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Professor Zofia Bielańska – Osuchowska

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Professor Zofia Bielańska-Osuchowska was born on December 6, 1919, in Cracow. 1952, she obtained a master's degree from Jagiellonian University (supervisor Prof. Z. Kraczkiewicz). From 1953 – 1990, I worked (1963 – 1990, head of the department) at Warsaw University of Life Sciences (SGGW). In 1964 – 1969, she served as a deputy dean of the Veterinary Medicine Faculty. Her research concerned, among others, histochemical and ultrastructural aspects of the development of the male and female gonads and placenta in the domestic pig, as well as the fine structure of Sertoli cells and spermatogenesis in the bull. The author of the handbooks: *An outline of general embryology* (1970), *Embryology* – for students and veterinarians (1993), *An outline of organogenesis* (2004), *Sperm, the only cell of this kind* (2018), published after death. For many years, together with Professor Jerzy Kawiak, she organized a very popular Autumn School *Progress in cell biology.* In 1971 – 1977, Professor Osuchowska was the president of the Polish Anatomical Society, and in 1986 – 1992, vice-president of the Polish Histochemistry and Cytochemistry Society. In 1994, she was awarded an *honorary doctorate from the University of Life Sciences and an honorary member* (1986) and *Bene Meritus* (2001) by the Polish Histochemistry and Cytochemistry Society. In the year of her death, her last work, *Extracellular vesicles – system of cell–cell communication*, was published in *Advances in Cell Biology* (2017, 44, 1, 3-32).

Professor Zofia Bielańska – Osuchowska was a widow for 38 years, and in the last years, she lived in the Polish Academy Science Seniors' Home in Konstancin, where she died on November 26, 2017. She had an extraordinary personality, and was a great lady of Polish embryology and histology.

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Memories of Professor Czesław Jura, embryologist, zoologist and long-time head of the Department of Systematic Zoology and Zoogeography at the Jagiellonian University

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The speech will highlight the most essential facts from the biography of Prof. Czesław Jura. The professor's scientific achievements, how he runs the Department, his attitude towards students and subordinates, and some of his views on life will also be discussed (Klag, 2020). Prof. Czesław Jura was born in 1927 in Nowy Sącz. He finished secondary school in this town and went to Kraków to study biology at Jagiellonian University (1948). After his graduation, he started working as an assistant. He prepared his doctoral thesis in Wrocław in 1956. After that, he returned to Kraków and worked in the Department of Zoology, where Prof. Smreczyński was a leader. However, he soon went to the Netherlands and the USA, where he studied the embryology of insects. His work at the Yale University made him known among embryologists of the world. In 1969, he started his work as the head of the Department of Systematic Zoology. From that time, he prepared some students' textbooks (Jura, 1971, 1988; Jura et al. 1983; Jura and Krzanowska, 1998; Jura and Klag, 2005) and organized editions of several textbooks written by many authors (in sum, 18 books). Under his direction, many students prepared their doctoral theses. He was always kind and helpful to his students.

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Plenary Lectures

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From 'metal-loving' violets to disappearing pasque-flowers – a relay of generations at the Jagiellonian and Silesian Universities with embryology in the background

Elżbieta Kuta[1], Aneta Słomka[1], Grzegorz Migdałek[2], Klaudia Sychta[1], Justyna Żabicka[1], Piotr Żabicki[1], Monika Kwiatkowska[1], Błażej Ślazak[3,4]

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The *Viola* L. is rich in species tolerant to high concentrations of heavy metals in soil. Some species are metallophytes or even *hyperaccumulators* of trace elements. The long-standing cooperation with University of Silesia started in 2002 on "zinc violets" (*V. lutea* subsp. *calaminaria*, *V. lutea* subsp. *westfalica*) and was successively extended to other species of violets and pansies (nonmetallophytes and metallophytes) (Słomka et al., 2012). The approach to the research was diverse (morphology, histology, taxonomy, cytology, phylogeny, physiology, embryology) and different techniques were applied (e.g., SEM, TEM, LSCM, *immuno*histochemical, AAS, LC-MS and MALDI-MS, FISH, AFLP, ITS, ISSR, cell and tissue *in vitro* culture), confirming the innate tolerance to heavy metals in the genus, microevolutionary process and adaptation to heavy metal stress, cyclotides as a part of plant defence mechanism against biotic and abiotic factors.

In recent years, joint research on the endangered *Pulsatilla* Mill. has been successfully completed. Extinct population of P. patens (L.) Mill. was recovered by re-introduction of plants propagated *via* different techniques (Żabicka et al., 2022).

Embryology, regardless of the topic or level of research, is always one of the goals and sparkles with colors (various staining techniques) because sexual reproduction allows the preservation/renewal of plant populations and maintain biodiversity.

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The authors thank drs: Monika Jędrzejczyk-Korycińska, Teresa Nowak, Elżbieta Wolny; professors: Ewa Kurczyńska, Jolanta Małuszyńska, Adam Rostański, from the University of Silesia for long-term cooperation in research.

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GONOCYTES, THE GERMINAL STEM CELLS IN ANURAN AMPHIBIANS

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The germline in amphibians originates from specific cells called primordial germ cells (PGCs) situated far from the somatic gonad primordia, to which they must actively immigrate. PGCs move over the basal lamina of inner surface epithelial cells of the mesentery until they reach the primordia of the gonad, where they start to proliferate and lose yolk, giving rise to gonocytes (Gs). Amphibians, like mammals, are gonochoristic, meaning that their initially undifferentiated (bipotential) gonads differentiate directly into ovaries or testes. Early gametogenesis in amphibians is not studied as thoroughly as in mammals, therefore

we may rely on models developed for mice and humans and compare them with the results obtained for frogs.

In the ovary, the mitotic divisions of the gonocytes (equal to primary oogonia) are asynchronous and extended in time. After several cell cycles, primary oogonia ceases the mitotic cycle and enters the next round of mitoses with incomplete cytokinesis. The resulting cells (secondary oogonia) are connected by cytoplasmic bridges. After the last mitotic cycle they enter meiosis and remain connected until the pachytene stage. Afterwards, the bridges disappear resulting in single diplotene oocytes that will wait for ovulation until adulthood. These processes are similar in amphibians and mammals.

In the mammalian testes, gonocytes (named prospermatogonia) multiply mitotically (M-), enter a transient period (T1-), also called quiescence (Q-), and finally resume mitotic proliferation as T2-prospermtogonia. In both mammals and amphibians, they do not enter meiosis, but remain quiescent until puberty when they transform into adult spermatogonial stem cells (SSCs). During the quiescent period, the chromatin of prospermatogonia undergoes extraordinary change known as genomic imprinting, i.e. genome-wide epigenetic reprogramming. Genomic imprinting occurs also in oogenesis, however in spermatogenesis it is more distinctive. Another example of extraordinary chromatin reorganization comes from several hybridogenetic hybrids of fish (*Poeciliopsis* and *Hypseleotris*) and amphibians (*Pelophylax*). Gonocytes of these hybrids eliminate one of the parental chromosome sets and reduplicate the remaining one to restore diploid state. The eliminated chromosomes are extruded from the interphase nuclei of gonocytes in the form of micronuclei. These two examples of extraordinary chromatin reorganization during corresponding stages of early gametogenesis (in frogs also oogenesis) may suggest that genome remodelling in gonocytes is a more common phenomenon also in other animals.

