ASCIDIAN NEWS*

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A big thank-you to all who sent in contributions. There are **84** New Publications listed at the end of this issue. Please continue to send me articles, and your new papers, to be included in the June 2020 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 R**osana Rocha** (rmrocha@ufpr.br): In 2018, Dr Baslavi Cóndor Luján invited me to offer a workshop on ascidian taxonomy in Peru and after a great effort from her and her University to put this together, the course "Taxonomía de Ascidias de la costa Pacífica Sudamericana y de la Antártida" was held in Universidad Científica del Sur, Lima in the first week of December. Dr **Marcos Tatián** from National University of Córdoba, and the doctoral candidate **Anabela Taverna** were also instructors in the course. We had 19 students enrolled from six different countries in Latin America!

Currently the knowledge of ascidians in the shallow waters of Peru is very poor and during the course we identified species from the University scientific collection of ascidians from Antarctic, and species previously collected by some students in Peru, Equador and Costa Rica. We also did some field work at Pucusana, 60 km south of Lima, during low tide. Although the site has a local port for fishing boats, and there are a great number of small boats moored in the small bay, diversity and abundance of ascidians was surprisingly low! As expected, most of them introduced species. Thus, this is a very interesting region for future biogeographical and ecological studies aimed to explain this gap in biodiversity!

In the end, everybody learned a lot, including us instructors, dealing with a fauna that we do not see in our routine work. We gained many Peruvian friends, and had the opportunity to try all kinds of exquisite dishes of the Peruvian cuisine (that does not include ascidian in their menu, kkkk). And, of course, we look forward to many new students joining the Tunicate community!

Instructors enjoying the Peruvian food. Left to right: Marcos Tatián, Anabela Taverna and Rosana Rocha.

2. From **Luciana Granthom** (lu.granthom@gmail.com): I would like to introduce my blog https://lugranthom.wixsite.com/ascidiasdorio about ascidians from the southeastern Brazilian coast still under construction (available in English soon) but with amazing images in order to help students or experts in the field.

WORK IN PROGRESS

From your editor, **Gretchen Lambert**: I participated in a two week intensive comprehensive bioblitz of the Los Angeles/Long Beach southern Calif. region during the last two weeks of August with the goal of recording as many species of marine invertebrates as possible. The survey was organized by Gustav Paulay of the Florida Museum on Natural History who has organized many bioblitzes (paulay@flmnh.ufl.edu). Many taxonomists participated as well as many graduate students. As mentioned in the June AN, I had been actively recruiting one or more ascidiologists to train with me to better learn the west coast species, with the goal of becoming my permanent replacements. While I have greatly enjoyed these surveys during the past several decades, they have become too physically challenging for me, as are the very long hours required at the microscope, and it seemed the right time to make my announcement that this will probably be my last year for field work. Lauren Stefaniak (lauren.stefaniak@gmail.com), who has become expert on the east coast ascidians, stepped up to the challenge and worked with me in Oregon. Marie Nydam (sunrise417@gmail.com) joined me for the August survey. She recently moved from the east coast to take a new faculty position at Soka Intl. Univ. in southern Orange County near Los Angeles and is anxious to learn all the southern Calif. ascidians so this was a perfect opportunity. There were so many ascidians! I could not have managed the recording, photographing, relaxation and fixation without her help. Many of the specimens were obtained by scuba by Gustav and others. For me the most exciting find was several specimens of *Ascidia vermiformis* collected by Gustav, a very large species previously collected and named by Wm. Ritter in 1913! A total of 40 species plus several unidentified didemnids were collected.

ABSTRACTS FROM RECENT MEETINGS

1. The 10th Intl. Tunicata Meeting was held July 7-12 in Villefranche sur Mer, France, on the Mediterranean. See https://2019-tunicate-meeting.obs-vlfr.fr/ for the downloadable abstract book pdf of the talks and posters presented. So many interesting presentations!! I am very sorry I could not attend. Billie Swalla lent me her program book a few weeks ago and I enjoyed reading the whole thing. She also gave me her souvenir bag which I am enjoying using, so I have felt some participation

in this special meeting for all ascidiologists. I hope to be able to announce the date and location of the next meeting.

