

ASCIDIAN NEWS*

Gretchen Lambert
12001 11th Ave. NW, Seattle, WA 98177
206-365-3734 gretchen.lambert00@gmail.com
home page: <http://depts.washington.edu/ascidian/>

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ISSC 2018 (the latest Intl. Invasive Sea Squirt Conference) was held May 2-4 in Woods Hole, MA at the Woods Hole Oceanographic Institution's excellent facilities. The meeting was organized by Mary Carman, as she has done so well before, and without whom there would not be an ISSC! At the conference website you can find the downloadable Program, Abstracts and list of participants with their contact information. <https://web.whoi.edu/sea-squirt-conference/> Stephan Bullard's summary talk, A Recap of 13 years of the International Invasive Sea Squirt Conference, reminded us of the importance of this meeting to the many participants over the years.

Conference proceedings will be peer reviewed and published in a special issue of the REABIC journal Management of Biological Invasions. Submit your manuscripts to Stephan Bullard (bullard@hartford.edu) or Mary Carman (mcarman@whoi.edu) by October 1. They are Associate Editors for the journal and will be overseeing the review process for the manuscripts. Manuscripts should be prepared according to the author guidelines for the journal Management of Biological Invasions <http://www.reabic.net/journals/mbi/Submission.aspx>.

While this year's attendance was smaller than at previous meetings, this was primarily due not to a lack of interest but sadly to a lack of funding for many potential attendees. We hope that this situation is only temporary!

A big thank-you to all who sent in contributions. There are 89 New Publications listed at the end of this issue. Please continue to send me articles, and your new papers, to be included in the December issue of AN. It's never too soon to plan ahead.

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

NEWS AND VIEWS

1. From **Evangelina Schwindt** (schwindtcnp@gmail.com):

The **10th Intl. Conference on Marine Bioinvasions** will be held in Puerto Madryn, Patagonia, Argentina, **October 16-18, 2018**. We already received abstracts from 22 countries from all continents and the list keeps growing! The deadline for abstract submission was May 30th but if you have not yet sent yours in, or plan to attend without presenting, contact Evangelina and she hopefully will add you to the list. Please visit the conference website (<http://www.marinebioinvasions.info/index>) Don't forget to check out our **TRAVEL AWARDS**, (tell your students to do so too) for the opportunity to apply for up to 1600 USD!! www.marinebioinvasions.info/travel_awards

The co-chairs of the meeting (Evangelina Schwindt and Ale Bortolus) write: We are glad to share with the Ascidian News followers that Ascidians will be one of the hot topics in Patagonia during ICMB X.

The ascidian expert from Brazil Dr. Rosana Rocha is one of the wonderful Keynote Speakers at the conference. Don't miss her talk "Ascidians in the anthropocene - invasions waiting to happen" .

Under the Program menu there is a link to the complete list of Keynote Speakers, with short bios of each, including Dr. James T. Carlton the special invited conference speaker. The Program menu also includes a link to the field trip, with some amazing photos.

2. From Stefano Tiozzo (tiozzo@obs-vlfr.fr) and Remi Dumollard (dumollard@obs-vlfr.fr): We are happy to announce the **10th Intl. Tunicata Meeting**, which will be held at the citadel of Saint Helme in Villefranche sur Mer (France), **8-12 July 2019**. The web site with all the information will be soon available, in the meanwhile save the date. We are looking forward to seeing you here in the Riviera. A bientôt! Remi and Stefano

3. From **Françoise Monniot** francoise.monniot@mnhn.fr . Museum national d'histoire naturelle (MNHN), 55 rue Buffon, FR 75005 Paris.

The MNHN European expedition « Madibenthos » in Martinique (Sept-Oct. 2016) has investigated all kinds of coastal habitats. The inventory was especially focused on molluscs, crustaceans and algae but very abundant ascidians have also been collected, both solitary and colonial forms. Unfortunately all were fixed in formalin on board and no genetic analyses can be done. The ascidians have been sorted and divided into several groups to be successively identified. The solitary ascidians have already been studied, the Phlebobranchia and Styelidae published [see New Publications in AN80 and this issue] and the Pyuridae-Molgulidae in press. The abundance of specimens has allowed us to evaluate the intraspecific variability of the anatomical characters but has enhanced the difficulty in separating species, especially for taxa with a wide geographic distribution. In spite of careful and intensive collecting around Martinique, few species are new and the diversity remains relatively small, compared to all species already known in the Western tropical Atlantic.

5. Euichi Hirose (Luigi) (euichi@sci.u-ryukyu.ac.jp) sent a very interesting photo of the “*Halocynthia roretzi* house” which is actually a restaurant in Tongyeong, Korea.



He writes: This city is famous for fisheries and there are large aquaculture facilities for *H. roretzi*. However, “soft tunic syndrome” is a serious problems for the fishermen. “Monge (= *H. roretzi*) House”

is just a cafe. They appeared to serve coffee and some sweets but not seafood. I took this photo but did not enter the shop. There is a large market for seafoods near the ascidian house, and we could find many fresh ascidians, *H. roretzi* and *S. clava* there.

