I apologize for the later than usual posting of this issue. May was especially busy for me, and June has also been much too busy but I knew I could not wait any longer. A big thank-you to all who sent in contributions. There are 101 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 2019 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. The **10th Intl. Tunicata Meeting** will be held at the citadel of Saint Helme in Villefranche sur Mer (France), 7-12 July 2019. This is coming up right away! Organizers are Stefano Tiozzo (tiozzo@obs-vlfr.fr) and Remi Dumollard (dumollard@obs-vlfr.fr). Web links to the program and abstracts will be included in the December issue of AN. See the following link for the list of invited speakers and the meeting topics. [https://2019-tunicate-meeting.obs-vlfr.fr/](https://2019-tunicate-meeting.obs-vlfr.fr/)

2. From **Anabela Taverna** Univ. Nacional de Córdoba, Argentina (anabelataverna@gmail.com): There will be a **new training course in ascidian taxonomy, in Peru, in December 2019**. The teachers will be Rosana Rocha and Anabela Taverna. The one week course “Taxonomía de Ascidias de la Costa Pacífica Sudamericana y la Antártida" will be held at Universidad Científica del Sur in Peru. Applications will be opened on July. More information will be available at [https://www.cientifica.edu.pe/ciencias-veterinarias-y-biologicas/biologia-marina](https://www.cientifica.edu.pe/ciencias-veterinarias-y-biologicas/biologia-marina). For more information contact Báslavi Cóndor Luján and Anabela Taverna: curso.ascidias.peru@gmail.com.

3. From the staff at WoRMS (World Register of Marine Species, info@marinespecies.org): **The digitized “Challenger Report” now hosted at VLIZ.** All the Reports of the Scientific Results of the" Voyage of H.M.S. Challenger, Zoology" are again available through [www.19thcenturyscience.org](http://www.19thcenturyscience.org), now hosted at the Flanders Marine Institute, home of WoRMS. Maybe some of you had seen the superb website where Dr. David Bossard, then Professor at Dartmouth College, New Hampshire, had digitized all the Reports of the Scientific Results of the Voyage of H.M.S. Challenger", Zoology. At some time in 2018, this site went offline and the WoRMS Editorial board contacted Dr. Bossard to enquire about what happened. It turned out that the place where the site had been hosted for years changed for an unsustainable price and Dr. Bossard was willing to hand it over to a non-profit organization. At the same time, the WoRMS Steering Committee was discussing the issue of “data rescue” and this seemed a good case to start with. Thanks to the efforts of the WoRMS Data Management Team who are funded through the LifeWatch.be project, the
site is now restored “as-is” but hosted at the Flanders Marine Institute (VLIZ), which is also the home of the World Register of Marine Species (WoRMS).

The H.M.S. Challenger Expedition, conducted between 1873 and 1876 was one of the first, and perhaps the most thoroughly documented, of many expeditions devoted to the investigation of ocean physics and biology. It was a major breakthrough in marine science, demonstrating that life could be thriving at all depths and definitively setting aside the XIXth century “azoic theory”. All of the 90-odd Zoology and Botany reports published by the Expedition have been digitized and are available here for browsing. The reports include over 30,000 pages of text and many thousands of illustrations.

Direct Access to the reports is through an index, from which the user can navigate to the text and plates. The next challenge, now in the hands of the WoRMS Editors, is to link all the original descriptions (or frequently redescriptions, when a preliminary diagnosis had been published previously in a journal). Link: http://www.19thcenturyscience.org/

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From your Ascidian News editor:
W. A. Herdman produced a large format 2 volume set of the ascidians from the Challenger Expedition. I am fortunate to own a hard copy of this set, but it was very expensive and is rarely available. Herdman was an amazing ascidian taxonomist and I frequently use these volumes; they are still very relevant today.

The web link for this volume (including all the marvelous plates of drawings) is http://www.19thcenturyscience.org/HMSC/HMSC-Reports/Zool-17/README.htm


4. James Carlton (jcarlton@williams.edu) alerted me to the following new publication on a novel predatory behavior on Ciona by gulls; I include the reference here at his suggestion because we think many of you will find this newly learned behavior very interesting. Holman, L. E., Rius, M. and Blackburn, T. M. 2019. Observations of a novel predatory gull behavior on an invasive ascidian: A new consequence of coastal urban sprawl? Ecosphere 10(3) e02636: 1-5. The paper includes some amusing photos of the gulls removing the Ciona tunic before eating them.


