

# ASCIDIAN NEWS\*

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Forty years ago Charley and I took over the editorship of *Ascidian News* from Dr. Lu Eldredge of the Univ. of Guam. We did not realize what a long-term commitment it would turn out to be! Let me know if you still find it useful. The first issue was May 1966, the second in April 1967, and the third Nov. 1969. Our first issue, #4, was in August 1975 and we continued with 1 or 2 per year ever since. My website includes links to all the newsletters since #31 in 1991. I think the original aims have been well fulfilled. From issue #1: "This newsletter is designed to bring together the various ascidian researchers throughout the world. It is intended to be a bulletin board for news of recent and present investigations, titles of recent publications, exchanges of ideas, questions concerning nomenclature, notices of expeditions, etc." Dr. Donald Zinn of the Univ. of Rhode Island, USA, wrote the following for issue 2:

Ascidologists of the world unite!  
Gather under the mantle of Eldredge's light;  
Surround yourselves with the tunic of NEWS  
Written with an (endo) style to cover all views.

I will participate in teaching a two week ascidian course at Nagoya University's Sugashima Marine Lab from the end of June to July 10, and then attend the Intl. Tunicata meeting in Aomori, Japan from July 13-17; I look forward to seeing many of you there.

There are **128** 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. *Ciona intestinalis* now shown to be 2 separate species.** Because so many researchers work on *Ciona intestinalis*, and so many papers are published on this species, I draw your attention to 2 new publications showing at last that *Ciona intestinalis* A and B are different species and designating the correct names to be used in all future publications:

Brunetti, R., Gissi, C., Pennati, R., Caicci, F., Gasparini, F. and Manni, L. 2015.

Morphological evidence indicates that *Ciona intestinalis* (Tunicata, Ascidiacea) type A and type B are different species. *Journal of Zool. Systematics & Evolutionary Research* (in press). Type A is now designated *C. robusta*; type B retains the name *C. intestinalis*.

A second new publication describes larval differences between the two species:

Pennati, R., Ficetola, G. F., Brunetti, R., Caicci, F., Gasparini, F., Griggio, F., Sato, A., Stach, T., Kaul-Strehlow, S., Gissi, C. and Manni, L. 2015. Morphological differences between larvae of the *Ciona intestinalis* species complex: hints for a valid taxonomic definition of distinct species. *PLoS One* 10(5): 1-22.

2. Another ascidian that continues to be the subject of hundreds of research projects is ***Botryllus schlosseri***. The following important new publication should be of great interest to many: Genetic evidence that the colonial ascidian *Botryllus schlosseri* is native to North America. Yund, P. O., Collins, C. and Johnson, S. L. 2015. Biol. Bull. 228 (3): In press (June).

3. The **8th Intl. Tunicata meeting** will be held July 13-17, 2015 in Aomori city, Japan, immediately following the summer ascidian course at Sugashima lab. Aomori is located at the northern end of the Japan main island of Honshu. Nearby is the Asamushi marine biological laboratory, one of the planned sites to visit on excursion day. The organizers are Kazuo Inaba, Director, Shimoda Marine Research Center, University of Tsukuba ([inaba@kurofune.shimoda.tsukuba.ac.jp](mailto:inaba@kurofune.shimoda.tsukuba.ac.jp)) and Gaku Kumano, Asamushi Research Center for Marine Biology, Tohoku University, Asamushi ([kumano@m.tohoku.ac.jp](mailto:kumano@m.tohoku.ac.jp)). More information can be found at <http://tunicatemeeting.info/Aomori2015/?q=top>.

4. The **9th Intl. Conference on Marine Bioinvasions** will be held in Sydney, Australia January 19-21, 2016. Abstract submission is now open until August 7<sup>th</sup>. For more information, go to [www.marinebioinvasions.info](http://www.marinebioinvasions.info) or contact Dr. Emma Johnston: [e.johnston@unsw.edu.au](mailto:e.johnston@unsw.edu.au).

5. **Christian Sardet** ([csardet@gmail.com](mailto:csardet@gmail.com)) has just published **Plankton: Wonders of the Drifting World**. It was previously published in French and Japanese; the English version will be published in June by the Univ of Chicago Press.

<http://www.press.uchicago.edu/ucp/books/book/chicago/P/bo19415930.html>

Some of the content can be seen at: <http://www.planktonchronicles.org/book>. The book was awarded Best Underwater World Publication by the World Festival of Underwater Images (Marseille 2014). Christian writes: There are pages devoted to salps, pyrosomes, appendicularians and the very last photos are about my favorite ascidian embryos.

