify tentative date.

Alexis & Kevin

Checking if payment went through.

CONTINUING BUSINESS

Notetakers

From now on, every shared lab meeting will have a notetaker keeping track of what's discussed as well as the progress and planning of tasks. Each meeting will have a different notetaker, rotating alphabetically.

Drafts

Each member will prepare the draft of a paper of primary interest for sharing with the lab. The suggested structure is the classical: Introduction -> Methods -> Results -> Discussion. The draft should be ready by March 8, and will vary from member to member depending on how advanced the project is (for starting projects, headlines with the structure and key references of the project are OK). A potential journal should also be presented along with the draft (ideally, the draft should follow the format of such a journal). By the following shared lab meeting, members will prepare a figure with expected results (or similar conceptualization) for such a draft. The potential topics of the drafts are as follows:

- *Ana:* Tardigrade behavior. Potential backup: RNA-Seq (Jason suggests focusing on behavior as there is more to explore here, given that the RNA-Seq subject takes care of itself more easily).
- *Alexis & Kevin:* Tardigrade tissue culture. Currently in the stage of reading literature.

- *Jesualdo*: Adaptation-inertia software review. Potential backup: Micro-macro simulation study.
- *Hannan*: Sex determination in *Aiptasia*. Potential backup: *Aiptasia* conservation.
- *Jess*: Microbiome characterization. Potential backup: RNA-Seq (it could also be a large MS with many elements combined to be split later in different papers).
- *Justin*: Genome announcement and RNA-Seq.

Reference manager

To those lab members not currently using a reference manager, Jason suggested to start using one; Zotero in particular.

NEW BUSINESS

Hannan

Potential high school student to volunteer in the lab, interested in marine science. Possibly monitored by Hannan. Jason and Hannan in charge of figuring out the logistics first.

Jess & Ana

Jess and Ana will explore activities for the lab to share, possibly kickball or volleyball. Other potential activities for the future are indoor climbing, surfing, kayaking, gaming and group dinner.

REMINDERS

- **Feb 23 (potential)**: Designate notetaker for shared lab meeting.
- **Mar 8**: Draft of paper and potential journal.
- **Mar 22 (potential)**: Draft figure.

For Jason:

- Figure out logistics for the high school volunteer.
- Figure out why Jess is not getting reminders.

LAB MEETING NOTES: March 1st, 2024

Notetaker: Jessica González

OLD BUSINESS

Scheduling committee meetings, Qualls

Justin

Qualls: Sent email; waiting to hear back. Need to get chair/members to share topics (Janna and Jason). Will send Jason topics from other members to Jason

Current: Troubleshooting code (exhaustive BLAST search). Dont have log file to tell what went wrong. Attempting to run through CIDER. Consult Tori: contigs. Those that were blasted -> server issue -> space issue

To Do: need to meet with Tori to complete the process.

Goal (this week): Info on contigs blasted and start blasting remaining w/ Tori's assistance.

Evolution: do experiments with moss for rna seq (separate reps on species). Expected results: any results from culturing (with explanation why doing this) or rna seq (if have results by then)

Jason's suggestion: subculture moss (clonal); need phenotypic markers for stages of dessication. 6 technical replicates to get DGE data.

Design: grow 6 technical replicates (separate petri dishes) with minimum 3 time-series treatments (1. no water stress, [DESSICATE] 2. soon after rehydration, 3. a while after rehydration). TOTAL: 18 SUBCULTURES.

To Do

1. Figure out if have pure culture (one moss)
2. Subculture 40
 - a. Set up in Narnia
 - i. Empty shelves.
 1. Purple lights with different light cycles
 - a. 12/12 LD
 - b. Other cycle
3. Optimize culturing conditions
 - a. Lit review on moss culturing
 - b. Alexis and Kevin to help with maintenance (watering, etc)
 - i. Need schedule/plan

Ana

Not present

Jess

Quals: Emailed Lima to reschedule for the following week (March 12, 13, or 14th). Will see if he is amenable to changing the current date of March 5th (next Tuesday!)