WORK IN PROGRESS

1. From your editor, Gretchen Lambert: I participated in a one week Sea Grant-sponsored ascidian survey of Puerto Rico marinas in March with Susanna López-Legentil (Univ. of N. Carolina Wilmington), her graduate student Olivia Streit, and Patrick Erwin and Jim Evans; our main emphasis was to document non-natives while Patrick and Jim’s goal was collection of microbiota from ascidians and sponges. It was a lot of work and long hours, and I am now identifying the numerous samples we
brought back; perhaps we will include a short article on what species we found in the next issue of AN.

I also participated in an Oregon intensive bioblitz this month, headquartered at the Oregon Institute of Marine Biology at Coos Bay marine lab, with the goal of recording as many species of marine invertebrates as possible in a two week period. Many taxonomists participated as well as many graduate students and staff at OIMB. I have 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 replacement. While I greatly enjoy these surveys 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, who has become expert on the east coast ascidians, stepped up to the challenge and worked with me in Oregon.

There will be a second west coast bioblitz for the last two weeks of August to cover Los Angeles/Long Beach and perhaps other nearby areas of southern California, again with a large group of taxonomists. Lauren is unavailable for this one, but Jenn Dijkstra from Connecticut who also has become expert on east coast ascidians especially in the New England area will participate with me to learn the west coast species, as will Marie Nydam, and Tony Phillips who has already been dedicated to learning the southern Calif. species since he attended my ascidian workshop in March of last year at the LA County Museum of Natural History. So now instead of dreading my participation in this year’s surveys I am eagerly looking forward to them! And hoping that everyone collects an incredible number of species to provide suitable challenges for the new recruits.

2. From Patrick Frank (pfrank@slac.stanford.edu). Bob and Elaine Carlson and I have extended our studies of tunicate blood cell sulfur to *Ascidia ceratodes* in Bodega Bay, California. We have inventoried the sulfur functional groups in whole blood cells and cleared blood plasma using x-ray absorption spectroscopy (XAS). We also measured the sulfur XAS spectra of fresh Henze solution from *A. ceratodes* collected from Monterey Bay, California. In blood cells, low valent sulfur is dominated by thiol/disulfide. This redox couple gives the cytosol a net reducing emf of about -0.2 V. Other blood cell sulfur functional groups include benzothiophene and thianthrene. A systematic relationship emerged between blood pH and the fraction of sulfonate sulfur. Sulfate and bisulfate are also present in the hundreds of mM range. Their ratio indicated pH values of 2-2.5 within the vacuoles of the signet ring cells. Similar sulfur types were found for fresh Henze solution, including a low pH. Sulfate and disulfide dominate blood plasma. Total plasma sulfur is in the 30 mM range. Our eventual paper will also discuss elements of vanadium biochemistry. We also will present an extended hypothesis for the site, mechanism, and regulation of the biological reduction of vanadium in the Phlebobranch ascidians.

ABSTRACTS FROM RECENT MEETINGS


**Characterization of the complement system in a colonial protochordate: C3 complement receptors and opsonic role of C3.** Peronato A., Franchi N., Schiavon L., Ballarin L. Dept. of Biol., Univ. of Padua, Italy. ballarin@bio.unipd.it

The complement system is one of the most ancient immune modulator mechanism of bilaterian metazoans, able to influence ancient cells and factors of both innate and adaptive immunity. 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. As an invertebrate, *B. schlosseri* relies
only on innate immunity for its defense and immunocytes. Recently, in the same species, we demonstrated the presence 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 that involves proteolysis and polymerization occurs resulting in the cleavage of the third complement component (C3) 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 microbial surfaces.

In the present work, we described, in *Botryllus schlosseri*, two new genes showing homology with vertebrate C3aR and CR1, respectively, and studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that their mRNAs are located in different immunocyte types suggesting the presence of an important cross-talk 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 immunocyte types, were labelled by the antisense probe for BsC3aR, whereas phagocytes and young, undifferentiated cells known as hemoblasts were the cells stained by the probe for BsCR1. Both the bsc3ar and bscr1 genes are constitutively transcribed as almost all morula cells and phagocytes, respectively, resulted labelled by the antisense probe in the ISH assay, independently of their previous challenge with zymosan, a known activator of *B. schlosseri* hemocytes. 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, when 20-30% of hemocytes undergo cell death by apoptosis and are replaced by new, differentiating cells entering the circulation in the same period.

2. *Benthic Ecology Meeting, St. John’s, Newfoundland, April 3-6, 2019.*

**Moving to the reef: historical and present observations of the worldwide introduced species *Styela plicata* in the Northwest Atlantic continental shelf.** Lauren Stefaniak¹, Susanna López-Legentil². ¹Dept. of Marine Sci., Coastal Carolina Univ., ²Dept. of Biology and Marine Biology, Univ. of North Carolina Wilmington. lauren.stefaniak@gmail.com, LopezLegentils@uncw.edu.

The long-standing cosmopolitan distribution of the solitary ascidian *Styela plicata* (Lesueur, 1823) has effectively obscured the historical record of the native range of this species. *Styela. plicata* distribution is usually restricted to artificial substrates – an indication of a non-native population. An extensive literature review found one major exception to this trend: the Northwest Atlantic continental shelf along the southern United States, where *S. plicata* is a common member of live bottom temperate reef communities. Here, we investigated whether the Northwest Atlantic continental shelf could be the native region of *S. plicata* by sequencing a fragment of the mitochondrial cytochrome oxidase I (COI) gene from 51 individuals collected from North Carolina (NC) and Georgia reefs as well as a NC harbor. Comparison to COI haplotype sequences available in GenBank showed a high number of private haplotypes for this region; however, all haplotypes clustered within one of two clades described in Pineda et al. (2011). These results suggest that, despite the extended and well-documented presence of *S. plicata* on natural substrates for this region, it is not native to the northwest Atlantic continental shelf. Rather, this species was able to colonize and establish sustainable populations in this area a long time ago.

**Update:** In our original analysis, we found a high number of total and private CO1 haplotypes in the *Styela plicata* populations on natural substrate, offshore reefs of the South Atlantic Bight (SAB), but they all belonged to just one of two haplotype groups found by previous researchers. After
Ascidians hold a promising potential for detection of PhACs, before causing irreversible ecological damage. Since ascidians are the closest living relatives of vertebrates, there is an advantage to exploit the cosmopolitan distribution of invasive ascidian species in order to develop a uniform method using the same bio-indicator species, across a wide geographic scale. Our objectives are to evaluate the spatial and seasonal variation of ascidians’ ability to accumulate PhACs and to identify protein expression in response to PhACs accumulation using a proteomic approach. We developed an analytical method for detection of PhACs in ascidians’ tissues using pressurized liquid extraction and analysis by high-pressure liquid chromatography. We detected three PhACs, Carbamazepine, Bezafibrate and Diclofenac, during preliminary testing. We are currently analyzing collected samples in order to present our results at the SETAC Europe 29th annual meeting. Analysis of protein profiles of ascidians under laboratory controlled exposure experiments is also in progress. To our knowledge, this is the first time ascidians are used as bio-indicators for PhACs. Our goal is to identify the spatial and temporal variation of ascidians’ ability to accumulate PhACs and to identify protein expression in response to PhACs accumulation using a proteomic approach.

The use of pharmaceutically active compounds (PhACs) is increased every year in human and animal medicine worldwide. Their presence in the marine environment presents a threat to marine organisms and ecosystems. Measures of PhACs concentrations in marine waters and sediments are not sufficient to predict exposure concentration by marine organisms, as they provide only a snapshot in time. Additionally, it does not illustrate the harmful effects and the fate of these compounds within living organisms. Some types of PhACs have been intentionally designed to affect living systems at relatively low doses. As a result, PhACs at environmentally relevant concentrations (µg L⁻¹ to ng L⁻¹), have been recently identified as potentially toxic for aquatic organisms. In addition, bioaccumulation is possible for some specific compounds. This study aims to investigate the use of solitary ascidians, highly efficient filter-feeding sessile invertebrates, as biological indicators for PhACs. Our goal is to exploit the cosmopolitan distribution of invasive ascidian species in order to develop a uniform method using the same bio-indicator species, across a wide geographic scale. Our objectives are to evaluate the spatial and seasonal variation of ascidians’ ability to accumulate PhACs and to identify protein expression in response to PhACs accumulation using a proteomic approach. We developed an analytical method for detection of PhACs in ascidians’ tissues using pressurized liquid extraction and analysis by high-pressure liquid chromatography. We detected three PhACs, Carbamazepine, Bezafibrate and Diclofenac, during preliminary testing. We are currently analyzing collected samples in order to present our results at the SETAC Europe 29th annual meeting. Analysis of protein profiles of ascidians under laboratory controlled exposure experiments is also in progress. To our knowledge, this is the first time ascidians are used as bio-indicators for PhACs contamination. Ascidians hold a promising potential for detection of PhACs, before causing irreversible ecological damage. Since ascidians are the closest living relatives of vertebrates, there is an advantage studying their response to PhACs contamination over other marine invertebrates, giving the opportunity to better understand the possible effects on marine vertebrates and on humans.

