# 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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This has been a busy spring. In February I attended a WoRMS (World Register of Marine Species) workshop for the 8 Ascidiacea editors of the database in Oostende, Belgium; see the Work in Progress article below. I then stayed several days in Paris with Francoise Monniot, and we worked on several of my problematic Singapore ascidians in her lab at the Museum National d'Histoire Naturelle. That was fun, and I learned some new helpful tricks for identifying ascidians.

In late April I joined a group from the Smithsonian Invasions lab to survey for invasive marine species on the main island of Santa Cruz in the Galapagos. My first visit to these unique islands, and like all other visitors I was thrilled to see a blue footed booby, lots of marine iguanas, a few giant tortoises at the Charles Darwin Research Station where we were working, and some of the endemic species of finches and mockingbirds.

After two major international trips already this year, I'm satisfied to stay home for a while, work more on the Singapore collection and hopefully identify the rest of the Galapagos ascidians I could not do on the spot without my extensive collection of monographs.

There are 90 New Publications listed at the end of this issue.

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

## NEWS AND VIEWS

**1.** The **9th Intl. Tunicata meeting** will be held in 2017 in New York City, USA, sometime in July, dates not yet decided. You can help decide by participating in the poll: http://doodle.com/poll/w6epanhawp9mfxan. For more information contact Lionel Christiaen (lc121@NYU.edu ).

**2.** The next **Ascidian Taxonomic Workshop** at the Smithsonian's Bocas del Toro Tropical Research Institute in Panama will be **June 20-July 4, 2017**. More information will be in the December issue of Ascidian News.

**3.** The **next Intl. Invasive Sea Squirt Conference (IISSC)** will be May 2-4, 2018 at Woods Hole Oceanographic Institution, Massachusetts. Information on past conferences can be accessed at http://www.whoi.edu/main/sea-squirt-conference-v and the link to the published papers from the previous IISSC conference, held in 2014, can be accessed at http://www.reabic.net/journals/mbi/2016/Issue1.aspx More information will be posted in future issues of AN. You can contact Mary Carman (mcarman@whoi.edu).

**4.** We are greatly saddened to report the death of **Prof. Armando Sabbadin** on March 18. Lucia Manni writes: “He was full professor of Comparative Anatomy of Vertebrates, and then professor Emeritus of the University of Padova. He introduced *Botryllus schlosseri* as model organism more than 60 years ago in the laboratories of the Department of Biology of the University of Padova. He formalized the staging and rearing method of this colonial ascidian, and made pioneering studies on self-non self recognition, stem cell recycling, genetics, bud competition in asexual reproduction, and self/cross fertilization. His researches represented the basis for successive and still actual studies. Together with his pupils, Loriano Ballarin, Riccardo Brunetti, Paolo Burighel, Francesca Cima, Gianbruno Martinucci and Giovanna Zaniolo, I thank him for having passed on to us his great passion for ascidian biology and for his scientific rigor.”

Dr. Billie Swalla adds: “He was a great man and excellent scientist and pioneered the use of colonial ascidians for important studies. The many labs around the world that study *Botryllus schlosseri* is a testament to his expertise and mentoring.”

A complete obituary was published in the following two journals:
Ballarin, L., Burighel, P., Cima, F., Manni, L. and Zaniolo, G. 2016. In memorium Armando Sabbadin 1920 - 2016. Invertebrate Survival Journal 13: 66-67 and Developmental and Comparative Immunology 60: 228.

Dr. Manni writes that the complete bibliography (103 papers) of Armando Sabbadin is now available at
https://sites.google.com/site/ascidianbiologylab/home/prof-a-sabbadin-s-bibliography
All the publications can be downloaded as pdf files. The website also contains a very moving tribute by Prof. Nicolò Parrinello.