## **ABSTRACTS FROM RECENT MEETINGS**

### **1. 44th Benthic Ecology Meeting, Univ. Laval, Quebec City, Canada, March 4-7, 2015.**

**a. Genetic evidence that the colonial ascidian *Botryllus schlosseri* is native to North America.** Yund, P.O.<sup>1</sup>; Collins, C.<sup>2</sup>; S.L. Johnson<sup>2,3</sup>. <sup>1</sup>The Downeast Institute, P.O. Box 83, Beals, ME 04611 USA [pyund@downeastinstitute.org](mailto:pyund@downeastinstitute.org); <sup>2</sup>Dept. of Anatomy, Univ. of Otago, Dunedin NZ; <sup>3</sup>Dept. of Zoology, Univ. of Otago, Dunedin NZ

Evidence for classifying the colonial ascidian *Botryllus schlosseri* as an invader is extremely circumstantial. Nevertheless, over the last 15 years most research groups have accepted the scenario of human-mediated dispersal. Meanwhile, others have continued to consider this species native or cryptogenic. We address the invasion status by adding 174 sequences to the growing worldwide database for the mitochondrial gene cytochrome c oxidase subunit I (*COI*) and analyzing 1,077 sequences to compare the genetic diversity of a clade of haplotypes in the Northwest Atlantic with two hypothesized source regions (the Northeast Atlantic and Mediterranean). Our results lead us to reject the prevailing view of the directionality of transport across the Atlantic. We argue that the genetic diversity patterns at *COI* are far more consistent with the existence of at least one haplotype clade in the Northwest Atlantic (and possibly a second) that substantially pre-dates modern human colonization from Europe, with this native North American clade subsequently introduced to

three sites in Northeast Atlantic and Mediterranean waters. However, we concur that some sites in the Northwest Atlantic have more recently been invaded by alien haplotypes, so that some populations are currently composed of a mixture of native and invader haplotypes. IN PRESS, BIOLOGICAL BULLETIN JUNE 2015.

**b. Morphological and genetic characterization of introduced ascidians and their distribution patterns in North Carolina harbors and marinas.** S. Villalobos<sup>1</sup>; G. Lambert<sup>2</sup>; S. López-Legentil<sup>1</sup>. <sup>1</sup>Univ. of N. Carolina Wilmington, <sup>2</sup>Univ. of Washington Friday Harbor Labs, Friday Harbor, WA; [smv7274@uncw.edu](mailto:smv7274@uncw.edu) (SV).

Marine introduced species have become increasingly prevalent around the world, in both natural and artificial environments. Ascidians are successfully introduced in many areas due to their high reproductive capacity, population growth rates and tolerance for a wide variety of environmental factors, including those that characterize many artificial substrates. In this study, we assessed the biodiversity and distribution of ascidians in 17 marinas along the North Carolina coast. A fragment of the mitochondrial gene cytochrome oxidase I (COI) was sequenced for each sample, analyzed, and then compared to those in GenBank using BLASTn. The distribution patterns of native, cryptogenic, and introduced ascidians in North Carolina harbors and marinas as well as the similarities between sites were examined and analyzed in computer software "PRIMER 6" and "R".

**c. Does settlement plate material matter? The influence of substrate type on fouling community development.** Chase, A.L.<sup>1</sup>; Dijkstra, J.A.<sup>2</sup>; Harris, L.G.<sup>1</sup>

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Benthic community composition and ascidian abundance can differ dramatically between adjacent man-made and natural substrates. Although multiple factors, including light exposure, surface orientation, predation exposure, and habitat type, are known to contribute to these patterns, few studies have directly tested the influence of substrate identity on community development. We compared fouling communities on settlement plates composed of commonly occurring natural (granite) and artificial (concrete, high density polyethylene, and PVC) marine materials deployed from late May to mid November 2014 from a floating dock in Newcastle, NH. We sought to determine if observed patterns resulted from differential recruitment onto substrate materials or post-settlement survival and growth. To do this, half of the plates were cleaned during bi-weekly examinations, and half were left un-cleaned. Preliminary analyses indicate that community composition on concrete plates differs from that on all other substrate materials. These results will help us to understand how substrate features contribute to nonnative species establishment and habitat dominance, and may inform decisions regarding material usage in marine construction. These findings also underline the importance of settlement substrate choice in scientific studies, as plate material may influence experimental conclusions.

**d. Novel commensal amphipod species discovered in endemic tunicate *Cnemidocarpa bicornuta* in New Zealand.** Brucker, K.; Thomas, J. D.; Messing, C.G.; Arena, P.

**2. 16th symposium in memory of Merav Ziv, "Species Invasion and Range Expansion", Sede Boker, Israel April 30, 2015.**

**a) All aboard! Marine vessels as a vector for non-indigenous ascidians dispersal in the Mediterranean Sea.** Mey-Tal Gewing, Noa Shenkar. Dept. of Zoology, Tel Aviv University. [meytalyaniv@gmail.com](mailto:meytalyaniv@gmail.com)

Ascidians (Phylum: *Chordata*, Sub-phylum: *Tunicata*) are considered to be very successful invaders, exploiting marine vessels as their main path of introduction. Non-indigenous ascidians are now commonplace in both tropical and temperate waters, thus creating severe damage both to natural fauna and to aquaculture by overgrowth. The Mediterranean Sea is also part of the global ascidian invasion. However, ship-mediated introductions have been only incidentally documented in the Mediterranean.

The main goal of our work is to acquire better understanding of marine vessels role in alien ascidians' dispersal in the Mediterranean. We have conducted surveys in five Israeli shipyards in which recreational, commercial and military vessels are docked. To date thirty two marine vessels have been examined. Crafts usage, size, home port, sailing trails, and last shipping date were recorded, along with ascidians detected and their location on the craft (hull, propeller, rudder, seachest and thruster). Samples were taken to the laboratory for morphologic and molecular identification.

Alien ascidians were detected on 47% of the vessels, two of them for the first time in the Israeli coast. Two thirds of the ascidians were found on large commercial crafts. In many cases, despite the fact that the vessel was in constant movement and well maintained, ascidians were located in the protected area of the seachest.

The results of our research verify that marine vessels are a vector for non-indigenous ascidians. In addition, it provides an early detection tool which will enable a rapid response once alien ascidians are observed. More importantly, the current research emphasizes the need to prevent ascidians' fouling on specific areas of the vessel, e. g. the sea-chest. In this manner both ship owners and marine local fauna will benefit from more efficient, environmental and economic treatments against ascidians fouling.

**b) How important are specialization and competitive abilities for becoming invasive?** Itai Granot, Noa Shenkar, Jonathan Belmaker. Dept. of Zoology, Tel-Aviv University, Israel. [itai811@gmail.com](mailto:itai811@gmail.com), [noa.shenkar@gmail.com](mailto:noa.shenkar@gmail.com)

Invasive species are one of the most acute environmental problems worldwide. Here we wish to understand what makes a species a successful invasive. We experimentally examine two key hypotheses: (1) invasive species are generalists compared to the other species in their native range, meaning they are able to thrive in a wider range of environment conditions. (2) invasive species are less likely to be influenced by competition in the introduced region. We focus on the Mediterranean coast of Israel, which is a major entry route of tropical Red Sea fauna through Suez Canal. We used Israel's unique position between the Red Sea (tropic environment) and the Mediterranean (temperate environment) to test these hypotheses using fouling communities. We designed a unique settlement plate composed of three types of substrate, and two lighting conditions which create six different niches. This heterogeneity is used to assess the degree of niche specialization of species. Initial analyses in the Red Sea indicate that, in contrary to our hypothesis, there are no significant differences in the specialization level of invasive species compared to other species in the invasives' native range. In order to test the second hypothesis we estimated the ratio between the realized and the fundamental niche of native and invasive species in the Mediterranean. This was done by removal experiments where species abundance and specialization were compared between complete fouling communities and plates where all species excluding the focal species were removed. The results again run contrary to our hypothesis, as initial analyses of the results indicates that invasive species are actually more

sensitive to competition compared to other species in the invaded region. These findings suggest that invasive do not seem to have an inherent ecological advantage over other species, in their native and invasive ranges alike, that can explain their success.

**3. XVIth scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADCI), 18 - 20 February 2015. Dept. of Life Sciences, Univ. of Trieste, and Natl. Inst. of Oceanography and Experimental Geophysics, Trieste, Italy.**

**a) New data on *Botryllus schlosseri* AMP: a transcript analysis.** N Franchi, L Ballarin Dept. of Biology, Univ. of Padua, Padua, Italy. [nicola.franchi@unipd.it](mailto:nicola.franchi@unipd.it), [ballarin@bio.unipd.it](mailto:ballarin@bio.unipd.it)

The innate immune system provides an immediate response against infections in animals and plants. Endogenous antimicrobial peptides (AMPs) are essential effector molecules of this first line of defense allowing the direct killing of invading micro-organisms. Moreover AMPs have attracted increasing interest because of their potential as new antibiotics. To date various natural peptides from all kinds of organisms and synthetic derivatives have been characterized for their potential use as novel therapeutics. However, the number of candidate peptides undergoing preclinical or clinical evaluation is still very low. Marine invertebrates are source of several AMPs and some of these have been isolated and characterized from different urochordate such as *Styela plicata* (clavanins and styelins) and *Ciona intestinalis* (Ci-MAM).

In the colonial ascidian *B. schlosseri*, exploiting the transcriptome and the genome, we have been able to identify a styelin-like AMP. We also carried out some preliminary experiments to investigate the chemical properties and the expression pattern of such gene in the presence of different PAMPs.

**b) New data on phagocytosis-induced apoptosis in the colonial ascidian *Botryllus schlosseri*.** N Franchi, F Schiavon, L Ballarin, Dept. of Biology, Univ. of Padua, Padua, Italy.

Colonies of the ascidian *Botryllus schlosseri* undergo cyclical generation change or take-over (TO) during which diffuse apoptosis occurs in zooid tissues, as indicated by morphological, biochemical and molecular investigations. Tissues are rapidly infiltrated by circulating phagocytes, selectively recruited by dying cells, which recognize and greedily ingest them. Previous observations led us to suggest that phagocytes, once ingested apoptotic cells or corpses, undergo phagocytosis-induced apoptosis (PIA). We already demonstrated, by Western blot and immunocytochemical analyses, the release of cytochrome c by hemocytes and the expression of apoptosis-related molecules, such as BAX and caspases, in phagocytes during the TO. In order to corroborate the above assumption, we carried out new morphological analyses at the transmission electron microscope (TEM) and started a molecular analysis of apoptosis in *Botryllus* phagocytes looking for transcripts differentially expressed in phagocytes at TO. We identified and characterized transcript sequences for BAX, AIF and PARP (poly ADP ribose polymerase), and studied their expression and the location of the corresponding mRNA in hemocytes. The collected data clearly indicate the diffuse occurrence of PIA among phagocytes which guarantee the disposal of apoptotic cells or corpses. In addition, they extend the classical view of PIA, intended as a mechanism for the prevention of microbial diffusion within the organism, and reveal an undescribed role of the process in the control of asexual development and colonial homeostasis.

**c) Expression study of molecular markers involved in staminality and differentiation in the colonial ascidians *Botryllus schlosseri*.** F Ballin, N Franchi, L Ballarin.

Dept. of Biology, Univ. of Padua, Padua, Italy.

All multicellular organisms originate from a small set of totipotent embryonic cells that differentiate into a structured body plan during embryogenesis. The ability to generate an embryo from a single cell and the regenerative capabilities of metazoans suggest the presence of cell types with stem cell attributes. Compound ascidians, like *Botryllus schlosseri*, offer the opportunities to investigate the biology of both embryonic and adult stem cells thanks to the presence of sexual and asexual reproduction. In *B. schlosseri* new buds emerge as thickening of the peribranchial epithelium in a process call palleal budding. Sometimes, a vascular budding occurs, with the development of new buds formed by circulating multipotent or pluripotent cells. These two kinds of budding processes render *B. schlosseri* a good research tool for the study of staminality.

In *B. schlosseri*, during the cyclical generation change, an increase in the number of hemoblasts occurs which will replace, after their differentiation, the circulating cells undergoing apoptotic cell death. Ascidian hematopoiesis occurs in close proximity to the pharyngeal vessels, in the so-called “hematopietic nodules” and in the endostyle, the cells of which proliferate and migrate to regenerating organs in 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 first results on the characterisation of staminality and differentiation molecular markers such as ABCG2, CD133 and GATA2/3 considered hematopoiesis molecular marker in other deuterostomes.

**4. In the Light of Evolution IX. Clonal Reproduction: Alternatives to Sex.** January 9-10, 2015; Irvine, CA.

Cooper, E.L. Pathogens and cancer: clonal processes and evolution.

**Ph.D. PROJECTS IN PROGRESS**

**Ecological patterns and processes that determine success in the colonization of invasive species in port areas.** Clara Giachetti, Centro Nacional Patagonia, CONICET, Puerto Madryn, Argentina. Supervisors: Evangelina Schwindt (Centro Nacional Patagonia, CONICET, Argentina) and Marcos Tatian (Instituto de Diversidad y Ecología Animal, CONICET, Cordoba, Argentina). [gjachetti@cenpat-conicet.gob.ar](mailto:gjachetti@cenpat-conicet.gob.ar)

Artificial habitats favour the recruitment and survival of exotic species. In this way, the study of port areas is important to understand the ecological patterns and processes that are involved in the success of invasive species. The ascidians are one of the most important groups in fouling communities with high recruitment and growth rate. During my Ph.D. studies I will use *Asciidiella aspersa* and *Ciona intestinalis* as models for testing predation and competition hypothesis in marine bioinvasions. The goal of this project is to understand the ecological patterns and processes that are involved in colonization success of the invasive ascidians in different artificial structures in the cold-temperate port of Puerto Madryn, Argentina. I will study this through different lab and field experiments over different artificial substrata. I will identify the potential predators of the dominant ascidians, their temporal and spatial variations, and their effects on artificial structures. I am also interested to study the differences in the timing in reproduction and recruitment of these ascidian species together with the variations according to environmental parameters. The results of this study will

contribute not only to better understand the biological invasion processes but also will provide essential information for a sustainable coastal management.

### **NEW PUBLICATIONS**

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