2. Proc. 65th Congress of the GEI-Italian Soc. of Development and Cell Biology (GEI-SIBSC) and 38th Congress of the Italian Soc. of Histochemistry, 24-27 June 2019. Published in Europ. J. Histochem. 63, supp. 2, 2019.

Identification and expression studies of putative stem/progenitor cell lmarkers in the urochordate *Botryllus schlosseri*. A. Peronato, N. Franchi, L. Ballarin, Dept. of Biol., Univ. of Padua, Italy. ballarin@bio.unipd.it

In the colonial ascidian *Botryllus schlosseri*, a cyclical generation change guarantees the recurrent (weekly at 20°C) renewal of the zooids. During the blastogenetic cycle (i.e., the interval of time between a generation change and the following one), buds progressively grow to the adult size before replacing the old zooids. With the aim of better elucidating the process stem cell differentiation, with particular reference to the genesis of haemocytes during the of the colonial ascidian, we screened the B. schlosseri genome and transcriptome, looking for transcripts/genes showing similarity to vertebrate molecular markers of haematopoietic stem/progenitor cells. On these sequences, after an in silico translation, we performed the phylogenetic reconstruction that, always, returned us the tunicate relevant position, within the protochordates cluster, of vertebrate sister group. The four mammalian orthologous genes, used as markers for the recognition of haematopoietic stem/progenitor cells, identified in B. schlosseri, are bsabcg2, bscd133, bsgata1/2/3 and bsgata4/5/6. The ISH assay, performed by antisense specific riboprobes, on haemocyte monolayers and colony sections, resulted in a labelling of the sub-endostylar haemolymph lacunae. This results matches previously morphological data that identified the endostyle as a stem cell niche, strengthening our idea to use bsabcg2, bscd133, bsgata1/2/3 and bsgata4/5/6 genes for the identification of haematopoietic stem/progenitor cells in B. schlosseri. Quantitative real time PCR (qRT-PCR) highlighted the overexpression of the considered genes in the mid-cycle phase of the blastogenetic cycle. During this phase, there is the formation of new secondary buds emerging from the primary buds. The higher transcription levels of bsabcg2, bscd133, bsgata1/2/3 and bsgata4/5/6 in the mid-cycle phase reflect the presence of undifferentiated cells involved in proliferative and differentiation events required for the formation of the new blastogenetic generation.

3. 8th Meeting of the Italian Soc. of Evolutionary Biology, Padua 1-4 Sept. 2019.

a)Characterization of the complement system in a colonial tunicate: C3 complement receptors and opsonic role of C3. Anna Peronato, Nicola Franchi, Laura Drago, Loriano Ballarin. Dept. of Biol., Univ. of Padova, Padova, Italy.

The compound ascidian *Botryllus schlosseri* is a reliable model organism for the study of immunobiology. As an invertebrate, it relies only on innate immunity for its defense. We already demonstrated the presence, in Botryllus, of homologues of mammalian C3, Bf, MBL and MASP1, referred to as BsC3, BsBf, BsMBL and BsMASP, respectively. 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, a cascade of reactions occurs resulting in the cleavage of the third complement component (C3) to C3a and C3b, the former exerting a chemotactic activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. The best-known receptor for C3a in mammals is C3aR, whereas CR1 is the receptor able to recognize and bind C3b on the microbial surfaces. Here, we describe, in B. schlosseri, new genes showing homology with vertebrate C3aR and CR1, respectively, and studied their transcription in the course of the colonial blastogenetic cycle. In addition, 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. Results indicate that only morula cells, and no other immunocytes type, are labelled by the antisense probe for BsC3aR, whereas phagocytes and young, undifferentiated cells, known as hemoblasts, are the cells stained by the probe for BsCR1. Both the

bsc3ar and bscr1 genes are constitutively transcribed. However, a modulation in the extent of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at TO, whereas no differences were observed when EC and MC were compared. This is probably related to the renewing of circulating cells at TO, that are replaced by new, differentiating cells entering the circulation in the same period.

b) Stem cell based survival strategies in the marine environment: alternative developmental and regenerative pathways in star ascidians. Gasparini F., Vanni V., Anselmi C., Manni L.

