Symposium Book

JAGIELLONIAN UNIVERSITY IN KRAKÓW

AUDITORIUM MAXIMUM
KRUPNICZA 33

tardigrada.uj.edu.pl

This Symposium is dedicated to the memory of

Professor BARBARA WĘGLARSKA

(1922–2020)

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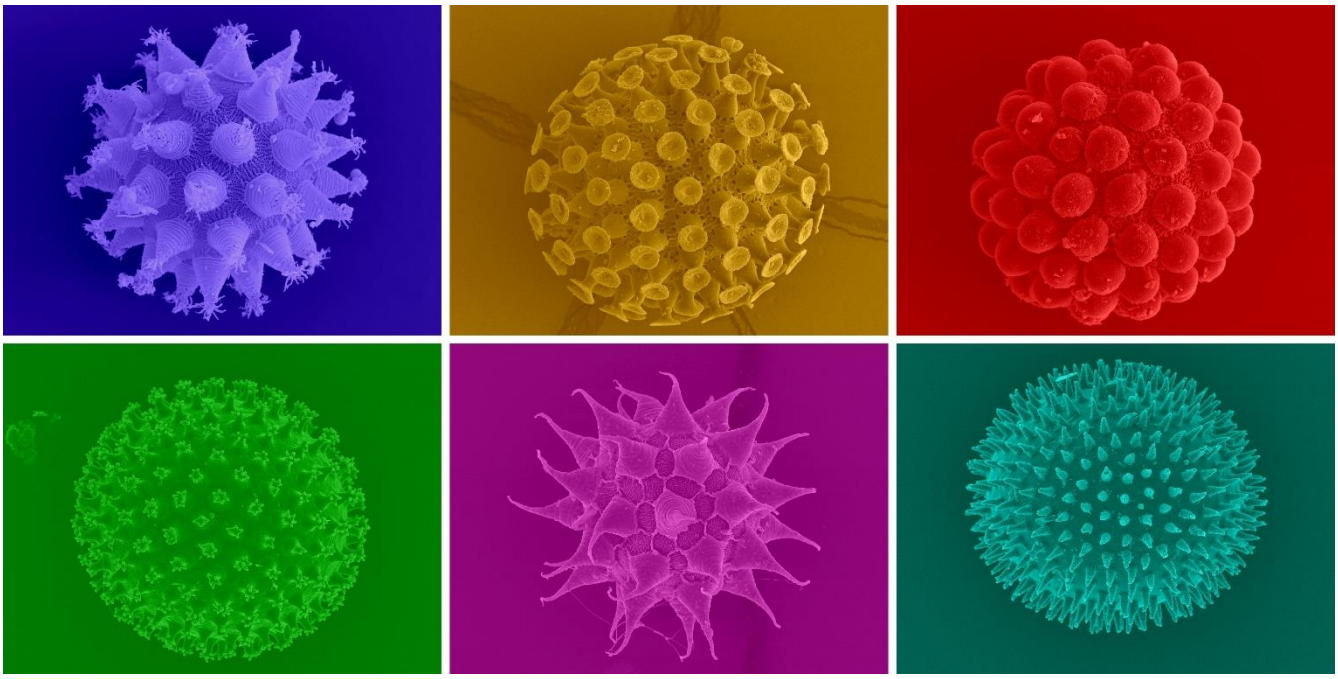
INTRODUCTION

This August, tardigrade researchers and enthusiasts from all continents will come to the Royal City of Kraków to present and learn about the newest and unpublished discoveries concerning tardigrade taxonomy, systematics, phylogeny, biogeography, ecology, evolution, behaviour, reproduction, development, anatomy, physiology, astrobiology, molecular biology (all of the "omics"), and to hear about potential medical applications of the remarkable tardigrade abilities to survive extreme conditions.

The main theme of the Symposium will be the biology of tardigrades, lovingly known as water bears or moss piglets. These microscopic (usually well under 1 mm in length) animals can be found in a great range of ecosystems throughout Earth, from ocean floors to mountain tops and from the poles to the tropics. Tardigrades constitute a phylum within the megaclade Ecdysozoa (moulting animals), thus they are closely related to insects, velvet worms and round worms. To a wider public, tardigrades are known because of their remarkable cryptobiotic abilities that allow some of them to survive desiccation, freezing down almost to absolute zero and heating up to high temperatures, vacuum and high pressures, megadoses of ionising radiation, and even quantum entanglement or exposure to outer space. Water bears owe their worldwide fame also to their undeniable and irresistible cuteness – by all measures they are the most adorable of all microscopic animals – just look at their lovely faces and amazingly sculptured eggs! ♥

Hugs and kisses from tardigrades. © ŁUKASZ MICHALCZYK, www.tardigrada.net, all rights reserved.

The Symposium will be held at the Auditorium Maximum, a modern and spacious conference centre of the Jagiellonian University in Kraków, Poland. The Jagiellonian University, founded in 1364 and Alma Mater of NICOLAS COPERNICUS, is the oldest university in Poland and one of the oldest functioning universities in the world. The JU's home is Kraków, a millennium-old city and a medieval capital of Poland, located in the southern part of this central European country. Thus, apart from all the exciting tardigrade research that will be presented and discussed at the Symposium, participants will have an occasion to enjoy the charms of Kraków and its magical atmosphere, as well as to try Polish cuisine (such as savoury or sweet *pierogi*, *barszcz*, *żurek*, *zapiekanki* and many others!). We have prepared a number of various attractions for our guests: there will be a Welcome Reception flowed by an Icebreaker, visits to unique medieval architectural monuments and museums, including the oldest JU building, underground passages of the Main Square that reach down to the prehistoric times, and an excursion to the exceptional Wieliczka Salt Mine with sculptures and chandeliers carved from salt, as well as a banquet in an original setting. Finally, discoveries presented at the Symposium will be published in the Zoological Journal of the Linnean Society, one of the most respected zoological journals in the world.



A variety of tardigrade eggs. © ŁUKASZ MICHALCZYK, www.tardigrada.net, all rights reserved.

Although there is no formal tardigradological society that would be responsible for organising periodical meetings, international conferences dedicated exclusively to tardigrades are held regularly. So far, there have been fourteen tardigrade Symposia, each organised by different volunteer tardigradologists. Previous Symposia were held in Italy,

USA, Germany, Denmark, Poland, Portugal and in the UK. On average, Symposia are held every three years and each congress is an extremely important event in our tardigrade world. The Symposia are rare occasions to meet in one place almost all tardigradologists, who, for most of the rest of the time, are scattered all over the globe.

This is the second time that a tardigrade conference is held in Poland. The 2nd International Symposium on Tardigrada took place in 1977 and it was hosted by the late Professor BARBARA WĘGLARSKA (20.02.1922–02.10.2020). The 15th Symposium was originally planned for 2021, but – due to the COVID-19 pandemic – it has been postponed to 2022. Thus, after 45 years, the Symposium comes back to Kraków and to the Jagiellonian University. In order to honour Professor BARBARA WĘGLARSKA and her contribution to tardigrade science, both the Symposium and the Symposium Proceedings will be dedicated to her memory and legacy.

Prof. dr hab. BARBARA WĘGLARSKA in her Kraków apartment in winter 2018. © ŁUKASZ MICHALCZYK, www.tardigrada.net, all rights reserved.

Stay tuned for Announcements, which are published on the Symposium Website and are also circulated via the Tardigrada Newsletter Mailing List (if you haven't joined the TN Mailing List yet, you can do it here; please remember to add the email domains **@tardigrada.net** and **@uj.edu.pl** to your white list, so our messages don't end up in your spam folder).

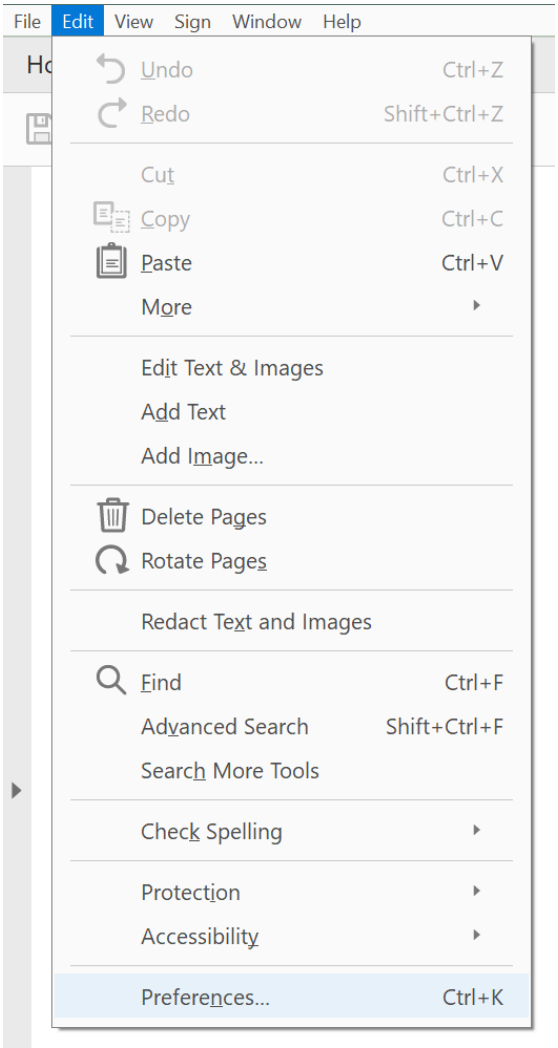
Last but not least, below is our Symposium logo, which depicts an individual of *Doryphoribius dawkinsi* stylised as the statue of the Wawel Dragon at the foot of the Wawel Hill in Kraków. Font and the outer flame colours represent the colours of the Jagiellonian University coat of arms. Concept and design: ŁUKASZ MICHALCZYK (Jagiellonian University), artwork: KAMIL JANELT (University of Silesia) & Ł.M.:

HOW TO USE THE SYMPOSIUM BOOK

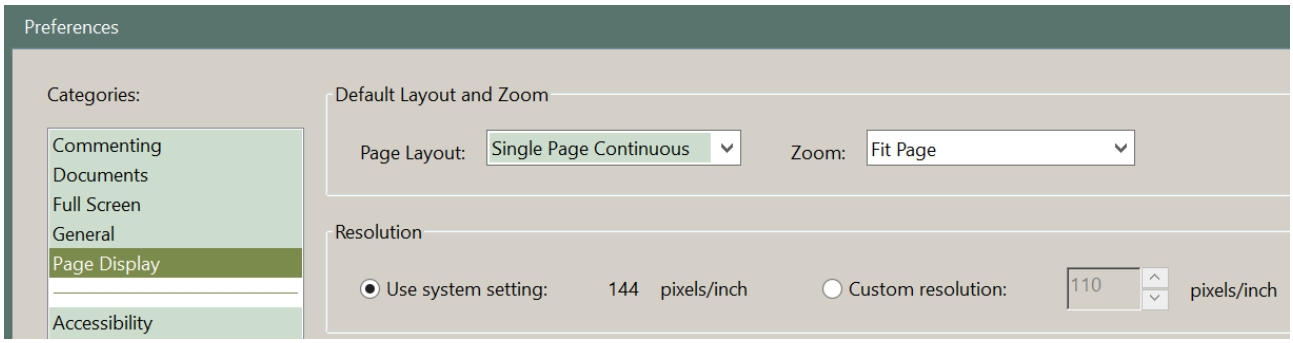
To aid with navigation through the numerous pages, many sections in the Symposium Book are **cross-linked**. First of all, clicking any line in the Table of Contents will take you to the corresponding place in the Book. Secondly, clicking any talk or poster title in the Programme will take you to the abstract of the given presentation. Finally, clicking the header on any page will bring you back to the Table of Contents.

Moreover, you may customise your Adobe Reader® (free software) to make the navigation even more user-friendly. Here are some useful functions and a short manual on how to use them:

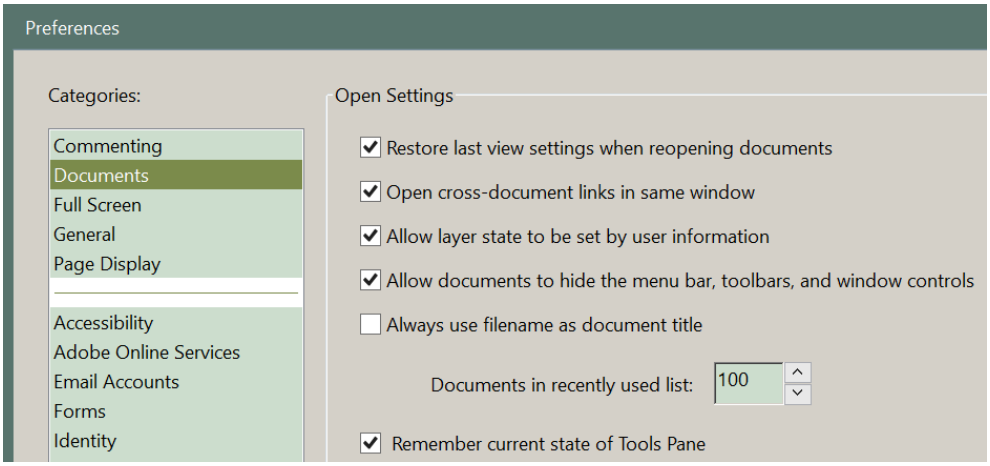
1. Continuous scrolling allows you to go smoothly from one page to another and you can force all PDF files to open this way by default. Go to *Edit* in the main menu, choose *Preferences* (or press Ctrl+k):



Then, select the *Page Display* section and choose *Single Page Continuous* from the scroll-down menus:

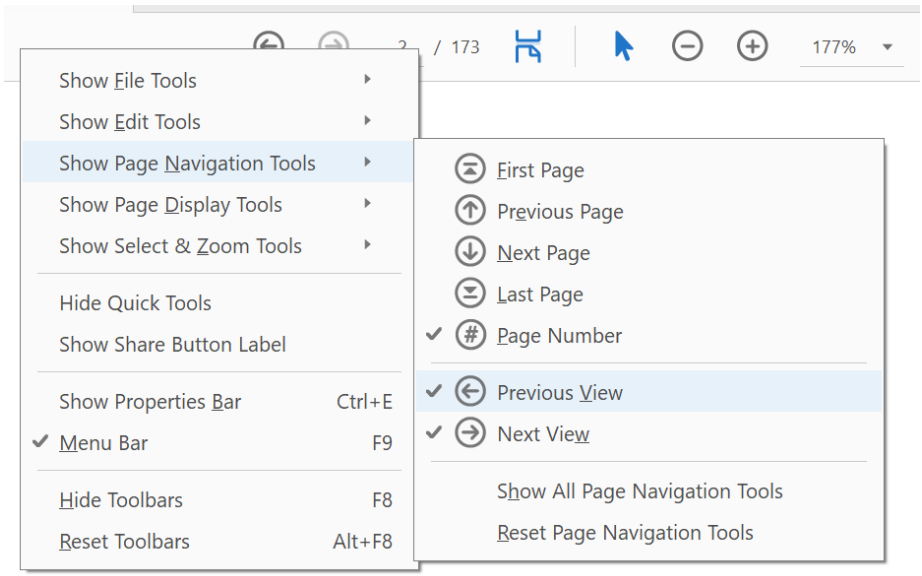


2. Another useful option is to tell Adobe Reader to remember the place in the document that you viewed before closing it. To do this, select the *Documents* section and select the first option, *i.e. Restore last view settings when reopening document*:

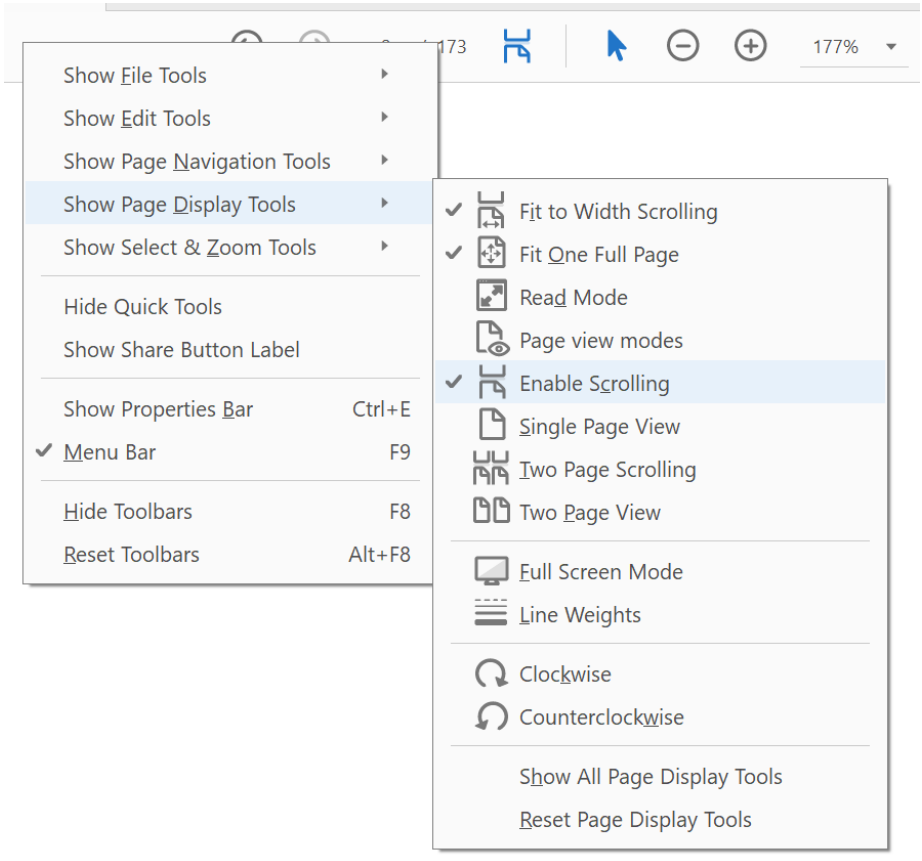


3. It can also be helpful to customise your toolbar to be able to quickly return to previous views (left and right arrows in circles in the picture below), as well as to rapidly return to the continuous scrolling mode (a two-page symbol in the picture below):

In order to add these icons to the toolbar, right-click on your toolbar and select *Show Page Navigation Tools*, and click *Previous View* and *Next View*:



Analogously, select *Show Page Display Tools*, and click *Enable Scrolling*:



ORGANISATIONAL INFO

Emergency number

In case of emergency please seek help from Volunteers (yellow badges) and/or Organisers (orange badges) and/or **call 112** (free of charge).

ID types

Each category of participants has a different badge colour (*Accompanying Persons* – pink, *Invited Speakers* – mint, *IT Support* – black, *Organisers* – orange, *Participants* – white, *Press* – red, *Sponsors* – green, and *Volunteers* – yellow). Moreover, badges contain information on Symposium Package, *Basic* (★), *Regular* (★★), and *Full* (★★★), first name and surname, institution name (if applicable) and country name. All badges will be placed in transparent sleeves with customised double-sided printed ribbons. The back of the sleeve has a small pocket for the 12-page Symposium Booklet which contains an abridged Programme. **Please wear your badge at all times during the Symposium**, as apart from allowing other participants to identify you, the badge also serves as a ticket to social and cultural events.

Symposium badge types (in alphabetical order).

Talks

Invited Talks: **60 minutes in total** (preferably 50 minutes of presentation + 10 minutes for discussion).

Regular Talks: **15 minutes in total** (preferably 12 minutes of presentation + 3 minutes for discussion). The Symposium agenda is quite tight and we would like all Presenters to have the same amount of time to present their talks. **Thus, please make sure that your talk does not take longer than the allocated time slot.**

We suggest preparing presentations in the PPTX or PDF format (it is a good idea to have the presentation saved in both formats).

Important information regarding talks: Please name your presentations according to the following key: **session number** (1–9), a **hyphen** (-), **talk number** within your session (1–6), a **hyphen** (-), and the **surname of the presenting author** without special characters/accents (basic Latin alphabet only, no underscores please). For example, the first talk on Monday should be named as "**1-1-Mapalo**.pptx" (and/or "**1-1-Mapalo**.pdf"), the last talk on Monday: "**2-5-Pust**.pptx", the second last talk in the morning session on Tuesday: "**3-4-Lopez-Lopez**.pptx", the first talk in the second session on Friday: "**9-1-Concordet**.pptx", *etc.* This will greatly help us with session organisation.

Please contact the IT Support (two guys, KONRAD and SZYMON, with black Symposium badges) to upload your presentation onto the Lecture Hall computer (using your laptop is possible but not recommended). **Please upload your talk no later than 30 minutes before your session.**

Talk Session Chairs

Talk Session Chairs are kindly requested to keep the time of the talks. If a Participant exceeds a total of 15 minutes, Chairs must interrupt the presentation to allow the next Participant to give their talk. It is advised to give the Presenter a visual sign when 11 minutes of the talk pass and an audio sign when 12 minutes are reached (there will be a small tardigrade bell available to the Chairs).

Posters

No larger than the B1 format (ISO 216), *i.e.* 100.0 cm high × 70.7 cm wide. Poster stands are two-sided and they will be numbered from 1A to 17B. Please hang and remove your poster(s) at designated times. Double-sided sticky tape and scissors will be available in the Exhibition Room.

Presenting Authors are kindly requested to be at their posters during the Poster Sessions.

Poster Session Chaperones

Poster Session Chaperones and Volunteers will be happy to help you with mounting and removing your poster(s).

Coffee & Lunch Breaks

All drinks and meals will be served in the Exhibition Room on the second floor (just above the Lecture Hall). Apart from water, Participants are kindly asked not to bring drinks and food to the Lecture Hall.

Young Scientist Awards

We are planning to grant a total of four awards: one for the best oral presentation and one for the best poster, each in two main fields: *Zoology* (Biodiversity, Taxonomy, Biogeography, Phylogeny, Evolution, Ecology, Life Histories, Behaviour, Morphology, Anatomy, Reproduction & Development) and *Physiology* (Physiology, Omics, Cryptobiosis & Astrobiology). Candidates will be evaluated by an ad hoc panel of senior scientists appointed by the Symposium Chair. The ranking list will be based on the originality and quality of the presented work, talk structure/poster design, aesthetics, as well as on the communicative skills of the presenter and the ability to answer questions.

Surnames of YSA Contestants are marked with a golden star (☆) in the Symposium Programme. Golden stars will also be mounted on respective poster stands.

In addition to a monetary prize, YSA Winners will also be presented with Symposium medals (golden for best talks and silver for best posters):

Medals to be presented to Young Scientist Award Winners for best zoological and physiological talks (golden medals) and posters (silver medals).

Travel Grants

TG Awardees are kindly asked to sign their reimbursement forms at the Registration Desk on 26.08.2022 and provide their travel and/or accommodation documents or send them via email to konferencje@uj.edu.pl.

Welcome Gifts

All Participants will be given a Welcome Gift. Additionally, Participants with the Regular Package (★★) will receive an extra gadget and Full Package Participants (★★★) will also get a VIP gift (the VIP gift is 28×24×10 cm in size and weighs 3 kg, so please keep some free room in your luggage for your trip back home).

Certificates

On the last day of the conference, all Participants will receive a printed Attendance and Presentation (if applicable) Symposium Certificate with a matte holographic Symposium seal. Moreover, digitally signed PDF Certificates will be sent individually to the email addresses provided at registration.

A printed and a digital Symposium Attendance & Presentation Certificate.

Symposium Proceedings

Symposium Proceedings will be published in the *Zoological Journal of the Linnean Society* (ZJLS), one of the most prestigious zoological journals in the World, published by the **Linnean Society of London**, one of the oldest scientific societies, and by the **Oxford University Press**, one of the most recognised scientific publishers on the planet. Proceedings will be edited by ŁUKASZ MICHALCZYK, KAZUHARU ARAKAWA & VLADIMIR GROSS.

The deadline for manuscript submission to the *Zoological Journal of the Linnean Society* is **31.10.2022.**

POLISH ESSENTIALS

A few (obvious and less obvious) facts and tips about Poland that may make your stay here easier and more enjoyable:

- Universal **emergency** phone number is 112 (free of charge).
- The Republic of Poland (Poland in short) is a member state of the **European Union** (EU), Schengen Area and the **North Atlantic Treaty Organisation** (NATO).
- Time zone in August is the **Central European Summer Time** (CEST) – BTW, please remember, we are a Central (not an Eastern) European country ☺
- Official language: **Polish** (see also below).
- **Street traffic is right-sided**, so always look left first when crossing the street! At pedestrian/zebra crossings, pedestrians have priority over cars **but not over trams**. Nevertheless, please always look both ways before crossing the street. Crossing the street when the red light is on is illegal.
- The currency is **Polski Złoty (zł. or PLN)**. There are many bureaux de change ("*kantor wymiany walut*" or simply "*kantor*" in Polish) in the Old Town, but we advise to avoid those in the most touristy places (*e.g.* Floriańska Street or the Airport), as they tend to have unfavourable exchange rates and spreads, and they also often charge commission fees. However, there are places with good exchange rates, narrow spreads and no commission fees very close to the City Centre and they are worth a few minute walk from the Main Square (*e.g. Kantor Merkury* or *Kantor Va Banque*, both at Wielopole Street). Current average exchange rates are 1 PLN ≈ 0.23 USD / 0.22 EUR / 0.18 GBP. Paypass payments with credit/debit cards and smartphones are very popular.
- Debit/credit cards in other currencies are accepted in most places. In those cases, you can select if you want to pay in Polish Złoty or in your own currency. It is always advisable to select Polish Złoty because in that case it is applied the standard exchange rate. If you select your own currency, your bank may apply an inflated rate or fees.
- We use the **Metric System**, *i.e.* grams/kilograms (g/kg) for mass, metres/kilometres (m/km) for distance, and centigrade (°C) for temperature.
- Electric current is 230 (220–240) Volts and 50 MHz; **electric sockets/power plugs are of the Type E** (but Types C and F also work).
- **Tap water is drinkable.**
- Supermarkets and most **shops are closed on Sundays** and public holidays.
- Drinking alcohol in the streets (*i.e.* outside beer gardens) is prohibited.
- There are several useful websites and mobile phone applications that may help you to get around the city using **public transportation** (***MPK*** in Polish). You may want to try https://jakdojade.pl/krakow website or their mobile phone application, or mobileMPK mobile phone application. This website shows the current position of buses and trams as toys in a playful way: https://www.mapakrakow.pl. Tickets can be bought from ticket machines at bus/tram stops and also on buses/trams themselves. Please be aware that some ticket machines only accept payments by card. You may also rent city bicycles and electric scooters to swiftly get around the city (we don't recommend renting a car, as traffic can be bad and finding a parking spot may also be frustrating).

Pronunciation survival guide

Due to exotic letter combinations, strange diacritic signs and too few vowels, Polish may seem unpronounceable at the first sight. However, if you master a few basic rules, most Polish words may suddenly become more friendly, and – believe it or not – pronounceable! ☺

Here're some basic pronunciation rules that may help you to get around in Poland and/or even impress someone:

- **a** as "*a*" in the English "*sm<u>a</u>rt*"
- **ą** is a nasal sound that is similar to the French "on" in "*garç<u>on</u>*"
- **c** is similar to the Italian "*z*" in "*<u>z</u>ucchero*" or to the Greek "*tz*" in "*<u>tz</u>a<u>tz</u>iki*"
- **ć** (= **ci**) is similar to the Italian "*ci*" in "*<u>ci</u>ao*"
- **ch** (= **h**) is similar to the English "*h*" in "*<u>h</u>ouse*"
- **cz** as the English "*ch*" in "*cat<u>ch</u>*"
- **dz** sounds like a hard version of c
- **dź** a softer version of **dż** (see below)
- **dż** as the English "*g*" in "*<u>g</u>entle*"
- **e** as in the English "*e*" in "*m<u>e</u>t*"
- **ę** is a nasal sound similar to the French "*en*"
- **g** as "*g*" in the English "*<u>g</u>arden*"
- **i** as a short version of "*ea*" in the English "*b<u>ea</u>ch*"
- **j** as "*y*" in the English "*<u>y</u>es*"
- **ł** as the English "*w*" in "*<u>w</u>ould*"
- **ń** as the English "*<u>new</u>*" or Spanish "ñ" in "*Espa<u>ñ</u>a*"
- **o** as in the English "*o*" in "*p<u>o</u>rt*"
- **ó** (= **u**) it's a short version of the English "*oo*" (as in "*w<u>oo</u>d*")
- **rz** (= **ż**) as in the English "*su*" in "*pleas<u>u</u>re*" or in the French "*j*" in "*<u>j</u>ournal*"
- **si** (= **ś**) as in English "*su*" in "*<u>su</u>re*"
- **sz** as in the English "*sh*" in "*<u>sh</u>op*"
- **ś** (= **si**) – see **si** above
- **u** (= **ó**) – see **ó** above
- **w** as in the English "*v*" in "*<u>v</u>ictory*"
- **y** as in the English "*y*" in "*sy<u>l</u>lable*"
- **ź** (= **zi**) a softer version of **rz**/**ż** (see above)
- **ż** (= **rz**) – see **rz** above

Thus, for example, Kraków is pronounced in Polish as "Krakoov", not as "Krakau" as it might seem at first sight ☺

GETTING TO KRAKÓW

Visas

Before planning your trip, **please check whether you need a visa to enter Poland** and, if you do, what kind of visa it is – one that you can get at the Kraków Airport or one that you need to apply for in advance. If it's the latter scenario, please apply for your visa as soon as possible. Information on visa types can be found on the website of the Polish Ministry of Foreign Affairs.

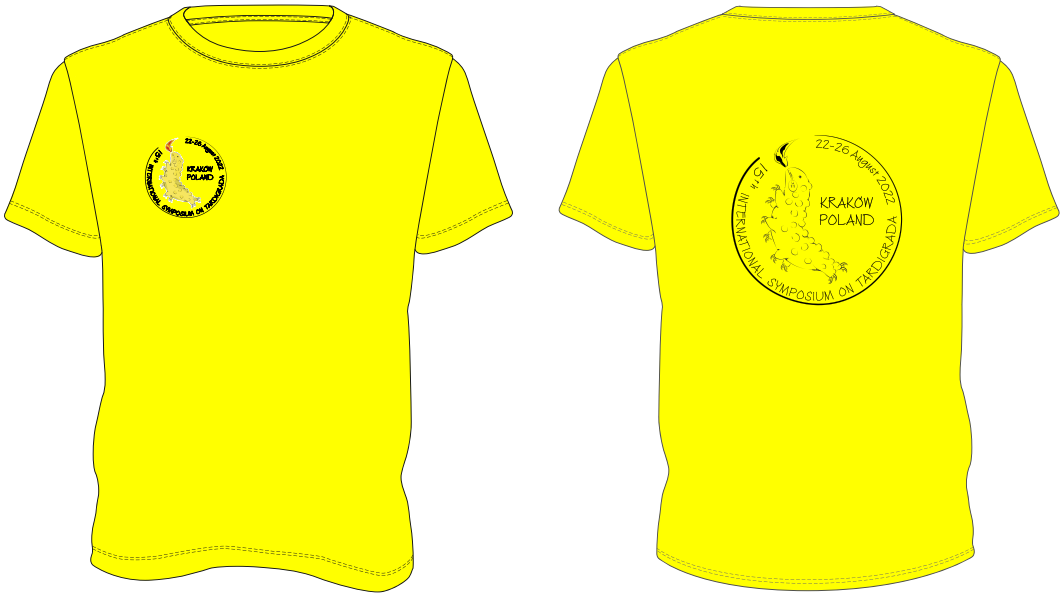
Getting to Kraków

Depending on the distance between your residence and Kraków, you can get to the Royal City by aeroplane, train, or coach/bus.

There are three most important transportation hubs you should get acquainted with prior to coming to Kraków:

- **The Kraków Airport** (IATA code: **KRK**), also known as the **Balice International Airport** or **John Paul II International Airport** (in Polish: *Port Lotniczy im. Jana Pawła II* or simply *Lotnisko w Balicach*), which is situated ca. 15 km from the Symposium Venue that is located in the Old Town (City Centre) and can be reached by the SKA1 Train, Airport Taxi, Uber/Bolt or City Bus (see below for more details on how to get from the airport to the Kraków Old Town). The Kraków Airport has good and frequent connections with larger European airway hubs, such as Frankfurt (Germany), Amsterdam (the Netherlands), or London (UK).
- **The Main Train Station** (in Polish: *Dworzec Główny Kraków* or simply *Kraków Główny*) is located in the immediate vicinity of the Main Coach Station and both are located close to the Old Town (ca. 1.5 km = within walking distance).
- **The Main Coach Station** (in Polish: *Małopolski Dworzec Autobusowy* or simply *Dworzec Autobusowy*) is located in the immediate vicinity of the Main Train Station and both are located close to the Old Town (ca. 1.5 km = within walking distance).

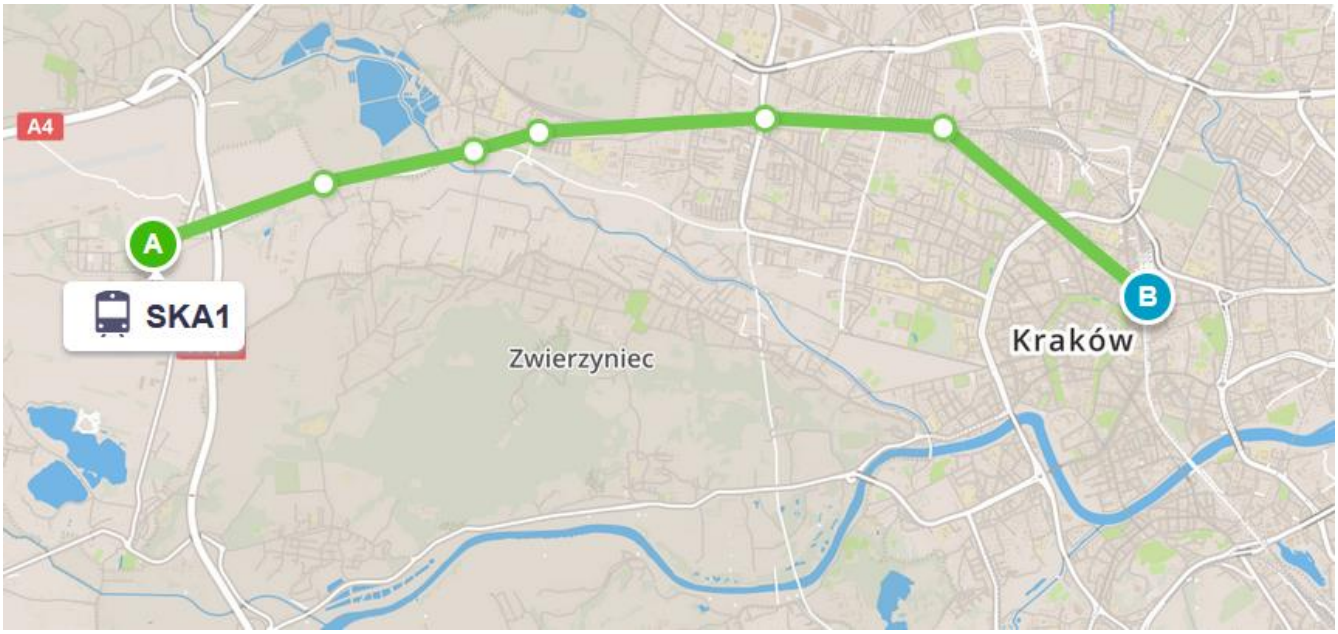
In order to make your arrival as comfortable and as smooth as possible, our Student Volunteers, dressed in yellow Symposium T-shirts (to make them easy for you to spot), will be at the Airport throughout Sunday the 21st of August to help you choose and get on the means of transportation of your choice. Please fill this yellow form to let us know what time, with what airlines and from which airport you will be arriving to Kraków.



FRONT **BACK**

TRAIN

- This is the easiest, fastest and the second cheapest way to get to the Main Train Station in Kraków.
- We suggest it if your accommodation is within walking distance of the Main Train Station.
- There is only one train line (**SKA1**) going from the airport to the city, so there is no risk of getting on a wrong train.
- Sometimes the train terminates at the Main Train Station in Kraków and in such cases the destination is "**Kraków Główny**", but some services terminate further, at the Wieliczka Salt Mine. In such instances, the destination is "**Wieliczka Rynek-Kopalnia**" or simply "**Wieliczka**" and you need to make sure to get off the train at the right station ☺ (the Main Train Station is the seventh stop, with the initial Airport stop being the first stop).
- The timetable can be accessed here.
- A one-way ticket costs 16 PLN and can be bought on the train from a ticket machine (you can pay with a debit/credit card or a smartphone with the paypass/NFC function on) or from the conductor (cash only) who passes through the train and punches the tickets during the journey.
- Please remember that passengers travelling on public transport in Poland must wear face masks covering the mouth and nose at all times.
- The ride takes ca. 15 min and the route is as follows:



UBER or BOLT

- Easy to book (you need to install the appropriate application on your smartphone) and will take you directly to your accommodation.
- It takes ca. 20–40 min to get to the city, depending on traffic, which depends on the day of the week and time of day). If you choose to fly in on Sunday the 21st of August (which we recommend), the traffic shouldn't be too high.
- Prices vary depending on the demand, car quality, and traffic, but usually they are within the range of 25–75 PLN.
- Given that payments are processed via smartphone apps, you will pay with your own currency (smartphone apps will do the currency exchange automatically for you).
- Please remember that passengers travelling by Uber/Bolt in Poland must wear face masks covering the mouth and nose at all times.

TAXI

- The Kraków Airport offers official taxi service.
- It takes ca. 20–40 min to get to the City (depends on traffic, which depends on the day of the weak and time of the day). If you choose to fly in on Sunday the 21st of August (which we recommend), the traffic shouldn't be too high.
- You can take the taxi in front of the Airport or book it online in advance.
- It should cost you ca. 90–100 PLN, depending on where your accommodation is (in this Airport Taxi Service world, the Kraków Old Town lies in "Zone 4").
- Please remember that passengers travelling by taxi in Poland must wear face masks covering the mouth and nose at all times.

CITY BUS (MPK)

- Although public transportation in Kraków (buses, trams and river trams – all operating under the city-owned MPK company) are generally reliable, run frequently and there is a dense connection grid, getting from the Airport to Kraków by city bus is not the best choice.
- Even though it is the cheapest, it is also definitely the slowest way to get to the Main Train Station in Kraków. Thus, we do not recommend it, unless there really is no other option available.
- The tickets can be bought from ticket machines at the airport and in the buses.
- You must get a 60-minute ticket for 6 PLN and punch the ticket as soon as you board the bus.
- Please remember that passengers travelling on public transport in Poland must wear face masks covering the mouth and nose at all times.

If you want to use cash, we suggest bringing ca. 100–300 PLN for starters with you or exchange a small amount at the Airport (we don't recommend exchanging larger amounts at the Airport, as the rates are not the best). In the section Polish Essentials, there are some tips on where to best exchange your currency.

KRAKÓW MAPS

Kraków and its surroundings. Map source: https://streetmap.pl.

Kraków's Old Town (*Stare Miasto*) and the Jewish Quarter (*Kazimierz*). Map source: https://streetmap.pl.

VENUE (AUDITORIUM MAXIMUM)

All scientific activities as well as coffee breaks and lunches will take place in the **Auditorium Maximum of the Jagiellonian University**. The venue, "AudiMax" in short, is located at **33 Krupnicza Street**, within Kraków's second ring road, within walking distance from the centre of the Old Town, *i.e.* Main Square (ca. 1 km/12 min by foot).

The **Registration Desk**, where you need to register to collect your ID badge and your Welcome Gift Package, is located on the **Ground Floor**, at the end of the corridor which is on the right side when entering the building.

The **Lecture Hall**, where all talks will be given is located across the **Ground and the First Floor**. The Lecture Hall can be entered both from the Ground Floor (via side doors at the bottom row level) and from the First Floor (via back doors at the top row level).

The **Exhibition Room**, where all Coffee Breaks, Lunches and Poster Sessions will be held, is located on the **Second Floor**, to the right from the staircase.

Light Contrast Microscopes (LCMs) will be located on the **First Floor**, left to the staircase.

Toilets are located on **all levels**.

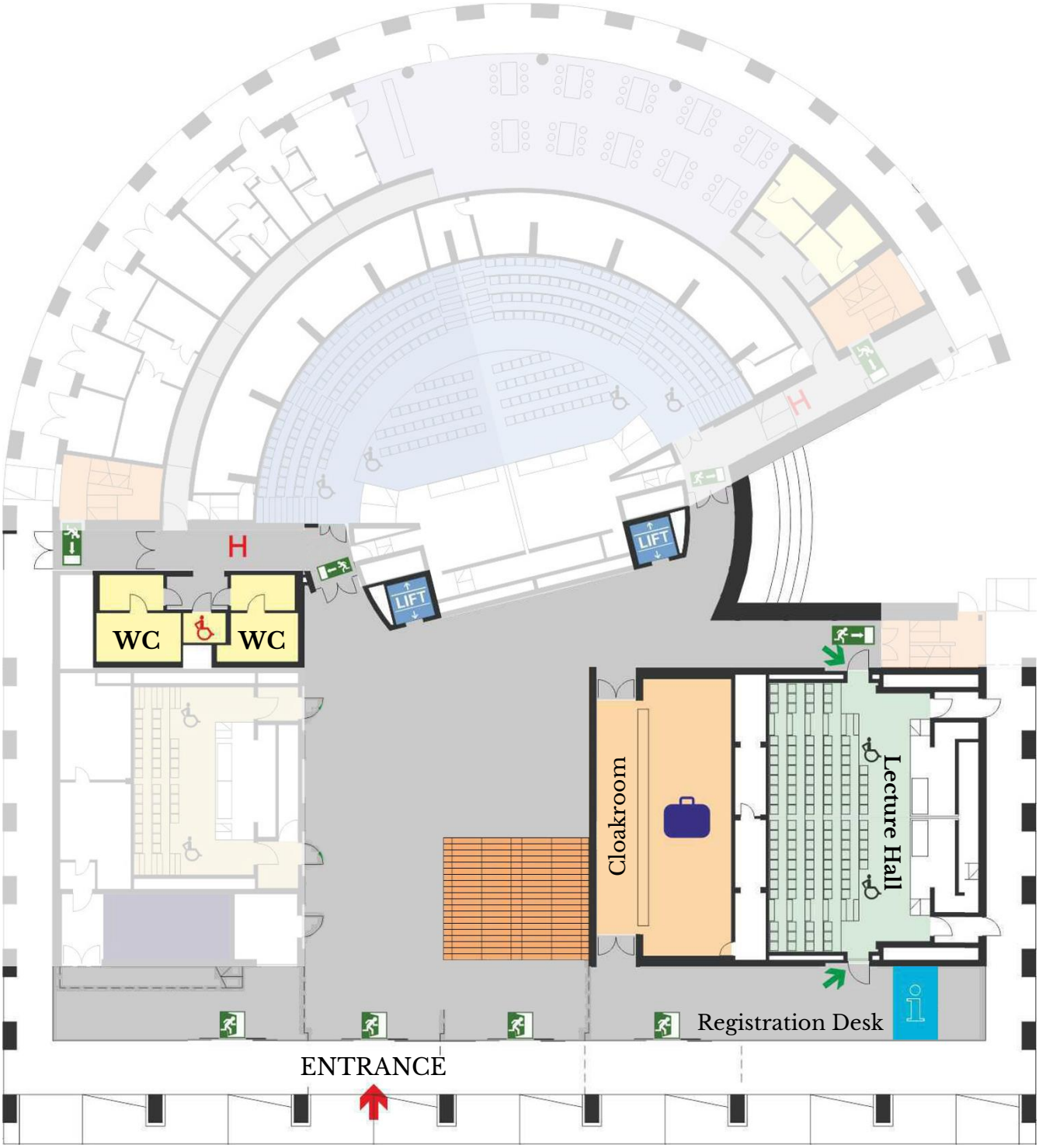
All levels can be accessed by both stairs and lifts.

There is **Wireless Internet** in the AudiMax, available freely to all Symposium Participants (Wi-Fi: **maximumwifi@uj.edu.pl**; **Tardies#15**).

On the following three pages are AudiMax blueprints with spaces available to Symposium Participants marked in vivid colours and the remaining spaces with dimmed colours. The fourth blueprint presents the arrangement of poster stands as well as positions of cocktail tables and tables with beverages and meals.

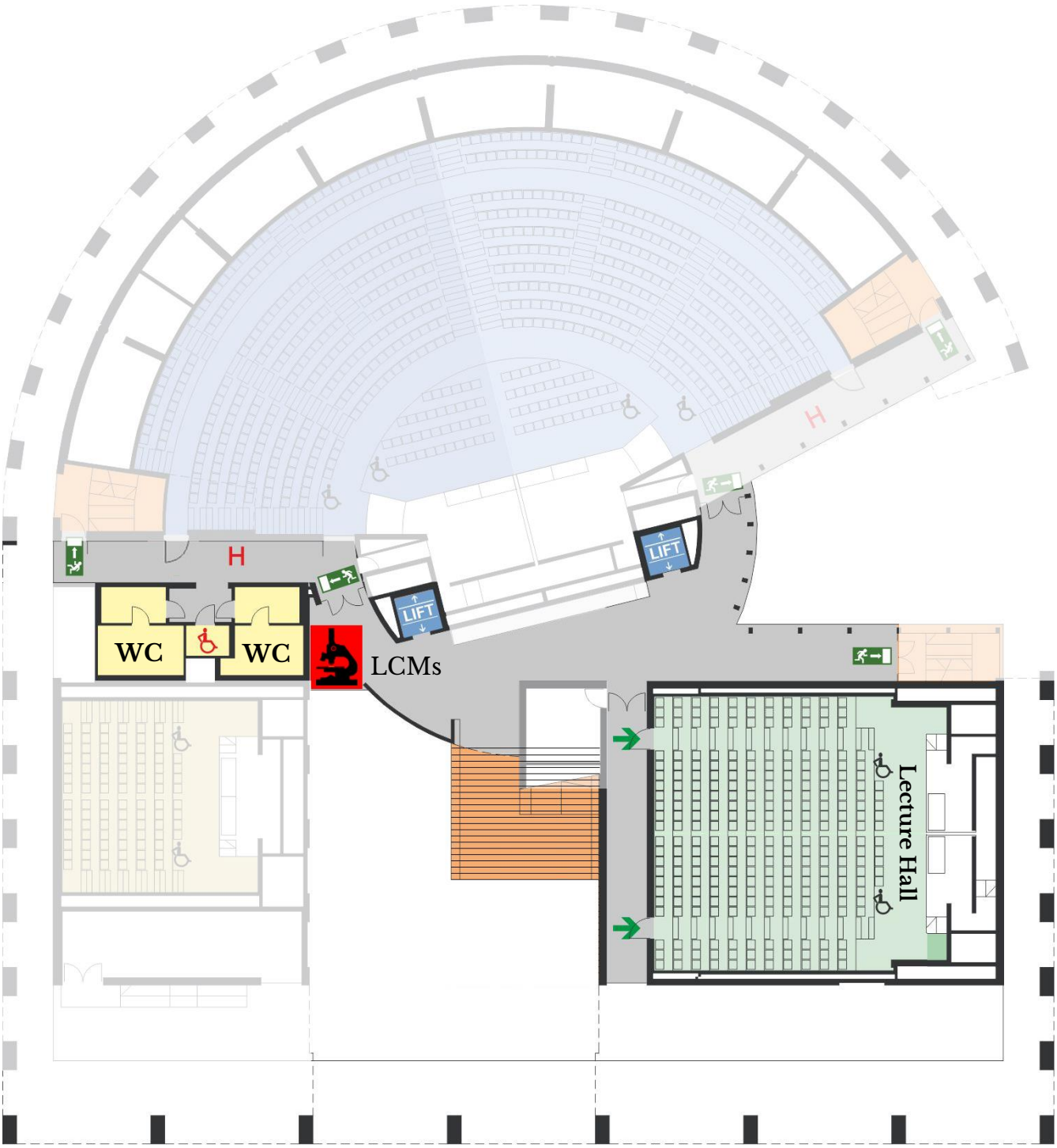
AudiMax

GROUND FLOOR



AudiMax

FIRST FLOOR



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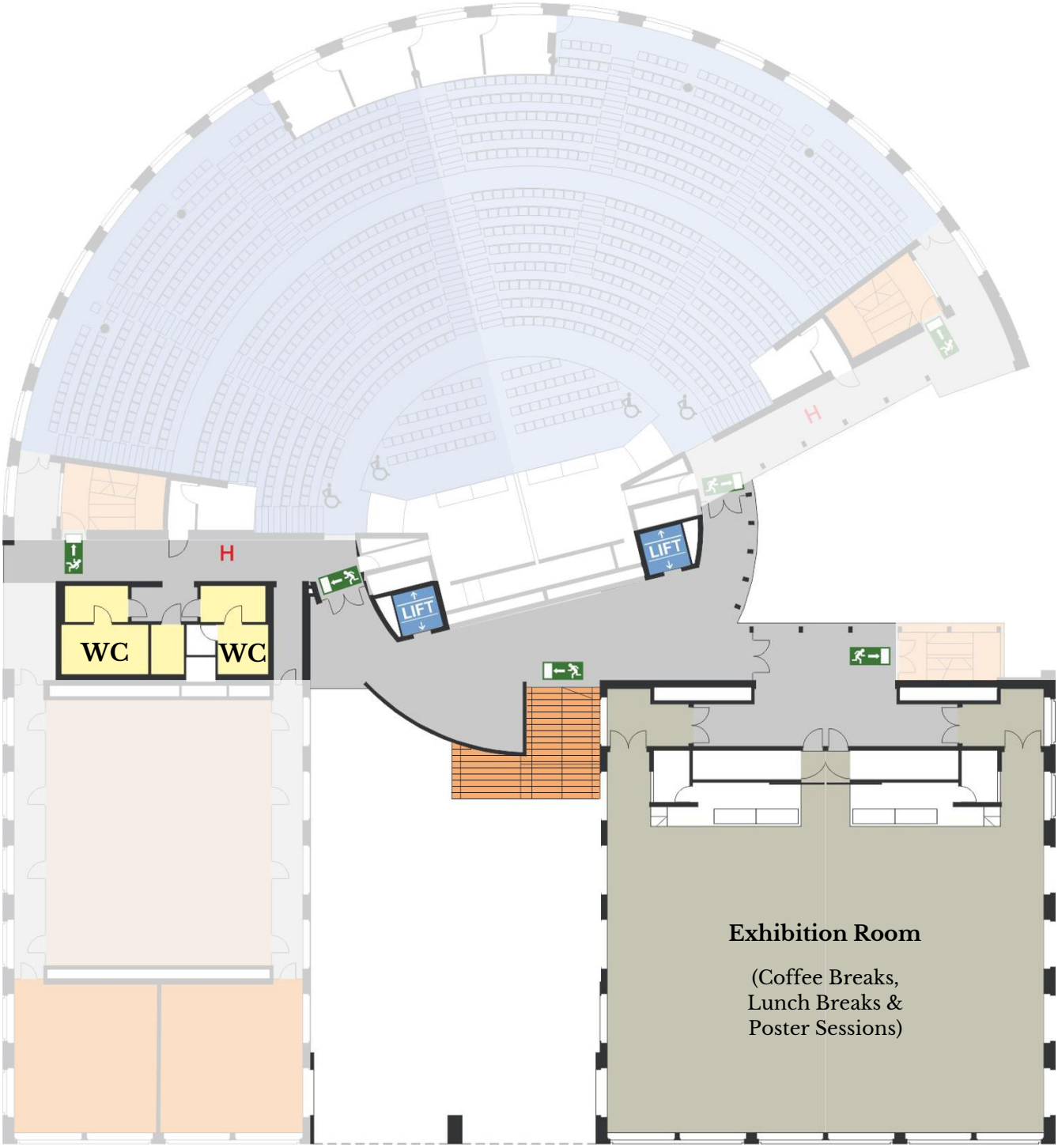
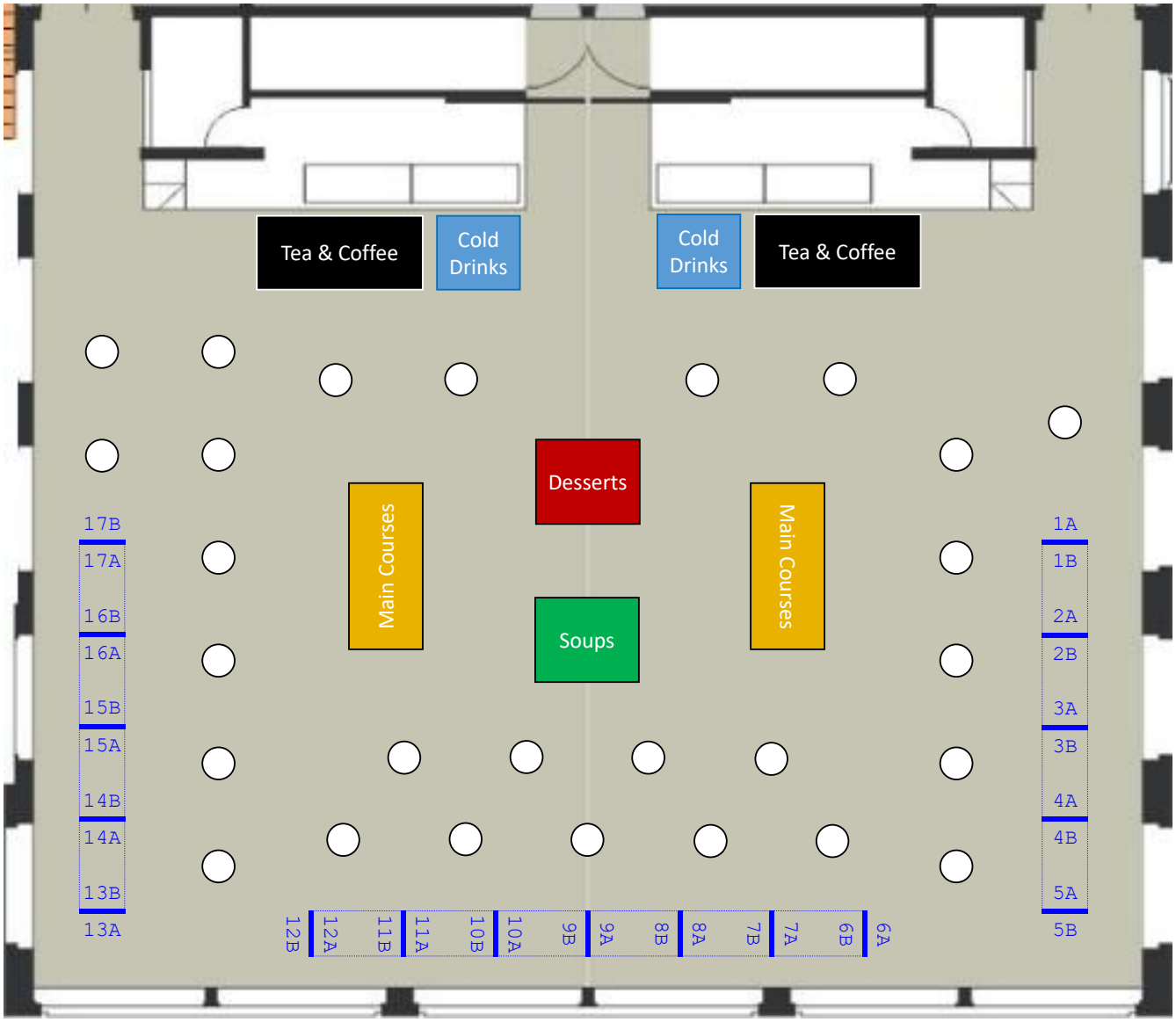
SECOND FLOOR



AudiMax

EXHIBITION ROOM ARRANGEMENT



PROGRAMME OVERVIEW (PARTICIPANTS)

DAY / TIME	Monday 22.08	Tuesday 23.08	Wednesday 24.08	Thursday 25.08	Friday 26.08	DAY / TIME
08:00	Registration & Poster Installation 1	Free Time	Free Time	Free Time	Free Time	08:00
08:15						08:15
08:30						08:30
08:45						08:45
09:00			Poster Installation 2			09:00
09:15	Opening Ceremony					09:15
09:30	**Invited Lecture 1** *Phylogeny*	**Invited Lecture 2** *EvoDevo*	**Invited Lecture 3** *EvoDevo*	**Invited Lecture 4** *Cryptobiosis*	**Talk Session 8** [4 talks] *Physiology*	09:30
09:45						09:45
10:00						10:00
10:15						10:15
10:30	Coffee Break 1	Coffee Break 3	Coffee Break 5	Coffee Break 7	Coffee Break 8	10:30
10:45						10:45
11:00	**Talk Session 1** [6 talks] *Biodiversity*	**Talk Session 3** [5 talks] *Biodiversity*	**Talk Session 5** [6 talks] *Morphology*	**Talk Session 7** [6 talks] *Physiology*	**Talk Session 9** [5 talks] *Physiology*	11:00
11:15						11:15
11:30						11:30
11:45						11:45
12:00						12:00
12:15		Group Photo			Closing Ceremony	12:15
12:30	Lunch 1	Lunch 2	Lunch 3	Lunch 4	Lunch 5	12:30
12:45						12:45
13:00						13:00
13:15						13:15
13:30						13:30
13:45	**Talk Session 2** [5 talks] *Biodiversity*	**Talk Session 4** [5 talks] *Ecology*	**Talk Session 6** [5 talks] *Physiology*	**Poster Session 2** & Poster Deinstallation 2	**Microscope Slide Examination**	13:45
14:00						14:00
14:15						14:15
14:30						14:30
14:45						14:45
15:00	Coffee Break 2 & **Poster Session 1**	Coffee Break 4 & **Poster Session 1** & Poster Deinstallation 1	Coffee Break 6 & **Poster Session 2**	Wieliczka Salt Mine Excursion ★★★	Free Time	15:00
15:15						15:15
15:30						15:30
15:45						15:45
16:00						16:00
16:15	Welcome Reception [Collegium Nowodworskiego Courtyard] ★	Collegium Maius Tour ★	Rynek Underground Tour ★		Kraków Old Town Tour ★★	16:15
16:30						16:30
16:45						16:45
17:00						17:00
17:15						17:15
17:30						17:30
17:45		Free Time				17:45
18:00	Icebreaker [paid bar] ★					18:00
18:15			Free Time			18:15
18:30					Symposium Banquet Young Scientist Awards ★★	18:30
18:45						18:45
19:00						19:00
19:15				Free Time		19:15
19:30						19:30
19:45						19:45

PROGRAMME OVERVIEW
(ACCOMPANYING PERSONS)

DAY / TIME	Monday 22.08	Tuesday 23.08	Wednesday 24.08	Thursday 25.08	Friday 26.08	DAY / TIME
08:00	Registration	Free Time	Free Time	Free Time	Free Time	08:00
08:15						08:15
08:30						08:30
08:45						08:45
09:00			Tardigrading...			09:00
09:15	**Opening Ceremony**					09:15
09:30	Tardigrading...	Tardigrading...		Tardigrading...	Tardigrading...	09:30
09:45						09:45
10:00						10:00
10:15						10:15
10:30	**Coffee Break 1**	**Coffee Break 3**	**Coffee Break 5**	**Coffee Break 7**	**Coffee Break 8**	10:30
10:45			**Oskar Schindler's Enamel Factory**			10:45
11:00	Tardigrading...	Tardigrading...		Tardigrading...	Tardigrading...	11:00
11:15						11:15
11:30						11:30
11:45						11:45
12:00						12:00
12:15		**Group photo**			**Closing Ceremony**	12:15
12:30	**Lunch 1**	**Lunch 2**	**Lunch 3**	**Lunch 4**	**Lunch 5**	12:30
12:45						12:45
13:00						13:00
13:15						13:15
13:30		**Wawel Royal Castle**				13:30
13:45	**Saint Mary's Basilica**		Tardigrading...	Tardigrading...	Tardigrading...	13:45
14:00						14:00
14:15						14:15
14:30						14:30
14:45						14:45
15:00				**Wieliczka Salt Mine Excursion** ★★★		15:00
15:15	**Coffee Break 2**		**Coffee Break 6**		Free Time	15:15
15:30		**Coffee Break 5**				15:30
15:45						15:45
16:00	Tardigrading...	Tardigrading...	Tardigrading...			16:00
16:15	**Welcome Reception [Collegium Nowodworskiego Courtyard]** ★	**Collegium Maius Tour** ★	**Rynek Underground Tour** ★		**Kraków Old Town Tour** ★★	16:15
16:30						16:30
16:45						16:45
17:00						17:00
17:15						17:15
17:30						17:30
17:45		Free Time	Free Time			17:45
18:00	**Icebreaker [paid bar]** ★					18:00
18:15						18:15
18:30					**Symposium Banquet Young Scientist Awards** ★★	18:30
18:45						18:45
19:00						19:00
19:15				Free Time		19:15
19:30						19:30
19:45						19:45

PROGRAMME DETAILS (PARTICIPANTS)

[☆ – Young Scientist Award Contestant]

MONDAY | 22.08.2022

08:00–09:15 **REGISTRATION & POSTER INSTALLATION #1** | Jagiellonian University Auditorium Maximum, Krupnicza 33

09:15–09:30 **OPENING CEREMONY** | AudiMax Lecture Hall

09:30–10:30 **INVITED LECTURE #1** | AudiMax Lecture Hall
A timeline for ecdysozoan diversification
EDGECOMBE, HOWARD, GIACOMELLI, LOZANO-FERNANDEZ & DONOGHUE
Chair: MICHALCZYK

10:30–11:00 **COFFEE BREAK #1** | AudiMax Exhibition Room

11:00–12:30 **TALK SESSION #1** | AudiMax Lecture Hall
Biodiversity, Taxonomy, Biogeography, Phylogeny & Evolution
Chairs: BARTELS & LISI

11:00–11:15 Solving the six decade-long mystery: phylogenetic relationships of the Cretaceous Canadian tardigrade amber fossils
MAPALO☆ & ORTEGA-HERNÁNDEZ

11:15–11:30 New fossil Eutardigrada from Rovno Eocene amber (Ukraine, 34–38 MYA)
INSHYNA, KACZMAREK, PERKOVSKY, HAMMEL, HEß & HAUG

11:30–11:45 A revision of the marine Orzeliscinae (Arthrotardigrada, Tardigrada)
KRISTENSEN, MØBJERG, MØBJERG & JØRGENSEN

11:45–12:00 Phylogeny and biogeography of the genus *Xerobiotus* (Macrobiotidae, Eutardigrada)
VINCENZI☆, CESARI, KACZMAREK, ROSZKOWSKA, MIODUCHOWSKA, REBECCHI, KIOSYA & GUIDETTI

MONDAY | 22.08.2022

12:00–12:15	Phylogeny of the superfamily Isohypsibioidea (Parachela, Eutardigrada) GUIDETTI, SALA, GIOVANNINI, REBECCHI & CESARI
12:15–12:30	Systematist's nightmare: an updated classification of Isohypsibioidea (Eutardigrada) GĄSIOREK, MOREK & MICHALCZYK
12:30–13:45	**LUNCH #1** | AudiMax Exhibition Room
13:45–15:00	**TALK SESSION #2** | AudiMax Lecture Hall **Biodiversity, Taxonomy, Biogeography, Phylogeny & Evolution** **Chairs: KRISTENSEN & ZAWIERUCHA**
13:45–14:00	Research on diversity of tardigrades in Northern China XUELING, JINGYU, NING, HEPING, MIN & XUEGANG
14:00–14:15	Riding on wind, birds or staying home? – Phylogeography of Nearctic *Milnesium* (Eutardigrada: Apochela) WAŁACH☆, LOEFFELHOLZ, MOREK, LÓPEZ-LÓPEZ, PIENAAR & MICHALCZYK
14:15–14:30	A first look into peatland moss tardigrades in Finland MÄENPÄÄ☆, BORG, SILENIUS, SOININEN, ELO, KOTIAHO & CALHIM
14:30–14:45	Current state of knowledge of the limno-terrestrial tardigrade fauna of the Republic of Argentina OSTERTAG, ROCHA, DOMA, GONZALEZ-REYES, CAMARDA, GRABOSKY & LISI
14:45–15:00	Environmental DNA metabarcoding of Danish soil samples reveals new insight into the hidden diversity of eutardigrades in Denmark PUST☆, MØBJERG, KRISTENSEN & FRØSLEV
15:00–16:15	**COFFEE BREAK #2 & POSTER SESSION #1** | AudiMax Exhibition Room **Physiology, Morphology & Ecology** **Chaperons: GĄSIOREK & MOREK**

MONDAY | 22.08.2022

16:15–18:00

WELCOME RECEPTION ★ | Collegium Nowodworskiego Courtyard (Św. Anny 12)

We'll all go from the Auditorium Maximum to the Collegium Nowodworskiego on foot together, so don't worry if you don't know where the Collegium is. Our Volunteers will show the way, so you'll just need to follow the yellow T-shirts. The distance is ca. 900 m and the walk should take ca. 10 min (https://goo.gl/maps/5sU3tF3LhtfEgKht8). We'll stop by Collegium Novum, the main building of the Jagiellonian University, on the way. **In the case of heavy rain, Welcome Reception will be held at the AudiMax.**

18:00–??:??

ICEBREAKER ★ | Budda Bar (Rynek Główny 6) | Paid Bar with Tardigrade Cocktails ☺

Those of you who would like to continue to party after the Welcome Reception are invited to join the Icebreaker at Budda Bar in the Main Square. This is a paid bar, but all Symposium Participants and Accompanying Persons will have a 20% discount upon showing their Symposium Badge. There will be Polish vodka, liqueurs and beer, also in the form of tardigrade cocktails. Our Volunteers will show you the way, so you'll just need to follow the yellow T-shirts. The distance is ca. 450 m and the walk should take ca. 5 min (https://goo.gl/maps/YE3Kpr2edmWY5hYCA). **In the case of rain, we'll go to the Bar directly from the AudiMax.**

TUESDAY | 23.08.2022

09:30–10:30 **INVITED LECTURE #2** | AudiMax Lecture Hall
Tardigrades in development and evolution
HEJNOL
Chair: MICHALCZYK

10:30–11:00 **COFFEE BREAK #3** | AudiMax Exhibition Room

11:00–12:15 **TALK SESSION #3** | AudiMax Lecture Hall
Biodiversity, Taxonomy, Biogeography, Phylogeny & Evolution
Chairs: MCINNES & SUZUKI

11:00–11:15 A new pantropical genus exposes the challenges of ramazzottiid phylogeny (Eutardigrada: Parachela)
DEY, MOREK, LÓPEZ-LÓPEZ & MICHALCZYK

11:15–11:30 Development of morphological data matrices for the use in integrated taxonomy
MARLEY & MCINNES

11:30–11:45 A new species of *Paramacrobiotus* from Hawaii with a high level of allometric variation in key morphometric traits
MEYER & JACKSON

11:45–12:00 First time-calibrated multilocus phylogeny of the phylum Tardigrada (Ecdysozoa: Panarthropoda)
LÓPEZ-LÓPEZ, GĄSIOREK, MOREK, DEY & MICHALCZYK

12:00–12:15 Broad sampling and HTS spark a revolution in our understanding of tardigrade biogeography
MICHALCZYK, MOREK, GĄSIOREK, DEY & LÓPEZ-LÓPEZ

12:15–12:30 **GROUP PHOTO** | AudiMax Front Steps

12:30–13:45 **LUNCH #2** | AudiMax Exhibition Room

TUESDAY | 23.08.2022

13:45–15:00 **TALK SESSION #4** | AudiMax Lecture Hall
Ecology, Life Histories & Behaviour
Chairs: Nelson & Calhim

13:45–14:00 The importance of soil structure for soil-inhabiting tardigrades in polar habitats
Tůmová, Jílková, Macek & Devetter

14:00–14:15 Control of tardigrades over cryoconite hole ecosystems
Zawierucha☆, Buda, Novotna Jaromerska, Ambrosini, Franzetti, Poniecka, Klimaszyk, Rozwalak, Richter, Pietryka & Bagshaw

14:15–14:30 Life cycle of *Acutuncus antarcticus* and acclimatory adaptation during exposure to increasing temperature
Giovannini, Manfrin, Altiero, Greco, Vincenzi, Guidetti, Giulianini & Rebecchi

14:30–14:45 Semiochemical-based mate searching behaviour in tardigrades: comparing the sexes
Chartrain☆, Knott, Michalczyk, Puro, Tynkkynen & Calhim

14:45–15:00 Effect of juvenile hormone, methyl farnesoate, on sex determination in *Paramacrobiotus metropolitanus*
Matsumoto, Uchida, Minato, Suzuki, Xie, Miyakawa & Sugiura

15:00–16:15 **COFFEE BREAK #4 & POSTER SESSION #1** | AudiMax Exhibition Room
Physiology, Morphology & Ecology
Chaperons: Gąsiorek & Morek
!!! Please remove your posters at the end of the Session !!!

16:15–17:30 **COLLEGIUM MAIUS TOUR** ★ | Jagiellońska 15
We'll all go from the Auditorium Maximum to the Collegium Maius on foot together. Our Volunteers will show the way, so you'll just need to follow the yellow T-shirts. This time we'll take a slightly shorter route: the distance is ca. 800 m and the walk should take ca. 10 min (https://goo.gl/maps/vEP2vMPyLqcYyn3JA). In addition to seeing the interiors of Collegium Maius, the oldest standing building of the Jagiellonian University, you'll also have a chance to see a Nobel Prize Medal and an Oscar Award.

WEDNESDAY | 24.08.2022

09:00–09:30 **POSTER INSTALLATION #2** | AudiMax Exhibition Room

09:30–10:30 **INVITED LECTURE #3** | AudiMax Lecture Hall
The tardigrade *Hypsibius exemplaris* as a model for body plan evolution
SMITH, CHAVARRIA, GAME & HARRISON
Chair: MICHALCZYK

10:30–11:00 **COFFEE BREAK #5** | AudiMax Exhibition Room

11:00–12:30 **TALK SESSION #5** | AudiMax Lecture Hall
Morphology, Anatomy, Reproduction & Development
Chairs: GUIDETTI & POPRAWA

11:00–11:15 Reproductive strategy of tardigrades: gamete motility and morphology
SUGIURA☆ & MATSUMOTO

11:15–11:30 Evolution of sperm morphology in Macrobiotoidea
VECCHI☆, RYNDOV, MICHALCZYK, NELSON, REBECCHI, STEC & CALHIM

11:30–11:45 Morphology of light perception: localisation of opsins in the eutardigrade *Hypsibius exemplaris*
GROSS, EPPLE, KLEIN & MAYER

11:45–12:00 Three-dimensional reconstruction of the eye in *Hypsibius exemplaris* based on nano-computed tomography and ultrastructure
KLEIN, GROSS, GREVING, LONGO & MAYER

12:00–12:15 Cuticle and cuticular capsule in freshwater tardigrade *Thulinius ruffoi* – formation and shedding
JANELT☆ & POPRAWA

12:15–12:30 New insights on chemical composition and morpho-functional adaptations of tardigrade feeding apparatus
MASSA, REBECCHI & GUIDETTI

12:30–13:45 **LUNCH #3** | AudiMax Exhibition Room

WEDNESDAY | 24.08.2022

13:45–15:00	**TALK SESSION #6** | AudiMax Lecture Hall **Physiology, Omics, Cryptobiosis & Astrobiology** **Chairs: Arakawa & Jørgensen**
13:45–14:00	Sequencing genomes from single tardigrade individuals Morek, Stevens, Blaxter & Michalczyk
14:00–14:15	*In vivo* gene expression in tardigrades, a technical breakthrough coming to tardigrades Tanaka☆ & Arakawa
14:15–14:30	Gene mining of cytokine-like molecules in the eutardigrade *Mesobiotus philippinicus* Itang☆ & Mirano-Bascos
14:30–14:45	Doyère-Pouchet controversy (1859–1860) as the first stage of the spontaneous-generation controversy (1859–1864) Suzuki
14:45–15:00	Identification of promoters associated with tardigrade anhydrobiosis-related genes Ishikawa☆, Tanaka & Arakawa
15:00–16:15	**COFFEE BREAK #6 & POSTER SESSION #2** | AudiMax Exhibition Room **Biodiversity, Taxonomy, Biogeography, Phylogeny & Evolution** **Chaperons: López-López & Dey**
16:15–18:15	**RYNEK UNDERGROUND TOUR** ★ | Rynek Główny, Sukiennice (Main Square, Cloth Hall) We'll all go from the Auditorium Maximum to the Main Square Underground Museum on foot. The route is ca. 1 km long and the walk should take ca. 12 min (https://goo.gl/maps/hexYkXdnPPx25YZw7). The Underground Museum offers a unique insight into the medieval (and earlier) history of Kraków: by going several metres under the surface of the Main Square, you take a journey back in time.

THURSDAY | 25.08.2022

09:30–10:30 **INVITED LECTURE #4** | AudiMax Lecture Hall

Unravelling the mechanisms underlying extreme stress tolerance in tardigrades

MØBJERG

Chair: MICHALCZYK

10:30–11:00 **COFFEE BREAK #7** | AudiMax Exhibition Room

11:00–12:30 **TALK SESSION #7** | AudiMax Lecture Hall

Physiology, Omics, Cryptobiosis & Astrobiology

Chairs: MØBJERG & JÖNSSON

11:00–11:15 Tardigrade autofluorescence: UV protection or spandrel?

BARTELS, COFFEY, PINEAU, NELSON & KACZMAREK

11:15–11:30 Investigation of osmobiosis in the limno-terrestrial eutardigrade *Ramazzottius varieornatus*

EMDEE☆, MØBJERG, GROLLMANN & MØBJERG

11:30–11:45 Why are not all tardigrades good models for anhydrobiosis studies?

KACZMAREK, POPRAWA & KMITA

11:45–12:00 Tardigrades are shining models of anhydrobiosis research

ARAKAWA & TANAKA

12:00–12:15 Examining the resistance of active tardigrades to cyanide poisoning

BARTYLAK, BUDA, KMITA & KACZMAREK

12:15–12:30 Are invertebrates capable of surviving Martian concentrations of perchlorate?

KAYASTHA, GOŁDYN, ROBOTNIKOWSKI, ZACHARYASIEWICZ, NAGWANI, FIAŁKOWSKA, PAJDAK-STÓS, RZYMSKI & KACZMAREK

12:30–13:45 **LUNCH #4** | AudiMax Exhibition Room

13:45–15:00 **POSTER SESSION #2** | AudiMax Exhibition Room

Biodiversity, Taxonomy, Biogeography, Phylogeny & Evolution

Chaperons: LÓPEZ-LÓPEZ & DEY

!!! Please remove your posters at the end of the Session !!!

THURSDAY | 25.08.2022

15:00–19:00

WIELICZKA SALT MINE EXCURSION ★★★

The half-day excursion for Participants and Accompanying Persons who registered with the Full Package. You'll be taken to Wieliczka by coach, which will be waiting in front of the AudiMax. The coaches will also bring you back to the AudiMax in the evening. Since there'll be no afternoon Coffee Break on Thursday, you'll be given a take-away snack in a paper bag (a bottle of water, something savoury, something sweet and some fruit). The route is ca. 3.5 km long, takes up to 3 hrs, includes ca. 800 stairs, 320 of which are at the start, and the temperature underground (-135 m) is ca. 18 °C, thus please wear comfortable shoes and appropriate clothing.

FRIDAY | 26.08.2022

09:30–10:30 **TALK SESSION #8** | AudiMax Lecture Hall
Physiology, Omics, Cryptobiosis & Astrobiology
Chairs: ALTIERO & KACZMAREK

09:30–09:45 Physiological roles of tardigrade-unique heat-soluble proteins
KUNIEDA, YASUI & TANAKA

09:45–10:00 Mechanisms of cold tolerance in tardigrades (*Hypsibius exemplaris*)
LYONS☆, ROBERTS, CHENG, SHANG, SEE, RITCHIE, WU, STOCK & WILLIAMS

10:00–10:15 Factors affecting survival of repeated anhydrobiosis in a dioecious tardigrade *Paramacrobiotus experimentalis* Kaczmarek *et al.*, 2020
NAGWANI☆, KACZMAREK & KMITA

10:15–10:30 Soft electrophiles inducing dormancy in tardigrades
MOMENI☆, PHILLIPI, PIENAAR & CIESLA

10:30–11:00 **COFFEE BREAK #8** | AudiMax Exhibition Room

11:00–12:15 **TALK SESSION #9** | AudiMax Lecture Hall
Physiology, Omics, Cryptobiosis & Astrobiology
Chairs: SMITH & GROSS

11:00–11:15 DNA damage response of *Hypsibius exemplaris* to genotoxic stress
DE CIAN, ANOUD, DUVERNOIS-BERTHET, JOURDREN, ADRAIT, HEINRICH, BLUGEON, COUTE, GIOVANNANGELI, DELAGOUTTE & CONCORDET

11:15–11:30 Tardigrade phenotype classification using neural networks
VÍTEK☆, FRYČÁK, FÜRST, LACEY, VAVRUŠA & VOLLER

11:30–11:45 Tardigrade community microbiomes in North American orchards (Iowa, USA) include putative plant pathogens and endosymbionts
TIBBS-CORTES☆, TIBBS-CORTES & SCHMITZ-ESSER

11:45–12:00 Using fluorescence *in situ* hybridisation to visualise *Rickettsia* in tardigrades
TIBBS-CORTES☆, TIBBS-CORTES, SCHULTZ & SCHMITZ-ESSER

12:00–12:15 Identifying the core microbiome of tardigrades: what is really there?
SURMACZ☆, STEC, MICHALCZYK & ŁUKASIK

FRIDAY | 26.08.2022

12:15–12:30 **CLOSING CEREMONY & INVITATION TO TSURUOKA 2025** | AudiMax Lecture Hall

12:30–13:45 **LUNCH #5** | AudiMax Exhibition Room

13:45–15:00 **MICROSCOPE SLIDE EXAMINATION** | AudiMax Hall (1st floor)

Sponsored by Evident | Olympus

Evident | Olympus Polska will provide two Light Contrast Microscopes (LCM), with Phase (PCM) and Nomarski (NCM) contrasts, equipped with digital cameras and computers. The LCMs will be available to all Participants throughout the Symposium. Additionally, on Friday, we will bring type specimens deposited at the Jagiellonian University, according to your requests. Since there'll be a possibility to take and record accurate measurements and high-quality photomicrographs, we strongly encourage you to bring your own slides. This way you'll be able to discuss your specimens with other tardigradologists. If you'd like to save measurements and/or photos, please bring your USB drive with you too.

16:30–18:30 **KRAKÓW OLD TOWN TOUR** ★★ | Main Square – Wawel Hill – Wawel Dragon – Kazimierz (Jewish Quarter) – Banquet Venue

Guided Tour for Participants and Accompanying Persons who registered with the Regular or Full Package. Guides will show you and tell you about the most important and iconic sites in the Kraków Old Town, Wawel Hill (where you'll have a chance to see the statue of the Wawel Dragon that inspired the Symposium Logo), and parts of Kazimierz (the Old Jewish District). **We will meet at 16:30 at the Town Hall Tower in the Main Square (please don't be late!).** The route is ca. 5 km long and the Tour will take up to 2 hrs (https://goo.gl/maps/wn1B8JPigG5JQ4sL6). The Tour will end at the Banquet Venue (see below for more details). In the case of rain, the Tour will be cancelled.

18:30–??:?? **SYMPOSIUM BANQUET & YOUNG SCIENTIST AWARDS** ★★ | Stara Zajezdnia Restaurant (Św. Wawrzyńca 12)

Banquet for Participants and Accompanying Persons who registered with the Regular or Full Package. The Banquet will be held in a part of an old tram barn in Kazimierz. After the dinner, there will be an open bar and those of you who would like to continue the fun are welcome to stay for an afterparty at the Banquet Venue. During the Banquet, YSA for the best talk and poster both in the field of Zoology and Physiology will be presented.

PROGRAMME DETAILS
POSTER SESSIONS

MONDAY-TUESDAY | 22-23.08.2022

1 | A Biomedical applications of Tardigrada: prospects and perspectives
KASIANCHUK☆, RZYMSKI & KACZMAREK

1 | B Tardigrades and their future biotechnological potential
REHAMNIA & LEE

2 | A Building a framework for tardigrade transgenics: identification and evaluation of candidate genetic promoters
LYONS☆, CHIU, GEMRICH & KATO

2 | B Protein modelling and in silico characterisation of two putative TIR-binding proteins in the limnoterrestrial tardigrade *Mesobiotus philippinicus*
PANLAQUI & MIRANO-BASCOS

3 | A Characterisation of tardigrade tubulins
NOVOTNÁ FLORIANČIČOVÁ☆, BALTZIS, SMEJKAL, CZERNEKOVÁ, NOTREDAME & VINOPAL

3 | B Stress-dependent cell stiffening by tardigrade tolerance proteins CAHS through reversible formation of cytoskeleton-like filamentous network and gel-transition
TANAKA☆, NAKANO, WATANABE, MASUDA, HONDA, KAMATA, YASUI, KOZUKA-HATA, WATANABE, CHINEN, KITAGAWA, SAWAI, OYAMA, YANAGISAWA & KUNIEDA

4 | A Could mitochondrial DNA copy number be a marker of successful anhydrobiosis?
ANTOS-KRZEMIŃSKA, KOSICKA, KULPA, BARTYLAK, KACZMAREK, KARACHITOS, & KMITA

4 | B Tardigrade proteins – molecular tools in anhydrobiosis phenomenon
KRAKOWIAK, KMITA, KACZMAREK & NAWROT

5 | A Metabolic markers of different stages of tardigrade anhydrobiosis
ROSZKOWSKA, KACZMAREK, KARACHITOS & KMITA

5 | B Geomagnetobiology and tardigrades – current state of knowledge
ERDMANN, KOSICKI & KACZMAREK

MONDAY-TUESDAY | 22-23.08.2022

6 | A Assessing anhydrobiotic performance – a new analytical method
VECCHI & CALHIM

6 | B Development of a novel desiccation chamber for induction of tardigrade anhydrobiosis
CZERNEKOVÁ, MAJEROVÁ, SMEJKAL, NOVOTNÁ & VINOPAL

7 | A TarMass – a fast, accurate and easy to use tool for tardigrade biomass calculation
BUDA, GRYCMACHER & ZAWIERUCHA

7 | B Evaluating diversity of terrestrial tardigrades in different localities: do we need new standards of sampling?
MATSKO & KIOSYA

8 | A Method for obtaining genetic material of single tardigrade by ultrasonic fragmentation technology
JINGYU, XUELING, ZITONG, LANBI, HEPING & MIN

8 | B The gaps in the tardigrade database
REHAMNIA, PEDROS, QUEDRUE & LEE

9 | A Hidden *Wolbachia* infections: Peekaboo with a "master manipulator" and the discovery of multiple *Wolbachia* infections in water bears (Tardigrada)
MIODUCHOWSKA, KACZMAREK & BARTOSZEK

9 | B Do somatic and germline cells of *Dactylobiotus dispar* ovary respond in the same way to ibuprofen?
KARNÓWKA☆, BLONKOWSKA, WIECZORKIEWICZ, DŁUGOSZ, SOJKA & POPRAWA

10 | A Effect of ibuprofen on the midgut of tardigrade *Paramacrobiotus experimentalis*
MIERNIK☆, POPRAWA & FIAŁKOWSKA

10 | B Does the presence of paracetamol in the environment affect the ultrastructure of the midgut of *Hypsibius exemplaris*?
SOJKA☆, WIECZORKIEWICZ, KARNÓWKA & POPRAWA

11 | A Effect of paracetamol on the storage cells ultrastructure of *Hypsibius exemplaris*
WIECZORKIEWICZ☆, SOJKA, KARNÓWKA & POPRAWA

MONDAY-TUESDAY | 22-23.08.2022

11 | B Structural analysis of the cuticle and cuticular capsule in freshwater tardigrade *Thulinius ruffoi*
JANELT☆ & POPRAWA

12 | A Effects of anthropause on tardigrade urban communities during COVID-19 pandemic restrictions in Salta, Argentina
GRABOSKY☆, GONZÁLEZ REYES, ROCHA, CORRONCA, RODRÍGUEZ ARTIGAS, DOMA, BALLARDINI & OSTERTAG

12 | B Urban green spaces as tardigrade biodiversity hotspots in cities: a case study in Salta (Argentina)
GONZÁLEZ REYES, GRABOSKY, CORRONCA, ROCHA, RODRÍGUEZ ARTIGAS, BALLARDINI, OSTERTAG & MEIER

13 | A Do microclimatic conditions affect the body size of terrestrial and freshwater tardigrades?
KACZMAREK, BARTELS, FONTANETO, NELSON, ŁACKA, BUDKA & RZYMSKI

13 | B Invertebrate habitat gaps in the terrestrial cryosphere
NAHIMOVA☆, SHAIN, ONO & ZAWIERUCHA

14 | A Tardigrade abundance, diversity and habitat preference in Treforest, United Kingdom
SUDWORTH☆

14 | B Occurrence of tardigrades and physiochemical conditions in rock pools by the Baltic Sea
TROELL & JÖNSSON

15 | A Using the evaluations of ecological niche models to estimate tardigrade species ranges
JINGYU, XUELING, DONG, HEPING & MIN

15 | B The dispersal of soil tardigrades in the gut of earthworms
VESELÁ☆ & TŮMOVÁ

16 | A At school with tardigrades – a microscopic model for science education
GIOVANNINI, BRANDOLI, VINCENZI, GUIDETTI & ALTIERO

WEDNESDAY-THURSDAY | 24-25.08.2022

1 | A Diversity of Swedish Macrobiotidae: preliminary results from Southern Sweden
ATHERTON

1 | B Diversity of the tardigrade communities in the Norwegian forests
GUIDETTI, KACZMAREK, ROSZKOWSKA, MEIER, PRESTØ, JÖNSSON, SPEED, STUR, GJERDE, TOPSTAD, HASSEL & EKREM

2 | A Terrestrial Tardigrada (water bears) of the Słowiński National Park (Northern Poland)
KACZMAREK, POLISHCHUK, ROSZKOWSKA, GÓRNA, RUTKOWSKI, ZACHARYSIEWICZ, MIODUCHOWSKA & BARTYLAK

2 | B Terrestrial tardigrades of the Black Sea Biosphere Reserve (Southern Ukraine) revisited
POLISHCHUK☆ & KIOSYA

3 | A Stratification of tardigrades in the Czerniejewskie Forests – a preliminary report
ERDMANN, KACZMAREK & KOSICKI

3 | B Diversity of tardigrades in Tenerife Island (Spain)
GÓRNA, BARTYLAK, RZYMSKI & KACZMAREK

4 | A High diversity of Tardigrada in Chile, South America
MARLEY, MCINNES & JIMÉNEZ

4 | B Update on the List of Available Names (LAN), Tardigrada Project
MARLEY, BERTOLANI, DEGMA, FONTOURA, GROTHMAN, GUIDETTI, GĄSIOREK, HANSEN, KACZMAREK, MCINNES, MICHALCZYK, MILLER, PERRY, TUMANOV, NELSON & ZAWIERUCHA

5 | A New records of two species of marine tardigrades, *Echiniscoides sigismundi* and *Styraconyx haploceros*, collected from barnacles on the coast of Korea
CHOI, JUNG, KIM, LEE & JO

5 | B An integrative description of a new *Richtersius* species (Tardigrada: Eutardigrada: Richtersiusidae) from Greece
POGWIZD & STEC

WEDNESDAY-THURSDAY | 24-25.08.2022

6 | A Completing the evolutionary tree of Macrobiotidae: the phylogenetic position of the genus *Calcarobiotus*
STEC & VECCHI

6 | B New records of Tardigrada from the Madeira Island (Portugal) with an integrative description of a new *Macrobiotus* species (*hufelandi* group)
KAYASTHA, GAWLAK, MIODUCHOWSKA, SŁUGOCKI, ARAÚJO, SILVA & KACZMAREK

7 | A *Paramacrobiotus fairbanksi* Schill, Förster, Dandekar & Wolf, 2010 – a cosmopolitan tardigrade
KAYASTHA, SZYDŁO, MIODUCHOWSKA & KACZMAREK

7 | B Two new species of the genus *Minibiotus* from an urban area of Northern Argentina
ROCHA, DOMA, CAMARDA, OSTERTAG, GRABOSKY, GONZÁLEZ-REYES & LISI

8 | A Native fauna of tardigrades from two natural areas of the Argentina Republic
ROCHA, OSTERTAG, GONZÁLES REYES, GRABOSKY, DOMA & CORRONCA

8 | B Preliminary study on tardigrades taxocenosis in epiphytic cryptogams of the Patagonian steppe, Nahuel Huapi National Park, Argentina
MEIER☆, ROCHA, OSTERTAG, GRABOSKY & MESSUTI

9 | A New tardigrade records from Montana, USA
MEYER, JACKSON, JABUSCH & FONTENOT

9 | B Is there a universal DNA barcode gap in tardigrades?
CESARI, VINCENZI☆, GIOVANNINI, REBECCHI & GUIDETTI

10 | A A tardigrade in Dominican amber
MAPALO, ROBIN, BOUDINOT, ORTEGA-HERNÁNDEZ & BARDEN

10 | B Quantifying the shape of the tardigrade claws
MAPALO☆ & ORTEGA-HERNÁNDEZ

11 | A *Milnesium guanyinensis* sp. nov. (Eutardigrada: Apochela: Milnesiidae) from Yunnan, China
YUAN, LIU, WANG, LIU, CHEN & LI

WEDNESDAY-THURSDAY | 24-25.08.2022

11 | B Frozen treasures: extraordinary *Milnesium* species (Tardigrada: Apochela) from the Antarctic
McInnes, Morek & Michalczyk

12 | A Phylogenetic position and validity of the genus *Milnesioides* Claxton, 1999 (Eutardigrada: Apochela)
Wałach, Sumlińska, Lamond, Morek & Michalczyk

12 | B Rough backs: taxonomic value of epicuticular sculpturing in the genus *Milnesium* Doyère, 1840 (Tardigrada: Apochela)
Morek, Wałach & Michalczyk

13 | A A stowaway or parasite? Insights into the nature of *Pyxidium tardigradum*
Wałach☆ & Blagden

13 | B Integrative description and phylogenetic position of a new Afro-Oriental *Viridiscus* species complex (Heterotardigrada: Echiniscidae)
Dey, Gąsiorek & Michalczyk

14 | A Neotropical jewels in the moss: biodiversity, distribution and evolution of the genus *Barbaria* (Heterotardigrada: Echiniscidae)
Wilamowski☆, Vončina, Gąsiorek & Michalczyk

14 | B Lost in the Arctic and in the mountains – the (dis)entangled classification of *Claxtonia* (Heterotardigrada: Echiniscidae)
Gąsiorek, Degma & Michalczyk

15 | A Phylogenetic position of (*Test*)*Echiniscus meridionalis* (Murray, 1906) (Heterotardigrada) revealed
Gąsiorek & McInnes

15 | B A new, catfish-headed *Cornechiniscus* (Heterotardigrada) illuminates evolution of the genus
Gąsiorek

16 | A Unique dorsal cuticular sculpture and phylogenetic position of *Cornechiniscus holmeni* (Petersen, 1951) from Ella Island, East Greenland
Kihm & Park

WEDNESDAY-THURSDAY | 24-25.08.2022

16 | B Investigation of arthrotardigrade phylogeny with the inclusion of *Actinarctus doryphorus* and Echiniscoididae sequences
GROLLMANN☆, JØRGENSEN & MØBJERG

17 | A Structure and diversity of Tardigrada communities from the deep South China Sea
BAI, WANG & FONTOURA

17 | B From pole to pole – biodiversity gradients and biogeographic patterns of marine Tardigrada across the Atlantic
TROKHYMCHUK☆, KIENEKE, SCHMIDT-RHAESA & UTEVSKY

PROGRAMME DETAILS
(ACCOMPANYING PERSONS)

MONDAY | 22.08.2022

13:45–15:15

SAINT MARY'S BASILICA | Main Square

All Accompanying Persons are welcome to see the interior of the most iconic of Kraków's churches, the St. Mary Basilica, with the famous and unique wooden altar from the 15th century. The tour will be guided. A Volunteer will take you on foot from the Auditorium Maximum to the Basilica and back to the AudiMax by foot: ca. 1 km/15 min of walking each direction (https://goo.gl/maps/p2iHgRyQhB5qKjpz8). **Please be ready at the AudiMax Reception Desk at 13:45.**

TUESDAY | 23.08.2022

13:30–15:30

WAWEL ROYAL CASTLE | Wawel Hill

All Accompanying Persons are welcome to see some of the interiors of the Wawel Royal Castle. The tour will be guided. A Volunteer will take you on foot from the Auditorium Maximum to the Wawel and back to the AudiMax by foot: ca. 1.5 km/20 min of walking each direction (https://goo.gl/maps/LSfxDd6AUee3YoRBA). **Please be ready at the AudiMax Reception Desk at 13:30.**

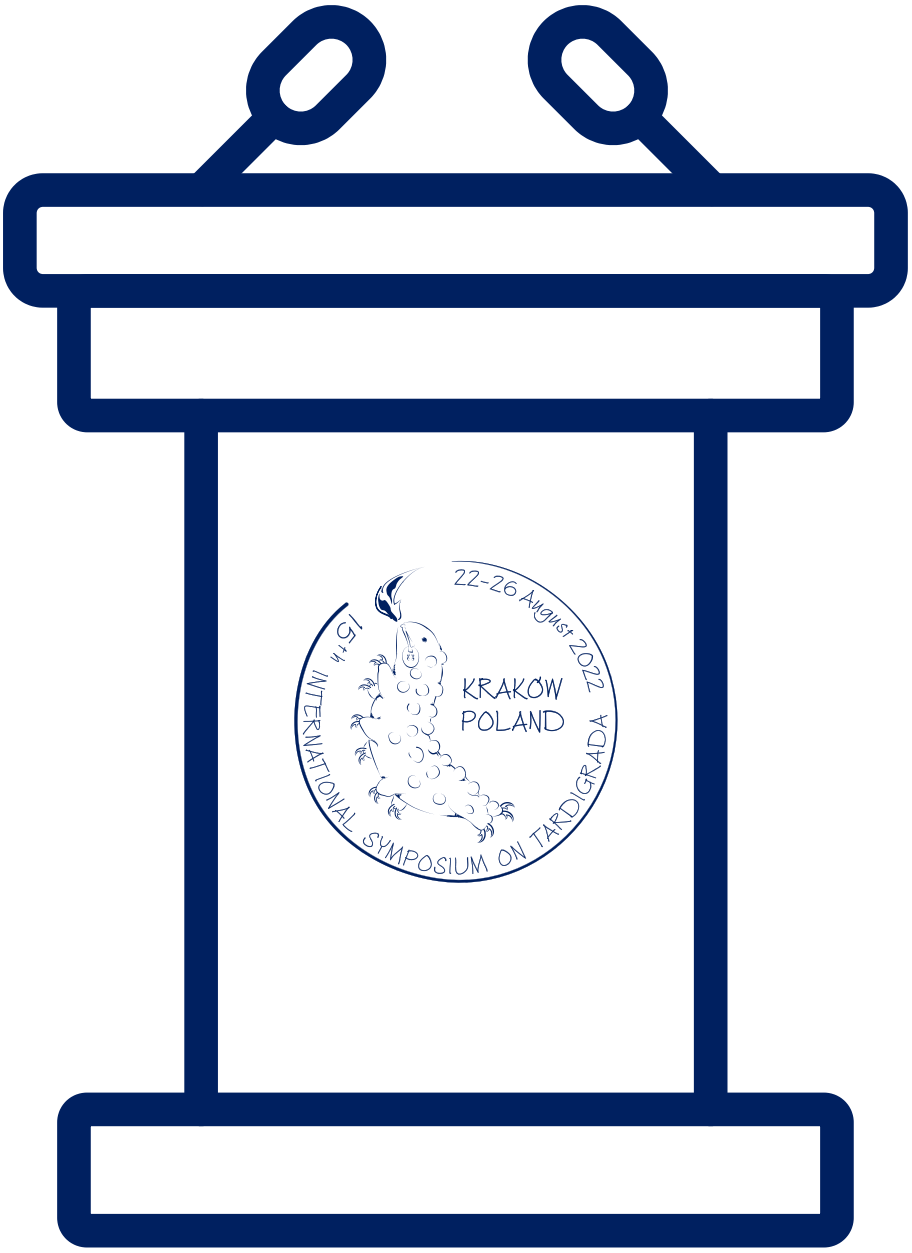
WEDNESDAY | 24.08.2022

10:45–12:45

OSKAR SCHINDLER'S ENAMEL FACTORY | Lipowa 4

All Accompanying Persons are welcome to see the Oskar Schindler Enamel Factory in the Podgórze (Foothills) District. The Factory was made famous by the widely acclaimed Oscar-winning (7 awards) "Schindler's List" by Steven Spielberg who shot the movie in Kraków in spring 1993. The tour will be guided. A Volunteer will take you from the Auditorium Maximum to the Factory and back to the AudiMax by a taxi: ca. 6 km/20 min each direction (https://goo.gl/maps/1qNgLbY8aTEyMQLe9). **Please be ready at the AudiMax Reception Desk at 10:45.**

TALK ABSTRACTS



INVITED TALKS

We are extremely honoured and happy to introduce our superb Invited Speakers (listed in the order of appearance):

Professor GREGORY D. EDGECOMBE
Natural History Museum
London, **United Kingdom**
GoogleScholar

Professor ANDREAS HEJNOL
Friedrich Schiller University
Jena, **Germany**
GoogleScholar

Professor FRANK W. SMITH
University of North Florida
Jacksonville, Florida, **USA**
GoogleScholar

Professor NADJA MØBJERG
University of Copenhagen
Copenhagen, **Denmark**
GoogleScholar

Monday | Invited Talk #1 | 09:30-10:30

A timeline for ecdysozoan diversification

GREGORY EDGECOMBE[1], RICHARD HOWARD[1,2], MATTIA GIACOMELLI[3], JESUS LOZANO-FERNANDEZ[4], PHILIP DONOGHUE[3] & DAVIDE PISANI[3]

[1]*The Natural History Museum, UK*

[2]*University of Exeter, UK*

[3]*University of Bristol, UK*

[4]*University of Barcelona, Spain*

The tough, periodically moulted cuticle of ecdysozoans predisposes them to exceptional fossil preservation. Ecdysozoans dominate the oldest exceptionally-preserved bilaterian animal biotas in the early- to mid-Cambrian (~520–508 Ma), with possible trace fossils in the latest Ediacaran (<556 Ma). The fossil record of Ecdysozoa is among the best understood of major animal clades and is widely considered to document their origins and evolutionary history well. However, molecular clock analyses have implied a considerably deeper Precambrian origin for Ecdysozoa, much older than their earliest fossils. Using a refined set of fossil calibrations, we performed Bayesian analyses to estimate an evolutionary time-tree for Ecdysozoa, sampling all eight phyla, including new transcriptomes for heterotardigrades and nematomorphs. Unlike most previous phylogenomic analyses, Scalidophora is recovered as a clade (albeit with only moderate support) and is sister group to Nematoida + Panarthropoda; tardigrades are sister group to Onychophora + Arthropoda. The dated trees suggest an Ediacaran (rather than earliest Cambrian) divergence of Ecdysozoa, predating the first potential ecdysozoan trace fossils by at least 23 million years. An Ediacaran origin of ecdysozoans is robust to the use of all plausible phylogenies, fossil prior distributions, evolutionary rate models and matrix partitioning strategies. Arthropods exhibit more precision and less incongruence between fossil- and clock-based estimates of clade ages than other ecdysozoan phyla.

Keywords: Ecdysozoa, fossil calibration, molecular dating, phylogenomics

Tuesday | Invited Talk #2 | 09:30-10:30

Tardigrades in development and evolution

ANDREAS HEJNOL[1,2]

[1]*Friedrich-Schiller University Jena, Germany*

[2]*University of Bergen, Norway*

Tardigrades were always puzzling scientists for very different questions. The still debated phylogenetic position of tardigrades triggered questions regarding miniaturisation and the evolution of organ systems. Their development is unique and not easy to compare with other lineages. In addition, several misleading descriptions of *e.g.* the enterocoelic development of the mesoderm have been published in the beginning of the 20th century. Recent advances in molecular methods have delivered progress to the knowledge about their phylogenetic position and their developmental biology. The encoding of the genome of some species brought some insights about the gene content and gene loss. I will summarise recent advances on placing tardigrades in the Ecdysozoan clade and discuss possible next steps to solve the remaining questions. Further, I will introduce shortly the historical debates about tardigrade development and recent insights. These characters I will compare with the development of other ecdysozoans and conclude about ancestral states of some major features at different nodes of the Ecdysozoan tree of life. I will end with some urgent remaining questions that need to be answered to gain the whole picture about tardigrade development and evolution.

Keywords: cleavage, development, genomics, mesoderm formation, nervous system development, phylogenomics, segmentation

Wednesday | Invited Talk #3 | 09:30-10:30

The tardigrade *Hypsibius exemplaris* as a model for body plan evolution

FRANK SMITH[1], RAUL CHAVARRIA[1], MANDY GAME[1] & TAYLOR HARRISON[1]

[1]*University of North Florida, United States of America*

The origin and diversification of animal body plans is difficult to study because the earliest steps in these processes occurred in ancient periods that are not recorded in the fossil record. Evolutionary developmental biology (evo-devo) complements palaeontology to illuminate these early steps. The tardigrade *Hypsibius exemplaris* is an attractive model for evo-devo studies due to its sequenced genome and available techniques to visualise embryonic gene expression patterns and experimentally disrupt gene function. Our comparisons of embryonic gene expression patterns have revealed surprising patterns of homology between the tardigrade body plan and the body plans of their relatives. For example, nearly the entire tardigrade body axis appears to be homologous to just the head of an arthropod, and tardigrades appear to have lost a mid-trunk region. Our more recent functional studies reveal conservation of mechanisms that regulate anteroposterior (AP) axis polarity during *H. exemplaris* development. These results suggest that tardigrades retain early steps in AP axis development. Therefore, the loss of a mid-trunk region most likely corresponds to the loss of posterior growth, a later acting development mechanism in many bilaterians. Intriguingly, we identified a similar loss of mid-axis identities along the proximodistal leg axis, suggesting a common pattern of axis simplification associated with miniaturisation. We have also identified a highly conserved mechanism that controls eye development in *H. exemplaris*. This mechanism controls development of both rhabdomeric photoreceptor cells and pigment cells. Comparisons to distantly related outgroups of Tardigrada suggest that this mechanism controlled eye development in the ancient bilaterian ancestor. We will present our results in a phylogenetic framework to reveal important insights into the origin and diversification of animal body plans.

Keywords: body plan evolution, canonical Wnt signaling, eye development, leg gap genes, segment polarity

Thursday | Invited Talk #4 | 09:30-10:30

Unravelling the mechanisms underlying extreme stress tolerance in tardigrades

NADJA MØBJERG[1]

[1]*University of Copenhagen, Denmark*

Tardigrades are renowned for their stress tolerance and many species have adapted to extreme and changing environments. Despite of their minute size and a body length of less than a millimetre, these eight-legged ecdysozoans are complex organisms with well-developed organ systems. This includes an integument equipped with sensory organs, complex digestive, muscular, nervous and reproductive systems and a fluid filled body cavity containing specialised cells involved in nutrient storage and possibly immune responses. Nervous and digestive organs likely release hormones to the body cavity and osmoregulatory and excretory functions are maintained by epithelia of the integument and digestive system. This complex biology is sustained by around 1 000 somatic cells that express a wide range of well-known membrane associated channels, aquaporins, solute carriers and ATPases.

Importantly, the integument is highly permeable to water and tardigrades, therefore, require a film of water to be in their metabolically active state. Species living in ephemeral aquatic habitats, such as moss-cushions, endure periods of desiccation by entering the highly resilient ametabolic "tun" state. Maintaining structural integrity, while in the tun state, is essential for resumption of life following rehydration. Tun formation depends on muscle contractions and muscle filaments play a vital role for stabilising the three-dimensional structure of the ATP depleted tun, possibly locking in a *rigor mortis*-like state. Interestingly, when facing elevated external osmolalities, limno-terrestrial as well as marine tidal species form tuns, indicating that tun formation evolved in the sea millions of years ago.

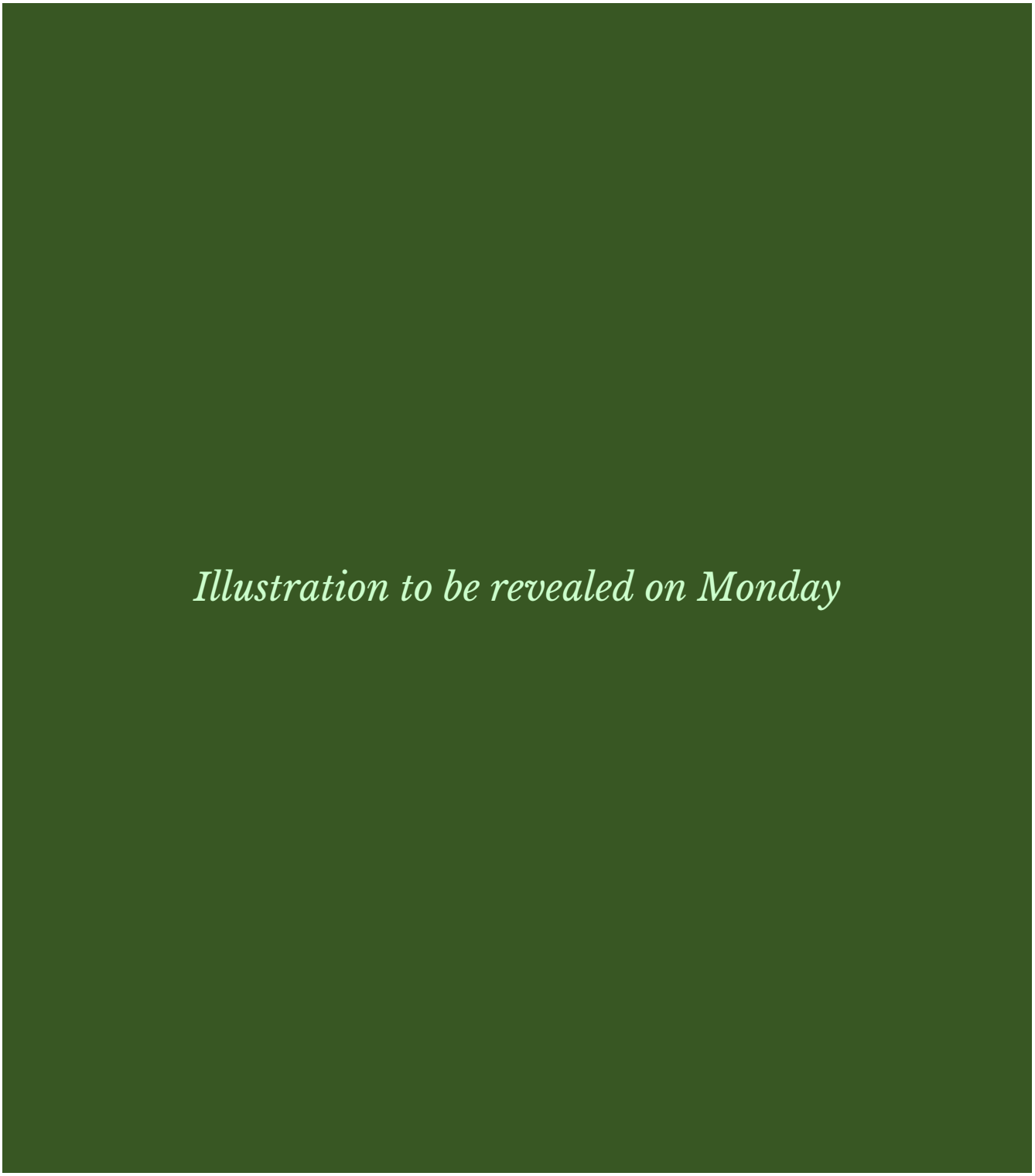
At the cellular level, intrinsically disordered proteins may provide structural stabilisation and *e.g.* shield DNA from reactive hydroxyl radicals. An effective antioxidant defence machinery and high fidelity DNA repair systems are likely crucial for survival following metabolic shutdown. Accordingly, tardigrades seem to have a comprehensive number of genes encoding proteins involved in antioxidant defence.

While tardigrades may survive temperatures close to absolute zero, metabolically active tardigrades are generally vulnerable to temperatures above 35 °C. Heat stress seems to induce a major transcriptome shift and even the highly resistant tun seems to have a restricted timeframe for high temperature tolerance, indicating that essential macromolecules are heat-labile. Thus, while the secrets of tardigrade stress tolerance in many aspects remain a mystery, recent research is beginning to unravel some of its underlying mechanisms.

Keywords: extreme environments, metabolism, muscle filaments, physiology, tun

TALK SESSIONS 1-3

Biodiversity, Taxonomy, Biogeography, Phylogeny & Evolution



Monday | Talk Session #1 | 11:00-11:15

Solving the six decade-long mystery: phylogenetic relationships of the Cretaceous Canadian tardigrade amber fossils

MARC A. MAPALO[1] ☆ & JAVIER ORTEGA-HERNÁNDEZ[1]

[1]Harvard University, USA

Background: Fossils have an important role for better understanding the evolutionary history of animals. Paleontological data can be used to estimate the time of divergence of clades of interest, to determine the ancestral and derived morphological structures, and to track their changes along different lineages, but this can only be done only if the phylogenetic relationships of the fossils relative to extant groups are known. Molecular estimates suggest that tardigrades diverged from other panarthropods before the Cambrian, but the fossil record of these microscopic animals is extremely sparse. Out of the four crown-group fossils know to date, the phylogenetic identities of only two are well resolved (*i.e. Milnesium swolenskyi* and *Paradoryphoribius chronocaribbeus*). The remaining two – *Beorn leggi* and a yet undescribed putative heterotardigrade – were the first discovered tardigrade fossils and are both embedded in the same Cretaceous amber. Since their initial discovery along Lake Cedar in Canada, their relationships to other extant tardigrades have remained controversial due to difficulty of imaging the taxonomically important morphological characters, such as the claws and cuticle.

Methods: Here, we used confocal fluorescence microscopy to visualise the two fossils and obtain high-quality images of their claws and cuticular features. We used geometric morphometric analysis to compare the shape of the claws of the fossils to extant tardigrades to assess their similarities and to improve character coding. We performed total evidence-based phylogenetic analyses to determine the relationship of the two fossils relative to extant tardigrades.

Results: Our analyses showed that *Beorn leggi* morphologically and phylogenetically belongs to an extant tardigrade family. We were also able to obtain high-quality images of the second tardigrade synclusion that allowed us to visualise important taxonomic characters, provide a detailed morphological description, and determine that it represents a new species, different from *Beorn leggi*.

Conclusion: Ultimately, we were able to resolve the phylogenetic relationships of *Beorn leggi* and the second undescribed tardigrade, which allowed us to use them as calibration points for molecular clock estimations and to hypothesise the evolution of some taxonomically important characters in tardigrades.

Keywords: *Beorn leggi*, Eutardigrada, invertebrate palaeontology

Monday | Talk Session #1 | 11:15-11:30

New fossil Eutardigrada from Rovno Eocene amber (Ukraine, 34–38 MYA)

VALENTYNA INSHYNA[1], ŁUKASZ KACZMAREK[2], EVGENY PERKOVSKY[3], JÖRG U. HAMMEL[4], MARTIN HEß[1] & JOACHIM T. HAUG[1]

[1]*Ludwig-Maximilians-University of Munich, Germany*

[2]*Adam Mickiewicz University in Poznań, Poland*

[3]*Schmalhausen Institute of Zoology, Ukraine*

[4]*Helmholtz Centre Hereon, Germany*

Background: Small, soft-bodied organisms, such as Tardigrada, Rotifera, Gastrotricha and/or other meiofaunal animals are very rare in the fossil record. It is mostly due to the fossilisation process being biased towards large organisms with hard skeleton. Ambers, fossil resins formed because of the plants' stress reactions, do have potential to preserve very small and fragile animals in exquisite details. It is therefore unsurprising that out of five known body fossils of the Tardigrada, four were found in amber. In this study we report an exciting new record of the Eutardigrada from Eocene Rovno amber.

Methods: We have applied several imaging techniques to get a best possible coverage of all the relevant morphological characters of the specimen. We have conducted synchrotron radiation-based x-ray micro-CT (SR-µCT) imaging, using Imaging Beamline P05 (operated by the Helmholtz-Zentrum Hereon at the storage ring PETRA III (Deutsches Elektronen Synchrotron – DESY, Hamburg, Germany) using a photon energy of 18 keV and a sample to detector distance of 80 mm. We also have used VHX-6000 digital microscope, BZX-900 fluorescent light source microscope and confocal laser scanning microscopy (Leica SP5 AOBS at 488, 496 and 514 nm excitation) to get additional images of certain characters. The holotype specimen is deposited in the Schmalhausen Institute of Zoology, Kyiv, Ukraine.

Results: We have found that the diminutive tardigrade (ca. 235 µm long) is likely a member of the Eutardigrada, based on the combination of autapomorphies of this clade present in the studied specimen. It exhibits unusual protrusions on the dorsal side of the head, similar to the elliptical organs of the genus *Ramazzottius* and related genera. Our specimen also exhibits a characteristic textured cuticle, similar to the *Ramazzottius agannae*.

Conclusions: This rare record of the Tardigrade from amber provides a unique glimpse into the Eocene stage of the group's evolution. This first record of a tardigrade from the Eocene is falsifying a long-standing hypothesis of the "morphological stasis" in Tardigrada, as this relatively young fossil shows a unique and even transitional combination of characters unknown in other Tardigrada.

Keywords: elliptical organs, Europe, evolution, fossil, Tardigrada

Monday | Talk Session #1 | 11:30-11:45

A revision of the marine Orzeliscinae (Arthrotardigrada, Tardigrada)

REINHARDT M. KRISTENSEN[1], SIF MØBJERG[1], NADJA MØBJERG[2] & ASLAK JØRGENSEN[2]

[1]*Natural History Museum of Denmark, University of Copenhagen, Denmark*

[2]*Department of Biology, University of Copenhagen, Denmark*

Background: Du Bois-Reymond Marcus described the marine genus *Orzeliscus* with the species *O. belopus* in 1952 from the island of São Sebastião in Brazil. In 1953 Schulz described the first European species *O. septentrionalis* and established Orzeliscidae in 1963. Pollock (1982) questioned the two species and subsequently different scientists have reported several findings of the genus, *e.g.* from Bermuda, France, Galapagos, New Caledonia, Scotland and Virgin Islands, with a significant variation in morphology. Kristensen & Higgins (1984) supported the family; however, also in 1984 the orzeliscids were included in the Halechiniscidae as Orzeliscinae by Renaud-Mornant.

Methods and Results: The large collections in the Zoological Museum of Copenhagen, Denmark include many specimens from Australia, Bermuda, Egypt, France, Japan, Tobago and USA. Close examinations have revealed the presence of several new species, *e.g.* a new species from Japan with a protruding mouth cone and one from Egypt, which is hermaphroditic and thus represents the first arthrotardigrade species with both ovary/testis and seminal receptacles. The characteristics of the new species will be presented together with a revision of the subfamily. Specimens from Queensland, Australia represent three new species comprising a common new species of the genus *Orzeliscus* and a new genus with two new species. The latter includes a species with only three toes on each leg and lateral projections on the body with long pillars in the cuticle, and another very similar species, which has four toes on each of the legs.

Conclusions: Based on the characters from the new genus, the subfamily Orzeliscinae is rediagnosed. A map of the world distribution of Orzeliscinae (including the genera *Mutaparadoxipus*, *Opydorscus*, *Orzeliscus*, and *Paradoxipus*) with all known records is also included. The enigmatic subfamily Quisarctinae Fujimoto, 2015 will be briefly discussed.

Keywords: distribution, heterotardigrades, new genus, new species

Monday | Talk Session #1 | 11:45-12:00

Phylogeny and biogeography of the genus *Xerobiotus* (Macrobiotidae, Eutardigrada)

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Background: Specimens of the genus *Xerobiotus* are relatively rare and little studied. To date, only four species were attributed to this genus and all of them were reported from arid environments: grass roots, mosses and lichens on sandy dunes or rocks exposed to sun. Recently, based on molecular phylogenetic data, it was proposed to suppress the genus and transfer its species to the genus *Macrobiotus*, despite the presence of synapomorphic characters that support its taxonomic validity. Indeed, no other tardigrades possess: first leg pair reduced compared to the second/third one, a cuticular "garter-like" structure girding legs, different claw morphology between the last and the first three leg pairs, absence of lunules in claws of first three pairs, cuticular pores only dorso-laterally (visible only with SEM). This study aims to enrich the knowledge related to biodiversity, biogeography, and phylogeny of the genus *Xerobiotus*, using an integrative approach.

Methods: Samples from several European, Caucasian, and Australian sites were used to collect tardigrade specimens of *Xerobiotus* and *Pseudohexapodibius* genera (including type locality of *Xerobiotus pseudohufelandi* in Austria). Light and scanning electron microscopy allowed to conduct detailed morphological analyses. Molecular data were obtained sequencing *cox1*, *18S*, *28S* and *ITS2* genes for a characterisation of populations, species delimitation, and phylogenetic analyses. Karyotype and reproductive modes of the populations were also investigated.

Results: In addition to the distinctive morphological structures of the taxon, microscope investigations showed new or still little known characters for *Xerobiotus*. *Xerobiotus pseudohufelandi* was redescribed and several taxa were identified within *Xerobiotus* supported by morphological/karyological and molecular data. Moreover, molecular data showed that not only parthenogenetic taxa have a widespread distribution and that *Xerobiotus* and *Pseudohexapodibius* are closely related.

Conclusion: Obtained results, other than to identify new taxa, underline how biogeography patterns of tardigrades are unclear and influenced by their reproductive mode. They also show that integrative approach (morphological, molecular, karyological and reproduction data) is highly useful to identify new taxa. The phylogenetic line of *Xerobiotus* results diversified in several taxa and closely related to *Pseudohexapodibius* and *Macrobiotus* species.

Key word: biodiversity, integrative taxonomy, karyology, *Pseudohexapodibius*, systematics

Monday | Talk Session #1 | 12:00-12:15

Phylogeny of the superfamily Isohypsibioidea (Parachela, Eutardigrada)

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Background: Isohypsibioidea is one of the larger superfamilies of Eutardigrada and, at the same time, the most basal evolutionary lineage of the order Parachela. In a recent pivotal paper, the systematic organisation of the superfamily has been recently modified based on phylogenetic molecular analyses: two new families were established (Doryphoribiidae and Halobiotidae), Isohypsibiidae and Hexapodibiidae families were confirmed, while the polyphyletic genus *Isohypsibius* (Isohypsibiidae) was split into four genera. Nevertheless, some internal phylogenies of the taxa remain unresolved and solid statistical support is lacking for some of these systematic rearrangements. For these reasons, we added new taxa to the analysis with the aim to solve some of these lasting problems in the Isohypsibioidea systematics.

Methods: Several populations of Isohypsibioidea genera have been collected from lichens and mosses collected in Europe and analysed with an integrative approach. Light and scanning microscopy observations and molecular analysis, on 18S and 28S, genes, were carried out.

Results: New Isohypsibioidea taxa have been identified. Halobiotidae and Doryphoribiidae were not supported by our data, while the Isohypsibiidae and Hexapodibiidae families were confirmed. Incongruences between the morphology of the new species and the definition of the genera at which these species have been assigned by the molecular phylogeny were detected.

Conclusion: In spite of the previous studies and our analyses, the phylogenetic relationships among families, genera and species within Isohypsibioidea need to be further studied to be completely resolved, and the synapomorphies (and the associated morphological characters) defining the genera within the superfamily are not always easy to identify.

Key word: Doryphoribiidae, Halobiotidae, Hexapodibiidae, Isohypsibiidae

Systematist's nightmare: an updated classification of Isohypsibioidea (Eutardigrada)

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Background: The superfamily Isohypsibioidea has undoubtedly the worst-known phylogeny compared to the remaining three eutardigrade superfamilies. This is not surprising given the rarity of many isohypsibioid taxa, but should be addressed in depth with influx of new genetic data since this lineage is morphologically most diverse among eutardigrades, and probably constitutes an earliest off-branch of Eutardigrada. Recent erection of several new families and genera underlined obscure phylogenetic position of several isohypsibioid genera.

Methods: We sequenced (18S rRNA, 28S rRNA, COI) new taxa representing various genera and species groups of Isohypsibiidae (*Fractonotus*, *Isohypsibius prosostomus* group (*Isohypsibius* s.s.), *Isohypsibius dastychi* group, *Isohypsibius undulatus* group), Doryphoribiidae (*Doryphoribius bertolanii* group, *Doryphoribius flavus* group), Hexapodibiidae (*Haplohexapodibius*, *Hexapodibius*, *Parhexapodibius*), and Ramajendidae, and updated phylogenetic reconstructions for the superfamily. Findings are discussed in the context of morphology of all isohypsibioid taxa.

Results: The phylogeny is poorly resolved at the family level, indicating non-monophyly of Isohypsibiidae (paraphyletic with respect to Halobiotidae + Ramajendidae), Doryphoribiidae (paraphyletic with respect to Hexapodibiidae), and Hexapodibiidae (polyphyletic). *Isohypsibius*, *Doryphoribius*, and *Hexapodibius* remain polyphyletic. For the first time, we found some support for the monophyly of *Thulinius*. The closest relatives of *Doryphoribius*-like taxa share the number of placoids (2 *vs* 3) in the pharynx with respective isohypsibioid lineages.

Conclusions: The number of placoids in the pharynx together with the sculpturing of dorsal cuticle are the key traits usable in the genus-level classification of Isohypsibioidea. Both *Doryphoribius* and *Isohypsibius* are still polyphyletic. *Doryphoribius bertolanii* and *Doryphoribius flavus* groups are separate genera, unrelated to *Doryphoribius macrodon* (*Doryphoribius* s.s.) group. *Fractonotus* is embedded within *Isohypsibius* and should be perhaps included within the latter, whereas *Isohypsibius dastychi* and *Isohypsibius undulatus* groups are independent genera. Hexapodibiidae and Doryphoribiidae are non-monophyletic and require modifications of their diagnoses: the first comprise isohypsibioid descendants of *Doryphoribius*-like ancestors with highly reduced claws that preserved isohypsibioid shape in some cases, and the second should be narrowed to *Apodibius* + *Doryphoribius macrodon* group. The validity of *Parhexapodibius* is questioned in the light of the finding of transient forms between *Hexapodibius* and *Parhexapodibius*-like morphotypes.

Keywords: claw reduction, morphology, pharynx, placoids, taxonomy

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417).

Monday | Talk Session #2 | 13:45-14:00

Research on diversity of tardigrades in Northern China

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Background: The forests in Northern China are remote and inaccessible, and the resource survey is limited. In order to fill the gap of tardigrade resources in the cold regions of China, we selected some locales and conducted tardigrade surveys through a period of several years.

Methods: We chose typical cold locations in northern China. The tardigrade animals in Liangshui National Nature Reserve, Yichun Fenglin National Nature Reserve, Normin Mountain, Inner Mongolia, Okridui Mountain and Changbai Mountain were selected as the research sites. Phase contrast microscope, differential interference microscope and scanning electron microscope were used for morphological observation, and combined with molecular biological detection method, *i.e.* PCR amplification sequence alignment analysis of 18S rRNA, COI and 28S rRNA of individual tardigrades were used for species identification.

Results: 26 species, 13 genera, 6 families, 3 orders, 2 classes of tardigrades were found in these areas, among which two species were widely distributed, namely *Adropion scoticum ommatophorum* and *Mesobiotus harmsworthi.* Endemic to Fenglin reserve is *Diphascon* sp. Endemic to Norminda mountains is *Pilatobius nuominensis*. *Pilatobius recamieri* is endemic to Oakley mound. Endemic to Changbai mountain is *Murrayon dianeae*.

Conclusions: Our survey provides new insights into the knowledge of the Chinese tardigrade fauna.

Keywords: cold regions, diversity, Northern China, Tardigrada

Monday | Talk Session #2 | 14:00-14:15

Riding on wind, birds or staying home? – Phylogeography of Nearctic *Milnesium* (Eutardigrada: Apochela)

KAROL WAŁACH[1]☆, JACOB LOEFFELHOLZ[2], WITOLD MOREK[1], ALEJANDRO LÓPEZ-LÓPEZ[1], JASON PIENAAR[3] & ŁUKASZ MICHALCZYK[1]

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Background: Tardigrade biogeography has been thought to be explainable by the 'Everything is everywhere but environment selects' (EiE) hypothesis. However, recent phylogeographic analyses of the genus *Milnesium* strongly suggested limited long distance dispersal (LDD) abilities of apochelan species, and a restricted explanative power of the EiE hypothesis for this tardigrade lineage. Nevertheless, the EiE seems to apply partially to the tropic and subtropics, where trade winds may explain latitudinal LDD resulting in pantropical distributions of some species. In parallel, zoochory by birds has been postulated as a means of LDD, although without direct evidence. Adding North American *Milnesium* populations to existing data from other continents provides an excellent opportunity to test hypotheses for LDD. Specifically, three scenarios can be predicted: *(i*) Exclusively Nearctic clade(s) would indicate the lack of LDD; *(ii*) Holarctic clade(s) would be evidence for latitudinal LDD mediated by westerlies and point to wind as the predominant LDD factor; and *(iii*) clade(s) with mixed Nearctic/Neotropic species would suggest longitudinal LDD via bird migrations.

Methods: A total of 39 *Milnesium* populations from across the USA were analysed. We sequenced four molecular markers: 18S and 28S rRNA, ITS-2 and COI. Only populations with at least three available barcodes (N=39) were added to the most recently published *Milnesium* dataset (N=127). The phylogenetic analysis was carried out in BEAST and the phylogeographic analysis in RASP.

Results: The newly obtained phylogeny revealed new clades and subclades. Although the clades are mostly well-supported, relationships between some of them are uncertain. The majority of Nearctic species formed an ancient clade (ca. 36 MY old), which, unexpectedly, was nested within the pantropical clade.

Conclusions: Our study further confirms that the distribution of *Milnesium* taxa does not follow the EiE hypothesis, as it shows the majority species are geographically restricted, LDD is rare, and seems to be mediated mainly by wind and humans. The Nearctic appears to be colonised by a single ancient dispersal from South to North. Last but not least, the study indicated the existence of several unrecognised species, clearly showing the considerable hidden *Milnesium* species diversity.

Keywords: biogeography, cosmopolitism, EiE, North America, USA, zoogeography

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417 and Preludium 2019/35/N/NZ8/04487).

Monday | Talk Session #2 | 14:15-14:30

A first look into peatland moss tardigrades in Finland

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Background: Boreal peatlands are ecologically important yet endangered ecosystems that host a range of specialised and unique biota. As moist and moss rich habitats, peatlands could offer ideal conditions for tardigrades. However, virtually nothing is known about tardigrade ecology in peatlands. Here we take a first look into tardigrade abundance and diversity in Finnish peatland mosses. Our aims are to investigate (1) whether tardigrades inhabit certain types of mosses, and (2) their seasonal variation in abundance and diversity.

Methods: We collected 289 samples from 18 peatlands across three different seasons in Central Finland. Samples represented 22 moss species that occur in bogs, spruce mires and pine mires. Samples were processed systematically using funnels. We recorded the number of tardigrades (corrected by dry weight of processed moss) and identified to genus level a maximum of 50 tardigrades per sample. We used a subset of 132 samples to study the within-moss-species seasonal variation in abundance and diversity.

Results: Tardigrades were present in 60 % of the samples and in 20/22 moss species. We found a total of 11726 (2671 identified) tardigrades belonging to 15 genera. The highest densities were found in Hylocomiaceae mosses from spruce mires (average 176 tardigrades per gram) and the lowest in *Sphagnum* mosses from bogs (average 10 tardigrades per gram). *Macrobiotus* was the most abundant (47 % of identified specimens) and widespread genus across sites and seasons. However, we still do not know whether we found several species, which themselves could be habitat specialists. *Crenubiotus* was the second most abundant and almost exclusive to *Sphagnum* mosses. The seasonal variation was most notable in wooded mires, whereby the highest abundance and number of genera were found in the autumn. We found that *Sphagnum fallax* moss in the autumn was particularly diverse substrate, hosting 10 genera that include *Pilatobius*, *Mesobiotus*, *Mesocrista* and *Adropion*.

Conclusions: We show that peatland tardigrades are affected by seasonal and micro- (moss species) and macro-spatial (peatland type) influences. Our results highlight the need for a more comprehensive and systematic sampling of these ecosystems.

Keywords: ecology, moss, peatland, seasonal variation, tardigrade

Monday | Talk Session #2 | 14:30-14:45

Current state of knowledge of the limno-terrestrial tardigrade fauna of the Republic of Argentina

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Background: Regarding the knowledge of limno-terrestrial tardigrades of Argentina, they had been studied in their beginnings (starting with 1908) and for a long time by European researchers. Since the 2000s and until today, methodical, continuous studies have been carried out in the framework of various scientific projects at the National University of La Pampa. Since then, we have tried to contribute to the knowledge of the limno-terrestrial tardigrade fauna (including new species descriptions) as well as to provide information about ecology (distribution, preference, population dynamics). So far, 135 species have been registered, of which 14 are due to Argentine researchers.

Methods: Samples of moss and lichen growing on tree bark from urban, undisturbed natural, and disturbed areas have been studied. Different environmental variables have been recorded in these areas in order to obtain data on the preference of tardigrade assemblages.

Results: Until now, investigations have been carried out in order to know and characterise the taxocenosis of tardigrades from cities in the north of Argentina (Salta, Tucuman, Jujuy) and in the centre of the country (Santa Rosa, General Pico, Doblás, Guatraché, Intendente Alvear, all of the province of La Pampa). Natural areas were also studied: one of the province of Salta for the North, one of the province of La Pampa for the centre, as well as the Nahuel Huapi National Park located in the south of the country (Patagonian region). In the last years, ecological studies have been published, as well as 5 new species descriptions (and several others are in preparation).

Conclusions: Despite the studies carried out so far, it is necessary to continue studying the different aspects regarding the limno-terrestrial tardigrades of the regions under study, and to complete the knowledge of the whole country by adding areas still not investigated.

Keywords: La Pampa province, natural areas, Neotropics, Patagonia, Salta city, urban biodiversity

Monday | Talk Session #2 | 14:45-15:00

Environmental DNA metabarcoding of Danish soil samples reveals new insight into the hidden diversity of eutardigrades in Denmark

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Background: Tardigrades are rarely included in biodiversity studies, mainly due to the impracticalities that follow larger sampling and identification of these microscopic animals. Yet, tardigrades are important members of the meiofaunal community and deserve a proper biogeographical mapping. This study aimed to investigate how well environmental DNA from 130 Danish soil samples could identify eutardigrades, using the V4 region of the 18S rRNA marker gene (~400 bp). A short single-marker metabarcoding protocol able to identify tardigrades without prior isolation would supplement current sampling techniques and perhaps provide a more comprehensive list of species inhabiting different areas.

Methods: Identification of unknown short query sequences are usually performed using BLAST or other similarity-based searches against a reference database. However, an issue arises in the metabarcoding field, where it is common that query sequences belong to unknown species or species with no reference sequences available. Assignments to higher taxa (genus or family) thus rely on other approaches, such as phylogenetics. Taxonomic assignment was performed by a combination of three different methods: 1) similarity-search (VSEARCH) on a quality-checked local reference database, 2) Maximum Likelihood phylogeny of the local database and query sequences and 3) using a new Phylogenetic Placement algorithm (APPLES*) to insert query sequences into a reference tree using genetic distances.

Results: Even though eutardigrades are currently inadequately represented in GenBank, it was possible to assign most query sequences to genus and family. Some query sequences came out with 100% match using all three approaches, of which some are new species to Denmark. These include *Microhypsibius bertolanii*, *Dianea sattleri* and *Macrobiotus polonicus.* As it is currently unclear exactly how well eutardigrades can be distinguished using the V4 region, further studies, preferable combining morphology and sequences, are needed.

Conclusions: The V4 region of the 18S rRNA marker gene has the potential to identify eutardigrades from environmental samples; however, the quality is only as good as the quality of the database.

Keywords: metabarcoding, eDNA, 18S, tardigrades, biodiversity, phylogenetic placement

Tuesday | Talk Session #3 | 11:00-11:15

A new pantropical genus exposes the challenges of ramazzottiid phylogeny (Eutardigrada: Parachela)

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ALEJANDRO LÓPEZ-LÓPEZ[1] & ŁUKASZ MICHALCZYK[1]

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Background: Dorsal gibbosities are a distinct morphological trait, rarely found in the genus *Ramazzottius*. So far, they have been recorded only in two species, *Ramazzottius szeptyckii* Dastych, 1980 from Africa (South Africa, Kenia, Tanzania) and *Ramazzottius saltensis* Claps & Rossi, 1984 from Argentina. The presence of dorsal gibbosities is considered an important diagnostic trait in many other parachelan lineages, suggesting that the *Ramazzottius* species exhibiting this trait could constitute a separate phylogenetic lineage. In order to test this hypothesis, we analysed six populations of *Ramazzottius* with gibbosities: five from South Africa and one from India.

Methods: Specimens from all populations were subjected to light contrast microscopy analysis and DNA barcoding of three nuclear markers (18S rRNA, 28S rRNA, ITS-2). Newly sequenced species were included in a Bayesian Inference phylogenetic analysis of the family Ramazzottidae.

Results: Phylogenetic trees obtained from different algorithms showed a similar topology. The phylogenetic relationships obtained from our analysis indicated that the populations with dorsal gibbosities constitute a clade that is a sister lineage to all the remaining species of the genus *Ramazzottius* and *Cryoconicus* represented by DNA sequences deposited in GenBank. Moreover, the analysis showed that the remaining *Ramazzottius* species form two clades and that the genus *Cryoconicus* is most likely paraphyletic with respect the clade containing *Ramazzottius oberhaeuseri* s.s. (Doyère, 1840). The majority of *Ramazzottius* sequences in GenBank are erroneously labelled as "*R. oberhaeuseri*" with no associated morphological data, which prevents a conclusive taxonomic analysis.

Conclusions: Our results of molecular phylogeny and a clear apomorphy support the erection of a new genus, embracing the previously described *Ramazzottius* species and two new species with dorsal gibbosities within the family Ramazzottiidae. In addition to gibbosities, species representing the new genus have toad-like body shape, whereas all other known *Ramazzottius* species exhibit a more elongated and slender body. The gibbosities can vary among different species in shape, size, arrangements and appearance. The surface of the gibbosities can be reticulated, delicately wrinkled or completely smooth. Moreover, our analysis raises doubts about the validity of *Cryoconicus*. However, integrative data for a larger number of species is needed to elucidate its status and ramazzottiid taxonomy in general.

Keywords: Africa, dorsal gibbosities, India, phylogeny, *Ramazzottius*, Ramazzottiidae

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417).

Tuesday | Talk Session #3 | 11:15-11:30

Development of morphological data matrices for the use in integrated taxonomy

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Background: The move to using both morphological and molecular data within integrated research approaches has highlighted the clear requirement to increase the usability of morphological data. The morphological characteristics need to be used more flexibly; both within data analysis but also in online keys.

Methods: The use of dichotomous keys requires all characters to be evident and needs updating / re-writing regularly with new taxa. There is a need to develop morphological datasets that are independent of the taxonomy of the phylum but flexible enough to incorporate the addition of new morphological character as these become available, *e.g.*, novel microscopy techniques. The main impediment to developing a flexible dataset is about how to codify the plethora of morphological characters. We explore the use of several schemas working at higher taxonomic ranks and then at family-group, genus-group, and species-group ranks.

Results: We offer an initial definition of "what is a tardigrade" based on its morphology, which may assist broad ecological diversity dataset where non-Tardigrada taxa are frequently encountered. Within the phylum, we then give examples of data matrices for some family-group, genus-group, and species-group taxa, examining how these grids can be combined into larger matrices. We propose to make these prototype character matrices available for other researchers to use and amend for their own studies, in the same way that molecular data is now openly available. The initial matrices will cover: Echiniscidae – represented by *Pseudechiniscus* spp. and *Antechiniscus* spp.; Apochela – represented by current genera; Isohypsibioidea – represented by most taxa. We are still to work on the templates for Arthrotardigrada, Hypsibioidea and Macrobiotoidea, but these should follow next year.

Conclusion: We have produced example prototype morphological matrices for inclusion into integrated datasets. These matrices are flexible, enabling the inclusion of new taxa and morphological characters at independent levels of detail. We propose to make these datasets available for colleagues use and adaptation. As well as being useful for integrated dataset with molecular data, they can also be used in the production of online keys, replacing dichotomous keys where specific morphological features can be difficult to view, *e.g.*, specimens in the wrong orientation, or characters that appear different depending on the microscopy technique.

Keywords: Apochela, Echiniscidae, example datasets, Isohypsibioidea, morphological character matrices, Tardigrada

Tuesday | Talk Session #3 | 11:30-11:45

A new species of *Paramacrobiotus* from Hawaii with a high level of allometric variation in key morphometric traits

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Background: Thirty-four limno-terrestrial Tardigrada species have reported from the Hawaiian Islands. Only one peer-reviewed publication has recorded a species (*Claxtonia goni*) from the island of Maui. The genus *Paramacrobiotus* has not been recorded in Maui.

Methods: Moss samples were collected from Waihou Springs Forest Reserve, Maui, Hawaii, USA. After soaking samples overnight in water, numerous specimens and eggs of an undescribed species of *Paramacrobiotus* were found. Specimens were measured for morphometric analysis using phase contrast microscopy. The COI genes of 7 animals were sequenced.

Results: COI genes exhibited a p-distance range from 0.000-0.002. Gen Bank sequences for eight *Paramacrobiotus* spp. were compared to this new species and were found to be between 72-76% similar. Egg processes are long, sharply tapering cones, some with bifurcated tips. SSI *pt* values from 77 specimens (BTL 25-64 µm) did not vary much (mean *81.8*). BTEW was highly variable (5 to 20 µm, mean 13 µ; *pt 17.1-36.0*, mean *27.5*; allometric corrected value *28.0*). Mean *pt* and allometric BTEW exceed the maximum value of all described *Paramacrobiotus* species except *P. halei* (maximum *pt 28.6*). SSI, microplacoid length, and ventral lamina length were not significantly allometric. Macroplacoid lengths and most claw measurements were significantly allometric.

Conclusions: The new *Paramacrobiotus* adds a second species endemic to Maui. At least seven published descriptions of *Paramacrobiotus* species with maximum BTEW *pt<23* used fewer than 10 specimens for morphometric analysis; their reported BTEW *pt* values are within the range of the smallest specimens from Maui. Given the high level of allometric variation in BTEW and other characters of the Hawaiian species, we recommend when only a few specimens, all of small size with narrow BTEW, are available for morphometric analysis, that characters likely to vary allometrically not be used as criteria for differentiating species in this genus without using other, non-morphometric data (*e.g.*, qualitative morphological traits, egg characteristics, molecular data).

Keywords: allometry, COI, morphometry, Pacific diversity, *Paramacrobiotus* sp.

Tuesday | Talk Session #3 | 11:45-12:00

First time-calibrated multilocus phylogeny of the phylum Tardigrada (Ecdysozoa: Panarthropoda)

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Background: While numerous studies have explored the phylogenetic relationships within specific groups of Tardigrada, only a few works have addressed the systematics and evolution of the phylum, albeit using incomplete or flawed matrices or unreliable methods and models. Thus, we lack of a framework on which assess evolutionary patterns along the history of this phylum.

Methods: In this work, we carry out a phylogenetic analysis of the phylum Tardigrada using a carefully assembled matrix composed of five nuclear and mitochondrial DNA fragments, including all groups of tardigrades for which reliable molecular data is available. Our analysis is also the first time-calibrated phylogenetic reconstruction carried out for the phylum, showing the ages of the main clades.

Results: Our results support almost all the major traditional taxonomic groups of tardigrades, but also raise some concerns about the monophyly of some of them, and also show relationships that contradict previous works. Moreover, we unveil some major problems that affected previous studies, such as the presence of multiple ribosomal DNA paralogs with ancient origin.

Conclusions: Our tree also allows us to reconstruct major events of morphological and ecological evolution of tardigrades, by correlating the acquisition of characteristics or the colonisation of new niches with geological events that affected the ecosystems along the Phanerozoic. To conclude, our phylogenetic tree sheds a new light on the evolution of these organisms and may serve as a framework for future studies.

Keywords: 28S rRNA, evolution, land colonisation, morphology, paralogs, phylogeny, Tardigrada, time-calibration, trait evolution

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Tuesday | Talk Session #3 | 12:00-12:15

Broad sampling and HTS spark a revolution in our understanding of tardigrade biogeography

ŁUKASZ MICHALCZYK[1], WITOLD MOREK[1], PIOTR GĄSIOREK[1], PRITAM K. DEY[1] & ALEJANDRO LÓPEZ-LÓPEZ[1]

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Background: Tardigrade taxonomy is burdened with numerous insufficiently detailed species descriptions and a low percentage of species diagnoses comprising genetic data. Inadequate species descriptions result in unreliable and often unverifiable faunistic records (also typically based solely on phenotypic traits) and these, in turn, lead to uncertain and erroneous biogeographic inference. Recent findings seem to undermine the "Everything is Everywhere, but environment selects" ("EiE") hypothesis as a universal explanation for geographic ranges of limnoterrestrial tardigrade species. The aim of this study is to introduce a new quality framework using reliable/verifiable data to tardigrade zoogeography, which will allow us to solve some long-standing puzzles of tardigrade distribution, dispersal and evolution.

Methods: We have sequenced COI for 1364 parachelan specimens isolated from 322 moss and lichen samples collected in 91 localities across Europe and South America. Illumina High Throughput Sequencing (HTS) was used to obtain the sequences. Operational Taxonomic Unit (OTU) delineations based on clustering and ABGD were used to analyse the data. A Median Joining haplotype network was computed for each OTU.

Results: Among the 322 sequenced eutardigrade populations, we found 127 OTUs. Some OTUs were found in numerous locations, signifying common taxa: 1% OTUs were recorded in at least 20 locations (the most common species, *Macrobiotus hufelandi* C.A.S Schultze, 1834, was found in as many as 95 samples from 21 localities). However, there was also a substantial fraction of OTUs that were found occasionally, suggesting that they represent rare species (64% OTUs were recorded in a single locality). None of the analysed species was found in more than one zoogeographic realm.

Conclusions: We show that HTS, even limited to a single barcode, is a powerful means for studying tardigrade biogeography. Initial results suggest that the majority of limnoterrestrial eutardigrades have limited geographic distributions, *i.e.* their biogeography cannot be explained by the "EiE" hypothesis. The results of this research programme also provide an occasion to discuss some of the current taxonomic and systematic practice, and pose questions about the directions of tardigrade science.

Keywords: COI, dispersal, DNA barcoding, limnoterrestrial Eutardigrada, zoogeography

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417).

TALK SESSION 4

Ecology, Life Histories & Behaviour

Illustration to be revealed on Tuesday

Tuesday | Talk Session #4 | 13:45-14:00

The importance of soil structure for soil-inhabiting tardigrades in polar habitats

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Background: The results of studies that deal with driving factors of tardigrade communities are often contradictory and inconclusive. The reason is that many studies lack a balanced statistical design or have not enough replicates to see clear patterns in the huge variability observed among soil samples. Moreover, most of the studies are focused on moss-inhabiting tardigrades. Here, we relate soil tardigrade communities from several polar habitats to physico-chemical soil properties in order to find the drivers of tardigrade distribution.

Methods: We sampled the soil in five contrasting habitats that provided a gradient of various factors – moisture, nutrient content, organic matter content, soil structure, and vegetation cover. Each habitat was sampled at four localities with five replicates per locality. We measured physico-chemical soil properties at the sites as well as characteristics of tardigrade communities, including species and trophic group composition. Using multivariate ordination, we identified environmental factors that corresponded to the distribution of tardigrades in these habitats.

Results: We observed more diverse and abundant communities in habitats that have developed over thousands of years and provided higher surface stability. The most important factors that influenced tardigrade communities were bulk density, water holding capacity, stoniness, and soil texture which affected most of the tardigrade community characteristics, *i.e.* abundance, species richness, diversity, species, and trophic group composition. On the other hand, chemical properties, *i.e.* content of total organic carbon and total nitrogen, affected only overall tardigrade abundance.

Conclusions: Soil physical properties were more important than chemical properties for the distribution of species among habitats. Contrary to expectations, even the role of tardigrades in the soil food web was primarily linked to the soil structure rather than to the soil nutrient content. In future research, we should invest more effort to investigate the effect of the soil physical properties on tardigrade communities.

Keywords: bird cliff, glacier foreland, polar tundra, soil crust, soil microfauna

Tuesday | Talk Session #4 | 14:00-14:15

Control of tardigrades over cryoconite hole ecosystems

KRZYSZTOF ZAWIERUCHA[1]☆, JAKUB BUDA[1], TEREZA NOVOTNA JAROMERSKA[2], ROBERTO AMBROSINI[3], ANDREA FRANZETTI[4], EWA PONIECKA[5], PIOTR KLIMASZYK[1], PIOTR ROZWALAK[1], DOROTA RICHTER[6], MIROSŁAWA PIETRYKA[6] & ELIZABETH BAGSHAW[7]

[1]AMU, Poland, [2]CUNI, Czechia, [3]UNIMI, Italy, [4]UniMiB, Italy, [5]UW, Poland, [6]UPWR, Poland, [7]CF, UK

Background: Cryoconite holes are small, water-filled reservoirs in the glacial ice. Due to their simplicity (stable temperature, truncated food webs, few species of grazers) they serve as a natural laboratory for studies of the biogeochemistry and ecology of the glacial biome. Although tardigrades are one of top consumers in the cryoconite holes, their impact on these ecosystems has not been recognised so far. Therefore, we tested the impact of water bears on (i) biomass of primary producers, (ii) organic matter (OM) production, (iii) oxygen concentration, and finally, (iv) the role of tardigrades in the trophic web.

Methods: We used both field and laboratory approaches. The relationship between biomass of primary producers and biomass of tardigrades was assessed in the material from five sampling campaigns during one ablation season. The impact of invertebrates on OM content was investigated by the incubation of cryoconite (sediments from cryoconite holes) with and without animals under laboratory conditions. Finally, we used stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to establish tardigrade position in food webs. The relationship between invertebrate density and oxygen conditions in cryoconite was investigated using microsensors for measurements of O_2.

Results: We found that biomass of tardigrades negatively correlated with the biomass of green algae. Incubation of cryoconite from the Arctic and Alpine glaciers revealed that invertebrates trigger OM production in the Arctic but do not influence the alpine cryoconite. In the Arctic, presence of tardigrades decreases O_2 concentration in cryoconite holes. Finally, we found presumptions that tardigrades on glaciers have different food requirements than the coexisting rotifers in the Arctic and coexisting springtails in the Alps.

Conclusions: Glaciers and ice sheets cover ca. 10% of lands where tardigrades are one of the most dominant grazers. Our study highlights the importance of tiny water bears in shaping of cryoconite hole environments. Our results indicate that photoautotrophs in cryoconite holes may be controlled by grazing and may increase their biomass as protection against overgrazing. Tardigrades may also have an impact on the organic matter content in cryoconite and on oxygen conditions.

Keywords: Alps, Arctic, cryoconite holes, cryosphere, top-down control

Tuesday Talk Session #4 14:15-14:30

Life cycle of *Acutuncus antarcticus* and acclimatory adaptation during exposure to increasing temperature

ILARIA GIOVANNINI[1], CHIARA MANFRIN[2], TIZIANA ALTIERO[1], SAMUELE GRECO[2], JOEL VINCENZI[1], ROBERTO GUIDETTI[1], PIERO GIULIANINI[2] & LORENA REBECCHI[1]

[1]*University of Modena and Reggio Emilia, Italy*

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Background: Due to global warming, temperature in Antarctica is predicted to increase by 0.34 °C per decade. This could affect Antarctic organisms, especially animals with limited dispersal capability, such as tardigrades. The aims of this study were to analyse the life cycle of the tardigrade *Acutuncus antarcticus*, to investigate the molecular mechanisms involved in the acclimatory adaptation during heat stress, and to identify the genes in charge of the responses to heat exposure.

Methods: Specimens of *A. antarcticus* collected from a temporary freshwater pond at Victoria Land (Antarctica) were individually reared from birth to death at 5 °C (12h/12h, L/D) with algae as food source. Data on life history traits of successive generations were collected: life span, age at first oviposition, eggs/life span, eggs/clutch, time interval among oviposition, egg hatching time, and egg hatching percentage. Moreover, groups of adult animals were exposed to gradually increasing temperatures (from 5 °C to 10°, 15°, and 20 °C) and kept at each temperature for 1 day. A reference transcriptome for the species was *de-novo* assembled and analysed to find differentially expressed genes under the different stress conditions.

Results: Reared specimens at 5 °C reached a maximum of life span of 686 days, laying a maximum of 93 eggs, which hatched in about 22 days. First oviposition occurred at the mean age of 34 days. Females laid eggs once a fortnight and the highest number of ovipositions per life span was 34. The transcriptome was constituted by 95511 contigs with a mean GC content of 45.82%. In relation to the increasing temperature, the expression of transcripts for Me31b (RNA-binding protein), protein-argonautel, protein-Gawky and UCRSFS1P1 (Ubiquinol-Cytochrome C Reductase) was down-regulated, while that of ND3, ND4 and ND5 was up-regulated. The Cox-2 was down-regulated until 10 °C, but up-regulated after 15 °C.

Conclusions: Comparing data on life history traits of *A. antarcticus* between 5 °C and 15 °C (obtained previously), specimens at 5 °C survive longer, lay more eggs, but reach sexual maturity later and egg hatching time is longer. The first transcriptome from the genus *Acutuncus* will be useful in understanding the metabolic and molecular response to heat stress in tardigrades.

Keywords: Antarctica, climate changes, differentially expressed genes, heat stress, transcriptome

Tuesday | Talk Session #4 | 14:30-14:45

Semiochemical-based mate searching behaviour in tardigrades: comparing the sexes

JUSTINE CHARTRAIN[1]☆, EMILY KNOTT[1], ŁUKASZ MICHALCZYK[2], SIMO PURO[1], RIIKKA TYNKKYNEN[1] & SARA CALHIM[1]

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Background: Semiochemical communication is widespread and used across multiple contexts, from finding food to attracting mates. Animals up to 1mm can use (1) trail pheromones laid on the substrate by a moving animal and/or (2) short-range diffusion of molecules from an origin (diffusing signals). Tardigrades are a phylum of microscopic animals largely neglected in behavioural ecology. Yet, there is evidence that both types of chemical cues are used: in one species, immotile receptive females attracted males using diffusing signals, whereas a predator-prey species dyad detected each other's trails.

Methods: In this study, we aimed to test the role of (1) trail pheromones and (2) diffusing signals in the mate searching behaviour of both male and female *Macrobiotus polonicus*. To this end, individuals were either (1) allowed to move more freely and simultaneously or (2) were placed in a designed double choice chambers.

Results: We found that males move more and preferentially associate with the female *vs* the male stimulus for diffusing signal. In contrast, females did not behave differently towards each sex. In the trail pheromone experiment, as with the choice chamber trials, males were more behaviourally active: they followed trail cues, approached, and then followed the opposite-sex individual more often than females.

Conclusions: These sex-specific behaviours are in agreement with previous descriptive mating observations of tardigrades, where males initiated the interaction by tracking and approaching. More importantly, our study suggests that multiple signalling cues – deposited and diffused – could be involved in mate attraction and/or finding behaviour in this animal group.

Keywords: chemical communication, choice chambers, mating behaviour, *Macrobiotus polonicus*, water bears

Tuesday | Talk Session #4 | 14:45-15:00

Effect of juvenile hormone, methyl farnesoate, on sex determination in *Paramacrobiotus metropolitanus*

MIDORI MATSUMOTO[1], RYUSEI UCHIDA[1], HIROKI MINATO[1], HIKARU SUZUKI[1], XIAONAN XIE[2], HITOSHI MIYAKAWA[2] & KENTA SUGIURA[3]

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[3]*Gunma University, Japan*

Background: Tardigrades are Ecdysozoa that grow by repeated molting. The molting mechanism in insects is regulated by the molting hormone, 20-hydroxyecdysone, and juvenile hormone (JH). JH in arthropods acts on various biological phenomena, such as sex determination and caste differentiation, and responds to some environmental cues. Although the hormone reportedly has functions in hatching in parthenogenetic species of tardigrades, its structure and other functions are still unknown. We considered that sex determination was driven by environmental cues, but not by genetics, because sex chromosomes have not been confirmed and the sex ratio is not fifty-fifty in the sexually reproducing tardigrade, *Paramacrobiotus metropolitanus*. In this study, we identified the presence and structure of JH and clarified its sex-determination function in *P. metropolitanus*.

Methods: To identify the molecular species of JH in *P. metropolitanus*, a liquid chromatography tandem-mass spectrometry analysis was performed using different mobility spectrometry methods for methyl farnesoate (MF) and JHIII. To clarify the effect of MF for the developmental process, tardigrades were exposed to various concentrations during development. The expression of JH-related genes in *P. metropolitanus* were measured with the reverse transcription-polymerase chain reaction.

Results: An analysis of *P. metropolitanus* extracts found MF but not JHIII. Thus, the molecular species of JH in *P. metropolitanus* is MF. Following exposure to MF, there was no effect on hatching, molting, or morphogenesis in *P. metropolitanus*, but there was an effect on the male-to-female ratio. The female percentage was approximately 30% in the control group (Volvic), but it increased significantly, to approximately 60%, when *P. metropolitanus* were exposed to 400 pM MF ($p < 0.05$). It indicates that MF acts on sex determination at the optimal concentration of 400 pM. JH-related genes that are highly homologous to *methoprene-tolerant (Met)* and *juvenile hormone acid methyltransferase (JHAMT)* in arthropods were found from the transcriptome data of *P. metropolitanus*. In addition, the obtained *Met* and *JHAMT* homologs were expressed in both the embryonic and larval stages.

Conclusions: The JH type of *P. metropolitanus* was identified as MF, and it acts on sex determination. This is the first study showing that JH has the potential to regulate sex determination in tardigrades.

Keywords: juvenile hormone, sex determination, sexual reproduction

TALK SESSION 5

Morphology, Anatomy, Reproduction & Development

Illustration to be revealed on Wednesday

Wednesday | Talk Session #5 | 11:00-11:15

Reproductive strategy of tardigrades: gamete motility and morphology

KENTA SUGIURA[1]☆ & MIDORI MATSUMOTO[2]

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[2]*Keio University, Japan*

Background: Egg morphology is often utilised in taxonomic studies of eutardigrades because of its species-specific characters. All species of tardigrades are oviparous; the animals therefore must produce eggs to proliferate regardless of reproductive mode. On the other hand, sexually reproducing species must produce spermatozoa. The morphology of the spermatozoa has also been reported in some eutardigrade species, with those of *Paramacrobiotus* species and *Macrobiotus* species having a longer and a shorter acrosome, respectively. Although it was previously observed that the spermatozoa of *Paramacrobiotus* sp. and *Macrobiotus shonaicus* were released into the water and swam to reach to the female, the detailed motility of the male gametes has not been revealed. In addition, it was still unclear where and how the male and female gametes fuse.

Methods: The specimens, *Paramacrobiotus* sp. or *M. shonaicus*, were placed into a droplet in a 0.5 mm gapped coverslip. To observe the motility of the spermatozoa, we used a high-speed camera attached to an inverted-phase microscope. After mating, the female specimens were fixed to observe with SEM or dissected to obtain spermathecal spermatozoa. The females oviposited approximately one hour after mating, then the laid eggs were also fixed immediately and observed with SEM.

Results: Comparison of the spermatozoon motility between the two species indicated that the longer male gametes released by *Paramacrobiotus* sp. swam faster than those of *M. shonaicus*, but showed equal beat frequency. Quantitative curvature of the head part, consisting of a nucleus and an acrosome, and the tail demonstrated that the longer head part of *Paramacrobiotus* sp. showed larger variance than that of *M. shonaicus*, suggesting that the morphology of the head affects spermatozoon flexibility and stability in swimming. The spermatozoa aggregated around the female's cloaca after mating, then entered the female's body to be stored in the spermatheca. The tail of the spermathecal spermatozoon was shortened in both species. Then the spermatozoon, without the flagella, attached to the surface of the laid egg by its acrosome.

Conclusions: This study is the first to demonstrate the detailed motilities of spermatozoa in different species of eutardigrades. Morphological variations and the connection between the egg and the spermatozoon with reduced flagella are described. The reproductive strategies of tardigrades are gradually becoming clearer.

Keywords: fertilisation, mating, morphology reproduction, spermatozoon

Wednesday | Talk Session #5 | 11:15-11:30

Evolution of sperm morphology in Macrobiotoidea

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Background: Spermatozoa are the most diverse cell type known. Comparative studies are a powerful tool to assess what forces drive the evolution of sperm size and shape. The incredible variation in morphology across animal taxa is the product of both fertilisation modes and pre- and post-copulatory selection.

Methods: Here we present the first phylogenetically controlled comparative study on sperm morphometry of the tardigrade superfamily Macrobiotoidea. We collected sperm morphometric data using confocal fluorescent microscopy for 32 species, spanning 8 genera and 3 families to evaluate how the spermatozoon components (acrosome, nucleus, midpiece, tail) differ and how they evolved.

Results: Total sperm length ranges from 15 µm in *Macrobiotus hannae* to 137 µm in *Paramacrobiotus spatialis*. As previously described, the general sperm design is constant within the superfamily, with most of the length variation occurring between rather than within genera. All sperm components show a strong phylogenetic signal and the patterns are most consistent with evolutionary models of decreasing evolution rate over time (early burst). Acrosome, nucleus, and tail lengths are correlated with each other, but not with midpiece length. This correlation pattern persists also when the effect of phylogeny is accounted for.

Conclusions: The pattern of macrobiotoid sperm evolution is compatible with an early burst of diversification and consequent higher inter- than intra-generic divergence. Furthermore, the strong correlation between all traits except the midpiece suggests that head and tail components are somewhat evolutionary constrained. Neither the causes nor the nature (genetic, physiological, etc.) of these constraints are yet known. Respectively, the tail is lost soon after insemination in representative species of three macrobiotid genera, and the complex eggshell morphology provides an elaborate, and species-specific, sperm-egg interaction context. Our results highlight the importance of exploring non-model systems for the study of gamete evolution and sexual selection.

Keywords: comparative study, Macrobiotoidea, phylogeny, sperm

Wednesday | Talk Session #5 | 11:30-11:45

Morphology of light perception: localisation of opsins in the eutardigrade *Hypsibius exemplaris*

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[4]*Hessian State Museum Darmstadt, Germany*

Background: The general composition of the eutardigrade eye has been clarified in at least two species using transmission electron microscopy. In these species, the eyes contain both rhabdomeric and ciliary sensory cell types as well as a single cell containing a dark pigment of various colours, depending on the species. Meanwhile, studies in the past decade have begun to elucidate the molecular basis of light perception and vision in tardigrades by analysing their genomes and transcriptomes. Such analyses have identified a total of nine opsins (light-sensitive proteins) in the transcriptome of *Hypsibius exemplaris* – a remarkable number considering the morphological simplicity of the eye and the apparent miniaturisation of the body. Taken together, these findings beg the question of where all these opsins are expressed in the body and what their possible functions may be.

Methods: In order to address these questions, we generated antibodies against the nine opsins we identified in the transcriptome and used these antibodies to localise the opsins in whole mounts of adult specimens of *H. exemplaris* via immunohistochemistry. To better understand the eye – the main light-perceiving organ – and the distribution of opsins within it, we additionally performed ultrastructural analysis via electron microscopy as well as nano-computed tomography.

Results: Our data show opsin expression in almost every major organ system of the body including the eyes, central nervous system, sensory fields, digestive tract, Malpighian tubules, and storage cells. Within the eye, immunolabelings confirm the presence of both rhabdomeric and ciliary cell types, while sensory fields on the head and legs express various rhabdomeric opsins but lack ciliary opsins. Rhabdomeric opsins are present in the ovary and gut as well.

Conclusions: Our discovery of widespread opsin expression throughout the body underscores the diverse roles that opsins are known to play in other organisms not only in vision but also in various physiological processes. Based on our results, these may include circadian rhythmicity, mechanosensation, and oogenesis, as well as structural functions like stabilising ciliary ultrastructure. Analysis of the ultrastructure and opsin distribution within the eye offer additional support for monochromatic vision in tardigrades and the rhabdomeric cell type as the one responsible for vision.

Keywords: ciliary, opsins, photoreception, rhabdomeric, vision

Wednesday | Talk Session #5 | 11:45-12:00

Three-dimensional reconstruction of the eye in *Hypsibius exemplaris* based on nano-computed tomography and ultrastructure

MERCEDES KLEIN[1], VLADIMIR GROSS[2], IMKE GREVING[3], ELENA LONGO[4] & GEORG MAYER[2]

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[3]*Helmholtz Centre Hereon DESY, Germany*

[4]*Elettra Synchrotron Trieste S.C.p.A., Italy*

Background: The structure of the tardigrade eye has been the subject of few studies, resulting in a general consensus of its morphology. A single pigment cup and one rhabdomeric cell with microvilli processes running towards the concave surface of the pigment cell, as well as one or two ciliary cells have been reported. Open questions remain regarding the three-dimensional reconstruction and origin of the eye.

Methods: Morphological features of the visual system in *Hypsibius exemplaris* were investigated through histological analysis of series of ultrathin sections combined with electron microscopy. Nano-computed tomography was performed at Imaging beamline P05 at Petra III (DESY), operated by Helmholtz-Zentrum Hereon. The results allowed for three-dimensional reconstructions of the head and ultrastructure of the eye.

Results: Situated inside the outer lobe of the brain, the dorsolateral eyes exhibit an ovoid shape with a ventral extension. We could identify three cells comprising the eye, with evidence indicating a fourth cell. Apart from one pigment cell, a rhabdomeric cell arises from a position ventral to the pigment cup. It sends microvilli towards the concave surface of the pigment cup while simultaneously building the optic cavity anteriorly through a dome-shaped evagination. A cilium emerging from a dorsally situated ciliary cell enters the optic cavity and branches off to form an interlaced labyrinth of lamellae. Filled by a ramified cilium and microvilli, an additional supporting structure – the optic cavity support – enters the otherwise closed optic cavity from a dorsal direction. Upon entering, the extension becomes flattened and spans the lateral surface of the dome-shaped evagination.

Conclusions: The position of the rhabdomeric cell with microvilli protruding into the concave side of the pigment cup suggests a role in directional light perception. Due to the sheet-like structure of the optic cavity support, a stabilising function was proposed to maintain the shape of the fluid-filled optic cavity. We cannot say whether the optic cavity support represents a cellular structure, as we did not identify any associated nucleus. These findings represent the most detailed morphological description of the tardigrade to date and lay the foundation for future comparative studies aiming to characterise this structure in other species.

Keywords: ciliary/rhabdomeric photoreceptors

Wednesday | Talk Session #5 | 12:00-12:15

Cuticle and cuticular capsule in freshwater tardigrade *Thulinius ruffoi* – formation and shedding

KAMIL JANELT[1]☆ & IZABELA POPRAWA[1]

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Background: The integument is an integral part of the body. It separates the inside of the body from the outside environment but, at the same time, allows contact with it. It provides mechanical support, protective function, participates in transport from and into the body (*i.e.*, of water and gases) and enables the locomotor activity of the tardigrades. Also, the exuvium is used as a container for laying eggs. Present preliminary research is dedicated to the course of the new cuticle formation in relation to oogenesis, moulting process and analysis of the process of the cuticular capsule formation.

Methods: The present study involved the analysis of animals kept at 19.0 °C and 6.5 °C. The non-encysted animals at different oogenesis stages and exuviae from control cultures as well as the cysts freshly formed at a lower temperature, older cysts, and animals that do not take the form of a cyst yet were analysed. Analysis was performed using light, scanning and transmission electron microscopy.

Results: The cuticle is produced by the underlying epithelial cells. Its formation proceeds with the progress of the events within the ovary. The eggs are laid within the shed cuticle underneath which a new cuticle is present. The analysed exuviae visibly differs from the typical cuticle that covers the epithelial cells of the integument. Also, the cuticular capsule formed by individuals entering the encystment is synthesised by the integument cells. Changes in the integument are visible even if the animal has not yet morphologically assumed the form of a cyst but entering into this process was started. The process of the cuticular capsule formation showed that it is related to releasing enormous amounts of the material. The newly formed cysts still did not show the typical organisation of the cuticular capsule which demonstrated visible differentiation in time. The animal remains in the cuticular capsule.

Conclusions: Presented research compares the events related to the new cuticle formation and shedding of the cuticle with the course of oogenesis. Moreover, new data on the cuticular capsule formation allowed us to better understand this aspect of tardigrade biology related to encystment.

Keywords: cuticle, cuticular capsule, encystment, exuviae, integument

The study was supported by the Polish National Science Centre (NCN Preludium 2020/37/N/NZ8/00170).

New insights on chemical composition and morpho-functional adaptations of tardigrade feeding apparatus

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Background: Tardigrade feeding apparatus is an endodermal derivate system that can be divided into foregut, comprising cuticular annexes derived from ectodermal stomodeum invagination, midgut, and hindgut; latter lined with cuticle derived from ectodermal proctodeum invagination. The buccopharyngeal apparatus is part of the foregut and is one of the structures majorly studied and described due to its taxonomic relevance. The cuticular nature of this structure has been confirmed by the studies on its chitinous composition performed in the second half of XX century. Since then, its chemical nature has been accepted and no further investigated. Recent use of advanced microscopies opens new perspectives for biochemistry studies and allows to deepen the knowledge on morphology, morpho-functional adaptation, and evolution of buccopharyngeal apparatus.

Methods: Live animals of several species of different genera belonging to both Heterotardigrada and Eutardigrada have been stained with Calcofluor White [CFW], specific chitin dye, and analysed under Confocal Laser Scanning Microscope [CLSM] with two laser line excitation (λ: 405 nm, 488 nm) and three acquisition ranges: blue (λ 425–470 nm), green (λ 509–614 nm), and far red (λ 690–740 nm). Spectral analyses have been used to distinguish the different signals. In addition, moulting specimens at different stages were analysed to detect the distribution patterns of spectra during the buccopharyngeal apparatus reconstruction. Species belonging to nematodes and rotifers were used as comparative materials.

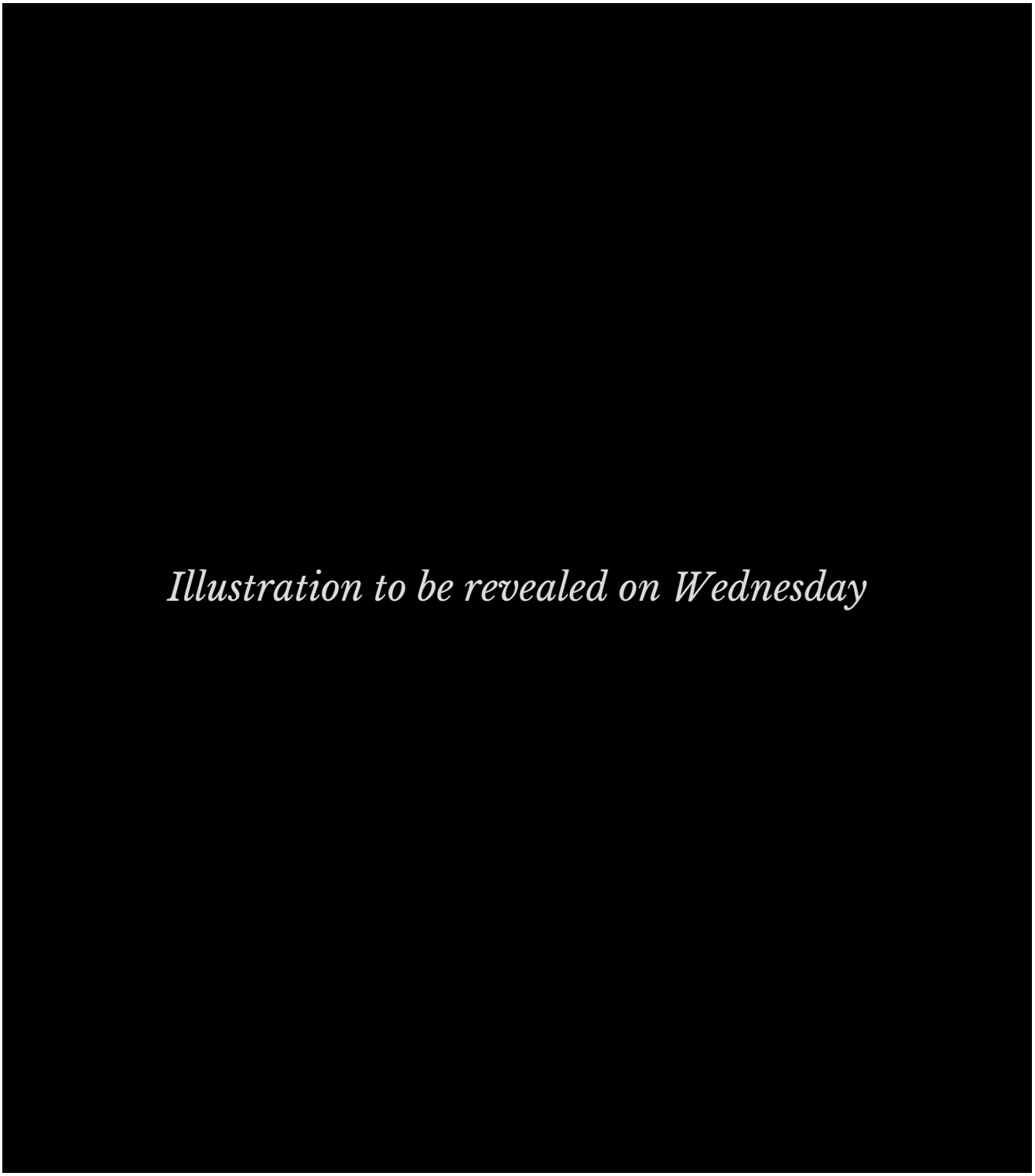
Results: The presence of chitin in the feeding apparatus of tardigrades as component of derivates of stomodeum and proctodeum was confirmed. Nevertheless, in the buccopharyngeal apparatus a combination of three non-colocalised acquisition signals was present. This confirmed the occurrence of at least two fluorochromes beside chitin, one of which with probable proteic nature, and a new organisation in the structures of chitin. Furthermore, the patterns of distribution and localisation of the three signals (very probably related to three different molecules), together with the previous knowledge on aragonite distribution in the feeding apparatus, further clarifies the relation between morphology and functioning of different parts of this autapomorphic apparatus.

Conclusions: The structures becoming clearly identifiable with CFW and CLSM combination can become relevant for taxonomic purposes. The nature of the detected molecules will provide the ground for future investigation on tardigrade evolution and miniaturisation pattern.

Keywords: buccopharyngeal apparatus, confocal microscopy, histochemistry, *in vivo* staining

TALK SESSIONS 6-9

Physiology, Omics, Cryptobiosis & Astrobiology



Wednesday | Talk Session #6 | 13:45-14:00

Sequencing genomes from single tardigrade individuals

WITOLD MOREK[1], LEWIS STEVENS[2],
MARK BLAXTER[2] & ŁUKASZ MICHALCZYK[1]

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Background: Despite numerous scientists working on tardigrades, there is still a scarcity of available genomic data, which is hampering our understanding of their cryptobiotic machinery or phylogenetic relationships. This is mainly caused by technical issues, with the main problems being small size of these animals, resulting in low input of DNA, difficulties in pooling several specimens for sequencing (require stable clonal, long-lasting cultures) and contamination (due to pooling and small size of animals), which is making the subsequent bioinformatics analysis challenging. Thus, developing a reliable method that would yield high quality genome assemblies from single individuals is a prominent challenge.

Methods: For the purpose of this study we used four species belonging to three genera: *Diploechiniscus*, *Macrobiotus* and *Milnesium* (two species), representing the three limnoterrestrial orders. For each species, two specimens were sequenced using a protocol developed by developed by Chris Laumer, NHM, London (with modifications). In short, the DNA was extracted form single tardigrade and in subsequent steps the entire genome was amplified using long-range PCR, tagged, size selected and prepared for PacBio HiFi sequencing. Moreover, in the initial steps the mRNA was separated using dT beads, reverse-transcribed, amplified using long range PCR, and sequenced to provide the transcriptome from the same individual.

Results: The sequencing of all individuals was successful, with the coverage ranging from 15–40× after removing PCR duplicates. The read N50 ranged from 8.5 to 10.2 kbp. The resulting genomes were highly contiguous, with contig N50s ranging from 0.6 to 2.4 Mb. The completeness of the genomes estimated with BUSCO, ranged from 75 to 85%. By comparison, the current reference genome for *Hypsibius exemplaris*, which was sequenced from a pool of nearly 900 000 tardigrades, has a contig N50 of 0.3 Mb and completeness of 83%.

Conclusions: We showed that high-quality sequencing of entire genome from a single tardigrade individual is feasible. As a result, our methods allow for sequencing species that are difficult or impossible to culture. The future goal is to use this approach to sequence the genomes of further tardigrade species, representing various lineages, and use the resulting dataset to study tardigrade genome evolution.

Keywords: *de novo* assembly, genome sequencing, PacBio, Tardigrada, transcriptome

The study was supported by the Polish National Science Centre (NCN Etiuda 2020/36/T/NZ8/00306 and Sonata Bis 2016/22/E/NZ8/00417).

Wednesday | Talk Session #6 | 14:00-14:15

In vivo gene expression in tardigrades, a technical breakthrough coming to tardigrades

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Background: Here, we introduce a novel analytical tool for the advancement of tardigrade biology, which enables the introduction of exogenous genes into tardigrade cells. So far, biochemical assays and omics analyses have provided various evidence for the molecular machineries of anhydrobiosis in Tardigrada; however, to date, there have been no method to introduce exogenous genes to tardigrades. Our new tool allows to observe dynamics of targets proteins fused with a fluorescent tag, and may even be utilised to confer tolerance to non-anhydrobiotic species.

Methods: To establish a vector system that works in tardigrade cells, we first designed simple plasmids consisting of a promoter, GFP, 3'UTR, selection marker, and a replication origin for amplification in *E. coli*. To easily visualise whether vector systems work, we used GFPs with nuclear localisation signal (NLS) to concentrate the signal on the nucleus. These plasmids were introduced by a microinjection-based method, and then the fluorescence was observed under fluorescence microscopy.

Results: We conformed our vector systems to work successfully in tardigrade cells. Tissue specificity and difference in expression levels were observed in these systems. The GFP fluorescence was observed from 24 hours after injection, lasting for more than 10 days. Furthermore, inter-specific expression was also confirmed for most promoters tested.

Conclusions: We have developed a new technology to introduce exogenous genes based on the novel tardigrade vector system. It will enable to perform various molecular biology experiments actually within tardigrades.

Keywords: anhydrobiosis, fluorescence live imaging, *in vivo* gene expression, vector system

Wednesday | Talk Session #6 | 14:15-14:30

Gene mining of cytokine-like molecules in the eutardigrade *Mesobiotus philippinicus*

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Background: Tardigrades are known to interact with microorganisms such as fungi and bacteria in parasitic and non-parasitic relationships. This implies the presence of an immune system that regulates these interactions. Efforts to identify the molecular players in the tardigrade immune response through gene mining have been undertaken previously, and the analysed tardigrade transcriptomes were found to lack homologs of several canonical *Drosophila melanogaster* and *Caenorhabditis elegans* immune response genes. To date, the key components of the tardigrade immune system remain unclear.

Methods: Sequences of genes encoding the cytokine-like molecules transforming growth factor-β (TGF-β), tumour necrosis factor (TNF), and LPS-induced TNF-α factor (LITAF) in *D. melanogaster* and *C. elegans* were used as BLAST queries to search for similar sequences in the transcriptome of the eutardigrade *Mesobiotus philippinicus* Mapalo *et al*, 2016. The Rel homology domain-containing protein nuclear factor activating T cells (NFAT) was also mined in the *M. philippinicus* transcriptome. Endpoint PCR was performed to validate the mined transcripts.

Results: One NFAT-like, three TGF-β-like, and six LITAF-like transcripts with complete putative ORFs were identified in the *M. philippinicus* transcriptome. No TNF superfamily member was found in the search. Of the six LITAF-like transcripts found, two pairs were found to be highly similar to each other. One pair possesses a single-base indel as well as a different nucleotide identity in one base pair. Both differences occur outside of the putative ORFs and therefore do not affect the predicted protein product. The second pair differs in the presence of a 73-bp indel that results in a nonsense mutation in one transcript. These transcripts, along with previously identified TIR and Spätzle transcripts, were successfully amplified from *M. philippinicus* cDNA. This verifies their presence in the *M. philippinicus* transcriptome.

Conclusions: The presence of transcripts encoding cytokine-like molecules in *M. philippinicus* provides insight into possible mechanisms of immune response in this tardigrade species. These transcripts also represent possible candidates for expression analysis to further characterise the immune response of this species to pathogens. Quantitative PCR and protein-level expression assays will be important in confirming the role of these genes in immunity, particularly for those that are known to be differentially expressed and/or undergo changes at the protein level (*e.g.* cleavage) during an immune response in other animals.

Keywords: cytokine-like molecules, LITAF, tardigrade immunity, TGF-β

Wednesday | Talk Session #6 | 14:30-14:45

Doyère-Pouchet controversy (1859–1860) as the first stage of the spontaneous-generation controversy (1859–1864)

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Background: The scientific controversy between Doyère and Pouchet in 1859, related to the desiccation and revivification of tardigrades and rotifers, have been shown mainly by two articles, one of which is the contemporary report written by Broca in 1860. The other by Keilin was published in 1959, just a century after the controversy, in which the term *cryptobiosis* was coined for the phenomena on the latent life. Pouchet at that time also had another controversy (1859–1864) against Pasteur on the theme of the spontaneous generation of living organisms. The results of Pouchet's work were severely criticised at the meeting of the Académie on 3 January 1859. Keilin recognised these responses as the first stage of the controversy on the spontaneous generation and suggested that the controversy between Doyère and Pouchet was intimately linked with the problem of spontaneous generation. Then, a question arises: Why and how Doyère joined the controversy?

Methods: In order to find the details of the beginning, records on the controversy were thoroughly searched among primary sources including many documents found in scientific presses published in Paris at that time.

Results: Among large number of documents related to the controversy, a published document and an unpublished autograph letter referred that Doyère himself once planned some experiments to clarify the problem on spontaneous generation. He expressed, at first, his worry about the rejection without further experiments. The details on 'the first stage' will be shown with these primary sources.

Conclusions: Doyère joined the debate *on the spontaneous generation* with his own interest to solve the problem with reliable experimental facts. Therefore, Doyère was one of the important members of the first stage of the controversy on the spontaneous generation.

Keywords: Académie des sciences, desiccation tolerance, revivification, spontaneous generation

Wednesday | Talk Session #6 | 14:45-15:00

Identification of promoters associated with tardigrade anhydrobiosis-related genes

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Background: Promoters are DNA motifs located upstream of a gene, functioning as a target site for transcription factor binding, required for accurate transcription initiation. In tardigrades, highly desiccation tolerant species are known to constitutively express genes for anhydrobiosis, while species with relatively low desiccation tolerance rely on *de novo* expression. Therefore, through the identification of promoter regions of tardigrade anhydrobiosis-related genes, we aim to elucidate the genetic evolution in transcriptional machinery between tardigrade species. However, promoter identification in eukaryotic species has been a challenge, due to its wide variety in motif and location relative to the transcription start site. Thus, we tried to elucidate promoter regions in tardigrades through *in silico* analysis and *in vivo* experiment.

Methods: Several tardigrade promoter candidates were searched, subsequently to determining syntenic genes between four species with different desiccation tolerance: *Ramazzottius varieornatus*, *Hypsibius exemplaris*, *Acutuncus antarcticus* and *Thulinius ruffoi*. Syntenic relations were identified by clustering collinear blocks, and matching genes with relatively high amino acid sequence similarity. The upstream sequences of syntenic anhydrobiosis-related genes were analysed for motif sequences to catalogue promoter candidates. Promoter candidates were then compared for similarity with motifs from promoter databases. Furthermore, to examine promoter function, exogenous mEGFP expression was observed *in vivo* under a fluorescent microscope.

Results: Only a few of the anhydrobiosis-related genes had conserved synteny across all four species used in the study, due to short contigs in some of the tardigrade genomes; thus, not enough sequence samples were obtained for most genes, required for an accurate motif search. Only genes with conserved synteny among 3 or more species were used, revealing several possible promoter candidates. Furthermore, the functional promoter region was experimentally validated in the tardigrade.

Conclusions: Our study established a method to determine functional promoters regulating tardigrade anhydrobiosis-related genes, combining *in silico* analysis and *in vivo* expression experiment. Currently identified promoter regions can help in understanding the evolutionary divergence of transcription machinery between tardigrade species, and in the discovery of other anhydrobiosis-related genes that may be coregulated.

Keywords: anhydrobiosis, promoter, transcriptional regulation

Thursday | Talk Session #7 | 11:00-11:15

Tardigrade autofluorescence: UV protection or spandrel?

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Background: While many studies have used fluorescent markers to examine tardigrades, autofluorescence remains poorly studied. In 2020, a paper purported to show that pigments in a tardigrade conferred protection from UV radiation via fluorescence. Stephen Jay Gould used an architectural feature called a spandrel as a metaphor for non-functional biological traits. Using three research modalities, we explored autofluorescence across numerous species of tardigrades to examine the nature and extent of inter- and intraspecific variation in autofluorescence and to chart a path forward to discern whether tardigrade autofluorescence is functional or a spandrel.

Methods: Using confocal microscopy on 11 tardigrade species, we characterised interspecific variation in fluorescence in response to violet, blue and green illumination, and examined how various body parts contributed to fluorescence. We used epifluorescence microscopy using ultraviolet illumination to examine intraspecific variation in 13 specimens of *Milnesium* sp., and examined how body size and pigmentation relate to this variation. Finally, and as far as we know for the first time, we used a plate reader to examine fluorescence in whole tardigrades to determine its efficacy for the measurement of autofluorescence.

Results: Tardigrade species show a wide range of fluorescent signatures that differ in response to wavelength of stimulation. In most species fluorescence was relatively weak. The most fluorescent species we measured appears to incorporate carotenes from the diet into storage cells and/or non-cellular structures. Intraspecific variation in *Milnesium* sp. was not large, and it was neither correlated with body size nor pigmentation. The plate reader was able to measure fluorescence emission spectra if animals were above a minimum size threshold, and so offers an alternative experimental approach. These results collectively allow us to propose a set of experimental guidelines necessary to determine whether UV autofluorescence conveys a selective advantage for tardigrades.

Conclusions: All examined tardigrades autofluoresced, but in most species this response was weak. Intraspecific variation was not excessive. Three complementary tools were used for examining fluorescence, but we believe the potential functionality of autofluorescence in tardigrades is still undetermined.

Keywords: fluorescence, Tardigrada, ultraviolet, UV

Thursday | Talk Session #7 | 11:15-11:30

Investigation of osmobiosis in the limno-terrestrial eutardigrade *Ramazzottius varieornatus*

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Background: During exposure to severe osmotic stress, limno-terrestrial tardigrades contract into a tun and enter osmobiosis. Although recognised as the most ancient form of cryptobiosis, osmobiosis is surprisingly understudied. Thus, we aimed to explore osmobiosis in *R. varieornatus*, in terms of tolerance extent, tun formation and underlying molecular mechanisms.

Methods: Groups of ca. 20 *R. varieornatus* were either exposed gradually to sucrose solutions of increasing osmolality (~100 mOsm·kg^{-1} to ~3,000 mOsm·kg^{-1}) or directly to ~3,000 mOsm·kg^{-1}. The morphology and activity of single specimens were monitored during increase in osmolality and following retransfer to moderately hard reconstituted water (MHRW) or ultrapure water. Further, RNA sequencing (RNA-seq) was conducted to identify differentially expressed transcripts in osmobiotic tuns induced by ~3,000 mOsm·kg^{-1} as compared to active tardigrades in ultrapure water. The RNA-seq datasets were subjected to a batch effect correction. The latter likely relates to a size difference observed among tardigrade specimens obtained from different batches.

Results: During gradual exposure to increasing osmolarity, *R. varieornatus* specimens started tun formation at ~200 mOsm·kg^{-1} and all specimens were in the tun state at ~500 mOsm·kg^{-1}. The activity of tardigrades following exposure to a gradual increase in osmolarity as well as acute exposure to ~3,000 mOsm·kg^{-1} could be recovered to almost 100% upon retransfer to MHWR or ultrapure water. Transcriptome profiling revealed 3,322 differentially expressed transcripts in the osmobiotic tuns as compared to active tardigrades. A gene ontology enrichment analysis suggested a shift in proteostasis as well as neural and hormonal signalling. Numerous transcripts are hypothesised to play a specific role in osmobiosis, including various channels, transporters, heat shock proteins, and eutardigrade specific proteins (Dsup, CAHS, MAHS, SAHS).

Conclusions: The high recovery rate following severe osmotic stress emphasises the fascinating capability of tardigrade to withstand extreme conditions. Moreover, the osmobiotic tun displayed a substantial transcriptomic change. The latter is in contrast to the constitutive expression previously reported from anhydrobiotic tuns of *R. varieornatus*. The newly identified transcripts provide a fundamental insight into osmobiosis, offering the possibility for future research.

Keywords: cryptobiosis, osmobiosis, osmotic stress, RNA sequencing, transcriptomics, tun

Thursday | Talk Session #7 | 11:30-11:45

Why are not all tardigrades good models for anhydrobiosis studies?

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Background: Anhydrobiosis is considered to be an adaptation of important applicative implications because it enables resistance to the lack of water. The phenomenon also occurs in invertebrates but is still not well understood at cellular level. Thus, a good model invertebrate species for the research is required. The best known anhydrobiotic invertebrates are water bears (Tardigrada), considered to be toughest animals in the world. Tardigrade anhydrobiosis includes entry, dormant and exit stages, that corresponds to the dehydration (*i.e.*, tun formation), tun and rehydration stages, respectively. The phylum Tardigrada currently consists of ca. 1 400 species that inhabit terrestrial and aquatic environments throughout the world. Among them *Hypsibius exemplaris* is one of the best studied species, with its name "*exemplaris*" referring to the widespread use as a laboratory model for various types of research, including anhydrobiosis.

Methods: We analysed available data as well as our unpublished results on anhydrobiosis of few different Tardigrada species. The relevant experiments were performed in plastic Petri dishes lined with filter paper. To test anhydrobiotic abilities, specimens were dehydrated and then rehydrated after different duration of the tun stage (0-, 7-, 14-, 30-, 60-, 120-, 240-days).

Results: Anhydrobiotic capability of *Hypsibius exemplaris* may be overestimated, as many specimens of that species just died during the tun stage (survival rate maximally about 50%). Moreover, *Milnesium inceptum*, *Ramazzottius subanomalus*, *Echiniscus testudo*, *Paramacrobiotus experimentalis*, *Pseudohexapodibius degenerans* and *Xerobiotus xerophilus* had much higher survival rate oscillating around 70–95%. Similar survival rate were also obtained for other species like *Ram. varieornatus*, *Pam. areolatus*, some species of *hufelandi* group and genus *Echiniscus*, by other authors.

Conclusions: Despite formation of tuns of proper appearance, some tardigrade species are not good anhydrobionts. This may result from impairments occurring in the tuns at cellular level and resulting in impairments of energetic support.

Keywords: cryptobiosis, interspecies differences, model, Tardigrada, tun

The study was supported by the Polish National Science Centre (NCN Opus 2016/21/B/NZ4/00131).

Thursday | Talk Session #7 | 11:45-12:00

Tardigrades are shining models of anhydrobiosis research

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Background: New tools allow us to view a new perspective. Limitation in the miniscule amount of samples in unculturable tardigrades as well as the lack of genetic toolkit other than RNA interference has been a bottleneck in tardigrade research. We therefore developed two new technologies to overcome these hurdles, namely, in ultra-low input genome sequencing and in tardigrade *in vivo* gene expression.

Methods: *Droplet-MDA*: We utilised a microfluidic device to encapsulate a fragment of genomic DNA in a microdroplet, and perform MDA (multiple-displacement amplification) in these droplets for unbiased, long fragment amplification from genomic DNA obtained from a single specimen. Combined with nanopore sequencing, this new method allows high quality genome sequencing of any tardigrade, including unculturable species. *Vector expression system*: We have created a plasmid vector that allows the expression of any protein of interest in tardigrades with the help of microinjection.

Results: We have sequenced, assembled, and annotated high-quality genomes for various tardigrades, including *Milnesium inceptum*, *Echiniscus testudo*, and *Viridiscus perviridis*. These genomes from Heterotardigrada and Apochela elucidated the conserved repertoire of anhydrobiosis machineries specific to Eutardigrada or that are common among the phylum Tardigrada, and provide clues to their evolution. For example, homology of the core machineries of anhydrobiosis and cyst formation is suggested from multi-omics study of *Thulinius ruffoi*. Structural clustering of these proteins with Alphafold2 predictions and cell-specific expression patterns allowed us to view a comprehensive picture of the anhydrobiosis machinery.

Conclusions: Both of Droplet-MDA as well as vector expression system have a wide applicability in tardigrade research, where the former provides a foundation for molecular biology researches in the form of high quality genomes, and the latter visualises the molecular actions within tardigrades with fluorescent proteins. The findings from these new technologies are beginning to show the comprehensive picture on the anhydrobiosis machineries in tardigrades.

Keywords: anhydrobiosis, evolution, gene expression, genome

Examining the resistance of active tardigrades to cyanide poisoning

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Background: Potassium cyanide is a potent toxin, affecting respiratory chain complex IV. Tardigrades are noted to exhibit a resistance to its effects. Despite this, very little studies has been done on this phenomenon. The extent of this ability has not been adequately tested, especially on active specimens. In present study we tested the influence of the cyanide on two tardigrade species from terrestrial and freshwater habitats.

Methods: In the experiments we used species *Hypsibius exemplaris* and *Paramacrobiotus experimentalis*. Groups of 20 specimens of the respective species were placed in glass staining blocks, into which KCN solution of concentration ranging from 0.5 to 5 mM was added. They remained submerged in the solution for the duration of the entire experiment. Following the initial exposure, tardigrades were observed for signs of activity under the stereomicroscope.

Results: Following exposure to KCN solution, specimens of both species ceased outwardly observable activity and appeared dead. However, within 300 minutes after the exposure, the majority of specimens returned to full activity. Their rate of recovery varied depending on KCN concentration, with specimens placed in lower concentration returning to active state significantly faster than those in higher concentrations. Moreover, *Paramacrobiotus experimentalis* appears to have higher resistance to KCN than *Hypsibius exemplaris*, requiring higher dose for all specimens tested to cease movement. Despite this discrepancy, the recovery rate from lowest number of active specimens to full recovery between those species remains comparable for all concentrations.

Conclusions: Obtained results shed light on poorly studied ability of tardigrades to resist cyanide poisoning. They are highly perplexing, as, unlike other invertebrates possessing this trait, tardigrades do not subsist on cyanide-rich food and are unlikely to encounter concentrations used in the present study in their natural habitats. The results presented act as the first benchmark of this ability and show that it is present amongst different Eutardigrada taxa, adapted to different habitats and food types. They also serve as a crucial steppingstone for future studies on the subject.

Keywords: anoxybiosis, Cyanide, *Hypsibius exemplaris*, *Paramacrobiotus experimentalis*, toxin resistance

Thursday | Talk Session #7 | 12:15-12:30

Are invertebrates capable of surviving Martian concentrations of perchlorate?

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Background: Experiments confirmed that some Earth microorganisms could potentially survive under Martian physical and chemical conditions. However, highly reactive and toxic chemical perchlorates (ClO_4^-), globally distributed in Martian regolith at the mean level of 0.6 wt %, are considered to be a large challenge for the survival of terrestrial life forms. Therefore, in this experimental study, the survivability of Crustacea, Nematoda, Rotifera, and Tardigrada under high perchlorate concentrations was tested. Understanding whether small invertebrates can cope with perchlorate-induced stress is pivotal in planning the potential use of these organisms in Mars exploration.

Methods: The following invertebrate species were used: *Artemia salina*, *Caenorhabditis elegans*, *Lecane inermis*, *Hypsibius exemplaris*, *Milnesium inceptum* and *Paramacrobiotus experimentalis*. All species were exposed on three solutions (0.25%, 0.5% and 1%) of magnesium perchlorate ($Mg(ClO_4)_2$) by 24, 48 and/or 72h. After exposition for specific solution number of active specimens were counted. Moreover, inactive tardigrades and nematodes were later put into clean water to check if they are able to return to normal activity. Survivability, mortality, and time necessary to return to activity after perchlorate exposure of different species were statistically compared.

Results: All studied species were able to survive in 0.25% solution of perchlorate although we observed some differences *e.g.* 100% of tardigrades were active even for few hours, but only ca. 50-60% of nematodes and crustacean. Moreover, even when tardigrades, rotifers or nematodes were inactive after exposure on 0.5 and 1% of perchlorates, were capable to return to normal activity when transferred to clean water.

Conclusions: Species from different taxonomic groups, inhabiting various habitats and having different diets can survive under perchlorate concentrations (from 0.25 to 1.0%) even higher than expected in the Martian regolith.

Keywords: Crustacea, Mars exploration, Nematoda, perchlorate, regolith, Rotifera, Tardigrada

Friday | Talk Session #8 | 09:30-09:45

Physiological roles of tardigrade-unique heat-soluble proteins

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Background: Several tardigrade-unique heat soluble proteins have been identified as prominent examples of tardigrade protective factors for enzymes, DNA and cellular integrity against several stresses including dehydration, irradiation and oxidative stress. Originally, heat soluble property was linked with the hydrophilic nature of the proteins and the resulting unstructuredness are believed to play important roles in a protective action. However, some unique heat soluble proteins are largely and highly structured, and thus should have a distinct mode of action for protection. For example, the extracellular SAHS proteins form a rigid beta-barrel structure resembling those of fatty-acid binding proteins (FABPs), suggesting that SAHS proteins could also bind fatty-acid or related molecules to function. However, nothing was known about the ligands and the physiological roles of SAHS proteins.

Methods: The binding between RvSAHS1 proteins and putative ligands were quantitatively measured using a fluorescent probe *in vitro*. RvSAHS1 protein complex was immunoprecipitated from the lysate of *Ramazzottius varieornatus*. The amount of heme in co-immunoprecipitant was quantified by measuring restoring activity of apoHRP. Suppressive activity of SAHS protein against oxidative stress generated by heme was measured by using fluorescent ROS indicator.

Results: We first revealed comprehensive profiles of lipids and fatty acids of the tardigrade *R. varieornatus* by lipidome analyses in both wet and dry conditions. Based on these data, we selected several candidate molecules as putative ligands of SAHS proteins. *In vitro* binding experiments revealed that among the examined molecules, RvSAHS1 exhibited the highest affinity to hemin. The binding between RvSAHS1 and heme was also confirmed *in vivo* by co-immunoprecipitation assay. We further demonstrated that RvSAHS1 protein successfully suppressed the ROS generation by heme.

Conclusions: Our data indicated that RvSAHS1 can bind heme *in vivo* as well as *in vitro* and play roles in suppression of ROS generation via sequestering heme. This mode of action is in a good agreement with the rigid FABP-like beta-barrel structure of SAHS proteins. Other heat-soluble proteins such as CAHS or Dsup are supposed to work in a largely unstructured state, but some recent evidence suggested certain structures could be important for their functions. The relationship among structuredness, sequence conservation and the mode of action in tardigrade-unique heat-soluble proteins will also be discussed.

Keywords: heat-soluble, oxidative stress, SAHS, structured

Friday | Talk Session #8 | 09:45-10:00

Mechanisms of cold tolerance in tardigrades (*Hypsibius exemplaris*)

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Background: Compared to other environmental stressors, little is known about tardigrade tolerance to cold and ice formation. Methodological limitations of prior survival-based assays prevent accurate scoring of large numbers of tardigrades required to interrogate key environmental conditions with statistical significance. Underlying molecular mechanisms, such as ice-binding proteins or proteome-level responses to cold, are similarly uncharacterised.

Methods: Firstly, we develop new methods to investigate the interaction of exposure time, low temperature, and ice formation on the survival of hydrated, adult *Hypsibius exemplaris*. Visualisation of SYTOX Green uptake in cold-exposed tardigrades allows us to quickly and accurately quantify the survival of thousands of animals under a range of ecologically-relevant low temperatures (-10 ˚C, -15 ˚C, -20 ˚C), exposure times (2–120 hours), and induced ice formation. Secondly, we implement a comparative proteomics pipeline to characterise how protein abundances in *H. exemplaris* shift in response to non-lethal cold (-10 ˚C for 24 hours) and perform ice-affinity purification to identify novel candidate ice-binding proteins.

Results: Visualisation of SYTOX Green uptake more accurately predicts survival outcomes of tardigrades post-cold exposure compared to locomotion. Using this improved scoring methodology, tardigrades exposed to -10 ˚C show high survival (absent ice formation). Tardigrades froze at -20 ˚C and began to accumulate cellular damage within 2h, experiencing increasing mortality with exposure time. Survival of tardigrades at -15 ˚C varied depending on exposure time and ice formation. Interestingly, we found that exposing tardigrades to ice-nucleating bacteria significantly improves survival, indicating selective tolerance to ice.

High-sensitivity DIA mass-spectrometry on cold-treated tardigrades identified 2776 proteins, with 89 differentially abundant (q-value < 0.1) compared to 20 ˚C controls. GO enrichment analysis of differentially abundant proteins suggests changes in neural function, calcium balance, cell division processes (such as apoptosis), glucose and lipid metabolism, and cuticle maintenance in response to cold. Ice-affinity purification and bioinformatics methods identified a ranked list of novel candidate ice-binding proteins.

Conclusions: This work offers a framework with new tools for performing large-scale physiological assays and comparative proteomics studies in tardigrades. Survival assays indicate sensitivity to freezing under select conditions, with significantly improved survival with environmentally-inoculated ice formation. Proteomics methods identify key biological processes and uncharacterised proteins which may underlie cold tolerance.

Keywords: cold tolerance, ice, ice-binding proteins, proteomics, SYTOX Green

Friday | Talk Session #8 | 10:00-10:15

Factors affecting survival of repeated anhydrobiosis in a dioecious tardigrade *Paramacrobiotus experimentalis* Kaczmarek *et al.*, 2020

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Background: Tardigrada are considered as one of the toughest animals on Earth due to their high ability to undergo anhydrobiosis. However, tardigrades' survival of repeated anhydrobiosis has rarely been studied. The same applies to research on sex and age effects anhydrobiosis survival as well as differences in anhydrobiosis survival between single and grouped specimens that we termed "social effect". To study the mentioned factors, we used a dioecious Madagascan eutardigrade *Paramacrobiotus experimentalis* (the average lifespan of ~360 days) as a model species.

Methods: Specimens of the *Pam. experimentalis* were extracted from stock laboratory cultures, performed at 20°C and 40% RH, and maintained in mixture of ddH_2O and spring water (3:1) with rotifers as a food. Females and males of different age groups were extracted and further cultured in Petri-dishes alone or in small groups. Specimens were then subjected to repeated episodes of short (3 days) and long (30 days) term anhydrobiosis. The differences in the determined survival rate were statistically tested using two-way ANOVA (GraphPad Prism).

Results: Younger specimens displayed higher survivability of repeated short and long term anhydrobiosis. Females appeared more capable to survive the anhydrobiosis episodes than males. Additionally, animals cultured in groups showed higher survivability than animals cultured individually. Furthermore, our results indicated that repeated anhydrobiosis episodes declined survival rate, with increasing number of dehydration events.

Conclusions: Age, sex and "social effect" as well as number of anhydrobiosis episodes affect *Pam. experimentalis*, anhydrobiosis. Accordingly, our study indicates that tardigrades living alone are more susceptible to death due to anhydrobiosis. Moreover, anhydrobiosis is more deadly for males as well as older specimens of both sexes.

Keywords: age, anhydrobiosis, sex differences, social effect, survivability, Tardigrada

The study was supported by the Polish National Science Centre (NCN Opus 2016/21/B/NZ4/00131 and Preludium 2021/41/N/NZ3/01165).

Friday | Talk Session #8 | 10:15-10:30

Soft electrophiles inducing dormancy in limno terrestrial heterotardigrades

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Background: Tardigrades tolerate various extreme conditions. They have different survival strategies, such as cryptobiosis which help them stay dormant until the situation is favourable again. Cryptobiotic tardigrades shrink their bodies and form tun-state during stress. Many studies have been performed on cryptobiotic tardigrades, especially during desiccation. However, the mechanism that leads to cryptobiosis is still not fully discovered. Recent studies on different species of tardigrades revealed that tardigrade intrinsically disordered proteins (TDPs) are upregulating during dehydration, helping them survive desiccation. According to our observations, some organic compounds on tardigrade bryophyte habitats (mosses and lichens) induce tun in tardigrades.

Methods: Moss/lichen samples were processed using Dionex ASE 150 machine. The solvent/extraction mixture evaporated, and the remaining residue was used to test the different concentrations of sample extracts on tardigrades isolated from the same samples. These tardigrades mainly were belonging to *Echiniscus* and *Viridiscus* genera. The HPLC machine confirmed the presence of plant metabolites. For further investigation, different concentrations of various organic compounds were also tested on more individuals of *Viridiscus viridianus* tardigrades, and their reactions were checked every 12 hours for four days. The proteomic study should perform for active tardigrades *versus* tardigrades in dormancy after applying the chemicals for TDPs comparison.

Results: The preliminary results show that *V. viridianus* tardigrades only react to the compounds known to be soft electrophile and can react with some proteins. More percentage of tardigrades react to higher concentrations, and the number of individuals in dormancy decrease with decreasing the compounds' concentration. Another critical factor is the time, especially for lower concentrations of the chemicals. It might take tardigrades a few days to react to lower concentrations, while they respond to high concentrations in less than thirty minutes.

Conclusions: The preliminary results support our initial hypothesis. According to these results, soft electrophile compounds affect some species of limno terrestrial tardigrades. Therefore, they can be an essential factor in upregulating TDPs and help tardigrades survive harsh conditions as they are found in their bryophyte habitats. Further investigation on TDPs and the effect of these molecules on tardigrades can explain the importance of TDPs in tardigrade dormancy and survival.

Keywords: heterotardigrades, intrinsically disordered proteins, organic compounds, soft electrophiles, tardigrade dormancy

Friday | Talk Session #9 | 11:00-11:15

DNA damage response of *Hypsibius exemplaris* to genotoxic stress

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Background: Many tardigrades species have the capacity to withstand remarkably high doses of UV or ionising radiation (IR). DNA protection by the *R. varieornatus* Dsup protein was first identified as a novel, powerful mechanism conferring resistance to DNA damage in cultured cells. We chose to investigate the potential contribution of DNA repair as an additional mechanism of resistance to DNA damage.

Methods: As a model study, we focused on *H. exemplaris* subjected to Cs^{137} γ-irradiation or bleomycin, a radiomimetic drug. We investigated the physical integrity of DNA by agarose gel electrophoresis and indirectly assessed levels of DNA double strand breaks by western blot using a phospho-H2AX specific antibody. Using RNA-seq and differential proteomics, we also examined gene expression in response to each genotoxic stress.

Results: We detected DNA damage and DNA double strand breaks which reached their highest rates from 30 min to 4 h after IR. DNA protection mechanisms therefore appeared insufficient to maintain genome integrity and DNA repair mechanisms may also play an important role in tardigrade resistance to IR. Differential expression at the RNA level showed a common pattern of highly induced genes. In particular, we observed a massive induction of mRNA levels of DNA double-strand break repair genes, including from classical Non-Homologous End Joining (such as XRCC5 (Ku80), XRCC6 (Ku70), Lig4), and Homologous Recombination (such as Rad51, BRCA-like-1 and PCNA). DNA polymerases such as Polθ involved in Microhomology-Mediated End Joining, or the trans-lesion DNA polymerase polζ were also overexpressed. Differential proteomic analysis confirmed the induction of a majority of DNA repair genes at the protein level, albeit fold inductions detected were much smaller.

Conclusions: We showed that the increased expression of DNA repair genes appears to help *H. exemplaris* cope with high levels of DNA damage induced by IR. We are now aiming to compare the DNA damage responses of *H. exemplaris* to other tardigrades species in order to identify common features and/or differences and to better understand their resistance to genotoxic stresses.

Keywords: DNA double-strand break repair, IR

Friday | Talk Session #9 | 11:15-11:30

Tardigrade phenotype classification using neural networks

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Background: Tardigrades show remarkable resilience to diverse forms of stress, including desiccation, gamma irradiation, and heat shock. Construction of dose-response curves requires laborious counting of the animals in many samples under the microscope. In order to increase the throughput of such experiments, we developed a method of counting living and dead individuals of the species *Hypsibius exemplaris* using automated microscopy and image analysis.

Methods: *Hypsibius exemplaris* is exposed to stress in 384-well microtitration plates, and the individual wells are imaged using an automated microscopy system CV7000S (Yokogawa) at low magnification. Classification of live/dead phenotypes is performed by a YOLOv5 type convolutional neural network using the Pytorch platform. Several image augmentation methods were tested, including software approaches and taking the images of the same view field under different illumination and/or with a z-stack. To facilitate the analysis, the animals can be optionally stained with SYTOX green stain.

Results: Neural network approach is superior to standard deterministic thresholding methods, especially in complex view fields with clusters of algae food. Sensitivity and specificity of over 90 percent can be attained with a limited number of examples. Image augmentation further improves performance.

Conclusions: Automated microscopy in conjunction with image analysis using convolutional neural networks greatly facilitates analysis of stress response in *H. exemplaris*. We are currently using the method to study the cross-resistance of *H. exemplaris* to various stress modalities.

Keywords: automated microscopy, deep learning, *Hypsibius exemplaris*, neural network

Friday | Talk Session #9 | 11:30-11:45

Tardigrade community microbiomes in North American orchards (Iowa, USA) include putative plant pathogens and endosymbionts

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Background: The tardigrade microbiome is poorly studied worldwide and is completely unknown both in agricultural settings and in North America. At least one tardigrade species (*Macrobiotus hufelandi* C.A.S. Schultze, 1834) can act as a vector of the plant pathogenic bacteria *Xanthomonas campestris* in the laboratory, demonstrating the importance of understanding tardigrade-associated microbiota in an agricultural context. In addition, recent surveys have identified putative endosymbionts of the order *Rickettsiales* associated with tardigrades on other continents.

Methods: We used 16S rRNA gene amplicon sequencing to analyse the microbiota of tardigrades from six apple orchards in Iowa, USA. We characterised the tardigrade community microbiome across four contrasts: location, substrate type (moss or lichen), collection year, and tardigrades *versus* their substrate.

Results: Alpha diversity of the tardigrade community microbiome differed significantly by location and year of collection but not by substrate type. Our work also demonstrated that tardigrades harbour a distinct microbiota from their environment, corroborating earlier findings. In addition, we identified tardigrade-associated taxa that belong to genera known to contain phytopathogens (*Pseudomonas*, *Ralstonia*, and the *Pantoea/Erwinia* complex). Finally, we observed members of the *Rickettsiales* genera *Rickettsia* and *Wolbachia* in the tardigrade microbiome; because these are obligate intracellular genera, we consider these taxa to be putative endosymbionts of tardigrades.

Conclusions: This study is the first microbiome analysis of wild tardigrade populations in an agricultural setting and is also the first microbiome study assessing North American tardigrades. The association of multiple putative phytopathogens with tardigrades in apple orchards suggests that tardigrades could act as reservoirs or vectors of these pathogens. Additionally, the presence in the tardigrade microbiome of *Rickettsia* and *Wolbachia*, genera known to manipulate reproduction in other ecdysozoans, may have implications for tardigrade reproduction.

Keywords: endosymbiont, microbiota, phytopathogen

Friday | Talk Session #9 | 11:45-12:00

Using fluorescence *in situ* hybridisation to visualise *Rickettsia* in tardigrades

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Background: Members of the bacterial genus *Rickettsia* are obligate endosymbionts of a broad range of invertebrates. While some *Rickettsia* species provide a benefit to their host, others manipulate their hosts' reproduction causing cytoplasmic incompatibility, male-killing, and parthenogenesis. Interestingly, parthenogenesis is a prevalent method of reproduction in the phylum Tardigrada. Using 16S rRNA gene amplicon sequencing, our group recently identified *Rickettsia* in the microbiome of tardigrades isolated from apple orchards. If *Rickettsia* are present in tardigrades, then it is possible that parthenogenesis in certain tardigrade species is due to reproductive manipulation by *Rickettsia*. However, additional experiments are necessary to confirm that *Rickettsia* is present within the tissues of tardigrades.

Methods: Lichen samples used in our previous microbiome analysis were rehydrated in water overnight. Tardigrades were subsequently isolated using a dissection microscope and washed in sterile water. Washed tardigrades were then placed on a slide and fixed for 2 hours in 3% paraformaldehyde before being dehydrated using an ethanol wash series. Fluorescence *in situ* hybridisation (FISH) was then conducted using the probes RickB1 (targeting the genus *Rickettsia*) and Eub338 (targeting all bacteria). Hybridisation was conducted in hybridisation buffer with a formamide concentration of 35% for 1.5 hours at 46 °C. After washing with wash buffer, tardigrades were mounted in an antifadent solution and imaged using a Leica fluorescence microscope at 40X and 100X magnification.

Results: In preliminary experiments, *Paramacrobiotus tonollii* (Ramazzotti, 1956) were isolated and visualised. FISH allowed the visualisation of numerous small ~1 µm cocci with fluorescent signals from both RickB1 and EUB338. The cocci were present throughout the tissue of the tardigrades, primarily towards the posterior of the animal between legs III and IV. Based on the binding of both the *Rickettsia*-specific probe as well as the general bacterial probe, these cocci are most likely *Rickettsia*.

Conclusions: Our preliminary results indicate that *Rickettsia* are present in *P. tonollii*. However, *P. tonollii* is not known to be parthenogenetic, meaning that the *Rickettsia* observed in our samples are unlikely to induce parthenogenesis. We are in the process of fine-tuning the FISH protocol and collecting and visualising additional samples. Future work will involve the use of confocal microscopy to achieve higher resolution images.

Keywords: FISH, *Rickettsia*, Tardigrades

Friday | Talk Session #9 | 12:00-12:15

Identifying the core microbiome of tardigrades: what is really there?

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Background: Bacterial symbioses have been hypothesised as an essential part of tardigrade biology. Although earlier reports of an unusually high degree of horizontal transfer of bacterial genes into tardigrade genomes were shown to be wrong, associations with microbes are evident in at least some marine arthrotardigrades. Recent studies on tardigrade microbiomes provided different sets of bacterial taxa in tardigrade samples and focused mainly on detecting intracellular endosymbionts. Here, we confront the previous findings by identifying the core tardigrade microbiome in a large, replicated experiment under controlled conditions.

Methods: We characterise the microbiota associated with 44 cultured strains of tardigrades using 16S rRNA amplicon sequencing. We applied different sample preparation strategies, multiple types of negative controls, and spike-in quantifications. We carefully decontaminated the obtained dataset and tracked the sources of bacteria found in tardigrades. Finally, we re-analysed published data, assessing the extent of contamination and identifying shared patterns.

Results: The microbial community profiles of cultured tardigrades were dominated by bacterial strains originating from food, medium, and laboratory reagents. Only a few microbial strains were consistently enriched in some of the analysed tardigrade species compared to culture media and controls, indicating possible symbiotic associations. We also identified methodological problems in previous studies, making some of their conclusions questionable.

Conclusions: Our results suggest that tardigrades are not universally dependent on specialised bacteria. However, specific associations are present but require a careful approach to identify them correctly. We caution against possible misinterpretations and propose future directions and recommendations for studies on the tardigrade microbiome.

Keywords: contamination, cultures, microbiome, reanalysis

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417 and Opus 2018/31/B/NZ8/01158).

POSTER ABSTRACTS

MONDAY-TUESDAY POSTER SESSION

Physiology, Morphology & Ecology

Physiology, Omics, Cryptobiosis & Astrobiology

Morphology, Anatomy, Reproduction & Development

Ecology, Life Histories & Behaviour

Biomedical applications of Tardigrada: prospects and perspectives

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Background: Active and cryptobiotic tardigrades reveal various adaptations to survive extreme environmental conditions. This, in turn, makes them and their chemical constituents promising candidates for different biomedical applications.

Methods: Literature search of PubMed database using a combination of keyword "tardigrade" with "health", "medicine" "Dsup", "pharmacy", "disease", "cancer". All retrieved articles were further inspected to select only the original research and review articles, which were relevant to the biomedical subject.

Results: Overall, l1 articles were selected, among which eight were original research papers, while three presented a literature review on the potential biomedical use of tardigrades. Most attention has been paid to the protective role of tardigrade proteins in human cells. As shown, mitochondria-targeted heat-soluble proteins RvLEAM and MAHS from *Ramazzottius varieornatus* improved the hyperosmotic tolerance of human cells. The HEK293T cells expressing DNA-associated damage suppressor protein (Dsup) revealed higher tolerance to X-ray. It has also been shown that Dsup has the potential to counteract oxidative stress, which is an important contributor to human diseases. Studies focused on tardigrade-derived cytosolic abundant heat soluble protein (CAHS) demonstrated their protective role for cellular components during desiccation. CAHS can also successfully mitigate the deactivation of various medically-relevant enzymes. This, in turn, has the potential to improve the stability of different pharmaceuticals, including protein-based biological drugs. No toxic or pro-inflammatory responses were observed in vivo following CAHS administration highlighting its safety for use as a stabiliser of different therapeutics. On the other hand, CAHS has antigenic properties, leading to the production of anti-CAHS antibodies. Finally, trehalose, a non-reducing disaccharide produced by some tardigrades, and trehalose-based polymers, remain the focus of cryopreservation of animal and human cells.

Conclusions: Literature review indicates that the field on biomedical use of tardigrade-derived chemical components is still almost unknown. Particular attention is paid to the protective role of Dsup as it may play a role in mitigating oxidative stress that underlies the aetiology of human diseases (*e.g.*, cardiovascular, neurological, cancer) as well as CAHS protein which may be useful for the pharmacological industry to preserve protein-based biological therapeutics.

Keywords: biostabilisation, cancer, desiccated enzymes, Dsup protein, radiotolerance

Tardigrades and their future potential for biomimetics and biotechnology

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Background: With the discovery of extremophilic microorganisms in the late 1960s, the biotechnological potential of these organisms was quickly recognised. Similarly, the biotechnological potential of tardigrades was suggested over 50 years ago, but the realisation of these visions has so far been slow. However, during the last decades, tardigrade research has provided several novel insights in fundamental biological science with regard to different forms of cryptobiosis, thereby providing new means to reconsider the potential of tardigrades for biomimetics and biotechnology.

Methods: We have surveyed over 260 articles related to fundamental insights into tardigrade biology and the biotechnological potential of tardigrades for five different fields, from agriculture, astrobiology, environmental science, food science to medicine.

Results: The potential for novel applications in biomimetics and biotechnology can be divided into two directions: (i) identification of suitable preservative biomolecules and processes that can then be added to samples that need to be protected, or (ii) identification of suitable genes that can be engineered into another host and expressed. The molecular processes associated with different forms of cryptobiosis are based on different types of preservation molecules, however, many of these have not yet been fully explored. Similarly, our understanding of the evolution, biodiversity, biogeography as well as molecular biology of tardigrades is far from complete. Thus, the true potential of tardigrades for different types of applied applications is most likely still underestimated.

Conclusions: The ability of tardigrades to maintain the integrity of cells and tissues under extreme environmental conditions makes them interesting for a number of applied fields in biotechnology. However, more research in fundamental science of tardigrades is needed in order to enable realistic applications in biomimetics and biotechnology.

Keywords: biomimetics, biotechnology, tardigrades

Monday-Tuesday stand **2** | side **A**

Building a framework for tardigrade transgenics: identification and evaluation of candidate genetic promoters

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Background: Tardigrades are an exciting emerging model system, given their unique ability to provide insight into foundational questions in physiology, neuroscience, and beyond. Tardigrades' microscopic size, transparent bodies, and ability to locomote in coordinated gaits make them interesting candidates for neuroscientific study using cutting-edge live-animal microscopy techniques. Genetic tools and transgenic methods, however, are lacking. Identifying and evaluating various tardigrade-compatible genetic promoters, with a diversity of expression patterns, is a first step to establishing transgenic tardigrades.

Methods: Leveraging existing promoters in sister taxa (*C. elegans, D. melanogaster*, and other invertebrates) and the *Hypsibius exemplaris* genome, we establish a list of candidate promoters for use in tardigrades. We propose promoters for three purposes: those with widely-expressed constitutive expression for easy validation, those that affect subtle but easily identifiable phenotypes, such as eye colouring for future positive controls, and finally neuronal and muscle-expressing promoters, for use in neuroimaging assays. In addition to promoters identified via literature search, we identify *H. exemplaris* homologs of functionally important genes in each of these three categories using BLASTp (E-value < 0.05). We subsequently generate an additional list of tardigrade-specific candidate promoters by taking 100–1000bp upstream of these homologs' start codons. Lastly, we propose methods to functionally verify expression patterns of each candidate promoter by expressing plasmids containing GFP, which are transfected into wild-type *H. exemplaris* and *C. elegans*, via a range of cargo delivery methods (injection in adults, embryos, feeding, and/or electroporation).

Results: Our curated list of candidate promoters for tardigrade transgenics provides dozens of sequences for future experimental validation and applications. Our experimental methodology to validate promoters provides a framework for exploration of numerous other genetic tools, needed to establish tardigrades as a tractable model system for molecular biological and neuroscientific studies.

Conclusions: Establishing functional promoter sequences and molecular cargo delivery methods for tardigrades is a first step into their untapped potential for an assortment of future genetic and behavioural studies. We propose methodological resources to the tardigrade community and other scientists interested in adopting tardigrades as prospective model organisms. Methods established here will enable the generation of transgenic animals (via CRISPR-Cas9, transposase-based, or other methods), enabling future exploration of tardigrades' neurological function and numerous other applications.

Keywords: genetic tools, phenotypic-screen, promoters, transgenics

Protein modelling and *in silico* characterisation of two putative TIR-binding proteins in the limnoterrestrial tardigrade *Mesobiotus philippinicus*

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Background: The tardigrade response to biotic stresses has not yet been well-elucidated. One of the potential candidates for a tardigrade immune pathway is the canonical Toll signalling pathway, with recent studies having uncovered two out of three Toll receptor domains in the limnoterrestrial tardigrade *Mesobiotus philippinicus*. Its intracellular binding partners, however, have yet to be identified. This study thus aimed to model and characterise two putative *M. philippinicus* TIR-binding proteins to further investigate the Toll signalling pathway as a potential tardigrade immune pathway.

Methods: Previous work identified five proteins from the *M. philippinicus* transcriptome that contain TIR-binding motifs that could facilitate their interaction with the tardigrade Toll-like receptor. Two were characterised *in silico* in this study. I-TASSER protein modelling predicted the structure of these putative proteins. Subsequent characterisation was done through pairwise sequence alignment with the putative homologs of the targets via EMBOSS Needle, Gene Ontology analysis via I-TASSER, and domain prediction through SMART and InterPro. Finally, docking to the Toll TIR domain was tested *in silico* using ClusPro 2.0.

Results: The first putative protein was found to be similar to the tardigrade C2 domain protein (TC2P) in sequence and structure and was hence called the TC2P-like protein. Characterisation suggests that it may bind to the Toll TIR domain and tether it to the cell membrane in place of the canonical Toll receptor transmembrane domain. The second putative protein was found to be structurally similar to pre-mRNA splicing factor SYF-1 and was thus called the SYF1-like protein. Subsequent characterisation suggests its role in the assembly of protein complexes involved in RNA processing. *In silico* docking of both putative proteins to the Toll TIR domain suggests their possibility of interaction.

Conclusions: The results suggest possible roles of the putative TC2P-like and SYF1-like *M. philippinicus* proteins in the tardigrade Toll-like receptor pathway. The TC2P-like protein possibly tethers the intracellular Toll TIR domain to the cell membrane, while the SYF1-like protein may be involved in the assembly of RNA processing machinery that may target nucleic acids of pathogens or that result from other immune cell signalling pathways. These results provide further insight into the different mechanisms that may be involved in the tardigrade immune system.

Keywords: *Mesobiotus philippinicus*, tardigrade immune response, TIR domain, Toll signalling

Monday-Tuesday stand 3 | side A

Characterisation of tardigrade tubulins

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Background: Tardigrades are microscopic ecdysozoans that can withstand extreme environmental challenges. Several tardigrade species undergo reversible morphological transformations and enter an ametabolic state called cryptobiosis that helps them to survive periods of unfavourable conditions. However, underlying molecular mechanisms are mostly unknown. Tubulins are evolutionary conservative components of microtubule cytoskeleton that plays critical roles in many cellular processes. We hypothesise that microtubules are necessary for morphological changes associated with entering and exiting cryptobiosis. Molecular composition of microtubule cytoskeleton in tardigrades is unknown. Therefore, we decided to analyse and characterise tardigrade tubulin genes.

Methods: We prepared a local BLAST+ database from available genomes and transcriptomes of eight tardigrade species. After removing redundant sequences, we performed sequence and phylogenetic analysis of found putative tardigrade tubulins that allowed us to assign them to individual isotypes and isoforms. To clarify whether *in silico* identified tubulin proteins are present in tardigrades, we isolated tubulin coding sequences from adults of *Hypsibius exemplaris*. We amplified and sequenced predicted coding sequences and tagged them with fluorescent proteins for visualisation in mammalian cells.

Results: We identified 80 unique tardigrade tubulin sequences including three ε-tubulins. We found three α-tubulin and seven β-tubulin isoforms, one γ-tubulin and one ε-tubulin isoform in tardigrades. We were able to amplify and sequence 9 out of 10 predicted tubulin coding sequences from *H. exemplaris*, including the ε-tubulin. Sequence and splice site analyses revealed that γ-tubulin was incorrectly annotated and we isolated a different coding sequence, a product of which localised properly to the centrosomes in human U87 MG cell line.

Conclusions: We developed a simple-to-use pipeline for tardigrade gene analysis based on a local BLAST+ database that can be expanded for newly sequenced omics data. Although tardigrade tubulins are highly conserved, they possess several unique sequence signatures. The phylogenetic position of tardigrades within the Ecdysozoa is still controversial. The presence of tardigrade ε-tubulins is interesting, since Nematoda lost their δ- and ε-tubulin, however, some groups of Arthropoda still possess them. Thus, our current data suggest that the placement of tardigrades within Panarthropoda is correct.

Keywords: cytoskeleton, tardigrades, tubulin, isotypes

Monday-Tuesday stand 3 | side B

Stress-dependent cell stiffening by tardigrade tolerance proteins CAHS through reversible formation of cytoskeleton-like filamentous network and gel-transition

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Background: Tardigrades are able to tolerate almost complete dehydration by entering a reversible ametabolic state called anhydrobiosis and resume their animation upon rehydration. Dehydrated tardigrades are exceptionally stable and withstand various physical extremes. Although tardigrades produce high amounts of tardigrade-unique protective proteins, CAHS, which are essential for their anhydrobiotic survival, the precise mechanisms of their action in this protective role are not fully understood.

Methods: Stress-dependent intracellular distribution changes were examined using human cultured cells engineered to express CAHS-GFP fusion proteins by exposure to hyperosmotic stress. We also performed protein mutational analyses to reveal the structural basis for CAHS filamentation in cells and gelation *in vitro*. To demonstrate the tolerance mechanisms of CAHS protein, the effects of CAHS filament formation on mechanical resistance and cell viability was measured.

Results: We revealed that CAHS proteins reversibly polymerise into many cytoskeleton-like filaments depending on hyperosmotic stress in animal cells and undergo reversible gel-transition *in vitro*. CAHS filamentation increases cell stiffness to resist deformation and improves resistance to dehydration-like stress. The conserved putative helical C-terminal region is necessary and sufficient for filament formation by CAHS proteins, and mutations disrupting the secondary structure of this region impaired both the filament formation and the gel transition.

Conclusions: On the basis of these results, we propose that CAHS proteins are novel cytoskeletal proteins that form filamentous networks and undergo gel-transition in a stress-dependent manner to provide on-demand physical stabilisation of cell integrity against deformative forces during dehydration and also contribute to the exceptional physical stability in a dehydrated state.

Keywords: CAHS, cytoskeleton, desiccation tolerance, gel transition

Monday-Tuesday stand 4 | side A

Could mitochondrial DNA copy number be a marker of successful anhydrobiosis?

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Background: Anhydrobiosis is one of the most prevalent adaptations to water deficiency. The phenomenon occurs in different organisms but among animals the best known example are tardigrades, indicated lately as an emerging source of knowledge of importance for medical sciences. Tardigrade anhydrobiosis includes entry, dormant and exit stages, that correspond to the dehydration (tun formation), tun and rehydration stages, respectively. On the organismal level, the tun formation and subsequent revival have been well understood but the role of mitochondria remains unexplored, although the limited data indicate the important role of these organelles. Firstly, uncoupling the mitochondrial respiratory chain from ATP synthesis eliminates the capability of tun formation and subsequent return to activity. Secondly, mitochondrial alternative oxidase increases tun revival, although does not affect the rehydration stage itself. Thirdly, mitochondrial integrity is proposed as crucial for tun survival. Finally, available differential transcriptomics data for active animals and the tun stage indicate at transcripts of protein encoding genes located both in mitochondrial and nuclear genome, the latter coding for proteins located in mitochondria. Thus, mitochondria appear to be an underestimated field in studies on cellular/molecular mechanisms of tardigrade successful anhydrobiosis. Therefore we decided to study mitochondrial DNA (mtDNA) copy number known as a strong biomarker for mitochondrial functionality.

Methods: Different PCR techniques were used for quantitative detection of transcripts of selected hallmark genes. Finally, qPCR was chosen as well as AOX and COXII encoding genes. The analysis were performed for *Hypsibius exemplaris* and *Paramacrobiotus fairbanksi*. Active animals, tuns of different duration as well as animals unable to recover from the tun stage (dead specimens) were applied.

Results: The applied approach enables addressing of mitochondria functioning during the tun stage. The calculated numbers of mtDNA copy number appear to be indicative for the possibility of revival from the tun stage.

Conclusions: The obtained results contribute to better understanding of mitochondria contribution to tardigrade successful anhydrobiosis and indication of the phenomenon biomarker(s).

Keywords: anhydrobiosis, biomarker, mitochondria, mtDNA copy number

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Tardigrade proteins – molecular tools in anhydrobiosis phenomenon

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Background: Tardigrades display the ability to undergo cryptobiosis at any stage of their life, which allows them to survive under extreme environmental conditions. Anhydrobiosis, characterised by survival of almost complete loss of body water, is the most prevalent form of cryptobiosis. Tardigrades have developed multiple molecular mechanisms enabling protection of cellular molecules and structures against dehydration. One of the mechanisms is the production of trehalose, however, it is not applied by all species able to undergo anhydrobiosis. Other mechanisms are based on proteins involved in molecular protection. Numerous of such proteins have been discovered, like intrinsically disordered proteins (MAHS, CAHS, SAHS, LEA proteins), chaperones (Hsp40, Hsp60, Hsp70, small Hsp), and damage suppressors (Dsup protein). However, none of them work independently, so to secure the molecular background of anhydrobiosis they are involved in multiple direct and indirect protein-protein interactions. Thus, the proteomic analysis seems to be an efficient method to unravel cellular mechanisms of anhydrobiosis.

Methods: Databases, such as UniProt and GenBank as well as original articles published in the range of 2000-2022 and transcriptomic data, were searched and analysed in order to present the most recent knowledge of tardigrades' anhydrobiosis proteome.

Results: The description of available data on tardigrades' anhydrobiosis proteome was prepared. Almost 300,000 records of proteins or protein encoding genes were found that functionally can be divided into two major groups, *i.e.*, cellular metabolism and cytoprotection. The majority of records assigned to the first group concern proteins participating in mitochondrial metabolism. The second group contains proteins included in DNA protection and repair, encapsulation and protection of functional proteins, prevention of aggregation of denatured proteins, organelles protection, extracellular components' protection, membrane stabilisation, modulation of hyperosmotic tolerance, and rehydration.

Conclusions: The knowledge about tardigrades' anhydrobiosis proteome is expanding very quickly. The discovery of new, including tardigrade-specific, proteins will allow for more precise explanation of the molecular mechanisms underlying successful anhydrobiosis.

Keywords: anhydrobiosis, proteins, proteome, tardigrades

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Metabolic markers of different stages of tardigrade anhydrobiosis

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Background: Anhydrobiosis is known as a desiccation tolerance that denotes the ability to survive almost complete dehydration without sustaining damages. It has been reported for some organisms, the best known of which are rotifers, nematodes and tardigrades. Comprehensive research based on metabolites' comparative analysis for different stages of anhydrobiosis has not been carried out yet. The same applies to the knowledge on the survival ability of anhydrobiosis in various species of tardigrades that is still very poorly understood.

Methods: Two species were selected as model organisms for our research: *Paramacrobiotus experimentalis* Kaczmarek, Mioduchowska, Poprawa & Roszkowska, 2020 and *Paramacrobiotus fairbanksi* Schill, Förster, Dandekar & Wolf, 2010. Experiments focused on anhydrobiosis survival rate were performed in covered plastic Petri dishes lined with a filter paper. After the drying process (72 hours), specimens were rehydrated after different duration of the tun stage (3, 30 and 45 days). Differences in a number of active and motionless animals as well as the moment of the first movement and recovery to full activity were recorded. To compare metabolic profiles of active and anhydrobiotic tardigrades we applied an untargeted metabolomic profiling based on gas chromatography-mass spectrometry (GC-MS).

Results: Experiments on anhydrobiosis survival rate showed that *P. experimentalis* displayed much better survivability although both species revealed high decrease in survival after 45 days of the tun stage when compared to 30 days. The GC-MS metabolomic approach allowed for detection of metabolites regarded as markers of known processes, *e.g.* Krebs cycle, gluconeogenesis or proteolysis and for determination of metabolic differences between different anhydrobiosis stages and the studied species. The detected unscrambled metabolites represented mainly amino acids, monosaccharides, carboxylic acids, membrane lipids and some products of Krebs cycle.

Conclusions: The chosen methodology leads to detection of various metabolites in active and anhydrobiotic specimens representing two tardigrade species belonging to the same genus and differing in anhydrobiosis capability. It gives us a first look at metabolic markers assigned to specific stages of anhydrobiosis in tardigrades.

Keywords: anhydrobiosis, metabolic markers, mitochondria, *Paramacrobiotus experimentalis*, *Paramacrobiotus fairbanksi*, survivability

The study was supported by the Polish National Science Centre (NCN Opus 2016/21/B/NZ4/00131).

Geomagnetobiology and tardigrades – current state of knowledge

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Background: Tardigrades show significant resistance to a number of environmental stressors, including exposure to the space vacuum. But even though, studies on the influence of hypomagnetic conditions or even geomagnetic field in general, on tardigrades were rare, until recently. We present the current state of knowledge on the influence of hypomagnetic conditions on the anhydrobiotic abilities of tardigrades based on our previous studies.

Methods: To study the effect of hypomagnetic conditions on the anhydrobiotic abilities of tardigrades three types of experiments were conducted on three tardigrade species: *Echiniscus testudo*, *Milnesium inceptum* and *Hypsibius exemplaris*. In first we tested the effects of the hypomagnetic conditions on anhydrobiotic tardigrades. In second, the influence of hypomagnetic conditions on the process of entering anhydrobiosis. In third, the influence of the hypomagnetic conditions on anhydrobiotic tardigrades returning to the active state. All experiments were conducted in controlled environmental conditions in CIMF (Chamber Isolated from Magnetic Field), constructed from a 1 mm thick µ-metal alloy. In every experiment, the mean mortality (expressed as a percentage of dead individuals) in both for test and control groups, of tardigrades of each species were calculated and compared.

Results: Hypomagnetic conditions significantly increased the mortality rate of *Ech. testudo* and *Mil. inceptum* in the cryptobiotic state, but not *Hys. exemplaris*, for which differences between test and control groups were insignificant. The return to the active state (experiment III) seems to be the most critical process for tardigrades of all tested species. Mean mortality in test groups in experiment III reached around 98% for *Hys. exemplaris*, 59% for *Ech. testudo* and 61% for *Mil. inceptum*.

Conclusions: Despite the unequivocal results proving the negative influence of hypomagnetic conditions on the anhydrobiotic abilities of tardigrades, it should be also noted that this factor did not lead to the death of all individuals. This is obviously a consequence of the intrapopulation variability in survival and resistance of individuals to physical factors.

Keywords: astrobiology, dehydration, extremophiles, geomagnetic field, invertebrates

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Assessing anhydrobiotic performance – a new analytical method

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Background: Anhydrobiosis (the ability to survive environmentally induced loss of water) is the most widespread and studied form of cryptobiosis in tardigrades. Unfortunately, not only do laboratory protocols vary considerably between research groups and across study species, but there is also a generalised use of statistically problematic approaches. The latter stems from (i) using multiple, often post-hoc and biologically unnecessary, pairwise comparisons that are prone to Type I errors, and (ii) ignoring the non-independence of the data. Therefore, we propose a novel analytical method to assess anhydrobiosis ability based on time-point survival monitoring data.

Methods: Our approach involves the Bayesian fitting of a cumulative exponential distribution function on the anhydrobiosis recovery: proportion of motile individuals against time point. This curve estimates both the overall survival (where the curve flattens) and recovery speed (the rate of increase of the proportion of motile individuals with time). These two measures can also be combined into a single index as the area under the fitted recovery curve. Three time points of monitoring data (*e.g.,* at 1h, 4h and 24h) were found to be enough for obtaining an adequate credible interval around the mean estimate of these indices.

Results: Using new (a comparative study in Macrobiotoidea) and published (an experiment using molecular inhibition on a single species) datasets, we first demonstrate that our method can not only objectively estimate reliable anhydrobiosis indices, but also do so for any combination of anhydrobiosis protocol, study species and manipulative experimental setup. Secondly, we illustrate how these indices can be used to produce easy-to-interpret plots, such as scatterplots of recovery speed *vs* maximum survival. Thirdly, since the output is in the form of posterior distributions (*cf.* single point estimates), we describe how these indices can themselves be used as more appropriate response variables in statistical models where the aim is to test for differences in anhydrobiotic performance between (experimental) groups.

Conclusions: Our approach provides two distinct (plus their combination) indices of anhydrobiotic performance. These indices are objective, reliable, and have both biologically meaningful interpretation and wide applicability. For example, this curve fitting approach can be easily employed in other types of cryptobiosis recovery data.

Keywords: anhydrobiosis, Bayesian statistics, recovery speed, survival

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Development of a novel desiccation chamber for induction of tardigrade anhydrobiosis

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Background: Tardigrades are micrometazoans that can survive desiccation, frequently by entering an ametabolic state, anhydrobiosis. Different species have various desiccation tolerance. Some tardigrades, *e.g.* the laboratory model *Hypsibius exemplaris* Gąsiorek, Stec, Morek & Michalczyk, 2018, require a specific desiccation protocol with precisely controlled relative humidity (RH) for their survival. A desiccator with different salt solutions is often used to set appropriate RH in desiccation studies. Different RHs allow "slower" or "faster" desiccation and induction of anhydrobiosis. This technique has limitations, including insufficient RH control. Therefore, novel desiccation tools with adjustable and controllable parameters including RH and temperature are needed. Such devices could greatly accelerate the research on tardigrade desiccation tolerance, since it is known in only a handful of species.

Methods: Desiccation chamber is built and programmed on basis of Arduino microcontroller, utilising dedicated temperature and humidity sensors. Humidity is regulated through solenoid valve, which regulates flow of pressurised humid air into the system. 3D printing was implemented to create top part of the desiccation chamber with interface for humid air supply tube and microtube holder for samples. Temperature control is done via several Peltier devices (TEC) with PID regulation. Basic machining was used to create bottom part of the desiccation chamber from aluminium plate to facilitate uniform spread of the temperature inside the desiccation chamber.

Results: Here, we present a first 3D printed desiccation chamber, which allows setting desiccation time and 1% RH stepping (from room RH to 100%) with stable RH (σ=0.5%). Precise control of temperature ensures stable conditions. Pilot testing of three various soil species (genera *Macrobiotus*, *Ramazzottius* and *Milnesium*) desiccated in the chamber showed successful tun formations and subsequent survival.

Conclusions: We expect this chamber will be very useful for comparative studies, providing data comparable among laboratories. It might help answering questions such as: "Can the time required for tardigrade desiccation be shortened?", „Can desiccation sensitive tardigrades adapt to desiccation?" or „Can tardigrade cells be desiccated outside their body?". Furthermore, comparative analysis among "slow" and "fast" or "strong" and "weak" anhydrobiotes and desiccation-sensitive species by using precise and controlled desiccation techniques can provide good clues to unveil the molecular basis of anhydrobiosis.

Keywords: desiccation, humidity, anhydrobiosis, tardigrades

Monday-Tuesday stand 7 | side A

TarMass – a fast, accurate and easy to use tool for tardigrade biomass calculation

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Background: Estimation of biomass instead of their count or density is crucial for understanding the role of organisms in ecosystem functioning like nutrients flow or oxygen consumption. Moreover, data on the biomass distribution of population reflects its demographic structure, which, together with environmental parameters compared between populations or over time, may be indicative of evolutionary processes like down or upsizing under acts of predatory pressure, temperature or food source change. Regrettable, data on the biomass of tardigrades, which are ubiquitous invertebrates and play multitrophic level functions in ecosystems, are scarce. Here, we present preliminary results of an efficient, simple use tool that calculates tardigrade biomass, based on stereomicroscopic pictures.

Methods: Artificial neural network has been trained to detect tardigrades and calculate their biomass. The estimation is carried out by measuring the volume of the body using its length and width and assuming that specific gravity is equal to 1.04, as estimated by Edwards (1967). According to Jennings (1976) for Eutardigrada the following formula is used $W = L \times \pi \times \left(\frac{L}{2R}\right) \times 1.04 \times 10^{-6}\,\mu g$, while for the representatives of Echiniscoidea $W = \frac{1}{12} \times L \times \pi \times \left(\frac{L}{R}\right)^2 \times 1.04 \times 10^{-6}\,\mu g$, where W is weight in μg, L is the length in μm, while R is the length to width ratio.

Results: The convolutional neural network has been trained on a pilot dataset. Preliminary tests show, that the model is able to detect a tardigrade and estimate key points on its body. Algorithm results were compared to the manually calculated biomass, and the difference turned out to be negligible: the mean difference was less than 2%. The automated detection was done in a few seconds (depending on image size), while manual calculation took about 10 minutes.

Conclusions: TarMass tool can increase the speed of biomass analysis compared to traditional, manual techniques, which can be useful in the studies on the weight structure of a population and might be of crucial importance in understanding ecological or evolutionary processes in ecosystems.

Keywords: biomass, ecology, neural networks, Python, software

Monday-Tuesday stand 7 | side B

Evaluating diversity of terrestrial tardigrades in different localities: do we need new standards of sampling?

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Background: There exists a problem of comparing species diversity of tardigrades in different studies. It was mostly resolved at the species level with the implementation of integrative taxonomy standards which allowed reliable identification. However, at the levels of localities, ecosystems and tardigrade communities such comparison still poses a challenge because of different sampling approaches used by different researchers. There are more or less standard ways to collect a particular sample, but what about deciding how many samples and which samples to take from the locality?

Methods: In this study a review of different sampling approaches used in the recent studies of terrestrial tardigrade biodiversity and ecology was made. We read Tardigrada-related articles published within the last decades and checked if they contained a detailed description of sampling approach and whether this approach was somehow justified. The obtained information was processed and systematised. We also investigated the experience of solving similar problems in some other branches of biodiversity studies (botany, hydrobiology *etc*.).

Results: The main result of this research is a database ordering current approaches to sampling for terrestrial tardigrades in the taxonomic and ecological studies. It specifies a list of parameters taken (or not taken) into account during the collection of samples. According to our data, most taxonomic studies seem not to follow any formal approach for sampling (or at least do not have it described in the papers). Quite likely, every research team or even each particular collector have their own and sometimes intuitive standards. This makes the interpretation of differences found between these studies a hard task.

Conclusions: There is a "white spot" in the tardigrade biodiversity research standards: the reasoning for sample selection. We believe that incorporating certain standards for it would increase the amount of information we get from taxonomic studies. Data about the strategies of different research groups, ecological features of tardigrades and approaches used in other branches may serve as the basis for creating a unified methodology and developing some simple real-world strategies for sampling and the description of it.

Keywords: biodiversity, microclimate parameters, mosaicism, sampling, tardigrade communities

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Method for obtaining genetic material of single tardigrade by ultrasonic fragmentation technology

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Background: Extracting tardigrade DNA and RNA is particularly difficult because of the microscopic size of these animals. However, many studies that aim to identify genera and species require special work in molecular biology. We have discovered a method to efficiently obtain DNA or RNA using an ultrasonic cell disruptor.

Methods: A single tardigrade individual was frozen and thawed three times at a low temperature of -80 ˚C, and the proteinase solution was added. After standing for 5 mins, we put it into an ultrasonic disrupter, ultrasonically broke it for 5 seconds and silence for 30 seconds, repeated 6 times, then referred to the traditional extraction method to obtain DNA or RNA.

Results: By this method, the nucleic acid content is increased, the enzyme content is lower, and the impurity content is lower.

Conclusions: Ultrasonic fragmentation significantly reduces the activity of other interfering biological enzymes, and the nucleic acid integrity is hardly affected, which is more conducive to the acquisition of PCR results.

Keywords: DNA and RNA extraction, ultrasonic breaking, Tardigrada

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The gaps in the tardigrade database

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Background: During the last decades, our knowledge about the tardigrades has expanded in an amazing way, from description of novel taxa, identification of novel study sites to the methods employed to study tardigrades. Many new methods have been introduced to describe tardigrade taxonomy, phylogeny, and molecular biology to complement the classic morphological approach. However, there is also a big discrepancy between these various methods. Today, genome sequencing coupled with various other advanced omics (transcriptomics etc.) studies provide the most comprehensive information. However, so far, these methods have only been employed on a few model species. Furthermore, various gene targets have been suggested as suitable markers for simplified and quick phylogenetic studies, but the problem is that these gene targets have not been tested on all tardigrade taxa.

Method: We downloaded all gene sequence targets suggested for tardigrade phylogenetic studies from the NCBI and the Silva database. All partial gene sequences were evaluated in the bioinformatics software package ARB software with regard to sequence quality, alignment quality and ecological data. All data were correlated with the classic taxonomical descriptions performed by so far published studies on different tardigrade taxa (Degma *et al.* 2018, GBIF database). Google Mymap was used to create interactive maps to display all data together.

Results: There is a big discrepancy between available data on morphological descriptions, ecological descriptions and molecular biological data for tardigrades. Only few tardigrade species have been fully described with all three categories of information. There is also a risk that some descriptions may be incomplete or even invalid. The highest amount of gene sequences for a phylogenetic marker gene has been retrieved for the ribosomal genes, while the full genome sequence has only been retrieved for a few tardigrade species. Thus, more gene sequencing is needed to fill many gaps in the molecular database of tardigrades.

Conclusions: This database may serve as an aid to visualise obvious gaps and needs in future tardigrade research. We intend to release the data on an open platform, so that everybody can access these databases (the ARB sequence files and the GoogleMyMap data).

Keywords: biogeography, database, molecular markers, tardigrade taxonomy

Monday-Tuesday stand **9** | side **A**

Hidden *Wolbachia* infections: Peekaboo with a "master manipulator" and the discovery of multiple *Wolbachia* infections in water bears (Tardigrada)

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Background: The most widespread intracellular bacterium in broad range of phylogenetically diverse terrestrial invertebrate hosts is *Wolbachia* – belonging to the class Alphaproteobacteria and closely related to *Rickettsia*. This bacterial endosymbiont has intrigued biologists since its discovery in 1924 by Hertig and Wolbach due to its significance to the evolution and reproductive biology, as well as, the ecology of host species. Nowadays they are infamous as "master manipulators" with documented effects including induced parthenogenesis, male killing, feminisation and cytoplasmic incompatibility. Nevertheless, molecular detection of *Wolbachia* is challenging. We hypothesised that *Wolbachia* detection is more complex when different strains occur in low frequency in the microbiome community of the host and the abundance of different strains prevented us from obtaining good quality sequences using the Sanger method.

Methods: We designed Wolbachia-specific primers, performed high-throughput sequencing based on the V3-V4 hypervariable region of the bacterial 16S rRNA gene and built a Python script to target and extract *Wolbachia* sequences from the microbiome communities in two tardigrade species.

Results: Our metabarcoding method – effective detecting low-density of multiple *Wolbachia* infections occurring within the host (*i.e. Paramacrobiotus experimentalis* and *Macrobiotus basiatus*) – allowed us to find the co-occurrence of DNAs representing different *Wolbachia* strains. Phylogenetic analyses revealed multiple infections within a very diverse *Wolbachia* supergroup A.

Conclusions: The method described in the present study offers new perspectives for detecting multiple infections in single hosts. Our findings open new frontiers in biology, ecology and DNA barcoding distortion of investigated tardigrades, as well as, indicates that the range of *Wolbachia* host species is much wider than previously thought.

Keywords: 16S rRNA gene, bacterial endosymbiont, metabarcoding, microbiome communities

Monday-Tuesday stand 9 | side B

Do somatic and germline cells of *Dactylobiotus dispar* ovary respond in the same way to ibuprofen?

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Background: Ibuprofen is one of the most popular over-the-counter medications with anti-inflammatory, analgesic, and antipyretic properties. The consumption of ibuprofen by one person ranges from 600–1200 mg/d for short-term inflammations and pain up to 2400 mg/d during long-term treatment of rheumatic inflammation and other severe musculoskeletal disorders. Its consumption increases significantly every year. Increasing ibuprofen consumption increases environmental contamination with this drug, which impacts invertebrates' and vertebrates' reproduction.

Methods: Adult specimens of *Dac. dispar* were incubated in two different ibuprofen concentrations (1 and 10 mg/l) for seven days. The concentrations were chosen based on research conducted on *Daphnia magna*. After incubation, the material was fixed for transmission electron microscopy according to a standard protocol. Ultrastructural analysis of the ovary was performed using a transmission electron microscope Hitachi H500.

Results: The ultrastructure of germline cells in the ovary of animals treated with ibuprofen at a concentration of 1 mg/l was similar to that seen in control animals. All cell organelles showed typical structure. Only a few mitochondria have lost their cristae. However, in the somatic cells of the ovarian wall, distended cisterns of the rough endoplasmic reticulum (RER) and single small vacuoles were observed. In the cells of the germline of animals from the second experimental group, damage of numerous cell organelles was found. Mitochondria were losing their cristae, their matrix was electron-lucent, and the cisterns of RER were distended. The cytoplasm of ovary wall cells was electron-lucent and filled with short distended cisternae of RER, mitochondria without cristae, and vacuoles.

Conclusions: The germline cells show less ultrastructural changes induced by ibuprofen than the somatic cells of the ovarian wall. The cells of the ovarian wall, like the midgut and integument, constitute a barrier that protects germline cells against environmental stressors. These are the first studies that show the protective role of the ovarian wall for germline cells against environmental stressors such as ibuprofen.

Keywords: germline cells, ibuprofen, ovary, ovary wall cells

Monday-Tuesday stand **10** | side **A**

Effect of ibuprofen on the midgut of tardigrade *Paramacrobiotus experimentalis*

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Background: Numerous studies conducted around the world show the presence of ibuprofen – both its metabolites and in its pure form in the environment. This issue is linked to the broader problem of inappropriate drug disposal and ineffective removal methods in wastewater treatment plants. There are no studies of the effect of this drug on tardigrades yet. One of the organs that acts as a barrier against environmental stressors that can affect the body is the midgut. The present research is dedicated to the ultrastructural analysis of the midgut in ibuprofen-treated carnivorous specimens.

Methods: Adult animals of *Pam. experimentalis* were incubated with three different concentrations of ibuprofen (0.1 µg/l, 16,8 µg/l and 1 mg/l). After 7 days, the individuals were fixed according to the standard protocol for transmission electron microscopy. The analysis of the midgut was carried out with the use of a transmission electron microscope Hitachi H500. In addition, several analyses were performed using a confocal microscope (JC1, LysoTracker Red).

Results: In the digestive cells of the animals from the first experimental group (0.1 µg/l), no significant ultrastructural changes were found, and only a few mitochondria began to lose their cristae. In midgut cells treated with ibuprofen at a concentration of 16.8 µg/l, many damaged mitochondria (without cristae) were found.. Moreover, distension of the cisterns of the rough endoplasmic reticulum was found. In the cytoplasm of digestive cells in the third experimental group (1 mg/l), apart from altered mitochondria and distended cisterns of the rough endoplasmic reticulum, the appearance of numerous autophagosomes was observed. Analysis performed using confocal microscopy confirmed increased inactive mitochondria in the second and third experimental groups and a significant increase in autophagic structures in the third experimental group.

Conclusions: Autophagy is a defence process activated to remove damaged cell organelles in midgut cells. This process is responsible for the survival of the cell. The middle intestine is one of the first barriers protecting *Pam. experimentalis* against ibuprofen presented in the environment. The research presents the first results concerning autophagy as a defence mechanism against environmental stressors such as ibuprofen in digestive cells in tardigrades.

Keywords: autophagy, environmental stressor, ibuprofen, midgut

Monday-Tuesday stand **10** | side **B**

Does the presence of paracetamol in the environment affect the ultrastructure of the midgut of *Hypsibius exemplaris*?

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Background: The consumption of pharmaceuticals, especially over-the-counter non-steroid anti-inflammatory drugs (NSAIDs), increases significantly from year to year. The increase in the consumption of drugs translates into an increase in contamination of the aquatic environment with medicinal substances and their degradation products. The presence of pharmaceuticals in surface waters has an impact on aquatic organisms. One of the most commonly used NSAIDs is paracetamol. This pharmaceutical is considered safe in the recommended doses.

Methods: The present study involved the analysis of the effect of short-term (7 days) exposure to paracetamol on the midgut of herbivorous tardigrade *Hys*. *exemplaris*. Three paracetamol concentrations (0.2 µg/l, 230 µg/l, 1 mg/l) were used in the experiment. The research was carried out using a transmission electron microscope. The material for analysis was prepared according to the standard TEM protocol.

Results: The first ultrastructural changes were found in the digestive cells of the midgut treated with ibuprofen at a concentration of 0.2 µg/l. Few of the mitochondria lost their cristae, and single autophagosomes appeared in the cytoplasm of these cells. A significant increase in the number of autophagic structures (autophagosomes, autolysosomes) was observed in the digestive cells after incubation in ibuprofen at a concentration of 230 µg/l and 1 mg/l. The number of mitochondria that had lost their cristae also increased. Despite the accumulation of autophagic structures, digestive cells retained their integrity.

Conclusions: The midgut cells constitute the first barrier against environmental stressors such as paracetamol, which enters the animal's body with food and water. The activated process of autophagy is a defence mechanism that allows cells to survive by eliminating damaged cell organelles. The research presents the first results concerning the influence of paracetamol present in the environment on tardigrades, with particular emphasis on the ultrastructure of the midgut cells. In addition, the data provided shed new light on autophagy as a defence mechanism against environmental stressors in tardigrades.

Keywords: autolysosome, autophagosome, midgut, mitochondria, NSAIDs, paracetamol

Effect of paracetamol on the storage cell ultrastructure of *Hypsibius exemplaris*

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OSKAR KARNÓWKA[1] & IZABELA POPRAWA[1]

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Background: Paracetamol is an organic chemical compound used as a medicine to relieve pain and fever. Unlike painkillers from the group of NSAIDs, it has a fragile anti-inflammatory effect. Its consumption increases every year, resulting in increased environmental contamination with this pharmaceutical. The source of paracetamol in the environment is municipal and medical wastewater, as well as improper disposal of drugs. Paracetamol was detected in surface waters, groundwater, and/or tap waters from 29 countries worldwide, with a global average and maximum measured environmental concentrations of 0.161 µg/l and 230 µg/l, respectively. The presence of paracetamol in surface waters has an impact on aquatic organisms.

Methods: The present study involved the analysis of the effect of short- (7 days) and long-term (28 days) exposure to paracetamol on the storage cells of *Hys. exemplaris*. Tardigrades were treated with three concentrations of paracetamol (0.2 µg/l, 230 µg/l, 1 mg/l). Ultrastructural analysis was performed using transmission electron microscopy.

Results: Short-term exposure to the paracetamol concentrations used in the experiment caused changes mainly in the ultrastructure of the storage cells' mitochondria. No changes in ultrastructure were observed in storage cells treated with 0.2 µg/l paracetamol solution. Due to the concentration of 230 µg/l and 1 mg/l, mitochondria began to lose their cristea. Changes in mitochondrial ultrastructure similar to those found in short-term exposure to the drug were observed in storage cells treated with long-term treatment with paracetamol. In addition, shortening of the cisterns of the rough endoplasmic reticulum and the appearance of autophagic structures were also observed. All changes intensified with the increase in the concentration of the pharmaceutical.

Conclusions: Paracetamol in environmental concentration causes relatively small changes in the ultrastructure of storage cells. Long-term exposure to the drug triggers the process of autophagy, which is responsible for the removal of damaged organelles and cell survival. The midgut and the integument are barriers against environmental stressors such as paracetamol. The presented research is the first analysis of the influence of paracetamol on the ultrastructure of storage cells in tardigrades, famous for their high resistance to environmental stressors.

Keywords: autophagic structures, mitochondria, NSAIDs, paracetamol, storage cells

Structural analysis of the cuticle and cuticular capsule in freshwater tardigrade *Thulinius ruffoi*

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Background: The integument is significant playing among other structural and protective functions. It is composed of the cuticle and underlying epithelial tissue. The cuticle of tardigrades may be differently developed, and its organisation is an important clue in investigating the systematic position of the species. The basis for this analysis was the lack of information about the hatchling cuticle structure and insufficient knowledge about the organisation of cuticular capsules of cysts.

Methods: The present study involved the analysis of both the cuticular structure and the cuticular capsule in the freshwater tardigrades *Thu. ruffoi*. Both active (non-encysted) animals and cysts were analysed. Within non-encysted animals, hatchlings up to 24h after hatching and adult individuals were used. Exuviae with eggs were isolated and incubated with daily observation to detect hatched individuals to obtain the hatchlings. To get the cysts under laboratory conditions, non-encysted animals were incubated at a low temperature. Structural analysis was performed using scanning and transmission electron microscopy.

Results: The cuticle in such young animals was thin and had the outline of a layered organisation typical for older animals. The analysis of the structure of individuals after hatching showed that the cuticle develops and visibly changes over 24 hours. The cuticle analysis of non-encysted individuals was also used for the comparative analysis with the structure of encysted individuals; however, no structural deviations were noted. Besides the analysis of the cuticle that covers the body, the structure of the cuticular capsule of the cysts was analysed. The capsule was composed of three distinctive sheaths. Within them, morphological and structural differences could be observed.

Conclusions: The integument of the hatchlings, similarly to the integument of the adults, plays many essential functions. Proper development of the body integuments, including the cuticle, is crucial for a safe start to life outside the egg. The research presented the first results concerning the integument structure of tardigrade hatchlings. Moreover, the given data sheds new light on the cuticular capsule organisation in encysted tardigrades.

Keywords: cuticle, cuticular capsule, encystment, integument

The study was supported by the Polish National Science Centre (NCN Preludium 2020/37/N/NZ8/00170).

Monday-Tuesday stand **12** | side **A**

Effects of anthropause on tardigrade urban communities during COVID-19 pandemic restrictions in Salta, Argentina

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Background: To manage and prevent coronavirus illness (COVID-19), restriction measures that heavily affected social activities were implemented. This period of restricted human movement, or anthropause, ensured an unprecedented reduction of anthropogenic emissions, resulting in a significant improvement in air quality in many cities.

Methods: We use the reduction of vehicular traffic in Salta as a reference for evaluating the impact of the anthropause on tardigrade populations. We used samples taken during the 2019 winter season with high (H) and low (L) vehicular traffic, as well as those collected during the 2020 winter season under the restrictions (HC and LC). The samples were processed like the usual methodology. The programmes PAST, iNEXT, SPADE, and R were used to analyse the data.

Results: A total of 1,887 specimens were collected, representing 13 species. Habitat H had the highest number of individuals (N = 1086), although habitat L was 1.34, 1.20, and 1.13 times more diverse than communities H, LC, and HC, respectively. In addition, habitat HC was found to be 1.19 times more varied than habitat H (prior to the restrictions). The Whittaker curves reveal that all urban communities are dominated by *Macrobiotus* sp. nov., *Milnesium quiranae*, and *Viridiscus rufoviridis*. *Doryphoribius* was only found in H and L, and *Mesobiotus* was only found in HC and LC. *Mesobiotus* sp. nov. 1 is a new species for science. The beta diversity partition indicated that species turnover was higher than nesting. The ordination (CCA) explained 64% of the total variance (Axis 1), distinguishing the L community from the H, HC, and LC communities, showing increasing similarities across the habitats during the restrictions.

Conclusions: We infer that limits on vehicular traffic during the COVID-19 pandemic mitigated the previously observed consequences of species extinction due to biotic homogeneity, allowing the colonisation of novel species such as *Mesobiotus* sp. nov. 1. Nested urban patterns of diversity could be reversible gradients over time. To summarise, we stressed that the diversity of tardigrades in Salta and a variety of variables, the most important of which is vehicular activity, influence their assemblages.

Keywords: anthropause, covid-19, ecology, urban tardigrades

Monday-Tuesday stand **12** | side **B**

Urban green spaces as tardigrade biodiversity hotspots in Salta city (Argentina)

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Background: In urban landscapes, fragmentation is widely regarded as a major threat to biodiversity, as it significantly alters landscape structure and decreases landscape connectivity. As a result, fragmentation restricts the dispersal of many species as well as the formation of strongly structured metacommunities. In Salta, Argentina, we tested the hypothesis that urban green spaces function like tardigrade biodiversity hotspots.

Methods: A total of 36 samples were collected. Green Areas (GA) and three neighbouring urban habitats were sampled: high (H), medium (M), and low (L) vehicular traffic. Specimens were handled following the normal research protocols. PAST, PC-Ord, iNEXT, SPADE, and R were used to analyse the data.

Results: Were collected 2380 specimens, representing 14 species/morphospecies. Green Areas was 1.20, 1.63, and 1.67 times more diverse than the L, H, and M communities, respectively. The GA community was more equitable, with a wider range of guilds. However, three species dominated the urban communities: *Macrobiotus* sp. nov, *Milnesium quiranae*, and *Viridiscus rufoviridis*. The partition of beta diversity revealed a species turnover gradient from GA to M. GA and L communities had the highest turnover (68%), whereas the difference in assemblage composition between GA and H was attributed to both components: species turnover (48%) and nesting (52%). The DCA analysis revealed a nested pattern between tardigrade assemblages in disturbed habitats and urban green spaces, with the first axis explaining 69.3% of the total variation observed and the second axis being despicable (8%). The Mantel test revealed that geographical distance between sites might explain some of the variations in assemblage similarity ($R = 0.3406$, $p = 0.0262$). As a result, urban sites closer to green spaces share more species than those farther away.

Conclusions: We found that green spaces in Salta city have a higher diversity of tardigrades, but urban communities have a propensity to establish a general nested pattern and a loss of species, resulting in biotic homogeneity in urban areas. Despite this, urban green spaces can act as biodiversity hotspots by supplying species to the urban areas nearby.

Keywords: biodiversity hotspot, ecology, green spaces, metacommunities, urban

Monday-Tuesday stand **13** | side **A**

Do microclimatic conditions affect the body size of terrestrial and freshwater tardigrades?

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Background: Body size is the 'single most important axis of biodiversity' with large impacts on everything from basic cellular processes to global biogeographical patterns in animals. It is also obvious is that microclimatic and macroclimatic conditions influence the morphology of animals. For example, it has been shown that homeotherms and some poikilotherms display a positive relationship between body size and latitude, however this has been rarely investigated in invertebrates. In marine tardigrades, a significant increase in body size with latitude was found for the Northern Hemisphere. Species with the smallest body sizes disappeared at high latitudes. However, specific studies on how microclimatic conditions affect body size have never been conducted on limnoterrestrial tardigrades. Here, we tested whether tardigrade body size was affected by altitude, temperature, and precipitation.

Methods: Body size data of limnoterrestrial tardigrades from their type localities were used. Exact localities were also extracted from original descriptions or redescriptions or estimated based on available original data of the place of the collection (*e.g.*, region, mountain name, city). Bioclimatic data like precipitation and temperature were downloaded from Worldclim 2 reference database (http://www.worldclim.com/version2). Statistical analysis was performed using Principal Component Analysis (PCA) and Pearson's correlation coefficients between body size of tardigrades and microclimatic and macroclimatic parameters.

Results: Both in the N and S Hemisphere, a significant correlation was found between temperature and body size of the Apochela. No relationship was found for Parachela in the N Hemisphere, while in the S Hemisphere, the relationship was observed. A statistically significant correlation between temperature and body size was found for the terrestrial Echiniscoidea both in the N and S Hemisphere.

Conclusions: In general, the lower mean temperature seems to correlate with the larger body size of tardigrades, in line with what is known for larger organisms according to Bergmann's rule.

Keywords: biogeography, precipitation, Tardigrada, temperature

Invertebrate habitat gaps in the terrestrial cryosphere

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Background: Despite the harsh conditions of snow and glacier habitats including low temperatures, short vegetative periods and high doses of UV irradiation, they are inhabited by a myriad life forms. Tardigrades are one of the dominant animal groups in the terrestrial cryosphere, however, the knowledge about "cold-loving" water bears is limited. Cryoconite holes, water-filled reservoirs on the glacier surface, are one of the best characterised habitats in terms of cryophilic tardigrade diversity while other habitats remain unexplored. Here we present a review and novel information on glacial and snow habitats requiring further investigation in terms of tardigrade diversity.

Methods: We used original observation and literature data about tardigrades in the cryosphere as well as emphasising the potential habitats that require urgent attention due to global warming.

Results: Although cryoconite holes are considered biodiversity hotspots in the glacial biome, other habitats are promising environments for studies on the diversity of tardigrades. We found that the weathering crust (*i.e.*, the upper few centimetres of hydrated surface ice) on New Zealand glaciers hosts novel tardigrade species. We discovered water bears in blooming snow algae that were feeding and reproducing. Water bears were previously found in Icelandic glacier mice (moss balls rolling on ice surface), and we confirm this observation in Norway. Mosses covering the Ugandan glacier (so-called glacial moss gemmae aggregation) are also inhabited by tardigrades. Other surface glacial habitats include the supraglacial debris (gravel covering glacial surface), moraines and supraglacial lakes which may provide suitable conditions for tardigrades, but these remain unexplored. Likewise, the englacial (subsurface) and subglacial (under glacier) zones have never been studied in terms of tardigrades, which hinders our understanding of cryophilic tardigrades.

Conclusions: Glaciers and snow form biomes across continents and hemispheres. They are inhabited by unique tardigrade species that are adapted to hostile conditions. Regrettably, this biome is shrinking and requires taking urgent actions in the search of potentially endangered metazoans including tardigrades. It is especially urgent in light of the current Sixth Great Extinction, making studies on biodiversity of high priority.

Keywords: glacier mice, weathering crust, moraines, supraglacial debris

Tardigrade abundance, diversity and habitat preference in Treforest, United Kingdom

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Background: Our understanding of the phylum Tardigrada is growing exponentially as technology and interest advance, but there are still many gaps in our knowledge regarding their ecology and specific habitat preference. This study aims to investigate the relationship between bryophyte species, the substrate and bearing those bryophytes are found, and the abundance and diversity of the associated tardigrade communities.

Methods: Moss samples were taken from a 500 m^2 area in the village of Treforest in South Wales, United Kingdom. The bryophyte species were identified under a MX7T Brunel light microscope using keys from Watson (1981) and Bosanquet (2010). Tardigrades were extracted from the samples by hand, mounted onto slides using Hoyer's medium and identified using a Leica EM500 microscope with phase contrast lighting and a key from Morgan and King (1976). Simpson diversity metrics were calculated, and the significance of their relationships were investigated.

Results: Within 16 bryophyte samples, 9 moss species were identified, 7 of which contained tardigrades. In total 239 individual tardigrades were observed, comprising of 3 genera of tardigrades across 2 orders. 132 individuals were identified to at least genus level. *Macrobiotus* was the most common genus, and 34% of all identified tardigrades were *M. hufelandi. Echiniscus* was the second most common genus. The bryophyte *Orthotrichum diaphanum,* which was only found on the stone substrate, accounted for only 2 of the moss samples but yielded 97 of the observed tardigrades, containing over 75% of all identified *E.* spp. Significant results could not be drawn for the factor of bearing, however correlations were observed between the presence of *Echiniscus* and the bearing of moss.

Conclusions: There are observable links between bryophyte species and tardigrade diversity, but few statistically significant conclusions can be drawn based on the current sample size. When possible, the study should be continued using a larger sample size with more replications to increase statistical significance. Nonetheless, the presence of multiple tardigrade species can now be confirmed within Treforest, United Kingdom, and can be used as a basis for future studies.

Keywords: abundance, diversity, distribution, South Wales, tardigrade, Treforest

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Occurrence of tardigrades and physiochemical conditions in rock pools by the Baltic Sea

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Background: Rock pools are eroded depressions in bedrock that provide temporary aquatic habitats. Their frequency of desiccation depends on geographic location and shape, and temperature, salinity, pH, and dissolved oxygen are important physiochemical variables that affect communities of organisms in rock pools. Tardigrades have evolved evolutionary adaptations that allow them to survive in many habitats with varying and extreme abiotic conditions, including desiccation. Their occurrence in rock pools, however, have rarely been investigated. The purpose of this study was to investigate the occurrence of tardigrades and the physiochemistry conditions in rock pools by the Baltic Sea in southeast Sweden.

Methods: Samples of benthic material were collected from 32 rock pools at three sites near the town Karlshamn in late May and early July 2021. Physiochemical measurements of pH, temperature, salinity, and dissolved oxygen of the water in the pools were made in the field using a portable mustimeter.

Results: Tardigrades occurred in 19% of the rock pools and represented five eutardigrade genera (*Ramazzottius*, *Hypsibius*, *Macrobiotus*, *Isohypsibius*, *Milnesium*). In most of the pools where tardigrades were found only few (<10) specimens were recorded, but in one pool >100 specimens were found (*Ramazzottius*). Rotifers were observed in 56% of the samples and were often abundant, while nematodes were observed in only 9% and were less abundant. Physiochemical variables showed some variation both within and among the three sites but there were no general differences between rock pools with or without tardigrades. However, rock pools with tardigrades tended to be shallower than pools without tardigrades, indicating that more desiccating-prone rock pools may be more favourable habitats for tardigrades.

Conclusions: The study shows that tardigrades are part of the micro-invertebrate fauna in rock pools. The tardigrade fauna in this habitat deserves more studies in general, as well as closer investigation into the tendency of more frequent occurrence of this animal group in shallow rock pools. The occurrence of tardigrades in rock pools provide interesting opportunities for many different experimental and non-experimental studies on, *e.g.*, environmental stress, population dynamics, and dispersal of tardigrades in a small-scale natural habitat.

Keywords: psysiochemical variables, rock pools, tardigrades, temporary waters

Monday-Tuesday stand **15** | side **A**

Using the evaluations of ecological niche models to estimate tardigrade species geographic ranges

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Background: Water bears (Tardigrada) are small and difficult to find. Moreover, some areas are not easily accessible. Thus, the research work on this phylum species is particularly unfavourable and the number of researchers in China working on tardigrade alpha taxonomy and distribution is low.

Methods: To solve this problem, we estimated the distribution of tardigrades in 13 genera and 26 species in China with the help of evaluations of ecological niche models (R package ENMeval), and found that temperature, precipitation and sunshine were the main factors affecting the distribution of the same species in different regions.

Results: We sampled according to the predicted locations, and found that the model had good accuracy.

Conclusion: Our method is feasible and provides a powerful tool for investigating the distribution of known species or discovering new species.

Keywords: distribution, ecological niche modelling, Tardigrada

Monday-Tuesday stand **15** | side **B**

The dispersal of soil tardigrades in the gut of earthworms

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Background: Tardigrades are limited in their active dispersal in soil because their movement is restricted to environments filled with water. When the soil dries, they can easily become enclosed in an isolated water drop or a single pore filled with water. Up to date research on tardigrade dispersal was mostly dedicated to moss-inhabiting species and dispersal by wind, birds, and snails. Here, we tested the possible transport of soil-inhabiting tardigrades in the earthworm gut.

Methods: First, we compared the abundance of tardigrades in freshly excreted earthworm casts with their usual numbers in the surrounding soil. Second, we inspected the content of the earthworm intestines extracted directly from the gut without further contact with soil inhabited by tardigrades.

Results: Earthworm casts and soil samples contained the same number of tardigrades. However, we did not find any tardigrade inspecting directly acquired content of earthworm intestines.

Conclusions: The numbers of tardigrades observed in the earthworm casts suggested that tardigrades can either survive the passage through the earthworm gut or colonise the casts after their excretion in less than 12 hours and by this time achieve the same densities as in the surrounding soil. We tried to confirm the survival of tardigrades after passage through the earthworm gut by inspection of dissected earthworms. However, this way we manage to examine only a small sample of earthworm gut content. In the future, larger volume of directly acquired gut contents from earthworms need to be examined to confirm the dispersal of tardigrades through the earthworm gut.

Keywords: earthworm casts, endozoochory, gut passage

At school with tardigrades – a microscopic model for science education

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Background: Tardigrades are very attractive not only to experts but also to the not-specialized audience and children, thanks to their biological fascinating features, especially those linked to their capability to cope with environmental stresses such as desiccation, freezing and space conditions. Nevertheless, due to their microscopic body size, they are still little used as "objects of study" for didactic experiences in primary and low secondary schools. To overcome this limitation and help pupils to discover tardigrades, we produced a series of creative and hands-on activities to be tested in classrooms.

Methods: To make easy the tardigrade extraction and observation, a video tutorial (https://youtu.be/5q6-jR_M5h4) involving the use of everyday sieving materials was realised within a project of Public Engagement (granted by the University of Modena and Reggio Emilia and the "Società dei Naturalisti e Matematici di Modena APS"). The classroom activities were carried out in primary schools of Modena and Reggio Emilia (Italy). Narrative and hands-on approaches, the scientific method, and the small-groups work were applied as teaching strategies. Lessons in classroom started with the reading of a story where the main character is a tardigrade describing itself. Pupils were invited to draw their idea of tardigrades and discuss about their drawings. After the vision of the video, children collected samples in the garden, extracted tardigrades from the substrates, observed them under stereomicroscope, described and drawn the procedure phases and animals. To formalise children' experience and learning process, a final large-group discussion was undertaken.

Results: The story telling caught the pupils' interest. At the beginning, their creative drawings represented tardigrades shaped as other known animals. Nevertheless, fancy tardigrades were drawn in detail in their environment. After the experience and microscope observation of tardigrades, children drawings became more precise, detailed, and consistent with reality. Children graphically represented the procedures of the scientific method and actively engaged during the activities by asking questions.

Conclusions: The narrative approach and the hands-on activities stimulated the pupils' curiosity and developed critical thinking skills. Direct observation of tardigrades allowed children to learn by doing, as shown by their drawings, going from fanciful to realistic. Children improved their knowledges and skills about tardigrades.

Keywords: hands-on, primary school, pupils, science education, tutorial video

WEDNESDAY-THURSDAY POSTER SESSION

Biodiversity

Biodiversity, Taxonomy, Biogeography, Phylogeny & Evolution

Diversity of Swedish Macrobiotidae: preliminary results from Southern Sweden

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Background: Macrobiotidae is one of the larger families of Tardigrada and can be found in aquatic and limno-terrestrial habitats worldwide. There are currently 319 species of Macrobiotidae known globally, with 22 species known from Sweden. However, research into the taxon's diversity has been limited, and the few recent studies that have been published suggest these numbers reflect only a fraction of the true biodiversity this family actually comprises. Thus, Svenska Artprojektet assigned the highest research priority to Macrobiotidae and funded a two-year project aimed at assessing the taxon's diversity throughout Sweden.

Methods: As part of the project, over 188 samples of vegetation (*e.g.* mosses, lichen, grass, etc.) or leaf litter were collected from various habitats around southern Sweden between March and July 2022. Tardigrades were extracted using sieves (mesh sizes: 125 µm and 35 µm) after keeping the samples in distilled water for 0.5 to 1.5 hours. Specimens were manually isolated and preserved in Hoyer's fluid for further observations or 96% ethanol for later DNA analysis.

Results: Macrobiotid tardigrades were isolated from thirteen samples. Abundance levels were typically medium–high (50–100+ individuals per 500 cm^3 substrate) in moss samples and low (<20 individuals per 500 cm^3) in leaf litter, grasses and lichen. Preliminary morphological identification suggests that at least twenty putative species from four genera have been collected.

Conclusions: The results presented here are extremely preliminary, yet they never-the-less suggest the true tardigrade biodiversity of Sweden is much higher than previously recorded. In addition to processing the samples already collected, future work will include additional sampling in mid and northern Sweden, with emphasis in areas that have been previously overlooked or are known to contain high tardigrade diversity. Four genes will be sequenced for species delimitation and phylogenetic reconstruction. The goals of this project are to determine which species of Macrobiotidae occur in Sweden, and provide an updated phylogenetic classification and generate data on their distributions and phylogeography.

Keywords: biodiversity, Macrobiotidae, morphology, new taxa, phylogenetics, taxonomy

Wednesday-Thursday stand 1 | side B

Diversity of the tardigrade communities in the Norwegian forests

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Background: Tardigrades are common in most habitats, however few studies have focused on large faunistic survey, specifically on tardigrade diversity in forests. Up to now, only 61 species have been recorded in different types of forest in Norway with an additional 25 found in limnic environments in forests. Although little is known about the ecological preferences of many species, previous studies have found that tardigrade diversity and community composition are significantly affected by ecological variables. In this study we associate georeferenced tardigrade species records with forest type, substrate type and substrate composition in order to see if tardigrade diversity and species communities can be associated with ecological characteristics of Norwegian forests.

Methods: In total 390 moss, lichen and litter samples were collected from 12 forests in central and southern Norway in the summers of 2017 and 2018 and later stored in paper envelopes. For the identification modern literature and keys for specific genera and groups of species were used. For statistical analyses, moss and lichen substrate of each sample was classified according to the main species, life form, growth forms and habitat of substrate and associated with each tardigrade identification and sample metadata.

Results: A total of 17 407 specimens were identified, encompassing in total 132 species (including some new species). Species richness increases with precipitation, but does not change with temperature or precipitation seasonality. The distribution of species richness between life forms and forest types showed considerable variation within and among the variables. Disregarding variables with low sample numbers, among life forms only acrocarpous moss samples appeared to deviate with respect to species richness, containing less species than substrates with other life forms.

Conclusions: Tardigrades in Norwegian forest are extremely abundant, frequent and diverse. Moreover, it appears that that certain species and/or entire communities prefer specific microhabitats.

Keywords: biodiversity, Europe, fauna, Norway, Tardigrada, taxonomy

Wednesday-Thursday stand **2** | side **A**

Terrestrial Tardigrada (water bears) of the Słowiński National Park (Northern Poland)

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Background: Tardigrada (water bears) of the Słowiński National Park (SPN) are almost unknown, with only two species, *Mesocrista spitzbergensis* (Richters, 1903) and *Hypsibius pallidus* Thulin, 1911, reported from this area. Taking into consideration intensive changes in tardigrade taxonomy in recent years, large number newly described tardigrades and high diversity of microhabitats in the SPN, we should consider that number of tardigrade species in this region is much higher. The aim of this study was to determine the diversity of terrestrial tardigrades in different habitats of the SPN.

Methods: Moss and lichen samples were collected between 27th and 30th October 2017 and later stored in paper envelopes. Tardigrades and their eggs were extracted according to standard methods and mounted on permanent slides in Hoyer's medium. Specimens and eggs were studied under Phase Contrast Microscopy. For the identification modern literature and keys for specific genera and groups of species were used.

Results: In total 107 samples were examined and more than 1500 specimens and eggs were found belonging to a dozen genera of both Heterotardigrada and Eutardigrada classes. Based on preliminary taxonomic studies at least 20 different species were identified. One species from *Macrobiotus persimilis* group is new for science. A very rare eutardigrade *Pseudohexapodibius degenerans* (Biserov, 1990) was also found, which was never reported outside of its type locality.

Conclusions: The obtained data show a high diversity of tardigrades in studied area. Furthermore, sandy areas were found to be characterised by their own unique tardigrade fauna with a large number of xerophilous species from the genera like *Xerobiotus*, *Milnesium*, *Ramazzottius, Eremobiotus* and a very rare *Pseudohexapodibius*.

Keywords: biodiversity, Europe, fauna, taxonomy, xerophilous species

Wednesday-Thursday stand **2** | side **B**

Terrestrial tardigrades of the Black Sea Biosphere Reserve (Southern Ukraine) revisited

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Background: There are around 120 species of Tardigrada reported from Ukraine. However, the territory is still poorly and unevenly studied. Up to now, only 37 species were found in the mainland of Southern Ukraine. This area used to represent an arid steppe zone, but historically it underwent significant anthropogenic transformation and now is largely occupied by fields and pasture lands. That is why the Black Sea Biosphere Reserve (BSBR) preserving some of the last natural ecosystems of the region became our main interest. In 2008-2011 few samples from the BSBE were studied (Pilato *et al.*, 2011). The main goal of present study was an exploration of the diversity of terrestrial tardigrades of the Black Sea Biosphere Reserve.

Methods: In total 77 moss and lichen samples were collected from arid sandy areas of the Biosphere Reserve in the period from 2019 to 2021 and stored in paper envelopes. Tardigrades and their eggs were extracted according to standard methods under the stereomicroscope (on a dark field) and mounted on permanent slides in Faure's medium. Specimens and eggs were studied using phase contrast microscopy. For the identification modern keys for tardigrade families and genera were used.

Results: Tardigrades were found in 61 out of 77 examined samples. Altogether 771 specimens of tardigrades (apart from eggs) belonging to 18 genera of both Heterotardigrada and Eutardigrada were detected. Both species described from BSBR earlier *Notahypsibius pallidoides* (Pilato, Kiosya, Lisi, Inshina & Biserov, 2011) and *Xerobiotus euxinus* Pilato, Kiosya, Lisi, Inshina & Biserov, 2011 were found again.

Conclusions: The obtained data show a high diversity of terrestrial tardigrades in studied region. Black Sea Biosphere Reserve needs further research on tardigrade diversity with the use of traditional and molecular taxonomy.

Keywords: biosphere reserve, Black Sea biodiversity, taxonomy, Southern Ukraine, water bears

Stratification of Tardigrades in Czerniejewskie Forests – a preliminary report

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Background: Faunistic study in this area of Czerniejewskie Forests was the first stage of a larger project "Micro- and macrofactors affecting vertical distribution of Tardigrada in temperate forest", which aims to identify the mechanisms which determine the vertical distribution of tardigrades inhabiting epiphytic mosses growing on trees. Which was inspired by studies on stratification of tardigrade populations in the tree canopy, published by Chang *et al.* in 2015. We present here the first results of this study, *i.e.* analyses of 184 samples collected from 21 deciduous trees.

Methods: Moss samples were collected from bases of the trees and from the tree trunks in Czerniejewskie Forests in Western Poland, Samples processing was conducted using a method based on the standard procedure. Tardigrades and their eggs were extracted with the use of Olympus stereomicroscope. The obtained tardigrades and eggs were mounted on microscope slides in Hoyer's medium, and further examined and measured under the Phase Contrast Microscope. Later, tardigrades were identified based on the modern literature and keys for the specific genera and species groups.

Results: In total 2111 specimens (adults and eggs) representing 16 species were found in studied area. The three most abundant taxa were *Notahypsibius* sp. (in total 490 specimens found in 80 samples), *Macrobiotus hufelandi* group (475 specimens in 52 samples) and *Minibiotus intermedius* (332 specimens in 66 samples). We found a vertical stratification of species distribution as manifested by changes in abundance and number of species.

Conclusions: Our analysis confirmed that our research area, in terms of tardigrade diversity, is a typical mixed forest of western Poland. What makes it a perfect area for studies on the mechanisms determining the distribution of terrestrial tardigrades inhabiting epiphytic mosses growing on trees.

Keywords: canopy, ecology, fauna, Tardigrada, temperate forest

The study was supported by the Polish National Science Centre (NCN Preludium 2016/21/N/NZ8/01023).

Wednesday-Thursday stand **3** | side **B**

Diversity of tardigrades in Tenerife Island (Spain)

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Background: Tenerife Island is a part of the Canary Islands Archipelago and it is characterised by varied geological structures which forms a great variability of the potential ecological niches. The relief of the island influences also the weather conditions, leading to a great variety of microclimates which are conducive to the occurrence of many endemic species of plants and animals. Tardigrades from this island are almost completely unknown and only 15 species were recorded from the Canary Island up to now. What is more not all species should be, at present, considered as correct taxa, according to modern taxonomy.

Methods: In 2022, ten samples of mosses were collected in El Pijaral Integral Nature Reserve by Piotr Rzymski. Tardigrades were extracted from the samples according to standard methods. For the correct species identification modern literature and keys for genera and species groups were used.

Result: In total, 142 specimens and 14 eggs were extracted from all samples. All species were preliminary identified and classified to nine genera *i.e. Ramazzottius*, *Paramacrobiotus*, *Pseudechiniscus*, *Doryphoribius*, *Echiniscus*, *Macrobiotus, Mesobiotus*, *Milnesium* and *Minibiotus.* Analysed samples revealed a high species diversity, despite the small collection area. Moreover, at least three or four species should be temporarily considered as new for science, but the final identification need a further morphological and genetic studies.

Conclusion: Our results indicate that tardigrade fauna of Tenerife Island is rich, diverse and very poorly known. It is obvious that on this island the number of different tardigrades species is much higher than it was reported in previous and rather scanty studies.

Keyword: Eutardigrada, fauna, Heterotardigrada, Tardigrada, taxonomy

Wednesday-Thursday stand 4 | side A

High diversity of Tardigrada in Chile, South America

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Background: There are very few records of terrestrial water bears, Tardigrada, in Chile. Early records relied upon European literature, subsequently underreporting the higher endemic biodiversity in Chile.

Methods: A range of terrestrial habitats have been sampled across Chile, as part of a broader ecological research programme. This multihabitat investigation was launched during the pandemic lockdowns and has relied on extensive field sampling, utilised a combination of photography, and online collaboration using Zoom, to review thousands of specimens collect by JEJ. So far, we have described specimen images into Operational Taxonomic Units, OTUs.

Results: We completed the initial sift of all the specimen images, approximately 8250 images, resulting in 82 OTUs being recognised. We are now reviewing each OTU description against example slide specimens trying to confirm potential taxonomic names for each specimen. In the next steps we will try to match these descriptions against taxa reported from elsewhere in South America.

From our initial results, so far, it is clear that there is a strong relationship with the fauna reported from Chile and Argentina, as well as the more distant fauna in northern location such as Peru, Ecuador, Colombia, and Venezuela.

Early records for South America were represented by many European taxa due to the lack of alpha-taxonomic studies in South America. Our data indicates species within our OTUs are similar to some European taxonomic genera functional groups but have additional taxa distinct to South America.

Conclusion: The diversity of Chilean tardigrades is much higher than previously reported. This project is on-going, having just reached the end of its first stage. Stage two will focus more on the *alpha* and *beta* taxonomy. Stage three will make further investigations into the biogeography and community ecology of tardigrades from southern Chile.

Keywords: Chile, rainforests, Tardigrada, terrestrial ecology

Wednesday-Thursday stand 4 | side B

Update on the List of Available Names (LAN), Tardigrada Project

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Background: The List of Available Names Project (LAN) Tardigrada was initiated at the 13th International Symposium on Tardigrada, Modena, Italy. Where a presentation by Diego Fontaneto *et al.*, was received about the LAN Rotifera project, the first project of its kind to validate taxonomic names and descriptions. Our symposium, in a plenary evaluated if we wanted/needed a similar project to the Rotifera researchers. The symposium voted unanimously in favour of initiating the LAN Tardigrada project.

Methods: The Commission for the International Code of Zoological Nomenclature was informed about the vote to initiate LAN Tardigrada. This was acknowledged by the Commission, with advice to start with Family-group names in order to build up experience team member's experiences. There was no single research publications collection available with every required publication. Individual researchers were approached and have shared access to their private collections for the purposes of this project, and a copy of required publications has been taken lodged into the project's archive, together with any translations which the project team has accessed.

Results: The first two indexes: Family-group names, and Genus-group names, are now approaching their first consultation releases in 2022. Whereas the first edition of the Species-group names indexes should be released in early 2023. Each listing will have two sections, firstly, the names judged to be valid and available under the Code, followed by a second index of names judged to be invalid, or unavailable. A clear justification statement should be available for each decision on a nomial.

Conclusions: We have more than 50 Family-group names, and more than 160 Genus-group names collated. Whereas the Species-group names have exceeded 1 500, but this metric is expected to continue to rise steeply depending on the progress with translating papers before they can be evaluated by the team's specialist taxonomists. Some recent publications will also mean significant changes to the indexes.

Keywords: *alpha*-taxonomy, *beta*-taxonomy, *gamma*-taxonomy, nomenclature, systematics

Wednesday-Thursday stand **5** | side **A**

New records of two species of marine tardigrades, *Echiniscoides sigismundi* and *Styraconyx haploceros*, collected from barnacles on the coast of Korea

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Background: About 200 species of marine tardigrades are known to occur worldwide. Among them, 15 species have been reported in relation with other invertebrates such as sea sponges, bryozoans, barnacles, and cucumbers.

To date, eleven species in the three genera have been reported from Korea. Previous studies on the marine tardigrades from Korean waters have been conducted mainly in the sandy intertidal zone. For the discovery of more species of marine tardigrades from Korea, we investigated the unexplored field such as sedentary barnacles in the rocky shores.

Methods: Six species of barnacles (*Amphibalanus improvisus, Tetraclita japonica, Perforatus perforatus, Fistulobalanus albicostatus, Chthamalus challengeri,* and *Balanus trigonus*) were collected on the coast of Korea, fixed with 10% formalin, and then transported to the laboratory. The formalin-fixed barnacles were rinsed carefully with tap water, and tardigrade samples were sorted and identified as species under a DIC microscope. Their morphological features were made with Wacom and an Illustrator program.

Results: Among six species of barnacles examined in this study, association with tardigrades was confirmed only in two species, namely *F. albicostatus* and *C. challengeri*. The tardigrades were identified as *Echiniscoides sigismundi* from *F. albicostatus* and *C. challengeri*, and *Styraconyx haploceros* from *F. albicostatus*. No tardigrades were found in any other barnacle species. *Echiniscoides sigismundi* shows no clear or indistinct pattern of the dorsal epicuticle. Unlike *E. hoepneri*, there is no stylet accessory and shorts. And they have smooth-bent claws without toes. The claws are arranged 8-8-8-7 or 8-8-7-7 on the each leg. *Styraconyx haploceros* is characterised by a reduction and shortage of cephalic appendages. But primary and secondary clavae are indistinct or absent. These morphological characters are unique to *S. haploceros* among the genus *Styraconyx*. We also confirmed and illustrated the existence of cirrus E, which the original description did not describe.

Conclusions: In this study, we have identified two species of marine tardigrades, *E. sigismundi* and *S. haploceros*, representing new records for Korea. As a result, the Korean fauna of marine tardigrades is newly updated with 13 species and five genera.

Keywords: *Echiniscoides sigismundi*, intertidal barnacle, marine tardigrades, *Styraconyx haploceros*

An integrative description of a new *Richtersius* species (Eutardigrada: Richtersiusidae) from Greece

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Background: Although tardigrade taxonomy is challenging because of their microscopic size and a small number of taxonomically informative characters, over a dozen species new to science are described every year, further expanding our knowledge on their biodiversity. For many years the genus *Richtersius* remained monotypic, containing only *R. coronifer*, a species considered to have a cosmopolitan distribution. A century after it was described, the first analyses of molecular data obtained for *R. coronifer* indicated that there were more than one species hidden within the genus *Richtersius*. In this study, we re-examined a *Richtersius* population from Greece reported in one of earlier studies as a candidate new species, and we described it as a new species.

Methods: A mixed moss and lichen sample was collected on the Greek island of Crete (Omalos, Chania). A total of 171 animals and 73 eggs were extracted from the sample and split into several groups for specific analyses, *i.e.* morphological analysis in PCM and SEM, as well as DNA sequencing.

Results: The new species, *Richtersius tertius*, has a yellow body and cuticle without granulation. An evident stalk system connects the claws to the lunulae. Lunulae are large with a crown of long spikes. The buccal apparatus is massive and robust. Hatchlings exhibit the same phenotype as adults, except for smaller body size and pores in the cuticle. Eggs are large, light yellow, laid freely. The DNA sequences of four molecular markers for the species description come from one of the previous *Richtersius* studies.

Conclusions: Thanks to morphological and morphometric analysis, as well as integration of these data with the DNA sequences published previously, we described a new *Richtersius* species that constitute the third formally named species in the genus. The implementation of integrative taxonomic tools and the collection of extensive sets of morphological and morphometric data, linked to molecular markers, will allow for unambiguous delimitations of further species within the genus.

Keywords: biodiversity, integrative taxonomy, systematics

The study was supported by the Polish National Science Centre (NCN Preludium 2018/31/N/NZ8/03096 and Sonata Bis 2016/22/E/NZ8/00417).

Completing the evolutionary tree of Macrobiotidae: the phylogenetic position of the genus *Calcarobiotus*

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Background: The family Macrobiotidae is one of the most species-rich taxa in the phylum, consisting of nearly 300 species grouped within 14 genera. All of these genera have been established based on morphology, with a clear monophyly confirmed by morphological and genetic data only for some of them. Importantly, there are no molecular data for eight out of these 14 genera. These taxa are also considered extremely rare and, except *Calcarobiotus* and *Insuetifurca*, they are all monotypic. Therefore, elucidation of the phylogenetic position of these enigmatic and morphologically divergent macrobiotid taxa is especially important.

Methods: Here, we constructed an upgraded multilocus phylogeny of the family Macrobiotidae with taxa for which novel sequences have recently been published. The data set included four DNA fragments: 18S rRNA, 28S rRNA, ITS-2, and COI. We focus specifically on the genus *Mesobiotus*, supplementing our data set with three newly sequenced populations. Additionally, we enriched our data set with unique DNA sequences of a population that belong to the rarely found genus *Calcarobiotus*.

Results: Our phylogenetic reconstructions corroborated the topology in the previous revision of the family Macrobiotidae. Similarly, we also confirmed the lack of monophyly for two traditionally recognised unformal species groups within the genus *Mesobiotus*, namely the *M. furciger* and *M. harmsworthi* group. Finally, the conducted analyses recovered the genus *Calcarobiotus* to be nested (and possibly polyphyletic) within *Mesobiotus*, at the same time falsifying the previously well-documented monophyly of the later taxon.

Conclusions: The presented results further indicate that special effort should be made towards increasing the sample size for the remaining, rarely found macrobiotid genera. The direct action which should be undertaken according to our outcomes is to lump *Calcarobiotus* + *Mesobiotus* within one single taxon as no morphological characters that could split *Mesobiotus* into multiple genera can be found. *Calcarobiotus* being the older name, should have priority according to the ICZN rules. However, this could cause confusion as the name *Mesobiotus*, although much more recent, is mentioned in the literature much more frequently. We believe that the international symposium gives us the perfect opportunity to discuss at length the mentioned nomenclatural problems with the tardigrade community to provide satisfactory solutions.

Keywords: monophyly, nomenclature, phylogeny, species group, systematics

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417 and Preludium 2018/31/N/NZ8/03096).

New records of Tardigrada from the Madeira Island (Portugal) with an integrative description of a new *Macrobiotus* species (*hufelandi* group)

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Background: Madeira Island has total of 741 km^2 and goes up to highest elevation of 1,861 metres. It has mild to warm year-round temperatures and has few different bioclimatic zones. Up to now, 33 species *i.e.* 22 Eutardigrada and 11 Heterotardigrada have been reported from this island. The aim of this study was to determine tardigrade diversity of Madeira Island based on modern taxonomy and provide integrative description of the new *Macrobiotus hufelandi* group species.

Methods: In total 112 moss and lichen samples from different habitats were collected. Tardigrades were extracted from the samples according to standard methods. For the correct species identification modern literature and keys for genera and species groups were used. The new species description is based on integrative taxonomy *i.e.*, morphological and morphometric data altogether with multilocus molecular analysis.

Results: Tardigrades were found in 48 among 112 studied samples. In total 22 tardigrade species belonging to 15 genera of both Heterotardigrada and Eutardigrada classes were found. Although already 33 species have been reported from Madeira, we provide next 10 species never reported from this island and four possibly new to science. A new *Macrobiotus* was found in moss sample collected from riverbed of Ribera de Brava, Madeira (32°44'29.8"N 17°01'00.5"W) and it is most similar to *Macrobiotus* cf. *recens* (uncorrected genetic p-distance was 9.37%), based on COI sequences.

Conclusion: The data suggest a high tardigrade diversity on rather small area of Madera Island. It is highly probable that further research on this island will bring more new discoveries of tardigrade species.

Keywords: diversity, *Macrobiotus* sp. nov., Madeira Island, *hufelandi* group, taxonomy, water bears

Wednesday-Thursday stand **7** | side **A**

Paramacrobiotus fairbanksi Schill, Förster, Dandekar & Wolf, 2010 – a cosmopolitan tardigrade

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Background: *Paramacrobiotus fairbanksi* was described from Alaska (USA) based on genetic markers and later reported from Antarctic, Italy, Poland and Spain. Hypothesis 'Everything is Everywhere' (EiE) assumes that microscopic organisms have unique features which help them to inhabit many different environments and are considered cosmopolitan. The most frequently mentioned adaptations are the passive dispersion (through wind, sea currents, freshwater, other animals, etc.), the presence of very resistant spore stages (cysts, eggs or cryptobiotic individuals) that help to survive extreme conditions, as well as the presence of asexual or parthenogenetic reproduction, allowing rapid increase in the number of individuals. However, the cosmopolitanism of microscopic organisms is increasingly discussed and undermined also within tardigrades.

Methods: Four moss and lichen samples were collected in 2018 (Mongolia) and 2019 (Albania, Canada and Madeira). Tardigrades were extracted from the samples, studied and measured according to standard methods. In total, 178 specimens of the *Pam. fairbanksi* were identified using light microscopy and later used in morphological and genetic studies. Four DNA fragments differing in effective mutation rates were sequenced: nuclear sequences, *i.e.*, 18S rRNA, 28S rRNA and ITS-2 and mitochondrial COI. Also, statistical significance of the differences in morphometrics between the studied populations were analysed using the ANOVA test with Bonferroni correction for multiple comparisons.

Results: A four populations of the *Pam. fairbanksi* from Northern Hemisphere are reported and analysed. Moreover, we also compared (genetically and morphologically) all known populations of *Pam. fairbanksi*. We found some statistically significant morphological, genetic and morphometric differences between the populations of *Pam. fairbanksi* from different regions.

Conclusions: Parthenogenetic eutardigrade *Pam. fairbanksi* has wide distribution around a world which suggest that 'EiE' hypothesis is true, at least for some tardigrade species.

Keywords: cosmopolitism, dispersal, "Everything is Everywhere" hypothesis, water bears, zoogeography

Wednesday-Thursday stand **7** | side **B**

Two new species of the genus *Minibiotus* from an urban area of Northern Argentina

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Background: The limno-terrestrial tardigrade fauna of Argentina has been investigated methodically just in the last two decades, but current knowledge is still incomplete. So far, 135 species are known for the country, of which only 5 belong to the genus *Minibiotus* R.O. SCHUSTER, 1980. Until 1988, the genus *Minibiotus* was monotypic, with only *Minibiotus intermedius* (PLATE, 1888), but today, thanks to very numerous contributions, the number of species of the genus has risen to 55. In the present contribution, we describe two species of that genus from Salta city (Argentina), which gives also the opportunity to discuss some taxonomic and morphological aspects within the genus.

Methods: Samples of moss and lichens growing on sidewalk trees from Salta city (province of Salta, Argentina), were collected in 2014; tardigrades were extracted and mounted on permanent slides with polyvinyl-lactophenol medium and deposited, without diagnosis, in the Rocha and Doma collection of the Department of Natural Sciences at the National University of La Pampa, Argentina in 2016. The revision of that collection (using PCM microscopy), in the present case revealed the two new species here described, but we have obtained new specimens for ongoing SEM and DNA barcoding analyses.

Results: *Min*. **sp. nov.** 1 has ten transverse bands of variously shaped cuticular pores, arranged in transverse rows, with differences between smaller and larger specimens. Three macroplacoids and a microplacoid in the pharynx. The eggs have small conical processes and granulated chorion. *Min*. **sp. nov.** 2 has dotted cuticle without pores, three macroplacoids and a microplacoid, and egg of the *intermedius* type. The two new species are morphologically and morphometrically well differentiated from all other species of the genus.

Conclusions: The description of the two new species contributes to the broadening of knowledge on taxonomy, morphology and faunistics of the genus *Minibiotus*, and on the tardigrade fauna of Argentina and the Neotropical region.

Keywords: α-diversity, lichens, moss, Salta city, tardigrades, urban biodiversity

Native fauna of tardigrades from two natural areas of the Argentina Republic

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Background: The increase in the degradation and destruction of natural habitats has reinforced the need to know and conserve the biodiversity found in them. Some authors highlight the importance of the study of invertebrates as a key group in conservation biology, especially in the natural environments. Our objective is to contribute to the taxonomic and ecological knowledge of tardigrade communities in disturbed natural environments, identifying indicator species that can be used for the conservation and monitoring of disturbed natural environments.

Methods: The sampling was carried out in 2018 in disturbed natural environments, belonging to Parque Luro Provincial Reserve in La Pampa province and Polygon A Municipal Ecological Reserve in Salta province. The samples were taken from epiphytic communities that grow on the bark of trees. Different environmental variables of the microhabitat were considered.

Results: A total of 1538 specimens were extracted and 11 species were identified. The highest abundance of tardigrades was found in La Pampa (N=1326), while Salta had the highest species richness (S=9). Native communities did not show a statistically significant difference in abundance and species richness ($p > 0.05$). The dominant species in La Pampa was *Viridiscus rufoviridis*, while in Salta it was *Pseudechiniscus saltensis*. Ordination analysis shows that the communities of La Pampa were explained by the temperature and humidity of the microhabitat, meanwhile the communities of Salta by thickness of the microhabitat. Four indicator species were reported for La Pampa (*V. rufoviridis, Macrobiotus kristenseni, Ramazzottius* cf. *oberhaeuseri*, *Milnesium pelufforum*) and one for Salta, *P. saltensis*. Diversity partitioning β showed that species turnover was the main component in the difference between the Salta and La Pampa communities.

Conclusions: It represents the first contribution on the native fauna of tardigrades for Argentina, also providing information on tardigrades in disturbed natural environments.

Keywords: alpha-beta diversity, invertebrates, La Pampa, lichen, moss, Salta

Wednesday-Thursday stand **8** | side **B**

Preliminary study on tardigrade taxocenosis in epiphytic cryptogams of the Patagonian steppe, Nahuel Huapi National Park, Argentina

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Background: Knowledge of tardigrades in National Parks is poorly understood worldwide. Especially in Patagonia, records of tardigrades are sparse and mainly concentrated in Andean-Patagonian forests, so there is an information gap regarding the steppe areas. The main objective of this work is to study the structure and composition of the tardigrade taxocenosis in epiphytic cryptogams of the steppe in Nahuel Huapi National Park, Argentina.

Methods: The study area is characterised by dry and cool weather with strong winds from the west and annual precipitation averaging 200 mm. The predominant vegetation is spiny shrubs and grasses. Tardigrades were sampled from epiphytic cryptogams (bryophytes and lichens) growing on native tree trunks. Various biotic and abiotic environmental variables were considered.

Results: Five species of eutardigrades were identified and no heterotardigrade was detected. The species found belong to the following genera: *Macrobiotus*, *Mesobiotus*, *Milnesium*, *Minibiotus*, and *Ramazzottius*, the last genus being clearly the most abundant. Subsequently, the α-diversity was analysed and the indicator species were determined.

Conclusions: The present work represents a preliminary study on ecological and taxonomic aspects of tardigrades within the Patagonian steppe.

Key words: α-diversity, bryophytes, lichens, Patagonia, protected natural areas, steppe

New tardigrade records from Montana, USA

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Background: In 1987 Maucci recorded tardigrade species collected in Yellowstone National Park, Montana, USA. Five species were new to science.

Methods: In June 2022 the first author collected moss and lichen samples in an around YNP. Specimens were measured for morphometric analysis using phase contrast microscopy. Some specimens were sequenced for the ITS2 gene.

Results: None of the new species described by Maucci were found. Several species were present in moss on a rock face in Lava Valley, YNP. Many specimens and eggs morphologically consistent with *Paramacrobiotus richtersi* were present, a species recorded by Maucci in 1987. The ITS-2 region was 100% identical in three sequenced animals and P-distances for this sequence calculated from sequences obtained from GenBank were as follows: *Paramacrobiotus fairbanksi* (0.3%), *Paramacrobiotus lachowskae* (38.8%), and *Paramacrobiotus experimentalis* (54.2%). We are presently examining the ITS-2 region of more animals as well as the COI gene. Other specimens were morphologically consistent with *G. prorsirostre*, *Diphascon* sp., and *Platicrista brunsoni*. Molecular data for these three species are pending.

Conclusions: The tardigrade identified by Maucci in YNP as *Paramacrobiotus richtersi* is in fact *P. fairbanksi.* This is the first record of *P. fairbanksi* in USA other than Alaska. This correction underscores the importance whenever possible of re-examining, using integrative methodology, old records of species from taxa considered 'cosmopolitan', especially when collected far from their type localities. At this time, we conclude that the presence of *P. richtersi* in North America is not confirmed. Maucci noted the presence of *Guidettion prorsirostre* in YNP. Our *Diphascon* sp. is morphologically consistent with *D. alpinum.* Maucci reported the presence of *D. pingue*; based on literature descriptions the two species are very similar morphologically. *Platicrista brunsoni* from YNP is morphologically consistent with specimens from elsewhere in Montana.

Keywords: cosmopolitan species, integrative taxonomy, North American Tardigrada, species identification

Wednesday-Thursday stand **9** | side **B**

Is there a universal DNA barcode gap in tardigrades?

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Background: Nearly two decades have passed since DNA barcoding was introduced in 2003, and it still plays a vital role for species delimitation across all organisms. One of the main difficulties of DNA barcoding is defining a clear-cut method to determine species boundaries. Among others, the identification of the barcode gap (*i.e.* the limit between smaller intraspecific distances and larger interspecific distances) is a very fast and less computationally demanding tool. In tardigrades, the first application of DNA barcoding was on *Macrobiotus* species. It was proposed that the tardigrade barcode gap to distinguish between species was around 3% p-distance. Today, with the integrative taxonomic approach being adopted by many tardigrade research groups, there is a plethora of available *cox1* sequences. The analysis of these sequences will allow understanding if there is a universal value of DNA barcoding gap in Eutardigrada.

Methods: All *cox1* sequences pertaining to Eutardigrada (both Apochela and Parachela orders) were analysed using the Assemble Species by Automatic Partitioning method (ASAP). In ASAP, the sequences are sorted into hypothetical species based on the barcode gap. The program first detects the barcode gap as the first significant gap beyond a model-based one-sided confidence limit for intraspecific divergence, and then uses it to produce several partitions of the data. Then ASAP computes an *ad hoc* ASAP-score for each defining partition, with the lower score indicating the better partition, thus overcoming the challenge of *a priori* definition.

Results: The ASAP analysis pointed out a high variability of the threshold value of the barcoding gap, depending on the considered taxa. For example, in the *Macrobiotus* genus the threshold value was 4.5%, while, when considering all Macrobiotoidea taxa, the threshold value was highly different (13.9%).

Conclusion: The definition of a clear-cut barcode gap for tardigrades is presently very difficult to achieve. Present data point out that relying on a single method for species delimitation will probably give a potentially distorted assessment of species boundaries, thus suggesting that using different tools based on different methods and frameworks would possibly achieve a most comprehensive biodiversity assessment.

Key words: Apochela, *cox1*, Eutardigrada, Parachela, species delimitation, taxonomy

A tardigrade in Dominican amber

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Background: Tardigrades are a diverse group of charismatic microscopic invertebrates that are best known for their ability to survive extreme conditions. Despite their long evolutionary history and global distribution in both aquatic and terrestrial environments, the tardigrade fossil record is exceedingly sparse. Molecular clocks estimate that tardigrades diverged from other panarthropod lineages before the Cambrian, but only two definitive crown-group representatives have been described to date, both from Cretaceous fossil deposits in North America. Here, we report a third fossil tardigrade from Miocene age Dominican amber.

Methods: We used confocal fluorescence microscopy to visualise the taxonomically important morphological characteristics. We performed morphology-based phylogenetic analyses to determine the relationship of this new fossil relative to extant tardigrades. We also tested the ability of X-ray computed tomography microscopy to visualise the external and internal morphological structures of a tardigrade fossil embedded in amber.

Results: We were able to obtain high-quality images using confocal fluorescence and X-ray computed tomography microscopy that allowed us to visualise, for the first time, taxonomically important internal morphological structures in a tardigrade fossil such as claws and the buccal apparatus. This allowed us to describe *Paradoryphoribius chronocaribbeus* gen. et sp. nov. as the first unambiguous fossil representative of the diverse superfamily Isohypsibioidea, as well as the first tardigrade fossil described from the Cenozoic.

Conclusion: Our results highlight the usefulness of these imaging techniques in studying and determining the phylogenetic relationships of extant and extinct tardigrades. Lastly, we discuss that the patchy tardigrade fossil record can be explained by the preferential preservation of these microinvertebrates as amber inclusions, coupled with the scarcity of fossiliferous amber deposits before the Cretaceous.

Keywords: Eutardigrada, invertebrate palaeontology, Miocene, *Paradoryphoribius*

Quantifying the shape of the tardigrade claws

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Background: The shape of the claw is one of the most important characters in tardigrade taxonomy. Among parachelans, for example, the morphology of their claws can used to identify them at the superfamily or family level. However, the description of this important trait is normally done qualitatively via visual inspection, which can introduce worker biases. In this study, we aimed to quantify the shape of the claws to increase the reproducibility of species descriptions and facilitate their identification.

Methods: We focused on species from three tardigrade families (Hypsibiidae, Doryphoribiidae, and Isohypsibiidae), which represents two superfamilies (Hypsibioidea and Isohypsibioidea) that are known to have different claw morphologies. We used geometrics morphometrics (geo-morpho) to measure two characters: the angular connection of the secondary branch to the basal section, and the presence of a flexible connection between the primary branch and the basal section.

Results: Our results showed that our analysis was able to distinguish the two superfamilies in terms of the angles of their external claws, but not of their internal claws. This suggests that the shape of the internal claws in these two superfamilies are homogenous and cannot be used for distinguishing the two groups. Although not as diagnostic compared to the angular measurements, our analysis was able to distinguish the two superfamilies in terms of the presence of the flexible connection. We also saw discrepancies between the results of our quantitative geo-morpho analysis and the traditional morphological descriptions. This suggests cryptic morphologies in tardigrades that are not easily observed when using qualitative methods and could affect their taxonomic placements. These highlights the need of DNA barcoding to complement the systematic descriptions of newly discovered species or the acquisition of more high-quality images to aid in reproducibility and correct species identification.

Conclusions: In summary, our geo-morpho analyses provide an alternative way to describing important tardigrade morphological characters. This will be useful for increasing robustness in coding characters for morphology-based phylogeny and comparing with samples wherein DNA extraction is impossible, such as with tardigrade fossils.

Keywords: angles, claws, flexible curve, geometric morphometrics, quantification

Milnesium guanyinensis sp. nov. (Eutardigrada: Apochela: Milnesiidae) from Yunnan, China

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Background: *Milnesium guanyinensis* sp. nov. is a new species of Eutardigrada described from China.

Methods: We used the traditional morphology-based taxonomic analysis, supported by morphometrics, light microscopy imaging and scanning electron microscopy.

Results: The new *Milnesium* species is characterised by its reticulated dorsal cuticle and a [2-2]-[2-2] claw configuration (CC) in hatchlings and a [2-3]-[3-2] CC in juveniles and adults. *Milnesium guanyinensis* sp. nov. is most similar to *Milnesium pacificum* but differs by: the brownish body colour and more delicate dorsal cuticular reticulum; relatively longer spurs on claws III (*pt=12.6–15.5* in the new species *vs 7.5–12.4* in *M. pacificum*) in juveniles and adults; a higher *pt* of the stylet supports insertion point (*67.1–72.1*, mean *69.1* in the new species *vs 58.7–69.2*, mean *63.9* in *M. pacificum*) in hatchlings.

Conclusions: The discovery of the new species raises the number of first known tardigrade species of the genus *Milnesium* in China to three. This small number, compared to other regions of the world, suggests that further study of the tardigrade fauna in this area is needed. *M. guanyiensis* sp. nov. is also is also the 52th described species in the genus *Milnesium*.

Keywords: China, *Milnesium*, new species, Tardigrada

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Frozen treasures: extraordinary morphotypes of *Milnesium* species (Tardigrada: Apochela) from the Antarctic

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Background: Some years ago, it was noted that *Milnesium* species from the Antarctic had characters not seen or described for species outside the continent. Since then, there have been numerous studies that have provided the descriptions of new *Milnesium* species from around the World. However, the peculiar characters seen in the Antarctic species have not been noted elsewhere, suggesting the uniqueness of the southern fauna. The aim of our study is to describe in detail the unique morphological features of the Antarctic *Milnesium* species.

Methods: We analysed several microscope slides originating from the following localities: Terra Firma Islands, Charcot Island, Larsemann Hills, and Wakefield Mountains. In addition, we compared them with *M.* cf. *quadrifidum* Nederström, 1919 from Iceland. All specimens were imaged in Phase and Nomarski Contrast Microscopes.

Results: Four species new to science, characterised by distinct morphological traits, were identified. Surprisingly, many possess a band of pores or pseudopores in the buccal tube at the level of styles sheaths. These (pseudo)pores are most pronounced in the species from Wakefield Mountains. Such a character has never been reported for any tardigrade. The claws of the investigated species exhibited three different claw configurations: [3-3]-[3-3] in two species from Terra Firma Islands and Charcot Island; [4-4]-[4-4] in the species from Larsemann Hills (similar to but different in several characters when compared with *M.* cf. *quadrifidum* from the Northern Hemisphere); and a new configuration [3^{+}-3^{+}]-[3^{+}-3^{+}], where the highest point is split into 4–6 smaller points arranged in a comb (species from Wakefield Mountains), a character unseen in any other apochelan species. Moreover, this species possesses granules on the caudal part of the cuticle (this is the first observation of cuticular granulation in Apochela).

Conclusions: The apochelan fauna of Antarctica is still poorly investigated and almost certainly includes unique phylogenetic lineages, possibly harbouring even a new genus or genera, that are characterised by extraordinary morphological traits. These taxa may be of crucial importance to understand the evolution of morphological traits in the order Apochela and to untangle phylogenetic relationships within the family Milnesiidae.

Keywords: Antarctica, Milnesiidae, morphological traits, new species

The study was supported by the Polish National Science Centre (NCN Preludium 2019/35/N/NZ8/04487 and Sonata Bis 2016/22/E/NZ8/00417).

Phylogenetic position and validity of the genus *Milnesioides* Claxton, 1999 (Eutardigrada: Apochela)

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Background: The order Apochela comprises four genera, of which *Milnesium* Doyère, 1840 contains multiple species and the remaining ones, *Limmenius* Horning *et al.* 1978, *Milnesioides* Claxton, 1999 and *Bergtrollus* Dastych, 2011 are monotypic. More importantly, whereas there are ample genetic data for numerous *Milnesium* species, no other apochelan genus has been sequenced and their phylogenetic positions remain unknown. Moreover, the recent discovery of new African *Milnesium* species, characterised by an elongated head with a "snout" and a very long buccal tube, challenged the validity of *Milnesioides*. Thus, establishing the phylogenetic position of this genus become an urgent challenge.

Methods: Two populations of *Milnesioides* originating from Western Australia were sequenced (18S and 28S rRNA, ITS-2 and COI) and imaged in Light Contrast Microscopy (LCM). The Bayesian inference phylogeny was run in BEAST, with no priors concerning the tree topology. The morphological comparisons in PCM with the African long-tubed *Milnesium* were also conducted.

Results: Both populations represent a single *Milnesioides* species. The obtained molecular phylogenetic tree clearly indicated *Milnesioides* as a sister lineage to all remaining *Milnesium* species, supporting the validity of the genus. In line with the molecular evidence, the detailed morphological comparisons indicated pronounced differences between the analysed *Milnesioides* sp. and the African long-tubed *Milnesium*, especially in the buccal apparatus morphology as well as cuticle sculpturing.

Conclusions: The validity of *Milnesioides* is now genetically confirmed, and further supported by newly observed morphological traits of the buccal apparatus. The sister relationship of *Milnesioides* and *Milnesium* lends support to the Gondwanan origin of the order Apochela. Several morphological differences between the original description of *Milnesioides exsertum* Claxton, 1999 from the eastern part of the continent and the western Australian specimens suggest that the genus is not monotypic.

Keywords: bucco-pharyngeal apparatus, Eutardigrada, *Milnesium*, Milnesiidae, molecular phylogeny, morphological traits

The study was supported by the Polish National Science Centre (NCN Preludium 2019/35/N/NZ8/04487 and Sonata Bis 2016/22/E/NZ8/00417).

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Rough backs: taxonomic value of epicuticular sculpturing in the genus *Milnesium* Doyère, 1840 (Tardigrada: Apochela)

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Background: The genus *Milnesium* Doyère, 1840 (Eutardigrada: Apochela) is characterised by a scarcity of taxonomically meaningful morphological traits. One of the most important qualitative phenotypic characters is the dorsal cuticle sculpturing, dividing the genus into two morphogroups: *tardigradum* and *granulatum*. The first one is characterised by evident sculpturing, usually in the form of reticulation, visible in both light contrast microscopy (LCM) and scanning electron microscopy (SEM), whereas the cuticle in the *tardigradum* group appears as smooth in LCM but might be finely sculptured in SEM. Irrespective of epicuticular sculpturing, pseudopores and pseudoplates may also be present on the dorsal cuticle and their taxonomic value is currently a subject of vivid discussion.

Methods: In this study, we address several aspects of the cuticle morphology and its taxonomic value in the genus *Milnesium*. We imaged several species from both *granulatum* and *tardigradum* morphogroups in LCM as well as in SEM, including a so far undescribed species from Columbia, characterised by unique cuticle sculpturing.

Results: We present high resolution LCM and SEM images of the dorsal cuticle of species representing *granulatum* and *tardigradum* morphogroups. For the first time, we also show photographs of a *Milnesium* species in which the entire cuticle sculptured. Furthermore, we provide schemes and cross-sections of sculpture types to explicitly indicate the differences between the studied morphogroups. Finally, we demonstrate that some species exhibit no pseudoplates.

Conclusions: The new data clearly indicate, that the dorsal cuticle sculpturing is still understudied and even in a genus with strongly limited number of morphological traits, the so far undescribed variability is still yet to be discovered. Although the *granulatum* and *tardigradum* morphogroups are not monophyletic, they are useful in taxonomy for constructing differential diagnoses and taxonomic keys. The taxonomic value of pseudopores is recognised as very low due to large intraspecific variability. In contrast, pseudoplates are considered a promising morphological trait, potentially useful for delineation and identification of at least some species in the genus.

Keywords: dorsal cuticle sculpturing, Milnesiidae, morphology, pseudoplates, pseudopores, reticulum, taxonomy

The study was supported by the Polish National Science Centre (NCN Preludium 2019/35/N/NZ8/04487 and Sonata Bis 2016/22/E/NZ8/00417).

Wednesday-Thursday stand **13** | side **A**

A stowaway or parasite? Insights into the nature of *Pyxidium tardigradum*

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Background: *Pyxidium tardigradum* (Van der Land, 1964) is a ciliate peritrich belonging to the protozoan family Operculariidae (Faure-Fremiet, 1979). It is a rarely encountered phoretic species that utilises limno-terrestrial tardigrades as hosts for dispersal and transport to new resources. It has been reported from various European countries, Asia (Kyrgyzstan) and from Antarctica. Since its discovery, because of the perceived impact heavy infestation may have on individual tardigrade hosts, the symphoriont nature of the ciliate has remained in doubt. Our aim was to contribute to the understanding of this peculiar relationship.

Methods: In November 2020 a sample of the lichen *Xanthoria parietina* was collected in North Aberdeenshire, Scotland. The sample contained tardigrades and numerous *P. tardigradum*. All tardigrades with and without ciliates were mounted onto permanent slides, imaged, and measured. Statistical analysis and tests were performed. The literature regarding *P. tardigradum* was reviewed to facilitate the debate regarding the phoront-host relationship.

Results: A total of 116 eutardigrades representing 4 species were recovered, *i.e.* (in order of abundance): *Hypsibius* cf. *scabropygus*, *Macrobiotus scoticus*, *Ramazzottius* cf. *oberhaeuseri* & *Milnesium variefidum*, with most of them (77%) not being infested. A total of 35 *Pyxidia* were found, with the majority attached to *M. scoticus* (22 *Pyxidia*, giving an overall infestation rate of 39%). Statistical analysis showed that *Pyxidium* exhibited a preference for larger hosts. The existing literature assumes the phoront may indirectly and deleteriously impact host fitness by: *(i)* increasing the energy cost of locomotion; *(ii)* impeding the host's locomotion in dense environments; *(iii)* generating unfavourable water currents (through the rotary action of the aboral cilia) which may affect foraging by the tardigrade; *(iv)* reducing host predation avoidance; and *(v)* potentially reducing the host's reproductive success.

Conclusions: Our findings are in accordance with the published literature. There are still many questions regarding *P. tardigradum* biology and phenology to be answered, including: Is *P. tardigradum* a single species or a species complex? How does the ciliate locate its hosts? Does *Pyxidium* infest heterotardigrades or attach to non-tardigrade substrates? In the meantime, the first author would be grateful for any records, formal or informal, relating to the occurrence of *P. tardigradum* (e-mail: karwalach@gmail.com).

Keywords: Eutardigrada, parasites, phoresy, Peritrichia, *Pyxidium tardigradum*, Scotland, symbiosis, United Kingdom

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417).

Integrative description and phylogenetic position of a new Afro-Oriental *Viridiscus* species complex (Heterotardigrada: Echiniscidae)

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Background: In 2019, Bhakare & Pai published a summary on the freshwater tardigrades of Western Ghats of India. They reported *Barbaria*, a primarily Neotropical echiniscid genus with a unique sculpturing type, to be present in limnic habitats in the Oriental region. After the verification of microphotographs, it became clear that this suspicious record is incorrect and animals represented a new *Viridiscus* species of a phenotype diverging from the morphology typical for the genus. A similar species was found in 2018 in lichen samples from Tanzania, yet it remains undescribed due to extraction of only two individuals. The finding of more abundant populations in the Nilgiri Mountains (Tamil Nadu, India) allowed us to elaborate on this extraordinary new *Viridiscus* morphotype.

Methods: The new *Viridiscus* populations were examined with the application of light contrast (LCM), scanning electron microscopy (SEM), and DNA barcoding of five genetic markers (18S rRNA, 28S rRNA, ITS-1, ITS-2, COI). Some animals were photographed when alive and at subsequent time intervals after mounting in Hoyer's medium to test whether body colour is subject to ontogenetic variability. The species were placed on the generic phylogeny tree, constituting an update of the most recent published *Viridiscus* phylogeny.

Results: We investigated 31 specimens (29 from India, 2 from Africa) under LCM, 8 specimens under SEM and analysed genetic data obtained from 7 specimens representing two Indian populations. The morphology and phylogenetics suggested the presence of three new species and corroborated their affinity within *Viridiscus*, at the same time pointing to an isolated character of the new species among the congeners, and a stunning case of crypsis in the two Indian species.

Conclusions: Unique caramel-like body colour, extremely short cirri *A* (<10% of the body length), large pedal plates with pores identical to those in the dorsal armour, and dorsal cuticular sculpture comprising two distinct types of pores differentiate these new species from all other members of this genus. We provide the first *Viridiscus* record from Palaeotropics and India. Whereas the African and Indian spp. can be differentiated, the two Indian spp. seem to be phenotypically identical. In contrast to other *Viridiscus* spp. that typically occur in anthropogenic habitats, the new species dwell in undisturbed, natural environs.

Keywords: cuticle, integrative taxonomy, pantropical, phylogeny, species delimitation

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417).

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Neotropical jewels in the moss: biodiversity, distribution and evolution of the genus *Barbaria* (Heterotardigrada: Echiniscidae)

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PIOTR GĄSIOREK[1] & ŁUKASZ MICHALCZYK[1]

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Background: The genus *Barbaria*, recently established to accommodate the former *Echiniscus bigranulatus* group, is a tardigrade group emblematic for the South American tardigrade fauna. This unappendaged echiniscid lineage is widely recognised for the so-called 'double' sculpturing composed of endocuticular pillars and pseudopores or pores in the dorsal cuticle. The phylogenetic relationships in the genus have so far been completely unknown, but the discovery of two new species (*B. paucigranulata* and *B. weglarskae*), together with new genetic data for further six species (*B. bigranulata*, *B. charrua*, *B. danieli*, *B. jenningsi*, *B. madonnae* and *B. ollantaytamboensis*), created an opportunity not only to uncover phyletic relationships, but also to reconstruct morphological evolution in the genus.

Methods: To achieve this, we sequenced five genetic markers (18S rRNA, 28S rRNA, ITS1, ITS2, COI) for multiple populations of eight species of *Barbaria* (two-thirds of all known species) collected in Alabama (USA), Argentina and the Antarctic, and we analysed them in tandem with detailed morphological data.

Results: In our research, we presented reconstructions of ancestral character states, thanks to which we were able to formulate several hypotheses of the evolution of systematically significant features in this genus. We have also thoroughly described two new species from the genus: *B. paucigranulata* sp. nov. and *B. weglarskae* sp. nov. Based on our analyses, we also made several taxonomic transfers: *Barbaria pseudowendti* to *Claxtonia* (*C. pseudowendti*), *Echiniscus charrua* and *Echiniscus quitensis* to *Barbaria* (*B. charrua* and *B. quitensis*).

Conclusions: Our phylogenetic analyses and the reconstruction of evolution of morphological traits suggest that the ancestor of the genus inhabited the Neotropics, and it was morphologically most similar to *B. bigranulata*. We also analysed literature records of *Barbaria* and concluded that the genus is most likely limited to the Neotropics, Antarctica and southern parts of the Nearctic. The findings are discussed in the context of the phylogeny of the *Echiniscus* evolutionary line.

Keywords: cuticle, integrative taxonomy, morphometry, phylogeny, species delineation, tardigrades, trait evolution

The study was supported by the Polish National Science Centre (NCN Sonata Bis 2016/22/E/NZ8/00417 and Preludium 2019/33/N/NZ8/02777).

Wednesday-Thursday stand **14** | side **B**

Lost in the Arctic and in the mountains – the (dis)entangled classification of *Claxtonia* (Heterotardigrada: Echiniscidae)

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Background: *Claxtonia wendti* (Richters, 1903) is among the first-described tardigrade species, and belongs to a large group of tardigrades with type locales in Svalbard. Historically, this taxon posed a continuous and recurring problem in taxonomic identification, as the original description does not include even a brief description of the dorsal plate sculpturing. Consequently, the species was frequently misidentified with other unappendaged echiniscids, including the poorly described and dubious *Echiniscus arctomys* Ehrenberg, 1853. Therefore, an integrative redescription of *C. wendti* is needed.

Methods: We sequenced (18S rRNA, 28S rRNA, ITS-1, ITS-2 & COI) numerous populations of *Claxtonia* collected in Europe (Austria, Bulgaria, Iceland, Italy, Norway, Poland, Scotland), Asia (Malaysia), North (USA) and South America (Argentina, Colombia, Trinidad and Tobago), including the *terra typica* of *C. wendti* in Svalbard and of *C. pardalis* (Degma & Schill, 2015) in Italian Alps. Populations were also examined using the light and scanning microscopy in search for potential morphological signs of differentiation between the analysed taxa.

Results: We inferred a well-resolved generic tree that contains eight species: *C. molluscorum* (Fox & Garcia-Moll, 1962), characterised by a wide tropical distribution and probably more related to *Viridiscus* than to *Claxtonia*; *C. mauccii* (Ramazzotti, 1956), a Nearctic endemic, *C. wendti*, and five related species. These new species must currently remain unnamed due to problems with diagnoses of formerly described species of *Claxtonia* (*C. corrugicaudata* (McInnes, 2010), *C. goni* Degma *et al.*, 2021) that make species delimitation extremely difficult. We discuss the morphological variability within the genus and formally establish the neotype for *C. wendti*, what makes it the first step towards sorting out the taxonomy of this lineage.

Conclusions: Species of *Claxtonia* are potentially distinguishable based on subtle differences in sculpturing. Some of them are widely distributed tropical (*C. molluscorum*) or montane, intercontinental (a potentially new species that reminds *C. corrugicaudata* inhabits both Argentinian Patagonia and Italian Alps) species. The recovered southernmost locality of *C. wendti* are the Polish Tatras, and the species is confirmed from Great Britain, the Scandinavian Peninsula, and Iceland. *Claxtonia*, along *Pseudechiniscus*, probably represents one of the most taxonomically challenging echiniscid groups. This study highlights how the lack of DNA sequences may considerably impede description of tardigrade species diversity.

Keywords: crypsis, cuticle, Echiniscoidea, integrative taxonomy, phylogeny, species delimitation

The study was supported by the Polish National Science Centre (NCN Preludium 2019/33/N/NZ8/02777 and Sonata Bis 2016/22/E/NZ8/00417).

Phylogenetic position of (*Test*)*Echiniscus meridionalis* (Murray, 1906) (Heterotardigrada) revealed

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Background: While revising the heterotardigrade family Echiniscidae in 1987, Kristensen included all *Echiniscus*-like taxa with well-developed ventral plates in a newly formed genus *Testechiniscus*. This entailed the transfer of *Echiniscus meridionalis* (Murray, 1906), a remarkable dioecious species known from sub-Antarctic islands. However, it appeared in recent years that many sexually adult specimens of *Echiniscus* species exhibit subcephalic and/or genital plates, rendering the ventral armour a convergent/plesiomorphic trait. *Testechiniscus* should be thus diagnosed as a genus with black crystalline eyes, dorsolateral spicules in positions C^l and D^l, and the *blumi-canadensis* type of dorsal sculpturing. Only *T. spitsbergensis* (Scourfield, 1897) and *T. laterculus* (Schuster *et al.*, 1980) fulfil these criteria, whereas *T. macronyx* (Richters, 1907) and *T. meridionalis* should be removed from *Testechiniscus*.

Methods: Taking advantage of finding numerous and specimen-rich populations of *T. meridionalis* in King George Island (South Shetland Islands), we conducted an integrative study in order to clarify its phylogenetic affinities: five markers were sequenced (18S rRNA, 28S rRNA, ITS-1 and ITS-2, COI), and tardigrade individuals were analysed under light and scanning microscopy.

Results: The reconstructed phylogeny firmly placed *T. meridionalis* as a sister, closely related species of *E. aonikenk* Gąsiorek *et al.*, 2021, a peculiar Neotropical *Echiniscus* described from Patagonia. The two species have males in their populations, although they differ in the intensity of sexual dimorphism: pronounced in the former, and less in the latter. Morphology of *T. meridionalis* is re-described with modern taxonomic tools.

Conclusions: *Testechiniscus meridionalis* is transferred back to *Echiniscus*, and its affinity to *E. aonikenk* supports the hypothesis on the relictual (Gondwanan) character of some echiniscid taxa. *Testechiniscus* is re-defined accordingly. *Testechiniscus macronyx* should be re-analysed and withdrawn from the genus.

Keywords: Antarctica, Echiniscidae, Gondwanan ancestry, phylogeny, ventral sculpturing

The study was supported by the Polish National Science Centre (NCN Preludium 2019/33/N/NZ8/02777 and Sonata Bis 2016/22/E/NZ8/00417).

Wednesday-Thursday stand **15** | side **B**

A new, catfish-headed *Cornechiniscus* (Heterotardigrada) illuminates evolution of the genus

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Background: *Cornechiniscus* Maucci & Ramazzotti, 1981 is a species-poor heterotardigrade genus with peculiar, horn-shaped appendages *A*. It can be found in mosses and lichens growing on dusty soils on all continents except for Australasia and Antarctica, with presumably Central Asia as the main place of species radiation. A recent, COI and ITS-1-based phylogeny elucidated phylogenetic relationships between 5 out of 10 *Cornechiniscus* species, and aided the integrative description of *C. imperfectus* Gąsiorek & Michalczyk, 2020 from mountains of Northern Kyrgyzstan. However, the other 5 species remained unplaced on the generic evolutionary tree.

Methods: Using new samples from Northern Kyrgyzstan and Italy (Piz Boè, Dolomites, indicated by Maucci to harbour the only continental European population of cold stenothermic *C. holmeni* (Petersen, 1951)), I extracted new representatives of the genus and updated the genus phylogeny in the frame of integrated taxonomic approach (light and scanning microscopy, DNA sequencing of four markers: 18S rRNA, 28S rRNA, ITS-1, and COI).

Results: The genus is currently divided into three phyletic lineages: a clade of medium-sized species (adult females typically <<500 µm) with a posterior margin of the pseudosegmental plate IV' bearing lobated extensions (*C. lobatus* (Ramazzotti, 1943), *C. madagascariensis* Maucci, 1993, and *C. subcornutus* Maucci & Ramazzotti, 1981), a clade of large species (adult females ~≥500 µm) with appendages in the lateral positions *C–E* (*C. holmeni*, *C. imperfectus*, and a new Asian species most closely resembling *C. tibetanus* (Maucci, 1979), with extremely elongated peribuccal cirri that remind of a catfish head), and, finally, the type species *C. cornutus* (Richters, 1907) with unappendaged body and isonych claws, making it morphologically the oddest of *Cornechiniscus* species. *Cornechiniscus lobatus* is genetically verified from the Neotropics.

Conclusions: With new data, relationships between 7 out of 11 *Cornechiniscus* species are known. Morphology is congruent with reconstructed phylogeny. I demonstrated the sister relationship between *C. holmeni* and *C. imperfectus*, previously hypothesised based on their morphological similarity. The new Kyrgyz species is dioecious and necessitates modifying the genus diagnosis to accommodate its cirri prolongation that contrasts with bulbous peribuccal cirri typical for *Cornechiniscus*. Furthermore, it enhances the hypothesis on the Central Asian origin of the genus. *Cornechiniscus lobatus* can be considered a subcosmopolitan taxon since it certainly occurs in the Holarctic and Neotropics.

Keywords: Central Asia, Echiniscidae, phylogeny

The study was supported by the Polish National Science Centre (NCN Preludium 2019/33/N/NZ8/02777 and Sonata Bis 2016/22/E/NZ8/00417).

Unique dorsal cuticular sculpture and phylogenetic position of *Cornechiniscus holmeni* (Petersen, 1951) from Ella Island, East Greenland

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Background: *Cornechiniscus* is a rare genus, characterised by having horn-shaped cirri *A*. While the relationships among the members of Echiniscidae are debated, *Cornechiniscus* has been considered to be closely related to *Acanthechiniscus* and *Proechiniscus*. Of all the species of the genus, *C. holmeni* and *C. imperfectus* are the only species with long lateral appendices, cirri *C* and *D*; other species have no lateral appendices or only short spines.

Methods: New specimens of *C. holmeni* were collected from Ella Island, East Greenland during 2019 seasons by a KOPRI (Korea Polar Research Institute) research team. Five DNA fragments (18S, 28S, ITS-1, ITS-2, and COI) were sequenced. A phylogenetic analysis was conducted using two DNA markers (COI+ITS-1). The Maximum Likelihood consensus tree and the Bayesian Inference tree were recovered. Unique dorsal cuticle sculptures of specimens were observed through Field-Emission SEM.

Results: Morphometric data of three ontogenetic stages show that the *psc* indices of claws in the larva is larger, while the overall morphology between the juvenile and the adult is similar. The obtained phylogenetic tree suggests that *Cornechiniscus holmeni* is close to *C. imperfectus*, and *C. cornutus* forms a sister group of (*C. holmeni* + *C. imperfectus*). There are several shared morphological characters between *C. holmeni* and *C. imperfectus*: *i.e.* long lateral trunk appendices (cirrus *C* and cirrus *D*), smooth abdomen, strongly heteronych claws, as suggested by a recent study. Each granule on dorsal plates has several radiating branches, joined by several *striae*. Unlike the typical connections between granules in *C. madagascariensis* and *Pseudechiniscus titianae*, the connections of *C. holmeni* do not make a constantly regular pattern, and occur on the cuticular surface. Some granules have a spicule-like structure on the centre. The granules in the animals right after moulting hardly show the connections.

Conclusions: Integrative redescription of *Cornechiniscus holmeni* on the basis of newly collected specimens shows morphological intraspecific variability on trunk appendices, particularly on D^d, pseudosegmental spines and spine *E*. Despite the variability, three ontogenetic stages (larva, juvenile, and adult) show similar morphology, except the *psc* index of claws. *C. holmeni* exhibits a unique cuticular sculpture on the dorsal plates, and the unusual structures of granules may be developed soon after moulting.

Keywords: *Cornechiniscus holmeni*, cuticular sculpture, Greenland

Wednesday-Thursday stand **16** | side **B**

Investigation of arthrotardigrade phylogeny with the inclusion of *Actinarctus doryphorus* and Echiniscoididae sequences

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Background: The marine arthrotardigrades are known for their large morphological variation. Due to the difficulties of taxon sampling, particularly molecular data of Arthrotardigrada are scarce. Although the paraphyly of this clade has been indicated in several studies during the last decades, recent studies have argued that the clade might not be paraphyletic. Here we present new molecular data from *Actinarctus doryphorus* (Tanarctidae) and by including sequences from *Isoechiniscoides sifae* and *Neoechiniscoides aski* (Echiniscoidea, Echiniscoididae) for the first time, we readdress arthrotardigrade phylogeny.

Methods: Universal primer pairs were used to amplify COI, 18S and 28S rDNA sequences from *A. doryphorus* specimens from Roscoff, France. Individual data sets were generated from these sequences, as well as from the 18S and 28S sequences of *I. sifae*, *N. aski*, and other selected Tardigrada species available in public databases. The datasets were individually and combined analysed with Bayesian inference (MrBayes) and Maximum Likelihood (IQ-Tree), and genetic pairwise distances were calculated.

Results: We succeeded in amplifying COI, 18S and 28S sequences from *A. doryphorus* specimens. Prior to the phylogenetic reconstructions, the alignment of the 18S sequences revealed a unique region in the *A. doryphorus* sequence, which is not present in the other tardigrade sequences. In our tree reconstructions, Arthrotardigrada was always inferred as paraphyletic as the clade includes the monophyletic Echiniscoidea. The highest range in pairwise distances for 28S was calculated within the Arthrotardigrada. *A. doryphorus* was placed as a sister-group to *Tanarctus* (Tanarctidae), which was supported by the lowest genetic pairwise distance range among all arthrotardigrade taxa. The Echiniscoididae was inferred as monophyletic and placed as sister-group to the Echiniscidae.

Discussion: By including previously unused species in this study, the paraphyly of Arthrotardigrada was reconfirmed by phylogenetic analyses and pairwise distances. Since *Actinarctus* and *Tanarctus* were grouped in a distinct clade from Halechiniscidae, the recent elevation of Tanarctinae to family rank is supported. The high genetic distances between the Arthrotardigrada species suggest high heterogeneity and an old lineage age. To increase the support of the phylogenetic relationships within the Arthrotardigrada, more molecular data of the clade's representatives are needed.

Keywords: Arthrotardigrada, Bayesian inference, Maximum Likelihood, phylogenetics, 18S, 28S

Structure and diversity of Tardigrada communities from the deep South China Sea

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Background: The knowledge about marine tardigrades from the South China Sea is very scarce with only four species from shallow waters recorded until now.

Methods: The present study investigates the structure and diversity of tardigrade communities from the deep-sea (1517–1725 m bsl) at 8 stations in the South China Sea.

Results: A total of 151 collected arthrotardigrades belong to 11 genera (*Angursa*, *Batillipes*, *Coronarctus*, *Euclavarctus*, *Exoclavarctus*, *Halechiniscus*, *Moebjergarctus*, *Raiarctus*, *Rhomboarctus*, *Tanarctus* and *Tholoarctus*), representing 17 species. Six species are new to Science (five *Angursa* species and one *Halechiniscus* species). Two *Angursa* species (*Angursa* sp. 4 and *Angursa* sp. 3) were the most abundant (25.2% and 14.6% respectively), followed by *Moebjergarctus* sp. (13.9%). The total number of species recorded in the South China Sea is near the global estimate (estimators of Jacknife and Bootstrap predict 19 and 21 species respectively). Specimens were mostly (90.7%) distributed in the upper layer of the sandy-mud sediment (0–1 cm). In different stations the number of species, the Shannon-Winer diversity index and the Pielou's evenness index ranged from 4 to 10; 1.94 to 2.87, and 0.75 to 1.00, respectively.

Conclusions: Since the deep sea is increasingly threatened by some principal anthropogenic impacts (waste deposition, oil and gas extraction, deep-sea mining and fishing), we hope that the results obtained in this study can provide some basic information on the diversity and structure of tardigrade communities that, in the near future, can contribute to assist people to manage human activities and to protect the vulnerable deep-sea areas.

Keywords: deep sea, diversity, Heterotardigrada, marine Tardigrada, meiofauna

Wednesday-Thursday stand 17 | side B

From pole to pole – biodiversity gradients and biogeographic patterns of marine Tardigrada across the Atlantic

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Background: In the project we propose to identify and compare marine tardigrades sampled on expeditions to several biogeographically interesting regions. These regions include seamounts and oceanic islands of the Arctic and temperate North Atlantic and the deep-sea floor from polar to subtropical latitudes. Very few tardigrade records are currently known from these regions, therefore the data will add to the knowledge on global biodiversity of marine tardigrades. The sampling locations roughly span a gradient from northern polar regions over the central Atlantic to the southern polar ocean and will allow regional comparisons across the Atlantic, but also comparisons between seamounts of, *e.g.*, the north-western Pacific and those from the central Atlantic.

Methods: The tardigrade material was collected during different marine expeditions with R/V POLARSTERN and R/V METEOR. It was taken either with a multiple corer or with a sediment grab. Sediment was fixed with buffered formalin and specimens were subsequently extracted by density gradient centrifugation using colloidal silica (Levasil). The majority of tardigrade specimens will be prepared as permanent microscopic slides. Observation and identification of tardigrades takes place using optical, confocal laser scanning and scanning electron microscopy.

Results: At this stage of research, Tardigrada specimens of ANDEEP 2, ANDEEP 3 and ANDEEP-SYSTCO expeditions have been identified to genus or even species level. Highly abundant are species of the genus *Angursa*, less frequent are specimens of the genera *Megastygarctides*, *Coronarctus*, *Trogloarctus, Parmursa*. At a later stage, the results of the different expeditions will be analysed with statistical approaches for community analysis.

Conclusions: Unique biogeographic regions with a variety of sampling sites are a good target for studying large-scale distribution patterns of genera and species of Atlantic Tardigrada. With this unique dataset at hand, it will furthermore be possible to assess if there are latitudinal or bathymetric gradients of their biodiversity.

Keywords: Atlantic, biodiversity, deep sea, meiofauna, Tardigrada

PROCEEDINGS

Symposium Proceedings will be published in the **Zoological Journal of the Linnean Society** (ZJLS), one of the most prestigious zoological journals in the World, published by the **Linnean Society of London**, one of the oldest scientific societies, and by the **Oxford University Press**, one of the most recognised scientific publishers on the planet.

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The Symposium Proceedings will be edited by ŁUKASZ MICHALCZYK, KAZUHARU ARAKAWA and VLADIMIR GROSS.

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ADDITIONAL ACTIVITIES

Kraków, The European Capital of Culture 2000, is a city of a multitude of activities. Thus, if you would like to get to know some more of its charms, here's a short subjective list of recommendations.

City views

If you would like to see Kraków from above, we suggest these options:

- *Wieża Mariacka* (St. Mary's Basilica Tower/Bugle Tower) in *Rynek Główny* (the Main Square). The Towers is famous for the *Hejnał Mariacki* (St. Mary's Trumpet Call) which is played every full hour, in each of the four cardinal directions, by a trumpeter on the highest tower of the Saint Mary's Basilica. The noon call is traditionally broadcasted via Polish radio. The Tower is open Tuesday-Saturday, from 10:00 to 17:30, with entries 5 and 30 minutes after a full hour (a visit is max. 25 minutes). Tickets are 20 PLN and can be bought at the door. Please note that there are nearly 300 steps to get to the top.
- *Balon Widokowy* (The Balloon) at the *Wisła* (Vistula River) bank. If you don't feel like climbing up a tower, this option is for you. Moreover, whereas the Bugle Tower is 80 m high, the Balloon will take you to up as high as 150 m. Thus, although it's farther from the City Centre, the view is greater (you may even see the Tatra Mountains in the south if the visibility is good). The Balloon is filled with helium and it is attached to the ground by a rope, so it operates like a lift. Tickets are 99 PLN (you can buy them on the spot and also online). The Balloon may take up to 30 persons per ride, and it is hard not to notice it in the Kraków sky wherever in the City you may be). Flights are subject to weather conditions.

Pubs

There are numerous pubs, bars and restaurants in Kraków. Many of them are concentrated in *Stare Miasto* (the Old Town) and in *Kazimierz* (Old Jewish Quarter). Whereas the Main Square is a bit more posh (and expensive), Kazimierz is more messy but more laid-back and student-friendly. Although the shortest route from *Rynek Główny* to *Kazimierz* is via *Starowiślna Street*, we suggest a longer, but also a more scenic route via the *Wisła* (Vistula River) Boulevards (if you get tired on the way, you may consider having a drink on one of several barges moored at the river bank).

A short walk in the city will offer a wide choice of pubs in all sorts of styles, but here's just a handful of places we particularly like:

- *Piwnica Pod Baranami* ('The Three Rams Cellar') in *Rynek Główny* (Main Square) has both a beer garden in the Square and a quaint cellar where you can take a nice break from the crowds and summer heat.

- *Mleczarnia* ('Creamery') in Kazimierz at *Meiselsa 20* with its lively beer garden under chestnut trees across the street. The pub used to be a Jewish creamery before WW2.
- *Eszeweria* ('Echeveria') in Kazimierz at *Józefa 9* has a wonderful green beer garden in a yard enclosed between old buildings. The famous disadvantage is a single toilet inside...
- *Alchemia* ('Alchemy') in Kazimierz at *Estery 5* is one of the oldest pubs in Kazimierz and refers to the mysterious medieval history of this district.
- *Absynth* ('Absinth') in Kazimierz at *Miodowa 28* serves interesting drinks, including a range of legendary Absinth spirits served in a traditional way (using an Absinth spoon, sugar cubes and flame).

While you are at Kazimierz, you should try one of Kraków's most popular street food, *zapiekanki* (a foot-long piece of tasty bread with various toppings baked in an oven). There are many places that sell *zapiekanki*, but our favourite is "*NaMaxa Minibar*" in *Okrąglak* (old ritual Jewish slaughterhouse) in the centre of *Plac Nowy*. Chives on top are mandatory! ☺

Boat Cruises

It is good sometimes to take a different perspective on things. Boat cruises along the Vistula (*Wisła*) River offer such a different look at the Royal City. There are a number of companies offering short and long boat trips (some of them will take you to the medieval Benedictine Abbey in Tyniec built in the 11th century). Here are ten best offers according to Tripadvisor.com.

City Sightseeing

Walking around the city may be tiring, especially on a hot summer day. That is why there are many companies that offer city sightseeing with audio guides in small electric cars (so called *Melex* cars). It is hard to get through the Main Square not to be asked if you want a ride, so there should be no problem finding one if you would like to try it.

Museums & Art Galleries

Similarly to pubs and bars, we have a multitude of museums and art galleries in Kraków. A quick online search will provide you with a more comprehensive list, so below are a few we think are among the most interesting:

- *Muzeum Książąt Czartoryskich* (Princes CZARTORYSKI Museum) at *Św. Jana 19*. The most valuable collection in Poland, and one of the most valuable ones in Europe. In 26 exhibition halls, you can see the 'Lady with an Ermine' by LEONARDO DA VINCI or the 'Landscape with the Good Samaritan' by REMBRANDT VAN RIJN, as well as many other masterpieces of not only painting, but also sculpture, crafts, military, applied arts. Tickets are 35 PLN; audio guide is 10 PLN.

- *Muzeum Farmacji* (Museum of Pharmacy) at *Floriańska 25*. Collections are on display throughout all premises of a 14th century building, taking up all Gothic cellars, then spread out through the rooms on all three floors, and ending up right at the attic. Those comprise a variety of pharmacy furnishing in different styles (Baroque, Empire, Biedermeier), pharmaceutical vessels from different eras, including a vast collection of majolica from various European manufactures, old medicinal ingredients of vegetable, mineral and animal origin, old pharmacy utensils (mortars, presses, filters, pill-making machines, dragee-making drums, herb slicers and grinders), old prints (*e.g.* Greek-Latin edition of the complete works of HIPPOCRATES, medieval and early modern pharmacy textbooks, official and unofficial listings of medications, herbal books, handwritten manuals). A small but charming museum. Part of the Jagiellonian University. Tickets are 15 PLN; audio guide is 7 PLN.
- *Galeria Sztuki Polskiej XIX Wieku* (Gallery of 19th-Century Polish Art) in *Sukiennice* (The Colth Hall) *Rynek Główny* (Main Square). The Sukiennice (Cloth Hall) is a large market hall erected in the 13th century in the middle of the Market Square, extended in the 14th century in the Gothic style and remodelled in the mid-16th century after Renaissance fashion. The Gallery of 19th-Century Polish Art is a continuation of the Gallery of the Art of Old Poland from the 12th to the 18th Century, situated in *Pałac Biskupa ERAZMA CIOŁKA* (the Bishop ERAZM CIOŁEK Palace) at *Kanonicza 17*. The display exhibited in the Sukiennice is chronologically followed by works presented in the Gallery of 20th-Century Polish Art in *Gmach Główny Muzeum Narodowego w Krakowie* (the Main Building of the National Museum in Kraków) at *3 Maja 1 Avenue*. Tickets are 28 PLN; audio guide is 7 PLN.
- *Zamek Królewski na Wawelu* (Wawel Royal Castle). There are quite a few permanent exhibitions showing the lives of Polish kings and national treasures (Poland was a kingdom until 1795): Crown Treasury & Armoury, State Rooms, Royal Private Apartments, The Lost Wawel, Wawel Recovered, Royal Gardens, Courtyards & the Church of Saint Gereon, and Dragon's Den, as well as some temporary exhibitions. We suggest booking guided tours to get the most out of your visit. Tickets are 5–100 PLN depending on the exhibition.
- *Fabryka Emalia Oskara Schindlera* (Oskar Schindler's Enamel Factory) at *Lipowa 4* in the *Podgórze* (Foothills) District. The museum takes you on an emotional journey to the anxious pre-WW2 Kraków, throughout the horrors of the war and the post-WW2 Soviet occupation of Poland to the current times. The journey ends with hope. The Factory was made famous by the widely acclaimed Oscar-winning (7 awards) "Schindler's List" by STEVEN SPIELBERG, who shot the movie in Kraków in spring 1993. We suggest booking a guided tour. Tickets are 28 PLN.
- *Muzeum Sztuki Współczesnej w Krakowie (MOCAK)* (Museum of Contemporary Art. In Kraków) at *Lipowa 4* in the *Podgórze* (Foothills) District. MOCAK's concentrates on presenting the art of the last two decades in the context of the post-war avant-garde and conceptual art as well as clarifying the rationale of creating art by highlighting its cognitive and ethical value and its relationship with everyday reality. Tickets are 20 PLN.

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