WORK IN PROGRESS

1. From Billie Swalla (bjswalla@u.washington.edu) We have an undergraduate student, Lindsay Turner, who is working in the Swalla Lab on the Molgulid genomes and is going to work with Delphine Dauga to curate the Molgula development and genome information on Aniseed. If you have recent published papers with Molgula gene expression data, please send the paper to Lindsay Turner <lindsay.c.turner1@gmail.com>. We are current up to 2016. We are looking forward to getting Aniseed updated with the Molgulid gene expression!

2. From **Delphine Dauga** (contact@bioself-communication.com)

The Tunicate Web Portal has a new look! The tunicate web portal is an access point to databases, standards, key papers and announcements related to tunicates. I didn't really kept the website up to date during the last years (especially because it takes time) but during the last tunicate meeting, I realized that it is really important for the community and that I like to play this role. Nowadays, communication and visibility on the web are essential and I think that an active community, in addition to being friendly, is more likely to attract financers (endangered species).... So let's put all the chances on our side! I try, this time, to make something more collaborative allowing you to submit your announcements, with simple forms (each time you fill in a form I will receive an email to publish your announcement on the website). If you use it a lot, I will find another solution so as you can publish directly on the website. You can play here : <http://www.tunicate-portal.org/> You will see that on the page where I list the ascidian labs (non-exhaustive list), I used some pictures of you that I found on the web. Don't hesitate if you don't want to appear on the website or if you prefer another picture... : <http://tunicate-portal.org/life-community/ascidian-labs> I hope you will like and use it ! If you have any remarks, suggestions, questions, find bugs (I'm sure there are some).

3. From **Simon Blanchoud & Lluís Matas** (simon.blanchoud@unifr.ch)

Dear colleagues,

It is my great pleasure and honour to announce that there is now an additional lab studying these marvellous ascidians! Thanks to a career starting grant from the Swiss National Science Foundation, I have opened my own lab working on whole-body regeneration in *Botrylloides leachii* at the University of Fribourg, Switzerland. With my PhD student, Lluís Matas, we are studying this fascinating mechanism using a combination of microscopy, histology, image analysis and micro-manipulations. Our lab is currently collaborating with that of Pr. Ballarin (University of Padova, Italy) to collect our colonies, which we breed (well... "try to" for now!) in a dedicated aquarium with recirculating artificial seawater. We also collaborate with Dr. Wilson (University of Otago, NZ) regarding the genomic and transcriptomic analysis of this process, as well as Dr. Tiozzo (Sorbonne University, FR) for the involvement of haemocytes during regeneration. Albeit these are already quite numerous collaborations, I always welcome new interactions and exchanges with all of you and would even gladly start some more!

I have a background in computer science, with expertise in image analysis, modelling, microscopy and have participated in the assembly of the draft genome of *B. leachii* (now available on ANISEED, <https://www.aniseed.cnrs.fr/>). Lluís has expertise in biochemistry, vascular physiology and plant physiology. If any of these skills could be of some help to you, feel free to contact us and we would be

more than happy to help as much as we can. Please, feel free to visit us anytime, we would love to host some of you in our modest lab to discuss about our favourite animals. Plus, we are located very strategically next to the Villars chocolate factory !

We are extremely pleased to join the ascidian community and are looking forward to meeting you all soon. <http://www3.unifr.ch/bio/en/research-fields/regenerative-biology/blanchoud.html>

4.

ABSTRACTS FROM RECENT MEETINGS

1. Marine Evolution 2018. 15-17 May 2018, Strömstad, Sweden.

a. Anthropogenic transport of species across native ranges: unpredictable genetic and evolutionary consequences. Jamie Hudson J.Hudson@soton.ac.uk (1), Frédérique Viard (2), Charlotte Roby (2) Marc Rius (1,3). (1) Ocean and Earth Science, University of Southampton, National Oceanography Centre, European Way, Southampton, SO14 3ZH, United Kingdom; (2) Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR 7144, Dept. 'Adaptation & Diversity in Marine Environment', Team Div&Co, Station Biologique de Roscoff, 29682, Roscoff, France; (3) Centre for Ecol. Genomics and Wildlife Conservation, Univ. of Johannesburg, Auckland Park, 2006, Johannesburg, S. Africa.

Most research on the effects of anthropogenic transport on invasive species has focused on the genetic patterns introduced range, in an attempt to understand post-invasion dynamics and evolutionary consequences. Despite this, a thorough understanding of the genetic signatures in the native range is required to accurately infer the identity of the source population, reconstruct the history and routes of invasion, and test if anthropogenic transport is affecting the genetic composition of the native range.

