ASCIDIAN NEWS*

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I thank the large number of AN readers who sent in contributions and for letting me know how important AN continues to be! This issue marks **50 years** 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, perhaps mostly younger researchers than 50 years ago, who find it useful.

There are **102** 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. In this issue there are quite a few more papers on the pelagic groups than usual.

Please keep in touch and continue to send me contributions for the next issue: abstracts of 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. Hello Marine Bioinvasion enthusiasts! We (MARE-Madeira) are your hosts for the **12th International Conference on Marine Bioinvasions** and we're excited to welcome you to Madeira next year! The conference will be held in Funchal, the capital city of Madeira Island. Madeira, for those who haven't been before, is a beautiful, mountainous, subtropical island that is an autonomous region of Portugal and located in the northeast Atlantic Ocean. Portuguese is the native language of Madeira, but given a long history of tourism, English is also widely spoken. ICMB XII will be held **from 7-9 October 2025** at the beautiful **Hotel VidaMar Madeira.** There will also be a field trip on **6 October**, which we hope gives you an excuse to come the weekend before and explore our island and ocean!

Abstract submission closed on May 18. Early registration ends June 1. For more information including speakers, themes, field trips and much more, go to https://marinebioinvasions.info/

2. From Noa Shenkar (shenkarn@tauex.tau.ac.il): In March 2025, I had the privilege of teaching local scientists a two-day course on ascidian taxonomy and ecology, organized by Prof. Inti Keith at the Charles Darwin Foundation Research Station in Santa Cruz, Galápagos. I also gave a public talk in Puerto Ayura library on "Secrets of the sea squirts: The hidden world of ascidians".

3. Scallop fishers are assisting the Huntsman Marine Science Centre in New Brunswick, Canada, in a three-year project by collecting samples of the invasive *Didemnum vexillum* in the Bay of Fundy, where it has been spreading and causing big problems fouling cultured scallops. https://www.cbc.ca/news/canada/new-brunswick/fishers-collect-sea-vomit-bay-of-fundy-1.7427806

Work in Progress

1. From Stefano Tiozzo (stefano.tiozzo@imev-mer.fr): **First chromosome-level genome assembly of the colonial chordate model *Botryllus schlosseri* (Tunicata).** Olivier De Thier, Marie Lebel, Mohammed M. Tawfeeq, Roland Faure, Philippe Dru, Simon Blanchoud, Alexandre Alié, Federico D. Brown, Jean-François Flot, Stefano Tiozzo. Freshly accepted in *GigaScience*, our manuscript reports the chromosome-level genome assembly of *Botryllus schlosseri*. The sequences and annotations should be accessible on NCBI in the coming days.

Background: *Botryllus schlosseri* (Tunicata) is a colonial, laboratory model tunicate recognized for its remarkable developmental diversity, its regenerative abilities, and its peculiar genetically determined allorecognition system governed by a polymorphic locus controlling chimerism and cell parasitism.

Results: We report the first chromosome-level genome assembly of *B. schlosseri* sub-clade A1. By integrating long and short reads with Hi-C scaffolding, we produced both a phased diploid genome assembly and a conventional collapsed unphased consensus sequence of 533 Mb. Of this total length, 96% belonged to 16 chromosome-scale scaffolds, with a BUSCO completeness score of 91.46%. We then compared our assembly with other high-quality tunicate genomes, revealing some synteny conservation but also extensive genomic rearrangements and a general loss of collinearity.

Conclusions: The chromosome-level resolution of this assembly enhances our understanding of genome organization in colonial modular organisms. Comparative analyses highlight the dynamic nature of tunicate genomes, with conserved macrosynteny yet extensive microsyntenic rearrangements and scrambling, underscoring their rapid evolutionary trajectory. This high-quality genome assembly provides a valuable resource for exploring the unique biological features of colonial chordates, including their exceptional regenerative abilities and complex allorecognition system.

2. From Stefano Tiozzo (stefano.tiozzo@imev-mer.fr): **Multi-stage single-cell atlas of *Botryllus schlosseri* asexual development unveils dedifferentiating bud founder cells.** Marie Lebel, Tiphaine Sancerini, Sharon Rabiteau, Solène Marchal, Pragati Sharma, Tal D. Scully, Estelle Balissat, Laurel S. Hiebert, Anthony W. De Tomaso, Allon M. Klein, Alexandre Alié, Stefano Tiozzo. We are in the process of uploading this manuscript to *bioRxiv*, and it should be available in the next few days.

Colonial tunicates are the only chordates capable of forming fully functional bodies from somatic tissues through non-embryonic development, known as budding. In *Botryllus schlosseri*, this agametic process, termed peribranchial budding, generates new zooids in a stereotyped, cyclical manner from a cluster of cells within the peribranchial epithelium. Despite detailed morphological characterization, the molecular and cellular underpinnings of budding initiation remain poorly understood. Here, we present the first single-cell transcriptomic atlas of *B. schlosseri* peribranchial budding, encompassing multiple stages from pre-budding to near-mature zooid. This high-resolution atlas captures the diversity of cell types involved in budding and their dynamics enabling precise cluster annotation and lineage trajectory inference. While circulating mesenchymal cells exhibit transcriptional hallmarks of stem-like states, we found no definitive evidence of broad contribution to bud onset and early morphogenesis beyond hematopoietic and gonadal lineages. Instead, we identified a distinct founder cell population arising from peribranchial epithelium, marked by a unique transcriptional profile and progressive acquisition of developmental potency. This supports a model in which budding is initiated by dedifferentiation of committed epithelial cells rather than activation of multi- or pluripotent stem cells. Furthermore, we highlight signaling pathways, including GNRHR-like receptors, that may couple metabolic state with developmental progression. Altogether, our data suggest that peribranchial budding in *B. schlosseri* is driven by potential reprogramming within epithelial tissues. This work provides a foundational resource for studying non-embryonic development and the evolution of regenerative strategies in chordates.

3. From Federico Brown (fdbrown@usp.br): ongoing projects of his students in Brazil.

a) Coloniality and gene expression of stem cell markers during budding of the ascidian *Perophora viridis* Verrill, 1871. Ivan Andre Morillo Guerrero (MSc student)

Within tunicates, coloniality has evolved several times independently with colonial species in two of the three classes of the group. The budding mechanisms are different among colonial taxa. Colonial organisms are composed of semi-dependent modules, which correspond to clonal individuals of asexual reproduction (zooids). The different types of budding mechanisms facilitated the evolution of coloniality, however, the tissues, or cells, that give rise to the modular development of each individual of the colony, also known as zooids, remain not well defined. Zooids are always in continuous renewal through a process called budding. The budding process in some species presents developmental mechanisms regulated by "coordinated and synchronous" cycles, unlike other species in which development occurs "independently and asynchronously". In this study, we propose to explore how budding times and zooid lifespans of colonial ascidians may vary intra- and interspecifically. Which developmental processes are regulated by the colony's own modules? Which processes result from the interaction between colony modules? Which processes are governed at the colonial level? Comparing zooid development times and longevity among ascidian species will allow us to determine the degree of modular dependence in budding and identify the levels that act in

the processes of regulation of colonial organization. In budding, new complete bodies, including all somatic and germinal tissues, develop from adult cells and tissues. The colonial ascidian *Perophora viridis* can reproduce asexually by stolonial budding. Through histological studies of budding, the role of stem cells present in the blood of several ascidian species (hemoblasts) and of stolon septal cells has been studied. However, the characterization using molecular markers and the analysis of the proliferation of these cells have not yet been performed. Therefore, we propose to determine the location and dynamics of stem cells in the budding process in P. viridis.

b) The role of bioelectricity in establishing stolon growth polarity and shoot symmetry in *Perophora viridis* regeneration. Enzo Costa Frediani (undergrad student)

Ascidians are sessile filter-feeding tunicates that serve as important models for studies of animal regeneration and development. Among them, the genus Perophora, colonial ascidians composed of zooids interconnected via stolons, was studied for their ability to regenerate from stolon fragments. In this context, the present work seeks to understand a phenomenon described in the literature: stolon fragments excised between zooids do not have a predefined polarity, which is subsequently defined. Fragments of the stolon tip, on the other hand, present polarity. In addition, the symmetry of the developed shoots follows this polarity. Thus, the hypothesis of this project is that the observed polarity is caused by an electrical gradient between the tip and the base of the stolon. To analyze the existence of a gradient, experiments will be performed to observe the regeneration of dissected fragments increasingly close to the tip of the stolon and the branching regions. The bioelectric gradient will be verified with voltage-sensitive dyes and the Ion selective vibrating probe method, in addition to ionophores to disrupt the membrane potentials of the cells. Discoveries in this area will bring new perspectives on how regeneration works in this animal model, which can be expanded to other models and in the future be applied in regenerative medicine, bringing important benefits to human health.

Meetings Abstracts

1. XXV Meeting of the Italian Association of Developmental and Comparative Immunobiology, February 19-21, 2025, Alessandria, Italy

a) Effects of polyethylene nanoplastics (nPET) on immune responses of the colonial ascidian *Botryllus schlosseri*. Domenichi, S., Ballarin, L. Dept. of Biology, Univ. of Padova.

Plastic pollution represents an environmental problem, with an estimated 20 million tons ofplastic waste entering the world's aquatic ecosystems annually. Plastic particles smallerthan 1 µm are classified as nanoplastics (NPs). NPs are of particular concern as they havethe capacity to enter cells and cross the blood-brain barrier, accumulating in vital organs and potentially influencing their physiology over long periods of exposure and accumulation. Ascidians are invertebrate chordates, closely related to vertebrates, and their phylogenetic position renders them ideal organisms for the study of the evolution of various biological processes, with particular focus on the invertebrate-vertebrate transition. *Botryllus schlosseri*, a colonial ascidian. is a widely used model in studies of innate immunity and the effects of pollutants on physiology. The focus of our research is on innate immune responses following exposure to polyethylene NPs (nPET). Haemocytes were exposed directly to nPET, or the

latter were directly microinjected into the colonial circulatory system. Our analysis shows that exposure of haemocytes to NPs has a negative influence on the phagocytosis of yeast (*Saccharomyces cerevisiae*) cells, and is accompanied by a modification of phagocyte morphology, probably related to cytoskeletal alterations. NPs have also a negative effect on the degranulation of morula cells, the immunocytes with cytotoxic activity that trigger the inflammatory response. In addition, we analysed the expression of genes involved in immunity and oxidative stress scavenging, observing a higher expression of C3, RBL and SOD in treated animals than in the control group undercertain conditions.

b) The ascidian phagocyte: a multitasking cell. Ballarin, L., Dept. of Biology, Univ. of Padova.

Ascidian professional phagocytes represent an important fraction of circulating hemocytes.Together with cytotoxic, proinflammatory cells, they represent the cellular component of theimmune system and are collectively known as immunocytes. However, notwithstanding theincreasing number of contributes dealing with ascidian immunity, ascidian phagocytesremains a neglected cell type, its study being mostly limited to a morphological level. In thelast three decades, using the colonial ascidian *Botryllus schlosseri* as our reference species,we studied phagocyte behavior and physiology under various experimental conditionsaiming at revealing their true contribution to immune responses. We could demonstrate themolecular basis of the interaction with nonself, acquire new information on the receptorsinvolved in the recognition of foreign and effete cells, clarify their involvement in the secretionof humoral factors able to improve phagocytosis and affect the activity of cytotoxic cells. The emerging scenario suggests an active and continuous cross-talk between phagocytes andcytotoxic cells able to modulate the immune response(s) to nonself.

2. The Second International Symposium on Women in Tunicate Biology (virtual, by zoom) March 25-26, 2025 was co-organized by Marie Nydam, Sydney Popsuj, Lucia Manni and Anna Di Gregorio. Differently from its first edition two years ago, this second meeting was mainly aimed at highlighting scientific projects led by women in the early stages of their scientific careers. The meeting featured presentations by Atsuko Sato, Jhimli Mondal, Fatıma Nur Oğul Ünal, Devon Gamble, Oshrat Ben-Hamo, Tal Gordon, Megan Olhasso, Kaylee Moody, Lily Palomino-Alvarez, Júlia Serenato, Brenna Hutchings, Sydney Popsuj, Laurel Hiebert, Assunta Liberti, and Billie Swalla. It also included a tribute to the late Francoise Monniot and her 63 years of research on ascidian biology, which was presented by Sarah Samadi. A meeting report for the journal *genesis,* the journal of Genetics and Development, which published the proceedings of the first symposium, is being prepared.

Program: Day One: Tuesday, March 25th

1. Welcome Anna Di Gregorio, New York University, USA

2. Origin of vertebrates and the evolutionary process of chordates under a changing environment: Insights from *Ciona.* Atsuko Sato, Tohoku University, Japan

3. Ascidian diversity: perspectives from the Indian Coast. Jhimli Mondal, Estuarine and Coastal Studies Foundation (ECSF), India

4. An Integrative Study on Botryllus humilis and Botrylloides niger: Ecological, Histological, Cellular and Gene Expression Perspectives. Fatıma Nur Oğul Ünal, Middle East Technical University, Türkiye

5. Decoding Regeneration: Uncovering the genetic regulation driving whole-body regeneration in Botrylloides diegensis. Devon Gamble, University of Otago, New Zealand

6. Unveiling the Cycle of Aging and Rejuvenation in Botryllus schlosseri. Oshrat Ben-Hamo, Israel Oceanographic and Limnological Research, Israel.

7. Insights into the cellular and molecular mechanisms of invertebrate chordate regeneration. Tal Gordon, University of Padua, Italy and Stanford University, USA

8. Husbandry and culture techniques of Megalodicopia at Monterey Bay Aquarium. Megan Olhasso and Kaylee Moody, Monterey Bay Aquarium, USA.

Day Two: Wednesday, March 26th

1. Welcome Marie Nydam, Soka University of America, USA

2. New Botryllids from Brazil. Júlia Serenato, Universidade Federal do Paraná, Brazil

3. A Tribute to the Academic Journey of Françoise Monniot: Sixty-Three Years of Dedicated Research on Ascidians. Sarah Samadi, Muséum National d'Histoire Naturelle, France

4. From the field to the freezer: Clarifying methods of microbial characterization in ascidian hosts. Brenna Hutchings, University of North Carolina, Wilmington, USA (with Stefaniak L, Nydam M, Erwin PM, López-Legentil S.)

Ascidians host diverse microsymbiont communities that are species-specific and can assist in breaking down carbohydrates, processing pollutants, enhancing nutrient absorption, and increasing their host’s immune response. Since each research group uses its own methods, it is critical to assess how methodology may influence the microbial communities. Here, four methods to characterize ascidian microbiomes were investigated: (1) the use of menthol crystals to relax ascidian tissues, (2) implementing depuration to purge ascidian gut contents, (3) processing microbial sequences in either the mothur or QIIME 2 software packages, and (4) reporting the presence of macrosymbionts within the ascidian host. Sequencing the 16S rRNA (V4) of microbial communities in the ascidian tunic, branchial sac, gut, and/or hepatic gland tissues, we found that (1) menthol addition mitigates significant decreases in microbial diversity, (2) depuration significantly alters microbial communities and aids in identifying resident microbes, and (3) macrosymbionts can share compositionally similar microbiomes than the host and should be reported when directionality of horizontal bacterial transfer is unclear.

5. An Emerging Gene Regulatory Network for Larval Ascidian Cholinergic Fate and Motor Neuron Identity. Sydney Popsuj, Georgia Institute of Technology, USA

6. Dormancy in a botryllid tunicate: A state of deconstruction. Laurel Hiebert, University of California at Santa Barbara, USA

7. Host-microbe interactions in the digestive tract: Lessons from the protochordate, Ciona robusta. Susy Liberti, Stazione Zoologica “A. Dohrn”, Italy

8. Follow the Yellow Brick Road: From Myoplasm to MBL. Billie Swalla, Friday Harbor Laboratories and University of Washington, USA

9. Opportunity for Informal Conversation

3. 53rd Benthic Ecology Meeting, Mobile, Alabama, April 1 - 4, 2025.

a) The microbiome of a coral killing ascidian. Levine J, Erwin PM, Nydam M, Stefaniak L, López-Legentil S.

Ascidians are sessile invertebrates that form symbiotic associations with diverse microbes, contributing to host physiology. *Tridemnum solidum* is a colonial ascidian known for competing with and killing corals, as well as housing *Synechocystis* cyanobacterial symbionts. Here, microbial composition and diversity between body regions (zooid vs. tunic) and color variants (gray vs. white) of *T. solidum* were compared by 16S rRNA gene sequencing. No significant differences in microbiome alpha diversity (richness, evenness, and Shannon H’; ANOVA; $p>0.05$) occurred between color variations or body regions; however, significant differences in beta diversity were found between body regions (Bray-Curtis similarity, PERMANOVA, $p=0.002$). Differential analysis revealed that 42 OTUs were significantly more abundant in the zooids and 26 in the tunic. These microbes may fulfill unique roles within the two body regions, including some putative prey microbes detected in the zooids. This study is the first to show that colonial ascidians maintain distinct microbiomes between body regions, possibly to enhance host functionality and overall fitness.

b) Explore the core: depuration depletes transient bacteria without altering microbiome alpha-diversity in an ascidian. Hutchings BT, López-Legentil S, Stefaniak L, Nydam M, Erwin PM.

Depuration, the process of clearing impurities from the gut, effectively removes pathogens from shellfish and edible ascidians but may alter microbiome diversity in depurated animals. Here, we examined the impact of depuration on bacteria in the branchial sac, gut, and hepatic gland of the solitary ascidian *Pyura vannamei*. Replicates were kept in filtered seawater for four days prior to dissection (aquaria-depuration) and compared to aquaria controls and samples immediately processed upon collection (wild-no depuration). Depuration resulted in no significant differences in microbial alpha-diversity but significant shifts in beta-diversity, with the number of core bacteria markedly decreased (43 to 82%) across all body regions of depurated compared to wild individuals. Bacteria conserved in depurated and wild individuals comprised genera associated with enhanced host health and resilience to pollutants in other marine symbioses. Thus, depuration is unnecessary to obtain accurate microbial alpha-diversity profiles in ascidian microbiomes, but it can help differentiate transient from resident taxa in complex host-microbe symbioses.

c) Investigating nitrifying microbes within coastal ascidians. Kennedy G, López-Legentil S, Pjevac P, Tolar B.

Once considered ecologically insignificant, ammonia-oxidizing Archaea (AOA) are now recognized as major contributors to nitrogen cycling and removal from coastal marine environments. Yet, little is known regarding AOA within marine invertebrate microbiomes. Though most host-associated archaeal studies have focused on sponges, AOA have also been found in other marine invertebrates including ascidians. Here we expand this knowledge by analyzing microbiome diversity (16S rRNA) and AOA abundance (qPCR) within the tunic of ten ascidian species. Of the 46 microbial phyla observed, Crenarchaeota (AOA) and Proteobacteriota were the most abundant archaea and bacteria, respectively. AOA dominated the nitrifying communities in all samples. The genus *Polyandrocarpa* showed the greatest AOA relative abundance among all ascidians. Future nutrient incubations will be necessary to estimate the ecological impacts of ascidians and AOA on coastal nitrification rates.

Thesis Abstracts

1. AN INTEGRATIVE STUDY ON *BOTRYLLUS HUMILIS* AND *BOTRYLLOIDES NIGER*: ECOLOGICAL, HISTOLOGICAL, CELLULAR, AND GENE EXPRESSION PERSPECTIVES.

Fatıma Nur Oğul-Ünal ,Doctor of Ph.D., January 2025. Institute of Marine Science, Middle East Technical University, Türkiye. Supervisor: Assoc. Prof. Dr. Arzu Karahan

Colonial ascidians are unique among invertebrates, with their ability to regenerate entire bodies from small tissue fragments and to reproduce both sexually and asexually. These traits position them as exceptional models for studying fundamental biological processes. Their phylogenetic position as close relatives of vertebrates provide additional value for understanding the evolution of regenerative capabilities and reproductive strategies. Furthermore, their dynamic responses to environmental changes offer insights into ecological adaptability, stress resilience, and the balance between regeneration and reproduction. Understanding these processes not only sheds light on the biology of ascidians but also bridges knowledge gaps in evolutionary biology, and regenerative medicine.

This Ph.D. thesis provides a comprehensive investigation into the ecological, cellular, histological, and molecular mechanisms underlying asexual reproduction and whole-body regeneration (WBR) in two colonial ascidians, *Botryllus humilis* and *Botrylloides niger*, which are widespread along the Mediterranean coasts. By integrating diverse methodological approaches, the study aims to elucidate how these processes interact with environmental factors, the trade-offs between growth, maintenance, and reproduction, and the broader evolutionary and ecological implications of these mechanisms. The study integrates ecological, cellular, histological, and molecular approaches to uncover the mechanisms underlying these processes, offering novel insights into their evolutionary and ecological significance. The thesis comprises three interconnected chapters offering novel insights into their evolutionary and ecological significance.

The first chapter investigates the ecological preferences and adaptive strategies of B. humilis and B. niger in response to salinity and temperature variations, in a controlled aquaculture system with comparison to natural habitat. The findings highlight species-specific preferences, and response, suggesting distinct ecological niches and adaptive strategies, with potential coexistence mechanisms that reduce competition. The results also reveal the aquaculture system is simulating natural environmental conditions, for experimental studies.

This chapter emphasizes the ecological adaptability of these ascidians and their potential to thrive in changing environments.

The second chapter provides the first detailed histological and cellular characterization of the blastogenic cycle and WBR in B. humilis and B. niger. Using histological and immunohistochemical techniques, critical cell types, stages, and tissue remodeling dynamics were identified, revealing differences in regenerative and asexual developmental processes. Histological sections and cytological analyses revealed ten distinct hemolymph cell types for the first time for these species. These cell types showed dynamic changes during both blastogenesis and WBR. Key differences between the species were observed, with B. humilis displaying more rapid regeneration and higher hemoblast activity in response to injury. Immunohistochemical staining identified markers such as Piwi, PL10, and cadherin, linking them to progenitor cell maintenance, differentiation, and tissuere modelling. This chapter identifies critical stages in both processes and highlights the cellular mechanisms driving rapid regeneration, providing a foundation for future studies on stem cell biology and tissue dynamics in colonial ascidians.

The third chapter explores the molecular mechanisms underlying the WBR in B. humilis, using transcriptomic analyses to identify genes and pathways active at critical regenerative stages; immediately post-ablation (0 h), during cellular reorganization (16 h), and at the niche formation stage. The results revealed distinct temporal shifts across the stages in gene expression and processes such as stress response, cytoskeletal organization, apoptosis, cell differentiation, and extracellular matrix remodeling. Pathway analyses highlighted the roles of different pathways including Wnt, Notch, and TGF-β signaling in guiding regeneration. These findings provide insights into the orchestration of cellular and molecular mechanisms during regeneration.

This thesis integrates ecological, cellular, and molecular perspectives to uncover the interconnected processes of reproduction, regeneration, and environmental adaptation in colonial ascidians. It reveals the species-specific strategies that balance these processes under varying environmental conditions, offers new insights into the cellular and molecular mechanisms of rapid regeneration, and highlights the potential of B. humilis and B. niger as model systems. These findings have broad implications for understanding adaptation, stress resilience, the evolution of longevity, and the trade-offs between regeneration and reproduction. It offers valuable perspectives on their ecological roles, evolutionary strategies, and potential applications in regenerative biology, contributing to broader discussions on adaptation, resilience, and the evolution of complex traits. Additionally, this work provides a platform for future studies in regenerative biology, evolutionary ecology, and comparative studies of colonial organisms, offering a bridge between invertebrates and vertebrates.

2. Study of alternative developmental modes in tunicates: atypical embryogenesis of salps and non-embryonic development of colonial ascidians. Marie Lebel, Ph.D. thesis, CNRS - Sorbonne University. Stefano Tiozzo advisor. (marie.lebel@imev-mer.fr)

Metazoans exhibit a remarkable diversity in the ways they generate and regenerate their bodies. Traditionally, development has been viewed through the lens of embryogenesis, starting from gametes and proceeding through a zygote. However, an equally fascinating and widespread, yet often overlooked, category of development exists: non-embryonic development (NED), which relies on somatic cells to build new individuals or regenerate lost structures. These two major developmental strategies, sexual embryogenesis and NED, offer complementary perspectives on how animal body plans are produced and maintained across

evolutionary time. Tunicates, the sister group of vertebrates, offer an excellent system for exploring this developmental diversity. Despite leading to morphologically similar adult forms, tunicates employ a variety of developmental modes, both embryonic and non-embryonic. This makes them ideal for disentangling the evolutionary and developmental principles underlying body plan formation.

This thesis is structured into four main chapters and several annexes. Chapter 1 offers essential background on both embryonic and non-embryonic development in tunicates, while also establishing a broader theoretical framework rooted in current evolutionary and developmental biology (Evo-Devo) perspectives. This conceptual foundation informs and guides the experimental approaches and interpretations presented in the subsequent chapters. Chapter 2 revisits the embryogenesis of salps, a pelagic group of colonial tunicates known for their unusual and historically under-described developmental patterns. Using a combination of advanced imaging and molecular techniques, this chapter re-evaluates early observations and presents an updated, more accurate characterization of salp embryogenesis. This mode of development diverges significantly from the typical tunicate model, and more generally from any described metazoan embryogenesis, notably involving interactions between zygote-derived and maternal cells. In addition, standardized developmental staging tables are proposed for *Salpa fusiformis* and *Thalia democratica*, two species commonly found in the Bay of Villefranche-sur-Mer, providing a valuable reference for future research. Chapter 3 presents a collaborative effort that led to the assembly and annotation of a high-quality, chromosome-level genome for the laboratory model *Botryllus schlosseri*. This chapter also includes key analyses of the genome, providing insights into its structure and content. The availability of this reference genome was a crucial foundation for the downstream transcriptomic and developmental investigations described in the subsequent chapter. Chapter 4 shifts the focus to non-embryonic development, specifically investigating the process of peribranchial budding in *Botryllus schlosseri*. Through single-cell transcriptomic profiling across multiple budding stages, this chapter presents a comprehensive molecular atlas of the budding process. Particular attention is given to identifying the elusive founder cells of the inner vesicle and characterizing the transcriptional programs that define their identity and trigger organogenesis in a zygote-independent context. Finally, Chapter 5 provides brief conclusions and general perspectives.

NEW PUBLICATIONS

Abdoul-Latif, F. M., Mohamed, H., Houmed Aboubaker, I., Saoudi, O., Ainane, A., Ali, A. M., Cacciatore, S., Zerbini, L. F., Abourriche, A. and Ainane, T. 2025. Cynthichlorine extracted from ascidian *Cynthia savignyi* from Djibouti: optimization of extraction, in vitro anticancer profiling, and in silico approach. Mar. Drugs **23**: epub.

Anselmi, C., Ishizuka, K. J., Palmeri, K. J., Burighel, P., Voskoboynik, A., Hotta, K. and Manni, L. 2025. Speed vs completeness: a comparative study of solitary and colonial tunicate embryogenesis. Front. Cell & Dev. Biol. **13**: 1-20.

Anselmi, C., Sepe, R. M., Manni, L. and Sordino, P. 2025. Tunicates in evolutionary developmental biology [editorial]. Frontiers Ecol. Evol. **13**: 1-3.

Aranceta-Garza, F., Ramírez-Luna, S. and Seijo, J. C. 2025. Bioeconomics of a regime shift by invasive epibiont species affecting a bivalve small-scale fishery. ICES J. Mar. Sci. **82**: epub.

Bae, S. and Choi, K.-H. 2025. Predicting the potential habitat suitability of two invasive ascidian species in Korean waters under present and future climate conditions. Regional Studies in Mar. Sci. **81**: epub.

Baker, K., Halfter, S., Scoulding, B. et al. 2025. Carbon injection potential of the mesopelagic-migrant pump in the Southern Ocean during summer. Front. Mar. Sci. **12**: epub.

Baur, P. and Comba, P. 2025. Copper coordination chemistry of the patellamides - cyanobactins in the ascidian-*Prochloron* symbiosis. Dalton Trans. **54**: 3968-3976.

Bender, C. J. D., Moir, C. D., Hajibabaei, M. and Boulding, E. G. 2025. Morphological and metabarcoding dietary analysis of the cunner wrasse (*Tautogolabrus adspersus*) revealed significant regional variation, with large overlap between its common prey species and biofouling animals living on salmonid sea cages. J. Fish Biol. epub.

Berenshtein, I. and Shenkar, N. 2025. Modelling non-indigenous ascidian larval dispersal in the coastal waters of the eastern Mediterranean Sea. Diversity and Distributions **31**: 1-14.

Brunelli, N., Palle, S. D. and Cima, F. 2025. Possible evolutionary precursors of mast cells: the ‘granular cell’ immunocyte of *Botrylloides leachii* (Tunicata; Ascidiacea). J. Mar. Sci. & Eng. **13**: 1-22.

Canning-Clode, J., Gizzi, F., Braga-Henriques, A., Ramalhosa, P. et al. 2024. A pioneering longterm experiment on mesophotic macrofouling communities in the North Atlantic. Communications Biol. **7**: epub.

Casertano, M., Esposito, C., Bello, I., Barile, M., Izzo, L., Mitidieri, E., Sorrentino, R., Menna, M., Panza, E., Imperatore, C. and d'Emmanuele di Villa Bianca, R. 2025. The Mediterranean Sea on the bench: unveiling the marine invertebrate *Sidnyum elegans* as a source of novel promising therapeutic tools against triple-negative breast cancer. Mar. Drugs **23**: epub.

Castro, N., Monteiro, J. G., Gouveia, M. M. et al. 2025. Refining management strategies in marine protected areas in oceanic islands: a non-indigenous species risk index for strategic prioritization. Biol. Invasions **27**: epub.

Ceriello, H., Bazzo, R. A. O., Martinez, L. D. et al. 2025. Rocking together: a report on a biological interaction between a non-native sea anemone and a sea squirt in southeastern Brazil. Ocean & Coastal Res. **73**: 1-5.

Chen, D., Rouvier, F., Keyzers, R., Bourguet-Kondracki, M. L., Brunel, J. M., Cadelis, M. M. and Copp, B. R. 2025. Ohauamines A-D, structurally unprecedented tricyclic depsi-tripeptides from the New Zealand ascidian *Pycnoclavella kottae*. J. Nat. Prod. **88**: 871-876.

Chen, Z., Chen, X. Z. and Zhu, G. P. 2025. Ecological niche and habitat use of Antarctic krill (*Euphausia superba*) and the pelagic tunicate (*Salpa thompsoni*) under future climate change: further overlap or separation? Biodiv. & Conserv. **34**: 1771-1792.

Costa, R. J., Paiva, S. V., Braga, M. D. A. et al. 2025. How temperature and meteorological conditions trigger algal blooms on tropical reefs. Mar. Pollution Bull. **217**: epub.

Damian-Serrano, A., Walton, K. A., Bishop-Perdue, A., Bagoye, S. et al. 2025. Colonial architecture modulates the speed and efficiency of multi-jet swimming in salp colonies. J. Exp. Biol. **228**: epub.

David, G. and Faure, E. 2025. End-to-end 3D instance segmentation of synthetic data and embryo microscopy images with a 3D Mask R-CNN. Front. Bioinform. **4**: epub.

de Carvalho, P. F., Dias, C. D., Moreira, D. L. et al. 2025. Pelagic tunicates (Chordata) in two seasons in the Santos Basin, tropical Southwestern Atlantic: horizontal and vertical distributions. Mar. Biodiversity **55**: epub.

De Thier, O., Lebel, M., Tawfeeq, M. M., et al. and Tiozzo, S. 2025. First chromosome-level genome assembly of the colonial chordate model *Botryllus schlosseri* (Tunicata). GigaScience epub.

De Tomaso, A. W. and Rodriguez-Valbuena, H. 2025. Histocompatibility in *Botryllus schlosseri* and the origins of adaptive immunity. Immunogenetics **77**: epub.

Domenichi, S. and Ballarin, L. 2024. The days after: cellular events following the inflammatory allorejection reaction in the colonial ascidian *Botryllus schlosseri*. Invert. Survival J. **21**: 109-115.

Eliso, M. C., Corsi, I., Spagnuolo, A. and Dumollard, R. 2025. Nanoplastic-induced developmental toxicity in ascidians: comparative analysis of chorionated and dechorionated *Phallusia mammillata* embryos. J. of Xenobiotics **15**: epub.

Faria, J., Piazzese, L., Ramalhosa, P. et al. 2025. Biodiversity on the move: Epibiotic communities associated with pelagic sargassum in the northeast Atlantic. Mar. Env. Res. **207**: 1-10.

Fernández-Gutiérrez, J., Rubal, M., Sampaio, L. et al. 2025. Structure of non-indigenous fouling assemblages and biocontamination levels in Portuguese recreational marinas under different salinity conditions. Diversity-Basel **17**: epub.

Furlan, L., Zamuner, A., Riccioni, A., Sabbadin, G. et al. 2025. Comparative analysis of tunicate vs. plant-based cellulose in chitosan hydrogels for bone regeneration. Gels **11**: 1-18.

Galobart, C., Zarcero, J., Antich, A., Turon, X. and Cebrian, E. 2025. Metabarcoding identifies macroalgal composition as a driver of benthic invertebrate assemblages in restored habitats. Sci. Rep. **15**: epub.

Gamba, G. A. and Rocha, R. M. 2025. New Records of Stolidobranchia (Tunicata: Ascidiacea) from Brazil. Taxonomy **5**: 1-18.

Gonzalez-Pineda, M., Avila, C., Lacerot, G., Lozoya, J. P., Teixeira de Mello, F., Faccio, R., Pignanelli, F. and Salvada, H. 2025. Experimental ingestion of microplastics in three common Antarctic benthic species. Mar. Env. Res. **204**: epub.

Govindharaj, M., Salim, M. H., Dali, M. H. A. et al. 2025. Multi-scaled cellulosic nanonetworks from tunicates. Advanced Functional Materials epub.

Granthom-Costa, L. V., Messano, L. V. R., Altvater, L., Spotorno, P. and Coutinho, R. 2025. Non-native marine sessile benthic species from the coastal upwelling ecosystem of Arraial do Cabo, Brazil. Aquatic Invasions **20**: 181-198.

Greenman, W., Murillo, F. J., de Moura Neves, B., Kenchington, E., Jasperse, L., Fox, A., Walker, B., Edinger, E. and Sherwood, O. 2025. Metre-scale vertical zonation of corals and sponges on a deep-marine cliff reflects trophic resource partitioning. Sci. Rep. **15**: epub.

Gruner, H. N., Pickett, C. J., Bao, J. Y., et al. and Davidson, B. 2025. Neural signaling contributes to heart formation and growth in the invertebrate chordate, *Ciona robusta*. bioRxiv epub.

Han, N., Weon, S. H., Han, J., Cha, J. E. and Lee, S. H. 2025. Two-step carboxymethylation of cellulose from *Halocynthia roretzi* using ionic liquid. Biomacromolecules **26**: 2909-2921.

Hanif, N., Miftah, J. A., Yanti, H. D. et al. 2025. Integrated biological and chemical investigation of Indonesian marine organisms targeting anti-quorum-sensing, anti-biofilm, anti-biofouling, and anti-biocorrosion activities. Molecules **30**: epub.

Hiebert, T. C., Thompson, A. W. and Sutherland, K. R. 2025. Diverse microbial prey in the guts of gelatinous grazers revealed by microscopy. Mar. Biol. **172**: epub.

Hill, J. E., Hardin, S., Donahou, A. D. et al. 2025. Review of invasiveness screening kit use in the United States of America. Manag. Biolog. Invasions **16**: 45-72.

Huang, Q., Zang, X., Jin, X. and Liu, Q. 2025. Discovery of PD-L1 peptide inhibitors from ascidian enzymatic hydrolysates by affinity ultrafiltration coupled to nanoLC-MS/MS. Mar. Drugs **23**: epub.

Hutchings, B., Lopez-Legentil, S., Stefaniak, L. and Nydam, M. L. 2025. Microbial distortion? Impacts of delayed preservation on microbiome diversity and composition in a marine invertebrate. MicrobiologyOpen **14**: 1-11.

Jiménez-Solera, A., Arias-Zumbado, F., Garcia-Rojas, A. et al. 2025. First community level description of rocky reefs at the Cabo Blanco absolute natural reserve on Costa Rica's Pacific Coast. Revmar-Revista Ciencias Marinas y Costeras **17**: 19-42.

Johnson, A. N. T., Huang, J., Marishta, A. et al. 2025. Sensitive and accurate proteome profiling of embryogenesis using real-time search and TMTproC quantification. Mol. Cell. Biochem. **24**: epub.

Kim, D. U., Ahn, I. Y., Gal, J. K., Ha, S. Y. and Khim, J. S. 2025. Spatial variations of ascidian diets in a glacial retreated fjord of the West Antarctic Peninsula. Mar. Env. Res. **204**: epub.

Kim, K., Piekarz, K. M. and Stolfi, A. 2025. A gene regulatory network for specification and morphogenesis of a Mauthner Cell homolog in non-vertebrate chordates. Dev. Biol. **522**: 51-63.

Kourakis, M. J., Ryan, K., Newman-Smith, E. D., Meinertzhagen, I. A. and Smith, W. C. 2025. Motor neurons in the tunicate caudal central nervous system reveal homology to the vertebrate spinal cord. Development **152**: epub.

Kuroiwa, K., Mita-Yoshida, K., Hamada, M., Hozumi, A., Nishino, A. S. and Sasakura, Y. 2025. Tunicate-specific protein Epi-1 is essential for conferring hydrophilicity to the larval tunic in the ascidian *Ciona*. Dev. Biol. **520**: 41-52.

Lebel, M., Sancerini, T., Rabiteau, S., et al. and Tiozzo, S. 2025. Multi-stage single-cell atlas of *Botryllus schlosseri* asexual development unveils dedifferentiating bud founder cells. bioRxiv epub.

Legrand, E., Svensen, O., Husa, V., Lelievre, Y. and Svensen, R. 2025. In situ growth dynamics of the invasive ascidian *Didemnum vexillum* in Norway: Insights from a two-year monitoring study. Mar. Pollution Bull. **211**: epub.

Leonor, C. M. and Muxagata, E. 2025. Interannual variability in surface mesozooplankton in the Western Antarctic Peninsula during two anomalous years. Deep Sea Res. Part 1-Oceanog. Res. Papers **217**: epub.

Liang, Z., Hoyer, J. and Chatzigeorgiou, M. 2025. Anoctamins mediate polymodal sensory perception and larval metamorphosis in a non-vertebrate chordate. Cell Rep. **44**: epub.

Lüskow, F., Dubischar, C. D., Plum, C. et al. 2025. Seasonal variability of the energy content of *Salpa thompsoni*, a key metazoan in Southern Ocean food webs. Polar Biol. **48**: epub.

Mathiesen, B. T., Ohta, M., De Magalhaes, B. P., Castelletti, C., Perria, V., Schuster, K., Christiaen, L. and Ohta, N. 2025. A simple inland culture system provides insights into ascidian post-embryonic developmental physiology. Open Biol. **15**: epub.

Moura, C. J., Wirtz, P., Nhanque, F. T., Barbosa, C. and Serrao, E. 2025. Hotspot of exotic benthic marine invertebrates discovered in the tropical east Atlantic: DNA barcoding insights from the Bijagós Archipelago, Guinea-Bissau. Ecol. & Evol. **15**: epub.

Nakahara, Y., Fukuda, T., Fujii, R. et al. 2025. Formal synthesis of lobatamides A and C. Organic Letters **27**: 3066-3070.

Nishikawa, T. and Hiruta, S. F. 2025. Molecular phylogeny and morphology of *Culeolus tenuis* and *C. uschakovi* (Urochordata: Ascidiacea) collected near the Chishima-Kamchatka Trench, southeastern Hokkaido, Japan, with special reference to significant differences in the *Culeolus*-ridge. Species Diversity **30**: 55–69.

Nishikawa, T., Nomura, K., Ohya, K. and Kawamura, M. 2024. First records of the invasive European ascidian *Clavelina lepadiformis* (Mùller,1776) complex from Kii Peninsula, Honshu, Japan (in Japanese, English summary). Nanki Seibutsu: The Nanki Biol. Soc. **66**: 113-116.

Nogueira, N. D. S., Portal, T. M., Nogueira, T. D. S., Miranda, A. E. S., Campos, E. and Monteiro de Barros, C. 2025. Neuronal degeneration, mitochondrial dysfunction, and disturbance of movements induced by rotenone in the ascidian *Styela plicata*. Neurotoxicology **108**: 69-80.

Oda, I. and Satou, Y. 2025. A master regulatory loop that activates genes in a temporally coordinated manner in muscle cells of ascidian embryos. Development **152**: epub.

Pakhomov, E. A., Mole, R., Bahl, A. A. et al. 2025. Early spring population dynamics of *Salpa thompsoni* linked to physical oceanography in the Atlantic sector of the Southern Ocean. Polar Biol. **48**: epub.

Pakhomov, E. A., Müller, S. J. and Meyer, B. 2025. Environmental conditions modulate hermaphroditic pathways in *Salpa thompsoni* near the Western Antarctic Peninsula. Sci. Rep. **15**: epub.

Paola, A. C., Giachetti, C. B., Nydam, M. L. et al. 2025. First record of the non-native colonial ascidian *Botrylloides diegensis* (Ascidiacea, Stolidobranchia) in the Southwestern Atlantic. Mar. Biodiversity **55**: epub.

Pelageev, D. N., Sabutski, Y. E., Kovach, S. M., Balaneva, N. N., Menchinskaya, E. S., Chingizova, E. A., Burylova, A. L. and Anufriev, V. P. 2025. Synthesis and biological activity of glycosyl thiazolyl disulfides based on thiacarpine, an analogue of the cytotoxic alkaloid polycarpine from the ascidian *Polycarpa aurata*. Mar. Drugs **23**: epub.

Peters, K. and Meyer, S. 2024. Tracking the settlement patterns of marine biofouling communities. Manag. Biolog. Invasions **15**: 549-566.

Pilcher, J., Monteith, G., Hutchings, B. and López-Legentil, S. 2025. Ascidian fouling in North Carolina shellfish farms. Aquaculture Res. **2025**: 1-10.

Ramalhosa, P., Monteiro, J. G., Rech, S. et al. 2025. The role of marine debris as a vector, dispersal agent, and substrate for non-indigenous species on Oceanic Islands (Northeast Atlantic). Mar. Pollution Bull. **214**: 1-16.

Reis, E. S. R., Portal, T. M., Nogueira, N. D. S., Nogueira, T. D. S., Mello, A. A. and Monteiro-de-Barros, C. 2025. Paraquat neurotoxicity: Oxidative stress and neuronal dysfunction in the ascidian brain. Comp. Biochem. Physiol. C **290**: epub.

Riccioni, A., Famengo, A., El Habra, N., Ballarin, L., Manni, L., Zamuner, A. and Schievano, E. 2025. Nanocrystalline cellulose from the solitary tunicate *Phallusia mammillata*, a valuable nanocellulose precursor. Advanced Sustainable Systems **5**: 1-9.

Riisgard, H. U. and Larsen, P. S. 2025. Filtration and respiration of filter-feeding marine invertebrates are linked through allometric power-law functions - verification of hypothesis. Biol. Open epub.

Rinkevich, B. 2025. From seabed to sickbed: lessons gained from allorecognition in marine invertebrates. Front. Immunol. **16**: epub.

Rinkevich, B. and Goulet, T. L. 2025. Micro-to multi-chimerism: the multiple facets of a singular phenomenon. Seminars in Immunopathology **47**: 1-16.

Rinkevich, B. and Pomponi, S. A. 2025. Advancing marine invertebrate cell line research: four key knowledge gaps. In Vitro Cellular & Developmental Biology-Animal epub.

Rodríguez, E. A., Arcángel, A. E., de la Barra, P. et al. 2025. Colonizing the open sea: population dynamics of an abundant marine invader on a natural shoreline. Biol. Invasions **27**: epub.

Rubinoff, B. G. and Grosholz, E. D. 2025. Structural effects of seagrass on fouling communities involve more direct than indirect effects. Mar. Ecol. Prog. Ser. **752**: 69-82.

Sakai, D., Sensui, N. and Hirose, E. 2024. Ascidian larvae discriminate nano-scale difference in surface structures during substrate selection for settlement. Zool. Sci. **41**: 564–569.

Sánchez-Serna, G., Badia-Ramentol, J., Bujos, P. et al. 2024. Less, but more: new insights from appendicularians on chordate Fgf evolution and the divergence of tunicate lifestyles. Molec. Biol. & Evol. **42**: 1-21.

Sawada, H., Saito, T., Shimada, Y. and Nishimura, H. 2025. Fertilization mechanisms in hermaphroditic ascidians and nematodes: common mechanisms with mammals and plants. Curr. Top. Dev. Biol. **162**: 55-114.

Scully, T. D., Pickett, C. J., Gort-Freitas, N. A., Davidson, B. and Klein, A. M. 2025. Immune cell type divergence in a basal chordate. bioRxiv epub.

Shi, W., Liu, P., Yang, D., Zhuang, Y., Lin, B. and Dong, B. 2025. Transcriptome analysis reveals the requirement of the TGFÎ² pathway in ascidian tail regression. Cells **14**: epub.

Sourav, M. A. R., Jung, S. W., Lee, J. S. et al. 2025. Distribution, abundance and grazing impact of *Salpa fusiformis*, with response to environmental conditions and their effects on other zooplankton species in Korean waters. J. Plankton Res. **47**: epub.

Stefaniak, L. M., Nydam, M. L. and López-Legentil, S. 2025. First record of *Corella japonica* in California. J. Mar. Biol. Ass. U.K. **105**: 1-9.

Tachioka, M., Miyazaki, M., Tsudome, M., Tsuda, M., Uchimura, K., Takaki, Y. and Deguchi, S. 2025. *Marinicellulosiphila megalodicopiae* gen. nov., sp. nov., a deep-sea alkaliphilic cellulolytic bacterium isolated from an endemic ascidian *Megalodicopia hians*. Intl. J. Syst. & Evol. Microbiol. **75**: epub.

Taylor, E., Allen, J. D. and Heyland, A. 2025. Thyroid hormones reversibly inhibit metamorphic development in ophiuroid larvae. J. Exp. Biol. **228**: epub.

Temiz, B., Meier, M. and Wilson, M. J. 2025. Single-cell transcriptomic profiling of the whole colony of *Botrylloides diegensis*: Insights into tissue specialization and blastogenesis. Development epub.

Tobias, Z. J. C., Miller, G. and Tepolt, C. K. 2025. Variation of thermal tolerance during northward range expansion in the invasive golden star tunicate, *Botryllus schlosseri*. Conservation Physiology **13**: epub.

Topic, P. N., Hamer, B., Strunjak-Perovic, I. et al. 2025. Biological, biochemical and elemental traits of *Clavelina oblonga*, an invasive tunicate in the Adriatic Sea. Animals (Basel) **15**: epub.

Tsotsios, D., Alvanou, M. V., Papadopoulos, D. K. et al. 2025. Influence of key physicochemical factors on the temporal dynamics of invasive and native ascidian settlement. Water **17**: epub.

Ubagan, M. D., Lee, T. K., Kim, Y. et al. 2025. Evaluating the ecological impacts of dominant non-indigenous sessile invertebrates in peninsular coastal ecosystems. Front. Mar. Sci. **12**: epub.

Urabe, I., Matsuno, K., Sugioka, R. et al. 2025. Spatio-temporal changes in the macrozooplankton community in the eastern Indian sector of the Southern Ocean during austral summers: a comparison between 1996 and 2018-2019. Prog. in Oceanog. **231**: epub.

von Ammon, U., Jeunen, G. J., Laroche, O., Pochon, X., Gemmell, N. J., Stanton, J. L. and Zaiko, A. 2025. Investigating passive eDNA samplers and submergence times for marine surveillance. PeerJ **13**: epub.

Wang, K., Suyama, R., Mizutani, N., Matsuo et al. 2025. Transcriptomes of a fast-developing chordate uncover drastic differences in transcription factors and localized maternal RNA composition compared with those of ascidians. Development **152**: epub.

Wibisana, J. N., Plessy, C., Dierckxsens, N., Masunaga, A., Miao, J. and Luscombe, N. M. 2025. The complete mitogenome of an unidentified *Oikopleura* species. F1000Research **13**: epub.

Witte, E. S., Franken, O., Temmink, R. J. M. et al. 2025. Structural complexity of hard substrates shapes shallow marine benthic communities. Oikos epub.

Wolvin, S., Hamel, J. F. and Mercier, A. 2025. Rock bottom: colonization patterns of deep-sea Arctic and subarctic dropstones (200-1300 m). Deep Sea Res. Part 1-Oceanog. Res. Papers **217**: epub.

Xiang, P., Bai, X., Xing, B., Li, J., Zhang, C. and Li, M. 2024. Full-length transcriptome annotation of a pyrosome, *Pyrosoma atlanticum* (Chordata, Thaliacea). Scientific Data **11**: epub.

Xin, Y., Huang, Y. L., Cai, F. F. et al. 2025. Effects of seasonal *Ciona* macrofouling on corrosion and hydrogen permeation behavior of a low-alloy high-strength steel in marine environments. J. of Materials Research & Technology **35**: 7037-7050.

Xu, X., Luo, W., Ren, Z. and Song, X. 2025. Intelligent detection and recognition of marine plankton by digital holography and deep learning. Sensors (Basel) **25**: epub.

Xue, F. and Zhu, G. P. 2025. Shifts in food composition of Antarctic krill (*Euphausia superba*) enhance coexistence with the pelagic tunicate (*Salpa thompsoni*). Mar. Biol. **172**: epub.

Yan, W., Fu, R., Huang, X. and Zhan, A. 2025. Dynamic and functional miRNA-mediated gene regulations in response to recurrent environmental challenges during biological invasions. Molec. Ecol. Epub.

Zambon, G., Sabbadin, G., Manni, L. et al. 2024. The European DeuteroNoise project: impact of anthropogenic noise on marine invertebrate deuterostomes. INTER-NOISE and NOISE-CON Congress and Conference Proceedings **24**: 9957-9961.

Zenetos, A., Dogan, A., Bakir, A. K. et al. 2025. Non-indigenous species (NIS) know no geopolitical borders-an update of NIS in the Aegean Sea. Diversity-Basel **17**: epub.

Zhang, J., Tan, Z., Qin, Q., Peng, H., Shi, W., Yu, H. and Dong, B. 2025. Integrated multi-omics identify key signalling pathways for notochord lumenogenesis in ascidian *Ciona savignyi*. Open Biol. **15**: epub.

Zhang, Y., Qin, Y., Wu, Y. et al. 2025. Morphological characteristics, mitochondrial genome, and evolutionary insights into a new sea squirt from the Beibu Gulf. Ecol. & Evol. **14**: 1-13.