- March 12 or 13 are okay

Culturing: Challenges (algae density, contamination in dishes and algae)

Start new algae culture on following Monday after review of papers (sterile technique)

Evolution: Microbiome characterization (GRFP)

To Do: Research and order microbiome supplies

Alexis & Kevin

Request: Need “holder” for microinjection (see Sutter Cookbook Ch. 4); shift to chitinase treatment (refer to paper: **INSERT NAME AND HYPERLINK**).

Challenges: protocol for *C. elegans* so the buffer may not be suitable for tardigrades (Juan’s feedback).

Jason’s suggestions: Stain embryos with methylene blue (~30 min.);

Tasks to complete: follow up on Jason's email to department for consult

Jesualdo

Evolution: Visa issues; or attend virtually

CONTINUING BUSINESS

- Paper drafts, March 8th
 - Reference managers (everyone)
- High school volunteer (Hannan)?
- Outreach (Hannan)
- Fun lab activities (Jess, Ana)?
 - Ana spoke with Fierst Lab; next Friday dodgeball?
 - Reassign fun committee chair to Kevin?
 - Send invite to everyone and add to lab calendar

NEW BUSINESS

Organization and Documentation

Lab Calendar (***please check that you have access!***)

<https://calendar.google.com/calendar/u/0?cid=MjUyODU5MmE3MzEwMTdhZTAwMGVhNzQ2NGFmZjA5Zjk1MTVmMzRhM2JhNzk3YjkzZGU5MDEzZTBiNTdhNTY0NEBncm91cC5jYWxlbmRhci5nb29nbGUuY29t>

Created *Pienaar Lab* folder in Google Drive that all should have to to (*Bookmark this!*)

- All members check access to and read Lab Manual
- Add paper drafts to corresponding folder
- Working protocols should be placed here

Lab notebooks, protocols and research, Guidance !

- · Communication and documentation between graduate students and

- undergrads while maintaining shared cultures
 - Start algae notebook

Spend more time in the lab (Jason and grad students)

Lab Management

Jess and Justin are now lab managers

- ☐ EHS binder with SDS sheets
- ☐ Binder with lab manual and dishwashing/autoclaving procedures
- ☐ Research or contact chemistry dep. To ask how to properly separate
- ☐ chemicals and waste (or dilution is an option)

Ordering

To Do: Add people to become orderers for the lab

- Hydrophobic PPE for trizol
- Microbiome extraction kits
- 16s primers and other universal bacterial primers

RA vs TA vs Fellowship Funding

- TA rules for fellowship (BD or GRFP) w/o giving up fellowship stipend
 - Jess and Jason: Speak to Laura ASAP

Authorship on papers

- Comments requested as paper submission approaches

Imaging and Storage

- Research new mounting medium or staining
 - Hoyer's medium? (difficult to access)
- Look into freezing samples/individuals

Field work

??

Journal Club

- Recurring every other Monday at 3 pm starting 03/04
- Expectations: Powerpoint presentation; other members must read paper before next lab meeting
 - Next week (03/04): "Tardigrade cuticle paper" → Jason

Lab Meetings and Paper Drafts

- Provide comments by following lab meeting

Network Credentials and Library Access

Jesualdo (Jason suggests: Plan B) **INSERT MORE INFO HERE**

REMINDERS

- **Mar 8:** Draft of paper and potential journal;
Jason will present “The tardigrade cuticle” paper
Jess and Justin will update lab on lab management
- **Mar 22 (potential):** Draft figure.
- **May 1:** Evolution abstract due
- **DATE:** UGS Graduate Student Evaluations
DATE: Departmental Graduate Student Evaluations

For Jason:

- Figure out how to add lab members to order & trainings
- Make presentation for Journal Club next week
- Speak with Laura and Jess about TA requirements
- Update lab calendar

LAB MEETING NOTES: March 8th, 2024

Notetaker: Kevin Murillo

OLD BUSINESS

- ☐ Remember to consult lab calendar regularly
- ☐ Scheduling Qualls (please put committee meetings / qualls in lab calendar)
- ☐ Committee updates:
 - Justin: only missing one member, prefers last week of May. Missing topics from Janna and Jason.
 - Ana: waiting for two members, also waiting on Jason. Also prefers late May. Ask committee members about topics.
- ☐ Send more readings to Ana.

