Rosana Rocha and I will be teaching the next tunicate workshop June 20-July 4 in Panama, at the Smithsonian's Bocas del Toro Tropical Research Institute on the Caribbean. This is the 5th advanced workshop we have taught since 2006 at this lab; it is very gratifying to see that many of the participants are now faculty members at various institutions, with their own labs and students pursuing research projects on ascidians.

A big thank-you to all who sent in contributions. There are 113 New Publications listed at the end of this issue. Please continue to send me articles, and your new papers, to be included in the next issue of AN.

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

**NEWS AND VIEWS**

1. I hope to see many of you at the upcoming **Intl. Tunicata meeting** in New York City July 17-21, at New York University, hosted by Dr. Lionel Christiaen. There will be a welcome reception on the evening of July 16th. For more information see [https://2017-tunicate-meeting.bio.nyu.edu/](https://2017-tunicate-meeting.bio.nyu.edu/).

2. The next International Summer Course will be held at Sugashima Marine Biological Laboratory, Toba, Mie Prefecture, Japan, from July 7 to July 14, 2017. This course deals with experiments and lectures on basic developmental biology of sea urchins and ascidians, basic taxonomy, and advanced course of experiments on genome editing and proteomics.

3. The next **Intl. Invasive Sea Squirt Conference (IISSC)** will be **May 2-4, 2018** at Woods Hole Oceanographic Institution, Massachusetts. More information will be posted in the next issue of AN. You can contact Mary Carman (mcarman@whoi.edu). For information on past conferences, see [http://www.whoi.edu/main/sea-squirt-conference-v](http://www.whoi.edu/main/sea-squirt-conference-v).

4. The Swedish company **Marin Biogas** is culturing and harvesting *Ciona intestinalis* to produce a novel renewable energy source, both as biogas and organic fertilizer. For the complete story, see [http://www.marinbiogas.se/en](http://www.marinbiogas.se/en). The background informational links (Our idea; Facts) are very interesting, and definitely applicable to the control of invasive ascidians in areas where they are present by the millions, such as in eastern Canada.

5. **A new use for invasive Styela clava!** Just rub it on your skin?


6. From Evangelina Schwindt ([schwindtcnp@gmail.com](mailto:schwindtcnp@gmail.com)): The **10th International Conference on Marine Bioinvasions** will be held in Puerto Madryn, Patagonia, Argentina, **October 16-18, 2018**. For more information, go to [www.marinebioinvasions.info](http://www.marinebioinvasions.info) or follow us in Facebook @marinebioinvasions, Twitter @ICMB2018 or Instagram @MarineBioinvasions.

7. From Yuni Nakauchi, Mitsuaki Nakauchi’s son: my father passed away on September 12th, at the age of 86, from complications of pneumonia. [yuni@sci.kj.yamagata-u.ac.jp](mailto:yuni@sci.kj.yamagata-u.ac.jp)

Kaz Kawamura ([kazuk@kochi-u.ac.jp](mailto:kazuk@kochi-u.ac.jp)) recently sent the following:

**A tribute to the memory of the late Professor Mitsuaki Nakauchi**

It is with deep sorrow that we report the death of Mitsu Nakauchi on September 12, 2016. Mitsu had retired in 1995 after 26 years on the faculty of Kochi University and 6 years as the president of Kochi University. He was one of the genuine pioneers in the research field of colonial ascidians in Japan. He spent most of his research time at the Usu (not USA!) Marine Biological Institute, Kochi University to find new modes of asexual reproduction in Aplousobranchia and to facilitate the systematic understanding of asexual reproduction of ascidians. Not a few ascidiologists may have learned ‘modes of bud formation in propagative budding’ from Figure 1 of his review article (Nakauchi, M. 1982. Amer. Zool., 22: 753-763, pictures drawn by Kaz).

Mitsu loved Monterey, California where Dr. Don Abbott stayed and worked at the Hopkins Marine Station. Mitsu also liked Italy and Naples, and in 1993, he finally established the Kochi-Italy Friendship Association. About 100 members are still working. According to his will declared during lifetime, his memorial is in Tokyo where he lived in youthful days.

From Gretchen Lambert, AN editor: Mits was such a wonderful man, an excellent researcher, a good friend. In 1982 our family including our 2 daughters visited Kochi, and I often think about that memorable trip we made across Japan after our stay at the Shimoda marine lab. In November of 1987 Mits and Taeko came to California to attend the Don Abbott Memorial Symposium in Pacific Grove. Then they rode back with us in our car to our home in Fullerton in southern California, after which we took them to Los Angeles where they stayed a few days before returning to Japan. My Endnote bibliographic database includes 44 publications by Mits either as first author or co-author, many of them with Kaz.