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Oral presentations

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Pole (germ) plasm in thysanopteran oocytes

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Animal germline cells are specified either *via* zygotic induction or cytoplasmic inheritance (Extavour and Akam, 2003). The former process takes place during embryogenesis and requires cell-to cell signaling that leads to the acquisition of germline fate *de novo*. In contrast, the cytoplasmic inheritance involves early formation of morphologically recognizable ooplasm region, termed the germ, or pole plasm. This region contains a set of maternally provided germline determinants that cooperate to induce germline fate in a subgroup of embryonic cells, termed primordial germ cells. Accumulated data suggest that among insects, the cytoplasmic inheritance is a derived condition of Holometabola, *i.e.* insects undergoing complete metamorphosis (Quan and Lynch, 2016) or, alternatively, Holometabola and its sister taxon, Paraneoptera (Ewen-Campen et al., 2013). As among paraneopterans, the pole plasm have been described in three species only, the latter alternative apparently needs further studies and/or reinvestigation. In this report, we present description of gradual formation of the pole plasm in developing oocytes of thysanopterans at the level of electron microscopy. We show that this process starts as early as during initial stages of previtellogenesis and involves transportation of nuage aggregations from the perinuclear ooplasm toward the posterior oocyte pole. After the onset of oocyte maturation, *i.e.* after reinitiation of meiotic division, the pole plasm occupies the posterior extremity of the oocyte and comprises electron-transparent aggregations of nuage material interspersed with electron-dense, ribosome-rich cytoplasm.

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TRUNK MUSCLE DIFFERENTIATION AND GROWTH IN NON-MODEL VERTEBRATE SPECIES

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Vertebrate trunk muscles belong to the most ancient phylogenetic types of tissue. They originate from unsegmented mesoderm, which divides into metameric units called somites. Further somites differentiate into three compartments: the dermomyotome, sclerotome, and myotome. Only the myotome is the main source of trunk muscles. It has been demonstrated that in amniote embryos, the dermomyotome is also the source of satellite cells, which are known progenitors of adult skeletal muscles. Myogenesis is a developmental cascade orchestrated by numerous different intrinsic and extrinsic factors. The key step for the entry of somatic cells into the myogenic potential program is the induction (by signals secreted from surrounding tissues, such as a neural tube, notochord, dorsal, and lateral ectoderm) of bHLH (basic/Helix-Loop-Helix) myogenic regulatory factors (MRFs). The regulatory gene family includes *MyoD*, *Myf5*, *myogenin* (*MyoG*), and *MRF4* (*Myf6*). Although in vertebrates muscle fibers are multinucleated, the pathways leading to them vary between vertebrate taxa. In fish during early myogenesis myoblasts differentiate into multinucleated lamellae or multinucleate myotubes. In amphibians, myoblasts fuse to form multinucleated myotubes or, bypassing fusion, directly differentiate into mononucleated myotubes. Furthermore, mononucleated myotubes were also observed during primary myogenesis in amniotes. In all vertebrates, muscle mass growth occurs due to satellite cells. These cells participate both in hypertrophy (the growth of muscle fibers) and hyperplasia (the formation of new muscle fibers). Additionally, it has been demonstrated that muscle splitting can also lead to hyperplastic muscle growth (Lewandowski et al. 2020). The authors strongly believe that the results conducted on non-model vertebrate species might shed more light on the evolutionary processes underlying myogenesis.

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Germline cysts in terrestrial Parasitengona mites (Chelicerata: Acariformes)

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Mites are small chelicerates divided into two lineages: Parasitiformes and Acariformes. They show a great diversity in the ovary structure, for the confirmed presence of panoistic or meroistic ovary in various representatives of this group. In terrestrial Parasitengona, the meroistic ovary has been reported in a few representatives until present. Nevertheless, general knowledge of the meroistic structure of the ovary in mites is inconsistent, and the architecture of cysts has not been studied in detail (Derdak et al., 2023).

The aim of our study was to analyze the ovary structure in representatives of terrestrial Parasitengona from three families: Erythraeidae, Trombidiidae, and Microtrombidiidae by means of light, confocal, and transmission electron microscopy.

Our analyses showed that in all examined species the ovary is meroistic and consists of dozens of four-cell germline cysts, each differentiated into one oocyte and three nurse cells. In a mature ovary, oocytes protrude from the ovary surface as they grow, and nurse cells remain in the ovarian wall. In the cysts, cells are connected by cytoplasmic bridges, which, as a result of the position change of the oocyte, elongate and transform into trophic cords. Organelles are transported by trophic cords from nurse cells to the oocyte. The nurse cells in all analyzed species are the same with respect to morphology, their nuclei withdraw from meiotic division, and the cytoplasm contains a *nuage* aggregate associated with mitochondria.

Our research describes in detail and verifies literature data on the germline cysts of the Parasitengona (e.g. Shatrov, 2002; Witte, 1997). The results have revealed the great similarity of the germ clusters and point to the constancy of this trait in the terrestrial Parasitengona.

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GENOME ELIMINATION FROM THE GERMLINE CELLS OF THE *PELOPHYLAX GRAFI* HYBRIDOGENETIC TADPOLES

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Reproductive isolation results in species diversity, usually caused by meiotic arrest during the gametogenesis of interspecies hybrids. However, several exceptions to this rule are reported, one of which is hybridogenesis. This process relies on the hybrid (AB) ability to selectively eliminate one of the parental genomes (B) and transmit only the other parental genome (A) to the gametes. If a hybrid mates with the AA individual, the hybrid AB progeny will be restored as constant F1 generation. Hybridogenesis occurs in water frogs from the genus *Pelophylax*, in which 3 hybrid taxa were reported: *P. esculentus*, *P. grafi* and *P. hispanicus*. All of them share the *ridibundus* genome, which is transmitted when mating with the parental species: *P. lessonae*, *P. perezi* and *P. bergeri*, respectively. For a long time, only *P. esculentus* was studied by various researchers specialized in the environmental, evolutionary and cytogenetic approaches. Their findings showed that *P. esculentus* exists in various populations with one or two of the parental species or in pure hybrid populations. Hybrids are either diploid or triploid and the eliminated genome (*ridibundus* or *lessonae*) depends on the ploidy of an individual and type of population. The data obtained in our laboratory proves that *P. esculentus* is an overly complicated cytogenetic and cytological model for studies on genome elimination. In search of a simpler equivalent, our group chose another hybrid – *P. grafi*, which exists in only one type of population together with *P. perezi* and supposedly eliminates only one type of the genome (*perezi*) from its germline. Harnessing comparative genomic hybridization performed on early germline cells (gonocytes) from tadpole gonads, we discovered the gradual elimination of the *perezi* chromosomes and one-time endoreplication of the *ridibundus* chromosomes in the hybrid germline cells.

