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UNITAS MALACOLOGICA

Newsletter

Number 38
March 2024

Dear members,

Although it seems like yesterday, a year has passed since the publication of our last newsletter. This year 2024 is full of malacological activities since many societies will organize their meetings. An excellent opportunity to present our results as a warm-up for the main course that will be next year's WCM.

From Munich to Sao Paulo, we are in the halfway to the next World Malacological Congress. I encourage students to apply this year for research awards 2024, in order to present their results next year at the WCM. You can find all the information to apply on the following pages of this newsletter and also on the UNITAS website.

In this issue we can find the minutes of the last general assembly; the translation of the original German text of the new constitution for UNITAS as a scientific society, and the revised bylaws. If you want to propose any small adjustment send your remark to the secretary.

At the end of the newsletter we can read the reports of the student awards winners in 2021. And, we will also know the names of the winners in 2023 and the titles of their projects.

That's all for now, I hope you enjoy. Feel free to send me any malacological news or announcements from your country if you want.

All the best for 2024

JST

What's Inside

Our aim is to further the study of Mollusca by individuals, societies and institutions world-wide

Affiliated Organisations

American Malacological Society | Conchology, Inc. | Deutsche Malakozoologische Gesellschaft | Instituto Português de Malacologia | Latvian Malacological Society | The Malacological Society of Japan | The Malacological Society of London | Malacological Society of the Philippines | Sociedade Brasileira de Malacologia | Sociedad Española de Malacología | Society for the Study of Molluscan Diversity, Japan |

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Keeping the World of Malacology Informed

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President's Message

Dear associates,

In a year full of work and challenges, we continued with the activities of UNITAS Malacologica (UM). We spent a year in virtual meetings to decide on updates of the UM constitution and bylaws, in the evaluation of Student Research Awards and in organizing the next World Congress Malacology (22nd WCM, São Paulo, Brazil, 2025). In partnership with the societies SBMa (Brazilian Society of Malacology, Eliane Pintor de Arruda president and myself as vice-president) and AMS (American Malacological Society, Jingchun Li president), we will maintain the traditional events of the two societies joining the WCM. We also have the support of the Spanish Society of Malacology (SEM), the Argentine Association of Malacology (ASAM), the Malacological Society of Uruguay (SMU), the Malacological Society of Mexico (SMMAC), the Malacological Society of Chile (SMACH) and the Latin American Malacology Association (ALM), transforming the congress a multi-ethnic event with a wide scope in the Americas.

We submitted the project for organizing a global event to the National Council for Scientific and Technological Development of Brazil (CNPq) and obtained our first financial support to hold the congress. Furthermore, we receive important grants for students from UM's partner societies.

Two prizes will be awarded by SEM during the 22nd WCM for the best oral presentation and poster, and SBMa granted registration fee exemption to the 10 students winners prizes at WCM 2022.

I would like to thank all UM council members for their efforts and time, especially Bernhard Ruthensteiner for organizing our society's documentation to regularize the UM constitution and legal aspects, Yasunori Kano for structuring the Student Research Awards, Jesus Souza Troncoso for reactivating this newsletter and Kevin Kocot for help with website updates.

We hope to have the support of all UM members in the activities that will be developed at the WCM and in the event publicity. May we meet in São Paulo next year and toast to this great event world of malacology.

Lenita de Freitas Tallarico
UM President

Secretary´s Report

UNITAS MALACOLOGICA

MINUTES OF GENERAL ASSEMBLY

Zoom meeting, 14:30–15:30 CET
Friday, 3rd March 2023
Chair: President Lenita de Freitas Tallarico
Minutes: Secretary Yasunori Kano

Number of participating members: 27
Number of participating voting members: 26

1. Amendment to the Constitution
Gerhard Haszprunar and Yasunori Kano reported that the amendment to the UM Constitution, with the aim of establishing its legal status and foundation in Germany with no other major changes to its concept, was voted upon and unanimously accepted at a Council Meeting on 27th January 2023. A Draft New Constitution, both original German and translated English versions, was then circulated to UM members via email on 17th February 2023.
The amendment was ratified by a majority vote of the attending members with Zoom's poll function (23 in favour, 0 against and 2 abstain). It will soon be submitted for electronic vote to all personal members in good standing.

2. Amendment to the Bylaws
Tauana Cunha reported that the amendment to the UM Bylaws was also unanimously accepted by the Council. The most significant changes are related to: allowing electronic forms of communication and meetings instead of only paper, mail or in-person; transferring some passages from the current Constitution; simplifying deadlines and procedures for amendments in the future; the possibility to waive membership fees for members from low income countries. The amendment was ratified and accepted by a majority vote of the attending members (25 in favour, 0 against and 1 abstain).

3. Treasurer's report for 2022
Bernhard Ruthensteiner presented detailed accounts of the UM finances for the year 2022, including income and expenditure for WCM 2022 in Munich.

4. Auditors' report for 2022
Bastian Brenzinger, on behalf of Timea Neusser and himself, certified that the accounts were in order and that the financial statement was an accurate record of UM's financial position. Lenita moved to approve the treasurer's report and the motion carried with one abstain.

5. Any other business
Gerhard proposed future digital archiving of past volumes of malacological periodicals that are currently unavailable online and asked journal editors and societies to grant copyright permission.

Secretary Yasunori Kano

Other News from The Secretary

Unitas Malacologica Constitution

(Translation of the legally valid German version)*

Preamble: All masculine terms are to be understood as gender-neutral.

§ 1 Name, Seat, Fiscal Year
1 The name of the organization is Unitas Malacologica.
2. It shall be entered in the register of

organizations and thereafter bear the suffix "e.V.".
3. The organization has its seat in Munich.
4. The business year is the calendar year.

§ 2 Purpose

1. The purpose of the organization is the promotion of research on molluscs (members of the animal phylum Mollusca; research on molluscs = malacology) by individual scientists and relevant societies and institutions worldwide. Research on molluscs, one of the most species-rich animal phyla, is an important contribution to biodiversity research, which plays an important role in the age of global species decline - land snails are the most endangered animal group of all. Also, especially findings on the shells of recent, subfossil or fossil species can provide important insights into the climatic history of their environment.