We studied the population genetics of the native range of a widespread invasive marine invertebrate (*Ciona intestinalis*, Ascidiacea, Tunicata) with restricted natural dispersal capabilities. This species readily fouls artificial structures and is prone to movement via anthropogenic transport. We sampled 15 artificial habitats, collected 18-69 individuals from harbours and marinas and used microsatellite markers to genotype them. The results revealed highly disparate patterns. We identified low levels of genetic differentiation among sites separated by both short and large geographical distances, demonstrating the homogenizing effect of anthropogenic transport in the native range. We also identified significant genetic differentiation between certain sites, suggesting additional factors such as natural dispersal or premodern population structure besides anthropogenic transport may be shaping genetic patterns.

Our results suggest that in the native range of a species that is readily altered through anthropogenic means, a complex interplay between natural and artificial factors shape fine-scale genetic patterns, with unpredictable genetic and evolutionary consequences. Urbanised regions may promote an erratic signature of population connectivity, and may blur our ability to correctly identify specific source populations. Taken together, the genetic study of the native range is important to discern the prevalence of natural and artificial dispersal, and how this may affect the natural genetic landscape within the native range.

b. Improving our understanding of biological invasion processes with DNA-based studies: a brief overview and a case study. Frédérique Viard, Station Biologique Roscoff, France.

Non-indigenous species provide excellent case studies to explore important ecoevolutionary processes. In this context, DNA-based studies have been extensively used in the past thirty years. A brief overview of what we had been learnt from such studies (e.g. little evidence of founder events) will be provided. An illustration will then be provided regarding an important issue of marine biological invasion studies: the outcome of secondary contacts between native and introduced species that

diverged in allopatry. The outcomes of such secondary contacts may be diverse (e.g. adaptive introgression of the introduced species, establishment of hybrid zones that may stop propagation of the introduced species etc.) but are not yet well examined. We investigated the fate of such a human-mediated secondary contact between two tunicates, namely *Ciona intestinalis* and *C. robusta*, in the English Channel. Field surveys documented that the two species are found in syntopy, mature at the same period and display similar life-cycle in the study area. In addition, despite an ancient divergence (ca. 4 Mya) they were shown to be only partially reproductively isolated under laboratory conditions. And yet, using 312 SNPs developed from fulltranscriptomes, we showed that the introgressions, observed in the wild, are the outcome of historical gene flow (spread afterwards at a worldwide level) rather than contemporary hybridization. We then investigated the mechanisms that may explain the lack of hybridization between the two species. Post-zygotic ecological effects alone are unlikely to be effective against hybridization. The analyses of F2 hybrid and backcross progenies with ddRaq-sequencing finally suggested that genetic incompatibilities are the most likely mechanisms preventing hybridization between the introduced and native species in the English Channel. This case study illustrates that combining field work, experimental crosses and high-throughput sequencingbased studies can help improving our understanding of eco-evolutionary processes associated with biological introductions.

c. A new protein of ascidian *Styela rustica* and possible horizontal transfer of its gene.

Daugavet M. Ka6tanka@yandex.ru (1), Shabelnikov S. (1), Adonin L. (1), Shaposhnikova T. (2), Podgornaya O (1,2). (1) Inst. of Cytology, Russian Acad. of Sciences, St. Petersburg, Russia; (2) Dept. of Cytology and Histology, Saint-Petersburg State Univ., St. Petersburg, Russia.

Ascidians belong to phylum *Chordata*, the same group that contains vertebrate animals. On the grounds of a distinct body plan, tadpole larvae and cellulose tunic ascidians (Class *Ascidiacea*), are placed in *Tunicata* subphylum. According to the modern views *Tunicata* considered the nearest sister group of vertebrates (Delsuc et al., 2006). Unique taxonomic position made *Tunicata* a model group for studying chordate development (Passamaneck, Gregorio, 2005), genome evolution (Denoëud et al., 2010) and horizontal gene transfer (Nakashima et al., 2004). The new protein p26 from ascidian *Styela rustica* blood cells was described in the present study. Full length homologues of p26 were found only in lower chordates and in one representative of primitive multicellular animals *Trichoplax adhaerens*. Protein structure prediction demonstrated presence of two distinct domains in all p26 homologues. Using HHpred search in PDB database we showed the reliable similarity of C-terminal domain to L-alanyl-D-glutamate-peptidase of bacteriophage A500. p26 homologues genes were screened for intron presence. It revealed the existence of several introns characterized by nearly identical positions in all analyzed p26 genes including that of *T. adhaerens*. Detailed analysis of *Ciona intestinalis* p26 introns uncovered a specific nucleotide sequence that could be utilized by bacteriophage A500 for chromosome integration. Taken together our data demonstrate that at least C-terminal domain of the p26 gene originated from bacteriophage A500. It must have appeared in genome by means of horizontal gene transfer probably at the stage of early multicellularity.

d. Is environmental adaptation in the invasive species *Didemnum vexillum* due to epigenetic mechanisms? Kirsty Smith

kirsty.smith@cawthron.org.nz (1), Nicola Hawes (1,2), Achira Amadoru (3), Xavier Pochon (1,2), Louis Tremblay (1,4), Sylvain Agostini (5), Yasunori Saito (5), Andrew Fidler (2)(1) Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand; (2) Institute of Marine Science, University of Auckland, Auckland 1142, New Zealand; (3) Nelson Marlborough Institute of Technology, 322 Hardy St, Nelson 7010, New Zealand;(4) School of Biological Sciences, University of Auckland, Private Bag 349, Warkworth 0941, New Zealand (5) Shimoda Marine Research Center, University of Tsukuba, 5-10-1 Shimoda City, Shizuoka 415-0025, Japan (deceased).