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OVARY STRUCTURE AND CHANGES IN OOCYTE ORGANIZATION DURING EARLY OOGENESIS IN SOME SPECIES OF OSTEOGLOSSIFORMES (TELEOSTEI)

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Most teleosts possess cystovaries, which are saccular gonads with ovigerous lamellae extending into the ovarian lumen. Consecutive stages of oogenesis take place in the epithelium and stroma of the lamellae (Hoar and Randal, 1969). Bony-tongue fishes, Osteoglossomorpha, constitute an early diverging teleost lineage that exhibits diversity in reproductive biology, including different modes of reproduction and four types of sperm (Hilton and Lavoue, 2018; Koenig and Gallant, 2021). It is worth considering whether this diversity is also reflected in organization of the oocytes during oogenesis in these fish. The structure of the ovary and the formation of oocytes were analyzed in detail using light microscopy as well as electron transmission (TEM) and scanning (SEM) microscopes. The study revealed distinct differences and unique characteristics among the examined species: internally fertilizing *Pantodon buchholzi* (Pantodontidae), and externally fertilizing *Osteoglossum bicirrhosum*, *Arapaima gigas* (Osteoglossidae), *Campylomormyrus compressirostris*, *Marcusenius cyprinoides*, *Gnathonemus petersii* and *Mormyrus rume* (Mormyridae). The variation mainly involve presence of a few types of ooplasm (perinuclear and peripheral or with different electron density), the formation of a mitochondrial net, and various forms of endoplasmic reticulum (e.g. whorls, karmellae, lamellae, nets) found in oogonia and/or in primary growth oocytes.

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Comparison of adaptations for matrotrophy in pseudoscorpions (Chelicerata, Pseudoscorpiones)

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Matrotrophy is a common phenomenon of nourishing embryos with substances produced by the mother's body. One group of matrotrophic animals are pseudoscorpions, small chelicerates in which females carry developing embryos in a brood sac under the opisthosoma and feed them with secretions, called a nutritive fluid, produced by the female reproductive system. The synthesis of nutrients occurs cyclically, i.e., it takes place in the secretory phase of the ovary cycle, is followed by the oogenetic phase comprising the growth and maturation of the oocytes and is finished with ovulation. The nutritive fluid is secreted in two organs: the ovary and the oviduct.

Production of the nutritive fluid in the female reproductive system is the key adaptation for matrotrophy in pseudoscorpions. Our comparative analyses (Jędrzejowska and Garbiec, 2020; Garbiec et al., 2022) showed that representatives of four pseudoscorpions families from three superfamilies: Neobisiidae (Neobisioidea), Cheiridiidae (Cheiridioidea), Cheliferidae and Chernetidae (Cheliferoidea) exhibit quite a wide range of adaptations to matrotrophy that affect the efficiency of nutrients production mainly due to the variable number of cell populations involved in this process. Those differences correlate with the amount of reserve materials deposited in the ooplasm. The more efficient the production of nutritive fluid is, the oocyte is less equipped with yolk. We noted also differences in the site and duration of the nutritive fluid storage before it is delivered to developing embryos in the brood sac. The results of our observations might indicate that these dependencies in adaptations to matrotrophy are part of the evolutionary history of the group.

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Antimicrobial agents in cocoons and embryos of web-spinning, and non-web-spinning spiders

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The putative antimicrobial properties of spider cocoons is the topic that remains poorly understood. Cocoons often named as egg sacs or egg cases, are structures made up from Aciniform and Cylindriform types of silk glands (Ewunkem et al., 2022). It was established, that the eggs within cocoons are sterile (Babczyńska et al., 2019). However, it remains unclear whether it is largely attributed to the dense webbing of the cocoon forming a mechanical barrier against pathogens, or there are other elements which could provide additional protection to the developing embryos. This research used material from two types of model spiders: *Parasteatoda sp.,* and *Pardosa sp.,* due to their distinct model cocoon care strategies and different ecological niches occupied. Experiments were focused in researching potential FAs (fatty acids) and AMPs (antimicrobial peptides) which are within sphere of research exploring novel antibiotics derived from animal sources and were neglected due to the discovery and the development of traditional antibiotic drugs (Wei et al., 2023, Uddin et al., 2021). Analyses utilized GC-MS chromatography coupled with FAME analysis as well as ELISA (i.e., enzyme-linked immunosorbent assay) and Protein Immunoblotting. The results helped to shed more light related to this topic by documenting the existence of researched antimicrobial agents in the material.

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Prenatal development of vasculature of the thyroid gland in shorthair cats

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The developmental data about the differentiation of the mammalian thyroid gland are scarce. It's known that in domestic cat shorthair in mid-pregnancy, i.e. about 28-30th day p.c., the undifferentiated primordia of the thyroid gland is positioned near the ventral surface of the trachea by the common carotid arteries and vagal nerve. The structural changes of glandular parenchyma start from the 36th - 38th day p.c., forming the first glandular follicles. Up to birth, an increase in the amount and secretory activity of the thyroid is observed.

The study aimed to show the formation of specific nutritional and functional vascular systems from the 28th to the 63rd days p.c. fetuses. The microstructure of the thyroid gland was evaluated in LM and SEM. The 3D imaging of parenchyma with particular emphasis on the vascular system was performed on serial histological sections. The angioarchitecture was studied on vascular corrosion casts (VCC) for SEM after injection of Mercox resin was prepared.

The macro- and microscopical observations of the leading nutritional blood vessels thyroid gland in cat fetuses showed, in general, a typical pattern, as in adult animals. Up to the 35th-day p.c., the undifferentiated parenchyma of the primordial thyroid possesses a loose, irregular network of voluminous capillaries between epithelial bands of cells. On such embryonic capillary networks, signs of intussusceptive angiogenesis were recognised. Together with the appearance of the first glandular follicles around day 36th - 40th p.c., a superficial vascular network was found as interlobular and follicular capillaries with signs of sprouting angiogenesis. The intensive growth of glandular follicles with basket-like and mesh-like capillary networks starts after day 48th p.c. Stepwise, the proper histological structure of the wall of arterioles and venules appears after the 50th day p.c. The thyroid's microvascularisation pattern resembles that of adult cats.

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ENDOPLASMIC RETICULUM AND MITOCHONDRIA COMPLEXES – IN A GOOD COMPANY TO THE NEXT GENERATION – LESSONS FROM A PSEUDOSCORPION *CHELIFER CANCROIDES*

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Mitochondria are one of the key organelles which affect the proper oocyte growth, maturation, and fertilization. They are multifunctional cell components with central roles in ATP production, fatty acid oxidation, and calcium ion homeostasis realized during temporary interactions with other organelles. The quality of oocyte mitochondria is crucial for the course of oogenesis and embryo development, since in almost all animals the mitochondria are maternally transmitted.

In this study we focused on the mitochondria distribution, behaviour and activity during oocyte growth, and early stages of embryogenesis in a pseudoscorpion *Chelifer cancroides*, *C. cancroides* is a cosmopolitan species, easy and cheap to culture in laboratory conditions. In *Chelifer* like in other pseudoscorpions the embryo development relies on nutrients provided by a mother, which is common for all matrotrophic animals including mammals. The amount of reserve materials deposited in the ooplasm is negligible what significantly facilitates the analysis of the oocyte structure.