2 The purpose of the organization is realized in particular by:
- Awarding grants, scholarships and prizes to young researchers in the field of malacology, whereby the calls for proposals and decisions are broadly advertised and fair selection of grant recipients and awardees is ensured by neutral committees.
- Organization of international congresses (usually the World Congress of Malacology in 3-year intervals), which enables exchange and publication of scientific results of malacology on a large scale. These congresses are open to all interested parties.
- Other activities, such as making scientific literature and other data available in digital form and disseminating information via internet portals to promote research on the diversity and habitats of molluscan species.

§ 3 Non-profit status

1. The organization shall exclusively and directly pursue charitable purposes within the meaning of the section "Tax-privileged purposes" of the Tax Code.
2. The organization shall act selflessly; it shall not primarily pursue its own economic purposes.
3. The organization's funds may only be used for purposes in accordance with the constitution. Members shall not receive any benefits from the organization's funds.
4. No person may be favoured by expenses which are alien to the purpose of the organization or by disproportionately high remuneration.

§ 4 Membership
1. Individuals as well as legal entities (e.g., an associated organization)- may become members of the organization.
2. Withdrawal from the organization is possible at any time. It must be declared in writing to the Council (via the Secretary or Treasurer).
3. The membership does not end with the cessation of the payment of the membership fees. After 3 years of non-payment of membership fees, a member may be expelled from the organization by decision of the Council.
4. A member can be excluded from the organization if his behaviour is deemed grossly against the interests of the organization. The General Assembly decides on the exclusion.
5. Membership ends with the death of the member, in the case of legal entities with their extinction.
6. The resigned or excluded member has no claim against the organization's assets.

§ 5. Membership fees
Members shall pay membership fees, the amount of which shall be determined by the General Assembly. Usually, the membership fees are paid in advance for a period of 3 years.

§ 6 Organs of the organization

The organs of the organization are the Council and the General Assembly.

§ 7 Council

1. The entire Council of the organization consists of the 1st chairperson (President), the 2nd chairperson (Vice President), the Secretary, the Treasurer and four Councilors.
2. The executive committee authorized to represent the organization in the sense of § 26 BGB consists of the 1st chairperson (President), the 2nd chairperson (Vice President) and the Secretary. Each of them represents the organization individually.
3. The Council is elected by the General Assembly for a period of 3 years, whereby the position of the Vice President is taken over by the President of the previous period. At each general meeting with new elections, two new Councilors are elected; the other two remain in office until the next general meeting with new elections. The Secretary and Treasurer may serve multiple terms if their nomination for re-election is unanimously supported by the Council. The Council shall remain in office until an effective new election has been held.
4. The Council shall meet at least once a year (in person or by virtual conference) to discuss matters of organization policy, administration, or other matters that come before it. A quorum of the Council shall consist of at least 5 Council members present for the proper transaction of UM business. Resolutions of the Council shall be passed by a simple majority. In the event of a tie, the President (or in his absence, the Vice President) shall have the casting vote.

§ 8 General Assembly

1. A regular General Assembly is held every 3 years in the context of the World Congress of Malacology. Additional, usually virtual, General Assemblies can be called at any time according to the decision of the Council.
2. A General Assembly must be convened if the interests of the organization require it or if at least 10% of its members request it in writing, stating the purpose and the reasons.
3. Every General Assembly must be called by the Council in written form under observance of an invitation period of 2 weeks and under specification of the agenda.
4. The chairperson of the meeting is the President. If the latter is unable to attend, the chair falls to the Vice President or the Secretary. If none of them are present, a chairperson shall be elected by the General Assembly.
5. Every properly convened General Assembly has a quorum regardless of the number of members present.
6. The resolutions of the General Assembly shall be passed by a simple majority of the votes cast. A majority of ¾ of the votes cast is required to amend the Constitution.
7. The resolutions of the General Assembly shall be recorded in the minutes, which shall be signed by the chairperson of the meeting and the keeper of the minutes.

§ 9 Dissolution

1. The dissolution of the organization can only be decided in a General Assembly called for this purpose. A majority of ¾ of the valid votes cast is required to dissolve the organization.
2. In case of dissolution of the organization, withdrawal of legal capacity or discontinuation of tax-privileged purposes, the assets of the organization shall fall to a legal entity under public law or to another tax-privileged corporation for use in the promotion of research on biological diversity.

Munich, the 03 March 2023

(Thank you very much **Bernhard for translating this text from the original in German.)*

BYLAWS OF UNITAS MALACOLOGICA

(Adopted by the UM Online General Assembly on 03 March 2023, followed by a vote by email)

These following internal bylaws are complementary to the Constitution and do not replace it.

DUTIES OF COUNCIL MEMBERS

1. President - presides as Chair over Council meetings and General Assemblies, casts the determining Council vote when votes on each side are equal; sets and distributes Council agendas; organizes the triennial World Congress of Malacology (WCM) and associated abstract publications and/or proceedings; signs for Unitas Malacologica (UM) in legal matters jointly with Secretary.

2. Secretary - speaks for the society and interacts with other national and international bodies on behalf of UM; performs corresponding and recording duties for UM (including minutes of Council meetings and General Assemblies); maintains UM archives; signs for society in legal matters jointly with President; oversees electronic publications (e.g. website, newsletter, possibly led by other UM members) concerned with communication with members.

3. Vice President (the Immediate Past President) - serves in the President's capacity in his/her absence.

4. Treasurer - maintains all financial and membership records for the society; oversees investments of UM funds; prepares annual treasury reports.

5. Councilors - serve as additional voting members of Council.

ELECTIONS

1. Any member or potential new member of the society can be nominated for the Council, with written agreement of the nominee. Persons nominated and the capacity in which they are nominated shall be informed to society members at least 7 days before the General Assembly. The election can be electronic or by paper, by simple majority of all votes received by the Secretary before or at the General Assembly.

2. In the case of death or resignation of a member of the Council, the remaining members of the Council may appoint a successor to serve until the next General Assembly.

CONGRESSES

1. The Council shall seek potential countries to host the triennial World Congress of Malacology, which is voted on by the General Assembly. The congress shall be organized by the President with a local organizing committee. No two consecutive WCMs shall take place in the same country. Efforts shall be made to alternate between host continents. Additional (usually regional or subject-specialized) meetings or other events may be sponsored by UM. Non-members may attend the WCM and other such events (but may be subject to additional fees).

2. The Council must approve the proposed date, site, and general arrangements for each WCM prior to any formal commitments.

COMMITTEES

1. Committees for special purposes may be created by, and shall report to, the Council.

2. The Auditing Committee consists of two UM members in good standing. This Committee shall audit the Treasurer's operations by reviewing the annual treasury reports.

DUES

Current membership fees are 16 Euro per annum (preferably paid for three years), with students being assessed half that amount. Membership fees might be waived at the

decision of the Council for members from low income countries, as listed by the World Bank (https://data.worldbank.org/country/XM).
The Council may review membership fees as necessary and changes shall be voted upon by the General Assembly.

USE OF FUNDS
Funds may be used, at Council's discretion, to further the goals of the Society, specifically through publications, special projects, scholarships, travel funds, and administrative costs.

COMMUNICATIONS
Communications of UM containing announcements of meetings, accounts, lists of members, etc., shall be sent to all members. These communications shall not contain any matter that will qualify them as scientific publications.

AMENDMENTS TO BYLAWS
1. Proposals to amend these Bylaws shall be prepared by the Council, or may be submitted to the President or Secretary by petition bearing the signatures of at least ten (10) UM members in good standing.
2. Proposed amendments shall be circulated to all Council members for vote. If accepted by the Council, the amendment will take effect immediately (unless otherwise specified) and will be shared with UM members.

Yasunori Kano
UM Secretary

Treasurer's Report

With the **new constitution**, UM has been registered as a non-profit organization with the authorities in Munich/Germany. It is now officially a society with "e.V." status (eingetragener Verein - Registered Society), which offers advantages in terms of the legal status of finances and tax matters, among other things.

There were only minor financial movements in 2023. These were largely expenses (compensation booking as part of the WCM 2022 - approx. € 3,500 (already included in the total WCM cost breakdown of Newsletter No. 37), science grants - approx. € 4,470, tax consultancy and legal costs in the context of the registration of the society - approx. 500 €). Total assets at the end of 2023 amounted to about 50,000 €.

Bernhard Ruthensteiner
UM Treasurer

Keep in touch…

Keep an eye on our webpage and Twitter account for Unitas Malacologica news and some of our favorite molluscan content. If you have a new publication or announcement you would like us to help spread the word about, please direct message us on Twitter or e-mail kmkocot@ua.edu with information.

Webpage:
http://www.unitasmalacologica.org/

Follow us on **Twitter**: @malacologica

Call 2024 UM Student Research Awards

Unitas Malacologica has been supporting student research on molluscs since 2000. **Applications for the 2024 Unitas Malacologica Student Research Award are now open**. Up to three awards of up to 2,000

euro will be made.

The deadline for submission of applications and one letter of recommendation from the student's research mentor is 30 April 2024. A report summarizing the results of the work funded is to be submitted for publication on the UM website at the end of the award period.

More information and a link to the application form and instructions can be found on UNITAS website.

http://www.unitasmalacologica.org/projects.html

Kevin Kocot
Webmaster - Social Network Manager

Other issues

2024 Prize from Spanish Malacological Society to WCM

The Spanish Malacological Society (SEM) agreed in its last general assembly to grant two awards to the next WCM. One for poster format or another for oral format. The prize will consist of a subscription to the SEM for two years, 2025 and 2026. The winner will receive two copies per year of the Journal *Iberus* and the Newsletter.

Submitted by Ramón Álvarez-Halcón, Secretary of SEM

Student Research Awards 2023

We are pleased to announce the winners of the Student Awards 2023. Congratulations to all.
On behalf of the council we wish you much success in your scientific career. We looking for your reports in view to be publish in the next Newsletter.

They are:
Nocella, Elisa (Sapienza University) A molecular approach to the trophic specialization in Ovulidae (Gastropoda: Cypraeoidea).

Maitra, Akash (Indian Institute of Science Education and Research Kolkata) Malacological diversity of the Plio-Pleistocene (5.333-0.012 Ma) marine rocks of the Western India.

Foon, Junn Kitt (Western Sydney University) Mapping and studying the ecology of the endemic and Critically Endangered land snail *Advena campbellii* on Norfolk Island to inform conservation planning.

In next pages the Reports of Winners 2021

Laia Burgués Palau (Netherlands): Effects of light stress on tropical solar-powered slugs.
Alessia Carini (Hong Kong): Blueprints of biological complexity: Decoding molecular pathways and the evolution of molluscan shell biomineralization.
José Ramón Pardos Blas (Spain): Insights into conotoxin gene regulation in cone snails through DNA methylation analysis.

Research Award Report (2021 Winners)

Note: Students reports are not scientific publications to be cited. The results will be or have been published elsewhere as original articles.

Assessing the effect of light on tropical solar-powered sea slugs

Laia Burgués Palau
Unitas Student Research Award 2020, Report.
January 10th, 2023

INTRODUCTION

Some species of sacoglossan sea slugs, also known as "solar-powered sea slugs" have the unique ability to steal intact chloroplasts (kleptoplasts) from their algal food and retain them within their digestive glands cells (Rumpho et al., 2000; Serôdio et al., 2014; Laetz, Moris, et al., 2017). These acquired chloroplasts (kleptoplasts) do not divide, but may remain functional for periods ranging from a few days to several months providing nutritional benefits during periods of food scarcity through photosynthesis (Händeler et al., 2009; Hinde & Smith, 1975; Casalduero & Muniain, 2008; Pelletreau et al., 2014). Based on the period that kleptoplasts remain functional once incorporated in the slug, sacoglossan are classified in three main categories depending on chlorophyll a fluorescence measurements of the maximum quantum efficiency of photosystem II (Fv/Fm): i) non-retention (NR), if they cannot retain any functional kleptoplasts (no Fv/Fm) , ii) short-term-retention (StR), if they are able to incorporate functional kleptoplasts for a period of weeks of starvation (Fv/Fm of at least 0.4 over 14 days of starvation) and iii) long-term retention (LtR) if they can retain functional kleptoplasts for months (Fv/Fm of over 0.4 for more than 21 days of starvation) (Clark et al., 1990; Händeler et al., 2009; Rauch et al., 2018).

In algae and plants, most of the pigments and enzymes required for the general chloroplast function and maintenance are produced by nuclear encoded genes (Roy et al., 2011). Gene expression surveys of sacoglossan slugs have failed to detect nuclear-encoded algal gene transcription in slugs, so kleptoplast function and repair is likely limited to chloroplast-encoded genes in these species (de Vries et al., 2013). Although some photosystem repair mechanisms are chloroplast-encoded, and thus could be found in some kleptoplastic sacoglossans, numerous studies report declining kleptoplast function when slugs are deprived of food (e.g. Vieira et al., 2009), showing kleptoplast repair is limited in these slugs. A major source of damage to photosynthetic machinery comes from exposure to light intensities higher than the chloroplast is acclimated, however continued photosynthesis and the energetic benefits it provides require exposure to

Fig. 1 A) Laia scuba diving collecting light measurements next to a *Elysia crispata* specimen.

light, (Havurinne & Tyystjärvi, 2020; Franklin & Forster, 1997). Kleptoplastic slugs may therefore face a trade-off between getting enough light to maximize the amount of nutritional support they receive from photosynthesis, while prevent photodamage when exposed to high irradiances.

Numerous studies have investigated this trade-off by collecting slugs, standardizing their lighting under lab conditions and then examining the effects that higher light intensities have on kleptoplast function. These studies often drastically reduce the amount of light to which these animals are exposed (<150µmol $m^{-2}s^{-1}$), meaning the results produced in these studies cannot be extrapolated to slugs exposed to natural light levels which can easily exceed 1000µmol $m^{-2}s^{-1}$ (e.g. Giménez Casalduero & Muniain, 2006; Vieira et al., 2009; Cruz et al., 2015; Christa et al., 2018; Richards Donà et al., 2022). To understand the photophysiology of kleptoplasts in slugs under natural conditions, I measured a range of light intensities various sacoglossans are exposed in nature, termed *in situ* light measurements here. These measurements were compared with their optimal irradiance obtained from Rapid light Curves (RLCs), which are a measurement of photosynthetic relative Electron Transport Rate (rETR) versus the irradiance (E). I performed RLC's within 30 minutes each slug of collecting to determine the optimal irradiance under natural conditions, which reflects their natural light histories instead of the standardized lab conditions. Additionally, behavioural experiments were carried out to determine their preferred irradiance. This study was carried out in the island of Curaçao during ten weeks of fieldwork. I used four congeneric tropical species with different kleptoplast retention times: *Elysia crispata* (LtR), *E. velutinus* (StR), *E. ornata* (StR) and *E. subornata* (NR) (Fig. 1) and their associated algae (with the exception *of E. crispata* which was found in light exposed areas on coral reefs/rubble but never found feeding on macroscopic algae). I also assessed possible photoprotective mechanisms for each species to better comprehend how these species cope with the high light intensities that they are exposed to in nature.

RESULTS

The preferred, optimal and *in situ* irradiances different from each other (X^2 (2)= 21.30, $p < .001$) for *E. crispata* (Fig. 3A); A Dunn's *post hoc* test showed significant differences between preferred and optimal irradiance (Z= 3.55, $p <.001$) and *in situ* and preferred irradiance (Z= 4.20, $p <.001$), being, in both cases, preferred irradiance significantly lower than optimal and *in situ*. No significant differences were observed between optimal and *in situ* irradiance, however, the *in situ* irradiance varied more than the optimal irradiance (Fig. 3A).

Similarly, differences were found among preferred, optimal and *in situ* irradiances for *E. velutinus* (X^2 (2)= 9.43, $p = .008$) (Fig. 4B). Dunn's *post hoc* test showed significant differences between preferred and optimal irradiance (Z= 2.15, $p =.031$) and preferred and *in situ* irradiance (Z= 2.60, $p =.009$). Algal optimal irradiance was higher for both algal species than for the slug, E_{opt} = 804.70 µmol photons m^{-2} s^{-1} for *H. incrassata and* E_{opt}= 655.69 µmol photons m^{-2} s^{-1} for *H. opuntia* compared to E_{opt} = 342.32 µmol photons m^{-2} s^{-1} for *E. velutinus* (Fig. 3B).

Elysia ornata specimens presented differences among preferred, optimal and *in situ* as well (X^2 (2)= 11.21, $p = .004$). Dunn's *post hoc* test showed significant differences between preferred and optimal irradiance (Z= 3.19, $p =.001$). Contrary to *E. velutinus*, *E. ornata* showed higher optimal irradiance than *B. plumosa* (E_{opt} = 584.18 µmol photons m^{-2} s^{-1} for *B. plumosa* and E_{opt} = 943.005 for *E. ornata*) (Fig. 3C).

For *E. subornata,* no RLCs were obtained for *E. subornata* despite varying the light steps and probe sensitivity, therefore no optimal irradiance for their photosynthetic activity was obtained. Kruskal-Wallis test showed significantly lower values of preferred irradiance than in situ irradiance (X^2 (1)= 9.60, $p = .002$), following the same trend as the other species (Fig. 3D).

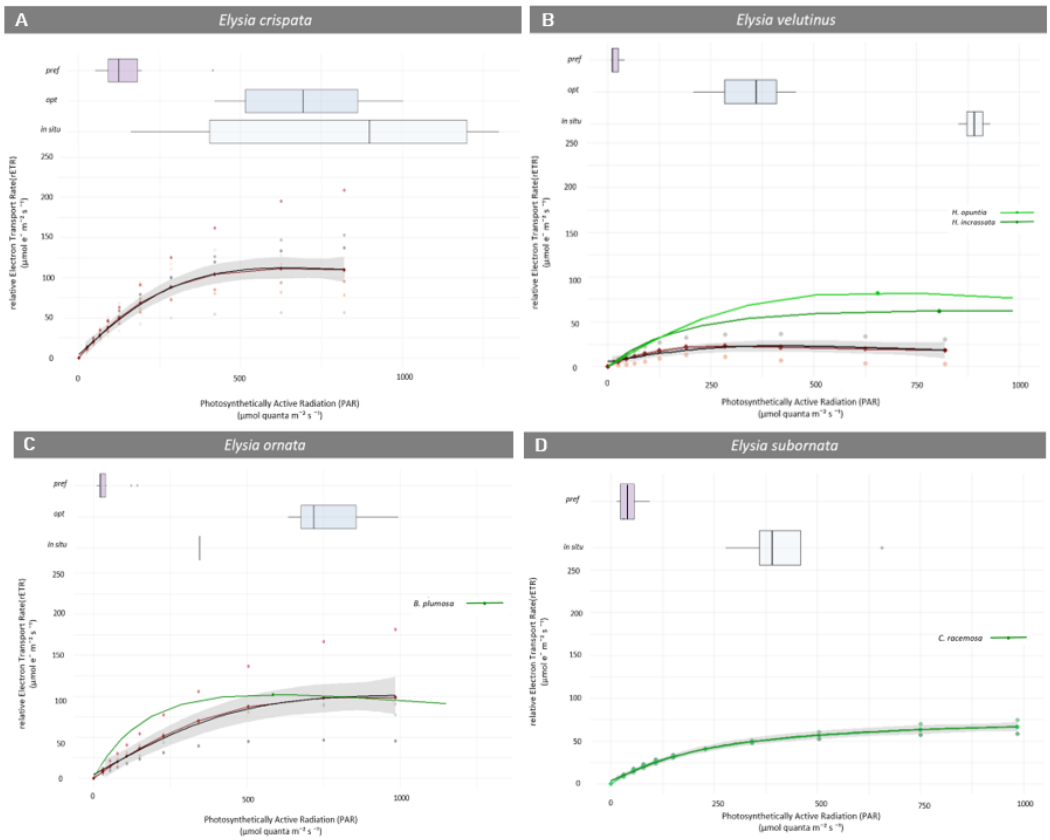

Fig. 2 Rapid light curves for the sea slug species and their macroalgal source of food. For *E. subornata* no RLC was created since no/very low signal was detected. In red, the averaged RLC among all the samples and in black the GAM fitted model. E_{opt} for the algal samples is indicated with a dot. Note that there is no E_{opt} for *C. racemosa* since the photoinhibition phase was never reached. On top of each graph preferred, *in situ* and optimal irradiances. Each panel contains all individuals of the final subsample. Sample sizes: $n_{E.crispata} = 9$, $n_{E.velutinus} = 3$, $n_{E.ornata} = 4$, $n_{E.subornata} = 0$, $n_{E.incrassata} = 1$, $n_{E.opuntia} = 1$, $n_{E.plumosa} = 1$, $n_{E.racemosa} = 2$.

DISCUSSION

All four species preferred lower irradiances than they were exposed to in situ, indicating a strong negative phototactic behaviour. The, *in situ* irradiance was always higher than the photosynthetic optimal irradiance, suggesting that they receive more light than can be used in photochemistry, and therefore either employ physiological or behavioural strategies to dissipate excess light or they must cope with any consequences of too much light. The preferred irradiance we measured for *E. crispata* was significantly higher than for the other species, which aligns with in situ observations where *E. crispata* specimens were found in light- exposed areas, strongly contrasting with the *E. velutinus*, *E. ornata*, and *E. subornata* which were found hiding in their macroalgal source of food. *Elysia crispata* seems to cope with excess of light by contracting their parapodia in order to cloak the kleptoplasts located in the digestive diverticula tubules (Fig. 4). *Elysia velutinus* and *E. ornata* presented strong light avoidance behaviours and they seem to cope with the excess of light by hiding in their macroalgal food, however the habitats of both species strongly differ from each other. While *E. velutinus* inhabits deeper waters (4-6 m) and highly light-stable environments, *E. ornata* was found in very shallow waters (0-2) and in an intertidal area with strong wave action creating a highly fluctuating light conditions. Thus, *E. ornata* might not only hide in their algae to prevent photodamage but also rely on photoprotective pigments

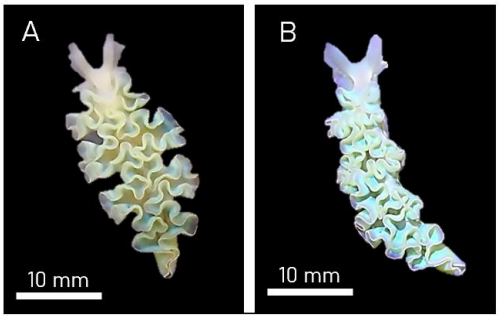

Fig. 4 **A)** *Elysia crispata* specimen with relaxed parapodia before being blasted with light. **B)** *Elysia crispata* specimen with contracted parapodia covering their kleptoplasts located in the slug's body.

(Richards Donà et al., 2022). Finally, *E subornata* also showed a strong light avoidance behaviour and was observed hiding in algal food.

This study clearly showed a link between the phototactic behaviour and the ecology of four *Elysia* species. Additionally, it underlined *Elysia sp.* employ species-specific strategies to cope with excess light. Lastly, we highlight the importance of investigating photophysiology under both lab conditions where each variable can be controlled and under natural conditions in order to understand the complexity of sacoglossan biology in nature.

AKNOWLEDGEMENTS

I am extremely grateful to Unitas Malacologica for funding and supporting this Master's project, to Dr. Ellie Laetz for her recommendation and confidence and to CARMABI Research for hosting this project. Also, I want to thank to Giulia Senna, my project partner, with whom was a truly pleasure to work with. I highly encourage other early career researchers to conduct their short-term research projects abroad if possible, since for me it has been an extremely enriching experience, both professionally and personally.

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DNA methylation landscape in the genome of the mediterranean cone snail *Lautoconus ventricosus* (Gmelin, 1791)

José Ramón Pardos-Blas

Unitas Student Research Award 2022. February 29th, 2024.

Keywords
Cone snails, genome, bisulfite sequencing, methylation.

Introduction

Cone snails are well known because of their species diversity and because they produce and inject cocktails of toxins for hunting and defense. The venom is a mixture of hundreds of different peptides, conotoxins, that act synergistically to produce general paralysis in the prey. The publication of the first cone snail genomes [1,2] revealed the architecture of venom genes and proposed a relation 1:1 between the number of conotoxin genes and transcripts. The analysis of *Lautoconus ventricosus* (Gmelin, 1791) conotoxin expression, also revealed low levels of expression of conotoxins in the muscle of the foot. Differences in the regulation of somatic gene expression rely on different mechanisms, like the interaction of transcription factors with the promotors and repressors, the maturation of the mRNA, and the epigenetic marks, which can regulate the accessibility to the loci in the chromatin. DNA methylation is arguably the most studied epigenetic modification that consists of the addition of a methyl group at the fifth cytosine's carbon. If this modification is located inside a gene or near its regulatory regions, it could act as an activator or suppressor of their expression. DNA methylation research in animals has been mainly focused on vertebrates whereas the epigenetic landscape in invertebrates remains poorly understood. For molluscs, few studies have analyzed methylation, although, it has been estimated that the group could have variable levels of methylation [3]. Whole-genome bisulfite (WGBS) is one of the most widely used and accurate techniques to detect methylation at a single base-pair level. Is based on the treatment of the genomic DNA with sodium bisulfite which modifies unmethylated cytosines into uracils. During the sequencing of the DNA, these uracils are detected as thymines. Methylated cytosines are not affected by the bisulfite treatment and therefore will be detected as cytosines. The objective of this study was to analyze for the first time the methylation landscape of the cone snail *L. ventricosus* using WGBS of the venom gland and muscle tissue and test for different methylated regions that would be related to venom regulation. In this report I show the results of the whole-genome bisulfite sequencing and the preliminary result of the methylation estimation in the genome of the cone snail *L. ventricosus*.

Methods

Three specimens of the Mediterranean cone snail *Lautoconus ventricosus* from Faro (Portugal) were sacrificed and removed from the shell using a needle. For each one of the snails, the venom gland and muscle tissue of the foot were dissected and stored in absolute ethanol. The DNA extraction of the venom gland and muscle tissue was carried out using the QUIAGEN DNS kit for blood cells. The extracted DNA was sent to Novogene company (UK) for whole-genome bisulfite sequencing (NovaSeq6000, paired-end 150 bp). The reads were first evaluated using FastQC v 0.12.1 and cleaned for adaptors and low-quality regions using Trim Galore v

0.6.10. Bismark v0.24.2 pipeline was used to perform the alignment of the reads (which internally runs Bowtie2 v 2.5.3), a deduplication process to reduce redundancy of identical reads using the script deduplicate_bismark and the analysis of CpG methylated positions with the script bismark_methylation_extractor.

Results and discussion

The venom gland and muscle methylome of three specimens of *L. ventricosus* were sequenced using WGBS. The sequencing results of the venom gland and the muscle tissue are summarized in Table 1. The sequencing raw data for the six samples varied from 37.1 to 43.2 Gb. Considering that the genome of *L. ventricosus* is 3.59 Gb, all of the samples were about 10x of sequenced depth, which falls into the recommended values for detecting differentially methylated regions [4].

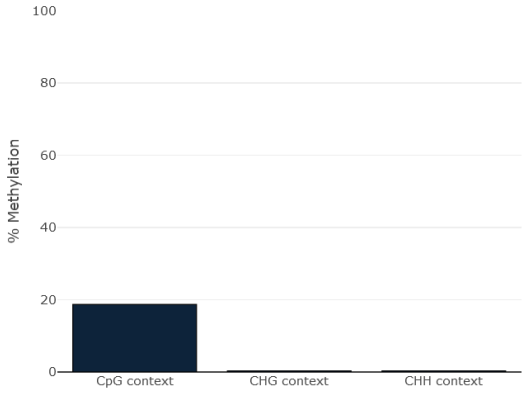
Species	Specimen code	Tissue	Number of raw reads*	Number of reads post trimming
L.ventricosus	LV1F	Foot muscle	287753306	287413234
	LV1G	Venom gland	251430220	251152564
L.ventricosus	LV3F	Foot muscle	254592794	254173806
	LV3G	Venom gland	247324806	246919480
L.ventricosus	LV8F	Foot muscle	247126436	246651980
	LV8G	Venom gland	249567510	249250484

Table 1. Samples of *L. ventricosus* sequencing in this work. (*) The number of raw reads equals the amount of R1 and R2.

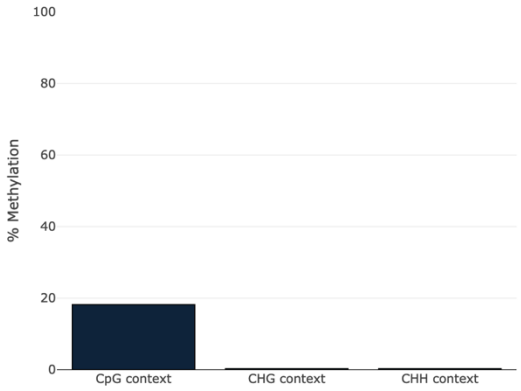
After cleaning the raw data, the reads were mapped against the genome of *L. ventricosus* with an average result of 32 % (SD=0.87) of unique alignments for the six samples in paired-end mode. Since this value was relatively low, I decided to evaluate the efficiency of the alignment for each set of reads independently, R1 and R2, to investigate for differences in mapping. The reads corresponding to the sample LV1F displayed different alignment scores, with R1 reaching a higher score than R2, 47.8% and 33.9% respectively. In the case of the venom gland of sample LV1G, I observed the same difference between the R1 (48%) and R2 (31.9%). Therefore, I decided to focus on the analysis of R1 reads for the estimation of DNA methylation. In our case, R1 analysis reported a methylation state of 18.7% of CpG sites for LV1F (foot muscle) and 18.2% for LV1G (venom gland) (Figure 1). Methylation does not occur randomly in the cytosines of the genome. Instead, they are frequently found in CpG sites that can also be present as arrays of multiple CpG sites, the so-called CpG islands. Therefore, it is important to analyze methylation in the context of CpG regions. Although methylation levels were similar between the venom gland and muscle tissue, it is not possible to differentiate whether the methylation is equally distributed in the genome for both tissues or has a different landscape.

a)

Total C's analysed	1756295450
Methylated C's in CpG context	43713771
Methylated C's in CHG context	1047273
Methylated C's in CHH context	2931863
Unmethylated C's in CpG context	189995265
Unmethylated C's in CHG context	386749533
Unmethylated C's in CHH context	1131857745
Percentage methylation (CpG context)	18.7%
Percentage methylation (CHG context)	0.3%
Percentage methylation (CHH context)	0.3%



Total C's analysed	1400598281
Methylated C's in CpG context	33799583
Methylated C's in CHG context	925835
Methylated C's in CHH context	2648368
Unmethylated C's in CpG context	151740538
Unmethylated C's in CHG context	305225281
Unmethylated C's in CHH context	906258676
Percentage methylation (CpG context)	18.2%
Percentage methylation (CHG context)	0.3%
Percentage methylation (CHH context)	0.3%



b)

Figure 1. Percentage of methylation according to the of C's in CpG, CHG, and CHH context (H represents either A, T or C) for a) *L. ventricosus* foot muscle (LV1F) and b) the venom gland of the same specimen (LVIG). For both analyses, only R1 reads were used.

Methylation is highly variable between different species, individuals, developmental stages, and even cell types from the same tissue [5]. Therefore, it is necessary to be conservative when comparing information about methylation among different samples. Genomes of invertebrates display wide variations among species in terms of how much and where DNA methylation occurs [3,6]. In molluscs, except for Monoplacophora, which showed no evidence of cytosine methylation, there is evidence of methylation for all the main lineages, including Gastropoda [3].

The data presented in this report represents the preliminary results for the methylation state in *L. ventricosus* genome, showing a moderate level of CpG methylation that agrees with previous estimates of methylation in gastropods. This is an ongoing project that will try to answer if the methylation detected co-localizes with some specific regions in the genome that would be related to venom regulation.

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Blueprints of biological complexity: Decoding molecular pathways and the evolution of molluscan shell biomineralization

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The University of Hong Kong

Bivalves are an evolutionarily successful group that has spread to virtually all aquatic habitats (1). One of the reasons bivalves are thought to be evolutionary successful is the secretion of an outer shell (2). Shell formation is controlled by a homologous organ, the mantle (3), through biologically controlled biomineralization, during which an extracellular organic matrix (ECM) is occluded within the mineral phase (4). A significant part of the ECM comprises proteins that can be recovered directly from the mineralized shells. Shell matrix proteins (SMPs) play active roles in calcification. SMPs assemblages can promote the formation of either calcite or aragonite (5), they can nucleate crystals (6), determine the CaCO3 phase (7), initiate and inhibit calcification (8) and control the crystallization patterns (9). Often, SMPs have negative charges that can interact with the charged ions of the mineral phase (10) and have repeating, low-complexity motifs that may mimic the crystal lattice (11). SMPs are also hypothesized to have immunology and antibacterial roles, making the bivalve shell not only a physical protection but also a chemical barrier (12).

Bivalve shells and their SMPs have been extensively studied; nevertheless, the complex mechanisms behind biologically controlled biomineralization remain elusive (2). In particular, identifying a core biomineralization molecular toolkit and common ancestry of bivalve SMPs has been challenging (12–14). Bivalve SMPs are accumulated through complex mechanisms involving multiple co-option and loss, de novo gene evolution, alternative splicing, and fast evolution of modular, repetitive SMP sequences (3, 13, 15, 16). Currently, bivalve shell formation is thought to be characterized by specific-specific proteomes (17).
Many studies have analyzed the shell proteins from adult bivalves (18). A few have directly compared SMPs from different species (12, 19, 20). However, comprehensive phylogenetic comparisons between bivalve species have relied heavily on mantle transcriptomic data instead (13, 21). Further, recent evidence suggests that the environment can affect organic contributions to mussel shells (22, 23) and their SMP diversity and regulation (24). However, the influence of the environment has generally not been considered in shell proteome comparisons. SMP studies have mainly focused on the Pteriomorphia (2, 18). Finally, many early studies could only use public databases as references for shell proteomics experiments (e.g., (25, 26)).

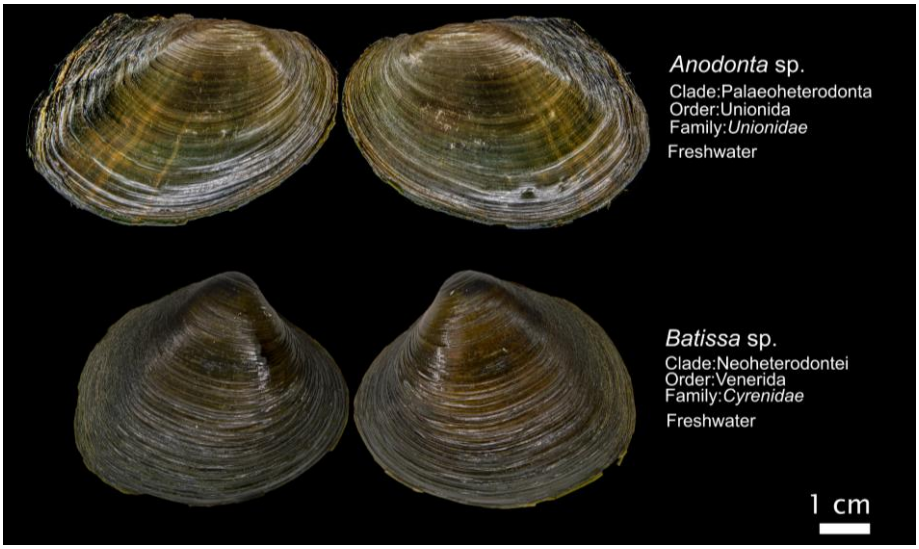

Figure 1 High-resolution images of the freshwater bivalve species used in this study. Shells are imaged from the outer side, and each species and habitat's taxonomic information is provided.

Therefore, in the last data chapter of my PhD thesis, I aimed to expand the current understanding of bivalve shell formation by investigating the shell proteomes of 11 novel bivalve species. This study constitutes the most extensive investigation of bivalve SMPs, supplemented with structural data from micro-computed tomography (micro-CT) imaging. Together, these analyses will significantly enhance our understanding of bivalve shell formation and its evolution.

To comprehensively investigate biomineralization processes in the Bivalvia, representative bivalve species from all major taxonomic clades (as in (27)) were targeted, and the environment was maintained as fixed as possible to isolate phylogenetic differences in shell formation better. Unfortunately, the Protobranchia were not collected in this study due to their rarity and remoteness (28). All other marine clades (Pteriomorphia, Archiheterodonta, Anomalodesmata, Imparidentia, Neoheterodontei) were represented with one or more species. Two species belonging to two different taxonomic orders within the same clade were collected where possible. However, a few phylogenetic constraints were applied to sample from only one site, Starfish Bay (海星灣), in Ma On Shan (馬鞍山), NE Hong Kong (22°25'55.3''N; 114°14'43.4''E).

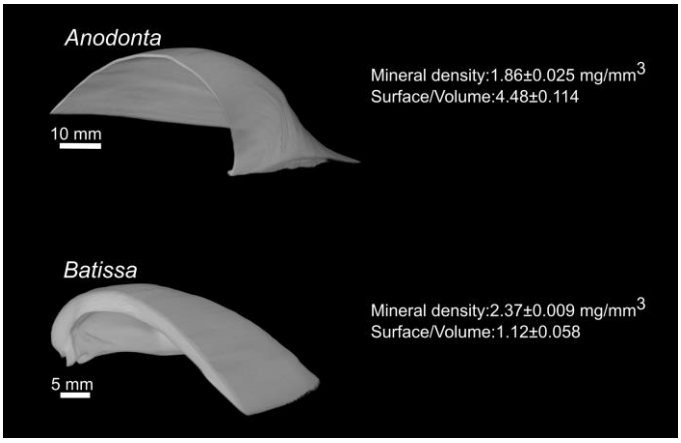

Figure 2 Examples of shell sections obtained with micro-computed tomography (micro-CT) imaging. The average mineral density and surface-to-volume ratio are provided as mean ± standard error for each freshwater species.

Nevertheless, the investigation would not have been complete without freshwater bivalves (Figure 1). The UM Student Research Award was instrumental in completing this part of the study. One bivalve clade, the Palaeoheterodonta, is a small clade represented exclusively by freshwater species. One species from the order Unionida, *Anodonta* sp., was obtained from a local goldfish market. Further, a second freshwater species, *Batissa* sp., from the Neoheterodontei clade, order Venerida, was also purchased. The species *Batissa* was included to 1) enrich the freshwater bivalve shell analysis with representatives from distant clades, 2) for comparisons within the order Venerida from different evolutionary trajectories and 3) to compare freshwater nacreous and crossed-lamellar structures (Figure 2).

To investigate bivalve shell formation, samples were collected, processed, and analyzed for:

- Bivalve shell protein characterization (shotgun proteomics)
- RNA sequencing (transcriptomics)
- Shell structural analyses (micro-computed tomography)

As a quick overview of our findings, there was a clear divide between bivalves with nacreous or crossed-lamellar microstructures. This study showed that bivalves with nacreous shells secrete much more diverse shell proteomes and share an extensive set of functional domains, suggesting that large, diverse, conserved proteomes are necessary for nacre secretion in distant clades and independently of marine or freshwater environments. Crossed lamellar species only shared six functional domains that were common to all investigated bivalves. Therefore, the shell proteome used for crossed-lamellar shell formation is more flexible. Interestingly, the bivalve shell structure and shell proteome data showed that mineral density and SMP diversity were more strongly correlated with shell shape and shell microstructure type, respectively, than with phylogenetic clade. These results suggest that the organic

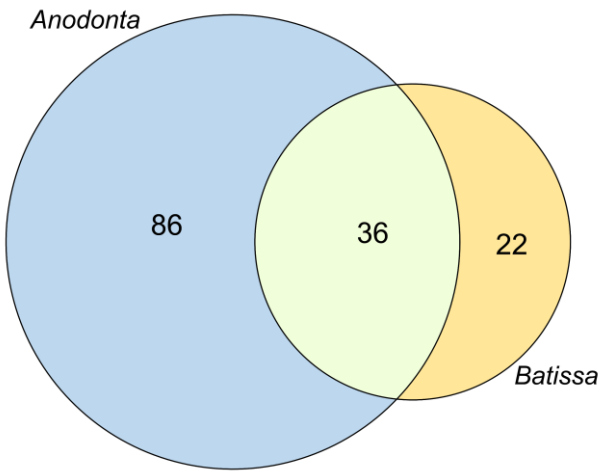

Figure 3 Protein functional domain comparison between the freshwater shell proteomes. The numbers in the circle represent the domain count.

and mineral components of the bivalve shell are more influenced by the adaptive morphology of the shell than its evolutionary history.

By comparing the shell proteomes of freshwater species and within the Neoheterodontei clade, no obvious pattern in domain distribution was observed (Figure 3). The shell proteome of *Batissa* was integrated within the larger *Anodonta* proteome. Still, the similarity was not higher than when compared to closely related species from the same site. Further, no SMP was unique to the freshwater species or to *Batissa* and the other *Veneridae,* suggesting the domain overlap is due to function rather than habitat or phylogeny. Therefore, a freshwater SMP toolkit was not identified. Together with other observations, the results suggest that shell type has a more decisive influence on the shell proteome than habitat or phylogeny.

No nuclear proteins were identified, supporting the traditional knowledge that bivalve shells occlude an extracellular matrix and can incorporate vesicles and transmembrane proteins involved in biomineralization (4). The bivalve shell proteomes investigated shared similarities with other phylogenetically distant calcifying ECMs (29, 30) and other co-opted structural proteomes, such as eye lenses, antifreeze proteins, and feather proteins (31). Therefore, the complex variety of mechanisms through which SMPs were recruited for shell formation is likely to follow similar directions as other animal ECMs.

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