Phylogenetic analyses of the globally invasive marine ascidian *Didemnum vexillum*, using the mitochondrial COI gene, have revealed two distinct clades: clade A is now found in temperate coastal areas around the world, while clade B is apparently restricted to its native region (NW Pacific). The distribution of clade B may reflect a lower thermotolerance than clade A or adaptation to warmer waters. Multiple mitochondrial genomes revealed significant intra- and inter-clade differences in the sequences for enzymes involved in oxidative phosphorylation. Laboratory experiments examined thermal stress tolerance and mitochondrial enzyme function of colonies from both clades within the native region. Clade A appears to be better adapted to lower water temperatures than clade B, consistent with clade A's abundance in temperate waters. Nonetheless, more recent clade A introductions (e.g., the Mediterranean Sea, southern India) suggest it has a remarkable ability to adapt to warm sub-tropical waters. The capacity of populations to adaptively evolve has generally been explained by existing heritable genetic variation that, until recently, has almost exclusively been identified as genomic DNA nucleotide variation. However, there is increasing acceptance that nonDNA nucleotide variation – collectively termed 'epigenetics' - can affect ecologically important traits. Environmentally induced epigenetic modifications (e.g., DNA methylation) have been proposed as one process underlying the rapid adaptation of invasive species. Global DNA methylation in introduced populations of clade A show distinct patterns and are significantly different to native populations. Introduced populations also show a significant reduction in global methylation levels, which has been suggested as a mechanism for increasing phenotypic plasticity. Epigenetic variation may act as a buffer against rapid environmental changes and compensate for low levels of DNA sequence-based variation. These processes are potentially important mechanisms in the success of many introduced species.

e. DNA methylation and species invasions: can epigenetic variation contribute to adaptation to new environments? Nicola Hawes Nicola.hawes@cawthron.org.nz (1,2), Achira Amadoru (3), Andrew Fidler (2), Xavier Pochon (1,2), Louis Tremblay (1,4), Brendon Dunphy (2,4) Kirsty Smith (1). (1) Cawthron Inst., Private Bag 2, Nelson 7042, New Zealand; (2) Institute of Marine Science, University of Auckland, Auckland 1142, NZ; (3) Nelson Marlborough Institute of Technol., 322 Hardy St, Nelson 7010, NZ;(4) School of Biol. Sci., Univ. of Auckland, Auckland 1142, NZ.

Environmentally induced epigenetic changes may contribute to phenotypic plasticity and increase adaptive potential in response to abiotic stress. Biological invasions provide a unique model to investigate adaptation and evolution within short timescales, as the introduced populations must rapidly adapt to new habitats. *Didemnum vexillum* is a highly invasive colonial ascidian that has become established in temperate and sub-tropical waters worldwide. The extensive distribution of *D. vexillum* demonstrates a significant capacity for rapid adaptation to new environments, despite invasive populations having strikingly low levels of genetic diversity. We hypothesised that epigenetic mechanisms, specifically DNA methylation, may play a key role in its success. *D. vexillum* is an excellent model organism in which to study epigenetic modifications in response to environmental stress as it reproduces asexually to produce colonies allowing replicate experiments to be carried out on genetically identical individuals. In addition, germ cell lineages can originate from somatic cells increasing the likelihood that stress-induced epigenetic modifications can be passed on through gametes. Global DNA methylation patterns of *D. vexillum* were determined to be significantly different between introduced and native populations. Introduced populations also displayed a reduction in global methylation levels, which has been suggested as a mechanism for rapidly increasing phenotypic plasticity. Using manipulative experiments with temperature and salinity stress treatments, we found that exposure to elevated temperature resulted in significant changes in DNA methylation over short time periods. Elevated temperatures also decreased growth rates in exposed colonies. In contrast, no differences in growth or methylation were observed in colonies exposed to a decreasing salinity gradient suggesting prior adaptation from the source environment. The results of this study

provide insights into potential role of DNA methylation as a rapid mechanism of adaptation in a globally invasive species.

f. Transitions to coloniality in the tunicates: new insights from phylogenomic and ecological studies. Laurel Sky Hiebert laurel.hiebert@gmail.com (1), Alexandre Alié (2), Paul Simion (3), Edson A. Vieira (4), Marta Scelzo (5), Maria-Mandela Prünster (6), Sonia Lotito (7), Frédéric Delsuc (8), Emmanuel J. P. Douzery (9), Christelle Dantec (10), Patrick Lemaire (11), Sébastien Darras (12), Kazuo Kawamura (13), Gustavo M. Dias (14), Stefano Tiozzo (15), Federico D. Brown (16). (1, 16) Departamento de Zoologia - Instituto Biociências, Universidade de São Paulo, 05508-090, São Paulo, Brazil; (2, 5, 6, 7, 15) CNRS, Sorbonne Universités, UPMC Univ Paris 06, Laboratoire de Biologie du Développement de Villefranche-sur-mer, Observatoire Océanographique, 06230, Villefranche-sur-mer, France; (3, 9) ISEM, Univ. de Montpellier, CNRS, IRD, EPHE, Montpellier, France ; (4, 14) Centro de Ciências Naturais e Humanas, Univ. Federal do ABC, Rua Arcturus 03 Jd Antares, São Bernardo do Campo, 09606-070, São Paulo, Brazil; (10, 11) CRBM, Univ. de Montpellier, CNRS, Montpellier, France; (12) CNRS, Sorbonne Universités, UPMC Univ. Paris 06, Biologie Intégrative des Organismes Marins (BIOM), Observatoire Océanologique, F-66650 Banyuls/Mer, France; (13) Laboratory of Cellular and Molecular Biotechnology, Faculty of Science, Kochi Univ., Kochi 780, Japan.

Asexual reproduction resulting in the production of connected modules (i.e. coloniality) has evolved independently in a number of animal taxa. We have undertaken a comparative and integrative approach to understand the origins of coloniality in the marine chordate subphylum Tunicata to address why, when, and how this transition occurred. First, we performed transcriptome-based phylogenetic analyses of the family Styelidae. We found that coloniality originated twice in this family; once in the clade containing species that undergo palleal budding (including the model species *Botryllus schlosseri*); a second origin was identified in the species *Polyandrocarpa zorritensis*, where we redescribed the budding mode and found it differs from that of the other styelids. Second, we conducted a three-monthlong manipulative field experiment to examine functional differences between solitary and colonial life histories. We performed this study in a southeast Brazil fouling community, where we found that colonial species dominate the substrate. We outplanted similar-sized starting individuals and colonies onto settlement plates and monitored their growth under predation- and competition-manipulation treatments. We found that colonial species were more susceptible to predation—both survivorship and growth of colonial species were more affected by predation than solitary species. Thus, the colonial species are more predated yet still dominate the substrate in this fouling community. We suggest that coloniality, which is more prevalent in the tropics, where predation is highest, may have evolved as a mechanism to tolerate predation. These studies are beginning to uncover the mechanisms and evolutionary forces that have led to a major evolution transition—the origin of coloniality.

2. Society of Integrative and Comparative Biology Meeting. San Francisco (USA) January 3-7, 2018. Symposium: Integrative Biology of Sensory Hair Cells.

Development and function of secondary mechanoreceptor cells in tunicates. Manni L. lucia.manni@unipd.it, Anselmi C., Pennati R., Mercurio S., Gasparini F. Dept. of Biology, Univ. of Padova, Italy; Dept. of Environmental Sci. and Policy, Univ. of Milan, Italy.

The presence of secondary sensory cells in tunicates was firstly signaled in 2003, when a new mechanoreceptor organ, the coronal organ, was described in the oral siphon of the ascidian *Botryllus schlosseri*. As tunicates are considered the sister group of vertebrates, coronal cells were immediately seen as the best candidate to address the controversial issue of hair cell evolution in chordates. Since then, the study of these cells has been active. Now, we know that the coronal organ derives from an anterior proto-placode, an ectodermal thickening expressing vertebrate placodal genes. The organ is considered a plesiomorphic feature of the taxon, as all tunicates analyzed so far possess it. Its sensory cells exhibit an apical bundle bearing cilia and microvilli (or stereovilli) and lack an axonal prolongation. Coronal cells check particle entrance into the oral siphon during filtering activity: in case of necessity, they evoke the typical "squirting reaction", i.e. the rapid body muscle

contraction that is used to eject dangerous particles from the branchial basket. Tunicate coronal cells share with vertebrate hair cells some developmental genes and neurotransmitters, the complexity of synaptic connectivity, and the susceptibility to ototoxic drugs. Some other features result specific of coronal cells, such as the absence of an ordinated pattern of stereovilli and mechano-electrical transduction based on tip-links, or the ability to divide mitotically, rendering them a unique chordate sensory system.

3. International Meeting on Marine Research 2018 Peniche, Portugal, 5-6 July.

Developmental performance of ascidian populations to an extreme warming event.

Elizabeth A. Clutton¹, Gastón Alurralde^{2,3} Tiago Repolho⁴. ¹Inst. of Marine Sci., Univ. of Portsmouth, United Kingdom. ²Univ. Nacional de Córdoba, Fac. de Ciencias Exactas, Físicas y Naturales, Depto. de Diversidad Biológica y Ecología, Ecología Marina, Córdoba, Argentina. ³Inst. de Diversidad y Ecología Animal (IDEA) CONICET., Córdoba, Argentina. ⁴MARE - Marine and Env. Sci. Centre, Laboratório Marítimo da Guia, Fac. de Ciências da Univ. de Lisboa, Portugal.

mitochondriarevelde@gmail.com

Coastal environments are increasingly experiencing an upsurge of anomalously high seawater temperature events, with unpredictable effects over marine biota. Marine heat wave episodes have been identified as a major climate change driver, which can prompt, to a more or less extent, detrimental outcomes to marine species. As a result of an increase in the frequency and timespan of short-term acute warming events, invasive species proliferation is expected to intensify, thus resulting in a loss of native biodiversity, within invaded ecosystems. Under this perspective, we investigated the effect of an acute temperature increase (simulating a marine heat wave), on the developmental performance of *Ciona intestinalis* early life stages (ELS), derived from populations of anthropogenically- modified and undisturbed environments. Fertilisation rate, embryo and larvae development, settlement and metamorphosis success, and juvenile heart beat rate were assessed. With the exception for fertilization rate and heart beat rates, temperature had a significant overall effect over analysed endpoints. *Ciona intestinalis* ELS, derived from undisturbed environments, were the most negatively affected by increased temperature conditions. Oposingly, anthropogenically impacted populations showed an overall consistency in their positive response to thermal stress. Interaction between temperature and population origin (*i.e.* anthropogenically impacted or undisturbed environments), was statistically significant for three of the ELS analysed endpoints, namely embryo and larvae development, and metamorphosis. A higher proportion of larvae development and settlement success was observed under increased temperature conditions. Additionally, a higher metamorphosis success rate was observed in individuals exposed to increased temperature conditions, derived from populations from anthropogenically impacted locations. Considering heart beat rates, no differences were observed between sampled populations and experimental temperature conditions. Our findings suggest that future marine heat wave events, together with stress resilience attained by *C. intestinalis* populations as a result of anthropogenic forcing exposure, could provide favourable breeding conditions towards their environmental proliferation, with unpredictable effects within invaded ecosystems.

4. Society for Developmental Biology 77th Annual meeting, Portland, Oregon July 20-24, 2018.

a. The SHARK gene *cymric* is truncated in the ascidian *Molgula occulta*. Alexander C. A. Fodor¹, zebinini@gmail.com, Kazuhiro Makabe², William R. Jeffery³ Noriyuki Satoh⁴ and Billie

J. Swalla^{1,3}. ¹Friday Harbor Laboratories and Biol. Dept., Univ. of Washington; ²Univ. of Tokushima, Tokushima, Japan; ³Station Biologique, Roscoff, France; ⁴Okinawa Institute for Sci. and Technology, Okinawa, Japan.

Ascidians share several developmental features with the vertebrates including pharyngeal slits, an endostyle, and a notochord inside a larval tail. During typical ascidian development notochord

precursor cells converge and extend to create a notochord that becomes flanked by muscle making a swimming tail. The molgulids are a monophyletic clade of ascidians in which a tailless phenotype has independently evolved multiple times. We are searching for the molecular basis of this tail loss by investigating the differential gene expression of two species, *Molgula oculata* and *Molgula occulta*. *M. oculata* has the tailed phenotype, but *M. occulta* has lost the tail and notochord, developing twenty notochord cells that do not converge and extend. The two species can be hybridized: if they will converge and extend into a non-functional half tail. We have sequenced the genomes and developmental transcriptomes of both species and the hybrid and are observing differential gene expression of notochord related genes to identify the developmental mechanisms responsible for tail loss. We first investigated *cymric*, a SHARK tyrosine kinase located in the myoplasm in *M. oculata*. *Cymric* is altered in *M. occulta* (Swalla et al. 1993); *M. oculata* makes the full transcript, but the *M. occulta* copy is missing the tyrosine kinase domain. Genome analysis confirms the transcriptome findings; the *M. occulta cymric* gene is on its way towards becoming a pseudogene like tail muscle structural genes (Kusabe et al. 1996) and the pigment gene *tyrosinase* (Racioppi et al. 2017). Our results suggest that the altered *cymric* is not the original mutation causing taillessness in molgulid ascidians, but rather a secondary effect observed in *M. occulta* so we are now investigating the differential effects of upstream genes such as Tbox transcription factors and ERK 1 & 2 to identify the initial change causing the tailless phenotype.

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b. Molgulid ascidians have a radical heterochronic shift in the metamorphic gene network.
Alexander Fodor^{1,2,3}, **Max Maliska**^{1,2,3}, **Elijah Lowe**^{1,3,4}, **Ceri Weber**^{1,2,3}, **C. Titus Brown**^{1,3,4} and **Billie J. Swalla**^{1,2,3,4}
¹Friday Harbor Laboratories, Univ. of Washington, Friday Harbor, WA 98250; ²Dept. of Biol., Univ. of Washington, Seattle, WA 98125; ³BEACON, Michigan State Univ., East Lansing, MI 48824; ⁴Station Biologique de Roscoff, Roscoff, 29682, France.

Transcriptomic and genomic data offer an exciting new approach to examine the genetic networks underlying the origin and evolution of the chordate body plan. One system for studying chordate body plan evolution is two tunicate species with radically different larval body plans—the tailed ascidian *Molgula oculata* and the tailless *M. occulta*. Tailed *M. oculata* embryos, like most solitary ascidians, have 40 notochord cells that are converged and extended in the tadpole larvae. The larvae also have tail muscle cells flanking the notochord in the tail, and, in the head, an otolith, a gravity sensory organ. The tailless *M. occulta* does not form a tail in their larval stage, and have only 20 notochord cells that do not converge and extend during larval development. We have sequenced the genomes and obtained developmental transcriptomes for both species and the hybrid embryos. We show by transcriptome analyses that the ascidian metamorphosis program begins earlier in molgulid ascidians. This radical heterochronic shift has been documented in another tailless ascidian, *Molgula tectiformis*, and is now reported for both the tailed, *Molgula oculata* and tailless *Molgula occulta*. Further functional data is necessary to determine if this pronounced heterochrony is the necessary preadaptation for tailless tadpole to evolve in molgulid ascidians. However, we forecast that these studies will facilitate the elucidation of the metamorphic signal in ascidian tadpole larvae, which is still currently unknown.

THESIS ABSTRACTS

1. Diversity, distribution and trophic ecology of ascidians (Chordata, Tunicata) from deep marine environments. Tamara Maggioni, Instituto de Diversidad y Ecología Animal (IDEA-CONICET), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sársfield 299, 5000 - Córdoba – Argentina. Ph.D. Supervisor: Marcos Tatián. Awarded March 2018.

The deep-sea includes a variety of ecosystems that range between 200 and 10900 m deep. Although it is the most extensive biome on Earth, the deep-sea still remains scarcely studied to this day. Abyssal plains (3000 – 6000 m) and continental slopes (200 – 3000 m) are the deepest deep marine environments. In particular, the Argentine continental slope (35°S – 55°S) is one of the most extensive in the world. Ascidians (Chordata, Tunicata) are important components of deep-sea benthic communities. However, the knowledge we have about the deep ascidian fauna is also limited. In the present thesis, ascidians were identified from the first Argentinean exploratory campaigns to the Argentine continental slope, around 38°S, onboard the Oceanographic RV A.R.A. *Puerto Deseado*. Ascidians from the Antarctic abyssal plains from expedition ANT XXII / 3 (ANDEEP III), onboard RV *Polarstern* in the Weddell Sea, were also identified. Knowledge about diversity, range of distribution and depth of these organisms was increased. The zonation pattern of ascidian assemblages in the slope area under study was also evaluated. The results obtained with data from the Argentinean slope campaigns and additional data from bibliography records revealed the existence of a zonation pattern determined by local topography and marine currents. In order to evaluate the distribution patterns of the deep-sea ascidian fauna, a list of ascidians with records >200 m worldwide was elaborated. In regard to the abyssal ascidian fauna, the hypothesis that establishes a direct relationship between the vertical distribution range and the horizontal geographic range was confirmed. In relation to ascidians from all deep marine environments, the largest proportion was widely distributed in a single ocean basin or corresponded to cosmopolitan species. These results could be indicating the capacity of ascidians to overcome topographical barriers. Finally, the types of organization (colonial, social and solitary) and the types of diets of the deep ascidian fauna from the Argentine slope were analyzed. An inverse relationship was detected between colonial organization and depth. Solitary ascidians, on the other hand, were frequent at all depths. Regarding the type of diet, an inverse relationship was found between microphage diet and depth and a direct relationship between mixed diet and depth. Ascidians with a macrophage diet were equally represented throughout the entire bathymetric gradient. These results reflect the preponderant effect of energy availability on the morpho-functional characteristics of deep marine ascidian fauna. The finding of micro-plastics fibers in the stomachs of some individuals was indicative of the contamination that the slope system receives through the Río de la Plata runoff and, more directly, from anthropogenic activities.

2. Benthic-pelagic coupling in an Antarctic coastal ecosystem affected by glacier melting: an ecological approach. Gastón Alurralde. mitochondriarevelde@gmail.com Faculty of Exact, Physical and Natural Sciences, National University of Córdoba, Córdoba, Argentina, 2017. Ph.D. supervisors Dr. Marcos Tatián, Dra. Verónica L. Fuentes.