8. The following announcement was added late to Ascidian News #78 (after it was already online), so it is repeated here:

From **Francesco Mastrototaro** ([francesco.mastrototaro@uniba.it](mailto:francesco.mastrototaro@uniba.it)): Riccardo Brunetti and I have completed a manual prepared for the Italian Zoological Society (UZI). It is part of the series of “Fauna d’Italia” : Asciacea of the European Waters by Riccardo Brunetti & Francesco Mastrototaro 2017. Fauna D’Italia Vol. LI, Calderini Publications, Bologna (Italy).

The aim of the volume is to provide a summary of the current knowledge of the ascidian fauna of the European waters delimited by the Arctic Ocean to the latitude 25°N with the western boundary marked by the Mid-Atlantic Ridge.
Every species is briefly described, paying attention to the morphological characters useful for species identification.

The work being addressed to a readership broader than the group specialists, a space devoted to the biology of the group has been felt useful to a better understanding of the original descriptions and illustrations, when available, integrated with the contributions of subsequent reviewers. Only consolidated synonyms as well as papers where more extensive descriptions of the individual taxa are given are included in the species accounts.

Dichotomus keys leading to the identification of all known families and genera and tabular keys with diagnostic characters for the European species within each genus are given.

We hope that our work will help young researchers in environmental studies, and excite renewed interest in taxonomy, a field of zoological research too much and too long neglected. The paper is divided in two parts:
Part one: Introduction; an overview of morphology and biology of Ascidians
Part two: Description of the species: a short description of 380 species (136 Aplousobranchiata; 67 Phlebobranchiata and 177 Stolodibranchiata) and their distribution in European waters.

9. From Hitoshi Sawada, director, Sugashima Marine Biological Lab, Japan (hsawada@bio.nagoya-u.ac.jp):

In February, Maki Shirae and Takaharu Numakunai and myself submitted a photo entitled "Spawning of the ascidian Halocynthia roretzi", which was taken by Numakunai-san many years ago, with our short comment in "VISION, the art of science" in the issue of "Hermaphroditism and sex determination" of Molecular Reproduction and Development: http://onlinelibrary.wiley.com/doi/10.1002/mrd.22776/full
(Comment from your AN editor: this is an amazing photo! Check it out.)

WORK IN PROGRESS

1. From John Ryland (j.s.ryland@swansea.ac.uk):

John announces that after many years of work, the second (and highly revised) edition of the Handbook of the Marine Fauna of North-West Europe (P J Hayward and J S Ryland, eds), is now out. The following was included in the December 2016 issue of AN, before the book was actually available, but I am repeating it here because the second edition of this book is sure to be highly useful to many of you. “The Ascidiacea comprise most of Chapter 13 (Acorn-Worms and Sea Squirts). The biggest changes to this chapter are a consequence of the influx of non-native species now found in southern marinas and harbours and, in some cases, also spreading along open shores. These, sometimes unwelcome, arrivals include Didemnum vexillum Kott, Perophora japonica Oka, Corella eumyota Traustedt, Asterocarpa humilis (Heller), Botrylloides diegensis Ritter & Forsyth, and B. violaceus Oka, now included in the book. Some nomenclature in Molgula has been corrected but the genus overall remains in desperate need of study in European waters. As in the first edition (1995), there are dichotomous keys and line drawings to aid identification, some included for the first time. During an ascidian workshop held in Portaferry, Northern Ireland, in August 2008, difficulties were experienced with the main key; a corrected replacement was prepared but seems not to have been incorporated until the 2012 reprint. Unlike its predecessor, the new edition will include some colour plates, including one with photos of newly introduced ascidian species.”
2. From Serena Teo (tmsteolm@nus.edu.sg) at St John’s Island National Marine Laboratory, Singapore.

Since 2010, (with guidance, encouragement and hard work by Gretchen Lambert), we have started to document the ascidian fauna around Singapore’s coastal waters. The following publication (open access) contains a checklist of what we have so far, in addition to an extensive table of all known ascidian species in the South China Sea: http://lkcnhm.nus.edu.sg/nus/images/data/raffles_bulletin_of_zoology/supplement34/S34rbz718-743.pdf

A large chest of specimens remain to be identified: we welcome help!

This year we have also secured a small research grant to establish laboratory cultures of a few selected tropical species (Phallusia nigra, Phallusia philippinesis and/or Ascidia sydneiensis) to support experimental research and education, and hopefully encourage a larger community of researchers to work with this truly amazing group of animals.

3. From Sarah Cohen (sarahcoh@sfsu.edu) and Marie Nydam (marie.nydam@centre.edu)

We are working on a multi-locus phylogeny of the Botryllus and Botrylloides genera within the Family Styelidae. We are using an anchored phylogenomics approach (anchoredphylogeny.com). This method generates hundreds of phylogenetically-informative loci by aligning transcriptomes from several different botryllid species; these loci are then used to develop a phylogeny of the botryllids. We are also developing markers in the fuhc locus to investigate the genetics of allogrecognition across the botryllids. The multi-locus phylogeny will complement a mtCOI phylogeny that is nearing completion in the Cohen Lab, as well as Beth Sheet’s published Master’s work on Botrylloides nigrum:


We would appreciate new tissue samples from botryllid species, as the DNA extraction protocol is specific for anchored phylogenomics. If you are able to collect botryllid species, please contact Sarah Cohen or Marie Nydam for preservation and shipping information. If you have already provided samples, thank you very much!!! And if you are unsure if your previous generously donated samples have proven appropriate for this extended project, please email Sarah, sarahcoh@sfsu.edu.

4. From Cristian Lagger (laggercristian@gmail.com): a new paper, Climate Change, Glacier Retreat and a New Ice-Free Island Offer New Insights on Antarctic Benthic Responses. This paper is about an unexpected (and a priori very fast!) colonization in a rocky island free of ice since 2003 in an Antarctic fjord. Ascidians were the most abundant and diverse group on the new island. Here, we present values of abundances an order of magnitude higher than previously reported in Antarctica, including this filter feeding. See the blogpost for some really interesting photos: http://www.ecography.org/blog/newly-ice-free-areas-antarctica-and-its-consequences-coastal-benthic-ecosystems.

ABSTRACTS FROM RECENT MEETINGS

a. A comparison of microbial symbiont community structure and host specificity in introduced and native ascidians from artificial versus natural habitats. Evans, J.1*; Erwin, P.M.1; Shenkar, N.2; López-Legentil, S.1 1University of North Carolina Wilmington, 2Tel-Aviv University. LopezLegentils@uncw.edu

Harbor systems are passive gateways for the introduction of non-native ascidian species. Once established, ascidians compete fiercely with the surrounding benthos and may spread through localized dispersal, even populating adjacent natural reef systems. To investigate the potential role of microbial symbionts in the success of ascidian introductions and spread, we evaluated the host-specificity of microbial communities within two ascidian species commonly found in harbors and natural habitats off the North Carolina coast. Replicate samples of the native ascidian *Eudistoma capsulatum*, the introduced ascidian *Distaplia bermudensis*, and ambient seawater were collected from artificial (harbor) and natural reef substrates in March 2016. Microbial communities in seawater samples and ascidian tunics were characterized with next-generation (Illumina) sequencing of 16S rRNA gene sequences. Ascidian microbial communities clustered strongly in response to host species, with significant differences in community structure between the two species and seawater. Further, symbiont community structure differed significantly between native ascidians collected from artificial and natural habitats, though this was not the case for the introduced species. These findings suggest that introduced ascidians form stronger associations with their microbial symbionts than native species, potentially contributing to the fitness, survival and spread of introduced ascidians across a wide range of environmental conditions.

b. The lesser known Caribbean ascidians: molecular and morphological identification of Bahamian species. Bailey Counts1*; Xavier Turon2; Susanna López-Legentil1. 1Dept. of Biology & Marine Biology and Center for Marine Science, Univ. of North Carolina Wilmington, Wilmington, North Carolina, USA; 2Dept. of Marine Ecology, Centre for Advanced Studies of Blanes (CEAB-CSIC), Blanes, Girona, Spain. LopezLegentils@uncw.edu

Ascidians or sea-squirts (Phylum: Chordata, Class: Asciidiacea) are sessile, filter-feeding organisms with numerous functions that render them crucial for a healthy ecosystem. The class Asciidiacea contains over 3,000 described species within three orders: Stolidobranchia, Aplousobranchia, and Phlebobranchia. Despite past and current sampling efforts to identify ascidians across the Caribbean Sea, the Bahamas region has been considerably undersampled. Currently, only a handful of species has been described from the Bahamas. In particular, Van Name (1945) listed eight species: two Stolidobranchia (*Pyura vittata, Halocynthia microspinosa*), two Phlebobranchia (*Ascidia interrupta, Ecteinascidia turbinata*), and four Aplousobranchia (*Trididemnum solidum, Polyclinum constellatum, Eudistoma capsulatum, Eudistoma ovilaceum*). This study aimed to identify the main ascidian species from the Bahamas. Two sampling events took place in May 2008 and July 2010 at fourteen different localities within eight islands in the Bahamas (Little San Salvador, San Salvador, Great Stirrup, Sweetings Cay, West Plana Cay, East Plana Cay, Exumus, New Providence). Samples were fixed in 4% formaldehyde for morphological identification and 100% ethanol for DNA barcoding of a fragment of the mitochondrial gene Cytochrome Oxidase subunit I (COI). In total, 115 samples were collected and successfully barcoded. Preliminary analyses indicate up to 51 species may exist, including several new species.
Rapid Assessment Survey of two contrasting marinas near Lisbon: Asciidiacea (Chordata: Tunicata). Ramos-Esplá AA, Berecibar E, Chainho PM, Castanheira AC, Frias P, Henriques FF, Henriques M, Jesus DC, Moreira PM, Pilar-Fonseca TC, Sá JN, Tavares S and. Ulman AH. alfonso.ramos@ua.es

Recreational boating is a major vector of introduction of non-indigenous species worldwide and ascidians are some of the most abundant introduced taxa. Nine ascidian species have been identified in two recreational marinas near Lisbon (Alcântara and Oeiras), sampled during February 2016, following rapid assessment survey (RAS) protocols. Different pontoon floats were sampled by scraping the surfaces with distinct orientations (east, south and west) and submerged structures, such as hanging ropes, buoys, chains and harbor walls were also surveyed. Both marinas are located nearby (about 10km distance) in the Tagus estuary and the dominant fouling species were the mussel Mytilus galloprovincialis and the non-indigenous bryozoans Watersipora subtorquata and Tricellaria inopinata. Nevertheless, significantly different fouling communities were identified within these marinas. M. galloprovincialis and the cnidarians Ectopleura crocea and Actinothoe sphyrodeta were abundant at the Oeiras marina, with very few ascidians recorded. On the other hand, a high abundance of ascidians were found at the Alcântara marina, with Styela plicata, S. clava, Microcosmus squamiger and Botrylloides leachii as the dominant species. The scarcity of ascidians in Oeiras marinas was noteworthy since a single specimen of Corella eumyota was recorded in this marina, while high abundances of this species had been observed in a previous study in February 2008.

European Evolution and Development, Uppsala (Sweden), 26-29 July 2016.

a. Neurotransmission and ion channels in the tunicate coronal organ and the evolution of mechanoreception based on secondary sensory cells. Manni L., Rigon F., Gasparni F. lucia.manni@bio.unipd.it

Tunicates, the sister group of vertebrates, represent an elective taxon for evo-devo studies aimed to clarify the origin of vertebrate sensory cells. Among tunicate sensory organs, the coronal organ is a mechanoreceptor located at the base of the oral siphon/mouth found in all species analyzed so far, thus representing a plesiomorphic feature of the subphylum. As peculiarity, the coronal organ is the only one constituted of secondary sensory cells (SSCs). The coronal SSCs are provided with an apical bundle bearing cilia and microvilli (or stereovilli) and lack an axonal prolongation. They are related to the feeding behavior, representing a filtering barrier in the mouth. In the perspective of an evolutionary comparison between tunicates and vertebrates sensory organs, we considered aspects of coronal SSCs relative to their neurotransmitters and ion channels during the development of the tunicate ascidian Ciona intestinalis. In particular, we studied the expression of a set of genes related to neurotransmission already surveyed in C. intestinalis larvae, such as Ci-Syn (synapsin), Ci-VACHT (Acetylcholine transporter), Ach (acetylcholin-esterase), Ci-GAD (Glutamic acid decarboxilase enzyme), Ci-GABA (γ-aminobutirric acid), Ci-TPH (Tryptophan hydroxilase enzyme), Ci-TH (Tyrosine hydroxilase enzyme). To enter in detail in the function of the coronal SSCs, we analyzed also the expression of two genes for ion channels: Ci-TRPA and Ci-TRPN, whose homologues in vertebrates are involved in the SSC (i.e., hair cell) signal transduction. Our data support the hypothesis that tunicate and vertebrate SSCs evolved from a SSC present in the common ancestor of the two sister groups.
b. Dynamics of the circulation system during development of a colonial chordate are driven by the activity of multiple vertebrate-like hearts. Gasparini F., Cognolato M., Salamon D., Donaggio E., Viviani L., Manni L. lucia.manni@bio.unipd.it