**5. Noa Shenkar** and her students, postdocs and colleagues continue to actively research and publish on interesting and diverse ascidian projects at Tel-Aviv University. There are several new publications listed at the end of this newsletter, including one on *Rhopalaea* confirming by molecular sequencing the placement of the Diazonidae in the Aplousobranchia, as well as the placement of the Cionidae as a sister group of the Aplousobranchia. She sent a photo of her new species named after two of her children (Ido and Neta) from Israel International Airport, where there is an exhibition on scientific breakthroughs http://www.ynetnews.com/articles/0,7340,L-4775952,00.html . “They really liked Gil Koplovitz's photo of the *Rhopalaea idoneta*, I am very proud that there is a photo of an ascidian in the airport!” shenkarn@post.tau.ac.il

## WORK IN PROGRESS

**1.** From **Gretchen Lambert and Billie J. Swalla**: During 15-17 February 2016, we attended the first-ever workshop for the 8 Ascidiacea editors of the World Register of Marine Species (WoRMS) database (http://www.marinespecies.org/ ), in Oostende, Belgium, at the Flanders Marine Institute, a modern outgrowth of the very first marine research station in the world (1843). We met to learn from the WoRMS data management team about the ascidian section (http://www.marinespecies.org/ascidiacea/) and discuss issues unique to ascidian taxonomy. Our goal is to provide the most authoritative list ever published of all ascidian species globally, with primary and secondary bibliographic references, distribution and other data, including the names of all species synonymized under each current valid name. This is a mammoth task, with nearly three thousand species of described ascidians and more being discovered every day during ocean exploration and biodiversity surveys. The three day workshop covered multiple aspects of the WoRMS web site; we all learned how to better enter the data and correct errors. WoRMS has become the "go to" site for marine biologists worldwide and also is increasingly linked to other marine databases, such as invasive species. The participants included (alphabetically) **Adriaan (Arjan**) **Gittenberger**, GiMaRIS Marine Research Inventory & Strategy and Museum of Natural History "Naturalis", Leiden, Netherlands; **Gretchen Lambert**, Univ. of Washington Friday Harbor Labs; **Françoise Monniot**, Museum National d'Histoire Naturelle; **Marc Rius**, Univ. of Southampton England (Marc organized this meeting and obtained the funding for it from the Belgian LifeWatch); **Rosana Rocha**, Universidade Federal do Paraná Brazil; **Noa Shenkar**, Tel Aviv University Israel; **Billie Swalla**, director, Univ. of Washington Friday Harbor Labs; and **Xavier Turon**, Dept. of Marine Ecology, Center for Advanced Studies of Blanes Spain.

Left to right: Wim Decock and Stefanie Dekeyzer of the WoRMS data management team; the 8 ascidian editors Arjan Gittenberger, Xavier Turon, Billie Swalla, Françoise Monniot, Noa Shenkar, Rosana Rocha, Gretchen Lambert, and Marc Rius. Photo credit: G. Lambert.

We first tackled the many question marks next to various fields on a number of entries in the Ascidiacea Database, indicating entries not yet checked for accuracy by one of the Ascidiacea editors. There seemed to be an impossible number of them, so we each volunteered to take on one or more ascidian families to check all species entries for accuracy and add additional information such as taxonomic and distribution bibliographic sources.

Some things can be checked rather quickly but others will take time and reading of old monographs.

Taxonomists tend to be "lumpers" or "splitters," and the splits can result in many genera with only one species each, or a family with only one genus. It was agreed that future molecular phylogenies will be used to create monophyletic groups and use new data to decide whether to combine some of these closely related families.

**PDFs of Critical Taxonomic Literature** — Rosana Rocha has been gathering hundreds of PDFs of taxonomic literature and this database was uploaded into WoRMS. This will allow everyone access to critical taxonomic literature if they would like to check something for themselves. **Families and Genera of Ascidiacea** — Claude and Françoise Monniot many years ago composed a tabular key to the ascidian families and genera of the world, which Françoise and Gretchen have been revising. This valuable tool will be transferred to Excel format and uploaded to the WoRMS website. **Taxagloss** — Rosana has created a glossary containing all the taxonomic terms for the Ascidiacea, which WoRMS will upload to the database, giving all users open access to it. These terms will be available in different languages, starting with English and Spanish and then adding French, Dutch and Hebrew, utilizing the expertise of the eight editors. It will be a lot of work for everyone, but a wonderful resource for all ascidiologists especially when used in conjunction with the Families and Genera tabular keys. There was a rousing and detailed discussion of some of the terms; it may be a while before the glossary is finished! **International Code of Zoological Nomenclature** — We discussed the conundrum of Family authorship before 1900, a period before Families were erected for all genera. For example, *Ascidia*, a genus described by Linnaeus; the authorship for the family Ascidiidae, erected later by a different taxonomist, is still Linnaeus. We have this problem in 9 ascidian families. Arjan and Billie will prepare a manuscript requesting from the Zoological Commission a revision to the code rules for family names in the International Code of Zoological Nomenclature.