CONTINUING BUSINESS

- Paper drafts -
 - Jason made shared drive on Onedrive called "Pienaar lab"
 - Transfer paper drafts from google drive to Onedrive.
 - Ana's paper: First step: correspondence analysis,/ or cross two dimensional bar plot
- Paper for SJ club Monday
- Fix onedrive issues (everyone is seeing different things)
- Meet to talk about Ana's dissertation work, Jason ask Adam.
- Ask Jess to help us with Zotero.
- Submit comments and figures in two weeks. (March 22nd)
 - After the two weeks focus on one or two papers at a time.
- Lab Management (Jess & Justin)
 - ☐ EHS binder with SDS sheets
 - ☐ Bigger binder with clear sheets.
 - ☐ Make a list for office supplies.
 - ☐ Binder with lab manual and dishwashing/autoclaving/ disposal procedures (working on it)
 - ☐ Research or contact chemistry dep. To ask how to properly separate (working on it)
 - ☐ chemicals and waste (or dilution is an option) (working on it)
 - ☐ Lab hygiene?
 - ☐ Be careful with beakers that were used for gels.Clean ALL equipment used, don't leave things in the sink.
 - ☐ PLEASE LABEL EVERYTHING USED ;)
 - ☐ Orders training - "Procure to Pay" course on FIU develop
 - ☐ Talk about who is ordering.
 - ☐ Hydrophobic PPE for trizol
 - ☐ Microbiome extraction kits (provide list)
 - ☐ 16s primers and other universal bacterial primers
 - ☐ Fridge: transfer things from incubator to fridge.
- ☐ Fun (Ana,Jess, Kevin)
 - ☐ Kickball? (put on lab calendar): March 15th, 3PM.
 - ☐ What's next? Field day with other labs
- ☐ Outreach (Hannan)
 - ☐ Fienberg fisher
 - ☐ Biscayne Beach Elementary
- ☐ Sample organization? (Kevin, Alexis, Jason) Ongoing.
- ☐ Field trip? Find a time that we can all agree on (preferably on a weekend)
- ☐ Communal culturing? Learn each other's system to help out with culturing.

☐ Culture learning schedule:

☐ March 15th Jess's cultures, March 22nd, Hannan's culture,

NEW BUSINESS

- Research Theme of Lab - suggest "Coevolutionary Processes" Create a cool name for the lab. **Ev.I.L Lab** (EVolutionary Interactions of Living systems Lab)
- **Ev.I.LS Lab** (EVolutionary Interactions of Living Systems Lab)
 - Coevolution
 - Phylogenetic comparative analysis
 - Mutualism
 - Microbiome
 - Symbiotic relationships
 - Evolution
 - Evolutionary ecology
 - Macro evolution
 - Game theory
 - Modeling
 - Tardigrades
 - Aiptasia
 - Bryophytes
 - Interaction
 - Fig wasps
 - Ficus
 - EvILab (Evolutionary Interactions Lab)
 - EVILS (EVolutionary Interactions in Living Systems)
- Grants
 - Aiptasia
 - With DeGennaro lab
 - New Tardigrade Grant
 - Macroevolutionary Processes
 - Figs and Fig wasps

Things to order / do

- Scissors (what kind)
- Clear sheets
- Ethanol?
- Hydrophobic PPE for trizol
- Purchase little air pumps for the Algae cultures
- Ask about internet access for Joao munos
- Jason to ask Adam
- Make a new Lab logo.

