ASCIDIAN NEWS*

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For this issue of AN, coming out at a very challenging time for everyone, I asked how and what you are doing, how you are coping, are you able to continue your research and teaching, has this been a good time to write up earlier work; what would you like to share with other readers of AN. I received an incredible response! A number of correspondences are included in the next two sections. Nearly everyone expressed that in spite of the frustrations, there was confidence at having met the challenges and a great feeling of accomplishment ; congratulations to you all! Many Ph.D. theses were recently awarded; the abstracts of 9 are included, plus an M.S. abstract, by far the most ever for a single newsletter. And there are many new publications! 111 are included at the end of the newsletter. I hope you enjoy reading this issue. This is now 45 years I have been doing AN! And it is so good to know how much it is read, used and appreciated. Please keep in touch and continue to send me contributions for the next issue. Keep safe, keep working, and good luck to everyone.

***Ascidian News is not part of the scientific literature and should not be cited as such.**

NEWS AND VIEWS

1. From **Lucia Manni**, Univ. of Padua: Here, in Italy, the situation is improving, hopefully. We have opened our lab and are making some experiments. It seems a dream!

2. From **Hiroki Nishida**, Osaka Univ.: We are happy to announce the date and venue of the 11th International Tunicate Meeting (ITM), which will be held in Kobe, Japan, from July 11(Sunday) to the 16th (Friday), 2021. Venue: Konan University, Kobe, Japan. https://www.konan-u.ac.jp/english/location/ 34°43'26.7"N 135°15'45.5"E

Local organizing committee: Takehito Kusakabe, Takahito Nishikata, Yasunori Sasakura, Hiroki Nishida. See you in Japan!

Tentative plans for the meeting after the next will be China, probably in Qingdao (2027).

3. From **Noa Shenkar** (noa.shenkar@gmail.com), Tel-Aviv Univ.: The COVID-19 crisis was combined with an extremely strong storm in Eilat, Red Sea coast of Israel. The storm caused enormous damage to the shallow coral reef, and the Interuniversity Institute (IUI). We also had to postpone all our field activities along the Mediterranean coast involving the use of ascidians as biological indicators. We could not go sampling until it was allowed to go to the beach. Gradually, at the beginning of May we returned to the lab and even to the field. The good news is that we all had plenty of time to write manuscripts, and hopefully by the next AN we will have some interesting work available.

4. From **Marcos Tatián** (marcostatian@gmail.com): Things here in Argentina are relatively well, in respect to the pandemic, owing to restrictive quarantine since March 16th. Nevertheless, the

economic situation is at the edge. Hopefully it will not be chaotic. We are working at home, fortunately everybody without health problems.

5. From **Mauro Pavao** mpavao@hucff.ufrj.br), Univ. Fed. Rio de Janeiro: We are all well, but worried about the situation. We are trying to stay at home as much as we can and taking all the preventive Measures to reduce the risk of infection. Unfortunately, great part of the population is not following the WHO recommendations, increasing the risk for others. Our government as you may be aware does not support the quarantine and wants all people at non risk to resume their business. Even knowing that the transmission rate hasn't been decreasing. Despite the gloomy situation we all see through the news, we are managing to stay physically and mentally healthy. Some days are betters that others.
Although our lab is closed at the moment, we are working from home. I am happy to share our recent accepted paper in Cancers, reporting a tunicate antimetastatic glycan. [see New Publications]

6. From **Euichi Hirose** (euichi@sci.u-ryukyu.ac.jp), University of the Ryukyus. In Okinawa, the faculty members are recommended to stay within the island now. Classes are basically online now and I am teaching via Skype.

7. Ryo Koyanogi (rk8052zh@gmail.com), former Ph.D. student of Thomas Honegger, who was retired from the Univ. of Zurich, gave me the very sad news that Thomas passed away rather suddenly in March, after a short illness. Thomas published many significant papers during his career, a legacy that will live on for a long time.

8. Hoya Fair promotes Japanese sea squirt consumption. Reprinted with permission from https://www.seafoodsource.com/news/foodservice-retail/hoya-fair-promotes-japanese-sea-squirt-consumption **,** Cliff White And the author Chris Loew (je5c-loew@asahi-net.or.jp) .

March 3, 2020

Photo by Chris Loew/SeafoodSource

The Hoya Hoya Association, with its secretariat in Ishinomaki, Miyagi Prefecture, Japan, and an office in Tokyo, was started in 2014 and became a formally incorporated association in November 2019 with the express purpose of raising awareness of the sea squirt, or sea pineapple (*hoya* in Japanese) in order to expand sales channels. Prior to the 2011 earthquake and tsunami disaster, hoya (*Halocynthia roretzi*) was a local delicacy along the Sanriku Coast – along the northeast part of Honshu Island – and Miyagi Prefecture produced 80 percent of the harvest. However, 70 percent of that was exported to South Korea, where it was often served as sashimi. The infrastructure and

production equipment for the species was destroyed by the tsunami. As it takes about three years to grow, harvests resumed in 2014, but a ban by South Korea on imports from the region due to concerns about radioactivity has left a lot of unsold hoya. In 2016, about 8,000 metric tons of sea squirts, roughly 60 percent of total production volume, was disposed of. As a result, producers are trying to introduce it to other areas of Japan that may be unfamiliar with it through the association. New recipes are being introduced and various processed forms are being marketed.
At the 17th Seafood Show Osaka 2020, held 19 and 20 February, the association had an attention-grabbing booth, with funny hats, plastic hoya-like action figures, products, and a questionnaire of hoya trivia. “About half of Japanese know about hoya," Hoya Hoya Association President Keiko Tayama told SeafoodSource. To spread the word, the association hosted a Hoya Fair during the month of February, with over 140 participating shops around Japan, featuring hoya in various recipes. Hoya processors with their own booths at Seafood Show Osaka 2020 displayed such products as hoya in rayu (chili oil), peeled hoya in a vacuum-pack, hoya-flavored salad dressing, dried powdered hoya (used as a spice), hoya with a soft-boiled egg inside, and breaded and deep-fried hoya.
The texture and color of hoya is similar to papaya, but with a salty rather than sweet flavor, and an aftertaste of astringency. It may be suitable as part of an appetizer set (*otoshi* or *tsukidashi*) served at Japanese izakaya restaurants. Other sea squirt processing companies at the show included Honda Suisan, Co., Ltd.; Sugetsudo Co., Ltd; Yamanaka Corp. (all based in Ishinomaki City, Miyagi prefecture); and Nihon Marine Ranch Co., Ltd., based in Shiogama City, Miyagi Prefecture.

9. From the Sydney Morning Herald online podcast, Australia: The Good Weekend Quiz May 30, 2020. By Stephanie Bull and Jacqui Martinez.
Starting with “C”, what Aboriginal name describes a sea squirt? Cunjevoi.