The structural and functional connections between the physical, chemical, biological and geological processes that occur between the water column and the seabed are generally known as the benthic-pelagic coupling (BPC). In the Western Antarctic Peninsula (WAP), the BPC is sensitive to the effects of environmental and climatic change currently ongoing in the Antarctic continent. For instance, higher levels of primary production in the WAP have been linked to the increase of the air

temperature in the region and the input of fresh water by melting ice from the continent. This contribution of fresh water transports high amounts of inorganic particles to the sea, increasing sedimentation in coastal environments with drastic effects on the ecosystem functioning. In such context, it has been argued that key ecosystem elements involved in the BPC might be extremely sensitive. In this sense, increased sedimentation, mainly lithogenic (inorganic) material could increase productivity (via iron fertilization) or prevent it (via light attenuation). At the same time, the lithogenic material could interfere in the trophic ecology of suspension-feeders species by diluting the available organic matter (OM), or by altering digestive process like absorption and faeces production. Evidence from different coastal systems of the globe, indicate that sedimentation mainly has negative effects at the autecological and community level, but their implications on the BPC are still scarce. The complexity of the BPC requires a multidisciplinary approach that allows a comprehensive vision of the marine system. The main objective of this PhD thesis is to analyze the BPC in an Antarctic coastal ecosystem (Potter Cove, South Shetland Island) affected by glacier melting by studying the year-round particle flux (i.e. downward flux, biogeochemical and microscopic composition), the effect of different food sources and sedimentation on the trophic ecology of suspension-feeder organisms, and the carbon movement across a sedimentation gradient. Three approaches that integrate multiple methodologies as well as spatial and temporal scales are taken into account. Oceanographic, geochemical and biological evidences are combined through experimental studies, field samplings and conceptual analyses.

Chapter 1 addresses the effect of sedimentation on the composition and temporal dynamics of downward particles flux in Potter Cove (collected in a sediment trap) and therefore, on the food provision to benthic species that rely on it. Consequently, in **Chapter 2**, the trophic response of two suspension-feeders is analyzed, one planktonic (the krill *Euphausia superba*) and one benthic (the ascidian *Cnemidocarpa verrucosa*); both are considered key in the ecosystem and in the Antarctic BPC. The absorption efficiency and faeces production rate in response to different diets and sediment concentrations are evaluated. The sedimentation in Potter Cove varies in relation to the distance to the glacier front, which can determine that the resources are distributed irregularly (varying their quality and quantity) conditioning the subsistence and development of the benthic community. For this reason, **Chapter 3** deals with the origin of carbon incorporated by organisms subjected to different sedimentation regimes, through the study of stable isotopes of carbon and nitrogen. Finally, **Chapter 4** integrates the evidence from the previous chapters and analyzes the role of faeces produced by zooplankton and zoobenthos in the BPC. The frequency of faeces in the composition of particulate material is weighted based on the observations of material collected in the sediment trap. Its nutritional value as a food source is determined experimentally from the supply of feces of krill as food to the ascidians. Its incorporation in the diet is evaluated in the isotopic composition of its tissues.

3. Morphological and molecular identification of ascidians from the Bahamas. Bailey Counts Master's Thesis. baileykcounts@gmail.com. Dept. of Biology & Marine Biology, and Center for Marine Science, Univ. of North Carolina Wilmington, Wilmington NC 28409. Advisor Susanna López-Legentil.

Ascidians or sea-squirts (Phylum: Chordata, Class: Ascidiacea) are conspicuous sessile, filter-feeding organisms with numerous instances of species introductions. The class Ascidiacea contains over 3,500 described species currently classified within three orders: Stolidobranchia, Aplousobranchia, and Phlebobranchia. To date, eighty of these species have been reported as globally introduced. Despite increased efforts to identify native and nonnative species around the globe, the Bahamas archipelago remains substantially under-sampled with only 8 species recorded by Van Name in 1945. This study aimed to identify common ascidian species in the Bahamas and to determine the prevalence of native and nonnative species in the area. Two sampling events took place in May 2008 and July 2010 at twelve different reef localities within five islands of the Bahamas.

Species were identified based on their morphology and barcoded by sequencing a fragment of the mitochondrial gene Cytochrome Oxidase subunit I. Twenty-two ascidian species were commonly observed: ten Stolidobranchia (*Pyura vannamei*, *Pyura* sp. 1, *Pyura* sp. 2, *Pyura* sp. 3, *Polycarpa spongiabilis*, *Eusynstyela floridana*, *E. tincta*, *Symplegma viride*, *S. stolonica*, and *Botrylloides* sp.), two Phlebobranchia (*Ecteinascidia conklini* and *E. turbinata*), and ten Aplousobranchia species (*Eudistoma hepaticum*, *E. obscuratum*, *E. amanitum*, *Aplidium* sp. 1, *Aplidium* sp. 2, *Diplosoma listerianum*, *Didemnum conchyliatum*, *Trididemnum savignii*, and *Trididemnum* sp.). All the species found are considered native to the area, except for *T. savignii* and *D. listerianum*, which were considered cryptogenic. Further monitoring efforts should include entry-points such as harbors and marinas to assess whether nonnative species are present there and their potential to spread to the surrounding reefs.

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