LAB MEETING NOTES: March 15th, 2024

OLD BUSINESS

- ☒ ~~Scheduling Qualls (please put committee meetings / qualls in lab calendar)~~
 - Committee updates:
 - ☒ ~~Justin: only missing one member, prefers last week of May. Missing topics from Janna and Jason.~~
 - ☒ ~~Ana: waiting for two members, also waiting on Jason. Also prefers late May. Ask committee members about topics.~~

May 22nd (Justin) May 29th (Ana)

- ☒ ~~Meet to talk about Ana's dissertation work, Jason asks Adam.~~

Ana and Jesualdo talk more about analysis

CONTINUING BUSINESS

Lab Management (Jess & Justin)

- ☒ ~~EHS binder with SDS sheets~~
- ☐ chemicals and waste (or dilution is an option) **(needs clear sheets)**
- ☒ ~~Lab hygiene?~~
- ☐ Orders training - "Procure to Pay" course on FIU develop **(no more in person sessions this semester, zoom session on march 20th, people enrolling: Justin and Jess)**
- ☐ Hydrophobic PPE for trizol **(Jason needs to order)**
- ☐ Microbiome extraction kits (provide list: **talk about more before ordering**)
 - ☐ 16s primers and other universal bacterial primers
- ☐ Make a excel sheet for new orders in lab one drive
- ☐ Fridge: transfer things from incubator to fridge.
- ☐ Fun (Ana, Jess, Kevin)
 - ☐ Kickball? (put on lab calendar): March 15th, 3PM.
- ☐ Outreach (Hannan)
 - ☐ Fienberg fisher
 - ☐ Biscayne Beach Elementary
- ☒ ~~Sample organization? (Kevin, Alexis, Jason) Ongoing.~~

Sample processing Monday at 3pm meeting

- ☐ Communal culturing? Learn each other's system to help out with culturing.

Culture learning schedule:

- ☐ March 15th Ana's cultures, March 22nd, Hannan's culture
- ☐ **Evolution conference:**
 - ☐ **Ana (DMV data)**
 - ☐ **Jesualdo: possibly virtual attending backup plan, main plan to go (Simulation study)**
 - ☐ **Justin (RNA seq of protonema and or moss cultures)**
 - ☐ **Jess (discussed Microbiome stuff, maybe switch to tardigrade cemetery thing)**
 - ☐ **Alexis and Kevin: volunteering is an option for travel**

NEW BUSINESS

- ☐ Jason finds out if a visitor (Joao) can have access to internet on campus
-

Lab Notes March 22

OLD BUSINESS:

- Ana is going to consolidate the different viridiscus morphotypes into species to clean up the data for the DMV analysis
 - One possible way of doing this is re extracting from the samples and identifying the viridiscus species present
 - PCRing the tardigrades right away after extracting could also be useful
 - Could try barcoding the moss itself as well at that point
 - Once this is setup we might need Alexis and Kevin to run pcr reactions regularly
- Did not do an analysis of association by tardigrade species yet, nor an association between different tardigrades
- Jess could think of making a tree based off the morphological data from Jacob's milnesium paper
- Basically everyone should be gearing up to begin PCR barcoding

CONTINUING BUSINESS

Figures

Justin

- Going to need quantitative pcr or microarray for quantitative RNAseq

- Should have venn diagrams for both the mature tissue and the protonema, as well as one showing the transcript in common between them

Jess

Ana

- Look into tardigrade density, ie: see if a certain moss hosts a lot more of a certain type of tardigrade than others
- Look into tardigrade presence/density across the different categories of urbanization

Kevin

- Standard error bars should be added, which will require replicates
- Viable cells could be quantified as growth rate (concentration over time) instead
- Survival curve can be used as well (more on that later)

NEW BUSINESS

- Everyone should incorporate their feedback on their papers and have an idea of the directions of the paper in two weeks 4/5

March 29th 2024 Lab meeting notes

OLD BUSINESS

Procure to Pay training (Justin)

One spreadsheet to rule them all

CONTINUING BUSINESS

Lab logo

Close to finalizing

Fridge?

Close to finalizing

Fun kickass?

Ana and Jess

Evolution conference (July 26th - 30th), Montreal

Volunteering applications? (April 15th)

Talk submission, early registration deadline (May 1st)

Dormitory room bookings (May 25th)

Hotel room deadlines (June 25th)

Poster submission deadline (July 1st)

TA's?

Grants

Aiptasia

With DeGennaro lab

New Tardigrade Grant (NASA with Lukasz), NSF

Send nasa grant to ana

Macroevolutionary Processes (Jesualdo)

Figs and Fig wasps (Justin van Goor)

NEW BUSINESS

Guest lecturing?

Email about library stuff

April 5th 2024 Lab meeting notes (Alexis Mas)

OLD BUSINESS:

Completion of the TA's for evolution

CONTINUING BUSINESS:

Start looking into PCR and genome sequencing of tardigrades (Alexis)

Submit TA's for evolution asap

NEW BUSINESS:

April 12th 2024 Lab meeting notes

OLD BUSINESS

PCR machine set-up

Hannan - set the timer for spanning, growing aptase,

2 of the aptasia resulted in high molecular DNA.

CONTINUING BUSINESS:

PCR primers

Figure out how the PCR machine works (ask Juan)

Kevin and Alexis - Look into embryo DNA sequencing

Ana - looking into the data from DMV, species found, moss ect.

Hanna - perform a gel on the extracted aptasia DNA, followed by a nanodrop to test quality of the DNA should also be checked to see if it is not contaminated.

Jesulado/Jason - continuing working on the grant

Justin - changing saiter script to decontaminate the genome, RNA sequence of protonema (try using a clonal lineage). Reading information about the wasp venom, go over this on monday, with Jason and Jesualdo.

NEW BUSINESS:

Order the materials needed for the tardigrade tissue culture

Ana - show the undergraduates how to look for tardigrades from different samples, make slides? and show them how to perform PCR.

Jesualdo - Coevolution might not necessarily be a connection between species species interaction but rather a trait trait interaction, the question then is how do we test for this? Look for additional data that might prove this, microbiome? reviewing paper (molecular ecology resources) on a topic of interest.

Hanna - Send over the forms that need to be filled out to the undergraduates.

Undergraduate - look to see if they rather be a volunteer or an intern? Can this affect the GPA, would a grade be given?

Deadline for the startup fund is in May, buy what we might need before the startup fund ends. Get 4 new computers, laptops.

CONTINUING BUSINESS

Travel plans evolution

Updates

Alexis / Kevin

Ana

Hannan

Jessica

Jesualdo

Justin

NEW BUSINESS

Undergraduate volunteers

April 26 2024

Note taker: Jason

Present: Jason, Jesualdo, Juao, Ana, Kevin, Alexis

OLD BUSINESS

PCR machine set-up?

CONTINUING BUSINESS

Presentations for evolution?

Undergraduate volunteer paperwork

High school student volunteer

Hannan travel

Girl scouts outreach

NEW BUSINESS

Temporary Replacement Lab manager?

Juao's visit

Marine tardigrades

June 14th Lab meeting

Present: Alexis, Ana, Hannan, Jessica, Jesualdo, Jason, Justin, Kevin, Samdrup

News:

- Got the consultation for the nasa artemis missions (might have to change plans and work with mice)
- Japanese research group created a CRISPR system for ramazzotious (we need to read the paper for next friday and see if there is an angle for us to do something)

CONTINUING BUSINESS

Ana

- Working on contingency tables with the DMV data to search for some interesting patterns for further analysis
- Could look to contrast DMV data with Alabama data
- Wants to see about biodiversity/co-occurrence
- Going to try and count the tardigrades in the alabama samples to have that data
- Ana, Kevin, and Alexis will try and check 3 samples a day to try and get this data
- Backup plan is to just test Adam's ecological hypothesis

Justin

Ideas for rna extractions:

- Try just leaves
 - Try some type of filter
 - Work in laminar flow
 - Use rnase away
 - Put everything in ice
- try blobology to decontaminate the genome while i wait for cider
 - look for proteins from lat years paper in my decontaminated genomes
 - do a phenotypic response measure of the mosses to desiccation
 - geomorph package in r can look at phenotypic trajectories of a structure
 - could do a coevolutionary test to see if tardigrades survive desiccation better with moss leaves present
 - another experiment with or without branch and with or without protonema to test for the chemical and physical components
 - are they feeding on the protenema while they dry
 - programs for landmarks tps, tpsg, imaj, and geomorph

- look at literature for moss leaf morphometrics
- do preliminary stuff so see if there is a morphometric angle
- go back to the original grant to see the ideas we had to do it
 - One slide two drops of water, one has a leaf with a tardigrade on it other one just a tardigrade. Video tape them drying out

Alexis & Kevin

- Working on tissue culturing, running into some trouble
- Going to keep working on in until next friday and then it might be time to consider a backup plan
- Could still make a poster with what they tried and didnt work
- Could be co-presenters with ana and justin
- Could present some other data coming from the alabama samples
- Could do observational stuff with the tardigrades reacting to the soft electrophile

Jason

- Going to present on methods to study co-evolution
- Could compare the MVSLOUCH, cophylogeny, and adam & nathan code

Jesualdo

- Presenting next week sometime between friday and sunday
- Presenting 4 different evolutionary processes
- If he has something to present by wednesday we can do a practice presentation

Jess

- Needed some help from kevin and alexis to 1. Collect or use florida samples with milnesium
- Can do the microbiome study on alabama milnesium
- Wanted to identify the core microbiome, so using the samples that have been frozen or just rehydrated and see which microbes persist
- Need to see what primers to order and what kit to use for the microbiome identification
- Looking into eurofins genomics to see if they do the 16s barcoding themselves

Hannan

- Going to present her GRFP proposal as her poster
- Might have to think about the evolutionary aspect, could do something like "working against evolution"
- Can play on how Dsup works with a general mechanism with DNA which is why it might work instead of playing on a specific evolved mechanism

CONTINUING BUSINESS

NEW BUSINESS

June 21st Lab Meeting

Present:

Jesualdo, Kevin, Alexis, Justin, Janna, Jason

1. Trip to John Pennekamp State Park (June 22nd)

11 am departure

Alexis, Kevin, Ana, Jason, Janna, Tory, Rohit maybe Jess.

2.

June 28th Lab poster

What is a tardigrade good for? Potentially everything.

J. Fuentes-Gonzalez; J.L. Gonzalez; A.A. Mas; K.J. Murillo; A.P. Perezsanchez; J.R. Rosario, H.O. Shabaan & J. Pienaar

In the EVIL (Evolutionary Interactions of Life) lab we study tardigrades using an integrative approach with the goal of saving the world, or in case of failure, figuring out how to live on an exoplanet. Tardigrades are renowned for their toughness, including the ability to survive space-like conditions, abilities we hope to use to achieve our goal. Corals are undergoing unprecedented bleaching events due climate change — the tardigrade Dsup protein may prevent the symbiont expulsion underlying bleaching. What allows communities of species to coexist through time? Tardigrades in their moss or lichen habitats represent a tractable experimental microcosm, co-occurring with nematodes, rotifers and various microbes. We can test for the negative frequency / density dependence required for coexistence as well as long standing hypotheses concerning sympatric speciation and coevolution. Furthermore, the tardigrade holobiont (tardigrade and its microbiome) is hypothesized to aid tardigrade cryptobiotic ability, but this hypothesis is in need of testing. Some tardigrades also interact with their bryophytic hosts in a way that increases their desiccation-resistance ability. It turns out these tardigrades enter their tun states when exposed to flavonoid-like compounds produced by their hosts. Most of the tardigrade's cryptobiotic abilities come about at the molecular and cellular level. Establishing cell cultures would provide an essential tool for understanding tardigrade cellular machinery. Here, we present ineffective methods of culturing cells along with

different approaches that may potentially lead to the successful culturing of tardigrade cells. We also present fruitless ways to produce high quality genomes along with possibly fruitful alternatives. Is this a one-person effort? Nope, this is a lab poster. **Yay tardigrades ! GO EVIL !!! camp !**

Key words

Microbiome interaction studies

Tardigrades coexist with microorganisms in a complex community (holobiont) in which their interactions are hypothesized to aid in the tardigrade's cryptobiotic ability. Our preliminary study aims to optimize extraction and barcoding protocols for downstream microbiome and tardigrade characterization, crucial for future multi-omic projects.

Tissue culturing

More cells, more good. Tardigrade no dna so more make better.

Many studies have been conducted on tardigrade's ability to survive in harsh environments , nevertheless they often fail to give an in depth understanding of tardigrades cellular mechanisms and proteins responsible for such abilities. This is often a result of their microscopic size, and the challenges of culturing them in excessive amounts, making it difficult to perform genomic and proteomic studies. Establishing a cell culture would serve as an essential tool to better understand tardigrade cellular machinery. In this study, we present ineffective methods of culturing cells along with different approaches that can potentially lead to the successful culturing of tardigrade cells.

useful

Saving coral reefs *Use tardigrade to save corals...maybe? No maybe, only yes.*

Anthropogenic climate change has led to severe consequences in our oceans, coral bleaching being one of the most notable impacts. Coral bleaching is the disruption of the symbiosis between corals and their dinoflagellate *symbiont*, Symbiodinium due to external stressors. Using Aiptasia sp. as a model system for coral algae symbiosis, we are proposing an idea to attenuate bleaching by introducing novel traits from tardigrades using CRISPR as means for assisted evolution.

Coexistence

Niche interactions

Limnoterrestrial tardigrades can be found in pockets of water held by moss or lichen. These habitats are their own organisms that share resistance to many different stressors with the tardigrades themselves, like desiccation. The close proximity of the tardigrades and their habitats, as well as the shared environmental conditions experienced provide the opportunity for interaction between them. To study this we are testing the effect of moss- produced compounds on tardigrades and the potential for interspecies signaling.

July 12th lab meeting

Present

Kevin, Jason, Jesualdo, Alexis, Ana, Samdrup

Agenda

Evolution poster - ongoing

Davalos stuff - Log transform all data, ordering of categorical data

Grad school application - ongoing

LAB MEETING NOTES: July 19, 2024

Notetaker: Jesualdo A. Fuentes-González

OLD BUSINESS

Alexis & Kevin

Inquiry if position for the fall (with more hours) involves a new application or an extension of the current contract. In Kevin's case, this might change depending on the admission to the graduate program (see below).

CONTINUING BUSINESS

Evolution 2024

Finalizing logistics (e.g. air tickets, hotel reservations).

Discussion of lab poster. General structure is roughly as follows: Title is a question, and headings (led by different lab members) reply to that question in a general way, with more information provided in a few brief sentences and further details provided as legends (of figures and tables). In this way, the idea is to emphasize on the visual aspect of the poster (figures). Sections discussed in detail:

- *Ana*: Discussion of tardigrade occurrence plots according to substrate and location. Coming up with a strategy to merge categories for the bars in the plots. It is unclear what some categories mean and how they can be merged, so Ana will search for more details to handle them more consistently (e.g. checking coordinates in a map, looking at pictures).
- *Alexis & Kevin*: Increase visual component by giving more space to figures and getting rid of table.

NEW BUSINESS

Backups

Everyone should try to create frequent backups of work and data in OneDrive.

Alexis

Platform seems to be set up for Fall admission, but attempts will be made to submit applications so that the program starts in Spring.

REMINDERS

- **Jul 26**: Lab trip to Montreal for Evolution 2024.

September 16th

Updates

Jason, Jesualdo Justin, Jess, Ana, Hannan, Alexis, Kevin, Samdrup

Financials

Sequencing, OPS, Rotifer

Manuscripts

Jason, Jesualdo Justin, Jess, Ana, Hannan, Alexis, Kevin, Samdrup

Michelle McCaully Visit

Scott Edwards Visit

October 7th

Scott Edwards Visit

Manuscripts

Jason, Jesualdo Justin, Jess, Ana, Hannan, Alexis, Kevin, Samdrup

Lab Presentations

Alexis Mas (14th October)

Jess (21st October)

Jesualdo (28th October)

Ana (November 4th)

Hannan (November 12th)

Kevin (November 18th)

Kevin/Alexis/Hanaan (December 2nd)

Samdrup (December 9th)

Sampling around FIU (as a lab) - Tuesday, October 15th

Journal club with undergrads ft. Alexis - Tuesday, October 15th

18 November 2024

December 2nd, 2024

Vacations

Jesualdo - 14th December, 14th January

Ana - 20th - 2nd

Justin 16 - 25

Jess -

Alexis

January 10th, 2025

- Tardigrade systematics workshop (30th - 4th)