Tunicates are the sister group of the vertebrates, and the only chordate taxon including species, such as Botryllus schlosseri, able to reproduce both sexually and asexually. A colony of B. schlosseri consists of several budding blastozoooids embedded in a common tunic. The latter is an extracellular matrix, which propagates following colony growth. The tunic is crossed by an extracorporeal vessel network, which links zooids each other. Each zooid has an open circulatory system and a single compartment heart. Peristaltic contractions passes from one end to the other and change direction cyclically, driving periodic reversals in blood flow. Despite the tunicate heart displays peculiar anatomical and physiological properties, there is a supported evolutionary picture of cell/tissue/organ homology with the more complex multi-chambered vertebrate heart. We studied the blood flow and the heartbeat dynamics in B. schlosseri evidencing that they are in relation to the colony development. When the colony is composed of a single zooid, the circulation is driven by a single heart and its dynamic is predictable. However, the predictability is absent in typical multi-zooid colonies. The heartbeat reversal period and the heart rate result highly heterogeneous, not only among colonies, but also among coexisting zooids at the same developmental stage in a same colony. The stage influences also the presence/absence of coordination between the heartbeat of a zooid and its bud. Heartbeat reversal period and heart rate are temperature- and development stage-dependent. Moreover, heart rate results responsive to human stabilizing and stimulating drugs (such as metoprolol and caffeine, respectively) in a predictable way. In conclusion, the contemporary presence of multiple hearts in a colony renders the system particularly complex. The high heterogeneity of results indicates that, similarly to vertebrate, a chaos theory may underline B. schlosseri circulation dynamics.


Ascidians as bio-indicators for micro-plastic and phthalates in marine environments. Gal Vered (Galvered1@mail.tau.ac.il ), Aviv Kaplan, Dror Avisar, N Shenkar (noa.shenkar@gmail.com)

Worldwide occurrence of micro-plastic in the marine environment is a major aspect of plastic pollution and an increasing threat to marine organisms and ecosystems. Ingested by different organisms, these particles can cause mechanical damage to tissue and release toxic chemicals into organism. One group of such chemicals is phthalate plasticizers, added to plastics during manufacture to soften and increase flexibility. Phthalates are found in many products and are potentially harmful, are known to bio-accumulate in organisms, and have been associated with endocrine disrupting effects. Therefore, scientific methods aimed at assessing phthalates accumulation in organisms will contribute to our understanding of its eco-toxicological impact. Our goal is to investigate the potential use of solitary ascidians (Chordata, Asciidiacea) as in-situ biological indicators of micro-plastic and phthalates. As sessile filter feeders, they filter high volumes of seawater and retain particulate matter. Ascidians have a wide global distribution in both polluted and pristine waters, and some are very successful invasive species. As so, they make ideal candidates for monitoring micro-plastic and their additives in a wide variety of marine environments. Our objectives are: (1)

Develop analytical methods for detection of micro-plastic and phthalates in solitary ascidians;
(2) Investigate the ability of solitary ascidians to accumulate micro-plastic and phthalates in polluted vs. non-polluted sites, and (3) Specify the organs in which micro-plastic and phthalates accumulate in the organism's body. By developing methods to detect and quantify micro-plastic and plastic additives, along with verifying the use of ascidians as suitable bio-indicators for micro-plastic and phthalates, this study will present a new and applicable tool for bio-monitoring these contaminants in the marine environment.

NEW PUBLICATIONS


Ananthan, G. and Murugan, R. 2016. Molecular phylogeny of four ascidian species inferred from mitochondrial cytochrome oxidase subunit I (COI) sequence. Mitochondrial DNA A DNA Mapp Seq Anal **epub**: 1-5 Dec. 27.


Dumollard, R., Minc, N., Salez, G., Aicha, S. B., Bekkouche, F., Hebras, C., Besnardeau, L. and McDougall, A. 2017. The invariant cleavage pattern displayed by ascidian embryos depends on spindle positioning along the cell's longest axis in the apical plane and relies on asynchronous cell divisions. Elife 6:


Manriquez, P. H., Castilla, J. C. and Ortiz, V. 2016. Empirical evidence for large-scale human impact on intertidal aggregations, larval supply and recruitment of *Pyura praeputialis* around the Bay of Antofagasta, Chile. Austral Ecol. **41**: 701-714.


Ryan, K., Lu, Z. and Meinertzhagen, I. A. 2016. The CNS connectome of a tadpole larva of Ciona intestinalis highlights sidedness in the brain of a chordate sibling. eLife 5:


Segovia, N. I., Gallardo-Escarate, C., Poulin, E. and Haye, P. A. 2017. Lineage divergence, local adaptation across a biogeographic break, and artificial transport, shape the genetic structure in the ascidian Pyura chilensis. Sci. Reports 7:


