ASCIDIAN NEWS*

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A very big thank you to the many AN readers who sent in contributions and for letting me know how important AN continues to be! This issue marks the end of <u>50 years</u> that I have been doing Ascidian News!! Charley and I took it over in 1975 after Don Abbott at Hopkins Marine Station did the first three issues. It is gratifying to know that it continues to be read by so many, who find it useful.

There are **108** new publications listed at the end of this newsletter. Although the title of this newsletter is Ascidian News, I also include papers on the pelagic Tunicata: the pyrosomes, doliolids, salps and appendicularians, and the subphylum Cephalochordata: *Amphioxus* and *Branchiostoma*, because these publications include important work on phylogenetics and evolution of the primitive chordates.

Please keep in touch and continue to send me contributions for the next issue: abstracts of recent meetings, work in progress, student thesis abstracts. To be assured of your new publications being included, always send me the pdf.

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

NEWS AND VIEWS

1. The 12th International Conference on Marine Bioinvasions was held from 7-9 October 2025 in Funchal, the capital city of Madeira Island, an autonomous region of Portugal and located in the northeast Atlantic Ocean.

The full program can be viewed at https://virtual.oxfordabstracts.com/event/73709/program. On this page there are two links to the abstract titles; click on each one to read the full abstract.

2. From Noa Shenkar (shenkarn@tauex.tau.ac.il):

To leave the audience with a positive impression, and a **good taste** of ascidians, during my talk at the XII International Conference on Marine Bioinvasions in Madeira, I prepared delicious butter cookies shaped like ascidians, made using a custom cookie cutter designed by my student, Amit Unger. It was also a pleasure to reconnect with fellow ascidian experts, including Arjan Gittenberger, Xavier Turon, Marc Rius, and others.



- 3. A New 'Red Meat' From the Sea Could Be Coming to Menus Near You. Imagine sitting down to eat pasta with meat sauce, but the meat doesn't come from an animal that walks on land, or even a plant-based alternative. Instead, it comes from a squishy marine invertebrate called a tunicate. Strange as this may seem, it's totally possible, especially if you're in Scandinavia. There, the startup Pronofa sells a convincing-looking ground beef replacement made of processed tunicates, also known as sea squirts [Ciona intestinalis]. People who've tried it say it cooks and tastes similarly to red meat. Scientists also say it has potential as a sustainable food choice. Gelatinous tunicates aren't often seen as food, and some balk at eating them even when they're processed into a beef-like product. They can also be invasive if grown outside of their natural habitat. Still, minced tunicate that's farmed and marketed with care could be an intriguing part of a sustainable diet. For some Scandinavian customers, it already is. https://triplepundit.com/2025/red-meat-replacement-tunicates-purply/
- 4. Plasmalogens in sea squirts may reverse aging. A study in China found that extracts from sea squirts, rich in plasmalogens, reversed certain age-related effects in mice. The marine lipids improved memory, increased neural stem cells, and boosted synaptic connections, suggesting potential benefits for brain health and aging research. Mice given extracts from a marine animal known as a sea squirt showed a reversal of certain age-related effects, according to a scientific study in China. The active substance in these extracts is called plasmalogens a type of lipid (fat) found in cells of many human organs, including the brain, kidneys, muscles, and lungs which guard against DNA damage. Researchers gave mice 300-500 times more plasmalogens than found in a chicken or scallop serving. They tested the animals' memory and several brain functions, and after two months, they showed higher numbers of neural stem cells and the number of synaptic connections between neurons. https://www.mid-day.com/news/world-news/article/seafood-could-help-reverse-aging-study-reveals-23600687 (see Watanabe et al. 2020. The impact of ascidian (Halocynthia roretzi)-derived plasmalogen on cognitive function in healthy humans: a randomized, double-blind, placebo-controlled trial. J. Oleo Sci.)

5. From Patrick Lamaire (patrick.lemaire@crbm.cnrs.fr): CNRS will not support ANISEED : urgent need for a systems administrator for its servers.

I am writing to update you on the situation of ANISEED.

Unfortunately, the Biology institute of CNRS sees no priority in supporting ANISEED, despite your many supporting letters and the fact that Aniseed may be the only worldwide organism database hosted in France. One more example showing that, despite their crucial importance for science, databases are *clearly* not institutional strategic priorities these days...

After 22 years of service, we therefore need to transfer the system as rapidly as possible either to a different country or to a distributed architecture. The only good news is that the system has a professional design and will not collapse overnight.

In the short term, however (within a couple of months), we need to find among Tunicate labs an administrator for Aniseed servers. The servers are hosted on a commercial cloud (their rental is paid for at least a year). They can therefore be administered from anywhere in the world. There are two Linux servers (bare metal VPS). All tools, OS and libraries have been updated last summer/beginning of autumn. The person in charge should be a computer scientist with systems administration experience.

If you know a system administrator in your team/institute who could take this on, please contact me. An initial investment of a couple of hours every week should be enough. I deeply apologise for this situation and thank you all for your continued support.

Best regards, Patrick Lemaire

Work in Progress

1. From Lionel A Christiaen (left:121@nyu.edu): We recently published Cell information vs gene expression on reduced dimensions. This paper contains a link to our scRNA-seq dataset for the whole embryo, from gastrula to pre-hatching, which can be mined online. http://christiaenlab-sars.com/scRNA-seq-datasets/whole_embryo/

Also, I was invited by the journal Nature Methods to write a short piece on the tunicate *Ciona*, which is in press and should come out on December 5 (the link does not work before then: https://www.nature.com/articles/s41592-025-02936-4

Meetings Abstracts

- 1. From Marcos Tatián (<u>marcostatian@gmail.com</u>): XII National Conference on Marine Sciences and XX Oceanography Colloquium. Puerto Madryn, Argentina, December 1-5, 2025.
- a) The ascidians of the Mar del Plata submarine canyon. Maggioni, Tamara; De Aranzamendi, Carla; Tatián, Marcos.

Ascidians are common, diverse, and abundant animals in all the world's oceans, from coastal areas to abyssal depths. In deep-sea environments they have evolved a great diversity of forms and feeding strategies, mainly due to the absence of hard substrates and the scarcity of phytoplankton. There, typical forms (active filter feeders) found in shallow environments coexist with others exclusive to the depths (passive filter feeders and carnivores). The Continental Slope (I-III) expeditions to the Mar del Plata submarine canyon, carried out aboard

the A.R.A. "Puerto Deseado" Oceanographic Vessel by the GEMPA group in 2012 and 2013, revealed a high diversity of ascidians, including two species new to science: *Aplidium marplatensis* and *Aplidium solitarium* (Maggioni et al. 2018). Additionally, a specimen of *Synoicum molle*, previously reported only once in 1886 during the Challenger Expedition (Maggioni et al. 2016), was collected and fully re-described. Analyses revealed that the assemblages are delimited by topography, but their boundaries are not abrupt. Species turnover between zones is gradual. This pattern has been associated with the possible export of surface phytoplankton production. The discovery of microplastic fibers in the stomachs of some individuals indicates pollution of the slope system from runoff from the Río de la Plata and, more directly, from human activities in the area. The Continental Slope Campaign IV, July-August 2025, allowed us to obtain high-definition images in this area and collect new specimens, whose morphological and genetic analyses will contribute to deepening the knowledge of the specific richness and onaspects related to trophic ecology, distribution and the factors that determine them.

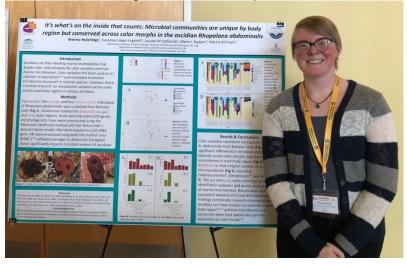
b) Redefining *Paramolgula* spp., a common ascidian in southern South America. Passetti, Juan Pablo; de Aranzamendi, María Carla; Tatián, Marcos.