- Harry Meyer, Diane Nelson, Courtney Clark-Hatchel, Lukasz Michalzyck, Piotr Gasiorek, Judy Dobles,
- Mark R still needs a place to stay
- Need people available to show guests around/make coffee/provide hospitality. May need people to help with transportation between airport and hotel, guests arrive on the 29th
- Mornings: theoretical work/conferences, afternoons: hands on work
- Have slides and tardigrade cultures (especially heterotardigrade) ready to present
- Have sequencing pipeline up and ready
- Leasi lab visit (29th - 4th) Rotifers
 - Francesca Leasi and 2 students
 - Opportunity to do field work
 - Kevin, Alexis, Georgina, Stephanie
- 2/8 Bio Symposium
 - No one ready to present yet
- Bird Evolution with Becca Hale
 - Collecting data for a comparative phylogenetic analysis on bird's parental effort
 - How to quantify parental effort?
 - Looking for people to be trained on bird ecology and taxonomy by a previous grad student - Chris, Kevin, Justin
- John Yin Virus coevolution with ancestors
 - Jess
- Research and Publications
 - Publish papers!
- Lab presentations
 - Hannan can no longer escape
 - Starting week after the workshop, Alexis and Kevin are ready
- Final notes
 - Need to order cybersafe and other things - Jesualdo (Ana will send a list)
 - Successful PCR imaging was performed!
 - Switch labs/deep clean day next Monday (all day)

Feb 7th, 2025

- Ordering supplies: Jesualdo and Justin - 2 phase contrast microscopes with 100x, 1-2 cameras for microscopes
- Consolidating information from the workshop into a single document - Ana, ask for slides from Piotr
- Relabeling sample envelopes with new labeling system

- Testing four different methods for collecting tardigrades from moss - normal, normal + Lukaz method with napkin, normal + Lukaz method without napkin, with beaker. Test with about 5-6 samples.
- Rotifers - Francesca method, first test salinity then use a plankton trawl to collect samples
- Almost ready to sequence genome of tardigrades, need to figure how to puncture them carefully - Alexis
- Potentially use electron microscope in AHC3 or other on campus location or send them to colleagues in Krakow
- Chemical experiment - incorporate advice from Lukaz and Piotr, need to order chemicals - Justin

Feb 21st, 2025

- Supply orders - still waiting on envelopes and microscopes - Jesualdo
- Currently planning outreach events, people authorized for outreach - Hannan, Justin, Ana, Jonathan is interested
- Jess will present and train on sample database at next group lab meeting on 3/7/25
- Male determination project - temperature does not seem to be a factor in gender determination - Alexis and Justin are wrapping this up
- Working on barcodes for tardigrades and on mosses - Ana, Alexis, Justin
- Bird project - Kevin and Georgina have worked on some entries, estimating an average of 15 entries per week. Chris and Stephanie will also hop on to help
- Permits for collecting in Florida state parks have come through!

March 7th, 2025

- Moss cultures - maybe cover with tape that allows gas exchange but limits contamination rather than using the lids, take pictures every 10 days for comparison starting today.
- Survey for sample collection database is working! Most people are able to submit, there is an app for Android users. Let Jess know if there are any issues or suggestions, we should all be using it moving forward.

April 4th, 2025

- New member of the lab! His name is Evan, everyone say hi!
- Presentations for evolution conference - Hannan and Alexis may have something ready to present. First deadline is on April 15th.
- Kevin and Stephanie saved the rotifers! They are going to try coming up with a protocol to extract DNA and come up with a genome. Stephanie will email Francesca with specific questions.

- Alexis is working on permits for Deering Estate in order to collect and sequence marine tardigrades.
- Georgina will follow up with Rebecca regarding setting up a meeting to talk about the bird project. We have completed roughly 60 entries and would like to discuss our methodology with her before continuing.
- We have a stable culture of Milnesiums, Ana is working on a Veridiscus culture.
- Jess will be demoing the barcodes she made at outreach today! She is looking into our options for data storage as there is a subscription fee for using a lot of storage on the program she is using.
-

SALACIOUS B. CRUMB



Salacious is always watching you.

Fear him.

He knows you know nothing, internalize this and prove them wrong !!