b) Ascidians (Chordata, Ascidiacea) as model organisms for assessing the extent of anthropogenic pollutants in marine environments. Noa Shenkar1,2*, Dror Avisar3, Aviv Ben-Tal1, Aviv Kaplan3, Zafrir Kuplik1, Gal Navon1, Lion Novak1, Adi Torfstein4, Roni Tzafriri-Milo1, Gal Vered1. 1School of Zoology, George S. Wise Faculty of Life Sci., Tel Aviv Univ., Israel. 2The Steinhardt Mus. of Natural History & Natl. Research Center, Tel Aviv Univ., Israel. 3The Water Research Center, Porter Sch. of the Environment & Earth Sciences, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel Aviv Univ., Israel. 4Institute of Earth Sci., Hebrew Univ., Jerusalem, & InterUniv. Instit. for Marine Sci., Eilat, Israel.

The Mediterranean coastline has been undergoing dramatic changes in the past few decades. Although environmental and governmental agencies invest much effort in general monitoring and protection of this environment, there is a gap in our knowledge of the physiological impacts of current...
environmental stressors on the marine fauna, and of the possible potential of marine organisms as bio-indicators of environmental health. Sessile marine organisms such as ascidians may provide invaluable information regarding a wide scale of pollutants and stress factors over time, in contrast to common chemical assays. As highly efficient filter feeders, ascidians are well known for their ability to accumulate heavy metals, and to concentrate harmful compounds that are present in low or even only trace concentrations in the water column. The wide geographic distribution of invasive ascidians in both polluted and pristine habitats make them ideal candidates for bio-monitoring a wide variety of marine habitats, and investigating the pathological effects of a variety of stressors. The main objectives of the current study are to explore the potential use of the solitary invasive species Phallusia nigra, Styela plicata, Microcosmus exesperatus, and Herdmania momus as biological indicators for micro-plastic, phthalates, heavy metals, and pharmaceuticals. By combining a suit of analytical methods including chemical, physiological, histological and proteomic analysis we aim to locate and describe 'hot-spots' of polluted zones along the Mediterranean and Red Sea coasts of Israel, and further understand the physiological effect of pollutants on benthic invertebrates.

**THESIS ABSTRACTS**


Ascidians are benthic invertebrates that inhabit virtually all marine ecosystems. These organisms are interesting bioindicators for the monitoring of environmental disturbances, especially due to the sessile life habit during adulthood. One of the regions under strong anthropic influence is the Gulf of Mexico, home to the largest number of oil and gas extraction platforms in the world, with eight major oil spills occurring in the last decades. The ascidians from the Gulf of Mexico has been registered through several studies and specimens in natural history museums, and assessing those records may real important changes occurred in the faunal composition of this region. The most recent accident in the Gulf of Mexico occurred on April 20, 2010, where a ruptured duct connected to the Deepwater Horizon semisubmersible rig caused the largest accidental oil spill in US marine regions. As part of the Deepwater Horizon leakage monitoring project, ascidians were collected off the west coast of Florida (U.S.A.) in May 2012. Through the analysis of the ascidians collected during this monitoring and also through the verification of part of the specimens acquired in the 1960s during the Hourglass cruises, 242 samples belonging to the three orders of the Ascidiae class were examined. A total of 56 species that make up the fauna of ascidians of the Gulf of Mexico was identified, distributed in eleven families. Considering this result, we can observe that the organisms registered in the present study represented 38% of the ascidians already registered for this area. In addition, 16 species unknown to occur in the Gulf of Mexico were identified, representing about 29% of the ascidians listed here, being: Ascidia aff. corallicola, Ascidia cf. munda, Ascidia xamaycana, Ascidia sp., Clavelina sp., Exostoma sp., Aplidium sp., Lissoclinum perforatum, Didemnum galacteum, Didemnum sp. 1, Didemnum sp. 2, Didemnum sp. 3, Tridemnum sp. 1, Tridemnum sp. 2, Molgula sp. and Metandrocarpa sterreri. Hence, despite the many works already done in the region it is still possible to observe new records and possible new species in the study area. Based on the various bibliographical references and online database, the temporal assessment of ascidians records in the Gulf of Mexico was also carried out. This work presents the description of all species recently collected, along with an identification key suitable for use in the study area.

**NEW PUBLICATIONS**
Tunicates: From humble sea squirt to proud model organism
Introduction to the issue. S.Q. Irvine, F. Ristoratore and A. Di Gregorio [thanks to Anna Di Gregorio for providing the table of contents.]


Transcriptional regulation of Rab32/38, a specific marker of pigment cell formation in Ciona robusta C. Racioppi, U. Coppola, L. Christiaen and F. Ristoratore

Positioning a multifunctional basic helix-loop-helix transcription factor within the Ciona notochord gene regulatory network J.E. Kugler, Y. Wu, L. Katikala, Y.J. Passamaneck, J. Addy, N. Caballero, I. Oda-Ishii, J.E. Maguire, R. Li and A. Di Gregorio

Multiple inputs into a posterior-specific regulatory network in the Ciona notochord M. Harder, W. Reeves, C. Byers, M. Santiago and M. Veeman

Ascidian notochord elongation Q. Lu, P. Bhattachan and B. Dong

Massive cytoplasmic transport and microtubule organization in fertilized chordate eggs T. Nishikata, T. Goto, H. Yagi and H. Ishii

Non-centrosomal microtubule structures regulated by egg activation signaling contribute to cytoplasmic and cortical reorganization in the ascidian egg T. Goto, K. Kanda and T. Nishikata

Wavy movements of epidermis monocilia drive the neurula rotation that determines left–right asymmetry in ascidian embryos S. Yamada, Y. Tanaka, K.S. Imai, M. Saigou, T.A. Onuma and H. Nishida

Papillae revisited and the nature of the adhesive secreting collocytes F. Zeng, J. Wunderer, W. Salvenmoser, M.W. Hess, P. Ladurner and U. Rothbächer

Initial characterization of Wnt-Tcf functions during Ciona heart development N.A. Kaplan, W. Wang and L. Christiaen

The Ciona myogenic regulatory factor functions as a typical MRF but possesses a novel N-terminus that is essential for activity L.E. Ratcliffe, E.K. Asiedu, C.J. Pickett, M.A. Warburton, S.A. Izzi and T.H. Meedel

Single-cell transcriptome profiling of the Ciona larval brain S. Sharma, W. Wang and A. Stolfi

Ci-hox12 tail gradient precedes and participates in the control of the apoptotic-dependent tail regression during Ciona larva metamorphosis G. Krasovec, K. Robine, E. Quéinnec, A. Karaiskou and J.P. Chambon

Spermatogenesis as a tool for staging gonad development in the gonochoric appendicularian Oikopleura dioica Fol 1872 F. Cima

Developmental atlas of appendicularian Oikopleura dioica actins provides new insights into the evolution of the notochord and the cardio-paraxial muscle in chordates A. Almazán, A. Ferrández-Roldán, R. Albalat and C. Cañestro

Cellular and molecular mechanisms of regeneration in colonial and solitary ascidians S.H. Kassmer, S. Nourizadeh and A.W. De Tomaso

Progenitor targeting by adult stem cells in Ciona homeostasis, injury, and regeneration W.R. Jeffery
The tail of the underwater phoenix  **B. Rinkevich**

Sixty years of experimental studies on the blastogenesis of the colonial tunicate *Botryllus schlosseri*  L. Manni, C. Anselmi, F. Cima, F. Gasparini, A. Yoskoboynik, M. Martini, A. Peronato, P. Burighel, G. Zaniolo and L. Ballarin

The biology of the extracorporeal vasculature of *Botryllus schlosseri*  D. Rodriguez, S. Nourizadeh and A.W. De Tomaso

IAP genes partake weighty roles in the astogeny and whole body regeneration in the colonial urochordate *Botryllus schlosseri*  A. Rosner, O. Kravchenko and B. Rinkevich

*De novo* neurogenesis in a budding chordate: Co-option of larval anteroposterior patterning genes in a transitory neurogenic organ  M.M. Prünster, L. Ricci, F.D. Brown and S. Tiozzo


evaluation of the effects against *Schistosoma mansoni* of the novel alkaloids polyaurines A and B. Mar. Drugs **17**: epub.


Palmquist, K. and Davidson, B. 2017. Establishment of lateral organ asymmetries in the invertebrate chordate, Ciona intestinalis [C. robusta]. EvoDevo 8:


Rudolf, J., Dondorp, D., Canon, L., Tieu, S. and Chatzigeorgiou, M. 2019. Automated behavioural analysis reveals the basic behavioural repertoire of the urochordate Ciona intestinalis. 9:


