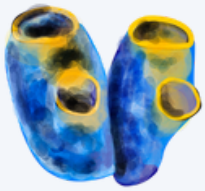
13TH INTERNATIONAL TUNICATE MEETING

BERLIN 2026

Humboldt University Berlin
in cooperation with
Tieranatomisches Theater

19.07.2026 - 24.07.2026

itm-berlin-2026.de



CONTENTS

DELEGATE INFORMATION

VENUES:

Registration and Coffee Breaks:
Café Flora, Campus Nord, Philippstraße 13/Haus 19

Presentations:
Campus Nord, Philippstraße 13/Haus 18, HS 2

Poster Presentation:
Campus Nord, Philippstraße 13/Haus 3

INTERNET ACCESS:

Free Wi-Fi is available via eduroam or Free Wifi Berlin/ Public Wifi Berlin

BADGES:

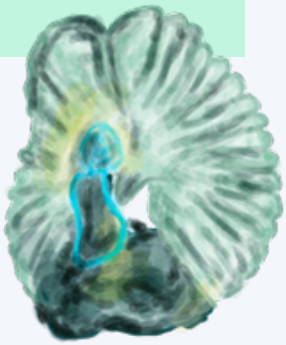
Name badges will be distributed on Sunday during registration

AWARDS FOR EARLY-CAREER PRESENTATIONS:

Thanks to our sponsor Springer, we award two book prices worth 100 € each to the two best oral presentations and poster presentations! Make sure to participate in our online-vote for these awards! (QR-code will be supplied during the conference.)
Eligible Early-Career-Scientists are marked by bold font and an asterisk in the program!

ON-SITE FACILITIES:

Looking for a workspace or other facilities? Ask one of our volunteers - we're happy to help!



DELEGATE INFORMATION

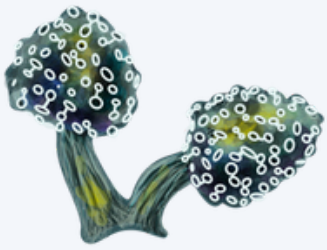
POSTER PRESENTATION:

Venue: Tieranatomisches Theater - (Campus Nord, Philippstraße 13/Haus 3)

Poster sizes: poster stands accommodate A0 format (= 84.1 cm × 118.9 cm) in portrait orientation.

Posters can be **put up** on Tuesday (July 21st) before the sessions and during the breaks.

Please **take down** the posters on Thursday during the coffee breaks or during the lunch break.

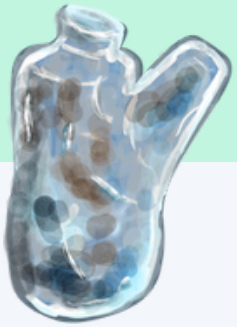


DELEGATE INFORMATION (ctd.)

ZOOM BROADCAST:

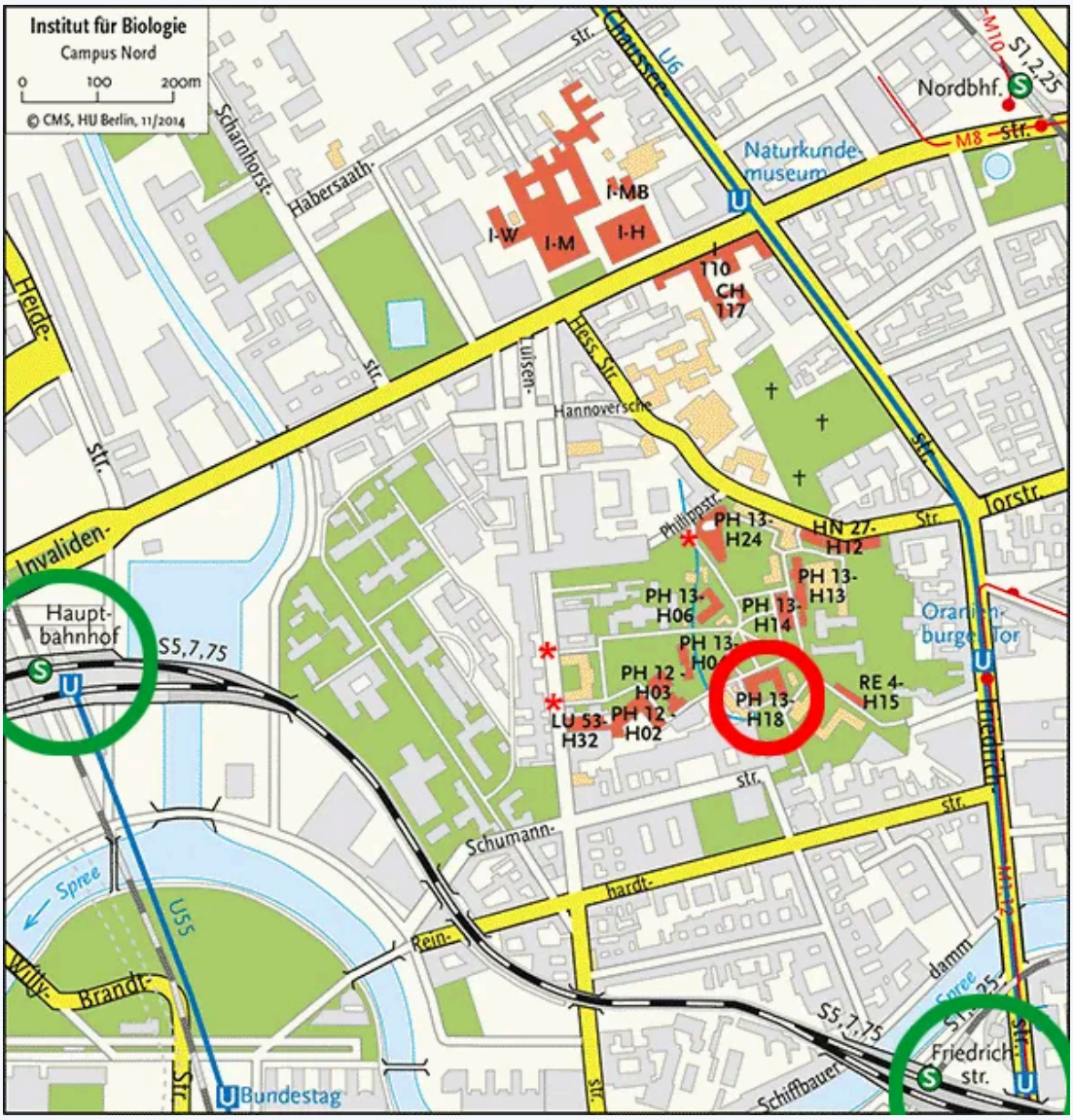
We intend to broadcast the oral presentations via zoom, for those interested, who cannot attend.
We will NOT record the presentations and of course we respect all requests to opt out of the broadcasting option.
Please contact thomas.stach@hu-berlin.de, if you wish to opt out!
In the detailed program, presentations that will not be available on zoom have their time slots framed in red. I repeat: presentations where the time slot is framed in red will not be available via zoom.

Here is the zoom link:
https://hu-berlin.zoom-x.de/j/69531102824?pwd=Cw3qHVpUwCPvbvaa8qmyWEcbZNjQRc.1



Scan this QR-code if you want to follow ITM13's talks online

MAP OF SURROUNDING AREA



Main location for presentations:
Campus Nord, Philippstraße 13/ Haus 18, HS 2 (highest floor)
For accessible entrance use the elevator (pink arrow, center of building) or feel free to ask our volunteers and we're happy to help!

Location of major public transport stations (Metro, Train, S-Bahn,Buses, Taxi)
Hauptbahnhof to the left, Bahnhof Friedrichstraße in the lower right corner.

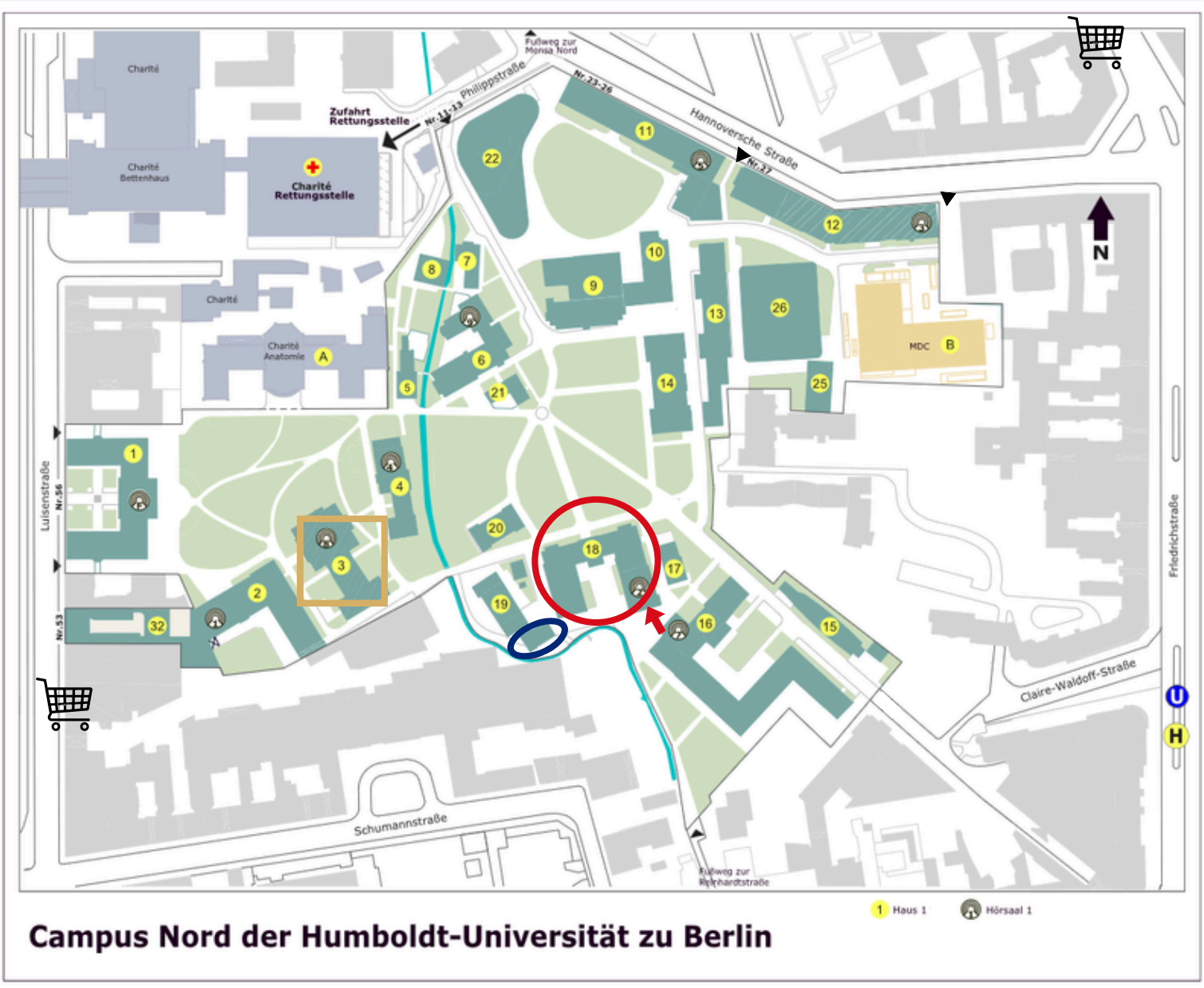
S-Bahn stations
Closest S-Bahn ('Schnell-Bahn', i.e. fast trains) stations

Metro stations
Closest Metro (Subway-) stations (lines U55 and U6)

Note:
In addition to the public transport options above, a dense net of bus lines exist. There are numerous lines and the bus stations feature the sign depicted to the right. Usually, the distance between bus stops is around 500 m. Official information can be found here: https://www.bvg.de/en

Another Note:
Ticket prices are designed to confuse visitors. Tickets are valid in all public transport vehicles (NOT taxis). For the whole city of Berlin, you need an AB-ticket. The airport is outside the city as is Potsdam. For these, you need an ABC-ticket.
A single AB-ticket is 4 € and is valid for 2 hours (in theory in one direction). Four single-trips can be purchased for 12,40 €., a day-ticket is 11,20 € and there are options 'for tourists' (so called 'city tour cards') for 2, 3, 4, 5, or 6 days.
The best is to check out the information here: https://www.bvg.de/en/subscriptions-and-tickets/all-tickets

CAMPUS MAP



Main location for presentations:
Campus Nord, Philippstraße 13/ Haus 18, HS 2 (highest floor)
For accessible entrance use the elevator (pink arrow, center of building) or feel free to ask our volunteers and we're happy to help!

Main location for registration, icebreaker, and coffee breaks:
Café Flora, Campus Nord, Philippstraße 13, Haus 19

Poster Presentation:
Campus Nord, Tieranatomisches Theater, Philippstraße 13, Haus 3

Supermarket close to campus:
Lidl, Luisenstraße 52
Rewe, Friedrichstraße 111

LUNCH OPTIONS AROUND CAMPUS

N.B.: There are MANY more in the vicinity - enjoy exploring them!

On Campus

- **Cafe Flora:** Student-run cafe on Campus Nord. Perfect for coffee, snacks and informal meetings.
- **Mensa HU Nord:** The University Cafeteria offers daily meals at affordable prices.

🌿 Vegan & Plant based

- **Mom's – Vegan Cooking:** Acclaimed all-vegan Vietnamese kitchen with inventive, generous dishes.
- **Ga Ya Ya:** Cozy fully-vegan Asian fusion spot with flavorful, wholesome curries and noodles.
- **SOY:** Plant-based Vietnamese restaurant loved for its soups and tofu satay.

☕ Cafés & Quick Bites

- **Oslo Kaffebar:** Warm specialty coffee shop with great flat whites and chocolate-chip cookies.
- **Café/Backshop Chausseestraße 51:** Friendly neighborhood bakery-café with fresh cakes and an evening beer selection.
- **Café Einstein Unter den Linden:** Elegant café ideal for coffee, pastries, and light meals.
- **Melody Nelson Bar:** Calm, relaxing wine bar with in and outdoor seating and specialty wine and cocktails.

🥨 German

- **Schnitzelei Mitte:** Beloved spot for top-notch schnitzel, with surprisingly good vegan options.
- **Berliner Republik:** Lively riverside beer garden with hearty German classics.
- **Hofbräu Wirtshaus Berlin:** Big, festive Bavarian beer hall with pork knuckle and live music.
- **Zur Letzten Instanz:** Berlin's oldest restaurant, serving classic German specialties.
- **Max und Moritz:** Traditional Berlin restaurant with local dishes and historic charm.

👨‍🍳 Other Cuisine

- **Casalot:** Authentic Arab food in oriental atmosphere.
- **Hoongy:** Vietnamese cuisine with vegan and vegetarian options and a cheaper lunch menu.
- **Kin Za Restaurant Cafe Bar:** Cozy restaurant serving traditional Georgian food and drinks.
- **Akito Sushi Bar:** Asian fusion kitchen, not only sushi in a cozy atmosphere.
- **Ristorante Nuovo Firenze:** Homey restaurant with homemade bread, fresh pasta, and good wine.
- **Coccodrillo:** Stylish, colorful Italian-Mediterranean spot known for truffle pasta and cocktails.
- **Machi Izakaya:** Japanese izakaya with great pho, wontons, and a solid cocktail list.
- **Golden Rice Mitte:** Spacious Vietnamese/sushi spot with an extensive, affordable menu.

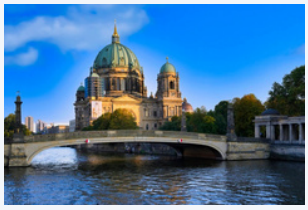
📍 Many of these recommendations are within walking distance or a short ride from Campus Nord. Click on the restaurant name to see the location and Menu.

❓ Looking for a specific cuisine or dietary option? Ask one of our volunteers—we'll be happy to help!

BERLIN SIGHTS AROUND CAMPUS

Brandenburger Tor

Berlin's most iconic monument and symbol of German reunification



Museumsinsel

Home to world-class museums including the Pergamon Museum, Neues Museum and Altes Museum

Humboldt-Forum

Housed in the reconstructed Berlin Palace. Features World cultures exhibitions, temporary exhibitions and a rooftop terrace with excellent views.

Berlin Wall Memorial

An open air memorial preserving part of the former Berlin wall and documenting the city's division during the cold war.

Futurium

Interactive museum exploring the future of technology, society, and sustainability.

Mori-Ōgai-Gedenkstätte

Museum dedicated to Japanese writer and physician Mori Ōgai, highlighting his life in Berlin and his contributions

Reichstag

Germany's historic parliament building, famous for its glass dome offering panoramic views of Berlin.

Hamburger Bahnhof

Contemporary art museum featuring works by international artists in a historic railway station.

Museum für Naturkunde

Natural history museum renowned for its impressive dinosaur and fossil collections.

Deutsches Technikmuseum

Interactive museum showcasing the history of transportation, aviation, and technology.

Berliner Medizin-historisches Museum

Museum showcasing the history of medicine, anatomy, and scientific discovery.

Volkspark Humboldthain

Historic park with scenic views, gardens, and a former WWII observation tower.

Public Transport Nearby

🚆 **S-Bahn:** Nordbahnhof (S1, S2, S25, S26) / S+U Friedrichstraße
🚇 **U-Bahn:** Naturkundemuseum (U6)/ Oranienburger Tor (U6)
🚋 **Tram:** Invalidenpark (M5, M8, M10) / Oranienburger Tor
🚌 **Bus:** Invalidenpark / Robert-Koch-Platz

Click on the picture to see the location and transportation options.
Have questions about the places? Please don't hesitate to ask our volunteers—we're here to help.

PROGRAM AT A GLANCE

Day 1: Sunday 19 July

15:30 - 19:00	**Registration, badge pickup & icebreaker**

Day 2: Monday 20 July

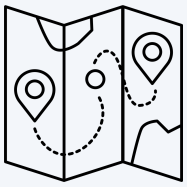
9:00 - 9:10	**Welcome Address**
9:10 - 9:50	**Keynote 1: Carsten Lüter**
9:50 - 10:40	**Developmental and Cellular Biology - Cellular Morphogenesis**
10:40 - 11:15	**Coffee Break**
11:15 - 12:00	**Developmental and Cellular Biology - Cellular Morphogenesis**
12:00 - 13:05	**Ecology**
13:05 - 14:45	**Lunch Break**
14:45 - 16:00	**Ecology**
16:00 - 16:30	**Coffee Break**
16:30 - 17:35	**Stem cells, asexual reproduction, regeneration and aging**
17:45 - 19:00	**Round-Table-Discussion: Online Resources and Computational Infrastructures Patrick Lemaire Carmela Gissi, Lionel Christiaen, Ayelet Voskoboynik, Takumi Shito**

Day 3: Tuesday 21 July

9:00 - 9:40	**Keynote 2: John Bishop**
9:40 - 10:30	**Developmental and Cellular Biology - Cell Fate Specification**
10:30 - 11:00	**Coffee Break**
11:00 - 12:15	**Developmental and Cellular Biology - Cell Fate Specification**
12:15 - 12:50	**Late Embryogenesis and Larvae**
13:05 - 14:45	**Lunch Break**
14:45 - 16:00	**Late Embryogenesis and Larvae**
16:00 - 16:30	**Coffee Break**
16:30 - 17:50	**Imaging and Modelling**
18:00 - 20:30	**Poster Presentation** (snacks and drinks provided)

PROGRAM AT A GLANCE

Day 4: Wednesday 22 July

Excursion Day : 

Potsdam	**Time:** 8:50 am **Location:** Meet at Berlin Main Station **Costs:** 40 € (plus commute and food) **A guided tour around Potsdam**
Stand Up Paddle-Boarding	**Time:** tba (likely around 3pm) **Location:** Meet at S-Bahn Station Friedrichstraße **Costs:** 40 € (plus commute and food) **Stand up paddling in the city**
Natural History Museum	**Time:** 9:50 am **Location:** Natural History Museum, Berlin **Costs:** free (donation welcome) **A guided tour through the Natural History Museum**
Egyptian Museum	**TIme:** 9:50 am **Location:** Neues Museum **Costs:** free (donation welcome) **A guided tour through the Egyptian Museum**

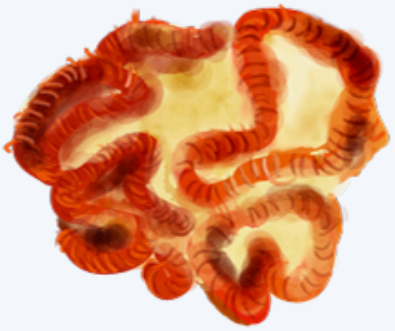
PROGRAM AT A GLANCE

Day 5: Thursday 23 July

9:00 - 9:40	Keynote 3: Billie J. Swalla
9:40 - 10:30	Developmental and Cellular Biology - Developmental Atlas
10:30 - 11:00	Coffee Break
11:00 - 11:45	Developmental and Cellular Biology - Developmental Atlas
11:45 - 12:50	Marine Pollution Effects
12:50 - 14:30	Lunch Break
14:30 - 15:30	Marine Pollution Effects
15:30 - 16:05	Evolution, Systematics, and Taxonomy
16:05 - 16:35	Coffee Break
16:35 - 17:35	Evolution, Systematics, and Taxonomy
18:30 - 22:00	Biergarten

Day 6: Friday 24 July

9:00 - 9:40	Keynote 2: Shigeki Fujiwara	
9:40 - 10:30	Gene Regulation	
10:30 - 11:00	Coffee Break	
11:00 - 12:00	Gene Regulation	
12:00 - 13:05	Evolution, Systematics, and Taxonomy	
13:05- 13:20	Farewell Address	
13:20 - 14:00	Short Break	
14:00- 18:00	Taxonomy Workshop	MorphoNet - Presentation & Workshop



DETAILED PROGRAM

DAY 1: SUNDAY 19 JULY

Registration

Badge Pick-Up

Excursion Sign-Up

Ice-Breaker

DAY 2: MONDAY 20 JULY

Time	Session	Chairs	Title	Presenter
9:00 - 9:10	**Opening of ITM13**	Thomas Stach	**Welcome address**	Mareis, Claudia (VP HU)
9:10 - 9:50	**Keynote 1**		**Historical collections as a resource for top-notch science**	Lüter, Carsten
9:50 - 10:10	**Developmental and Cellular Biology - Cellular Morphogenesis**	Alberto Stolfi & Atsuko Sato	Investigating the dynamic regulation and functions of claudins in *Ciona* morphogenesis	*** Hernandez, S.A**
10:10 - 10:25			Bidirectional redistribution of actomyosin drives epithelial invagination in ascidian siphon tube morphogenesis	Dong, Bo
10:25 - 10:40			Residual tail twisting in ascidian larvae is stabilized by asymmetric myofibrils that resist bilateral symmetry restoration	*** Nishio, Misaki**
10:40 - 11:15	**Coffee Break**			
11:15 - 11:30			From deterministic to regulative development in a variable ocean	Christiaen, Lionel
11:30 - 11:45			Multisensory dopaminergic neurons (coronet cells) control swimming behavior of Ciona larvae	Kusakabe, T. G.
11:45- 12:00			Morphological characterization of pre-fertilization elevation in the egg coat of Ascidiidae	*** Hanazaki, Kana**
12:00 - 12:20	**Ecology**	Noa Shenkar & Rodolfo da Silva Mazzarini Baldinotti	Toward the scalable cultivation of edible tunicates: Optimized protocol for *Styela plicata*	*** Platin, Raz**
12:20 - 12:35			Impact of invasive bioengineer ascidians in the rocky shore intertidal zone	da Rocha, Rosana
12:35 - 12:50			Quantifying early-life transition probabilities to estimate propagule pressure in invasive ascidians	*** Unger, Amit**
12:50 - 13:05			Threat-induced behavioral plasticity in two invertebrate chordates integrates sensory and neuromodulatory inputs	*** da Silva Mazzarini Baldinotti, Rodolfo**
13:05 - 14:45	**Lunch Break**			

Time	**Session**	Chairs	Title	Presenter
13:05-14:45	**Lunch Break**			
14:45 - 15:00	**Ecology**	Noa Shenkar & Rodolfo da Silva Mazzarini Baldinotti	Symbionts of didemnid ascidians from Turkish coastal waters and their bioactive secondary metabolites	Karahan, Arzu
15:00 - 15:15			Microbial origin of Palmerolide A in the Antarctic ascidian *Synoicum adareanum*	Murray, Alison E.
15:15 - 15:30			Ascidian biodiversity updates along the Israeli Mediterranean Coast and the arrival of the Caribbean species *Didemnum cineraceum* (Sluiter, 1898)	*** Gali, T.**
15:30 - 15:45			Isolation and identification of an agglutination factor in the hemolymph of *Halocynthia roretzi* against a parasitic flagellate *Azumiobodo hoyamushi*	Yanagida, T.
15:45 - 16:00			The zooplanktonic tunicate *Oikopleura dioica* reveals an adapted genetic-toolkit against climate change-derived biotoxins	Torres-Aguila, Nuria
16:00 - 16:30	**Coffee Break**			
16:30 - 16:50	**Stem cells, asexual reproduction, regeneration and aging**	Tal Gordon & Stefano Tiozzo	Regional signaling controls stem cell-mediated regeneration in an invertebrate chordate	*** Gordon, Tal**
16:50 - 17:05			Stolonial budding in the salp *Thalia democratica* relies on multiple lineage-restricted stem cells populations	Alié, Alexandre
17:05 - 17:20			The JAK/STAT pathway structure and its role in stemness in a basal chordate	*** La Torre, Federico**
17:20 - 17:35			Survival benefits outweigh germline competition costs in kin chimeras	Voskoboynik, Ayelet
17:45 - 19:00	**Round-Table-Discussion: Online Resources and Computational Infrastructures** (Patrick Lemaire, Carmela Gissi, Ayelet Voskoboynik, Takumi Shito, Lionel Christiaen, Stefano Tiozzo) (main venue: Philippstr. 13 / Haus 18)			

DAY 3: TUESDAY 21 JULY

Please feel free to drop off your posters also before 9:00 AM

Time	Session	Chairs	Title	Presenter
9:00 - 9:40	**Keynote 2**	Atsuko Sato	**A look back at work on sexual reproduction and chimerism in Diplosoma listerianum (Didemnidae)**	Bishop, John
9:40 - 10:00	**Developmental and Cellular Biology - Cell Fate Specification**	Lionel Christiaen & Jiankai Wai	The endoderm cell trajectory of urochordate *Styela clava* reveals the dual developmental origin and evolution of digestive tract	Wei, Jiankai
10:00-10:15			The cell lineage and developmental mechanisms of the glutamatergic neurons in the brain of the *Ciona* larva	Oonuma, Kouhei
10:15 - 10:30			Role of retinoic acid in cardiopharyngeal specification during embryogenesis of *Ciona robusta*	D'Aniello, Enrico
10:30 - 11:00	**Coffee Break**		**Turning in posters at "Tieranatomisches Theater"**	
11:00 - 11:15		Lionel Christiaen & Jiankai Wai	An NKX2-5 homolog is required downstream of BMP signaling to pattern the sensory-adhesive organ of a tunicate larva	Johnson, J. Chris
11:15 - 11:30			Opposing roles of BMP and FGF signaling in cardiopharyngeal progenitor maturation and fate choices in *Ciona intestinalis*	*** Perria, Vincenzo**
11:30 - 11:45			Exploring divergence of tunicate immune regulatory networks across cionids	Popsuj, Sydney
11:45-12:00			Self-Nonself Recognition and Reproductive Strategies in Ascidians	Saito, Takako
12:00-12:15			Germline Stem Cell Isolation, Lineage Tracing, and Aging in a Colonial Tunicate	*** Levy, Tom**
12:15 - 12:35	**Late Embryogenesis and Larvae**	Bo Dong & Kilian Biasuz	Tunicate underwater adhesion: cell types for sensory adhesion, their secretions and species comparisons	Zeng, Fan
12:35 - 12:50			Core mechanism of ascidian metamorphosis	Sasakura, Yasunori
12:50 - 13:05			Identification of protein precursor for thyroid hormone synthesis in basal chordate ascidian *Styela clava*	Zhang, Jin
12:50 - 14:30	**Lunch Break**		**Turning in posters at "Tieranatomisches Theater"**	

Time	**Session**	Chairs	Title	Presenter
12:50 - 14:30	**Lunch Break**			
14:30 - 14:45	**Late Embryogenesis and Larvae**	Bo Dong & Kilian Biasuz	A protocadherin mediates oral placode morphogenesis in the tunicate *Ciona*	*** Jadali, B.**
14:45 - 15:00			Antenna cells of ascidian larvae are gravity-sensing neurons	*** Chokki, A.**
15:00 - 15:15			On the organization of the *Ciona* tunic	Nakashima, Keisuke
15:15 - 15:30			Tail muscle evolution in *Oikopleura dioica* sheds light on the appendicularian transition to a fully free-swimming lifestyle	Torrus, Marc Fabregà
15:30 - 15:45			Navigating flow: cellular and behavioral basis of rheotaxis in *Ciona* larvae	Tolstenkov, Oleg
15:45 - 16:15	**Coffee Break**			
16:15 - 16:35	**Imaging and Modelling**	Roberta Pennati & Noah Bruderer	MorphoNet: segmentation, qualitative evaluation and curation of large-scale 3D+t datasets	Tao Laurent
16:35 - 16:50			Direct Live Imaging Reveals Transcellular Migration of Mesenchymal Cells during Ascidian Metamorphosis	Hotta, Kohji
16:50 - 17:05			Decoding Chordate Development through High-Content Morpho-Topological Analysis	Bruderer, Noah
17:05 - 17:20			3D imaging analysis of genes involved in notochord morphogenesis	*** Kashiro, Sae J.**
17:20 - 17:35			Spatiotemporal Dynamics of Neuronal Activity During the Initiation of Ascidian Metamorphosis	*** Doi, Manami**
17:35 - 20:30	**Poster Session**	**Location: Tieranatomisches Theater**	Location will be open until Thursday evening (23 July 18:00 o'clock) for additional viewing of posters	

DAY 4: WEDNESDAY 22 JULY

Potsdam – guided tour

We will meet in front of Berlin main station (Hauptbahnhof) 8:50 am. After a ca. 45 min. ride with the S-Bahn we'll meet our local guide Reinhard Müller at the Obelisk at Potsdam's central marketplace at 10 am. He has prepared a ca. 5h tour to see one of the most beautiful cities in Europe, a UNESCO World Heritage Site.
We'll collect 40 € per person at the registration. You will need two ABC-tickets (5 € each or 12,90 € for a 24 h-ticket) plus what you will need for meals and drinks. (I asked our guide to think about proper rest stops.)

Egyptian Museum Tour

We will meet at 9:50 am at Neues Museum (street address: James-Simon-Galerie, Bodestraße 10178 Berlin). Dr. Rebekka Pabst, curator of the museum will guide us through the depot of the sarcophagi, with in depth explanations on chronology, gods, religion, the Amarna-period, and details about the process of mummification and animal cults in ancient Egypt. You will also receive tickets for the general exhibition, to visit after the guided tour.
The tour is free of charge. Donations are welcome.

Archaeopteryx et al. at the Museum für Naturkunde in Berlin

We will meet at 9:50 am at the main entrance of the Museum für Naturkunde (street address: Invalidenstraße 43, 10115 Berlin). Clemens Zinner, a Master student at HU and paleontology enthusiast will show us some of the fossil treasures at the museum and of course will tell you much about the famous dinosaurs among which the most beautiful fossil of Archaeopteryx stands out. You will also receive tickets for the general exhibition, to visit after the guided tour.
The tour is free of charge. Donations are welcome.

Stand Up Paddling

The time of meeting is not fixed yet, probably early afternoon around 3pm. We'll take public transportation from the station Berlin, Friedrichstraße to the SUP station at the Funkhaus (street address: Nalepastr. 18, 12459). This will take approximately 45 minutes and you will need an AB-ticket (4 € one way). The friendly and relaxed people from StandUpClub Berlin (https://standupclub.de/sup-station-funkhaus/) will then introduce us to the quirks of stand up paddling, supply the equipment, and will guide us on the water in the middle of the city, where we will come face to face (or rather face to legs) with Molecule Man.
We'll collect 40 € per person at the registration. You will need two AB-tickets (4 € each or 11,20 € for a 24 h-ticket) plus what you will need for meals and drinks.

DAY 5: THURSDAY 23 JULY

Time	**Session**	Chairs	Title	Presenter
9:00 - 9:40	**Keynote 3**	Stefano Tiozzo	**Deuterostome ancestors and chordate origins**	Swalla, Billie J.
9:40 - 10:00	**Developmental and Cellular Biology - Developmental Atlas**	Kohji Hotta & Sabrina Hernandez	Principled construction of a variation-aware developmental atlas with cellular resolution for ascidian embryogenesis	Biasuz, Kilian
10:00 - 10:15			Substantial gene expression variation is compatible with highly stereotyped ascidian embryonic development	*** Falcioni, L.**
10:15 - 10:30			Identifying the biosynthetic origins of tunichromes, an enigmatic group of natural products	Zeke, Andras
10:30 - 11:00	**Coffee Break**			
11:00 - 11:15		Kohji Hotta & Sabrina Hernandez	An unbiased monoclonal antibody platform to map *Botryllus* hematopoiesis and allorecognition	*** Hirakata, Alyssa**
11:15 - 11:30			Role of Piwi in *Ciona* nervous systems	Yokogawa, Y.
11:30 - 11:45			A mitosis-coupled regulatory transition controls collective migration of the cardiopharyngeal progenitors in the tunicate *Ciona*	Ohta, Naoyuki
11:45 - 12:05	**Marine Pollution Effects**	Lucia Manni & Enrico D'Aniello	Ocean acidifications effects on calcifying and non-calcifying ascidians	*** Ayoub, Ahmad**
12:05 - 12:20			Transcriptomic and cellular basis of developmental thermal stress in the ascidian *Ciona intestinalis*	Brandenburg , Jonas
12:20 - 12:35			Plastic responses to environmental stress drove evolution of the chordate characteristics in the two species of *Ciona*	Sato, Atsuko
12:35 - 12:50			Multilevel responses to emerging contaminants in the tunicate *Ciona intestinalis:* a comparative perspective	*** Morgillo, Antonio**
12:50 - 14:30	**Lunch Break**			

Time	Session	Chairs	Title	Presenter
12:50 - 14:30	**Lunch Break**			
14:30 - 14:45		Lucia Manni & Enrico D'Aniello	Maritime low frequency noise impacts *Ciona robusta* physiology and behaviour	*** Panarari, Elettra**
14:45 - 15:00			Underwater noise negative impacts behavior and physiology of the solitary ascidian *Styela plicata*	*** Sabbadin, Giacomo**
15:00 - 15:15			Sound matters: evidence of noise perception and its effects on physiology and larval distribution in *Clavelina lepadiformis*	*** Blumer, Giorgio**
15:15 - 15:30			Sound perception in ascidians: behavioural and physiological responses to vibration but not sound pressure	*** Böttner, Til**
15:30 - 15:50	**Evolution, Systematics, and Taxonomy**	Marie Nydam & Mai-Lee Van Le	Comparative anatomy and phylogenetic analysis of the nervous system and endostyle in marine invertebrate appendicularians (Chordata, Tunicata) - paedomorphosis and ecology as evolutionary drivers	*** Le, Mai-Lee Van**
15:50 - 16:05			Colonial structure and budding mode in *Syncarpa composita* revealed by histology and micro-CT imaging	Hasegawa, N
16:05 - 16:35	**Coffee Break**		**Pick-up of posters from "Tieranatomisches Theater"**	
16:35 - 16:50		Marie Nydam & Mai-Lee Van Le	Nano-scale nipple arrays in tunicates and similar structures in other phyla	Hirose, Euichi
16:50 - 17:05			Unraveling the *Clavelina lepadiformis* species complex: from chromosomal rearrangements and genomic reduction, to cryptic speciation and portuary colonisations	*** Junkin, Liam**
17:05 - 17:20			The taxonomy of native and non-native ascidians of New Zealand	*** Olmedo-Rojas, Pame**
17:20 - 17:35			Genomic resources for ascidiid species and comparative analysis across tunicates reveal evolutionary diversification	*** Shito, Takumi T.**
17:35 - 18:30	**Break**		**Pick-up of posters from "Tieranatomisches Theater" (& commute to Loretta!)**	
18:30 - 22:00	**Biergarten**	**Location: Loretta (S Wannsee)**	Kronprinzessinnenweg 260, 14109 Berlin-Bezirk Steglitz-Zehlendorf	

DAY 6: FRIDAY 24 JULY

Time		Session	Chairs	Title	Presenter
9:00 - 9:40		**Keynote 4**	tba	**Retinoic acid, tunicate development, and chordate evolution**	Fujiwara, Shigeki
9:40 - 10:00		**Gene Regulation**	Ute Rothbächer & Yael Klirs	Deep evolutionary conservation of Hmx transcription factors and their role in sensory ganglia	Pennati, Alessandro
10:00 - 10:15				Evolutionary changes in the mechanisms regulating peripheral nervous system formation in *Molgula*	*** Leblond, Cécile**
10:15 - 10:30				Hypomethylation in *Oikopleura dioica* genome	*** Klirs, Yael**
10:30 - 11:00		**Coffee Break**			
11:00 - 11:15			Ute Rothbächer & Yael Klirs	Six3/6 acts downstream of canonical Wnt signaling to regulate the formation of anterior neural border-derived structures in ascidian embryos	Darras, Sébastien
11:15 - 11:30				*Lzts* is a new player in the gene regulatory network for *Ciona* Bipolar Tail Neurons	Coppola, Ugo
11:30 - 11:45				Repressive roles of Erf and Elk in FGF-regulated neural development in *Ciona intestinalis*	Rothbächer, Ute
11:45 - 12:00				MAPK signaling integrates immune and stress responses during colonial blastogenesis in *Botryllus schlosseri*	Ballarin, Loriano
12:00 - 12:20		**Evolution, Systematics, and Taxonomy**	Carmela Gissi	DNA barcoding survey of ascidians in the Ryukyu archipelago	*** Okaji, R.**
12:20 - 12:35				Sleep in solitary ascidians? Diurnal behavioral and neural rhythms in *Styela plicata*	Shenkar, Noa
12:35 - 12:50				*Botrylloides* and *Botryllus* from Victoria, Australia	Nydam, Marie
12:50 - 13:05				Hidden diversity in ascidians of the genus *Pyura* (Chordata, Ascidiacea, Pyuridae) in Belize	Stefaniak, Lauren M.
13:05 - 13:20		**Awards & Farewell Address**			
13:20 - 14:00		**Short Break**			
14:00 - 18:00	**MorphoNet Presentation & Workshop** (Philippstr. 13/Haus 18 - main venue, seminar room)		**Taxonomy Workshop** (Philippstr. 13/Haus 14 - Molecular Parasitology Building)		

Abstracts in order of program

MONDAY 20 JULY

Keynote: Historical collections as a resource for top-notch science

Presenting author (PA): Lüter, Carsten
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The advent of non-invasive morphological and molecular sampling methods has opened new avenues for research on historical natural history collections. Museum collections form the foundation of our knowledge of Earth's biodiversity and represent an indispensable resource for taxonomic, evolutionary, and ecological research. At the same time, they are an important part of our cultural heritage and must be preserved for future generations.
This dual role has long posed a challenge. While researchers seek access to morphological and molecular information preserved in museum specimens, curators are understandably reluctant to permit destructive sampling of valuable material, particularly type specimens, even when only minute amounts of tissue are required. Recent methodological advances, including micro-computed tomography (µCT) and novel DNA extraction techniques based on leaching procedures, now offer a solution by enabling the recovery of high-quality data without compromising specimen integrity.
This talk presents an overview of projects and research strategies established at the Museum für Naturkunde Berlin that use these approaches to unlock the scientific potential of its extensive collections. By providing access to previously inaccessible morphological and molecular information while preserving the specimens themselves, these methods are transforming historical collections into an increasingly powerful resource for investigating biodiversity and the evolution of life on Earth.

DEVELOPMENTAL AND CELLULAR BIOLOGY - CELLULAR MORPHOGENESIS

.Investigating the dynamic regulation and functions of claudins in *Ciona* morphogenesis

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Epithelial barrier junctions regulate diverse biological functions such as barrier formation, cell polarity, and paracellular ion transport. However, the evolution of vertebrate tight junctions from more invertebrate-like barrier junctions is still poorly understood. This underscores the importance of tunicates such as *Ciona* spp. as ideal organisms for investigating the function and evolution of tight junctions and their component proteins. Claudins are one such family of proteins that are integral to both tunicate and vertebrate tight junctions. We have recently profiled in greater detail the 13 claudin and claudin-like protein-encoding genes that have been identified in the genome of *Ciona robusta*. The surprisingly dynamic spatiotemporal expression of different claudin genes in *Ciona* embryogenesis suggested they could be contributing to development in a multitude of hitherto unappreciated ways. Here we demonstrate, using CRISPR/Cas9-mediated mutagenesis, that individual claudins have distinct, nonredundant roles in notochord morphogenesis in *Ciona*. We also present evidence of complex post-transcriptional regulation of these claudin genes, hinting at further functional diversity of claudin protein isoforms. Taken together, these findings reveal unexpectedly broad roles for claudins in chordate development, extending our understanding of these proteins beyond their canonical roles in establishing epithelial tight junctions.

Bidirectional redistribution of actomyosin drives epithelial invagination in ascidian siphon tube morphogenesis

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How epithelia perform a spatiotemporal heterogeneous force generating program to drive a sequential tissue morphogenesis remains unclear, particularly the underlying precise mechanical mechanisms. This study investigated dynamic actomyosin reorganization between apical and lateral membrane cortex regions during two sequentially invaginated stages during ascidian atrial siphon tube morphogenesis. At the initial invagination stage, the originally lateral-located actomyosin redistributed to the apical domains, while those actomyosin redistributed back to lateral domains at the accelerated invagination stage. Using genetic mutants to modulate myosin activities, the initial invagination was strengthened or abolished, indicating invagination is apical constriction dependent. Optogenetic inhibition of myosin activities in lateral domains after initial invagination stage blocked the further processes, suggesting lateral constriction of actomyosin is required for the accelerated invagination. Vertex model simulations uncovered a coupled mechanism underlying epithelial invagination driven by apicobasal tension imbalance and lateral contraction. We thus propose an actomyosin redistribution mechanical model: lateral actomyosin first redistributes apically to drive apical constriction and shape the initial invagination, then apical actomyosin redistributes laterally to promote lateral contractility and accelerate invagination. Our findings reveal a bidirectional reorganization of actomyosin network as a central mechanism driving epithelial invagination, providing insights on epithelial invagination and the organ morphogenesis during development.

Residual tail twisting in ascidian larvae is stabilized by asymmetric myofibrils that resist bilateral symmetry restoration

Presenting author (PA): *** Misaki Nishio**
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Bilateral animals are traditionally characterized by an inherently left-right (L-R) symmetric body plan, and developmental research have been predominantly focused on the mechanisms underlying symmetry breakdown (Hamada 2020). However, recent studies in *Drosophila* and zebrafish have revealed that embryonic symmetry is not merely a default state but is actively maintained or corrected during morphogenesis (Naganathan, Popović, and Oates 2022; Smits et al. 2023), yet the underlying mechanisms remain poorly understood. In *Ciona*, we previously discovered that the tail undergoes a clockwise twisting deformation and developed an image-based quantitative method to measure the twisting angle (Kogure et al. 2025). Quantitative analysis across developmental stages revealed that the twisting angle increases until the late tail bud stage and subsequently decreases toward the larval stage. Interestingly, while the clockwise twist is largely corrected during later development, but approximately 10° of residual clockwise twist persists in larvae. Inhibition of retinoic acid signaling or neuromuscular activity eliminated this residual twist, restoring full bilateral symmetry, and was associated with disruption of myofibril organization within bilaterally positioned tail muscles. Because myofibrils in both left and right muscles are known to adopt the same left-handed helical orientation (Matsuo et al., 2021), we constructed a mechanical model reflecting such anatomical architecture and demonstrated that it generates a net torque sufficient to stabilize the residual twist against full symmetry restoration, as observed in wild-type larvae. These findings suggest that the existence of mechanobiological mechanisms by which body symmetry and asymmetry are regulated during chordate development.

From deterministic to regulative development in a variable ocean

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At macroscopic scales, animal development appears markedly reproducible from one individual to the next, and across generations. However, the molecular and cellular events underlying vertebrate embryogenesis display greater stochastic variability, and species-specific genetic determinism only becomes apparent through multiscale integration. By contrast with their vertebrate relatives and fellow chordates, tunicates display highly stereotyped embryogenesis at single cell resolution, with invariant lineages. Here I will first explore the underlying molecular mechanisms driving deterministic embryogenesis, with a focus on the cardiopharyngeal lineage in the ascidian *Ciona*. Remarkably, Distinct species and populations of *Ciona* live across variable environmental conditions, especially sea surface temperatures, which the developing embryo seemingly buffers, exhibiting robustness that results in a canalized outcome : the tadpole-like swimming larva. I will present our approach aimed at disentangling the molecular and cellular basis of thermal adaptation, with a focus on tempo scaling and developmental canalization. Finally, once the larva settles and begins metamorphosis, the rules change: post-embryonic development become regulative, the animal acquires the ability to regenerate lost parts, and key functional systems complete development and differentiation, including the digestive, reproductive and circulatory systems. I will present progress in our understanding of post-embryonic regulative development of the whole animal at single cell resolution. Taken together, our studies leverage the unique features of the tunicate model Ciona to reveal fundamental aspects of animal development and evolution.

Multisensory dopaminergic neurons (coronet cells) control swimming behavior of *Ciona* larvae

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The life history of ascidians consists of the swimming larval stage and the sessile adult stage. The larval stage is usually very short; in the case of *Ciona intestinalis* type A (*Ciona robusta*), larvae start to metamorphose into juveniles within a half day after hatching. The behavior during the short larval stage is crucial for their life because it determines where they live throughout the rest of their life. Swimming behavior of the larva is controlled by photoreception and gravity sensing of the ocellus and otolith, respectively. The ocellus contains ciliary photoreceptor cells that use a vertebrate-type visual pigment opsin (Ci-opsin1) as the photoreceptor molecule. In addition to the ocellus and otolith, the dopaminergic coronet cells have been shown to play a role in the control of swimming behavior. However, how the activity of coronet cells is regulated remains unknown. Here, we report that the coronet cells are multimodal sensory neurons that detect temperature and light and control larval swimming in response to these environmental stimuli. The optimal temperature for development and reproduction of this species is known to be between 16-20°C and higher temperature is harmful. We tested whether *Ciona* larvae show thermotaxis around this range of temperature and found that they swim towards lower temperature. Calcium imaging revealed that coronet cells respond to a temperature change. Coronet cells express a putative thermosensor protein TRPA1 and its inhibitor suppresses the thermoresponse of the coronet cells. Photoreceptor molecules are also expressed in coronet cells. Ci-opsin2, a paralogue of Ci-opsin1, and Ci-opsin6, a unique opsin gene, are specifically expressed in coronet cells. Furthermore, optogenetic activation of coronet cells triggered tail movements. We discuss our findings in the context of the homology between the coronet cells and the vertebrate hypothalamus.

Morphological characterization of pre-fertilization elevation in the egg coat of Ascidiidae

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The extracellular matrix surrounding animal eggs is important for fertilization, serving as a site for species-specific gamete recognition. In sea urchins, the vitelline membrane elevates after fertilization to form the fertilization membrane, which contributes to the block against polyspermy. We found that in *Phallusia philippinensis* (Ascidiidae), the vitelline coat (VC) markedly elevates upon spawning into seawater regardless of fertilization. In this study, we examined whether pre-fertilization VC elevation is unique to *P. philippinensis* or shared among other Ascidiidae species, investigated its underlying mechanism, and assessed its role in fertilization.
Eggs from *P. philippinensis*, *Ascidiella aspersa*, *Ascidia sydneiensis*, and *Ascidia zara* were collected by dissection and transferred to seawater at pH 8.2. Marked VC elevation was observed in all four species, and the ratios of post-elevation to pre-elevation VC diameter were larger than the ratio previously reported in eggs of *Halocynthia roretzi*. Since the oviduct of *P. philippinensis* is maintained at a lower pH than seawater and eggs become fertilizable as exposure to seawater elevates their intracellular pH, we examined the effect of pH and found that VC elevation was inhibited in low-pH seawater. We also tested the involvement of osmotic pressure, since vitelline membrane elevation in sea urchins is driven by osmotic pressure generated in the perivitelline space. Elevation was suppressed in seawater containing high molecular weight molecules as well. Protease inhibitors revealed species-specific effects: leupeptin significantly suppressed membrane elevation in *P. philippinensis* and *A. aspersa*, but not in *A. sydneiensis*. Finally, eggs in which membrane elevation had been inhibited by high molecular weight molecules were successfully fertilized, with no significant difference from control eggs.
The presence of pre-fertilization VC elevation across multiple Ascidiidae species suggests that it may have been acquired in the common ancestor of this family. This phenomenon may provide insight into the fertilization strategy of Ascidiidae.

Toward the scalable cultivation of edible tunicates: Optimized protocol for *Styela plicata*

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Styela plicata is a globally distributed ascidian commonly regarded as a pest in aquaculture and marine infrastructures due to its tendency to form large aggregations on artificial substrates. This species is considered invasive in many regions and can thrive under variable environmental conditions. In contrast, in some areas it is consumed as seafood and has attracted growing interest as a potential source of bioactive compounds and other natural products. However, evaluating the potential of *S. plicata* as a source of natural products first requires establishing effective cultivation under controlled conditions. To assess its potential, we established a land-based rearing protocol for the post-settlement juvenile phase of *S. plicata*. Juvenile growth and survival were examined under different feeding regimes, light intensities, feeding frequencies, and temperatures, using two water systems: a recirculated-water system (RWS) and a running seawater system (RSS). Results showed that while juveniles reared in the RSS exhibited faster growth, survival was significantly higher in the RWS. Within the experimental timeframe, feeding frequency and light intensity did not significantly affect performance, suggesting that juveniles can develop without daily feeding or artificial illumination. In contrast, temperature had a pronounced influence, with optimal growth observed at 24-27 °C. These findings demonstrate that *S. plicata* juveniles tolerated the range of controlled culture conditions examined in the present study, supporting its potential for scalable, low-input and cost-effective aquaculture. Here, scalability refers to the feasibility of increasing juvenile production under standardized and simplified rearing protocols prior to full-scale industrial application. Given its wide global distribution and tolerance to warm and food-limited environments, *S. plicata* offers a promising candidate for sustainable aquaculture and future food security.

Impact of invasive bioengineer ascidians in the rocky shore intertidal zone

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The potential impacts of invasive ascidian species on rocky shores include competition for space and modification of the community associated with them. Two invasive species of the intertidal zone in South Brazil are *Eudistoma carolinense* and *Polyandrocarpa zorritensis*. The former was first registered in 1995 and the latter in 2019. Both are capable of covering extensive areas and their colonies form erected lobes among which other sessile and mobile fauna dwell. This study aimed to evaluate the impact of these invasive ascidians on the associated fauna community in relation to other native bioengineer species (*Anguinella palmata* - bryozoan, *Alsidium seaforthii* - algae, and *Polysyncraton amethysteum* - ascidian). We scraped four 25 cm2 samples dominated by each one of those species in Itapoá, Santa Catarina, removed the associated fauna which was identified as morphotypes and measured the biological substrates. 5310 individuals of 122 morphotypes were found, distributed in the taxa Annelida - 25 morphotypes, Arthropoda-28, Mollusca-23, Cnidaria-8, Bryozoa-9, Nematoda-6, Ascidiacea-9, Echinodermata-1, Foraminifera-1, and Platyhelminthes-1. With the exception of weight, area ($F_{4,14}$= 21.22; p=0.001), volume ($F_{4,14}$= 14.72; p=0.001) and height ($F_{4,14}$= 56.67; p=0.001) differed among substrates, and the ascidians showed lower height and surface area, what limits the space available for the associated fauna. Ascidians also showed lower values of abundance ($F_{4,14}$ = 22.46, $p < 0.001$) and richness ($F_{4,14}$ = 10.06, $p < 0.001$) than the other substrates. PCoA analysis revealed that the communities associated with ascidians are taxonomically distinct, grouping separately from those found in the macroalgae and the bryozoan. These later substrates showed a dominance of Arthropoda and Mollusca species, while the two invasive ascidians had more Arthropoda and Annelida species. We conclude that both invasive ascidians impose restrictions for the colonization of associated fauna, impacting biodiversity in the rocky shore.

Quantifying Early-Life Transition Probabilities to Estimate Propagule Pressure in Invasive Ascidians

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Ascidians are among the most notorious marine invaders worldwide, with global shipping acting as a primary vector for their introduction beyond native ranges. Increasing maritime traffic, coupled with changing environmental conditions, is expected to further enhance the likelihood of ascidian introductions. However, the arrival of an adult individual in a new region represents only the first step of invasion; successful establishment additionally requires reproduction, survival across multiple early life stages, and tolerance to local environmental conditions. The cumulative success of these stages determines propagule pressure, defined as the number of viable offspring released into the invaded environment, yet this process remains poorly quantified for invasive ascidians. Similar demographic bottlenecks during early life stages have been documented in other taxa, including the planktonic stage of sea urchins, larval stages of fishes, larval and juvenile stages of amphibians, and the larval stage of the invasive zebra mussel.
Here, we provide the first comprehensive quantification of transition probabilities across fertilization, larval development, metamorphosis, and juvenile survival in three solitary ascidian species frequently associated with shipping-mediated introductions: the tropical species *Herdmania momus* and *Microcosmus exasperatus*, and the temperate/sub-tropical *Styela plicata*. Using experimentally derived stage-specific success rates, we model potential propagule pressure produced per adult individual under multiple shipping-relevant introduction scenarios. Results indicate strong stage-specific bottlenecks in reproductive success. In *Styela plicata*, fertilization represents the most susceptible stage, with an average success of ~25%, whereas survival through subsequent stages is significantly higher, with nearly all fertilized eggs successfully developing through larval and metamorphic stages to juvenile settlement. By linking early life-stage performance to propagule pressure following adult transport, this study provides a mechanistic framework for assessing establishment risk of ascidian species introduced through global shipping.

Threat-induced behavioral plasticity in two invertebrate chordates integrates sensory and neuromodulatory inputs

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Threat detection and evasion are fundamental challenges across the animal kingdom. Here we examine threat detection and avoidance behaviors in two planktonic tunicates, *Ciona intestinalis* larvae and *Oikopleura dioica* adults, by quantifying locomotion and ecologically relevant behaviors in the presence of calanoid copepods. Both species exhibited marked increases in swimming speed, distance travelled, and roaming behavior upon predator exposure, whereas conditioned water alone had no effect. Despite this shared response, behavioral organization diverged: *Ciona* primarily reallocated time toward roaming with few state transitions, while *Oikopleura* increased behavioral switching, reduced dwelling, and prolonged roaming bouts. Pharmacological manipulations revealed species-specific neuromodulatory dependencies: in *Ciona*, bioamine, glutamatergic, and GABAergic perturbations disrupted threat-induced movement patterning, whereas in *Oikopleura* they suppressed motility increases. Threat presence also accelerated metamorphosis and altered settlement in *Ciona*, while in *Oikopleura* it triggered rapid house escape, delayed expansion, and reduced turnover. Finally, we show that *Ciona* larvae leverage a distributed network of polymodal sensory neurons to detect chemical cues emanating from the copepods. These findings uncover conserved yet mechanistically distinct anti-threat strategies in two evolutionary distant tunicates.

Symbionts of Didemnid Ascidians from Turkish Coastal Waters and Their Bioactive Secondary Metabolites

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Antimicrobial resistance has become a major global challenge, reducing the effectiveness of many existing drugs and increasing the need for novel bioactive compounds. Marine ecosystems, which cover 71% of the Earth's surface and contain an estimated 50-80% of global biodiversity, provide an immense source of chemical diversity. Among marine organisms, ascidians are particularly promising due to their ability to produce bioactive secondary metabolites directly or through their associated symbiotic microorganisms. To date, over 1000 bioactive compounds have been reported from ascidians, including around 400 from the family Didemnidae and more than 200 from the genus Didemnum. Despite their recognized importance, studies on Turkish didemnid ascidians have remained largely limited to taxonomy. In this study, we investigated didemnid species distributed along the Turkish coastline, their symbiotic communities, and their marine bioactive secondary metabolites (MBSMs). Special attention was given to terpenoids and polyketides, two metabolite groups of high pharmaceutical relevance known for their antimicrobial, anticancer, anti-inflammatory, and immunosuppressive properties. Since many of these compounds are produced by associated bacteria, fungi, and protists, symbiont communities were characterized using metabarcoding approaches with both prokaryotic and eukaryotic markers. Most identified metabolites showed potential symbiotic associations. Simbionts and metabolomic profiles of twelve didemnid species were compared across species, genera, and regional sea basins. Among the three genera analyzed (*Didemnum*, *Lissoclinum*, and *Diplosoma*), *Diplosoma* exhibited the highest number of novel metabolites, particularly within terpenoid and polyketide groups, while *Lissoclinum* also displayed unique chemical diversity. In contrast, metabolite profiles among *Didemnum* species were relatively similar, although certain locations, especially Kemer and Bozyazı, showed distinct regional signatures. This study represents the first comprehensive assessment of bioactive metabolites and host-symbiont chemical ecology in Turkish didemnid ascidians. The findings provide regionally unique and globally relevant insights into marine natural products with potential applications in medicine and biotechnology.

Microbial origin of Palmerolide A in the Antarctic ascidian *Synoicum adareanum*

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Palmerolides are macrolides isolated from the Antarctic ascidian *Synoicum adareanum*. The major metabolite, palmerolide A (PalA), has potent and selective *in vitro* activity toward melanoma cells via low nanomolar inhibition of vacuolar proton ATPases (VHA). In contrast to other VHA inhibitors, such as concanamycin and bafilomycin, PalA is well tolerated by mice and displays anticancer activity in the hollow fiber assay commensurate with activity levels of taxol. Further pharmacological evaluation of the palmerolides has been hampered by lack of a reliable supply. We have recently identified a microbial producer that lives in association with *S. adareanum* but the microbe, *Candidatus Synoicohabitans palmerolidicus*, has proven recalcitrant to cultivation. In Feb/Mar 2026 we conducted field work to, among other things, identify environmental or ecological factors supporting *Ca. S. palmerolidicus*. Four distinct morphologies of *S. adareanum* were identified and the presence, absence and relative abundance of the microbe and PalA were determined. Zooid thin sections were also imaged with immunofluorescence to identify sites of vacuolar H'-ATPase enzyme expression. Morphological and biogeographical influences on *Ca. S. palmerolidicus*, preliminary work with immunofluorescence, microbiome profiling as well as cultivation efforts of field-collected isolates, will be presented.

Ascidian Biodiversity Updates along the Israeli Mediterranean Coast and the Arrival of the Caribbean Species *Didemnum cineraceum* (Sluiter, 1898).

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The Eastern Mediterranean, particularly the Israeli coastline, is rapidly changing marine environment subjected to intense anthropogenic pressures, climatic shifts, and biological invasions. These factors drive the rapid alteration of local habitats and benthic communities. Consequently, updating the taxonomic and systematic registries of marine invertebrates is critical for effective ecological monitoring and conservation. While previous studies have estimated the presence of approximately 90 ascidian species in the Levantine basin, comprehensive contemporary baselines are lacking. This study aims to map ascidian biodiversity along the Israeli coast and to facilitate future species identification. We collected 150 samples from both natural and artificial substrates across 11 coastal locations, in addition to investigating The Steinhardt Museum of Natural History ascidian collection. Utilizing a combination of morphological taxonomy and molecular (COI) characterization, our preliminary analysis identified approximately 30 distinct species. Our current baseline data highlight a complex, shifting community structure comprising both indigenous and established non-indigenous species. Among the new records of species, we report the first Mediterranean record of the non-indigenous species *Didemnum cineraceum* (Sluiter, 1898). Native to the Western Atlantic, this colonial ascidian was discovered off the central Israeli coast, extensively overgrowing aquaculture fish cage facilities. Retrospective analysis of photographic citizen science data indicates this species was present at depths up to 40 meters as early as 2015 yet remained unidentified. To evaluate its invasive capacity, year-round monitoring was conducted, revealing that despite cooler winter temperatures reaching 16 degrees Celsius, *D. cineraceum* maintains developed gonads and reproduces continuously throughout the year. This sustained reproductive output supports a rapidly expanding population that is already colonizing natural habitats adjacent to artificial infrastructure. These findings underscore the dynamic nature of Levantine benthic communities and emphasize the critical need for continuous, integrative taxonomic monitoring to rapidly detect and track new introductions before they fundamentally alter local ecosystem dynamics.

Isolation and identification of an agglutination factor in the hemolymph of *Halocynthia roretzi* against a parasitic flagellate *Azumiobodo hoyamushi*

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Soft tunic syndrome (STS) is a lethal infectious disease of the edible ascidian *Halocynthia roretzi*. This disease is causing economic losses to the aquaculture of *H. roretzi* in Japan and Korea. The causative agent of STS is a parasitic kinetoplastid flagellate, *Azumiobodo hoyamushi*, which is exclusively found in the tunic. Our previous research demonstrated that the hemolymph and blood plasma of *H. roretzi* have an activity to agglutinate and disintegrate *A. hoyamushi*, suggesting the innate immunity protects the internal organs from the invasion of the parasite. This study aimed to isolate and identify the agglutination factor in the plasma of *H. roretzi.* As lectins were most plausible candidates, we conducted affinity chromatography to isolate the agglutination factor. To determine the optimal carbohydrate ligand for affinity chromatography, seven plant lectins were examined for their ability to agglutinate *A. hoyamushi*. Three lectins were found to agglutinate *A. hoyamushi*, and wheat germ lectin (WGA) showed the strongest agglutination activity. Affinity chromatography of plasma was conducted using the agarose bound with N-acetylglucosamine, which is recognized by WGA, and obtained fractions with the agglutination activity to *A. hoyamushi*. SDS-PAGE showed a single band of 37-kDa protein in the fractions with agglutination activity. Liquid chromatography quadrupole time-of-flight mass spectrometry of the protein revealed an amino acid sequence that matched (95.1%) a gene presumed to be an intelectin in the genome of *H. roretzi*. These results indicate that the intelectin is responsible for the agglutination of *A. hoyamushi* in the plasma of *H. roretzi*.

The zooplanktonic tunicate *Oikopleura dioica* reveals an adapted genetic-toolkit against climate change-derived biotoxins

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Global warming is increasing the frequency of harmful algal blooms in marine environments, leading to elevated levels of natural toxins such as polyunsaturated aldehydes (PUAs), which can impair animal fitness, fertilization and embryonic development. However, the cosmopolitan tunicate *Oikopleura dioica* shows an unexpected resilience to these compounds. Its highly compact and fast-evolving genome makes it a powerful model to investigate both conserved stress responses and lineage-specific evolutionary adaptations. We performed RNA sequencing on control and PUA-treated embryos of *O. dioica*, including stages from pre-zygotic transcription activation stage to mid-embryonic developmental stage (8-cell, 64-cell, and incipient tailbud). To address challenges associated with compact genome annotation, we developed a new framework (CORAL), which enabled improved gene annotation and the identification of differentially expressed genes (DEGs) across stages, revealing early PUA-response genes. Gene Ontology analysis revealed that 40–58% of DEGs lack functional annotation, indicating a substantial contribution of lineage-specific genes to the stress response. Nevertheless, Gene Set Enrichment Analyses across the different stages consistently identified energy metabolism as the most affected pathway, with early downregulation followed by later upregulation, suggesting a staged physiological response to toxin exposure and highlighting this essential physiological function as a potential conserved pathway affected by PUAs across marine animals. Notably, a previously uncharacterized glutathione S-transferase (GST), involved in detoxification, was consistently upregulated on 64-cell and incipient tailbud stages. Phylogenetic analysis indicates that this gene likely arose from a lineage-specific duplication, potentially contributing to the species' enhanced resilience. Together, these findings provide a foundation for understanding the impact of climate change-related stressors on marine animals and offer insights into lineage-specific innovations underlying resilience to climate change-related stressors.

STEM CELLS, ASEXUAL REPRODUCTION, REGENERATION AND AGING

Regional Signaling Controls Stem Cell-Mediated Regeneration in an Invertebrate Chordate

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A fundamental unresolved question in regenerative biology is why some animals can regrow lost or damaged organs while others cannot. Among the chordates, ascidians (Tunicata) are the closest living relatives of vertebrates and uniquely retain the ability for whole body regeneration, providing a valuable model for exploring the evolution of regeneration within the chordate lineage. Here, we use the solitary ascidian *Ciona robusta* to investigate how local signaling environments regulate stem cell mediated regeneration. Following bisection along the anterior-posterior axis, only the posterior fragment regenerates, whereas the anterior fragment fails to do so and degenerates. Comparative transcriptomic analyses of these regenerative and non-regenerative contexts reveal distinct transcriptional dynamics and signaling environments, with JAK/STAT and MAPK pathways emerging as hallmarks of a pro-regenerative state. To identify the cell types driving this process, we combined flow cytometry, single-cell RNA sequencing, transplantation, and fate mapping. These analyses uncovered candidate stem cell populations with robust proliferative and differentiation capacity following transplantation. While *Ciona robusta* possesses broadly distributed stem and progenitor cell populations, regenerative success depends on regional signaling cues rather than stem cell abundance alone. The local activation of metabolic, immune, and differentiation-related pathways highlights the importance of spatially distinct tissue environments in determining regenerative outcomes. Together, our findings reveal how a shared injury response can diverge into regeneration or failure, providing insight into the cellular and molecular principles that underlie regenerative capacity and offering perspectives relevant to tissue repair in other chordates.

Stolonial budding in the salp *Thalia democratica* relies on multiple lineage-restricted stem cells populations

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Colonial tunicates provide a compelling system in which multiple modes of asexual reproduction and whole-body regeneration have evolved convergently. These processes have been both associated to circulating putative stem cells (often termed hemoblasts) and to epithelial plasticity, including cell dedifferentiation. However, the precise cellular origin of regenerating and budding tissues remains unresolved, largely due to the difficulty of identifying, functionally characterizing, and lineage-tracing candidate stem cell populations in vivo. Using the salp *Thalia democratica* as a model, we constructed a cellular atlas of stolonial budding that reveals the contribution of lineage-restricted stem and progenitor cells to asexual reproduction. Salps are holoplanktonic tunicates with a complex life cycle that includes an obligate asexual phase, during which individuals generate extensive chains of clonal offspring through stolonial budding. The stolon functions as a spatially organized developmental axis along which all stages of bud formation are simultaneously represented, offering a powerful system to investigate the cellular and molecular basis of non-embryonic development in chordates. Using thymidine analogues pulse-chase labeling, we identified spatially segregated stem cells populations localized at the base of the stolon, which contribute to the formation of all major tissues during continuous bud production. Complementary single-cell transcriptomic profiling of the stolon helped to resolve multiple, transcriptionally distinct stem and progenitor cell populations, each characterized by gene expression signatures consistent with restricted differentiation trajectories.
Together, these findings support a model in which asexual reproduction in a colonial tunicate is driven by multiple lineage-restricted stem cell populations - reminiscent of a vertebrate-like system - that act in a coordinated manner to generate fully functional adults, rather than by a single pool of broadly multipotent stem cells, typical of many aquatic invertebrates.

The JAK/STAT pathway structure and its role in stemness in a basal chordate

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The JAK/STAT pathway plays a pivotal role in coordinating several processes, including stemness and immune response. Here, we present the first comprehensive structural and functional analysis of this pathway in the colonial ascidian *Botryllus schlosseri*. We identified two JAKs, two STATs, and three SOCS genes, which retain global conserved domain organization while also exhibiting lineage specific diversification. Phylogenetic analysis across deuterostomes reveals independent JAK duplication events in tunicates and in their closest relatives, the vertebrates, and supports a revised evolutionary framework for STAT diversification in vertebrates. Expression profiling shows that JAK/STAT components are broadly expressed in *B. schlosseri* circulating candidate stem cells, as well as in other haemocytes involved in immunity. We demonstrated by RT-qPCR the LPS induced activation of JAK/STAT target genes and the selective pathway inhibition by the JAK inhibitor Peficitinib, evidenced in silico to be compatible with *B. schlosseri*'s JAK. JAK inhibition further reveals a direct and conserved role for this pathway in regulating stem cell proliferation: the drug administration in colonies at different phase showed a significant decrease in cell proliferation across distinct stem cell niches, establishing Peficitinib as an effective inhibitor of *B. schlosseri* JAK activity. Together, these findings underscore the central involvement of the JAK/STAT pathway in stem cell proliferation and maintenance in *B. schlosseri*, positioning this colonial tunicate as a powerful model for dissecting the role of this conserved pathway in stemness in basal chordates. While our data also suggest a possible involvement of JAK/STAT signalling in immune related processes, this aspect remains to be fully explored in future studies.

Survival Benefits Outweigh Germline Competition Costs in Kin Chimeras

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Chimerism, the coexistence of genetically distinct cells within an organism, is a widespread phenomenon in nature. In the colonial chordate *Botryllus schlosseri*, fusion between compatible colonies can lead to stem cell competition with a single genotype taking over the gonadal and/or somatic tissue of the chimera. Over five years, we quantified the frequency and consequences of chimeric formation among 809 larvae, assessing the rates of fusion after natural settlement and its long-term impact on colony survival. We found larvae preferentially settle near kin, facilitating high rates of successful fusion. Fusion was concentrated around the first month of life, boosting survival during this period of high mortality. At all timepoints chimerism significantly increased survival relative to naive and rejected colonies and extended overall lifespan. However, fusion triggered intense internal competition: in all cases tested, a single genotype achieved dominance over both germline and somatic tissues within several weeks post-fusion, regardless of initial genotype contribution. We estimate that among kin, the survival benefits of chimerism outweigh the potential costs of germline loss, extending Hamilton's kin selection framework to colonial marine organisms. In this context, chimerism functions as an adaptive strategy that enhances early survival while enabling selection among competing stem cell lineages.

Keynote: A look back at work on sexual reproduction and chimerism in *Diplosoma listerianum* (Didemnidae)

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Diplosoma listerianum colonies can be cultured indefinitely and divided to provide multiple, transparent, clonal fragments for experiments, each with tens or hundreds of zooids. In the absence of a colony-wide blood vascular system, zooids are relatively independent, with neither budding nor sexual reproduction synchronised between zooids (unlike botryllid colonies). Experiments showed that sperm released into the water is taken up by conspecific colonies, entering the oviduct and moving to the ovary. Self-fertilization is limited or absent in reproductive isolation but cross-fertilization by water-borne sperm can occur over distance, despite severe sperm dilution, to be followed by brooding of young. Uptake of compatible allosperm triggers oocyte development. Allosperm are maintained in the ovary in very close proximity to small and developing oocytes throughout vitellogenesis. Levels of mating between different clones vary widely, suggesting an incompatibility system. Self sperm and incompatible allosperm are blocked and destroyed in a particular section of the oviduct. Some of the phenomena influencing mate choice and paternity in animals with copulatory mating behaviour have been detected in *D. listerianum*: sperm precedence, rare-male advantage, and differential maternal provisioning dependent on the identity of the male. This suggests very sophisticated discrimination between sperm of different origins by the female tract, despite the absence of any information about the providers of sperm beyond that offered by the sperm themselves. Being hermaphroditic animals, the triggering of maternal investment in eggs by allosperm suggests scope for sexual conflict between the acting male (sperm source) and the acting female (sperm receiver) over the level of maternal provisioning--the number/size of offspring. The acting male would benefit from maximising female investment by the recipient, while the acting female should uphold an optimal ratio of male to female investment to maximise fitness. Studies of chimerism through indiscriminate colony fusion in *D. listerianum* will also be outlined.

DEVELOPMENTAL AND CELLULAR BIOLOGY - CELL FATE SPECIFICATION

The endoderm cell trajectory of urochordate *Styela clava* reveals the dual developmental origin and evolution of digestive tract

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The digestive system exhibits extensive diversity in developmental mechanisms and morphology across metazoans, yet the evolutionary origins underlying its organ differentiation remain unclear. Here, single-cell RNA sequencing was employed to investigate endodermal cell lineage specification during metamorphosis in the urochordate *Styela clava*, a newly established model for chordate evolution. By profiling 26,099 cells across five stages, we identified 21 major cell clusters and reconstructed the endodermal differentiation trajectories. Our analysis reveals two larval endodermal progenitor populations with distinct differentiation potentials. Pseudotime and RNA velocity analyses indicate that these progenitors give rise to stomach and intestinal lineages, respectively. Cross-species comparisons reveal putative homologous relationships between ascidian endodermal lineages and mouse definitive and visceral endodermal lineages, suggesting dual origins of digestive tract in chordates. We also identified conserved TGF-β and FGF regulatory programs in digestive organ patterning and highlight earlier fate restriction of stomach and intestinal progenitors in ascidians compared to vertebrates. These findings provide insights into how chordate digestive organs evolved from ancestral endodermal patterning programs.

The cell lineage and developmental mechanisms of the glutamatergic neurons in the brain of the *Ciona* larva

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To understand brain development at single-cell resolution in chordates, *Ciona* is an ideal model organism. However, both cell lineage and developmental mechanisms of the brain vesicle of the *Ciona* larva remain unclear because removal of the chorion, which is a commonly used technique for manipulation of *Ciona* embryos, disturbs the structure of the brain vesicle. By improving the microinjection technique and developing a method to label single cells of *Ciona* embryos with the chorion, we have studied the cell lineage and molecular mechanisms of the brain vesicle. Glutamatergic neurons are major excitatory neurons in vertebrate central nervous systems. In the brain vesicle of *Ciona* larvae, glutamatergic neurons are thought to function primarily as sensory cells, including photoreceptor and gravity-sensing (antenna) cells, and are essential for larval sensory responses. We previously demonstrated that photoreceptor cells are derived from the right side of the neural plate and reported the regulatory mechanism of the transcription factor Rx, which is thought to regulate expression of photoreceptor-related genes such as Ci-Opsin1. However, the cell lineage and developmental mechanisms of antenna cells remain unclear. Here, we investigated their cell lineage using the photoconvertible fluorescent protein Kaede. We found that antenna cells are derived from a9.33 cell on the right side of the neural plate. This cell undergoes two to four rounds of division to produce eight cells, two of which differentiate into glutamatergic antenna cells. We also showed that Rx is required for expression of the glutamatergic neuron marker vGlut in both antenna cells and their sister cells. These findings revealed that both photoreceptor and antenna cells are derived from the right side of the neural plate and further suggest that Rx commonly regulates their development. In future studies, we aim to elucidate what differences in the developmental mechanisms give rise to distinct sensory cell types.

Role of Retinoic Acid in Cardiopharyngeal Specification during Embryogenesis of *Ciona robusta*

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Retinoic acid (RA), a metabolite of retinol (vitamin A), functions as a ligand of RA receptors (RARs) that regulate development of chordate animals. Many genetic studies revealed a key role of this signaling during development of many organs and tissues including the heart. The ascidian *Ciona robusta* has emerged as a significant model system for the study of chordate development. However, although it has been widely shown the existence of a RA signaling pathway, its role during cardiopharyngeal specification has never been investigated in *C. robusta*. Therefore, we decided to investigate if, also in *C. robusta*, RA plays a key role for the proper patterning of cardiopharyngeal development as in Vertebrates counterparts. Here, we show that *Ciona* gastrulae treated with low doses of exogenous RA at the onset of Mesp activation develop into larvae exhibiting Mesp-positive cells shifted anteriorly toward the palp region. To characterize this phenotype, we co-electroporated the Mesp>GFP construct together with an Ebf>H2B:mCherry pharyngeal marker and found that RA induces alterations in the migration of these cells rather than changes in their cell fate, as indicated by the persistence of Tbx1/10 expression in the second cardiopharyngeal progenitors (aka STVCs) after RA treatment. CRISPR/Cas9 knock-out experiments targeting enzymes involved in RA signaling (CYP26, RALDH, and RAR), as well as characterization of two different RAR isoforms, are currently underway to further elucidate the role of RA signaling in cardiomyocyte specification in *C. robusta*. Understanding the role of RA signaling and the transcriptional mechanisms that drive gene regulatory networks during heart and pharyngeal development in *C. robusta* will provide a unique opportunity to shed light on the complexity of cardiac development and morphogenesis in an ancestral chordate.

An NKX2-5 homolog is required downstream of BMP signaling to pattern the sensory-adhesive organ of a tunicate larva

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Tunicates are the sister group to vertebrates within the chordate phylum, yet unlike other chordate groups, they evolved a biphasic lifecycle alternating between motile larvae and sessile adults. The papillae of most tunicate larvae are the key sensory-adhesive organ regulating their settlement and metamorphosis. The papillae are nearly always arranged as a group of three morphologically identical organs that arise from an anterior neural plate border region nested between ventral epidermis and more dorsal/posterior neural tube progenitors. Due to their embryonic origin and molecular signatures, this anterior border has been evolutionarily linked to vertebrate placode regions. It was previously shown that the specification, patterning, and morphogenesis of the embryonic papilla region all depend on BMP signaling, though downstream mechanisms remain poorly understood. Here we show that the NKX2-3/5/6 ortholog NK4 is a key transcription factor that acts downstream of BMP signaling to pattern the papillae in the *Ciona* embryo. We present evidence that NK4 is activated by BMP signaling and encodes a transcriptional repressor that is required to restrict the expression of the papilla regulatory gene Foxg to three cell clusters that give rise to the three papillae. Loss of NK4 function results in the formation of a single large papilla. In contrast, overexpression of NK4 represses Foxg, eliminating the papillae. We also show that the expression of NK4 is restricted dorsally by the BMP antagonist Chordin, while the ventrally-expressed transcription factor Msx alleviates the repressive effect of NK4, potentially allowing for the characteristic tripartite patterning of the papillae.

Opposing roles of BMP and FGF signaling in cardiopharyngeal progenitor maturation and fate choices in *Ciona intestinalis*.

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In *Ciona*, bilateral pairs of Mesp+ founder cells divide asymmetrically to produce anterior tail muscles (ATMs) and multipotent trunk ventral cells (TVCs), which are the cardiopharyngeal progenitors. TVCs migrate collectively and undergo a process of maturation before dividing to give rise to committed first heart precursors (FHPs) and secondary TVCs (STVCs), which retain multipotency. FGF-MAPK signaling is necessary and sufficient for TVC induction and later drives a feed-forward circuit underlying successive cardiopharyngeal fate choices. By contrast, the role of BMP signaling in this lineage remains largely unexplored. We present evidence that BMP and FGF-MAPK play opposing roles during cardiopharyngeal development. Early perturbation of BMP signaling, through Noggin overexpression or DMH1 treatment, causes ectopic TVC induction at the expense of ATMs, suggesting that BMP may normally dampen FGF-mediated TVC specification in founder cell daughters. Later, BMP inhibition disrupts TVC migration polarity and alters the orientation of cardiopharyngeal cell divisions. During the subsequent TVC maturation phase, bulk RNA-seq profiling of FACS-purified cardiopharyngeal cells indicates that BMP inhibition and FGF inhibition shift cardiac versus pharyngeal muscle gene expression in opposite directions: BMP loss favors the pharyngeal muscle program, while FGF loss favors the cardiac program. We propose that this reflects a dichotomy in which BMP promotes commitment toward differentiated fates, whereas FGF maintains multipotent progenitor states. We are now investigating the mechanisms underlying this antagonism. Evidence from literature suggests that cross-regulation may occur through Smad linker phosphorylation and transcriptional suppression of opposing pathway targets. Using Mesp and FoxF enhancers to perturb BMP at distinct developmental stages, combined with pharmacological MEK inhibition and quantification of pSmad and dpERK patterns, we aim to test whether this mutual antagonism operates at successive cardiopharyngeal fate decisions, reinforcing the balance between BMP-driven commitment and FGF-maintained multipotency.

Exploring Divergence of Tunicate Immune Regulatory Networks across Cionids

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Divergence in gene regulatory networks (GRNs) plays a major role in evolutionary change including the acquisition of novel cellular functions. Interestingly, these GRN changes underlying novel functions can occur either through the co-option of distinct modules of genes or individual gene co-option. As some of the latest differentiating cell types, the immune system is a playground for studying divergence and the emergence of novel functions. However, studying these changes in vertebrate immune cells is challenging given the interplay between both innate (self from non-self) and adaptive (learned) immunity. Tunicates, the closest extant invertebrate relatives to the vertebrates, are an ideal taxa to explore how rewiring of gene networks can drive novel function. Here, we share findings surrounding immune cell divergence between three closely related tunicate species from the genus *Ciona*. Our analysis of single-cell RNA sequencing data from the blood cells from three Cionid species have begun to delineate co-expressed, functionally related groups of genes that display coordinated shifts in expression across blood cell types. Further analysis of these shifted groups of genes will allow us to determine whether they represent co-opted regulatory modules. More broadly, this work has the potential to illuminate the mechanisms that drive functional divergence of blood cells in response to changes in pathogen composition or other environmental immune challenges.

Self-Nonself Recognition and Reproductive Strategies in Ascidians

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Fertilization is a fundamental biological process that generates new life with a mixed genetic background. To ensure successful fertilization, sperm and egg must undergo a complex sequence of molecular and cellular events. Ascidians are hermaphroditic marine invertebrates that prevent self-fertilization through a self-incompatibility (SI) system, which functions as a gamete-level self/non-self recognition mechanism. Understanding the molecular mechanisms of SI provides key insights into the regulation of fertilization and the evolutionary diversification of reproductive strategies in ascidians. The Themis gene family has been identified as a key determinant of SI in ascidians; however, the extent of its polymorphism, its conservation across species, and the evolutionary dynamics of SI remain largely unresolved.
In this study, we performed comprehensive polymorphism analyses of Themis genes in *Ciona robusta*, together with comparative fertilization assays to evaluate the presence and characteristics of SI across species. We further used population genetic simulations to show how such systems can arise and be maintained.

Germline Stem Cell Isolation, Lineage Tracing, and Aging in a Colonial Tunicate

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Germline stem cells (GSCs), the source of gametes, are the only stem cells capable of passing genes to future generations via sexual reproduction and are therefore considered units of natural selection. Yet, the factors that influence GSC fitness, and thus govern GSC competition, which exist in both invertebrate chordates and mammals, remain poorly understood. We studied how aging affects GSC fitness in the colonial marine tunicate *Botryllus schlosseri*, an evolutionary crosspoint between invertebrates and vertebrates. In the absence of specific antibodies for this non-classical invertebrate chordate model, we employed a unique strategy to isolate and profile GSCs from Botryllus testes using index-sorting and scRNA-Seq (Smart-seq3), supported by a new PacBio genome assembly. Their function was validated through a novel lineage tracing approach that combines membrane-labeled GSC transplantation with scRNA-Seq. Leveraging our method to isolate them, single-cell transcriptomics showed significant age-related changes between young and old GSCs. Spermatids and sperm, however, showed minimal changes, suggesting that reproductive aging is governed mainly by GSCs rather than by gametes. Reduced expressions of markers like DDX4 and PIWIL1 in aged GSCs mirrored trends in mammalian datasets, pointing to a conserved GSC-driven aging mechanism across chordate evolution. This study provides new techniques that lay the foundation to investigate GSC competition and further drivers of GSC fitness. Moreover, this study highlights fertility-related genes as promising targets for therapies to preserve reproductive health.

Single-cell mapping reveals a dynamic neural complex in the colonial tunicate *Botryllus schlosseri*

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The colonial tunicate *Botryllus schlosseri* offers a unique opportunity to study how adult nervous systems are maintained, remodeled, and regenerated in an invertebrate chordate. During its weekly blastogenic cycle, each adult zooid undergoes degeneration and replacement by a new generation, implying repeated reconstruction of the neural complex throughout life. However, the cellular composition and molecular organization of the adult Botryllus brain remain poorly understood. Here, we combined Smart-seq3 single-cell transcriptomics, spatial gene expression analysis, proliferation assays, and cell-tracking experiments to define the cellular organization of the *B. schlosseri* neural complex. Clustering identified six transcriptionally distinct cell states revealing that the adult neural complex is a heterogeneous and dynamic tissue containing neuronal, progenitor-like, epithelial-like, mesenchymal-like, and transitional cell populations. Marker analysis identified neuronal and sensory-associated genes, including NALCN, UNC79, UNC80, CDH23, and cholinergic pathway components, together with regulatory and progenitor-associated genes such as SOX2, MSI1, HES1, NOTCH, and NEUROG1. Spatial validation by HCR RNA-FISH confirmed distinct gene expression domains within the adult brain, neural-gland-associated territories, surrounding mantle tissues, and developing buds. In addition, proliferation and cell-tracking experiments suggest that neural-complex-associated cell states are embedded within broader programs of adult tissue renewal and blastogenic remodeling. Together, these data provide the first single-cell view of the *Botryllus* adult neural complex and reveal a cellular landscape in which mature neural identities coexist with progenitor-like and remodeling-associated states. This work establishes *B. schlosseri* as a powerful comparative model for investigating the evolution of adult neurogenesis, nervous system renewal, and conserved mechanisms linking regeneration and degeneration in chordates.

Tunicate underwater adhesion: cell types for sensory adhesion, their secretions and species comparisons

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In ascidians (tunicates), larval settlement and attachment are mediated by specialized sensory adhesive papillae that enable substrate exploration, habitat selection, and the initiation of metamorphosis towards a sessile adult stage. The molecular mechanisms that allow for underwater adhesion are not yet understood, but they offers significant biomimetic potential for biomedical sealants and environmentally friendly antifouling strategies. Our work investigates the cellular and molecular mechanisms underlying ascidian larval bioadhesion using the model species *Ciona*. We previously demonstrated that larval adhesion relies on adhesive proteins and modified carbohydrates produced by specialized papillar cells, the glue-secreting collocytes [1], and characterized the substrate preferences of larvae on defined surface chemistries [2]. Building on this, single-cell transcriptomic datasets now allow the assignment of adhesion-related transcripts to specific papillar cell types [3]. By integrating papilla transcriptomes with proteomic analyses of adhesive prints, we identify and investigate candidate adhesive proteins in collocytes. We elucidate the roles of glycosylation and fibre formation, demonstrating how the sugar component and fibre crosslinking contribute to larval underwater adhesion. Comparative analyses across ascidian species, including *Phallusia mammillata*, also reveal species-specific differences in adhesive systems. High-resolution imaging (HPF-TEM) is resolving the papillar architecture and adhesive granule composition. Furthermore, our long-read genome assembly for *Phallusia* has enabled the identification of extremely large extracellular proteins which are typical of bioadhesive secretions.

Core mechanism of ascidian metamorphosis

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The sessile lifestyle is a defining characteristic of ascidians. Therefore, understanding the mechanisms of metamorphosis, which is the event converting swimming tadpole larvae into sessile adults, is essential for elucidating the evolution of ascidians. It is known that adhesion to a substrate by the adhesive papillae is the innate event that triggers metamorphosis. Because adhesive papillae are neuronal organs, their neuronal properties are believed to be required for sensing adhesive stimuli. However, which subcellular organelle is responsible for sensing the stimulus generated by the adhesion? We recently found that the endoplasmic reticulum (ER) owns this role. Molecules capable of inducing metamorphosis, especially those involved in the Gq pathway, induce ER stress in papilla cells. Once stress reaches a threshold, ER homeostasis is disrupted, which is the main trigger of metamorphosis. ER homeostasis disruption initiates metamorphic events by activating protein kinases, as exemplified by Src. When the metamorphosis initiation signal reaches the tail, the second round of ER homeostasis disruption is evoked to trigger tail regression. Xbp1.d, a tunicate-specific paralog of ER stress-relevant transcription factor XBP1, customizes the larval body so as to convert the stress into the developmental switch. The mechanism centered on the ER can unify existing knowledge on ascidian metamorphosis and explain how Ciona larvae respond to multiple chemicals and experimental conditions without assuming the presence of specific receptors for each of them.

Identification of protein precursor for thyroid hormone synthesis in basal chordate ascidian *Styela clava*

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Thyroid hormones (THs) are essential for development, growth, and metabolism in animals. Although TH synthesis is well described in vertebrates, it remains elusive in invertebrates due to the lack of identified thyroglobulin (TG) orthologs, the protein precursor for TH synthesis. Here, we identified a functional TG-like protein in ascidian S*tyela clava* via immunoprecipitation-mass spectrometry combined with phylogenetic and expression analyses. In vitro iodination demonstrated that ScTG-like provides hormonogenic sites for TH synthesis. In vivo ScTg-like knockdown significantly reduced THs and inhibited larval metamorphosis. An invaginated follicle-like structure in the larval trunk, deposited with ScTG-like, was identified as the location for THs synthesis and storage. Furthermore, structural analysis of ScTG-like and predicted TG-like proteins across bilaterian phyla suggests that endogenous TH synthesis may be an ancestral and synapomorphic bilaterian trait. This study reports the first identification of a TH precursor outside vertebrates, shedding lights on the evolution of the TH synthesis.

A protocadherin mediates oral placode morphogenesis in the tunicate *Ciona*

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In chordate embryos, placodes are ectodermal thickenings around the borders of the neural plate that give rise to various sensory organs and cell types. While generally thought to be a vertebrate-specific innovation, homologous placodes are proposed to exist in nonvertebrate chordates as well. In *Ciona robusta*, a solitary tunicate, the adult mouth (the oral siphon) is derived from one such 'cranial-like' placode in the larva, which we term the oral siphon placode (OSP). At embryonic and larval stages, the OSP consists of a small rosette of cells that form from the neuropore at the anteriormost extent of neural tube closure. While the morphogenesis of the OSP and its physical separation from other surface ectoderm structures have been described in detail, how this is regulated at the molecular level is currently unknown. Here, we show the involvement of protocadherin-mediated cell-cell interactions in the proper morphogenesis of the OSP. Protocadherin.e (Pcdh.e) is expressed specifically in the OSP, but not in other surface ectoderm cells. CRISPR/Cas9-mediated disruption of Pcdh.e in these cells results in the failure of the OSP to physically separate from other structures derived from the same cell lineage. Overexpression of Pcdh.e throughout the anterior surface ectoderm results in a similar loss of a physically separate and distinct OSP territory. This effect is likely mediated by homophilic adhesion in trans, as Pcdh.e with scrambled extracellular domains failed to recapitulate the phenotype. Finally, we show that Pcdh.e expression in the OSP depends on oral placode-specific transcription factors such as Six1/2 and Pitx. Our results suggest that OSP morphogenesis requires precise regulation of a homotypic cell-cell adhesion molecule, which might reflect a conserved mechanism for placode formation in chordates.

Antenna cells of ascidian larvae are gravity-sensing neurons

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In ascidian larvae, the otolith is essential for the gravity response, and antenna cells associated with it are candidate neurons that sense gravity. To directly determine that antenna cells mediate the gravity response, we performed a behavioral analysis of larvae lacking antenna cells, and a calcium imaging experiment of the antenna cells of larvae. The antenna cells are glutamatergic neurons expressing the vesicular glutamate transporter gene, Vglut. By a deletion analysis of the upstream regulatory region of Vglut, we identified a short regulatory region that drives expression specifically in the antenna cells of larvae. Using this regulatory region, we induced apoptosis specifically in antenna cells by expressing the Bax gene. Larvae lacking antenna cells did not exhibit normal gravity response. This observation demonstrates an essential role of the antenna cells in gravity response. To further confirm that the antenna cells mediate gravitational stimuli, we expressed GCaMP specifically in the antenna cells using the same regulatory region and visualized intracellular Ca^{2+} dynamics during tilt stimulus using a "tiltable objective microscope". In preliminary observations, Ca^{2+} transients in antenna cells were observed during multiple types of tilt stimuli. Thus, the cell ablation experiment and the live imaging experiment demonstrated that the antenna cells are gravity-sensing neurons.

On the organization of the *Ciona* tunic

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The animal group Tunicata was established based on its unique pharyngeal structure together with an organic integumentary tissue that is distinct from the inorganic shells of mollusks. This integumentary tissue, termed the tunic, contains a recalcitrant substance later identified as unique animal cellulose. There is a remarkable variation in tunic appearance and cellulose content among tunicates, as seen in the leathery, cellulose-rich tunic of *Halocynthia*. Here, we report the organization of the adult tunic of the phlebobranch ascidian *Ciona intestinalis* type A (aka *Ciona robusta*), which possesses a translucent, gelatinous tunic. Anatomically consistent with past microscopic observations, the adult *Ciona* tunic, which first appears as the inner layer of the double-layered tunic of the larval tadpole, covers the entire epidermis and thickens with body growth, while harboring numerous migratory free cells within. Histological staining suggested the nature of the *Ciona* tunic to be distinct from the fibrillar collagen-based connective tissue of vertebrates, the sister group of tunicates. Quantitative proteomics revealed an assembly of proteins broadly categorized into soluble immune molecules and matrix molecules. The former category supports the view that the complement system constitutes the major immune arm at this external interface of the body, while the latter suggests a lineage-specific proteome evolution parallel to the vertebrate matrisome. After chemical purification, the *Ciona* tunic, now reduced to its cellulosic skeleton, retains its original bulk morphology and exhibits transparency. Based on the measured specific surface area, porosity, and nanostructural features of this skeleton, we sought to account for its transparency despite the substantial thickness. We propose a model for the structural continuity of cellulose across hierarchies, from single glucan chains, via crystalline nanofibers, to the integrated tunic morphology.

Tail muscle evolution in *Oikopleura dioica* sheds light on the appendicularian transition to a fully free-swimming lifestyle

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In most tunicates, the tail is present only during the larval stage and is reabsorbed during metamorphosis, giving rise to a sessile adult form. This is not the case in appendicularians, which have a fully free-swimming adult lifestyle characterized by the lifelong maintenance of a chordate-like tail and the emergence of complex tail behaviours. Our work on tail muscle development in the appendicularian *Oikopleura dioica* identified a muscle organization that differs strikingly from that of other tunicates, suggesting extensive remodelling of tail muscle lineages. In addition, we uncovered a previously undescribed muscle lineage giving rise to the most distal muscle cells of the tail tip. At the molecular level, analysis of the myofibrillar repertoire revealed a massive, lineage-specific expansion of myosin and troponin gene families in appendicularians. Expression analyses of multiple paralogs uncovered complex spatial patterns of muscle identity along the tail axis, suggesting a high degree of functional specialization. Together, these results suggest that the evolution and diversification of axial musculature played a key role in the emergence of complex tail behaviours essential for the appendicularian lifestyle. They are inconsistent with an ancestral free-swimming condition in tunicates and instead support an evolutionary model explaining the transition from a biphasic, ascidian-like ancestor to the fully free-swimming lifestyle of appendicularians.

Navigating flow: cellular and behavioral basis of rheotaxis in *Ciona* larvae

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The oceans are filled with life forms exhibiting complex adaptations that enable navigation in fluid environments and the use of fluid motion for locomotion and dispersal. However, the neuronal and behavioral mechanisms underlying navigation in flow outside vertebrates remain poorly understood. Here, we examine rheotaxis in the protochordate *Ciona* intestinalis using a combination of quantitative behavioral analysis, computational modeling, and functional imaging. Video recordings were analyzed using DeepLabCut to extract trajectories and kinematic parameters. Microfluidic approaches were used to deliver controlled flow stimuli during behavioral assays and spinning-disc whole-brain calcium imaging, and a perfusion pencil was used for targeted stimulation during sensory neuron recordings. We find that animals modulate heading and angular velocities in response to oncoming flow and display positive rheotaxis. A distributed network of ciliated peripheral sensory neurons contributes to the detection of flow velocity and direction. Pharmacological removal of sensory cilia disrupts rheotactic behavior and reduces stimulus-evoked neuronal activity. Whole-brain calcium imaging shows that the central nervous system encodes flow onset and offset, direction, and velocity. These results indicate that a simple chordate nervous system can encode multiple features of fluid stimuli and support navigation in flow.

MorphoNet: segmentation, qualitative evaluation and curation of large-scale 3D+t datasets

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Thanks to recent advances in AI, automated segmentation of imaging dataset has improved significantly. However, assessing the quality and potentially curating complex 3D and 3D+t dataset remains a difficult, time-consuming task. With the latest version of MorphoNet, we present a platform to simplify the segmentation, evaluation and curation of 3D datasets. The platform is designed for non-programming biologists and is available on all major operating systems. MorphoNet allows upgrading segmentation accuracy and enhancing interpretability across a wide range of datasets. This new approach is crucial for producing ground-truth datasets of discovery-level scientific quality, that are also critical for training and benchmarking advanced AI-driven segmentation tools, as well as for competitive challenges.

Direct Live Imaging Reveals Transcellular Migration of Mesenchymal Cells during Ascidian Metamorphosis

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Metamorphosis in *Ciona intestinalis* type A (*Ciona robusta*) can be triggered by mechanical stimulation of the adhesive organ. During this process, mesenchymal cells acquire migratory capacity and differentiate into diverse adult tissues. A subset of these cells extravasates before tail regression and later contributes to adult immune function, but the spatiotemporal dynamics underlying this behavior remain poorly understood.
Based on electron microscopy, two modes of mesenchymal cell extravasation have been proposed: paracellular migration through intercellular spaces and transcellular migration (TCM) through individual epidermal cells. However, neither mode has been directly validated by live imaging in *Ciona*. To address this, we labeled mesenchymal or epidermal cells using the photoconvertible protein Kaede or the tight junction marker ZO-1, respectively. Metamorphosis was induced by mechanical stimulation, and cell movements were tracked by live imaging. Extravasation modes were analyzed by segmenting epidermal cell boundaries visualized by ZO-1. Mesenchymal cell behavior during tail regression was classified into six temporal phases based on migration speed, and cells were further categorized into long-range and local migrants. Cells located near the trunk and those near the adhesive organ exhibited distinct phase-specific migration profiles. In addition, a subset of mesenchymal cells underwent a morphological transition from ellipsoidal pseudopodia to lamellipodia, suggesting dynamic changes in migratory mode. Live imaging of ZO-1-labeled epidermal cells revealed transient transcellular openings spanning the basal-to-apical axis of individual epidermal cells. These openings were spatiotemporally correlated with mesenchymal cell passage, providing direct evidence that mesenchymal cells extravasate via TCM through the epidermis. These findings reveal heterogeneous mesenchymal cell dynamics during *Ciona* metamorphosis and identify TCM as an extravasation mechanism, providing insight into the evolutionary origins of immune cell migration.