We made a lot of progress, and left with our “to do” lists. We now have a closer relationship with the WoRMS Data Management Team, we understand the database better, and we all enjoyed the hospitable atmosphere in historic Oostende. We hope you enjoy using the Ascidiacea database in WoRMS and find it useful to your research. Please let us know of any errors, changes or additions that should be made.

This article was condensed from the April 2016 issue of the Univ. of Washington Friday Harbor Labs Tide Bites newsletter: http://depts.washington.edu/fhl/tidebites/Vol32/index.html

**2.** From **Lionel Christiaen** (lc121@NYU.edu ): My lab just published an update on our method s for CRISPR/Cas9 in *Ciona*, with tentative whole-genome predictions of efficient sgRNAs. We hope you will find this useful. http://biorxiv.org/content/early/2016/02/28/041632

## ABSTRACTS FROM RECENT MEETINGS

### 1.9th Int’l. Conference on Marine Bioinvasions, Sydney, Australia January 19-21, 2016.

**Unravelling the introduction and dispersal of a worldwide marine invader *Didemnum perlucidum* in Australia.** Joana Dias, Jason Kennington, Justin McDonald, Mike Snow. Joana.Dias@fish.wa.gov.au

The ascidian *Didemnum perlucidum* is a colonial tunicate with a reported worldwide distribution across the Atlantic, Pacific and Indian Oceans. Although *D. perlucidum* was first described from the island of Guadeloupe in the Caribbean, its native range is unknown. It is thought to have been introduced to many areas of the world including Western Australia (WA) where it seems to have become established and has demonstrated invasive characteristics. In Australia, *D. perlucidum* is listed as a target species under the introduced marine pest national monitoring network. Defining the status of a species as native or introduced is important as it can influence management decisions, but it is often challenging, particularly within communities which have been under the influence of human intervention. Molecular markers can be used to characterize species diversity at locations, providing important supporting evidence to identify native and introduced ranges, and investigate potential frequency and routes of colonization and dispersal. In the present work we characterised the COI gene region of *D. perlucidum* populations collected from around Australia and abroad. We found colonies at presumed introduced locations worldwide to consist mainly of a single COI haplotype. This has led us to further develop microsatellite markers to support invasion genetic studies on *D. perlucidum*. The more variable markers developed should prove useful in further resolving the origins of introduced populations of *D. perlucidum*, and potential high-risk routes of introduction. Such studies will support a science-based management approach to the future prevention, management and/ or eradication of this important species.

**2. Marine &Freshwater Invasive Species conference, Buenos Aires, May 2-4, 2016.**

**a. Resolution of ambiguous taxonomy of ascidians reveals high levels of introductions of invasive species at the SW Atlantic.** Marcos Tatián[1-2] , Gastón Alurralde[2], Clara Giachetti[3], Diego Giménez[1], Cristian Lagger[2], Tamara Maggioni[2], Evangelina Schwindt[3], Anabela Taverna[2] . [1]Depto. de Diversidad y Ecología, Fac. de Ciencias Exactas, Físicas y Naturales. Univ. Nacional de Córdoba; [2]Inst. de Diversidad y Ecología Animal, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)/Universidad Nacional de Córdoba, Argentina; [3]Grupo de Ecología en Ambientes Costeros, Puerto Madryn, Argentina. mtatian@unc.edu.ar

One of the poorly known regions in terms of its biodiversity, in particular in the case of ascidians, is the Southwestern Atlantic (SWA). Ascidiacea is one of the main groups in bioinvasion studies given that they are good indicators of anthropogenic transport and because they are usually very abundant in terms of richness. The accurate species identifications are crucial to clarify causes and consequences of the invasion processes. The aim of this work was to determine species richness and especially, to report those species considered as exotic and cryptogenic together with their present distribution along the SWA. We performed different field sampling at the SWA including coastal areas (natural and harbour localities) and oceanic areas. We also exhaustively studied old museum collections and the specific taxonomic and global literature that helped us to elucidate the status of native, cryptogenic or exotic in the area. The general survey of species revealed, at the moment, a total of 80 ascidian species, being 18% of them, species whose status is considered exotic and/or cryptogenic. The use of morphological and genetic tools to clarify the species identity is the first step into the evaluation of invasion processes and this would help to better understand the real scenario in terms of native biodiversity.

**b. Invasive ascidians: Do local predators play a key role in colonization success?** GIACHETTI, C.[1], BATTINI, N.[1], TATIÁN,M.[2],&SCHWINDT, E.[1] [1]Grupo de Ecol. en Ambientes

Costeros, Inst. de Biol. de Organismos Marinos, Argentina; [2]Inst. de Diversidad y Ecología Animal, Argentina. giachetti@cenpat-conicet.gob.ar

Invasive ascidians are usually considered as pests in the invaded areas including aquaculture facilities. Predation might control the establishment and further colonization depending on the habitat and the predator/prey species involved in the interaction. In this work, we evaluate the effect of different potential predators on the survival of the adults of *Ascidiella aspersa*, one of the most invasive ascidian species in Argentina. Predator and prey species were collected in Puerto Madryn port, transported to the aquarium and acclimated during seven days. As potential predators, we tested the exotic sea slug, as well as native sea stars and sea urchins. Several trials were made in individual tanks subdivided in two equal compartments (prey alone and prey plus one predator species). After a starvation period (2-9 days depending on the species), a predator was placed in a compartment with four ascidians during 2-4 days depending on the predator species. At the end, we quantified mortality percentage in both compartments; when it was zero, we offered to the predator his favourite prey to confirm that the rejection was not due to satiety. Ascidians were observed for a week to discard death due to tunic damage. Preliminary results showed that sea urchins and sea slugs feed upon the epibionts growing on the ascidian tunic, causing a superficial damage to the tunic but not killing the ascidians. Only sea stars were able to injure the tunic but the ascidians survived and lived with that injury. More experiments are being performed with a wider pool of predator species, including the voracious invasive green crab *Carcinus maenas*, and also evaluating the effects of predation on other invasive ascidians, including adults and juveniles. These results suggest that *A. aspersa* adults are strong enough for local predators, however the softer juveniles could be negatively affected by predation.

**c. Management of tunicate invasion on Prince Edward Island, Canada.** T. Landry[1] and J. Davidson[2]**.** [1]Dept. of Fisheries and Oceans, Gulf Region, Canada; [2]Atlantic Vet. College, Univ. of Prince Edward Island, Charlottetown, Canada. thomas.landry@dfo-mpo.gc.ca

Four invasive tunicates were first reported on Prince Edward Island (PEI) almost 2 decades ago. Their rapid proliferation had severe impacts on a shellfish aquaculture industry and associated ecosystems. Tunicates are important fouling organisms that compete with mussels, oysters and associated fauna for space and food. Untreated infestations have led to reduced growth rates and meat yields of farmed shellfish and significant loss in productivity due to fall-off. The main impact, however, is the disruption in the ecological function of the estuaries with the potential loss of productivity and biodiversity.
Science has played an important role in providing key knowledge and advice to identify management options. Relevant environmental, economic, social, and cultural values were considered to reduce the rate of spread and to mitigate the effects tunicate fouling on shellfish farms. Hazard Analysis and Critical Control Points (HACCP) principles were used to identify critical control points and potential control measures. The outcome has been highly effective for solitary tunicates but less so for colonial tunicates. Such results may guide the development of future control strategies with key stakeholders.

**d. Benthic non-indigenous species on the southern coast of Chubut (Argentina) at the onset of the 21st century.** Rico, A.[1], Riera, M.[1] &López Gappa, J.[2] [1] Depto. Biol. General, Fac. de Ciencias Naturales, Univ. Nacional de la Patagonia "SJB", Argentina; [2]Investigador Principal CONICET, Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina. aliciaerico@yahoo.com.ar

During the last decades, several studies have dealt with benthic marine organisms occurring on the abrasion platforms of Comodoro Rivadavia (45º52' S, 67º28' W) and Rada Tilly (45°57′ S, 67°32′ W). Benthic species were sampled using different methodologies. Stone fragments were glued to intertidal and subtidal substrates with epoxy putty and plastic panels were screwed to supporting structures at the subtidal level of Comodoro Rivadavia harbor. Intertidal research at Rada Tilly was based on monthly non-destructive observations. Species richness and diversity (Shannon-Wiener index) were estimated and differences among samples were then tested by two-way ANOVAs followed by post-hoc comparisons. Multivariate data were analyzed with the PRIMER package. The presence of *Balanus glandula*, a barnacle native to the Pacific coast of North America, was discovered in 2001 on intertidal hard substrates of the harbor. The species then continued its dispersion along Atlantic rocky shores down to Tierra del Fuego. The alien bryozoans *Cryptosula pallasiana* and *Bugula flabellata* were previously known from fouling assemblages in harbors of Buenos Aires province. Their geographic distribution was extended towards the southern coast of Chubut. Exotic ascidians were particularly abundant. They were mainly represented by solitary species such as *Ascidiella aspersa* and *Molgula manhattensis*, or by the colonial forms *Botryllus schlosseri*, *Diplosoma listerianum* and *Lissoclinum fragile*. The invading kelp *Undaria pinnatifida* was reported for the first time for Argentina in 1994 at Nuevo Gulf (northern coast of Chubut). In 2005, this species was found in Comodoro Rivadavia harbor. Its life-cycle and the macro- and microstructure of tagged individuals are currently being studied and compared with morphological features of populations from other geographic areas.

**e. First register of the invasive ascidian *Asterocarpa humilis* in the SW Atlantic.**

TAVERNA, A.[1], MAGGIONI, T.[1], ALURRALDE, G.[1], GIMENEZ, D.[2] &TATIÁN, M.[1]
[1] Instituto de Diversidad y Ecología Animal, Argentina; [2]Universidad Nacional de Córdoba., Argentina. anabelataverna@gmail.com

The ascidian *Asterocarpa humilis* (Heller, 1878), (Tunicata, Chordata), native to New Zealand, has expanded its original range of distribution. It is considered exotic in remote areas such as Chile, South Africa and more recently, Great Britain and France. Until now it had not been observed in Argentina. A similar species (considered synonym of it by Kott, 1985), *Cnemidocarpa robinsoni* (Hartmeyer, 1916) is native to the Juan Fernández archipelago. That species was reported in the Southwest Atlantic (Diehl 1977), but its status in the area remains uncertain. The examination of morphological characteristics in abundant specimens from different localities of Argentina (i.e., the number of oral tentacles, the amount and form of the gonads, the location of the gonoducts and the presence of larvae) showed similarities with those described for *A. humilis* the orientation of gonoducts and the presence of larvae, being the most relevant characterisitcs. The name *Asterocarpa humilis* has precedence over *Cnemidocarpa robinsoni*, so the latter should be discarded. The absence of true records prior to the description of Diehl and its current wide distribution, particularly in port areas, affirms the exotic status of *A. humilis* in the SW Atlantic.

**3. XVIIth scientific meeting of the Italian Association of Developmental and Comparative Immunobiology**, 11 - 13 February 2016, Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy. See http://www.isj.unimo.it/articoli/ISJ411.pdf for the complete program.

a. **New data on the expression of molecular markers involved in stemness and differentiation in the colonial ascidian *Botryllus schlosseri*.** F. Ballin, N. Franchi, L. Ballarin. Department of Biology, Univ. of Padua, Padua, Italy. ballarin@bio.unipd.it

Cell types are often identified by determining which genes they express specifically. The use of specific antibodies or complementary RNA probes allows the identification of the translational/transcriptional products: These molecules, also called “molecular markers”, show an unique expression pattern, frequently used to identify and isolate stem cell populations. In B. schlosseri, a compound ascidian, there are three important processes that suggest the presence of stem cells during the life cycle: i) embryogenesis, in which an embryo develops from a zygote, palleal budding where new buds emerge as thickenings of the peribranchial epithelium and vascular budding, i.e., the development of new buds within the vasculature by circulating multipotent or pluripotent cells. During the cyclical generation changes, which characterize the colonial blastogenetic cycle, an increase in the number of hemoblasts, i.e., undifferentiated circulating cells, occurs which will replace, once differentiated, the hemocytes undergoing apoptotic cell death. Ascidian hematopoiesis occurs in close proximity to the pharyngeal vessels, in the so-called “hematopoietic nodules” and in the endostyle, the cells of which proliferate and migrate to developing buds. Despite the morphologic suggestions that hemoblasts are the precursors of all the circulating cell types, immunocytes included, there is a general lack of biochemical and molecular data supporting this assumption. Here we report the identification of hematopoietic molecular markers in B. schlosseri, very similar to those of vertebrates, their localization and expression profile during the blastogenetic cycle.

**b. Growing complexity of the invertebrate complement system: evidences from colonial tunicates.** N. Franchi, L. Ballarin. Dept. of Biol., Univ. of Padua, Italy ballarin@bio.unipd.it

The evolutionary history of the complement system is not yet fully elucidated. Evolutionary studies suggest that the origin of the complement system can be traced back to the common ancestor of the Eumetazoa as the genes for the C3, factor B and MASP have been identified in sea anemones. Nonetheless, no complement genes are present in the genome of the sponge Amphimedon queenslandica or the choanoflagellate Monosiga brevicollis and, although their presence in invertebrate protostomes is generally accepted, complement genes are missing also in Drosophila and Caenorhabditis, probably due to a secondary loss. As regards deuterostome complement system, it is well studied in mammals, where more than 30 different proteins have been described, involved in the activation and regulation of this fundamental humoral system able to modulate immunocytes behaviour, belonging to both innate and adaptive immune response. However, the mere report of the presence of C3, factor B and lectin pathway in a species cannot help in elucidating the evolution of the complement system. Since adaptive immunity evolved in the presence of a functioning complement system, the presence of considerable and important interaction between complement and adaptive responses is not surprising. In particular, referring to the invertebrate-vertebrate transition, the description of the complement-mediated immune modulation requires the identification and characterization of additional factors, such as complement control proteins (e.g., factor H, C4bp, CR1, CD46, CD 55) and receptors in invertebrates chordates. Here we report on the identification and analysis of transcripts for CR1 (C3b receptor), C3aR and two factors H in the colonial ascidian Botryllus schlosseri that, for the first time are described in tunicates, the sister group of vertebrates. The localization of CR1 and C3aR on phagocytes and morula cells, respectively, open the possibility to use

such molecules as molecular markers for immunocytes. In addition, the presence of a complement regulator, such as factor H, in tunicates suggests a higher level of complexity than that expected in an invertebrate.

**4. Inaugural Meetings of the Pan-American Society for Evolutionary Developmental Biology. Augusto 5-9, 2015. Berkeley, CA, USA.**

**Modular development and evolution of clonal marine chordates.** A. S. Gutierrez (a), C. A. Velandia (b), C. Bermudez-Santana (b), A. Gittenberger (c), F. D. Brown (a,d) fdbrown@usp.br
(a)Depto. de Ciencias Biológicas, Univ. de los Andes, Bogotá, Colombia; (b) Depto. de Biología, Univ. Nacional de Colombia, Bogotá, Colombia; (c) GiMaRIS, Leiden, The Netherlands; (d) Depto. de Zoologia, Instituto de Biociências, Univ. de São Paulo, Brazil.

Colonial styelid ascidians form two types of organization. In derived species (i.e. botryllid ascidians) individuals of the colony are connected and integrated within a common tunic and new individuals form in weekly cycles of budding; in contrast, other colonial styelids present an aggregate organization, in which individuals are embedded within their own tunic and remain connected by extracorporeal tissues. The latter develop independently and by asynchronous budding. A sister species of the botryllids Symplegma brakenhielmi presents an intermediate form, i.e. individuals are well integrated, but development occurs in an apparently independent manner. To understand how major reproductive modes evolve during life history transitions, we experimentally disrupted asexual modules of development of a colonial ascidian Symplegma brakenhielmi by systemic bud or zooid removals in the colony and identification of putative circulatory progenitor cells involved in asexual reproduction. Budding in ascidians requires a permanent supply of progenitor cells likely regulated by ncRNA pathways. To associate specific ncRNAs to putative mechanisms of asexual reproduction, we compared the genomes of solitary Ciona intestinalis and C. savignyi and colonial Didemnum vexillum and Botryllus schlosseri. We generated ncRNA predictions and aCempted to associate specific loci to the evolution of asexual modes of reproduction and regeneration. Our results support a stepwise integration of budding synchrony and developmental interaction of individuals during the evolution of coloniality, and raise new questions about ncRNA regulation in stem cell function of colonial marine chordates.

**5. First Encuentro Nacional de Biologia del Desarrollo. September 24-16, 2015. Bogotá, Colombia.**

**Evolutionary origins of chordate stem cells. Federico D. Brown.** fdbrown@usp.br

Basal chordate species can live as solitary individuals that reproduce sexually, or alternatively live as colonies that reproduce both asexually and sexually. Colonial styelid ascidians form two types of organization. In derived species (i.e. botryllid ascidians) well integrated individuals of the colony share a common tunic and new individuals form in weekly cycles of budding; in contrast, aggregated individuals in other colonial styelids retain their own tunic and bud independently by asynchronous budding. Symplegma brakenhielmi, a sister species of the botryllids, presents an intermediate form, i.e. individuals are well integrated, but development occurs in an apparently independent manner. To understand how asexual modes of reproduction evolved in our phylum, we compare budding processes and genomes across several colonial ascidian species. Budding in colonial ascidians requires a permanent supply of progenitor cells likely regulated by ncRNA pathways. We studied hemocytes and analyzed proliferation in S. brakenhielmi to identify putative circulatory

progenitor cells. To associate specific ncRNAs to putative mechanisms of asexual reproduction, we compared the genomes of solitary Ciona intestinalis and C. savignyi and colonial Didemnum vexillum and Botryllus schlosseri. We generated ncRNA predictions and attempted to associate specific loci to the evolution of asexual modes of reproduction and regeneration. Our results support a developmental integration of individuals and developmental processes during the evolution of coloniality, and raise new questions about ncRNA regulation in stem cell function of colonial marine chordates.

**6. The 2015 Latin American Society for Developmental Biology (LASDB) Meeting. October 20-23, 2015. Santos, São Paulo, Brazil.**

**Vascular budding in *Symplegma brakenhielmi* and the evolution of coloniality in styelid ascidians.** Stefania Gutiérrez (1,2,3,4), Federico Brown (1,3,4)
(1) Lab. de Biología del Desarrollo Evolutiva (EvoDevo), Depto. de Ciencias Biológicas, Facultad de Ciencias, Univ. de los Andes, Bogotá D.C., Colombia; (2) Smithsonian Tropical Research Institute, Bocas del Toro, Republica de Panamá; (3) Depto. de Zoologia, Instituto de Biociências, Universidade de São Paulo, SP, Brazil; (4) Centro de Biologia Marinha, Univ. de São Paulo, SP, Brazil.

Coloniality is a life history that consistsSymplegma, Botryllus, and Botrylloides are colonial. Phylogenetic reconstructions of related genera show a stepwise evolution of colonial characters, i.e. higher integration and independence of zooids and buds during blastogenesis. Botryllus and Botrylloides zooids bud in a highly synchronous and periodic manner, whereas Symplegma is asynchronous. Therefore, Symplegma shows an intermediate state between basal solitary species and derived colonial species. Progenitor undifferentiated hemocytes are involved in asexual reproduction in Botryllus and Botrylloides. Due to the closer phylogenetic position and morphological similarities of Symplegma to botryllids, we hypothesized that colony-wide removal of zooids or buds would affect the development of the remaining individuals, and that circulatory progenitor cells would be present in the blood. Using our results, we establish this model that predicts the following sequence of events: (1) autonomous budding of discrete individual entities that remain united, (2) colony-wide dependency for budding of integrated individuals within a common tunic, (3) synchronous and cyclic budding of highly integrated individuals. For comparative studies, we first characterized blastogenic series of stages for Symplegma brakenhielmi using Botryllus schlosseri stages as a reference (Berrill, 1941; Sabbadin 1955). Buds formed at random sites throughout the vasculature of the colony, and blastogenic series of development showed that differentiation and growth of the branchial sac continued after siphons formation. To explore whether putative circulatory progenitor hemocytes were involved in the formation of new buds, we characterized hemocytes. We identified at least two populations of putative progenitors that expressed mitotic marker Phospho-histone H3. Macrophage-like cells that are involved in zooid resorption and colony turnover were the most abundant hemocytes in different colonies and at different stages, suggesting that tissue recycling permanently occursin in S. brakenhielmi. A diversification of circulatory hemocytes (i.e. stem cell progenitors for budding and macrophages for tissues recycling) in colonial styelids, including Symplegma, arguably support that the integration of individual development to orchestrated colonial blastogenesis is mediated a functional diversification of circulatory cells. To test independence in the asexual development of individuals in the colony we conducted systemic zooid or bud removal experiments. Symplegma brakenhielmi zooid and bud ablation experiments did not affect blastogenesis. In general, zooids showed a high degree of autonomous development, except in budectomized colonies in which the

development of new buds was faster than bud in controls. These results suggest that fully autonomous blastogenesis in this species is only partial, and that a small degree of colony-wide effects occurs. Thus, our results suggest that S. brakenhielmi represents an intermediate state of colony-wide dependence for bud development in the stepwise model of colonial evolution within the Styelidae. Our findings raise new questions on how developmental mechanisms may orchestrate the evolution of new life histories.

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## THESIS ABSTRACTS

**A model to study alternative developments: asexual propagation and regeneration in the basal chordate *Botryllus schlosseri*. Lorenzo Ricci**, Ph.D. thesis.Université Pierre et Marie Curie, Laboratoire de Biologie du Développement de Villefranche sur mer, France. Supervisor: Stefano Tiozzo. tiozzo@obs-vlfr.fr

Ascidians (sub-phylum: Tunicata), among many other metazoans, evolved alternative ways to develop an adult body. The colonial ascidian *Botryllus schlosseri*, in addition to embryogenesis, can give rise to an adult body, the zooid, adopting two different, non-embryonic ontogenies. The two “alternatives” to sexual reproduction, blastogenesis and vascular budding, consist of the formation of a new body starting respectively from a preexistent adult epithelium, and from the vasculature and blood cells. While blastogenesis occurs in a periodical and stereotyped fashion as part of *B. schlosseri* life cycle, vascular budding is a stricto sensu form of regeneration, and it is triggered by physical ablation of the adult body.In this manuscript, I described the results of my comparative study of *Botryllus* non-embryonic developmental pathways. Taking advantage of the extensively documented ascidian embryogenesis as a starting point, I intended a broad characterization of *Botryllus* blastogenetic and regenerative developments. First I adopted a gene candidate approach, took advantage of the well documented robustness of ascidian embryogenesis, and focused on a sub-set of conserved transcription factors that define the three germ layers in this class. Comparing embryonic expression patterns of GATAs, GSC, TBX2/3, OTX and FOXA TFs to those during asexual development and whole body regeneration. I observed a re-deployment of these genes in topological domains that resemble expression patterns observed during gastrulation. Using similar approach I started dissecting conserved neural and muscular developmental networks and analized the expression of Macho-1, Pax3/7, POU3 and 4, MRF and TBX6 to track the origin of neural and muscular tissues in blastogenesis. The preliminary data suggest that a particular anatomical structure, the dorsal tube, may have the potential to be a centre of neural and myogenic cell fate commitment. Whereas blastogenesis already presented described stereotyped stages, vascular budding needed discrete and recognizable staging. I then characterized the kinetics of early regenerative steps by using gene candidate gene approach, RTqPCR and live observations. Consistently with previous studies, I was confronted to the unpredictability of vascular bud appearance following surgery. Nonetheless, these observations allowed the identification of a time-frame where the formation of a vascular bud showed a maximal probability. I used this data provide a deeper morphological characterization of the early vascular buds morphogenesis by using immunohistological techniques and TEM. For both non-embryonic ontogenis I adopted an unbiased approach by characterizing the transcriptome profiles of: 1) budding and non budding tissues and 2) the first steps of whole body regeneration (WBR) via vascular budding. The analysis of differentially expressed genes and signalling pathways from the palleal bud transcriptomes

revealed the involvement of conserved developmental genes (i.e.: GATA, POU, Pitx, RALDH2) and potential novel *Botryllus*-specific genes whose function remain to be tested. Transcriptomic characterization of WBR is still undergoing analysis and will be available shortly. Together, these data bring new insights on the mechanisms that underlie non-embryonic developments, showing how new structures, different morphogenesis and regulatory mechanisms can arise from the same genome, in different biological contexts.

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