10. From **Xavier Turon** (xturon@ceab.csic.es), Dept. of Marine Ecology, Center for Advanced Studies of Blanes, Spain.
Here we continue with tele-working. My lab has a limited access, only for essential work that cannot be put off. I have managed to continue the field monitorings, at least. I was fortunate that, at this time, I have lots of data to analyze and mss to write. Of course, no option to work on taxonomy until the Institute is reopened (scheduled end of June, with restrictions).

11. Has anyone read the wonderful books on marine life by Jack Rudloe? He founded the Gulf Specimen Marine Company in Panacea, Florida, in 1963, and collected marine specimens for high school and university classes for many decades, as Ed Ricketts had done earlier in Monterey California. I read all Rudloe’s books starting in the 1960’s and thought he must be a lot older than I and had probably passed away. But no! Here is a recent article on this amazing guy who is actually a year younger than I am and still going strong. https://www.tallahassee.com/story/life/2020/05/30/pure-stubbornness-founder-wont-give-up-gulf-specimen-marine-lab/5244716002/ . From the article: “Rudloe launched Gulf Specimen in 1963 with encouragement from marine biology fan John Steinbeck”! This is amazing because Steinbeck was also a very good friend of Ed Ricketts during the 1930’s and 40’s, they went on collecting trips together (the most well known being their trip to the Sea of Cortez in Baja California) and published a book together. You can find much more information about this remarkable man at https://en.wikipedia.org/wiki/Jack_Rudloe .

WORK IN PROGRESS

1. From **Loriano Ballarin (**ballarin@bio.unipd.it), Univ. of Padua, Italy: We are slowly returning to normality. The Department is still without students and lessons but research has started again. I send you two abstracts presented at the last meeting we were able to attend (February).

2. From **Fabio Gasparini** (fabio.gasparini@unipd.it): To complete the information on the new species *Botrylloides crystallinus* (Bay-Nouailhat, A., Bay-Nouailhat, W., Gasparini, F. and Brunetti, R. 2020. *Botrylloides crystallinus* n. sp., a new Botryllinae Adams & Adams, 1858 (Ascidiacea) from Mediterranean Sea. Zoosystema **42**: 131-138), it has been also molecularly characterised using a COI fragment by Carmela Gissi. The COI entry was submitted at EMBL with AC number LR537145 and is available as an old version at the following link: https://www.ncbi.nlm.nih.gov/nuccore/1829779306. A new version of the entry, with the corrected metadata, has been also submitted to EMBL but is not yet available.

3. From **Dan Minchin** (moiireland@yahoo.ie): Julia Nunn and I are undertaking a review of all known NIS (tunicates) on the island of Ireland.

4. There is always so much interest in *Ciona* spp! Here is the newest progress in unraveling the taxonomy of this interesting genus. From **Francesco Mastrototaro (**francesco.mastrototaro@uniba.it) : "Our paper describes a new enigmatic species of *Ciona* with morphological and biomolecular intermediate features." Mastrototaro, F., Montesanto, F., Salonna, M., Viard, F., Chimienti, G., Trainito, E. and Gissi, C. 2020. An integrative taxonomic framework for the study of the genus *Ciona* (Ascidiacea) and description of a new species, *Ciona intermedia*. Zool. J. Linn. Soc. **20**: 1–24.

5. From **Tito Lutofo** (tmlotufo@gmail.com): I was thinking about you and other friends living in the USA, and wondering how are you doing during this odd time. Here in Brazil things are a bit worse than in the US. In São Paulo we are suffering with increasing deaths. Our governor and mayor instituted a quarantine for all non essential activities. We have been in this quarantine since the beginning of March, and still don't have a certain date for coming back to work. My lab and university is shut down since then, so no lab work is being done. We are writing and wrapping up things that were almost ready, while still teaching remotely and doing a lot of virtual meetings as well.

Last November I went to Koumac, New Caledonia, along with Chris Meyer and Sarah Tweedt to process 36 ARMS [Autonomous Reef Monitoring Structures] deployed around Koumac in 2018, as part of the La Planète Revisitée program, led by Philippe Bouchet, from MNHN [Muséum national d'Histoire naturelle] in Paris. We got plenty of nice ascidians, and lots of samples to work later. Right before the COVID we managed to process ARMS deployed on coastal islands off Sao Paulo state, and gathered many ascidian samples as well. Unfortunately we could not keep working on these samples. I am now finishing a paper with new *Ecteinascidia* species, as a result of Nadia Bonnet Ph.D., with Rosana Rocha's help too. We also have new descriptions of very small didemnids associated with crustose coralline algae, all from Alcatrazes Island.

6. From **Federico Brown** (fdbrown@usp.br), Instituto Biociências Universidade de São Paulo.

It has been some real challenging times for all of us indeed. I am currently doing a sabbatical in the Tiozzo Lab at the Institut de la Mer Villefranche. We are also returning slowly to our research activities but with many restrictions. Currently we are at 50% capacity, one person per room, and of course masks and social distancing. My daughter is attending school only two days per week so that the classrooms remain with low numbers of kids. My lab in São Paulo unfortunately had to lock down and we had to kill most of our colonial laboratory ascidians. We only have a few remaining juveniles of *Microcosmus exasperatus* that have survived in our acquaria despite very irregular visits (every twoo weeks). Members of my lab are focusing on bioinformatic projects and writing manuscripts at the moment. The current situation in São Paulo as in many Latin American cities is problematic at the moment as shops and businesses start to open up despite the constant and permanent rise of corona cases. And to top it off, many governors and politicians in the region have only worsened the situation by generating political and economical chaos.

Recent work: 1) a University of Magallanes media report (published in Dec 11th, 2019) on an ascidian evo-devo course that I. Cañete, S. Tiozzo, and I co-taught in Punta Arenas Chile at the end of last year. We had the opportunity to observe some very strange and large species of ascidians typical of many invertebrates in those lower and colder latitudes. See link in Spanish: http://www.umag.cl/vcm/?p=43433.
2) A Special Issue on Animal Coloniality and Modularity that I am currently serving as Editor for will be published soon in the Journal of Experimental Zoology - Part B. We had a great turn-out of manuscripts of different taxa and topics, and of course several articles will include research on our beloved tunicates. Stay tuned! Many accepted manuscripts can already be found as Early View at: https://onlinelibrary.wiley.com/toc/15525015/0/0
3) As a result from collaborative efforts among laboratories in Colombia (Bermudez lab), Germany (Stadler lab), Netherlands (Gittenberger lab), and Brazil (Brown lab), we now report a new re-assembly of the *Didemnum vexillum* genome. Although still highly fragmented, we used PacBio and transcriptomes this time to increase contig length by 8-fold (from the previous draft genome), and have better annotated protein-coding genes and non-coding RNAs. See pre-print at: https://www.researchsquare.com/article/rs-23360/v1. The link to the ongoing *D. vexillum* genome project: http://tunicatadvexillum.bioinf.uni-leipzig.de/Home.html.

7. From **Anne and Wilfried Bay-Nouailhat** (mer.littoral@gmail.com) : We are pleased to announce the publication of an identification guide entirely devoted to tunicates in western Europe. This is a field guide, in French, for all nature lovers, naturalist divers, students and specialists in marine environment. The authors are Anne and Wilfried Bay-Nouailhat and it is prefaced by the ascidiologist Xavier Turon. This guide presents the history of our knowledge about ascidians, describes the anatomy of sessile and pelagic Tunicates, their biology and ecology, and precisely describes, with many illustrations, the species most commonly seen along the coasts of Western Europe. It contains 113 species and more than 630 photos and colour illustrations, visual identification keys for quick and easy identification, information on the internal anatomy, distribution and ecology of each species, a glossary of terms used and a reference bibliography.
Anne Bay-Nouailhat, Wilfried Bay-Nouailhat (2020) Guide des Tuniciers de l'Europe de l'Ouest. M&L editions. 240 pages. ISBN: 978-2-9571115-0-3. For more info, https://www.mer-littoral.org/guide-tuniciers.php or livre.tuniciers@gmail.com.

This Guide des Tuniciers is very different from the excellent guide by Riccardo Brunetti and Francesco Mastrototaro, which is aimed at an informed public, capable of fixing and dissecting ascidians. [Brunetti, R. and Mastrototaro, F. 2017. Ascidiacea of the European Waters. In: (ed.), Fauna D'Italia. Bologna, Italy, Calderini Publications.] In our guide we address a wider audience, divers, naturalists but also specialists. For this we put forward the external anatomy of the species as encountered in their environment and we illustrate each species with photographs taken in situ. [Also, see their new publications in the New Publications section 1) a new species of *Botrylloides, B. crystallinus*, https://doi.org/10.5252/zoosystema2020v42a9. http://zoosystema.com/42/9; 2) the use of eugenol as a relaxing agent for ascidians. https://www.mer-littoral.org/eugenol-ascidians-anaesthetic.pdf.

8. From **Tamara Maggioni** (tamaramaggioni@gmail.com), Universidad Nacional de Córdoba, Argentina, and her colleagues Gastón Alurralde, Paola Reyna, Anabela Taverna, Clara Rimondino, Emilia Calcagno and Marcos Tatián:

Greetings from Argentina to all the community of "ascidian lovers"! We hope all our fellow ascidiologists are in good health and able to carry on with their activities. Although the COVID-19 pandemic has affected us all, different countries have responded in a variety of ways. With the health of its citizens as a priority and under the constant advice of national scientists and epidemiologists, Argentina decided to implement the quarantine quickly and prematurely. As probably in most of the

cases, Argentinian scientific activity has been focused on SARS-CoV-2 and COVID-19 related investigation lines. Some achievements in this regard are worth to mention, such as: the sequencing of the complete genome of the virus strain present in Argentina, the development of a rapid detection test and an innovative ventilator system.

For those of us whose research is not related to or could not be refocused to COVID-19, all activities have been interrupted since March 20th. Only a reduced number of personnel assigned with essential activities, such as feeding and maintaining living organisms, has had permission to attend their institutions. Thus, working from home has not only been encouraged, but also expected. Assistance to classes has also been suspended. At the Universidad Nacional de Córdoba, classes are on-line. Field trips or lab practices had to be suspended. In this context, researchers from the Biología de Ambientes Acuáticos group (@biologia.anb.acuaticos) at the Instituto de Diversidad y Ecología Animal (Córdoba, Argentina), have been telecommuting on the following projects:
1- Discussion of results on morphology and genetics of material from SW Atlantic, especially harbour areas and the Burdwood Bank (an oceanic Marine Protected Area)
2- Ascidian epibiosis in deep-sea environments
3- Ascidians as bioindicators of heavy metal contamination
4- Trophic ecology of two ascidian species and their impact on particle flux in a port area in Puerto Madryn city, Argentina.

9. From **Carmela Gissi** (carmela.gissi@uniba.it): In this bad pandemic period I was mostly teaching at home, which was even more complicated and took more time than teaching "in person". Writing papers was not so fast, because there were a lot of online meetings and the concentration was not so good. University and laboratories are now open [June 10], but for reduced time and with staff schedule, so I am still working at home. Currently I am almost at the end of the writing of the paper of Salonna's talk on Botryllids (the one presented at the Tunicate meeting in July 2019, see below). Proudly, we were able to publish two of the works presented at the last Tunicate meeting, both including the description of a new species. [See the New Publications section.]

10. From **Stefano Tiozzo** (tiozzo@obs-vlfr.fr): Ongoing projects:
a) Searching for master genes of budding: a comparative transcriptomic study in tunicates
Canon Foundation is supporting our team, in collaboration with Kazuo Kawamura and Takeshi Sunanaga, for a project that aims at finding putative master genes controlling NED (budding and whole body regeneration) in tunicates by a comparative transcriptomic analysis between four species of styelids: *Botryllus schlosseri, Polyandrocarpa zorritensis, Botryllus primigenus* and *Polyandrocarpa misakiensis*.
b) Comparative genomics among solitary and colonial (NED) species
To highlight genomic signatures linked to NED and to highlight other genotype-phenotype convergent associations related to characters linked to the capacity of undergoing NED, we are sequencing and comparing the genomes of four species of Styelid, two colonials (*Botryllus schlosseri* and *Polyandrocarpa zorritensis*) and two solitary (*Dendrodoa grossularia* and *Styela plicata*).
c) Art and Regeneration
Funded by the Advanced Research Program at the Université Côte d'Azur, the project ' If we were to look at regeneration with a different eye' has been initiated as an artistic-scientific collaboration with the artist Irene Kopelman and the Röttinger (IRCAN – UCA, CNRS, INSERM), and is driven by Irene long-term engagement with scientific research which underlies her artistic practice.

11. From **Marie Nydam** (mnydam@soka.edu), Soka Intl. Univ.:

a) Nydam, M.L. 2020. **Evolution of allorecognition in the Tunicata.** Accepted in Biology, Special Issue Ancient Immunity. Phylogenetic Emergence of Recognition-Defence Mechanisms.

Allorecognition, the ability to distinguishing self or kin from unrelated conspecifics, plays several important biological roles in invertebrate animals. Two of these roles include negotiating limited benthic space for colonial invertebrates, and inbreeding avoidance through self-incompatibility systems. Subphylum Tunicata (Phylum Chordata), the sister group to the vertebrates, is a promising group in which to study allorecognition. Coloniality has evolved many times independently in the tunicates, and the best known invertebrate self-incompatibility systems are in tunicates. Recent phylogenomic studies have coalesced around a phylogeny of the Tunicata as well as the Order Stolidobranchia within the Tunicata, providing a path forward for the study of allorecognition in this group.

b) Nydam, M.L., Lemmon, A.R., Cherry, J.R., Kortyna, M.L., Hernandez, C., Cohen, C.S. 2020. **Phylogenetic relationships among the botryllid ascidians.** *Manuscript in Preparation.*
To provide a framework for future studies of botryllid ascidians, we have developed 200 nuclear loci and constructed a well-resolved phylogenomic tree using these loci. Probes corresponding to the 200 nuclear loci were hybridized to 55 genomic libraries, and 17 OTUs were recovered. These 17 OTUs include previously undescribed species from the Caribbean Sea and the Philippines. A Principal Components Analysis using 31 taxonomic characteristics revealed that some species occupy a unique morphological space and can be easily identified using characteristics of adult colonies. For other species, additional information such as larval or life history characteristics may be required for taxonomy. Molecular barcodes are critical for delineating morphologically similar species in this group.

c) Nydam, M.L., Gissi, C., Palomino-Alvarez, L.A. As an expansion of the phylogenetic tree described in the above manuscript, we plan to include ~40 additional samples from Australia, Europe, Florida, the Gulf of Mexico and Japan.

12. From **Billie Swalla** (bjswalla@uw.edu): I had to teach 110 undergraduates Developmental Biology (on-line!) this spring quarter. It was truly a challenging experience, and took 110% of my time and energy. Teaching that many students on-line is a huge challenge when I rarely even saw their faces, instead it was a blur of Zoom calls, emails, on-line assignments and Google docs. The good thing is that the students actually did very well and learned quite a bit of Developmental Biology, even though I was exhausted much of the time. Obviously, the grant and papers that I thought I would be able to work on, are still languishing, but maybe I'll get to them this summer. We have a paper in review: Lowe, E.K., Racioppi, C., Peyriéras, N. Ristoratore, F., Ruggiu, M., Christiaen, L., Swalla, B.J., Stolfi, A. (2020) A cis-regulatory change underlying the motor neuron-specific loss of terminal selector gene expression in immotile tunicate larvae. Evolution and Development (in revision). bioRxiv preprint doi: https://doi.org/10.1101/567719.

13. From **Gretchen Lambert** (gretchen.lambert00@gmail.com): In 2013 I was a participant in a huge marine biodiversity survey in Singapore, one of many taxonomists invited from around the world. Samples were collected by various teams by snorkel, SCUBA, dredging, and intertidally, with a large team of students to do the preliminary sorting (Tan, KS, Koh, KS, Ng, JY and Goh, L 2016. The comprehensive marine biodiversity survey Singapore Strait International Workshop 2013. Raffles Bull. of Zoology Suppl. 34, Part 1: 1-7). I spent a month at the Univ. of Singapore marine lab, also took samples home and others were sent to me. Although many species remain unidentified in the Raffles Museum, with no further opportunities to work on them for the forseeable future, it seemed appropriate to write up the results so far for the ascidians. Indeed, the manuscript got so big it is now divided into two parts, Aplousobranchia and Phlebobranchia in the first part, Stolidobranchia in the second part. I hope it will soon be submitted to Zoosystema.

MEETINGS ABSTRACTS

1. Atti XXI Convegno della Società Italiana di Immunologia Comparata e dello Sviluppo (SIICS)/, Varese 12-14 febbraio.

a) Stress granules in C*iona robusta*: molecular evolution of TIAR and TTP and early evidence of their gene expression under stress conditions induced by metals. L Drago, L Ballarin, G Santovito, Dept. of Biology, Univ. of Padova, Padova, Italy. ballarin@bio.unipd.it

Stress granules are non-membranous cytoplasmic foci composed of messengers (not translated), ribonucleoproteins, translation initiation components and other additional proteins, that represent a primary mechanism by which gene expression is rapidly modulated when cells are subjected to adverse environmental conditions (KEDERSHA *et al.*, 2002; ANDERSON *et al.*, 2009; LAVUT *et al.*, 2012; WARIS *et al.*, 2014). Very few works have been devoted to study the presence of molecular components of stress granules in invertebrate animals. In this work, we characterized, for the first time in the solitary ascidian *Ciona robusta*, the genetic sequences of two important protein components of stress granules, TIAR (TIA-1 related to proteins) and TTP (tristetraprolin), and carried out the first studies on their gene expression. The sequences characterized for *tiar* and *ttp* genes have allowed to start a study on the molecular evolution of these proteins in animals: for TIAR the obtained results are consistent with recent phylogenetic analysis that place tunicates as sister group of vertebrates, whereas the phylogenetic position of TTP remains still uncertain. The data on mRNA expression, provided by qRTPCR analysis, are absolutely the first obtained in non-mammalian animals. As expected, the exposure to each metal (Cu, Zn and Cd) led to a generalized decrease in mRNA expression levels for both TIAR and TTP, suggesting that the metal accumulation induce acute stress and the inhibition of the transcription of *tiar* and *ttp* genes. The data presented here improved our knowledge about the molecular evolution anti-stress proteins in metazoans and emphasize the importance of the transcription of *tiar* and *ttp* genes, which represents an efficient physiological response allowing *C. robusta* to survive in the presence of metals in the marine environment (Supported by M.I.U.R. grant). References**:**

Anderson, P., Kedersha, N., 2009. Stress granules. Current Biology 19 (10): R397-398.
Kedersha, N., Anderson, P., 2002. Stress granules: sites of mRNA triage that regulate mRNA stability and translatability. Biochemical Society Transactions 30 (6): 963-969.
Lavut, A., Raveh, D., 2012. Sequestration of highly expressed mRNAs in cytoplasmic granules, P-bodies and stress granules enhances cell viability. PLoS Genetics 8 (2): e1002527.
Waris, S., Wilce, M.C.J., Wilce, J.A., 2014. RNA recognition and stress granule formation by TIA proteins. International Journal of Molecular Sciences 15 (12): 23377-23388.

b) Characterisation of the complement system of a colonial protochordate: study of the expression of C3, CR1, C3AR and their role in nonself recognition. A Peronato, N Franchi, L Drago, L Ballarin. Dept. of Biology, Univ. of Padova, Padova, Italy. ballarin@bio.unipd.it

The complement system is one of the most ancient immune effector mechanism of bilaterian metazoans. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways. In the compound ascidian *Botryllus schlosseri*, a reliable model organism for the study of immunobiology, we demonstrated the presence of the lectin and the alternative pathways. All the complement components identified so far, are expressed by morula cells, the most abundant circulating hemocytes. In mammals, once the complement system is activated, C3 is cleaved to C3a and C3b, the former exerting a chemokine–like activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. In the present work, we continued our analysis of the role of C3 in *Botryllus* immunity by studying the modulation of BsC3 transcription during the colonial blastogenetic cycle and the effect of bsc3 knockdown on immune responses. In addition, we looked for putative complement receptors. In mammals, the best-known receptor for C3a is C3aR, whereas CR1 is the receptor, on the phagocyte surface, able to recognize and bind

C3b. Here, we describe, in *B. schlosseri*, a gene showing similarity with vertebrate C3aR and three genes with similarity to CR1 (two soluble forms and one transmembrane). We also studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that complement receptor mRNAs are located in different immunocytes, suggesting the presence of a cross-talk between phagocytes and morula cells. Only morula cells, and no other immunocytes type, were labelled by the antisense probe for BsC3aR and the soluble CR1s, whereas phagocytes and young, undifferentiated cells known as hemoblasts were the cells stained by the probe for the membrane-linked BsCR1. Both the *bsc3ar* and *bscr1* genes are constitutively transcribed; however, a modulation of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at take-over, whereas no differences were observed when early-cycle and mid-cycle were compared. This is probably related to the renewing of circulating cells at TO, when 20-30% of hemocytes undergo cell death by apoptosis and are replaced by new, differentiating cells entering the circulation in the same period.

2. SETAC Europe 30th annual meeting, 3-7 May 2020, Dublin, Ireland. Virtual meeting!

Invasive ascidians as a tool for assessing the extent and effects of pharmaceutical contamination in marine environments. G. Navon, A. Kaplan, D. Avisar, N. Shenkar, Tel-Aviv University, Israel. noa.shenkar@gmail.com

Despite the global increase in the use of pharmaceutically active compounds (PhACs) in human and animal medicine, efficient treatment of PhACs in wastewater plants is still insufficient. This result in a chronic release of these substances into the marine environment, where their presence presents a threat to marine organisms. Measures of PhAC concentrations in marine waters and sediments are not sufficient to predict exposure concentration by marine organisms, as they provide only a snapshot in time. Additionally, it does not illustrate the harmful effects and the fate of these compounds within living organisms. We investigated the use of three solitary ascidian (Chordata, Ascidiacea) species, Herdmania momus, Microcosmus exasperatus, and Styela plicata, highly efficient filter-feeding sessile invertebrates, which are considered as cosmopolitan invasive species, as bio-indicators of three common PhACs in Israeli coastal waters. In addition, we exposed H. momus individuals to two environmentally relevant concentrations of Carbamazepine (CBZ) under controlled laboratory conditions, and examined their protein expression using a proteomic approach. Results show that both the Mediterranean and Red Sea coasts of Israel are contaminated with PhACs which were detected in twelve sampling sites. Four sampling sites - Haifa marina, Sdot Yam, Ashdod marina and Ashqelon marina, were contaminated with all studied PhACs. Diclofenac (DCF) was the most frequently detected compound, present in 75% of the sampling sites. The highest concentrations measured were 51.9 ng/g dry weight (dw) (of ascidian tissue) of DCF at Ashdod marina, 47.8 ng/g dw of BZF at ‘Akko, and 14.3 ng/g dw of CBZ at Sdot Yam. Finally, in response to exposure of H. momus individuals to CBZ, differential expression was detected in 34 proteins, 3 of them are related to stress responses. By exploiting the cosmopolitan distribution of invasive ascidian species, PhAC contamination can be examined using a comparable method with the same bio-indicator species in various locations around the world. The alarming amounts of PhACs detected in ascidians along the coasts of Israel emphasize the need for future research regarding PhAC contamination, in order to provide essential data on the extant of this matter in coastal environments and revealing the possible effects of these contaminants on marine organisms.

3. Workshop: Aquatics models for regeneration and aging research, Nice, France, December 16-17, 2019.

Understanding the roles of circulatory stem cells for the evolution of regeneration and budding in marine chordates. Jiménez-Merino J, Santos de Abreu I, Hiebert LS, Allodi S, Tiozzo S, De Barros C, Brown FD. fdbrown@usp.br

In marine chordates (i.e. ascidians), circulating putative stem cells (CPSCs) play a role in regeneration and colony asexual propagation. Studies of these cell population(s) have focused in whole body regeneration in colonial species, but few have investigated the evolutionary origins of CPSCs or occurrence in solitary species. In this study, we examine CPSCs in the solitary ascidian Styela plicata. We used imaging flow cytometry to analyze hemocytes, and morphological analyses (ultrastructure and immunohistochemistry) to find putative stem cell niches. In the hemolymph, we found one population of aldehyde dehydrogenase (ALDH+) cells with low granularity, suggesting the presence of CPSCs. Morphological analyses revealed aggregates of small cells with a large nucleus in the intestinal submucosa (IS). IS cells expressed mitotic and stem cell markers (i.e. pH3 and PIWI) supporting the IS as a putative niche. Thus, CPSCs were likely present in the solitary styelid ancestor and possibly diverged functionally to acquire new roles of asexual reproduction or whole body regeneration in the colonial styelids. Studies of CPSCs in ascidians allow us to explore basic mechanisms of stem cell functions conserved across the chordates.

THESIS ABSTRACTS

1. Alexander Fodor. Molecular Analysis of the Evolution and Development of Molgulid Ascidians. Ph.D. thesis December 2019 (posthumous), Univ. of Washington Biology Dept., Seattle, WA. Advisor Billie J. Swalla. Zander did much of his work at the U.W. Friday Harbor Laboratories on San Juan Island, and passed away very suddenly shortly before his degree was completed. He was gentle, smart, and a friend to everyone; he is very much missed.

2. Kevin Ma, January 2020. **Rare species detection and benthic recruitment across multiple scales of space and time with implications for early detection of marine invasive species.** Ph.D. Thesis, Dept. de Biol., Université Laval, Québec. Advisors Ladd E. Johnson and Christopher W. McKindsey. k.ma@ru.ac.za

As a consequence of anthropogenic activities, biological invasions have become a global problem that can cause ecological (e.g., biodiversity and habitat), economic (industries), and social (human wellbeing) harm. Prevention and early detection of new invasions are vital components of managing risks and impacts to ecosystems and economies. Prevention is, of course, preferred but early detection is a critical step that can ultimately stop future spread of invasive species because it provides an opportunity for eradication before population growth and spread. Despite being a cost- and time-effective management option, early detection requires considerably high sampling effort to detect incipient invasive populations at the early stages of their invasion.

Using the marine benthic system as a model, four inter-related studies were carried out to identify sampling strategies that could enhance our ability to detect rare invasive populations and to understand ecological patterns and processes of benthic recruitment across multiple scales of space and time. Specifically, these experimental field studies aimed to (1) evaluate the relationship between propagule supply and settlement in a closed invasive population, (2) determine the optimal sampling duration and frequency using settlement plates to detect rare species, (3) ascertain the relative importance of spatial and temporal sources of variation in benthic recruitment, and (4) examine how the spatial scale of sampling affects species detection by analyzing recruitment patterns at multiple scales across four orders of magnitudes ranging from tens of metres to tens of kilometres.

First study: Contrary to the expectation of a strong relationship between supply and initial settlement, larval supply was instead a limited determinant of settlement at mesoscales. This finding suggests that the strength of this relationship weakens as the spatial scale increased from previously reported small-scale field observations to mesoscales of the present study. Nonetheless, a quarter of the variation in settlement can still be explained by supply over short timescales (one week). Therefore, this relationship supports the utility of settlement plates as an effective tool for early

detection at mesoscales within a marina because low densities of recruitment on plates correspond to low abundances of invasive propagules in the water column.

Second study: Intermediate sampling timescales of one to two weeks (duration treatments ranging from one day to one month) was the optimal plate deployment duration for “rare” species detection (i.e., during the seasonal onset of recruitment). An assemblage-level analysis, however, showed that increasing the duration and frequency of sampling logarithmically increased the total number of rare species observed. These species-by-species and assemblage-level findings demonstrate that modifying temporal aspects of sampling, such as duration and frequency, can substantially affect species detection.

Third study: The seasonal timing of plate deployment was determined to be the greatest source of variability in benthic recruitment of rare species after evaluating several spatial and temporal sources (i.e., site, region, season, and year). In particular, the optimal seasonal timing for early detection may be in the autumn (a) when the seasonal recruitment of established invasive species tends to peak and (b) when the site-level detection of rare invasive species tends to occur.

Fourth study: The dominant scale in rare species recruitment is at the smallest spatial scale (hundreds of metres). This dominant scale can be interpreted as being the correct spatial scale for rare species detection. Further analysis showed that if sampling was structured randomly, then forcing sampling at intermediate scales (thousands of metres) is the optimal scale for rare species detection. These results elucidate differences in the natural variability in benthic recruitment across multiple scales of space and time for both rare and common species.

These ecological studies are part of an early detection toolkit that can inform marine invasive species management with respect to how sampling for rare species should proceed across multiple spatiotemporal scales. Similar field experiments optimizing for rare species detection (beyond the use of settlement plates to detect benthic organisms in the Canadian Maritimes) should be done for other taxa, regions, and sampling tools—especially for forecasted high-risk invaders, regions susceptible to future invasions, and newly-developed tools.

Kevin has now begun a postdoc at Rhodes Univ. in South Africa with Christopher McQuaid, working on biogeographic drivers of distribution and abundance of intertidal rocky shore species in South Africa, and with and Tamara Robinson on invasive mussels.

3. Clara Giachetti. Ecological patterns and processes that determine the colonization success of exotic species in port areas. Ph.D. March 2020, Universidad de Buenos Aires, Argentina. Advisors Drs. Evangelina Schwindt and Marcos Tatián. claragia88@gmail.com

Port areas are the gateway to numerous exotic species. Ports have a large number of artificial structures, such as buoys and pilings, which provide new habitat for several exotic species, differentially favoring their survival. Among these exotic species, ascidians are the most important taxa. Understanding the ecological patterns and processes that act on these fouling communities in port areas, together with the key environmental variables involved, is essential to efficiently apply management strategies. The goal of this thesis was to understand the ecological patterns and processes that determine the colonization success of the exotic ascidians, mainly *Ciona robusta* and *Ascidiella aspersa,* on different artificial structures in a port area. Through sampling, field and aquarium experiments, and the study of reproductive ecology of these ascidians, it is shown that the colonization success of these organisms varies among artificial structures, due to a differential predation pressure. Exotic ascidians successfully colonize floating structures where benthic predators are absent, while they do not colonize structures that are fixed to sea floor where benthic predators are present. Moreover, juveniles are more vulnerable to predation compared to adults. Different species of exotic ascidians have seasonal differences in their abundance on fixed structures, which could not be explained by the presence of predators, but by intrinsic differences in their reproductive

ecology. Predation has a key role in the functioning of marine-coastal ecosystems of port areas, regulating the colonization success of exotic species in different types of artificial structures.
I had my PhD dissertation last March 19th, just a day before the lockdown started in Argentina. During my PhD, I have obtained an important evidence about the important role of exotic and native benthic predators reducing the abundance of exotic ascidians and consequently their colonization success on artificial structures associated to sea bottom, e.g. pilings of the pier. These results were also supported by aquarium experiments with which I identified some of the main macropredators of adults and juveniles exotic ascidians. Finally, I studied the reproductive ecology of *A. aspersa* and *C. robusta*, and I established some hypothesis on how the differences between both species could influence their colonization success.

One of the things that I most enjoyed during this period was the course of Taxonomy and Biology of Tunicates, in Bocas del Toro. It was an excellent opportunity to learn and interact with Rosana Rocha, Gretchen Lambert and colleagues from around the world, in one of the most beautiful places I have met. The training that I acquired in the course was important not only for the developing of my PhD, but also for the detection of new exotic ascidians in my study area. I strongly recommend this course to all the PhD candidates studying ascidians.

4. Maria Casso. Genomic analyses of an introduced ascidian and implications for invasiveness. Ph.D. Dissertation, public defense: 12 March 2020, the last day before everything was closed! Dept. of Marine Ecology, Centre for Advanced Studies of Blanes (CEAB, CSIC), Catalonia, Spain. Supervisors: Xavier Turon (CEAB) and Marta Pascual (Dept. of Genetics, Microbiology and Statistics, University of Barcelona, Catalonia, Spain). xturon@ceab.csic.es

Invasive species constitute a major threat to global biodiversity and cause important economic losses and ecological impacts. In the marine realm, ascidians include several aggressive invasive species, some of which have worldwide colonisation ranges, such as *Didemnum vexillum*. In this thesis, some biological and ecological characteristics implicated in the invasiveness of this species are assessed. First, we performed a 20 month monitoring to determine settlement and growth preferences of invasive ascidian species in the Ebro Delta aquaculture facility, including *D. vexillum*. Our results indicated that *D. vexillum* has a preference for complex substrates. To minimise fouling on bivalves, spat immersion during fall and below 1 m depth is recommended. To detect new introduced species, a follow-up program based on occurrences would be sufficient. Second, a protocol for small DNA samples combining whole genome amplification (WGA) and genotyping-by-sequencing (GBS) was developed and applied to *D. vexillum* using a single zooid per colony to determine patterns of genomic diversity and differentiation, describe the colonisation history of the species, and study its capability to form chimeras. Our results confirmed that Japan is in the native area of the species and only one clade spread worldwide. We found that the two main mitochondrial clades are strongly differentiated at the genomic level suggesting reproductive isolation, we determined that three independent colonisation events shaped the global distribution of the species, and we found that populations are diverse and well differentiated indicating a high expansion potential of *D. vexillum*. Third we detected high prevalence of chimerism, and fusion was unlinked to global genetic relatedness. Finally, we analysed the microbiome of *D. vexillum* that showed markedly different composition than a congeneric species and water. The invasive clade had a small but abundant core and a highly diverse variable microbiome component with a strong capacity to enrich the symbionts from the environment. The microbiome structure correlated to host genetic distance, temperature and geographical distances, pointing to vertical and horizontal transmission. In conclusion, *D. vexillum* is an aggressive invasive species with a high adaptive capacity that may contribute to the invasiveness of this global pest.

5. Elisa Arroyo Martinez, Ph.D., University of Alicante, Spain. Advisor Dr. Alfonso Ramos-Espla. (elisa.arroyo.m@gmail.com) Elisa writes: My work is about soft-bottom circalittoral and bathial

ascidians of the Iberian Mediterranean. These funds are target for trawling and, apart from the knowledge of the ascidiofauna associated to the Mediterranean soft bottoms, another objective of this study is to consider them as possible bioindicators of the trawling impact. The samples I am working with come from MEDITS surveys.

Ascidians form aggregations in soft bottoms, thereby creating a complex habitat and increasing the biodiversity by providing new substrates, food, and refuge. As sessile and filtering organisms, they are extremely vulnerable to bottom trawling, which exerts a strong pressure on the circalittoral soft bottoms and could be used as bioindicators of this impact. During the MEDITS campaigns of 2010 and 2017, the ascidiofauna of the Iberian Mediterranean Sea was studied from Alborán Sea to Catalonia at depths ranging from 37.5 to 693 m. A total of 3810 specimens belonging to 10 families and 38 species were assessed. Taxocenosis demonstrated that Southeast (SE) and Northeast (NE) Iberian Peninsula host a high diversity, specific richness, and abundance of both colonial and solitary species; however, in the Gulf of Valencia, the rates were lower than those previously recorded, and the colonial strategies were scarce. This may have occurred as a result of the continental shelf being steeper in the Alborán Sea and the SE and NE zones coasts than in the shallow shelf of Gulf of Valencia, which is more prone to bottom trawling. Moreover, differences were found in terms of depth, with the highest values of the previous rates being lower than 50 m, where Spanish legislation prohibits bottom trawling at depths lower than that mentioned. For the results obtained, both the taxocenosis of the ascidians and their strategies could be good indicators of the condition of the circalittoral soft bottoms of the Mediterranean Sea impacted by bottom trawling.

6. Jamie Hudson. The Roles of Hybridisation, Contemporary Climate Change, and Recent Range Shifts on the Redistribution of Marine Biodiversity. Ph.D., School of Ocean and Earth Science, University of Southampton, UK. Supervisors: Drs. Marc Riu and Mark Chapman. jh25g11@soton.ac.uk

Global change as a result of human activities has caused unprecedented alterations to biodiversity. One consequence of such alteration is the redistribution of species. Understanding the mechanisms that determine and maintain species' ranges is at the forefront of ecological and evolutionary research. Current drivers of the redistribution of biodiversity include natural range expansions, anthropogenic transport of species, and contemporary climate change. These can alter both species ranges and evolutionary trajectories in multiple ways. A notorious example is when changes in species distributions bring divergent genotypes into secondary contact, facilitating hybridisation. Despite each of these topics being well studied individually, there remains a dearth of research studying these factors in combination to understand how they reshape biodiversity patterns in the marine environment. It is predicted that an integrative approach studying these factors in combination would enable further understanding how they interact to affect the redistribution of biodiversity.

This thesis used a multidisciplinary approach that combined population genomic data, controlled experimental crosses of divergent genotypes, and environmental datasets to investigate the role of different factors on past, current, and future changes in species distributions. My research has shown that whilst hybridisation can be beneficial for range expansions, this is not always the case. I found evidence that some species that have spread as a result of human mediated transport have undergone secondary contacts, facilitating the colonisation of distant habitats. Conversely, other species with transoceanic distributions may be expected to undergo secondary contact due to anthropogenic transport and the presence of two native lineages, though I found no evidence that range expansion had been preceded by hybridisation. Finally, species that are able to currently hybridise may not perform as well as parental crosses under thermal stress, suggesting that under CCC, hybridisation may not be such a dominant driver of species redistribution.

Taken together, this thesis demonstrates the utility in using a multidisciplinary approach to study factors that can combine to determine species ranges. I have shown that different mechanisms can

have dissimilar consequences on species ranges that can be difficult to predict, and that by studying multiple factors can one successfully disentangle the role each one plays in the redistribution of marine biodiversity. [See Jamie's new publication 2020 in the next section.]

7. Anabela Taverna. Ascidian biodiversity of the Argentine Sea and the Southeast Pacific: comparison from a multidisciplinary perspective. Instituto de Diversidad y Ecología Animal (IDEA, CONICET-UNC), Universidad Nacional de Córdoba. Argentina. Ph.D. Supervisors: Marcos Tatián and María Carla de Aranzamendi. In the middle of the pandemic, she had to present the oral defense of her Ph.D. thesis at a distance. The dissertation was successfully performed on-line March 2020. anabelataverna@gmail.com

Ascidians are among the dominant groups of macroinvertebrates in many benthic marine communities, both on natural and artificial substrates, and are often considered in biogeographic research. Biodiversity and changes in biota on both coasts of southern South America, the Southwest Atlantic (SO) (Argentine Sea) and the South East Pacific (SE), are interesting to compare, since both coasts differ, among other conditions, in topography and in the intensity of maritime traffic. The latter enables the introduction of exotic species, whose presence may be underestimated due to misidentification or poor understanding of their historical distribution. Although the biodiversity of ascidians in the area has been partially described by various authors, efforts to update and interpret novel data are still necessary. The development of molecular methods complements the morphological analysis in the identifications of cases of difficult resolution. In order to deepen the knowledge of the biodiversity of ascidians in the SW Atlantic and Pacific SE oceans, analyzes were made of a material diverse from ascidians of samples taken on the coasts of southern South America, with special emphasis on taxonomic problems associated with the determination at a specific level, the definition of biogeographic status and the evaluation of biogeographic affinities. A total of 42 species were identified in the two large areas studied. The richness for the SW Atlantic was 23 species, adding four new records: *Lissoclinum perforatum*, *Eudistoma magalhaensis*, *Ascidiella scabra* and *Botryllus* sp. For the SE Pacific, the richness was 27 species, adding a new record: *Eudistoma molle*. The combined use of morphological and genetic analyzes contributed to define the identity of the species. The presence of *Ascidiella scabra* and *Ciona robusta*, exotic to the SW Atlantic, was found. The synonymy of *Cnemidocarpa robinsoni* and *Asterocarpa humilis* was confirmed, a species that would have colonized large areas in a historical process of human transport. The presence of clade A of *Botryllus schlosseri*, an exotic species in the area, was determined. Of the 42 species identified, a total of 11 exotics (one of them invasive) and two cryptogenic were detected. Finally, the distribution of ascidians possible to evaluate the biogeographic affinities between the four provinces of the South American Region, defined by Briggs and Bowen (2012). Based on the fauna similarity and the percentage of endemisms, a separation of the South American Region into three provinces is proposed for the ascidian distribution: 1) southern Chile, 2) Tierra del Fuego, and 3) southern Argentina-Malvinas/Falkland Islands.

8. Marta Scelzo. **Vasal budding: characterization of a new form of non-embryonic development in the colonial ascidian *Polyandrocarpa zorritensis*.** Ph.D. thesis, Sorbonne University, CNRS, Laboratoire de Biologie du Développement de Villefranche-sur-mer, France. Advisor Stefano Tiozzo (tiozzo@obs-vlfr.fr).

Colonial tunicates are the closest relatives of vertebrates capable to build an adult body from an adult epithelia and/or mesenchymal cells via non-embryonic development (NED), i.e., asexual budding and whole body regeneration. Interestingly, this regenerative capacity has been gained and lost several times in this group, with species able to undergo NED (colonial), and closely related species where regenerative capabilities are absent or reduced (solitary). To understand the mechanisms of NED in tunicates and their evolution it is fundamental to start from a robust phylogenetic framework in order to select relevant species to compare. During my PhD I participated in the construction of a phylogeny of the tunicate family of Styelidae, which revealed an independent acquisition of NED in the colonial species *Polyandrocarpa zorritensis* and

highlighted an undescribed budding mode. I characterized the asexual life cycle and the morphology of the early stages of the NED of *P. zorritensis* , which I decided to name “vasal budding”. By in vivo, histological and ultrastructural analyses I observed that buds arise through a folding of the epithelia of vasculature with the contribution of undifferentiated mesenchymal cells. In order to assess the involvement of these two tissues I analyzed the proliferation dynamics via EdU labelling. Then, I characterized the hemocyte morphotypes and identified a population of undifferentiated cells, the hemoblasts, known as putative stem cells in other ascidian species and playing a role in the bud formation. By using Integrin alpha 6 as a marker, I started to map the distribution of hemoblasts during budding and isolate them by FACS. Though surveys in the field and in lab condition I observed that the budding tissues can alternatively develop into a round-shaped structure, the spherule, which represents a dormant state, able to survive low temperatures and harsh environmental conditions. Spherules have been described ultrastructurally and molecularly via transcriptome analyses and compared with the bud. The descriptions of NED in *P. zorritensis* provide the foundation for future comparative studies on plasticity of budding and whole body regeneration in tunicates.

9. James Evans. Microbial symbionts of sessile marine invertebrates. Ph.D. in Marine Biology, Univ. of N. Carolina Wilmington (defended April 24, 2020). Advisors: Susanna Lopez-Legentil & Patrick Erwin. jse5258@uncw.edu

Ascidians and sponges, while taxonomically very different, are both sessile filter feeders and common members of coral reef benthic communities worldwide. These benthic communities are not static, however; many are feeling the effects of anthropogenically induced climate change and introductions of non-native species. Microbial symbionts may help their host organism to survive under many of these fluctuating environmental pressures, but this role remains poorly characterized. In order to better understand how microbial symbionts may influence the survival of their sessile marine invertebrate hosts, I characterized the diversity, host-specificity, and overall community structure of the microbial symbionts of both ascidians and sponges. As different DNA extraction methods can yield highly variable results, I first investigated the effectiveness of major extraction methods in characterizing ascidian microbial communities, and determined that the two methods most commonly used did not yield significantly different results in the characterization of symbiotic microbial communities. I then worked to characterize the microbial communities of numerous ascidians commonly found on the North Carolina coastline. While the different native and nonnative ascidian species all possessed highly host-specific microbial communities, introduced ascidians were more likely to retain their microbial community structure across different environments, while the native species possessed more dynamic microbial associations that fluctuated in response to environment. Within ascidian symbiont communities, putative links to nutrient cycling and pollutant processing were detected, suggesting ways in which symbionts may benefit their ascidian hosts. I also investigated the use of microbial community characterization as a taxonomic tool for distinguishing between morphologically indistinct cryptic lineages. In a population of the cryptogenic ascidian *Distaplia bermudensis*, two distinct genetic lineages were detected based on the analysis of two separate genes. In the giant barrel sponge *Xestospongia muta*, we similarly detected two cryptic lineages, in agreement with previous studies. In both *D. bermudensis* and *X. muta*, differences in microbial community structure and diversity also aligned with these genetic differences, indicating that characterization of microbial symbionts could represent a useful mechanism for resolving the taxonomic status of the host organism.

10. Olivia Streit. Diversity and relative abundance of native and non-native ascidians in Puerto Rico. M.S. in Marine Biology, Univ. of N. Carolina Wilmington (defended April 21, 2020). Advisors: Susanna Lopez-Legentil & Patrick Erwin. ots6570@uncw.edu

Increased global connectivity has resulted in greater spread of organisms worldwide. Introduced species commonly inhabit harbors and marinas because constant ship traffic brings in new propagules, often released from organisms attached to ship hulls. Ascidians or sea-squirts are ideal species to study invasion processes. Ascidians require anthropogenic introduction vectors for long-

distance dispersal, are easy to collect and monitor, and are abundant on artificial substrates. In Puerto Rico, the last ascidian survey was conducted almost 90 years ago. To investigate current ascidian communities, 11 harbors around the coast of Puerto Rico were surveyed in March 2019. In total, 47 species were observed, 36 of which were identified to the lowest taxonomic rank: 11 of these species were further classified as introduced, 13 as cryptogenic, and 13 as native. Low salinity (25 ppt) in one harbor resulted in no ascidian establishment. Species richness in the remaining 10 harbors ranged from 11 to 26 species. Six introduced species with worldwide distributions were particularly abundant in Puerto Rico: Didemnum perlucidum, Didemnum psammatodes, Diplosoma listerianum, Ascidia sydneiensis, Polyandrocarpa zorritenis and Styela canopus. Surprisingly, only two tiny individuals of the globally distributed species Styela plicata were observed. Geographic location and distance between marinas did not have a significant effect on community composition, while marina size had a significant effect on species richness, presence and absence of species, and their relative abundances. Stakeholder involvement and continuous monitoring are now critical to detect the arrival of new species and prevent the spread of established introduced species to natural habitats.

NEW PUBLICATIONS

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