Decoding Chordate Development through High-Content Morpho-Topological Analysis

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Life emerges from a system of physical particles organised in time and space, encapsulated within a distinct physical boundary, observable as the organism's morphology. We hypothesise that gene expression of a cell and its neighbours over time harbours sufficient information to predict its morphology. Hence, a quantitative framework to analyse shape with the same systematic, high-dimensional analysis and resolution as gene expression is required. We address this gap by establishing 'Morphosnaker', a high-throughput computational pipeline designed to extract and analyse morphometric and topological features from 3D images of membrane- and nuclei-labelled *Ciona intestinalis* embryos. *Ciona* develops from a small number of cells undergoing a stereotyped invariant development. Despite this simple trait, its cells undergo a plethora of cell fate decisions and movements conserved in vertebrates, resulting in morphogenesis of various tissues. This simplified complexity makes it a highly reproducible biological system at a cellular level, optimal for measuring morphological traits at different spatiotemporal scales. Leveraging a massive dataset of thousands of embryos and millions of cells we construct a high-dimensional "Morpho-Space" that encodes the morpho-topological state of the organism. This space allows us to define the developmental trajectory of its morphological units (cells, cellular neighbourhoods and tissues) independent of chronological time. With this framework, we identify distinct morphological and topological signatures for various tissues, enabling label transfer from human-annotated embryos to unseen data. Our results demonstrate that geometric and topological features of whole embryos and single cells yield robust developmental trajectories. Ultimately, this establishes the potential of single cell and cell neighbourhood morphology as a computable data modality alongside the classic 'omics'.

3D imaging analysis of genes involved in notochord morphogenesis

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Abstract: The notochord is a rod-like structure running along the anterior-posterior (AP) axis that characterizes chordates. Because notochord serves as a source of inductive signals necessary for the fate determination and patterning of adjacent tissues such as neural tube and somites, proper notochord formation is crucial for normal chordate embryonic development. *Ciona intestinalis* type A (*Ciona robusta*), one of the ascidians most closely related to vertebrates, has a notochord composed of 32 cells of the A-line lineage and 8 cells of the B-line lineage (Nishida, 1987). Through cell-cell intercalation, these cells form the anterior and posterior part of the notochord, respectively, resulting in a tapered structure with the cells aligned in a single row (Veeman and Smith, 2013). However, the mechanisms linking gene expression and morphogenesis during the formation of such an ordered structure remain largely unknown.
In this study, we investigated the functions of RTTN, which is necessary for normal formation of notochord in mice (Faisst et al., 2002), and Ci-TGF-β, which is expressed in a gradient along the anteroposterior axis of the notochord in Ciona embryos (Reeves et al., 2014).
The severe phenotype caused by translation inhibition of Ci-RTTN by morpholino antisense oligo nucleotide was characterized by dispersed cells, and the tail region was not recognizable by the tailbud stage. The mild phenotype, observed in mutant embryos that developed to the tailbud stage, showed a shortened tail and a reduced number of notochord cells, suggesting that the cell proliferation was impaired. Ci-TGF-β knockdown embryos also showed notochord defects. Although notochord cell-cell intercalation appeared to occur normally, some duplicated rows running parallel to one another were observed, suggesting that Ci-TGF-β potentially regulates the arrangement of rows consisting of A-line and B-line notochord cells along AP axis. We are currently performing live imaging using notochord markers to investigate this hypothesis.

Spatiotemporal Dynamics of Neuronal Activity During the Initiation of Ascidian Metamorphosis

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The metamorphosis of ascidian *Ciona intestinalis* type A can be artificially induced by continuous mechanical stimulation of the papillae, leading to early metamorphic events such as tail regression, backward movement of epidermal cells, and mesenchymal cell extravasation from epidermis (Wakai et al., 2021; Totsuka et al., 2023). According to the connectome, primary sensory neurons (PSNs) in the papillae sensing mechanical stimulation connected to neurons in the brain vesicle (BV)--the central nervous system--via rostral trunk epidermal neurons (RTENs). Our previous study revealed that the specific activation of PSNs by optogenetics is enough to induce the initiation of metamorphosis (Totsuka et al., 2025). The aim of this study is to elucidate the neural circuits controlling metamorphosis initiated from PSNs through imaging analysis of neuronal activity before and after the onset of metamorphosis. We applied mechanical stimulation to Ciona embryos expressing GCaMP6s in neurons and measured neural activity before and after the onset of metamorphosis. In PSNs, intracellular Ca^{2+} levels increased immediately after mechanical stimulation and gradually decreased thereafter. In contrast, in RTENs, sporadic increases in Ca^{2+} were observed during mechanical stimulation until the onset of mesenchymal cell extravasation. Furthermore, in BV neurons, an increase in Ca^{2+} was observed prior to the backward movement of epidermal cells. These results revealed differences in the response of each neuronal subtype at the onset of metamorphosis. Considering the relationship between timings of each neural activity, the metamorphic events and neuronal connectivity, it was suggested that different neuronal subtypes may control the different initiation of early metamorphic event. Future studies using optogenetics will clarify the roles of these neurons in metamorphosis initiation.

THURSDAY 23 JULY

Keynote: Deuterostome Ancestors and Chordate Origins

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The Deuterostomia are a monophyletic group, consisting of the Ambulacraria, with two phyla, Hemichordata and Echinodermata, and the phylum Chordata, containing the subphyla Cephalochordata (lancelets or Amphioxus), Tunicata (Urochordata) and Vertebrata. Hemichordates and echinoderms are sister groups and are critical for understanding the deuterostome ancestor and the origin and evolution of the chordates within the deuterostomes. Enteropneusta, worm-like hemichordates, share many chordate features as adults, including a post-anal tail, gill slits, and a Central Nervous System (CNS) that deploy similar developmental Genetic Regulatory Networks (GRNs). Genomic comparisons show that cephalochordates share synteny and a vermiform body plan similar to vertebrates, but phylogenomic analyses place tunicates as the sister group of vertebrates. Tunicates have a U-shaped gut and a very different adult body plan than the rest of the chordates, and all tunicates have small genomes and many gene losses, although the GRNs underlying specific tissues, such as notochord and muscle, are conserved. Echinoderms and vertebrates have extensive fossil records, with fewer specimens found for tunicates and enteropneusts, or worm-like hemichordates. The data is mounting that the deuterostome ancestor was a complex benthic worm, with gill slits, a cartilaginous skeleton, and a CNS. Two extant groups, echinoderms and tunicates, have evolved highly derived body plans, remarkably different than the deuterostome ancestor. We review the current genomic and GRN data on the different groups of deuterostomes' characters to re-evaluate different hypotheses of chordate origins. Notochord loss in echinoderms and hemichordates is as parsimonious as notochord gain in the chordates but has implications for the deuterostome ancestor. The chordate ancestor lost an ancestral nerve net, retained the central nervous system, and also evolved neural crest cells.

DEVELOPMENTAL AND CELLULAR BIOLOGY - DEVELOPMENTAL ATLAS

Principled construction of a variation-aware developmental atlas with cellular resolution for ascidian embryogenesis

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Building cellular-resolution atlases of animal development is essential to understand the progressive emergence of order from the collective behavior of individual cells. Such an atlas fulfills two tasks: it provides a shared referential in which interindividual or interspecies variability can be quantified, while constituting a community tool to integrate experiments from various labs. Its construction, however, faces a fundamental problem: the construction procedure should be robust to variations, yet it should remain variation-aware. We present an automated framework that assigns cell homologies across independent three-dimensional time-lapse *Phallusia* embryo reconstructions by exploiting a key property of ascidian embryonic geometry: the pattern of contacts a cell forms with its neighbors is highly conserved, even when absolute positions vary. At the heart of the framework, cell identities are propagated through successive divisions based on these local neighborhood signatures. We leveraged this reference framework to extend the century-old Conklin nomenclature to internal tissues and later developmental stages, and reconciling over a century of fragmentary naming conventions into a unified reference nomenclature for over 700 cells up to the neurula stages. We used this framework to quantify natural variability. We found that small heterochronies in division timing generate marked but transient differences in cellular composition between individuals, and we uncovered unexpected variations in division orientation. We also showed that the framework generalizes across evolutionary scales to *Ascidiella aspersa*, and provides a sensitive assay for phenotyping experimentally-perturbed embryos. Together, these tools establish a quantitative foundation for population-level and comparative ascidian embryology.

Substantial gene expression variation is compatible with highly stereotyped ascidian embryonic development

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Gene expression is a stochastic process which leads to expression variation in otherwise "identical" cells. Ascidian embryos display an unusually high level of stereotypy at the cellular and subcellular levels. We wanted to test to what extent this phenotypic stereotypy was compatible with interindividual gene expression variation. To this aim, we established a single-molecule FISH protocol to count individual mRNA molecules in each embryonic cell which allows us to measure both intra-embryonic (i.e. left vs right) and inter-embryonic expression variation. We applied this procedure to two early direct target genes of β-catenin, FoxAa and FoxD, in 24-cell stage embryos of the phlebobranch *Phallusia mammillata*. The results highlight gene expression as a variable, quantitative trait which challenges the classical distinction between expressing and non-expressing cells. They also reveal that although homologous left and right cells show undistinguishable gene expression when averaged over several embryos, individual embryos can show substantial left-right expression differences. Bilateral symmetry is thus a statistical property of the population, not a cell-intrinsic precision mechanism. Comparison of the expression of the two genes reveals marked differences in the mean and variance of expression: the count distributions are overspread negative binomials of similar dispersion parameters but different means. These results suggest that transcription of these two genes is bursty, but that the mean burst size (around 3 transcripts/burst) is much lower than classically observed in Drosophila embryos. These results thus reveal that the unusually high cellular stereotypy of ascidian embryos is achieved despite substantial gene expression variation. To better define the transcriptional requirements for wild-type development, we are now lowering global transcription levels using transcription inhibitors, to test the notion that that wild-type gene expression in *Phallusia* is much higher than the minimal level of expression needed for development to proceed normally.

Identifying the biosynthetic origins of tunichromes, an enigmatic group of natural products

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Tunichromes are poorly understood marine natural products identified in the hemolymph cells of various tunicate species. These short, highly modified oligopeptides contain DOPA and dehydroDOPA moieties. These features make them oxidatively unstable and prone to covalent crosslinking but also render them excellent metal chelators. Here we present compelling evidence that tunichromes arise from much longer, genomically encoded, ribosomally produced and post-translationally modified precursor proteins (RiPPs). While RiPPs are common in bacteria and also occur in plants and fungi, they are almost unknown in the animal kingdom. A deeper exploration of tunicate genomes and transcriptomes also reveals a wealth of novel precursor proteins belonging to a large number of hitherto unidentified products. Surprisingly, antimicrobial peptides are often produced by the same precursors, strongly suggesting tunichromes to be defensive compounds against microbes. This is also supported by their expression patterns. What is more, even closely related species tend to produce different products, as shown by rapid evolutionary shuffling of sequences, gene duplications, de novo evolution of precursors, as well as proteomic analyses. Our findings suggest a major new, untapped source of marine natural products for antimicrobial discovery, present in tunicates.

An unbiased monoclonal antibody platform to map *Botryllus* hematopoiesis and allorecognition

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Natural chimerism provides a powerful biological context to study how organisms distinguish self, non-self, and compatible non-self. The colonial tunicate *Botryllus schlosseri* undergoes vascular fusion or rejection between individuals, controlled in part by the highly polymorphic BHF locus, making it a unique model for allorecognition, tolerance, and rejection. However, mechanistic dissection of *Botryllus* immunity has been limited by the lack of monoclonal antibodies that identify hematopoietic and immune cell populations. To overcome this bottleneck, we developed an unbiased monoclonal antibody discovery strategy using whole *Botryllus* blood cells as immunogen. Two rounds of immunization generated more than 130 hybridoma-derived monoclonal antibodies recognizing diverse antigens on *Botryllus* hematopoietic cells. This approach bypassed the need for prior antigen selection and created a broad reagent panel suitable for cell-type discovery, phenotyping, and future functional interrogation. We are now systematically characterizing this antibody library by flow cytometry, imaging, cell sorting, and antigen-identification approaches. Initial analyses indicate that the panel contains antibodies with distinct staining patterns across blood cell populations, suggesting that it can resolve cellular heterogeneity within the *Botryllus* hematopoietic system. We will use these reagents to map cell populations involved in fusion, rejection, and BHF-linked allorecognition. This work establishes a foundational antibody platform for *Botryllus* immunology. Beyond enabling mechanistic studies in a key marine chordate model, these reagents may help reveal conserved and divergent principles of allorecognition across evolution. By building the tools needed to study natural chimerism at cellular resolution, this platform provides a new entry point to understand how tolerance and rejection are encoded outside conventional vertebrate immune systems.

Role of Piwi in *Ciona* nervous systems

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Piwi proteins and Piwi-interacting RNAs (piRNAs) are required for the maintenance and formation of germline cells, the repression of transposable elements, and the epigenetic gene expression control. The Piwi/piRNA pathway has also been suggested to play roles in differentiation and function of somatic cells, including those in the nervous systems. For example, the epigenetic control by Piwi is involved in the synaptic plasticity of *Aplysia* sensory neurons. A murine homologue of Piwi (Mili) is expressed in adult neural progenitor cells in the hippocampus and required for sustainable neurogenesis. In this study, we explored a somatic role of Piwi in the ascidian Ciona intestinalis type A (Ciona robusta). The *Ciona* genome contains three piwi/argonaute family genes, piwi-like1, piwi-like2, and argonaute. Maternal Piwi-like1 proteins are localized during early embryogenesis, and later in primordial germ cells in the endodermal strand of the larva. Interestingly, piwi-like1 is also expressed in the larval brain, including photoreceptor cells and lens cells of the ocellus and glial ependymal cells. Two isoforms of Piwi-like1, a long form and a short form, differing N-terminal sequences, have been identified and the short form was shown to be specific to somatic cells. Brain ependymal cells in the *Ciona* larva are known to be stem-cell-like progenitor cells for the adult nervous system. Developmental fate analysis of the larval cells expressing piwi-like1 revealed that some of these cells were kept during metamorphosis and exhibited a neuron-like feature in the central nervous system of juveniles. Forced expression of Piwi-like1 in neuronal precursor cells suppressed differentiation of specific types of neurons, suggesting that Piwi-like1 may play a role in maintaining properties of undifferentiated neural precursor cells. We discuss putative roles of Piwi-like1 in the formation of the larval and adult nervous system of *Ciona*.

A mitosis-coupled regulatory transition controls collective migration of the cardiopharyngeal progenitors in the tunicate *Ciona*

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During early development of the cardiovascular system in vertebrates, dynamic gene expression underlies cell fate decisions and differentiation, as well as coordinated cellular behaviors that integrate into complex morphogenetic processes culminating into organogenesis. In mammals, the transcription factor Mesp1, one the earliest determinants of cardiogenesis, is expressed in migrating cells through gastrulation where it governs both early fate specification, by both promoting cardiovascular progenitors identities and inhibiting endodermal alternatives, as well as individual and collective behaviors. Remarkably, Mesp1 is transiently expressed and regulatory relays are thought to ensure continued deployment of the cardiogenic program, but it remains unclear whether the system requires Mesp1 to be downregulated. In the ascidian *Ciona*, a tunicate among the closest relatives to the vertebrates, a Mesp homolog is also expressed early, specifically and transiently in the earliest progenitors of the cardiopharyngeal lineage, which produced both heart and pharyngeal muscles, like its Mesp1+ mammalian counterpart. Here we began to revisit the roles of Mesp in *Ciona* and uncovered unexpected post-transcriptional regulatory mechanisms important for the transition from a naive Mesp+ progenitor state to a migration-competent Mesp- cardiopharyngeal progenitor. I will present our current understanding of this morphogenetic regulatory transition, which involves an unexpected interplay between Mesp function and retinoic acid signaling, as well as a tentative generalization of the post-transcriptional regulatory mechanisms that pace and couple successive cell divisions, regulatory transitions and fate choices in the cardiopharyngeal lineage.

MARINE POLLUTION EFFECTS

Ocean acidifications effects on calcifying and non-calcifying ascidians

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Ocean acidification (OA), a consequence of increasing atmospheric CO_2, poses a major threat to marine organisms through changes in seawater carbonate chemistry. Despite their significant ecological role, ascidians (Chordata, Ascidiacea) remain underrepresented in OA research. This study examined the physiological and structural responses to OA of two solitary ascidians: *Herdmania momus*, a calcifying species with vaterite-polymorph spicules, considered native to the Red Sea and invasive in Mediterranean Sea, and the globally distributed non-calcifying species *Styela plicata*. Individuals were exposed to three pH conditions 8.2 (ambient), 7.8, and 7.6 over a 23-day period under controlled laboratory conditions. We monitored survivorship, metabolic rates, and for *H. momus*, spicule morphology and density. Results revealed that both *S. plicata* and *H. momus* (native and non-native populations) maintained high survival and stable metabolic rates across treatments, indicating high resilience. In both populations of *H. momus*, spicule dimensions were largely stable across pH treatments, except for a shortening of tunic spicules in the Mediterranean population under lower pH level, spicule density showed only few changes under reduced pH. These findings suggest that ascidians may maintain biological fitness under near-future OA conditions, while also highlighting the need for future studies to examine whether this resilience extends across life stages, growth forms, and combined stressors such as salinity and temperature.

Transcriptomic and cellular basis of developmental thermal stress in the ascidian Ciona intestinalis.

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Multicellular organisms need to yield consistent developmental outcomes in the context of a variable environment. Therefore, patterns of gene expression are tightly regulated. Deviations from the optimal developmental temperature can be buffered in a certain permissive range, leading to a temporal scaling of developmental speed, but will become detrimental outside of this range. As the effect of thermal stress is often cell type-specific, investigations of the effects of such environmental perturbations need to be performed at a scale and resolution matching this non-uniform response, e.g. by using single-cell genomics. Here, we leverage a Norwegian population of the sea squirt *Ciona intestinalis* to investigate developmental thermal effects at the single-cell level. We constructed a thermal profile for this population and generated a de novo genome using long-read sequencing. Finally, we profiled over 80 000 cells of embryos developing at the reference temperature of 15˚C. This highly continuous atlas revealed the transcriptomic and cellular dynamics of *Ciona* development. Using a second scRNA-seq dataset (about 85 000 cells) of *Ciona* embryos grown at various permissive and non-permissive temperatures (12 to 21˚C) and sampled at developmental times matching the continous developmental atlas previously created, we investigated temperature-sensitive developmental dynamics in *Ciona*. In this temperature experiment, only the highest temperature (21°C) exhibited markedly reduced hatching rates and increased levels of developmental deformations, whereas the time from fertilization to hatching scaled from 36h (12°C) to 19h (21°C). While broad celltype identities appear to be maintained even in strongly perturbed embryos, more subtle, celltype-specific effects were observed. Using cell-type abundance, developmental progression and differential gene expression as quantitative metrics, we identify cell type-specific sensitivity to temperature stress with notochord cells appearing among the most perturbed cells. Finally, gene expression changes in response to temperature treatments are widespread and often cell type-specific and can further be releated to common stress phenotypes. Future work will aim to combine this dataset with high-content morphometric data to relate the transcriptional effects of temperature to its morphogenetic outcomes and to identify the underlying causes of temperature sensitivity in gene expression.

Plastic responses to environmental stress drove evolution of the chordate characteristics in the two species of *Ciona*

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The role of developmental plasticity and/or genetic assimilation in the evolutionary process has been a central interest for evolutionary biologists for decades. However, since the transgenerational culture of animals for many generations is not feasible apart from a few exceptions, testing related hypotheses is no easy task. To address this, we used the two sister species of tunicates, *Ciona robusta* (type A) and *Ciona intestinalis* (type B), which shows different levels of developmental buffering to thermal stress. By comparing the gene network of the two sibling species in control and stressed conditions, we found a distinction in the plastic response to thermal stress in high robustness batches (>80% normal development after thermal stress at neurula stage) compared to low robustness batches, and the distinction was clearly recognized by Random Forests, a Machine Learning technique. We specifically investigated the gene networks of CNS, notochord, cardiopharyngeal structures and muscle. As a result, we found a significant similarity of the gene network between different genotypes and different treatments in notochord and CNS, but not in heart and cardiopharyngeal developmental networks. We propose that evolution of the chordate characteristics such as notochord and CNS had biased evolution using plastic responses to environmental stress.

Multilevel responses to emerging contaminants in the tunicate *Ciona intestinalis*: a comparative perspective

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The increasing presence of contaminants of emerging concern (CECs) in marine coastal waters necessitates urgent investigation into their impacts on marine biota and human health. Antidepressants and micro- and nanomaterials, particularly fibers, have gained increasing attention, as both ranked among the most widespread and hazardous classes, especially during and after the COVID-19 pandemic. Despite their increasing presence in the coastal environment, their effects on marine biota remain poorly resolved. Here, we investigated the acute and chronic effects, alone and in combination, of the antidepressant Venlafaxine (VEN) and two types of cellulose nanofibers, non-oxidized (CNF) and TEMPO-oxidized (TOCNF), at environmentally relevant concentrations and under hotspot scenarios, on the urochordate *Ciona intestinalis* from early development to the adult stage. To enhance the ecological relevance of the study, similar analyses were also carried out on the bivalve mussel *Mytilus galloprovincialis*, a widely recognized sentinel species in marine ecotoxicology. Using an integrated phenotypic, molecular, and behavioural framework, juvenile stages of *Ciona* were investigated using phenotypic and transcriptomic analyses and larvae and adults were assessed using behavioural assessments. Our results show that single and combined exposure of VEN and CNFs induced significant, concentration-dependent, and stage/species-specific effects on developmental and for *Ciona*, also behavioral endpoints. Computational analysis of *Ciona* larval swimming dynamics reveals changes in behavioral responses and their transitions. This comparative analysis allows us to identify conserved and species-specific responses to CEC exposure across marine taxa. Overall, these findings highlight for the first time the ecological risks of mixtures of an antidepressant and cellulose nanofibers, supporting the need for integrative, multi-level approaches in ecotoxicological risk assessment of CECs.

Maritime low frequency noise impacts *Ciona robusta* physiology and behaviour

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Anthropogenic noise pollution is a recognised threat to marine wildlife, but most biological studies have focused on a few species, such as marine mammals and fish, as they possess specialized hearing organs and, in some cases, communicate using sound. However, most marine invertebrates are also able to perceive noise, particularly particle motion (i.e., oscillating particles in the medium) produced by noise, rather than noise pressure, by means of mechanosensory cells. Therefore, they may be affected by underwater noise. Ascidians have been shown to possess vertebrate-like mechanoreceptors on oral tentacles (the coronal cells), other than epidermal scattered mechanoreceptor cells, making them susceptible to noise pollution. Moreover, being sessile animals, they cannot escape the pollutant. Since noise effects are not known in detail, our study aimed to assess the impact of anthropogenic underwater noise from maritime traffic on *Ciona robusta* at juvenile stage. A playback in-lab experiment was designed to test the animals' response, using a digitally generated low frequency audio signal. The latter was based on the acoustic features proposed by the Descriptor 11 of the Marine Strategy Framework Directive as the signal representing maritime traffic noise. Before noise exposure, tanks were set up measuring both the sound pressure level and particle motion level to which animals were exposed. Morphological, physiological and molecular responses were tested after long- (1 day, during embryonic development) and short-term (1 h) exposure. Control animals were not treated with noise. Although no morphological changes were recorded in treated animals, there was a significant impact on animal physiology, with a decrease in heartbeat frequency and an increase in mechanoreceptor sensitivity, depending on noise pressure and particle motion levels. Moreover, transcriptomic analyses evidenced differences in gene expression related to physiological and cellular processes. Altogether, these results show that noise pollution negatively affects *C. robusta* juveniles.

Underwater noise negative impacts behavior and physiology of the solitary ascidian *Styela plicata*

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Underwater noises shape the marine soundscape, undergoing minimal attenuation and propagating over long distances, therefore affecting organisms far from their source. Among them, maritime traffic is the main widespread and threatening anthropogenic noise source affecting marine ecosystems. Although most studies on effects of underwater noise focus on vertebrates, increasing attention is being given to marine invertebrates. Underwater noise propagation occurs as sound pressure waves and as seawater particle motion. The latter, together with substrate vibrations, particularly affects sessile invertebrates, vulnerable because they cannot escape disturbance sources. Indeed, noise may directly damage their sensory structures, with repercussions on their behavior and physiology. In this study, we evaluated the effects of low-frequency noise on adult individuals of the solitary ascidian *Styela plicata*. Animals, collected in the Venice Lagoon (Italy), were treated with a pink noise signal enriched at 63 Hz and 125 Hz, using a 50 L tank previously calibrated with a hydrophone and an accelerometer. Noise was applied at five different sound pressure levels in the range 132-159 dB, corresponding to particle motion levels 1.6-40 cm/s2, nine animals were used per level. After 15 minutes of acclimation in the tank, two-hour videos were recorded, with the first hour serving as control (animals were not treated with noise) and the second hour for noise exposure. Videos were analysed both manually and automatically. The automatic analysis was performed developing a new strategy based on the open-source software ImagJ, allowing the detection of behavioral traits not recognisable by naked eyes. Moreover, noise effects on the filtration rate were evaluated. Results showed that noise exposure increased the time of oral siphon closure and decreased the siphon maximum opening diameter while reducing filtration activity. These findings evidence that low-frequency noise stresses animals and impairs feeding efficiency with possible negative repercussions on animal fitness.

Sound Matters: Evidence of Noise Perception and Its Effects on Physiology and Larval Distribution in *Clavelina lepadiformis*

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Anthropogenic noise in marine environments has increased markedly in recent years and is now recognized as an emerging pollutant (Camerlenghi, 2021). However, its effects on marine invertebrates--particularly sessile species that cannot avoid sound sources--remain poorly understood. *Clavelina lepadiformis* is a colonial aplousobranch ascidian widely distributed in the Mediterranean Sea. It exhibits an ovoviviparous reproductive strategy, with adults brooding asynchronously developing embryos within the atrial cavity (Berrill, 1950). In this study, gravid adults were exposed in controlled tank conditions to two different acoustic treatments using an underwater speaker: pink noise (peak bands 63-125 Hz) and a recorded ship passage. Analysis of larval spatial distribution under noise exposure suggested a tendency for larvae to settle farther from the sound source. In adults, oxidative stress biomarkers indicated physiological stress after only 1 hour of exposure, with pronounced effects after 24 hours. Noise exposure also reduced the filtration efficiency of adults, as evidenced by decreased algal uptake. Morphological analyses, performed using immunostaining and scanning electron microscopy (SEM), revealed potential alterations in sensory structures. Overall, these findings suggest that *C. lepadiformis* is capable of perceiving acoustic stimuli and that noise exposure can significantly affect its physiology and behavior. This study highlights the need to further investigate and monitor anthropogenic noise in marine environments, including its impact on vulnerable invertebrate species.

Sound perception in ascidians: behavioural and physiological responses to vibration but not sound pressure

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Anthropogenic underwater noise is increasing globally, yet its effects on benthic invertebrates, let alone ascidians, remain poorly understood. A key unresolved question is whether ascidians respond to waterborne sound pressure or to mechanically induced vibrations associated with underwater sound. For ascidians as species permanently attached to solid substrates, therefore, being directly exposed to mechanically transmitted vibrations, this distinction is particularly relevant. We investigated sound perception in the solitary ascidian *Halocynthia papillosa* using a combination of field and laboratory experiments. Individuals were exposed to acoustic stimuli between 50 and 1500 Hz while contraction responses were quantified from video recordings. When sound was played through an underwater loudspeaker producing both sound pressure and mechanical vibration, contractions occurred primarily at low frequencies (100-600 Hz). To disentangle the underlying stimulus components, we developed an experimental setup that allowed the controlled decoupling of sound pressure and vibration. Under these conditions, contractions were triggered exclusively by vibration stimuli, whereas pure sound pressure up to 130 dB re 1 µPa elicited no response. This experimental separation allowed us to directly assess which physical component of underwater noise is biologically relevant for this sessile species. In addition to behavioural responses, we assessed physiological and molecular stress responses. Vibrations at 100 Hz (0.354 m s^{-2} RMS) significantly reduced both filtration rate and respiration. Western blot analyses further revealed a significant upregulation of the heat shock protein HSP70 following exposure to ferry noise and boat hull slamming sounds, whereas pure 100 Hz sinusoidal tones did not induce a detectable stress response. Together, these results demonstrate that *H. papillosa* responds primarily to mechanically induced vibrations rather than to sound pressure itself. Our findings highlight the importance of vibration components in studies of underwater noise and suggest that the ecological impacts of anthropogenic noise on benthic organisms may have been underestimated.

EVOLUTION, SYSTEMATICS, AND TAXONOMY

Comparative Anatomy and Phylogenetic Analysis of the Nervous System and Endostyle in Marine Invertebrate Appendicularians (Chordata, Tunicata) - Paedomorphosis and Ecology as Evolutionary Drivers

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Appendicularians are marine, pelagic chordates that are widely regarded as a paedomorphic lineage within Tunicata which is derived from their retention of a larval-like body plan, small size and fast development. Although 70 species in three families are recognized (Oikopleuridae, Fritillariidae, Kowalevskiidae), a clade-wide phylogenetic analysis of phenotypic characters has not been attempted. This work addresses the gap through a comparative investigation of the nervous system and endostyle across twelve appendicularian species. The deep-sea giant appendicularian *Bathochordaeus stygius* has a brain which contains 102 neurons despite its considerable body size, a number similar to the far smaller model organism *Oikopleura dioica*. Broader comparison across all twelve species reveals striking, lineage-specific neural reductions. Oikopleuridae retain 80-102 brain cells, previously considered a functional minimum, yet Fritillariidae manage with 33-47 cells, fundamentally challenging this assumption. Families differ in caudal nerve cord routing with dominantly exhibiting right sidedness in the trajectory of the nerve cord in Oikopleuridae and left sidedness in Fritillariidae. Moreover, appendicularian endostyles exhibit lineage specific reductions: with two paired gland cell rows in Oikopleuridae, to a single row in smaller Oikopleurids and Fritillariidae, to complete absence in Kowalevskiidae. Additionally absolute numbers of gland cells in the endostyle reveal an interesting pattern of "halving": with approximately 80 gland cells in *Megalocercus huxleyi* and *Stegosoma magnum*, approximately 40 gland cells in *O. dioica*, *O. vanhoffeni* and Folia mediterranea and 18 and 24 gland cells in *Fritillaria borealis* and *F. pellucida*. Lastly, the phylogenetic analysis based on 63 characters including endostyle- and nervous system anatomy confirms the monophyly of all three families and recovers, albeit statistically weak, a sister-group relationship between Fritillariidae and Kowalevskiidae. Taken together, the findings support a progenesis-driven paedomorphic scenario in the stem lineage of Appendicularia and illuminate how miniaturization, anatomical efficiency, and habitat adaptation intersect within this marine invertebrate lineage.

Colonial structure and budding mode in *Syncarpa* composita revealed by histology and micro-CT imaging

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The ascidian genus *Syncarpa* (Stolidobranchia: Styelidae) was established by Redikorzev in 1913 with *Syncarpa oviformis* as the type species and has long been regarded as a colonial taxon. The genus currently includes two species, one of which is *Syncarpa composita*. Despite its recognition as a colonial ascidian, the colonial organization and budding mode of *Syncarpa* have not been thoroughly examined in previous taxonomic studies. Molecular phylogenetic analyses based on nuclear 18S rRNA and mitochondrial cytochrome c oxidase subunit I (COI) genes, as well as transcriptome-based phylogenomic analyses, consistently place *Syncarpa* as the sister group to the solitary genus *Dendrodoa*. In Styelidae, three major budding modes are known in colonial taxa: peribranchial, vasal, and vascular budding. However, *Syncarpa* is not included in any clade characterized by these established modes.These results raise three alternative hypotheses: (1) *Syncarpa* may not be truly colonial, but instead represent an aggregate form in which individuals are secondarily fused; (2) *Syncarpa* may possess one of the known budding modes, acquired convergently; or (3) *Syncarpa* may exhibit a previously unrecognized mode of budding. To evaluate these possibilities, we conducted histological and imaging-based analyses of multiple colonies of *S. composita*. Serial paraffin sections stained with hematoxylin were used to examine tissue continuity and budding structures, while CT scanning and MRI were applied to visualize internal three-dimensional organization. Although this study is ongoing, we present preliminary observations on the internal structure of *S. composita* and discuss their implications for understanding colonial organization and the evolution of budding modes in Styelidae.

Nano-scale nipple arrays in tunicates and similar structures in other phyla

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Tunicates, except for appendicularians, possess a cellulosic integument called a tunic. In some species, protuberances approximately 100 to several tens of nanometers in height densely occur on the tunic surface, forming a nano-scale nipple array. The presence or absence of nipple arrays appears to be associated with certain phylogenetic groups within ascidians, for example, Clavelinidae, Polyclinidae, Polystyelinae, and Molgulidae. In pelagic tunicates, the presence of nipple arrays has been reported only in some salp species that inhabit shallow depths, even during the daytime; thus, their function is possibly related to the light environment and visibility. Nano-scale nipple arrays are thought to have multifunctional properties. In addition to anti-reflective effects, the structure reduces the adhesion of bubbles, microparticles, cells, and fouling organisms. Nipple arrays of a similar size range have also been found on the body surface in other phyla. Those on the epicuticle in certain arthropods are often referred to as "moth-eye structures," and similar ones have been reported in some species of crustaceans and annelids. In some echinoderms and entoprocts, nipple arrays are formed by the tips of epidermal microvilli that penetrate the cuticle or mucus layer. In many metazoan phyla, the epidermis with a mucus layer is exposed at body surfaces. Even in these taxa, epidermal microvilli form nano-scale nipple arrays of several hundred nanometers high in some species of Cnidaria, Chaetognatha, and Pterotracheoidea (Gastropoda). These nipple array-like structures appear to have been acquired convergently in each lineage but may serve similar functions.

Unraveling the *Clavelina lepadiformis* species complex: from chromosomal rearrangements and genomic reduction, to cryptic speciation and portuary colonisations

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In the context of the sixth mass extinction, uncovering species complexes is particularly relevant for unraveling the status of species, as hidden biodiversity or currently threatened cryptic species might otherwise go unnoticed. This is especially relevant for ascidans, a clade where numerous species complexes have been reported previously. One such case is that of the colonial aplousobranch *Clavelina lepadiformis*, which is considered an invasive species distributed in harbours worldwide. However, it is also found outside harbours in the NE Atlantic and in the Mediterranean Sea, which is considered its native area of distribution. Previous studies have shown that Mediterranean and Atlantic inner-port populations and Mediterranean outer-port populations form distinct clades. Here, we use genomics to highlight differentiation at the genome level between these two clades. First, we assembled and annotated a chromosome-level reference genome for the Mediterranean outer-port form, and compared it through synteny analyses against one of the published genomes of the inner-port form of *C. lepadiformis*. We found various structural rearrangements at the chromosomal level, as well as a reduced genome in the outer form. Additionally, we used whole genome sequencing (WGS) techniques to evaluate genomic clustering of *C. lepadiformis* of 47 individuals from 9 populations to further showcase the differences between both forms of this alleged species complex. Through WGS analysis, we found a clear distinction between forms regardless of using nuclear or mitochondrial data. All inner-port samples clustered together and do not follow any isolation-by-distance pattern, although they were substructured by sampling site when analysed independently from the outer-port samples. The same pattern was also true for outer-port populations. Considering our results, we genomically confirm that the two clades of *C. lepadiformis* can be considered two cryptic species, and set the ground to formally describe the Mediterranean outer-port form as a new species of ascidian.

The taxonomy of native and non-native ascidians of New Zealand

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New Zealand is regularly absent in maps. Yet, it exists, it has a diverse biota, and ascidians are part of it.
In recent years, taxonomic research on New Zealand ascidians has indirectly benefitted from ongoing biosecurity surveys of non-native ascidians in ports and harbours. In this presentation early results of the description of a new endemic Hypsistozoa (Brewin, 1953) species will be shown. *Hypsistozoa* is currently formed by *H. fasmeriana* (Michaelsen, 1924), the type species and endemic to New Zealand; H. distomoides (Herdman, 1899), endemic to Australia; and *H. obscura* (Kott, 1969), known from the Peru-Chile Trench. New molecular and morphological evidence suggests that at least a new form of *Hypsistozoa*, endemic to the tail of the South Island of New Zealand, needs to be described and, consequently, the genus revised. A critique of taxonomic support albeit the apparent urgency for biosecurity management is also included in this presentation.

Genomic resources for ascidiid species and comparative analysis across tunicates reveal evolutionary diversification

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Ascidiid species possess highly transparent embryos, making them promising model organism for imaging analysis in developmental biology. However, genomic and transcriptomic resources for this group remain limited, with many available tunicate genomes lacking comprehensive annotation, and no large-scale comparative analysis conducted. In this study, we performed de novo genome assembly and transcriptome analysis of *Ascidiella aspersa* and *Phallusia philippinensis* (family Ascidiidae). We generated high-quality genome assemblies of 306.5 Mb (1,411 contigs; N50 = 1.2 Mb) for A. aspersa and 150.2 Mb (407 contigs; N50 = 909 kb) for *P. philippinensis*. In addition, we constructed transcriptomic datasets, including 9 adult organs and 6 embryonic stages for *A. aspersa,* and 5 adult organs and 9 embryonic stages for *P. philippinensis*. Using ab initio and homology-based approaches, we predicted 24,504 genes in A. aspersa (BUSCO completeness: 92.2%; 18,636 functionally annotated) and 20,928 genes in *P. philippinensis* (BUSCO completeness: 88.6%; 16,507 functionally annotated). We further conducted a genome-wide comparative analysis incorporating 35 publicly available tunicate genomes spanning three classes, five orders, and 12 families. Gene models were constructed for 27 species with BUSCO scores greater than 80%. Phylogenetic analysis suggested a revised hypothesis for the relationships among families within Phlebobranchia and Aplousobranchia. Gene family analysis revealed lineage-specific contractions, including the loss of several DNA repair related genes shared among Thaliacea. Tunicate genomes exhibited substantial variation in genomic GC content (28.0%-42.7%). Notably, A. aspersa showed the highest GC content in coding regions and the third position of codons among tunicate species, along with a distinct codon usage bias compared to other ascidiid species. Finally, we developed an online comparative tunicate genome database (TUNOME), which provides functional annotations and ortholog analyses based on these genomic and transcriptomic resources. These datasets establish a basis for developmental studies in non-model tunicates and enable broad comparative analysis.

Keynote: Retinoic acid, tunicate development, and chordate evolution

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Retinoic acid (RA) signaling plays essential roles in ascidian development. We investigated its functions in embryogenesis and budding. Genes encoding the RA synthase (Raldh2), the RA-degrading enzyme (Cyp26), and the RA receptor (RAR) have been identified in *Ciona robusta* (*C. intestianlis* Type A). Non-overlapping expression domains of mesodermal Raldh2 and anterior neuroectodermal Cyp26 are conserved between ascidians and vertebrates. Microarray analyses identified embryonic RA target genes such as Hox1. Both epidermal and neural enhancers of Hox1 contain a functional RA response element required for normal endogenous expression. Inhibition of Raldh2 or Hox1 resulted in individuals lacking the atrial siphon primordia. Thus, the RA-dependent regulation of Hox1 is conserved among chordates and is functionally linked to tissue differentiation and morphogenesis in ascidians. In contrast, the larvacean *Oikopleura dioica* lacks canonical RA pathway genes, yet shows a similar spatial expression pattern of Hox1. The 5' flanking region of *Oikopleura* Hox1 was transcriptionally active in the *Ciona* embryo, and the enhancer of *Ciona* Hox1 was activated in the *Oikopleura* embryo. These observations suggest that *Ciona* and *Oikopleura* share a common RA-independent mechanism for Hox1 activation. In *Ciona*, RA-dependent and RA-independent pathways coexist as redundant regulatory systems. RA is also required for bud development in the budding ascidian *Polyandrocarpa misakiensis*, where it promotes dedifferentiation and proliferation of multipotent epithelial stem cells, partly via induction of a plasmin-like protease. These findings suggest that ascidians have acquired a novel mode of RA utilization associated with asexual reproduction. Together, these results highlight both conserved and divergent roles of RA signaling in tunicate development and chordate evolution.

GENE REGULATION

Deep Evolutionary Conservation of Hmx Transcription Factors and their Role in Sensory Ganglia

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The evolutionary origin of vertebrates included innovations in sensory processing crucial for the development of a predatory lifestyle. Central to these innovations are cranial sensory ganglia (CSG), which arise predominantly from cranial placodes. The homeobox transcription factors Hmx are key regulators of vertebrate sensory ganglia development and play a conserved role in neural differentiation. In the tunicate Ciona intestinalis, Hmx drives the differentiation of bipolar tail neurons, suggesting homology of these cells with vertebrate CSG. Comparative genomics has revealed two conserved non-coding elements (CNEs), associated with the Hmx3-Hmx2 locus. These CNEs are exceptional for their size, their deep evolutionary conservation across jawed and jawless vertebrates, and tandem duplication in the stem-vertebrate lineage. Remarkably, these enhancers exhibit robust regulatory function in both vertebrates and invertebrate chordate, indicating that the upstream regulatory network governing Hmx predates vertebrates. By comparing downstream Hmx targets in the central and peripheral nervous system of both Ciona and chicken embryos, we are trying to uncover the mechanisms underlying the conserved regulation of Hmx. This cross-species approach will allow us to distinguish ancestral regulatory functions from lineage-specific adaptations, and to reconstruct the core gene network controlled by Hmx in early chordates. These findings will also provide critical insights into how deeply conserved regulatory systems contributed to vertebrate sensory innovations.

Evolutionary changes in the mechanisms regulating peripheral nervous system formation in *Molgula appendiculata*.

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The development of organisms relies on finely coordinated regulation of gene expression and cell-cell communication encoded by gene regulatory networks (GRNs). While these networks are globally conserved, their modification can lead to the emergence of new structures or phenotypic diversity. In ascidians, the peripheral nervous system (PNS) derives from the neural plate borders and shares evolutionary origins with vertebrate placodes and neural crest. The GRN underlying PNS specification has been extensively characterized in *Ciona intestinalis*, providing a reference framework to explore evolutionary variations. Using a comparative approach across four species (*Ascidia mentula*, *Phallusia mammillata*, *Clavelina lepadiformis*, and *Molgula appendiculata*), I examined how this regulatory program is deployed during PNS development. While the overall morpho-molecular organization of sensory neurons is largely conserved across species, Molgula appendiculata displays a marked reduction in neuron number. This reduction correlates with altered spatial expression of key transcription factors and atypical BMP signaling dynamics. These findings suggest that PNS reduction in *M. appendiculata* is not due to the loss of core regulatory components, but rather to modifications in the connectivity and signaling thresholds within a conserved network architecture. More broadly, this study illustrates how evolutionary changes in GRN deployment can generate trait modifications while maintaining the overall developmental scaffold, offering new insights into how biological complexity is rewired through evolutionary time.

Hypomethylation in *Oikopleura dioica* genome

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Cytosine DNA methylation is a conserved eukaryotic epigenetic modification that plays central roles in gene expression, cellular differentiation, and development. It is widespread across animal phyla, with only a few documented cases of secondary loss. Methylation patterns differ among animals: vertebrates typically exhibit high, genome-wide CpG methylation, whereas invertebrates display sparse or mosaic methylation, with some genes heavily methylated and others nearly unmethylated. Because methylated cytosines are prone to spontaneous deamination to thymine, methylated regions lose CpG sites over evolutionary time, producing CpG observed/expected (O/E) ratios below one; the mosaic methylation characteristic of invertebrates therefore gives rise to bimodal CpG O/E distributions. We investigated DNA methylation in tunicates and found that the appendicularian *Oikopleura dioica* exhibits a unimodal CpG O/E distribution centered around one, consistent with absence of cytosine methylation. To test this hypothesis, we searched tunicate proteomes for core enzymes required for cytosine methylation and demethylation. HMMER analyses indicate that *O. dioica* lacks canonical components of the methylation machinery. In parallel, analysis of Oxford Nanopore reads identified a small set of CpG sites with high apparent methylation frequencies, yet the overall distribution of fractional methylation differed markedly from that seen in other tunicates. In the absence of canonical methylation machinery, and considering the background noise inherent to Nanopore methylation detection, the biological relevance of these signals remains uncertain. Taken together, our results support loss of genome-wide cytosine methylation in *O. dioica*, placing this species among the rare animal lineages which underwent secondary loss of DNA methylation.

Six3/6 acts downstream of canonical Wnt signaling to regulate the formation of anterior neural border-derived structures in ascidian embryos

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Three protruding papillae at the anterior-most region of larva of the ascidian *Ciona intestinalis* constitute an adhesive, mechanosensory and chemosensory organ, known as the palps, essential for settlement and metamorphosis. The palps derive from the anterior neural plate border, formation of which is regulated through the combined actions of FGF, BMP and Wnt signaling pathways before neurulation. Here, we show that, following the formation of this precursor territory, canonical Wnt signaling acts again to regulate palp formation. Activating Wnt during neurulation abolishes palp formation. Reciprocally, inhibiting Wnt leads to the formation of an ectopic fourth palp-like structure posteriorly. Moreover, we show that Six3/6, expressed posteriorly to the palps in the anterior brain and oral siphon, is activated by Wnt and likely mediates its action. In other deuterostomes, Six3/6 is expressed in an equivalent anterior domain away from posteriorizing Wnt activity. We conclude that Wnt regulates the size of the anterior neurectoderm in *Ciona* as in other deuterostomes, but in a different manner. Deciphering changes in Wnt-mediated transcriptional regulation at the invertebrate/vertebrate transition is crucial for understanding the evolution of anterior nervous system patterning and the emergence of vertebrate unique characters.

Lzts is a new player in the gene regulatory network for *Ciona* Bipolar Tail Neurons

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In tunicates, there is still very limited knowledge on the molecular mechanisms orchestrating the formation of bipolar tail neurons (BTNs), which are a type of sensory neuron located along the tail nerve cord and are considered the functional equivalent of the vertebrate cranial sensory ganglia (CSG). Here, we investigate for the first time the role in the formation of BTN cells during development of Lzts genes, which belong to a family whose function is poorly understood. First, through a complementary evolutionary genomics approach combining phylogenetic, synteny and gene structure data, we defined the clear orthology existing among the sole invertebrate Lzts1/2/3/4 and the four gnathostome Lzts subfamilies (Lzts1, Lzts2, Lzts3, Lzts4). Thus, we characterized the dynamic expression pattern of the unique tunicate Lzts in the larval BTNs of *Ciona robusta*. Coupling RNA-seq datasets analysis and CRISPR/Cas9 approaches, we identified Lzts as a key player in the control of BTN formation. Functional analyses with specific markers (Rimbp, Gad) showed that the inactivation of Lzts increases the number of BTNs, suggesting a key function in the differentiation and biogenesis of anterior BTN cells. Additionally, functional approaches showed that Lzts gene is regulated by Neurogenin in the context of BTN differentiation. Altogether, our data support a major, previously unrecognized, role for the sole tunicate Lzts in the gene regulatory network underlying the control of the number of BTNs during development.

Repressive Roles of Erf and Elk in FGF-Regulated Neural Development in *Ciona intestinalis*

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The emergence of new cell types, such as the neural crest and placodes in vertebrates, often relies on conserved signaling pathways that are newly interpreted at the transcriptional level. The dynamic network of multiple ETS transcriptional effectors downstream of RTK/ERK signaling is poorly understood despite its central role in development and cancer. We examined how co-existing ETS repressors and ETS activators interpret FGF/ERK signaling at successive binary cell fate choices in the invariant neural developmental lineage of tunicates/ascidians. Genetic interference shows that both Erf and Elk act as repressors, playing successive roles at different transcriptional targets. We propose an ETS site occupancy model in which ETS activators and repressors compete for the same DNA binding sites, and whereby the different balance of this competition drives adjacent cells towards opposing fates. This mechanism provides a general framework for ERK/ETS factor-controlled cell fate switching in adjacent territories, also beyond ascidians. It may further the interpretation of other ETS activator and repressor activities including Elk-mediated repression in neural crest specification and deregulation downstream of ERK in stem cells and cancer.

MAPK signaling integrates immune and stress responses during colonial blastogenesis in *Botryllus schlosseri*

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Mitogen-activated protein kinase (MAPK) pathways are evolutionarily conserved signaling cascades that regulate key cellular processes such as proliferation, differentiation, and stress responses. In the ascidian *Botryllus schlosseri*, the colony consists of repeated functional units called zooids, which are continuously replaced through blastogenesis, a cyclical developmental process requiring immune-mediated clearance of aging cells. We identified two MAPK genes, bsmapk-1 and bsmapk-8, homologous to vertebrate erk and jnk, and examined their transcriptional dynamics throughout the blastogenetic cycle. The two genes showed distinct temporal patterns and differential responsiveness to immune stimulation and environmental stressors, suggesting pathway-specific roles. Functional inhibition of ERK and JNK signalling impaired hemocyte phagocytosis, altered the expression of genes involved in oxidative stress defense (sod, gpx-5) and stress granule dynamics (tiar, ttp), and disrupted normal blastogenesis, resulting in reduced zooid growth and developmental arrest. These findings demonstrate that MAPK pathways act as critical integrators of immune function, stress responses, and developmental renewal, ensuring the coordinated turnover and maintenance of colonial modules in a non-vertebrate chordate.

DNA Barcoding Survey of Ascidians in the Ryukyu Archipelago

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Ascidians (class Ascidiacea, phylum Chordata) inhabit a wide range of marine environments worldwide, from shallow coastal waters to the deep sea. Approximately 3,000 species have been described globally, of which about 300 species, roughly 10%, have been recorded from Japan, making the country one of the global hotspots of ascidian diversity. The waters surrounding the Ryukyu Archipelago are expected to harbor particularly high ascidian diversity due to their heterogeneous benthic environments shaped by Ryukyu limestone formations and the Kuroshio Current. Previous studies on ascidians in this region have mainly consisted of taxonomic and ecological research focused on photosymbiotic ascidians, with some more recent efforts included the description of several new species. However, comprehensive surveys of regional ascidian fauna remain insufficient. To address this gap, we conducted a DNA barcoding survey to assess ascidian diversity across the Ryukyu Archipelago. From May 2025 to March 2026, scuba-based field surveys were carried out at 28 sites, mainly around Okinawa Island. Specimens were photographed in situ, and piece of tissue were preserved in 99% ethanol for molecular analyses, while voucher specimens were fixed in 10% seawater formalin for morphological observation. A total of 220 samples were collected, representing 69 OTUs across 14 genera and 8 families. Among these OTUs are potentially undescribed species, as well as many novel DNA sequences, were obtained. This study establishes the first comprehensive dataset integrating specimen-based and molecular information for ascidians in the Ryukyu Archipelago, providing an essential taxonomic foundation for future studies and enabling a higher-resolution understanding of subtropical ascidian diversity.

Sleep in solitary ascidians? Diurnal behavioral and neural rhythms in *Styela plicata*

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The ability to anticipate daily environmental changes through rhythmic activity is a fundamental property of living systems, yet its neurophysiological basis in invertebrate chordates remains poorly resolved. Sleep, in particular, is a conserved biological phenomenon across diverse taxa, but its evolutionary origins and minimal neural requirements are still under debate. Here, we examine whether a solitary ascidian exhibits signatures consistent with a sleep-like state by investigating diurnal neural and behavioral rhythms in *Styela plicata* (Chordata, Ascidiacea, Stolidobranchia). We recorded extracellular neural activity from the cerebral ganglion over 24-48 h periods, alongside continuous behavioral monitoring of siphon dynamics under controlled light-dark and constant darkness conditions. Our results reveal robust diurnal patterns in both neural and behavioral outputs. Neural firing rates were significantly elevated during the daytime, accompanied by faster responses to mechanical stimulation, indicating heightened responsiveness. In contrast, during dark phases, individuals exhibited prolonged siphon opening bouts and reduced neural activity, suggestive of a quiescent state. Importantly, rhythmicity persisted under constant darkness, supporting the presence of an endogenous circadian clock. The observed reduction in responsiveness and neural activity during the dark phase, together with distinct behavioral quiescence, are consistent with criteria commonly used to define sleep-like states in invertebrates. These findings position *S. plicata* as a promising model for exploring the evolutionary origins of sleep and circadian regulation within chordates. More broadly, our study provides a framework for integrating neural recordings with behavioral analyses to investigate fundamental questions about rhythmicity and rest states in simple nervous systems. By bridging neurophysiology and behavior in this unique group of chordates, we offer new insight into how sleep-like states may have emerged early in chordate evolution.

Botrylloides and _Botryllus_ from Victoria, Australia

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Australia has over 700 described ascidian species, largely due to the immense efforts of Patricia Kott during her 50-year career. The Botrylloides and *Botryllus* species are not well-understood, however. Dr. Kott identified *Botrylloides magnicoecus* and *Botrylloides saccus*, but several other species with distinct morphological characteristics were classified as *Botrylloides leachii*, a species previously only known from Europe. Four of these species, *Botrylloides jacksonianus*, *Botrylloides leptus*, *Botrylloides pannosus*, and *Botrylloides purpureus* were originally described by Herdman. Samples collected from around Victoria, Australia, have been barcoded, and are presently being characterized morphologically. These samples are also being compared to the syntypes of the original Herdman species. Barcode sequencing has confirmed the presence of widely distributed species *Botrylloides diegensis* and *Botryllus schlosseri* in Victoria, as well as *Botryllus gaiae*. We believe this is the first record of *B. gaiae* outside Europe.

Hidden diversity in ascidians of the genus _Pyura_ (Chordata, Ascidiacea, Pyuridae) in Belize

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Ascidians (also known as sea squirts) are a group of marine benthic invertebrates in the Phylum Chordata. Proper identification of ascidians is important because many ascidian species have been introduced beyond their native ranges and can become invasive, posing a threat to marine environments and aquaculture. However, identifying ascidians using morphological techniques alone can be difficult. Many related species have very similar morphological characters, and there are few taxonomic experts. DNA barcoding uses molecular markers to aid in proper identification and increases accessibility to taxonomic information. Indeed, several morphologically cryptic ascidian species have been found using molecular-based phylogenetics. According to the published literature, of the 101 accepted species in the genus *Pyura*, only three are known from Belize. Here, we sequenced the mitochondrial Cytochrome Oxidase I (COI) gene from 65 samples collected from artificial (docks, marinas) and natural (mangrove, reef) habitats in Belize and conducted phylogenetic analyses. Our results indicate that there are at least six *Pyura* spp. in Belize, only one of which (*Pyura vittata*) had a DNA barcode match in GenBank, highlighting the continued need for integrative morphological and molecular taxonomy. Additional *Pyura* spp. from Belize were identified by combining molecular and morphological characters, providing information on the relative distribution of species on artificial and natural substrates.

Poster Abstracts

ASEXUAL REPRODUCTION, REGENERATION & AGING

Comparative transcriptome analysis of aging in botryllid ascidians

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Colonial tunicates offer a unique perspective on aging; as chordates, they do not exhibit typical vertebrate senescence. Despite regenerating their entire soma weekly via lifelong stem cells, colonies undergo precise morphological decline shortly before death. This study performs a comparative transcriptomic analysis of *Botryllus niger* and *Botryllus humilis* to identify conserved and species-specific molecular markers of natural aging. By comparing healthy young colonies with senescent old colonies, we aim to define the core genetic program of botryllid senescence. Using de novo transcriptome assembly and differential expression profiling (DESeq2), we identified the molecular drivers associated with senescence in *B. niger*. Preliminary results reveal significant upregulation of genes involved in structural remodeling and cellular defense in older colonies. The prominent expression of SCO-spondin and Actin homologs suggests a compensatory response to the physical degradation of the extracellular matrix and the colonial tunic. Simultaneously, the upregulation of Heat Shock Protein 90 (HSP90) indicates increased proteostatic stress. Conversely, aging was marked by a sharp decline in energy metabolism, specifically through the downregulation of ATPases and mitochondrial transport proteins, reflecting a systemic reduction in the colony's metabolic budget. Furthermore, several highly differentially expressed transcripts yielded no known homologs in the Swiss-Prot database, representing potential novel, tunicate-specific regulators of longevity. By integrating data from both species, we will distinguish between universal colonial aging markers and species-specific adaptations, providing a foundational framework for understanding the evolution of senescence in colonial chordates.

Molecular Characterization of Multipotent Cells in Botryllus primigenus

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Colonial tunicates provide a powerful model system for elucidating regulatory mechanisms governing the maintenance, proliferation, and differentiation of adult somatic multipotent cells. Under certain conditions, gonads develop in individual zooids, and germ cells differentiate in concert with asexual budding. Thus, colonial tunicates provide a unique experimental system for dissecting the cellular and molecular mechanisms underlying the establishment and maintenance of germline stem cells, as well as the regulation of gametogenesis. In our laboratory, we have used *Botryllus primigenus* as a model organism to study these processes. Among tunicates, colonial species in the family Botryllidae serve as valuable experimental systems. Previous studies have identified genes expressed in multipotent cells during palleal budding and vascular budding (whole-body regeneration). In addition, characteristic gene expression profiles of germline stem cells have been described, and their functions are increasingly being elucidated. In recent years, we have generated transcriptomic datasets and initiated genomic analyses. Our aim is to elucidate the unique biological features of *B. primigenus* and the conserved molecular genetic mechanisms shared among colonial ascidians in the family Botryllidae. Here, we present gene expression and functional analyses in B. primigenus and promote further discussion within the tunicate research community.

BEGINNING OF EMBRYOGENESIS

Conservation and variation in early embryogenesis: a comparison of cleavage patterns in *Botryllus schlosseri* and *Clavelina lepadiformis*

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Embryogenesis in ascidians has long been regarded as a textbook example of mosaic, highly determinate development. Cleavage patterns are remarkably robust and conserved across species that diverged hundreds of millions of years ago, with the Conklin nomenclature remaining applicable across major clades, including Stolidobranchia and Phlebobranchia. The stereotyped sequence of cell divisions is similarly conserved, at least up to gastrulation and early neurulation. However, these observations are almost exclusively derived from solitary species. Colonial ascidians exhibit markedly different reproductive strategies, including brooding, reduced embryo number, and the presence of placenta-like structures. Embryogenesis is generally slower (days versus hours in solitary species) and often produces larger larvae with advanced organogenesis. These differences raise the question of whether early embryogenesis is either largely conserved, as in solitary forms, or instead modified in ways that reflect the emergence of budding, potentially including shifts in cleavage dynamics or early lineage segregation. To address this, we started to compare early cleavage stages, up to early gastrulation, in two phylogenetically distant colonial ascidians, *Botryllus schlosseri* and *Clavelina lepadiformis*. We developed optimized in vitro fertilization protocols and combined confocal and light-sheet (SPIM) microscopy to generate high-resolution 3D reconstructions of early embryos. Our analyses reveal a largely conserved cleavage geometry during the first divisions, supporting a shared developmental framework. Video-microscopy also indicates that despite a greatly expanded cell cycle duration, the order of cell divisions and temporal sequence of mitotic domains are like in solitary species. However, we also detect subtle but reproducible differences, particularly in the behavior of germline-associated blastomeres (e.g., B6.3), suggesting potential shifts in lineage segregation. These preliminary results point to a conserved developmental backbone with localized divergence, opening the possibility that coloniality is accompanied by early embryonic modifications.

DEVELOPMENTAL AND CELL BIOLOGY

Protocadherin-1 controls endoderm invagination during ascidian gastrulation independently of cell fate specification

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Gastrulation is a fundamental morphogenetic process in which coordinated cell behaviors drive the internalization of embryonic tissues. In ascidians, this process is highly reproducible despite potential variability in gene expression and relies on a well-defined gene regulatory network (GRN) governing endoderm formation. Here, we investigate the role of protocadherins during gastrulation in the ascidian Phallusia mammillata. Although protocadherins belong to the cadherin superfamily, their functions in early embryogenesis remain poorly understood. We focused on protocadherin-1 (Pcdh1), which is specifically expressed in pre-endodermal blastomeres at the onset of gastrulation, with a conserved expression pattern between *Ciona* and *Phallusia*. Using a morpholino-mediated loss-of-function approach, we show that Pcdh1 is essential for endoderm invagination. Pcdh1-depleted embryos fail to undergo gastrulation and arrest at early stages, whereas control embryos develop normally. Quantitative analyses confirm a strong reduction in the proportion of invaginated embryos under Pcdh1 knockdown conditions. Endodermal specification remains unaffected, as assessed by the expression of canonical markers, indicating that Pcdh1 is dispensable for cell fate determination but required for morphogenetic progression. In parallel, PI3K inhibition also prevents endoderm invagination, but in this case with a broader impact on endoderm specification. Preliminary observations further indicate that FAK perturbation similarly disrupts invagination. Together, these perturbations suggest that endoderm invagination depends on multiple regulatory layers, including both fate-specification pathways and adhesion- or mechanics-related processes. More broadly, this work contributes to an ongoing effort to refine the endodermal GRN by incorporating adhesion and signaling components. It provides a framework to investigate how morphogenetic processes are coordinated with gene regulatory activity, and how robustness may emerge despite variability in upstream gene expression.

Optimizing CRISPR/Cas9 Genome Editing in *Ciona intestinalis*

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Ciona intestinalis is a powerful system for studying chordate development due to its simple body plan, rapid embryogenesis, and well-annotated genome. However, relatively low CRISPR/Cas9 editing efficiency remains a limiting factor for consistent gene perturbation in this species. Moreover, globally distributed and highly invasive Ciona intestinalis populations exhibit substantial natural genetic variation, further complicating guide RNA design and target site fidelity. To address these challenges, we are systematically optimizing key CRISPR/Cas9 components to improve genome editing efficiency and reproducibility in *Ciona intestinalis*. To enhance sgRNA design, we performed long-read nanopore sequencing of genomic DNA from individuals collected in the Gulf of Maine and compared these data to available genome assemblies from Roscoff and Plymouth, enabling assessment of population-level variation at candidate target sites. Building on previous work, we identified significant species-specific sequence divergence in regulatory elements, including promoters used to drive Cas9 expression and the U6 promoter commonly used for small RNA expression in tunicates. To improve sgRNA delivery and expression, we generated a U6>sgRNA (F+E) vector incorporating endogenous U6 promoter sequences from *C. intestinalis*. In parallel, to optimize Cas9 expression and enable robust, tissue-specific editing, we employed *C. intestinalis*-specific promoters. Together, these optimizations establish a more reliable and efficient CRISPR workflow in *Ciona intestinalis*, enhancing its utility as a model for functional genomics and developmental biology.

A conserved gene regulatory circuit underlies sensory neuron identity across chordates

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Vertebrate sensory ganglia contain diverse neuron types specialised for distinct sensory modalities. In vertebrates, epibranchial (viscerosensory) and auditory neurons arise from the posterior placodal domain and are specified by distinct transcription factor programs, including Pax2/5/8, Phox2 and Err. However, the evolutionary origin of this regulatory architecture remains unclear. We hypothesise that Pax2/5/8 controls the specification of posterior placode-like sensory neurons through an ancestral gene regulatory module that predates vertebrates, giving rise to distinct sensory neuron subtypes via downstream Phox2- and Err-dependent programs. To test this, we combine comparative single-cell transcriptomics, gene expression analyses, and CRISPR-mediated perturbations across amphioxus, *Ciona intestinalis*, chicken embryos, and mice. We assess whether Pax2/5/8 regulates Phox2- and Err-expressing sensory neuron populations across chordates and evaluate the extent to which this regulatory hierarchy is conserved. By reconstructing conserved and lineage-specific features of this gene regulatory network, we aim to determine whether modification and expansion of an ancestral posterior placode neuron-like module contributed to vertebrate sensory neuron diversity. This work provides insight into how regulatory rewiring and gene duplication facilitated the evolution of vertebrate sensory neuron diversity.

Reporter analysis of Foxa.a enhancers driving anterior expression at the 8-cell stage in *Ciona robusta*

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In *Ciona robusta*, Foxa.a is one of the earliest zygotic genes to be expressed. Its expression begins at the 8-cell stage and is restricted to the anterior blastomeres in both the animal and vegetal hemispheres. Foxa.a plays a central role in subsequent cell fate specification. However, the enhancer elements responsible for its anterior-specific expression at the 8-cell stage have not been identified. To identify enhancer regions required for 8-cell stage expression, we analyzed a series of LacZ reporter constructs containing 5' flanking sequences of Foxa.a. A 1.77-kb upstream region, previously shown to drive expression in tailbud embryos, also drove anterior-specific LacZ expression at the 8-cell stage. To further dissect this activity, a set of deletion constructs based on this 1.77-kb region was analyzed. Deletion of an internal region of approximately 500 bp markedly reduced reporter activity at the 8-cell stage. None of the deletion constructs tested caused precocious expression, expansion into posterior blastomeres, or restriction of expression to a single hemisphere. These results suggest that this approximately 500-bp region contains an enhancer required for 8-cell stage expression. The maternal transcription factor Gata.a is already present in the nuclei of all blastomeres at the 8-cell stage. The deleted region overlaps with a Gata.a ChIP-seq peak and contains a Gata.a recognition sequence, raising the possibility that Gata.a activates transcription through this region. Sox1/2/3 is also expressed in the anterior four blastomeres at the 8-cell stage. To further investigate transcriptional regulation in anterior blastomeres, we constructed a LacZ reporter containing a 2.3-kb upstream region of Sox1/2/3 and generated a series of deletion constructs. The results of this analysis will be presented.

Temporal control of gene expression by DNA replication in *Ciona* embryos.

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Gene expression in the ascidian embryo is tightly regulated in a stage-dependent manner. The acetylcholinesterase-encoding gene (AChE) is predominantly expressed in muscle-lineage cells from the gastrula stage onward. Treatment of embryos with the DNA replication inhibitor aphidicolin revealed stage-specific effects on this expression: continuous exposure beginning at the 32-cell stage abolished AChE expression even after the gastrula stage in *Ciona robusta*, whereas treatment initiated at the 110-cell stage had little effect. These observations suggest that progression through a certain number of DNA replication cycles is required to establish competence for AChE expression. When aphidicolin was applied from the 64-cell stage, Mrf mRNA, encoding a transcription factor that activates AChE expression, was expressed in B7.4; however, AChE expression was inhibited in this lineage despite the presence of Mrf. Similarly, when aphidicolin was applied from the 32-cell stage, Zic-r.b and Tbx6-r.b mRNAs, encoding transcription factors that activate Mrf expression, were expressed in the muscle lineage; however, Mrf expression was inhibited despite the presence of these upstream factors. To examine whether these transcripts are translated, we introduced GFP-fused constructs containing the endogenous regulatory and coding sequences (with GFP fused at the C-terminus) into embryos. GFP fluorescence was detected even under aphidicolin treatment, indicating that these mRNAs are translated. These results suggest that transcription of the Mrf gene is suppressed despite the presence of upstream transcription factors. We are also investigating whether similar DNA replication-dependent regulation occurs in other cell-lineages, and the results will be presented.

Analysis of a gene regulatory network to demystify the phenotypic robustness to gene expression variations

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Across the tree of life, many examples illustrate that similar phenotypes can be produced by different genetic contexts. The relationship between genotype and phenotype is thus degenerate. Ancestrality can produce conserved phenotypes despite genetic divergence, while convergence can produce similar phenotypes in distinct phylogenetic branches. The developmental mechanisms that allow different genetic configurations to produce the same phenotype remain poorly understood. The architecture of gene regulatory networks could be a major control step in buffering molecular variation between and within species. Our project aims to describe the role of a gene regulatory network in producing a reproducible complex morphogenetic process, gastrulation. We are using ascidian embryos, which develop with invariant cell lineages, show high robustness to gene expression variation and are perfectly suited for quantitative approaches. We focus on the gene regulatory network controlling the invagination of endodermal cells. We use light imaging to quantify both gene expression and phenotype in the same embryo. Using a sequential RNA-FISH approach, we are setting up methods to measure the inter-individual (co)variations in the expression of all genes of the regulatory network. This will allow us to determine how the architecture of the gene regulatory network buffers or amplifies transcriptional noise as it propagates through the network. To move beyond these correlative observations, we will test the effect of transcriptional modulations by targeting specific enhancers using a dead-Cas9 coupled to activators or repressors. This study will provide a mechanistic framework to better understand how organisms produce highly reproducible phenotypes despite inter-individual molecular variation.

The role of Cdkn1.b in cell cycle regulation and morphogenesis in *Ciona robusta* embryos

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Embryonic cells of *Ciona robusta* divide a strictly limited number of times before terminal differentiation. The notochord consists of exactly 40 cells aligned in a single row along the midline. During neurulation, the notochord cells undergo convergent extension movement. The planar cell polarity (PCP) pathway is known to regulate this process. The cyclin-dependent kinase inhibitor Cdkn1.b is expressed in the notochord-lineage cells from the gastrula stage. Inhibition of Cdkn1.b increases the number of notochord cells beyond 40 and prevents them from aligning in a single row. These observations suggest that Cdkn1.b has two distinct functions: one is the regulation of cell cycle exit and the other is the regulation of convergent extension. Premature expression of Cdkn1.b fused with a nuclear localization signal (NLS) reduced the number of notochord cells to fewer than 40 without affecting their alignment. In contrast, premature expression of Cdkn1.b fused with a nuclear export signal (NES) only slightly reduced the number of notochord cells and prevented their alignment into a single row. These results suggest that nuclear Cdkn1.b is involved in cell cycle arrest, whereas cytoplasmic Cdkn1.b is involved in notochord convergent extension. In mammals, the Cdk inhibitor p27 is involved in cell migration via regulation of Rho. Cytoplasmic Cdkn1.b may similarly regulate convergent extension via Rho. We are currently investigating the requirement for Rho activity in notochord convergent extension, as well as the interaction between Cdkn1.b and Rho.

Tunic Structure and Its Molecular Basis in Ascidians: Comparative Insights into Cellulose and CesA Genes

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Ascidians are unique animals that produce a cellulose-based extracellular matrix known as the tunic. The tunic serves as an important structural and functional component and exhibits substantial variation in morphology and properties among ascidian species. However, the molecular basis underlying this variation remains poorly understood. The tunic consists mainly of proteins and cellulose, and cellulose synthesis is mediated by the cellulose synthase enzyme CesA. Here, we performed a comparative analysis of tunic composition and structure across multiple ascidian species. Tunic proteins were identified and compared, and CesA genes were analyzed for sequence conservation. We also quantified cellulose content and examined tunic ultrastructure using scanning electron microscopy. Our results showed that tunic composition differs among species. While CesA genes were highly conserved, differences in protein composition were observed. In contrast, no significant differences were detected in cellulose content or fiber organization. These findings suggest that differences in tunic properties among species are mainly linked to variation in protein composition.

FROM DEVELOPMENT TO GENOMICS

Impact of genome scrambling on 3D genome organisation in *Oikopleura dioica*

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Gene expression is a complex process regulated at multiple levels. A key aspect is transcriptional co-regulation, where genes involved in related functions are co-transcribed to produce coordinated cellular responses. This is often achieved through linear clustering of co-regulated genes within Topologically Associating Domains (TADs). 3D genome organisation can also bring distant loci into close spatial proximity, enabling co-regulation across large genomic distances. However, the relative influence of these two levels of organisation, as well as how transcriptional regulation operates across the tree of life, remains largely unexplored. To address this, we are exploiting the unique genome of *Oikopleura dioica*. Whole genome assembly from *O. dioica* populations isolated in Barcelona, Osaka and Okinawa reveals an unprecedented level of genome scrambling with thousands of chromosomal rearrangements altering gene order. Despite this, no obvious differences in behaviour or morphology are visible between these populations (Plessy, et al., 2024). We have generated chromosome conformation capture data from just-hatched larvae from the Barcelona population (Cañestro lab) which demonstrates that the *Oikopleura* genome is devoid of TADs but features a large number of long-range (>Mb) interactions. These interactions occur, at least in part, between pairs of co-regulated genes. To visualise chromosome interactions and assess the expression of co-regulated loci simultaneously, we are developing whole-mount in situ hybridisation and spatial transcriptomics approaches in *O. dioica* embryos. In collaboration with the Chourrout (Bergen) and Luscombe (Okinawa) labs these techniques will then be applied to samples from the Norwegian and Japanese populations, to study co-regulated loci with changed linear location. Together, this data will reveal whether co-regulated genes cluster in 3D across populations despite a loss of gene collinearity. Such a mechanism could allow *O. dioica* to maintain robust gene expression despite genomic rearrangements and challenge the dogma regarding the structure function relationship of the genome.

Linking environmental drivers to asexual reproduction dynamic in the salp *Thalia democratica*

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Salps are holoplanktonic tunicates with a byphasic cycle alternating between sexual and asexual reproduction. While sexual reproduction yields a single embryo per individual, the asexual phase enables the rapid production of hundreds of clonal zooids released within days as chains of interconnected blastozooids. Under favorable environmental conditions, this explosive budding capacity drives the formation of seasonal blooms that contribute significantly to the biological carbon pump. Despite extensive field observations and the development of population models, the quantitative effects of environmental drivers on asexual reproduction rates remain poorly understood. We monitored the abundance of *Thalia democratica*, the sea surface temperature and the chlorophyl-a concentration in the Bay of Villefranche-sur-Mer from February 2023 onward. This revealed a positive correlation between phytoplankton concentration and the number of blastozooids per chain. In parallel, combining controlled laboratory experiments with in situ measurements of cell proliferation, we showed that favorable environmental conditions are associated with accelerated blastozooid production and release. Together, these results suggest that bloom formation is driven, at least in part, by an increase in both the rate of production and the length of blastozooid chains. These finding challenge prevailing theoretical frameworks and provides a basis for improving models of salp population dynamics.

Diversity, population structure, and holobiont organization of botryllid ascidians with a focus on *Botryllus schlosseri* in relation to environmental parameters along Turkish coasts

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Botryllid ascidians, particularly *Botryllus schlosseri*, are key models for immune evolution, development, and regeneration. As sessile filter feeders, they act as coastal bioindicators and holobionts with diverse microbiota, yet their diversity and population genetics in Turkish waters remain limited. This study investigates the diversity of botryllid ascidians and the population structure of *B. schlosseri* along the Turkish coastline, encompassing the Black Sea, Sea of Marmara, Aegean Sea, and Eastern Mediterranean. Specimens were collected between 2023 and 2024 from 26 stations representing diverse hydrographic conditions and microhabitats. Phenotypic variation was assessed using morphotype analyses, while mitochondrial COI sequences and microsatellite markers were used to evaluate genetic diversity and population structure. Host-associated microbial communities were characterized via 16S and 18S rRNA amplicon sequencing, and environmental variables were integrated to define holobiont components. Results revealed marked environmental variability across basins and microhabitats. Eight species; *Botrylloides niger*, *Botrylloides israeliensis*, *Botryllus humilis*, *B. schlosseri, Botryllus gaiae*, *Botrylloides* sp., *Symplegma brakenhielmi*, and Polyclinum constellatum were identified, alongside an unresolved botryllid lineage at Konacık. B. schlosseri was the only species with a continuous north-south distribution, although unevenly represented. Notably, this study reports the first record of *B. gaiae* in Turkish waters. Population analyses revealed high genetic diversity in B. schlosseri, with 83 COI haplotypes and strong spatial structuring. A total of 68 morphotypes were recorded, independent of mitochondrial variation. Microsatellites revealed strong Black Sea-Marmara connectivity but Mediterranean isolation, supporting a three-cluster structure (K = 3). Holobiont analyses demonstrated clear differences between tissue-associated and seawater microbial communities, identified potential bacterial symbionts, and revealed specialized tissue-associated eukaryotic organisms. This study presents the first integrative assessment of botryllid ascidian diversity in Turkish waters, synthesizing environmental, genetic, phenotypic, and microbial data to establish a foundation for future research.

First evidence of tolerance to vanadium in the solitary tunicate *Ciona robusta:* inflammatory and oxidative stress responses following sodium orthovanadate exposure

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Vanadium is naturally present in seawater mainly as vanadate, with typical dissolved concentrations of approximately 30-35 nM (~1.5-1.8 µg V L^{-1}). Tunicates are known to bioaccumulate vanadium up to 10,000-fold compared with surrounding seawater. In recent decades, anthropogenic sources have progressively increased vanadium concentrations in marine environments, raising concerns about potential toxic effects on marine organisms. This study investigated the effects to sodium orthovanadate exposure on the solitary tunicate *Ciona robusta* by evaluating inflammatory- and oxidative stress-related biomarkers. Animals were exposed for 7 and 14 days to two nominal concentrations of sodium orthovanadate, 100 and 1000 µg L^{-1}, corresponding to approximately 0.54 and 5.44 µM of dissolved V, respectively. The lower exposure concentration falls within the upper range reported for vanadium-contaminated seawater, whereas the higher one was selected as an experimental challenge condition. Indirect inflammatory-related biomarkers, including phenoloxidase, arylsulfatase, acid phosphatase, and alkaline phosphatase, were evaluated. Activity of antioxidant (catalase, superoxide dismutase) and detoxification (glutathione S-transferase) enzymes, as well as acetylcholinesterase activity and oxidative damage endpoints, including protein carbonyl content and lipid peroxidation, were also measured. Responses of most biomarkers in treated organisms did not differ significantly from controls, suggesting a remarkable capacity of C. robusta to withstand vanadium exposure without triggering broad inflammatory and oxidative or metabolic stress. Only alkaline phosphatase activity was inhibited at 1000 µg L^{-1} after 7 days, likely reflecting direct interference of vanadate with phosphate metabolism, though enzymatic activity recovered after 14 days. Superoxide dismutase activity increased significantly after 14 days at the highest concentration, suggesting activation of defense line against oxidative stress. Overall, these results support the hypothesis that *C. robusta* possesses considerable biochemical mechanisms that enhance its tolerance towards vanadium, highlighting its value as a biological system for better investigating the strategies that allow tunicates to tolerate and manage vanadium exposure.

Fouling ascidians (Chordata: Tunicata: Ascidiacea) in floating pontoons of the Portuguese Coast: still a place for natives?

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Marine bioinvasions have been considered a source of marine pollution causing ecological and economic harm. Ascidians are the most notorious invaders worldwide threatening native biodiversity and the infrastructures where they occur. The Portuguese coast remains underestimated in ascidian catalogues and databases for both native and non-indigenous species (NIS).To fill this knowledge gap, surveys were conducted in artificial habitats (floating pontoons) along the Portuguese coast across three regions: Northern (Leça de Palmeira, Póvoa de Varzim and Viana do Castelo), Central (Peniche) and Southern (Faro e Praia de Faro). A total of 701 colonies/individuals belonging to 12 species were collected. Of these, four species were natives (*Synoicum beauchampi, Clavelina lepadiformis*, *Molgula socialis* and *Perophora* aff. *listeri*), four were NIS (*Microcosmus squamiger*, *Molgula manhattensis*, *Styela clava* and *Styela plicata*), and two were cryptogenic (*Botryllus schlosseri* and *Diplosoma listerianum*). The remaining taxa (*Polyclinum* sp. and *Pycnoclavella* spp.) were considered of uncertain status due to unsolved species identity. Ascidian richness varied across the studied regions. The Northern region, where sampling effort was greater, exhibited the highest richness (8 species), particularly in Leça da Palmeira, which hosted 75% of the total recorded taxa. In contrast, the Central and Southern regions were less diverse, each with 3 species. Among NIS, the solitary ascidian Styela clava showed the widest distribution, appearing in three out of the six surveyed localities, two in northern Portugal and one in Central Portugal. Overall, our results highlight the prevalence of NIS across the studied regions, particularly in Central Portugal, where ascidian communities were exclusively composed of NIS. These findings underscore the role of artificial structures as vectors for the introduction and spread of NIS ascidians, highlighting the need for improved monitoring and mapping efforts to better assess and mitigate their impacts. The information provided here may assist stakeholders and policymakers in the development of NIS management strategies.

Getting the modest but important historical Ascidiacea (Tunicata) collection of the Royal Belgian Institute of Natural Sciences in Brussels documented through dedicated inventory

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The unmistakable value of natural history collections has been conclusively argued before. Such is especially true for old natural history collections of relatively poorly studied taxa that have been sampled in locations that were and still are hard to access and that have remained largely unknown because of lack of visibility on open-access platforms. The historical collection of Ascidiacea as conserved in the Royal Belgian Institute of Natural History is such a case. While modest in its size, it holds for instance important specimens that were sampled during the world-famous Belgian Antarctic Expedition of 1897–1899 with the research vessel Belgica. Equally interesting and important are those specimens brought home from the Belgian coast, sampled with dedication during repetitive events, some already over a century ago. Unfortunately, the existence and content of this collection have remained largely unknown, because it had not yet previously been fully inventoried and digitized. In the present effort we have retaken past inventories on paper, cross-checked these with the physical collection, and have started to render them fully accessible by databasing them with the collection platform used in the RBINS: DaRWIN (https://www.naturalheritage.be/darwin/rbins) So far, the present preliminary inventory revealed 399 jars, 309 of which with samples originating from the Belgian coast, especially belonging to the Styelidae, Polyclinidae and Molgulidae, and 99 with samples from non-Belgian waters. In the latter lot, the type material of no less than six different species have been detected. These being: (1) the holotype of *Amaroucium stellatum* Verrill, 1871, (2) the holotype of *Perophora viridis* Verrill, 1870; (3) the holotype of *Corella benedeni* Van Beneden & de Selys Longchamps, 1913; (4) two syntypes of *Ascidia complanata* Fabricius, 1780; (5) seven syntypes of *Boltenia antarctica* Van Beneden & de Selys Longchamps, 1913 and (6) two syntypes of *Sycozoa* (*Colella*) *racovitzai* Van Beneden, 1913. Surprisingly, we could not yet find the type material (=holotype) of *Corella dohrni* Van Beneden & de Selys Longchamps, 1913 which normally should also be present in the RBINS collection.

Climate change effects on colonial ascidians: an integrated approach to predict ascidians' future distribution in the Mediterranean Sea (EPICA)

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EPICA is a three-year Italy-Türkiye collaborative project aiming at assessing the effects of climate change on cosmopolitan colonial ascidians characterized by high invasive potential and representing key components of marine biofouling communities. The study will focus on Botryllus schlosseri and Botryllus gaiae, that will be seasonally sampled in the Lagoon of Venice (Italy) and the Sea of Marmara (Türkiye), taking into account seawater salinity, temperature, pH, and dissolved oxygen level. Seawater samples will also be collected to evaluate ascidian biodiversity by eDNA analyses. In parallel, controlled laboratory experiments will be conducted to evaluate the effects of different temperature and salinity conditions on colony growth, health status, and physiological responses Furthermore, gene expression analysis will be performed using RT-qPCR and transcriptomic analysis to characterize the molecular mechanism underlying responses to environmentally stressful conditions. The collected data will be used to develop Species Distribution Models (SDMs) aimed at predicting future botryllid distribution under climate change scenarios. The synergistic approach of EPICA will enable the optimization of a botryllid rearing system in Türkiye, leveraging the expertise of the Italian research group; reciprocally, the latter group will advance its knowledge in predictive modeling through the transfer of knowledge from the Turkish partner. By integrating field observations, experimental data, and molecular analysis, EPICA will contribute to a more comprehensive understanding of the responses of marine fouling organisms to climate change. The study will help in predicting species spread, assessing invasion risks, and improving biofouling management strategies in a rapidly changing environment.

Drivers of ascidian invasion in Brazilian artificial habitats

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Ascidians can be dominant organisms of the fouling communities, in which most of the species are exotic. Bioinvasion poses a significant threat to coastal ecosystems, especially around ports and marinas, where artificial structures favor the survival and spread of these species. This study investigated the patterns and drivers associated with ascidian bioinvasion along the Brazilian coast, focusing on variables related to environmental and landscape conditions. A comprehensive survey was performed at 35 sites across the Brazilian coast, including 8 piers, 18 marinas, and 9 ports. Data was based on field collections carried out between 2004 and 2024 and on complementary literature data. Species were identified and classified according to origin (native, cryptogenic, or exotic - detected, established, or invasive) and life form (solitary or colonial). Environmental variables considered included salinity, temperature, maximum current velocity, primary productivity, substrate type, and colonization time. Landscape variables included dock size, connectivity, and distance to the nearest port. A total of 62 ascidian species were recorded: 14 native, 26 exotic, and 22 cryptogenic. Native species were generally rare, with 29 of 35 sites showing ≤20% natives. Exotic species accounted for 20-100% of total richness, with 27 sites showing ≥50%. Among these, invasive exotics represented 55-100% of exotic richness, with 30 sites presenting ≥75%. Richness of exotic species decreased with distance from ports ($z = -2.06$; $p = 0.039$) and increased with maximum current velocity ($z = 2.079$; $p = 0.0376$). Species composition was not well explained by any of the variables tested suggesting that a lottery process based on the composition of propagule pressure might be the main driver in action. Overall, the results highlight that artificial and well-connected environments near major ports act as hotspots for introduction and secondary spread, and local environment drivers are not limiting or selecting ascidian communities.

EVOLUTION, SYSTEMATICS AND TAXONOMY

Evaluating sampling procedure and improving HMW-DNA extraction and HiC protocols for the generation of chromosome-scale reference genomes of colonial ascidians

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Ascidians are solitary or colonial marine chordates that play a central role in benthic ecosystems. They are widely studied for their invasive potential, to unravel the chordate evolution, and as model to investigate complex processes such as regeneration, stemness and development. However, ascidian biodiversity is currently underestimated due to the frequent occurrence of species complexes, identified mainly by integrative taxonomy, and to the intrinsic challenges in morphological as well as genomic characterization (due, for example, to the small body size, the presence of pigments/metabolites interfering with the DNA extraction, etc). Within the framework of the Biodiversity Genomics Europe project (https://www.erga-biodiversity.eu/erga-bge), we aimed to explore the ascidian biodiversity and advance ascidian genomic research by generating high-quality chromosome-level assemblies compliant with the Earth BioGenome Project standards. Here we describe the results of different protocols for High Molecular Weight DNA (HMW-DNA) extraction and the optimization of Arima HiC library preparation for nine species, mainly colonial ascidians of the Styelidae family. The quality of the HMW-DNA extraction and the final chromosome-level assembly was evaluated in relation to the species morphology, the sampling procedure, the specimen identification step, and the preservation method (99% ethanol, RNA later, or flash frozen) and time. Most specimens, collected either manually or by scuba diving, were transported alive to the laboratory and quickly analysed under the microscope before preservation for molecular analyses. Then, a subsample was anesthetized with menthol in seawater and fixed in 4% formalin for accurate morphological identification. For HMW-DNA and Arima HiC protocols, the entire colony was processes without tunic removal. Our results demonstrate that even RNA-later or ethanol preserved samples are suitable for generating high-quality chromosome-level assemblies and indicate that our HMW-DNA/HiC optimized protocols can be successfully extended to other challenging colonial ascidian such as aplousobranches. However, sampling procedures need to be improved.

First chromosome-level reference genome of *Botryllus gaiae* Brunetti 2020, formerly *Botryllus schlosseri* clade E, generated from a syntype specimen

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*Botryllus schlosseri (*Pallas, 1766) is a cosmopolitan fouling species and a widespread marine invader, as well as a model organism to study complex processes such as asexual development, regeneration and allorecognition. However, B. schlosseri is now recognized as a species complex comprising five genetically highly divergent clades, A to E, identified through COI barcode sequences. As it had no type specimen nor detailed morphological description, *B. schlosseri* was redescribed in 2017 by Brunetti, with a clade A specimen from Venice designated as neotype, thus providing a taxonomic reference. In 2020, a clade E specimen from Southern Adriatic Sea was described as a new species, *Botryllus gaiae*, integrating morphological and mitogenome data. Here, we present the chromosome-level genome of *B. gaiae* generated from the BT2 syntype, preserved in 99% ethanol since 2013, together with a comparison with available assemblies of *B. schlosseri.* A primary and a phased assembly were generated using Oxford Nanopore long reads and Arima HiC Illumina reads for scaffolding. The primary assembly consists of 16 chromosome-scale scaffolds, spanning 95% of the total assembly length, with an N50 of 17.4 Mb and 91.5% complete single-copy BUSCO genes. Despite time and preservation conditions of the syntype, which were of suboptimal quality for both HMW-DNA extraction and HiC library preparation, all metrics indicate high assembly quality. Remarkably, the *B. gaiae* assembly spans 298 Mb, thus nearly half the size of the chromosome-level assembly of *B. schlosseri* sensu Brunetti 2017 (formerly clade A), which is 533 Mb long. Both genome sizes were independently confirmed with an improved histochemical Feulgen protocol based on image analysis densitometry. Additional genomic differences between the two species will be presented and discussed. Overall, our results show a high nuclear divergence between *B. gaiae* and *B. schlosseri*, confirming the results obtained from morphological and mitogenome data.

Ascidians of Ponta do Ouro, South Mozambique

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The Marine Area of Maputo National Park in Mozambique is the first marine Transfrontier Conservation Area in Africa. Ascidians are exclusively marine sessile filter feeders and an ecologically important part of the benthic reef fauna. Previous research on the ascidian fauna of Mozambique was sporadic and knowledge about species composition, biodiversity and distribution of ascidians remains limited. Here, we present results of intertidal and SCUBA collections of ascidians from the submerged reefs of Maputo National Park, thereby updating the list of species reported for Southern Mozambique. We established the first Mozambican ascidian bio-bank at Maputo Natural History Museum by systematically depositing voucher specimens, cox1-mt-DNA sequence data, photographs of living specimens and of taxonomically important morphological structures using light- and electron microscopy. Of the 31 species described, 17 were recorded in Mozambique for the first time and 3 are probably new to science.

In silico network-based approach to tunicate immunome evolution

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The tunicate *Ciona robusta,* an invertebrate chordate closely related to vertebrates, represents a valuable model for investigating the evolutionary origin of immune molecular networks. Although it lacks an adaptive immune system, *Ciona* possesses a complex innate immune repertoire involved in microorganism recognition, immune regulation, and host-environment interactions. This study aimed to reconstruct and analyze an immune-related protein-protein interactome in *C. robusta*, moving from single-gene identification toward a pathway- and network-level interpretation of the tunicate immunome. A structure-, function- and conservation-based bioinformatic strategy was applied to identify *Ciona* molecules homologous or functionally related to vertebrate immune components. Candidate proteins were selected using sequence similarity searches, domain annotation, Gene Ontology classification, and comparative pathway analysis, with particular attention to TLR signaling, JAK/STAT pathway, complement-related molecules, lectin-mediated recognition, immunoglobulin-like and C-type lectin-like domain-containing proteins. The initial interaction network was generated using STRING-db as a reference platform for predicted and known protein-protein associations and was subsequently imported into Cytoscape for network construction and preliminary topology inspection. For poorly characterized candidates or proteins lacking sufficient structural information, de novo structural modelling was performed using structure-prediction tools, including I-TASSER-MTD, to support functional inference and comparative annotation. Network refinement, evolutionary-level clustering, and visualization were carried out in Python. Evolutionary-level cluster analysis was used to group immune candidates according to functional associations and comparative distribution across taxa. The interactome reveals a central immune signaling module involving TLR-associated molecules such as MyD88, TollIP, IRAK4/IRAK-like proteins, TRAF3, TAK1, NF-κB-related components, IκB, REL1, and p38/MAPK-related factors. Additional subnetworks include JAK/STAT elements, complement-associated molecules such as C3 and C3aR, pattern-recognition receptors including C-type lectin-like proteins, MBL-like molecules, TLR1/2/7, and several poorly characterized proteins bearing immune-related domains. The SYK-like molecule appears in an intermediate network position, close to JAK/STAT-related and receptor-associated components, suggesting a possible role as a kinase-mediated connector between immune recognition and downstream intracellular signaling. This may reflect the conservation of an ancestral signaling logic linking receptor engagement, lectin-like recognition, and transcriptional immune responses. The reconstructed interactome provides an integrated view of the *C. robusta* immune molecular landscape and suggests that the ascidian innate immune system is organized through conserved signaling hubs, receptor-like molecules, and lineage-specific expansions. By shifting from candidate-gene discovery to network-level interpretation, this analysis supports Ciona robusta as a valuable model for exploring the evolutionary assembly of chordate immune pathways and for identifying novel immune-related molecules involved in tunicate host-microbe interactions.

GENE REGULATION

A conserved gene regulatory circuit underlies sensory neuron identity across chordates

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Vertebrate sensory ganglia contain diverse neuron types specialised for distinct sensory modalities. In vertebrates, epibranchial (viscerosensory) and auditory neurons arise from the posterior placodal domain and are specified by distinct transcription factor programs, including Pax2/5/8, Phox2 and Err.
However, the evolutionary origin of this regulatory architecture remains unclear.
We hypothesise that Pax2/5/8 controls the specification of posterior placode-like sensory neurons through an ancestral gene regulatory module that predates vertebrates, giving rise to distinct sensory neuron subtypes via downstream Phox2- and Err-dependent programs.
To test this, we combine comparative single-cell transcriptomics, gene expression analyses, and CRISPR-mediated perturbations across amphioxus, *Ciona intestinalis,* chicken embryos, and mice. We assess whether Pax2/5/8 regulates Phox2- and Err-expressing sensory neuron populations across chordates and evaluate the extent to which this regulatory hierarchy is conserved.
By reconstructing conserved and lineage-specific features of this gene regulatory network, we aim to determine whether modification and expansion of an ancestral posterior placode neuron-like module contributed to vertebrate sensory neuron diversity. This work provides insight into how regulatory rewiring and gene duplication facilitated the evolution of vertebrate sensory neuron diversity.

IMAGING AND MODELLING

Modeling Morphogenetic Trajectories in Ascidian Embryos via Latent Space built with Autoencoders

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Understanding embryonic morphogenesis requires quantitative approaches capable of capturing both the complexity and variability of biological shapes. Ascidians provide a powerful model due to their rapid, highly stereotyped embryonic development and well-characterized cell lineages. However, analyzing morphological changes over time remains challenging, as classical shape descriptors-such as volume, eccentricity, or surface roughness-are often insufficient to capture the full complexity of biological forms. Moreover, raw 3D imaging data cannot be readily exploited due to its high dimensionality, storage requirements, and computational cost. In this project, we first leverage autoencoders, a class of unsupervised deep learning models, to learn compact latent representations of embryonic morphology from 3D time-resolved imaging data. These models encode complex shape information into a low-dimensional space, referred to as a latent space, while preserving key morphological features. Establishing such a representation is a crucial step toward enabling meaningful comparisons of embryonic forms across individuals. Building on this latent space, we then aim to investigate the temporal dimension of morphogenesis by incorporating cell tracking and time-resolved analysis. This approach will allow us to reconstruct continuous developmental trajectories, track morphological changes throughout embryogenesis, and compare developmental paths across individuals. By bridging developmental biology and machine learning, this work aims to provide a novel quantitative framework to study morphogenesis, allowing new opportunities to uncover patterns of shape evolution, quantify variability, and identify deviations associated with experimental perturbations.

Ciona bioresource project in Japan

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Ciona robusta/intestinalis type A is a splendid model tunicate for elucidating biological mechanisms. This superiority is supported by the availability of genetic technologies in this species. Transgenic lines established by transposon-mediated germline transgenesis provide ideal tissue-specific markers that stably express reporter genes and indicators of subcellular events. Wild-type animals maintained as closed colonies enable researchers to perform reliable experiments, ensuring reproducible results by using genetically isolated populations. To enhance its usability, we are collecting and distributing genetic tools in *Ciona* as part of a resource project funded by the Japanese government. In this poster, we would like to introduce this project to promote its use. Our project is not only about resource distribution but also about improving the experimental background in tunicates. For this purpose, we will present our recent advances in genetic techniques, including genome sequencing of closed-colony populations and extremely efficient germline transgenesis using transposons.

IMMUNOLOGY ALLORECOGNITION

Preliminary results on immune priming in the colonial ascidian *Botryllus schlosseri*

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Immune priming is the capability of the innate immune system to change the immune response following a previous experience with nonself molecules. It acts as a form of immune memory in invertebrates, differing from the long-term, lymphocyte-based, immune memory of jawed vertebrates. Immune priming can occur in the form of a "recall" response or potentiation, so that the second response is faster and more powerful than the first one, as a shift from one type of response to another, more efficient response, as a long-lasting upregulation of the defence response, or even as a state of tolerance with reduced immune activity. First evidence in the scientific literature points towards the possible presence of immune priming in colonial ascidians. The few available data refer to the possibility, in *Botryllus primigenus* and *Botryllus schlosseri*, to alter colony fusibility when colonies were previously fused, for a few days, to a compatible colony sharing only one allele at the Fu/HC locus. The phenomenon is due to the persistence, in a colony, of a cell population from the previously fused colony. With the present work, in the attempt to demonstrate the presence of immune memory in colonial ascidians, we primed colonies of *B. schlosseri* by microinjecting bacteria or LPS from E. coli in the colonial vasculature and compared the obtained response, in terms of transcription of immune-related genes, to that obtained following a second challenge. The responses in various phases of the colonial blastogenetic cycle were also considered. Results indicate the capability of *B. schlosseri* to enhance the transcription of immune-related genes after the second challenge and pave the way for further analyses on the molecular mechanism(s) underlying this phenomenon.

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