Ascidians (Chordata, Ascidiacea) are marine organisms with a remarkable capacity for adaptation and key roles in the structure of benthic ecosystems. With approximately 3,000 species, they constitute the most diverse group within the subphylum Tunicata. However, ascidian diversity in southern South America may be underestimated due to a scarcity of taxonomic studies. The genus Paramolgula (Molgulidae) comprises five species, whose delimitation is uncertain due to overlapping diagnostic characters. Furthermore, the validity of the genus has been questioned due to similarities with other Molgulidae. This study analyzed 53 specimens collected in the Southwest Atlantic (Argentina) and the Southeast Pacific (Chile), encompassing the shallow sublittoral zone and the continental shelf. The aim was to contribute to the knowledge of *Paramolgula* diversity by combining morphological and genetic analyses. Dissections were performed under a stereomicroscope, evaluating body proportions and qualitative characteristics. Through multivariate analysis, groupings were identified and diagnostic characters were established. DNA was extracted from the specimens, and the mitochondrial COI and 18S ribosomal genes were sequenced after amplification by PCR. Genetic analyses included calculations of nucleotide and haplotype diversity, genetic distances, haplotype networks, and phylogenetic trees using Maximum Likelihood and Bayesian Inference. The results suggest a clear separation of the genus *Paramolgula* within the family Molgulidae. Furthermore, the diagnostic characters of the species within the genus would represent the variability of a single entity, supporting the proposal of a monotypic genus.

2. North Carolina Branch of American Soc. for Microbiology (NC ASM), Nov. 1, 2025. It's what's on the inside that counts: Microbial communities are unique by body region but conserved across color morphs in the ascidian *Rhopalaea abdominalis*.

Brenna Hutchings¹ (bth7647@uncw.edu), Susanna López-Legentil¹; Lauren M. Stefaniak²; Marie L. Nydam³; Patrick M. Erwin¹. ¹Dept. of Biol. & Mar. Biol., and Center for Mar. Sci., Univ. of North Carolina Wilmington, Wilmington NC; ²Dept. of Mar. Sci., Coastal Carolina Univ., Conway South Carolina; ³Life Sci. Concentration, SOKA Univ. of America, Aliso Viejo, Calif.

Ascidians are filter-feeding marine invertebrates that display inter- and intraspecific color variation and host diverse microbiomes. While color variation has been used as an indicator of speciation and correlated to distinct microbiome structure in colonial species, there is limited research on microbiome variation across color morphs and body regions in solitary ascidians. To address these knowledge gaps, three color morphs (purple, orange, intermediate) of the solitary Belizean ascidian Rhopalaea abdominalis were collected for phylogenetic analysis (cytochrome oxidase I barcodinggene) and microbial characterization (16S rRNA gene, V4 region). R. abdominalis barcoding revealed two distinct phylogenetic lineages unrelated to color morphs, indicating that color variation represents intraspecific variation. Microbiome characterization showed no significant differences in alpha- or beta-diversity metrics across ascidian color morphs, but distinct microbiomes within each body region. The branchial sac harbored a higher number of core members (i.e., detected across all host individuals) that included known ascidian microsymbionts, while the gut and tunic were colonized by taxa identified in seawater and across multiple classes of marine invertebrates. These findings corroborate published research showing that solitary ascidians have unique microbiomes in each body region, while overall microbiome composition remains consistent when genetic differences do not align with color morphs.



- 3. The annual meeting of the European Aquaculture Society, Valencia, Spain, September. Beyond the dock: cultivating early stages of the ascidian *Styela plicata* for a sustainable marine economy. R. Platin, Noa Shenkar.
- 4. XII International Conference on Marine Bioinvasions (ICMB), Madeira, Portugal October 6-9.
- a)Climate Change Resilience in *Phallusia nigra*: A Comparative Study Between Native and Introduced Populations. Unger A, Lee S S C, Teo S L-M, Levy O, Shenkar N.
- b) Harnessing AI for developing novel approaches to control the spread of invasive species by marine vessels. N. Lev, Y Shimony, T Pupko, N Shenkar.
- c). Super invaders of the sea: unraveling the success of non-indigenous ascidians. Noa Shenkar.

Thesis Abstracts

1. Inside Belizean ascidians: how symbiotic amphipods, host anatomy, and study methodology shape microbiomes. Brenna Therese Hutchings, Univ. of North Carolina Wilmington Ph.D. thesis in Integrative, Comparative, and Marine Biology, Fall 2025. Advisors: Susanna López-Legentil & Patrick Erwin. bth7647@uncw.edu

Ascidians contain diverse, species-specific microsymbiont communities. The chapters herein address gaps in understanding how amphipod residence, field and sequenceprocessing methods, and host anatomy and color affect symbiont structure in Belizean ascidians. First, symbiotic amphipods were retrieved from the branchial sacs of Ascidia sydneiensis, and their microbiomes were compared with those of their ascidian hosts (tunic and branchial sac). Amphipod microbiomes exhibited significantly lower diversity and a different composition than tunic samples, with a similar microbial profile to the branchial sac, suggesting that environmental filtering and co-evolutionary processes determined microbiome composition. Second, the effects of delayed preservation and relaxation with menthol on microbiome diversity and composition were assessed in *Trididemnum solidum*. Replicate samples were either immediately preserved in ethanol, held in ambient seawater for 3 hours before preservation, or held in ambient seawater with menthol for 3 hours before preservation. Delay in preservation did not significantly alter community-level microbiome composition and dominant taxa, with menthol exposure counteracting minor microbiome shifts associated with preservation delays. Next, the impact of depuration (clearing of the host gut) on bacteria in the branchial sac, gut, and hepatic gland of Pyura vittata was assessed. Replicates were depurated in filtered seawater for 4 days prior to dissection and compared to samples that were immediately processed following collection. Microbial profiles of depurated and nondepurated animals did not substantially differ, but depuration did help differentiate transient from coretaxa. Finally, three color morphs (purple, orange, and intermediate-pink shades) of Rhopalaea abdominalis were collected for phylogenetic analysis and microbial characterization of the branchial sac, gut, and tunic body regions. Microbial sequences were processed using both operational taxonomic unit (OTU, mothur) and amplicon sequence variant (ASV, QIIME 2 with DADA2) pipelines to determine the resolution and consistency of both programs. Samples grouped in two phylogenetic lineages independent of color. No significant differences in microbial alpha- or beta-diversity were retrieved across color morphs, but both OTU and ASVbased pipelines showed distinct microbiomes in each body region. Together, these studies highlight the importance of host traits, methodological rigor, and symbiont localization in shaping ascidian microbiomes, offering new insights into host-microbe interactions in marine invertebrates.



2. Morphological and molecular taxonomic study of Korean Ascidiacea (Chordata: Tunicata) with emphasis on three newly recorded species. Soyeon Kwon, M.S. thesis, August 2024. Department of Environmental Horticulture, Graduate School of Sahmyook University, Seoul, Korea. 990409kwon@gmail.com

From April 2020 to April 2025, a total of 46 ascidian specimens were collected from 27 sites along the Korean coast to investigate the morphological and molecular taxonomy of Korean Ascidiacea. Among them, three unrecorded species in Korea (*Molgula* sp., *Herdmania pallida*, and *Symplegma reptans*) were examined in detail through integrative taxonomic approaches.

Morphological analysis focused on major anatomical traits, including branchial folds, structure of gonads, and the shape of anal lobes. Detailed illustrations of the three unrecorded species were produced using dissection microscopy and scanning electron microscopy (SEM). Updated dichotomous keys for Korean Ascidiacea were provided, and comparative morphological character tables were created for each genus containing the unrecorded species.

Molecular phylogenetic analyses were conducted using sequences from two genes: nuclear 18S rDNA and mitochondrial cytochrome *c* oxidase subunit 1(COI). Phylogenetic trees were constructed using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods, and pairwise genetic distances were calculated.

The unrecorded species *Molgula* sp. was distinguished from previously reported Korean species by a combination of morphological traits, including the number of branchial folds and the shape of the gonads. *Herdmania pallid* was consistent with its original description, but showed approximately 9.8 % genetic morphologically divergence from *H. pallida* registered in GenBank, suggesting the possibility of a cryptic species. *Symplegma reptans* was identified based on morphological congruence with its original description and high sequence similarity with GenBank entries.

Phylogenetic analyses revealed that the nuclear 18S rDNA marker effectively resolved relationships at the family level and above, while the mitochondrial COI marker was more informative at the species level but susceptible to phylogenetic artifacts such as long-branch attraction. A concatenated analysis combining both markers helped overcome the limitations of individual genes, resulting in a more robust and consistent phylogeny. This study contributes to refining the classification system of Korean Ascidiacea and expanding knowledge of species diversity, highlighting the importance of integrating morphological and molecular approaches in ascidian taxonomy.

3. Integrative Taxonomy and Phylogenomics of Pyuridae Ascidians (Ascidiacea: Stolidobranchia) from the Northeast Atlantic and Mediterranean Sea. Riccardo Virgili Ph.D. thesis, Stazione Zoologica Anton Dohrn and Univ. of Naples Federico II (riccardo.virgili@szn.it). Supervisor: Fabio Crocetta, Stazione Zoologica Anton Dohrn. Cosupervisors: Angelo Fontana, Univ. of Naples Federico II; Xavier Turon, Centre d'Estudis Avançats de Blanes, Spain.

Ascidians (Ascidiacea: Tunicata: Chordata) are a highly derived group of marine sessile filter-feeders that include important members of benthic communities in terms of diversity, distribution, and ecological relevance. Among these, the solitary ascidian family Pyuridae encompasses important habitat-formers, non-indigenous species, and species of commercial interest, of which at least 38 are reported from the Northeast Atlantic and the Mediterranean Sea. Although the ascidian fauna of this region is considered well-characterised, the diversity and evolution of pyurid taxa are still widely debated at the local and global scales. The limited application of integrated studies in the past has hindered a stable classification, leaving their evolutionary history poorly understood.

In view of these knowledge gaps, the present thesis aimed to investigate the diversity, ecology, and evolution of Pyuridae ascidians with an integrative approach. After a first introductory chapter on the topic's background, in the second chapter the majority of genera and species known from the area were included for preliminary species delimitation analyses. Species boundaries and a preliminary phylogeny of the family were inferred from four markers (cox1, 16S rRNA, 18S rRNA, and 28S rRNA genes) and used to test the accepted species hypotheses.

In the third chapter, specific cases of taxonomic inconsistencies were examined to stabilise the classification. The three subchapters deal with the taxonomy of the genera *Microcosmus*, *Pyura*, and *Heterostigma*, with a particular emphasis on their morphology and diagnosis. A dedicated focus on the behavioural ecology of *Heterostigma* was included, due to the peculiar adaptations observed in this atypical sand-living pyurid. Lastly, in the fourth chapter, the evolutionary history of the family was addressed through a robust phylogenomic framework based on whole genomes and mitochondrial genomes, to clarify the relationships and diversification patterns of these solitary ascidians. This Ph.D. thesis therefore represents an integrative effort to advance the knowledge on Pyuridae from the NE Atlantic and Mediterranean, providing new multiple insights on the taxonomy, phylogeny, and biology of these ascidians.

NEW PUBLICATIONS

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