Tunicates are the unique chordate species utilizing a stem cells-based asexual reproduction. Among tunicates, the star ascidian *Botryllus schlosseri* produces through embryogenesis a swimming tadpole larva, which metamorphoses into a sessile oozooid. The latter begins a cyclical palleal budding, forming a colony of genetically identical blastozooids joined by extracorporeal blood vessel in the tunic. The colony weekly renews its entire body by a synchronized process of resorption of adult zooids, which are replaced in filtering activity by their buds. Palleal budding, that allows a rapid expansion of a colony in the marine environment, is not the only stem cell-mediated developmental strategy exhibited by the species. The regeneration of a colony can also come from bud fragments isolated by their parents. New buds are emitted by bud fragments that, after healing of the cut surfaces, are progressively resorbed. Strategies for survival are further extreme when the vascular budding is experimentally forced. After the extirpation of all individuals from a colony, circulating stem cells clot close to a vessel epithelium initiating a budding process. The new zooid differentiates through the so-called whole-body regeneration, restoring a new colony. Stem cells involved in budding and regeneration can be considered unit of selection. When the vasculature of two colonies come into contact, a genetically controlled recognition process occurs. If compatibles, colonies fuse to form a chimaera. Here, circulating stem cells compete each other for the formation of somatic and germline organs in new buds. Stem cells from a looser partner in the chimera can be totally resorbed by those of the winner partner. Moreover, the winner partner for the somatic stem cells can be a germline looser (or *vice-versa*). Here we present these stem cell-mediated developmental strategies, comparing tunicate stem cell features to those of vertebrates.

4. 80th Meeting of the Italian Zool. Society, Rome, 23-26 September 2019.

Characterisation of complement system of a colonial protochordate: study of the expression of C3, CR1, C3AR and their role of C3 in nonself recognition. Anna Peronato, Nicola Franchi, Laura Drago, Loriano Ballarin, Dept. of Biol., Univ. of Padua, Italy. ballarin@bio.unipd.it

The complement system is one of the most ancient immune modulation mechanism of bilaterian metazoans. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways; all of them converge on the cleavage of C3. The compound ascidian *Botryllus schlosseri* is a reliable model organism for the study of immunobiology. It relies only on innate immunity for its defense and immunocytes. Recently, in the same species, we demonstrated of the lectin and 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. The best-known receptor for C3a in mammals is C3aR, whereas CR1 is the receptor able to recognize and bind C3b on the phagocyte surfaces. In the present work, we describe, in *B. schlosseri*, one genes showing similarity with vertebrate C3aR and three genes with similarity to CR1 (two soluble formsand one transmembrane), and studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that their mRNAs are located in different immunocytes suggesting the presence of a crosstalk between phagocytes and morula cells. In addition, 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. 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.

NEW PUBLICATIONS

(in some cases where the species is not in the title I have added it in brackets.)

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Casso, M., Tagliapietra, D., Turon, X. and Pascual, M. 2019. High fusibility and chimera prevalence in an invasive colonial ascidian [*Didemnum vexillum*]. Sci. Reports **9**: 1-10.

Casso, M., Turon, X. and Pascual, M. 2019. Single zooids, multiple loci: independent colonisations revealed by population genomics of a global invader [*Didemnum vexillum*]. Biol. Invasions **in press**: 1-18.

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Colgan, W., Leanza, A., Hwang, A., DeBiasse, M. B., Llosa, I. et al. 2019. Variable levels of drift in tunicate cardiopharyngeal gene regulatory elements. EvoDevo **10**: epub.
Confalonieri, S., Colaluca, I. N., Basile, A., Pece, S. and Di Fiore, P. P. 2019. Exon 3 of the NUMB gene emerged in the chordate lineage coopting the NUMB protein to the regulation of MDM2. Genes Genomes Genetics **9**: 3359-3367.
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Gallo, A., Boni, R., Buia, M. C., Monfrecola, V., Esposito, M. C. and Tosti, E. 2019. Ocean acidification impact on ascidian *Ciona robusta* spermatozoa: New evidence for stress resilience. Sci. Total Environ. **697**: 1-12.
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Matsubara, S., Shiraishi, A., Osugi, T., Kawada, T. and Satake, H. 2019. The regulation of oocyte maturation and ovulation in the closest sister group of vertebrates. **8**: 1-24.
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