Our observations show that in early previtellogenic stages of oocyte growth, mitochondria constitute the main component of the Balbiani body, the organelle assemblage, gathered in the juxtanuclear position. With the progress of previtellogenesis mitochondria of the Balbiani body gradually disperse in the whole ooplasm which is preceded by biogenesis of endoplasmic reticulum. At advanced stages of previtellogenesis, most mitochondria associate with the endoplasmic reticulum and begin to form endoplasmic mt-ER complexes whose structure changes significantly as a result of alterations in the shape of organelles and their arrangement. Finally, from late previtellogenesis, the mitochondria and regularly sandwiched by individual ER cisternae. In such complexes mitochondria are kept until the final stages of oocyte growth and maturation. Moreover, in such complexes, mitochondria are transferred to blastomeres of early embryos. Analyses of mitochondria activity by means of several mitochondrial markers (rhodamine 123, JC-1, Mitotracker CMX-Ros) revealed that active mitochondria are characteristic only of early (Balbiani body) stages, while from advanced previtellogenesis their activity is reduced.

We postulate that formation of mt/ER complexes in *Chelifer* oocytes, on the one hand, helps maintain low mitochondrial activity, and on the other hand, enables the transfer of basic organelles to the embryos in a state ready for their rapid activation and usage during early embryogenesis.

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Evolution of the premaxillary dentition in Lepidosauria, with special emphasis on the egg tooth

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The egg tooth of squamate reptiles is a true, premaxillary tooth that allows them to break or cut the eggshell during hatching. The presence of two egg teeth is a characteristic of extant gekkotans, and according to molecular phylogeny, this condition may represent an ancestral trait, contrasting with Unidentata, its sister clade distinguished by a single median egg tooth. This perspective challenges the traditional morphology-based phylogenetic framework, which typically rejects the monophyly of Unidentata. Additionally, the 'egg tooth' of *Sphenodon*, the sister group of squamates, is a keratinized structure known as caruncle. The same condition is present in crocodilians, and birds. Consequently, the evolution of the egg tooth and the remaining premaxillary dentition remains unclear. Here, we employed light microscopy and X-ray microtomography to explore the development of the premaxillary dentition in Lepidosauria, primarily focusing on the sand lizard (*Lacerta agilis*, Squamata, Unidentata) and *Sphenodon*, to construct their evolutionary history. The results showed that the large size of the squamate egg tooth can be attributed to a specific heterochronic shift, and its final morphology strongly relates to the development of the premaxilla. Moreover, per recently published studies for pleurodont squamates, we identified all dental tissues in the sand lizard that had previously been attributed to thecodont animals, such as extant mammals and crocodilians.

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Craniofacial malformations in squamate embryos: a 3D perspective

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The anatomy of the vertebrate head plays a crucial role in evolutionary processes, with modifications in shape serving as adaptations to the environment, particularly in relation to food intake and the development of specialized sensory organs. Here, we analyzed three brachycephalic, "short-snouted" embryos of squamates: the brown anole (*Anolis sagrei*) and the sand lizard (*Lacerta agilis*). These malformations occurred spontaneously, without any laboratory interventions, such as high incubation temperature. The analyzed embryos were observed under a stereomicroscope, and then the heads were embedded in paraffin and serially sectioned for anatomical and histological analysis. Based on the histological serial section, 3D reconstructions of the heads were performed to show the skull, chondrocranium, olfactory organs and palatal surface. In *Anolis sagrei* embryos, craniofacial defects co-occurred with abnormalities in other body parts, such as the limbs. This condition strongly resembles developmental abnormalities found in humans. In such cases, the limbs were additionally analyzed by scanning electron microscopy. The results were compared with normally developed embryos at the corresponding developmental stages and discussed in evolutionary and medical terms.

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How gonocytes can follow the gametogenic path full of traps and give rise to functional gametes in hybrid frogs

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Normal gametogenesis is tightly regulated to ensure production of haploid gametes and viable progeny, which makes hybrid animals usually sterile. Some hybrids acquired a way to overcome meiotic impairments in a modified reproduction - hybridogenesis. We unravel the process on the cellular level by tracking the germ line cells along the developmental sequence in two European hybridogenetic frogs: *Pelophylax esculentus* (*P. ridibundus* (RR) x *P. lessonae* (LL)) and *P. grafi* (*P. ridibundus* x *P. perezi* (PP)). Functional gamete production requires the removal of one of the parental chromosomal sets, endoreplication of the remaining one and formation of clonal gametes. After examination of the earliest stages of gametogenic cells we found that *P. esculentus* gonocytes had initially mixed genotypes containing 26 chromosomes (13R, 13L) in diploid and 39 chromosomes (13R, 26L or 26R, 13L) in triploid hybrids. Programmed genome elimination led to erasing one of the chromosomal sets (*lessonae* or *ridibundus*) from interphase nuclei by forming micronuclei subsequently degraded by nucleophagy. Simultaneously, we found mitotic abnormalities, multinucleation and degeneration of gonocytes that can sweep cells out of the pool (trap 1). Our cytogenetic data revealed abnormal endoreplication of mixed chromosomal sets, leading to the formation of polyploid cells. Among spermatogonial stem cells (descendants of gonocytes) in adult males, we found only 20% regular genomic compositions enabling meiosis and 80% aneuploid cells. Apparently, cell death removed aneuploids (trap 2), because the majority of spermatozoa showed regular genomic compositions ensuring male fertility. Micronuclei in *P. esculentus* were the marker of genome elimination in gonocytes, and we discovered a similar mechanism in another hybrid *P. grafi* when we detected micronuclei in the cytoplasm of their gonocytes followed by elimination of *P. perezi* chromosomes. We can conclude that the genome elimination process is evolutionarily conservative in various hybrid lineages of water frogs.

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TRANSPLACENTAL TRANSMISSION OF *BABESIA MICROTI* IN INTERMEDIATE HOSTS

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The goal of the presentation was to analyze the penetration of *Babesia microti* - a representative of Apicomplexa - through the rat placenta. This problem is of veterinary and medical significance because *B. microti* is a protozoan for which intermediate hosts can be not only animals, (mainly rodents) but also humans. The problem of transplacental transmission of *B. microti* has been confirmed many times, but the mechanisms of such infection have not been clarified. Using routine techniques of light microscopy, transmission electron microscopy, atomic force microscopy, and molecular methods, the structure of placentas, fetuses, and animals born with confirmed congenital babesiosis were observed. The study results were observations of *B. microti* migration between maternal and fetal blood. Despite the insignificant differences observed in the development of some organs during the organogenesis of healthy and *B. microti*-infected fetuses, the final development of the born-infected rats showed no malformations of these organisms. No reduction in reproduction was also noted. The presence of *B. microti* trophozoites was demonstrated in embryos - inside cells and in the intercellular tissue matrix. Degenerative processes of neuroepithelial cells caused by *B. microti* invasion are in balance with regenerative processes, which ensures the normal development of the nervous system during the fetal period. From the start of formation of the blood system, the adhesion of blood cells to each other and the wall blood vessels have been visible. This results in the formation of thrombus, and clot formation and vascular obstruction in organs in rats born with congenital babesiosis. Potentially implies little degenerative changes in the neuropilum of the cerebral cortex and cerebellum. Infection of the rats with the *B. microti* results in decreased placental weight and the proportion of the volume of the layers of this organ. Among other effects, it causes an increase in the basal layer a decrease in the size of the labyrinth, and also neovascularization of the marginal part of the placenta. Both fetuses and born animals exhibit distinct changes in the structure of the kidneys, which indicates their dysfunction.

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DIFFERENTIATION OF PANCREATIC CONNECTIONS WITH LIVER AND GALLBLADDER IN SELECTED SPECIES OF LIZARD EMBRYOS – COMPARATIVE STUDIES

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The pancreas connects to the duodenum to release enzymes into the digestive tract. A literature review shows considerable variation in the organization of the pancreatic ducts and their connections with the duodenum in vertebrates. The connection between the liver and the pancreas can also be categorized into different types. These types of connections are poorly studied in reptile embryos, so this study focused on the differentiation of pancreatic connections with the duodenum and liver in four lizard species representing three clades: the brown anole (*Anolis sagrei*) representing the Iguania clade, the sand lizard (*Lacerta agilis*) representing the Lacertoidea clade, and the leopard gecko (*Eublepharis macularius*) and the mourning gecko (*Lepidodactylus lugubris*) representing the Gekkota clade. The embryo isolation took place in regular time intervals until hatching. The embryonic tissues, including the pancreas and surrounding organs, were fixed in Bouin solution and, after dehydration, embedded in paraffin. Paraffin blocks were cut on the 7µm thick serial sections. Histological sections were stained using hematoxylin-eosin and Azan Heidenhan's methods. Embryonic tissues were analyzed by light microscopy, and 3D reconstructions were made. This study showed that the differentiation of pancreatic connections can be divided into a few steps. At the beginning of the pancreas development, all studied species have three pancreatic buds. Each of them entered the duodenum with a separate outlet. Connected ducts from the liver (hepatic duct) and gallbladder (cystic duct) entered the duodenum as a common bile duct. During the next steps of the pancreatic connection formation, all the pancreatic ducts joined the common bile duct before entering the duodenum. For this reason, only one large duct entered the duodenum. At the time of hatching, each duct (hepatic, cystic, and three pancreatic ducts - from the dorsal and two ventral buds) again entered the duodenum as separate ducts with a narrow lumen at the site called the ampulla of Vater.

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GOLD NANOPARTICLES AND SOMATIC EMBRYOGENESIS IN *ARABIDOPSIS*

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Somatic embryogenesis (SE) manifests the totipotency of plant cells. During this process, embryos are formed from the somatic cells, passing the fertilization stage. Mechanisms underlying embryo formation are not fully understood. The influence of various endogenous and external factors on this process is the subject of a lot of research from the molecular, ultrastructural, to histological levels. The main purpose is to search for the answer to the question of what lies at the basis of this process. During SE, there is a change in the direction of cell differentiation from the somatic to the embryogenic state, which is an excellent model for studying factors regulating cell differentiation.

The development of nanotechnology in recent years has been very rapid, and nanomaterials are commonly found in everyday life. Recent research has shown that nanostructures can have both positive and negative impacts. Therefore, the question arose as to whether and what effect nanoparticles (NPs) have on the process of SE, with particular emphasis on their impact on changes in the direction of cell differentiation.

The cell wall is an extremely sensitive indicator of the influence of various factors on the cells. The composition of the wall is specific to cells implementing different development programs. Therefore, the studies also focused on the chemical composition of the walls of cells implementing various developmental programs during SE in control and NPs-treated explants.

The study used the *35S:BBM Arabidopsis thaliana* as an explant. This line is characterized by the spontaneous formation of somatic embryos on the edges of the cotyledons. The explants were exposed to gold nanoparticles (AuNPs) with different surface charges during culture. The process of SE under the influence of AuNPs, as well as changes in the chemical composition of walls from domains composed of cells with different phenotypes were analysed. It turned out that AuNPs: 1/ block the entry of cells into the embryogenic pathway regardless of the surface charge of the NPs, 2/ the chemical composition of the cell walls were diverse in the cells realizing different developmental programs: SE (control) and non-SE (treated with Au NPs).

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AN OLD STORY WITH NEW INSIGHTS INTO THE ROLE OF MYOSIN VI IN *DROSOPHILA* BORDER CELL MIGRATION

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Myosin VI is the only actin-based motor known to move toward the minus end of actin filaments. This unique feature, and the fact that myosin VI activity in different cells and cellular processes is modulated by different adapter proteins, may explain many of the proposed functions of myosin VI, including endocytosis, secretion, and regulation of actin dynamics. Myosin VI has also been suggested to play a role in cell motility. Over 20 years ago, it was shown that this protein is essential for the migration of border cells during oogenesis in *Drosophila*, thus playing a crucial role in the morphogenesis of the egg chamber. These remarkable findings provided a starting point for considering the potential role of myosin VI in tumor invasion, with the collective migration of border cells emerging as a simple, genetically feasible model for studying the conversion of epithelial cells to migratory cells. However, since then, no results have been presented that could explain the mechanism of action of myosin VI during border cell migration. On the contrary, *Drosophila* females lacking myosin VI in all tissues have been shown to be fertile. We therefore decided to reassess the role of this unique motor protein in the collective migration of *Drosophila* border cells. Our research shows that myosin VI may be involved in forming clusters of border cells and initiating their migration, but it is not a key protein in the development of a mature egg.

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IBUPROFEN'S IMPACT ON SURVIVAL AND REPRODUCTION IN *PARAMACROBIOTUS EXPERIMENTALIS* (TARDIGRADA: EUTARDIGRADA)

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Tardigrades, often referred to as water bears, are a diverse group of invertebrates known for their extraordinary resilience. They can be found in various environments, from polar regions to tropical habitats. Their resilience makes them interesting subjects for research. Investigating different aspects of these organisms lives will lay the groundwork for future research, not only on other tardigrade species but also for projects exploring the effects of additional non-steroidal anti-inflammatory drugs.

Our study focused on the effects of ibuprofen, a drug increasingly detected in aquatic environments (aus der Beek et al., 2016), on the survival and reproduction of the tardigrade *Paramacrobiotus experimentalis*. This species is gonochoric. Females lay ornamental eggs freely in the environment, not within the exuvium (Kaczmarek et al., 2020). The specimens were incubated in two different environmental concentrations of ibuprofen: 0.1 µg/L and 16.8 µg/L, alongside 1 mg/L (an experimental concentration). The study comprised two incubation periods: one lasting 7 days and the other 28 days. The experiment involved daily observations of individuals to analyze the studied aspects. Both shorter and longer incubation periods showed no significant differences in survival or reproduction (number of eggs laid) between the experimental and control groups. This observation suggests that the oogenesis process remains unaffected by incubation in ibuprofen, even at significantly higher concentrations than those typically found in the environment. These findings demonstrate the resilience of the oogenesis mechanism in *P. experimentalis* to exposure to this non-steroidal anti-inflammatory drug. This observation may imply the existence of barriers against harmful substances or mechanisms that protect the gonad from potential damage.

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Syngamy in angiosperms – is the old question answered?

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Double fertilization, discovered in 1898, implied a question if two gamete fusions occur at random or are determined. Here we focus on gamete recognition and fusion in *Arabidopsis thaliana*, the model plant. GEX2 protein located in sperm plasma membranes is required for gamete adhesion (Mori et al., 2014). At sperm arrival the egg cell releases EC1 protein which activate the sperm cells to expose HAP2/GCS1 fusogen protein (von Besser et al., 2006; Sprunck et al., 2012). Usually the angiosperm sperm cells are isomorphic, and it appears that they are functionally equivalent. No preference in the female target appeared among sperm pairs differentially labelled with a photo-convertible fluorescent protein (Hamamura et al., 2011). Little is known about the sperm-central cell recognition. Differences in cytosolic Ca^{2+} spikes during plasmogamy occurred between the egg and central cell (Hamamura et al., 2014). Time gap of few minutes separates the sperm-egg and sperm-central cell fusions. Presumably, both sperm cells initially compete for the egg cell but only one gains plasmogamy. It appears that none of three models proposed by Knox and Singh (1987) is valid. More investigation on the syngamy of the central cell is needed to propose a new model.

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THE EFFECTS OF h-BN-OH ON REPRODUCTION AND DEVELOPMENT OF THE *TENEBRIO MOLITOR*

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Hexagonal boron nitride (h-BN) has unique physicochemical properties (high electrical resistance and incredible resistance to oxidation), which, according to experts, will allow the use of this nanomaterial in biomedicine, electronics and industry (Rafiei-Sarmazdeh et al., 2020). However, its nanoflakes are poorly soluble in aqueous solutions, which limits their therapeutic potential. This problem was overcome by adding hydroxyl residues to the surface of h-BN nanoflakes, which made them more hydrophilic. h-BN-OH in short-term *in vitro* tests performed so far showed either cytotoxic activity or no cytotoxicity (Lu et al., 2016). Our studies showed no morphological and functional changes in the *Tenebrio molitor* hemocytes but decreased survival of L929 cells exposed to h-BN-OH. In turn, in long-term *in vivo* studies, we demonstrated in *T. molitor* that h-BN-OH nanoflakes interfered with the cellular immune response against the *Staphylococcus aureus* bacteria (Czarniewska et al., 2019). In developmental studies, we showed that the h-BN-OH-injected larvae of *Tenebrio* showed lower survival, problems with pupation compared to the control, and adult insects resulting from larvae exposed to h-BN-OH nanoflakes had teratogenic changes. h-BN-OH also had a negative effect on the functioning of the ovaries of *T. molitor* females. These studies demonstrated that h-BN-OH nanoflakes disrupt reproductive and developmental processes in the insect. Therefore, in order to practically use h-BN in biomedicine, other surface modifications of h-BN nanoflakes should be considered, which will not only improve the hydrophilicity of the surface, but also the biocompatibility of this nanomaterial.

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THE EFFECT OF BOROPHENE ON THE DEVELOPMENT OF THE *TENEBRIO MOLITOR* BEETLE

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Borophene is a new monoelemental 2D supermaterial that has attracted extensive attention from researchers due to its excellent electrical, mechanical, chemical and thermal properties (Piazza et al., 2014). It is a promising drug delivery and theranostic material due to its material properties and fluorescent and photoacoustic contrast for imaging (Duo et al., 2021). However, it is necessary to understand its interaction with the organism of animals and humans. Recently, we showed that 2D borophene nanoflakes did not induce hemocytotoxicity in hemocytes of *T. molitor*: the cells' morphology, adhesiveness, ability to form long filopodia and viability were detected to be the same as in the control hemocytes. Nanoflakes increased the reducing power of hemocytes and did not generate intracellular reactive oxygen species in hemocytes or affect the mitochondrial membrane potential. The test of the immunological activity of hemocytes demonstrated that the nanoflakes did not influence phagocytosis (Czarniewska et al., 2023). The results of this long-term study indicate that borophene nanoflakes injected into larvae of *Tenebrio* did not disturb the metamorphosis, did not cause teratogenic effects, did not reduce the survival of larvae, pupae and adult insects and did not affect the ovary function. However, the long-term *in vivo* studies on insects and other animal models are still necessary to clearly confirm that 2D borophene does not disturb the development and reproduction processes of animals and is a biocompatible and biologically safe nanomaterial for use in industry and medicine.

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Is the formation of aflagellate spermatozoon in *Campylomormyrus compressirostris* (Osteoglossomorpha: Mormyridae) a new type of spermiogenesis in teleost fishes?

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Representatives of bony tongue fishes (Osteoglossomorpha) are the only vertebrates in which aflagellate, monoflagellate, biflagellate aquasperm and complex introsperm spermatozoa have been described (Jamieson 1991; Dymek & Pecio, 2020). Aflagellism is a common phenomenon in invertebrates, but is very unique in vertebrates, where it is restricted to species of the Mormyridae (272 species) and the monotypic Gymnarchidae. Spermatozoa in representatives of these two families exhibit ultrastructural variation, e.g. in *Gymnarchus niloticus*, the chromatin in the nucleus is fully uncondensed, and cytoplasm is rich in microtubules. In contrast, representatives of Mormyridae have condensed chromatin with little reduction of cytoplasm. Our analysis of spermiogenesis in *Campylomormyrus compressirostris* revealed that spermatozoa differentiation is extremely simple and takes place partially extracystic, in the lumen of tubules. The process involves only chromatin condensation in the central region of the nucleus, slight decrease in nuclear volume and the appearance of numerous vesicles forming a tubular-vesicular system (TVS) in the cytoplasm. The centriolar complex among mitochondria is grouped together with TVS and form midpiece. Translocation of centrioles, migration of mitochondria and nuclear rotation commonly observed during spermiogenesis in most teleost are absent in *C. compressirostris* and other species with aflagellate spermatozoa in Osteoglossomorpha.

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Influence of electromagnetic fields on *Parasteatoda tepidariorum* embryos

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Exposure to radio-frequency electromagnetic fields has increased significantly in recent decades. There are no research which show the influence of electromagnetic field's (EMF) parameters on spiders' embryos. Therefore, the mechanisms of EMF's acting are unknown. The direct targets of EMF in producing non-thermal effects have not been clearly established.

The main purposes of the project is to investigate the influence of 10MHz frequency of electromagnetic field on the oxidative stress level in spiders' cells, the level of heat shock proteins (HSP) and the process of apoptosis.

Parasteatoda tepidariorum embryos were used as research model. They were divided in two groups: eggs in cocoons and without cocoons' protection. Both groups were exposed to 10 MHz EMF for 72h. The level of oxidative stress in cells was examined by MUSE test, the level of HSP by test named ELISA.

The studies can explain some of the molecular mechanisms of EMF's acting on embryos and can develop knowledge about the influence of EMF on living organisms.

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ORGANIZATION OF THE FEMALE REPRODUCTIVE SYSTEM IN *EUDRILUS EUGENIAE* - A REPRESENTATIVE OF EARTHWORMS WITH INTERNAL FERTILIZATION

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Earthworms (Crassiclitellata) belong to the class of annelids known as oligochaetes, characterized by hermaphroditism, where each individual possesses both female and male reproductive gonads. So far, the female organs of earthworms have not been studied in detail. Therefore, this work is part of a broader project that aims to thoroughly study and describe the ovaries of earthworms at a morphological and ultrastructural level. In this way, the ovaries of earthworms from the families Hormogastridae, Megascolecidae, and Lumbricidae have already been characterized. In these earthworms, fertilization is external, and the ovaries appear as small paired structures attached to a septum in the XIII segment. In *Eudrilus eugeniae* (Eudrilidae), internal fertilization occurs, and thus, the female gonads are organized as a part of a complex female reproductive system. The system comprises four main parts: ovo-spermathecal duct, spermathecal diverticulum, spermathecal ampoule, and ovisac. Observations of female germ cells within the ovisac show the occurrence of oogonia and growing oocytes, which strongly suggest that the ovisac functions as the ovary. In addition, the germline cells within the ovisacs are organized similarly to those found in other earthworms, i.e., they form syncytial cysts. Clustering cells are connected by stable intercellular bridges to the central cytoplasmic mass – cytophore. Regarding oogenesis, we found that the vitelline envelope in *E. eugeniae* is considerably more developed than in other earthworms, probably due to the mode of fertilization.

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Expression profile of myosin VI in mouse epididymal epithelium – comparative studies in control mice and Snell's waltzer mutants

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Myosin VI (M6) is an actin-based molecular motor involved in clathrin-mediated endocytosis that is highly expressed in specialized mammalian epithelia, such as the sensory epithelium of the cochlea, epithelium of the intestinal microvilli, and the epithelium of the proximal renal tubules. These epithelia are characterized by apically located projections, and M6 is undoubtedly responsible for maintaining their structural integrity and function. For example, this protein is essential for the proper architecture of stereocilia in the inner ear, and loss of M6 function causes deafness in mice and humans. The role of M6 in the apical domain of polarized epithelial cells is related to endocytosis and anchoring of the cell membrane to the base of the microvilli. To date, no data are available on the involvement of M6 in the functional organization of the epididymal epithelium. Epididymal epithelial cells play different roles depending on their location in the epididymis, and most of them form numerous microvilli in the apical zone like other specialized epithelia. We recently demonstrated that M6 deficiency in Snell's waltzer mice causes structural abnormalities during spermiogenesis and reduced male fertility, which may also be the result of impaired sperm maturation in the epididymis. Here we show for the first time the expression profile of M6 in the epididymal epithelium of control mice and Snell's waltzer mutants and discuss the possible role of M6 in this highly specialized epithelium.

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How cell death affects morphology of the testis in frogs

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The studies presented here were conducted on various species of water frogs of the genus *Pelophylax* (*P. lessonae*, *P. perezi*, and *P. ridibundus*). The final shaping of the testes is closely related to degeneration of the distal parts of the undifferentiated gonad. Initially, germ cells (gonocytes) undergo apoptosis, followed by Sertoli and interstitial cells, ultimately leading to significant shortening of the entire gonad. In the anterior part of the testis, numerous degenerating gonocytes appear at stage VII of the gonad (prespermatogenesis). Degenerating gonocytes exhibited: discontinuity of the cytoplasmic membrane; accumulation of vacuoles and glycogen, organelle degeneration, and changes in the morphology of the cell nucleus and nucleoli. Changes were also observed in Sertoli cells, which participate in the phagocytosis of degenerating gonocytes. The presence of numerous desmosomes between the processes of Sertoli cells in cysts indicates the possibility of separating degenerating cells from healthy ones. The morphology of gonocytes, as well as the presence of active caspase 3, indicate cell death through necrosis and apoptosis. In stage VIII (active spermatogenesis starts), the number of gonocytes decreases significantly, correlating with the progression of spermatogenesis. Cell death, which affected gonocytes, also occurs less frequently.. In stage IX (meiosis begins in cysts), degeneration was observed in cysts containing germ cells at various stages of spermatogenesis. These phenomena were most pronounced in hybridogenetic hybrids *P. esculentus* and *P. grafi*. In gonocytes of these hybrids, the genome of one of the parental species (*lessonae* in *P. esculentus* and *perezi* in *P. grafi*) is eliminated, and the remaining genome (*ridibundus*) is duplicated. This complex process is not precise and often leads to the formation of aneuploid cells, resulting in their death. This intense degeneration affected not only the number of germ cells but also entire cysts, which lost integrity with the tubular wall and passed into the tubular lumen, as well as the morphology of the entire testis. Seminiferous tubules exhibited abnormal structure, from narrowed and devoid of germ cells to enlarged with massive degeneration of germ cells and hypertrophy of somatic tissue. In hybrids, degeneration was observed at every stage of germ cell development, even among spermatozoa.

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COMPARATIVE STUDIES OF PANCREATIC DEVELOPMENT IN DIFFERENT LIZARD CLADES

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Differentiation of the vertebrate pancreas is dependent on a systematic relationship. The characteristic pancreas structure for lizards generally consists of three lobes: upper, lower and splenic. However, the shape and size of each lobe, as well as their position relative to each other, varies according to the phylogenetic position. The embryonic development of the pancreas has not yet been studied in detail from a comparative point of view between the different groups of lizards. Thus, this study focused on pancreatic differentiation in four lizard species representing three clades: brown anole (*Anolis sagrei*) representing the Iguania clade, sand lizard (*Lacerta agilis*) representing the Lacertoidea clade, and leopard gecko (*Eublepharis macularius*) and mourning gecko (*Lepidodactylus lugubris*) representing the Gekkota clade. Embryonic tissues were analyzed using light microscopy, and 3D reconstructions were performed. The results of this study showed that at the time of oviposition, the embryonic pancreas of the two gecko species and the sand lizard consisted of three buds: one dorsal and two ventral: left and right. Of these primordia, the most advanced in differentiation was the dorsal bud, which was already terminated by cells forming the primary pancreatic islet at this stage of development. Unlike these species, the embryonic pancreas of brown anole consisted of fused primordia at the earliest stages of development. At the end of embryonic development, the pancreatic lobes of two species representing the Gekkota clade were very close together, and the pancreas was compact in shape.

Interestingly, these species' upper pancreatic lobe was curved and directed towards the spleen near the splenic lobe, which may be related to their phylogenetic proximity. The pancreas of brown anole and sand lizard was more elongated. The distribution of the islets within the pancreas of the studied species was also different. At the end of embryonic development, the pancreas of brown anole embryos had one large islet in the splenic lobe. In contrast, in the sand lizard, many smaller islets were present in the splenic lobe and the pancreas body.

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THE CONSEQUENCE OF ADVANCED MATERNAL AGE ON OFFSPRING MENTAL HEALTH: DISSECTING GENETIC, EMBRYONIC, AND MATERNAL FACTORS AFFECTING NEURODEVELOPMENT IN *MUS MUSCULUS*

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Genetic, maternal, and environmental factors influence neurodevelopmental trajectories since early life windows, thus affecting offspring mental health and neuropsychiatric disease predisposition (Boivin et al., 2015). Advanced maternal age (AMA) is a risk factor for neurodevelopmental disorders in offspring, including autism (Tearne, 2015; Zacchini et al., 2022), but the etiopathogenetic mechanisms underlying this association are not well known. The present research investigated the hypothesis that advanced age may arouse brain and behavioral changes reminiscent of autism in the offspring of different mouse strains. Furthermore, by using a model of reciprocal embryo transfer between old and young female mice, we evaluated the contribution of age-related embryonic and uterine factors on offspring behavioral outcomes.

AMA negatively affected female reproductive and pregnancy outcomes, and perturbed placental and fetal growth, inducing significant changes in the expression of several neurodevelopmental genes in the fetal brain. Postnatally, AMA exerted strain-dependent effects on adult sociability, learning, and repetitive behaviors, varying between male and female offspring. Moreover, when transferred to young recipients, the embryos conceived by aged females displayed altered ultrasonic vocalization and enhanced learning skills, even though they were both prenatally and postnatally fostered by young females.

Overall, these findings highlight the interplay between advanced maternal age, genetic variability, and embryonic programming, revealing that AMA can either exacerbate or alleviate neurodevelopmental conditions depending on the background genotype. Moreover, we demonstrated that these effects are already established at pre-implantation stages, consistent with maternal age-induced aberrations affecting the embryo.

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IHC analysis of alpha-keratin, keratin-associated proteins, and transglutaminase 1 during embryonic development of orto- and parakeratinized epithelium of lingual mucosa in domestic goose (*Anser anser f. domestica*)

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The lingual mucosa in birds is covered with two types of cornified epithelium, i.e. ortho- and parakeratinized epithelium, which evolved depending on mechanism of food intake. Examination of structure of these epithelia and way of cornification is important to estimate readiness of lingual mucosa at the time of hatching to fulfill protective function and in veterinary practice during the evaluation of pathological changes in oral cavity.

Therefore immunohistochemical studies (IHC) of specific for birds alpha-keratin, keratin-associated proteins (KAPs) i.e. filaggrin and loricrin and transglutaminase 1 (TGM-1) have proceededin ortho- and parakeratinized epithelium of tongue in domestic goose embryos to indicate their expression profile during three developmental stages: embryonic, transformation, and prehatching stage.

The results indicate that, at embryonic stage (9^{th}-$13/14^{th}$ embryonic day),alpha-keratin and KAPs occur only in cytoplasm of superficial cells in both epithelia, while TGM-1 is present in cytoplasm of all cells. At the beginning of transformation stage ($14/15^{th}$ - 17^{th} embryonic day), when embryonic epithelium differentiates into multilayered epithelium, alpha-keratin, KAPs, and TGM-1 are visible in all epithelial layers of both epithelia. However, superficial layer is characterised by stronger expression than lower epithelial layers. At the end of transformation stage (18^{th} - $20^{th}/22^{nd}$ embryonic day), specific granules, named periderm granules, are present in the cytoplasm of superficial cells, both ortho- and parakeratinized epithelium. This layer indicates strong expression with alpha-keratin and medium with loricrin and TGM-1, while no expression with filaggrin. In the pre-hatching stage ($21^{st}/23^{rd}$ - 25^{th} embryonic day), when cornified layer develops, alpha-keratin shows strong expression only in intermediate and basal layer and weak expression in cornified layer in both epithelia. In the case of KAPs and TGM-1, strong or medium expression is observed in basal and intermediate layer, while no expression is in cornified layer.

Summarizing, presence of alpha-keratin, KAPs and TGM-1 in cells of undifferentiated epithelia indicates formation of epithelial cell cytoskeleton. Initialization of cornification process of ortho- and parakeratinized epithelium of tongue of domestic goose embryos occurs during the transformation stage and is fully completed only in the parakeratinized epithelium. The presence of loricrin and TGM-1 in cornified layer of orthokeratinized epithelium will occur after hatching.

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Organization of ovaries in clitellate annelids closely related to earthworms (Crassiclitellata)

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In recent years, ovaries and oogenesis of Clitellata (Annelida) have been the focus of intense research, which has proven that they are plastic structures, so more than a dozen different ovary types have been distinguished (especially in leeches and minute oligochaetes referred to as Microdrile). As a rule, the ovary type is constant on the family/subfamily level. The data obtained have contributed to a better understanding of the reproductive biology of Clitellata and shed light on the evolution of the ovary. It also indirectly allows to determine the degree of relationships between taxa.

There was a gap in our knowledge of the detailed organization and function of ovaries in earthworms (Crassiclitellata = Megadrile) and the allied taxa. Here, we focus on ovaries and the oogenesis in two groups of annelids closely related to earthworms. The first are representatives of Haplotaxidae – a paraphyletic group in which the ovary composition was studied in Haplotaxidae *sensu stricto* (genus *Haplotaxis*) and other Haplotaxidae (genus *Delaya*). The second group, Moniligastridae, is represented by the genus *Drawida*. Moniligastrids are believed to be the closest relatives to Crassiclitellata, and both taxa are regarded as sister groups that form a clade called Metagynophora.

Ovaries of all studied clitellate groups are composed of syncytial germline cysts accompanied by somatic cells. Germline cysts are formed by germ cells connected via intercellular bridges with a common cytoplasmic mass (cytophore). In the genus *Haplotaxis*, the cytophore forms an extensive mass of cytoplasm. In contrast, in the genera *Drawida* and *Delaya*, cytophore is poorly developed, forming only extremely thin cytoplasmic projections. As oogenesis progresses, subsequent cell categories are observed in the ovaries, from oogonia, germ cells in meiotic prophase I, to nurse cells and oocytes. Oocytes grow considerably and become filled with cell organelles and yolk. Large vitellogenic oocytes freely flow in a coelomic fluid (as in *Haplotaxis*) or are deposited in ovisacs (as in *Drawida*).

The differences observed cause ovaries to differ morphologically. *Hapotaxis* has a 'Tubifex' ovary type (as many Micodrilide), while *Delaya* and *Drawida* have completely different ovarian systems that will soon be described and classified.

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Chromosomal inheritance of parental rDNAs distribution pattern detected by FISH in *Cobitis* allotriploids (Teleostei, Cobitidae)

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This study describes the major and the minor rDNA chromosome distribution in clonally reproduced *Cobitis* allotriploids of different genomes composed of parental species: spined loach *Cobitis taenia* (TT), danubian loach *C. elongatoides* (EE) and *C. tanaitica* (NN). It was tested by fluorescence *in situ* hybridisation (FISH) whether nucleolar organiser regions (NORs) were inherited from all parental species or nucleolar dominance can be observed in clonal progeny. We also tested if the karyotype structure with the number and location of 28S and 5S rRNA sites of allotetraploids reflects their parental species. The genome composition of 12 allotriploid females was determined by karyotype analyses, intron S7 sequencing and microsatellites.

Two females (ETT) exhibited a karyotype of 3n=73 chromosomes, composed of *C. elongatoides* genome (E) and two genomes of *C. taenia* (TT). Eight 28S rDNA loci located in seven uniarmed chromosomes corresponded to *C. taenia*, whereas those in biarmed chromosomes corresponded to *C. elongatoides*. Similarly, 5S rDNA sites detected in centromeric regions of four acrocentrics and on large submetacentric chromosome corresponded to *C. taenia* and *C. elongatoides*, respectively. Six *Cobitis* females had 3n=74 chromosomes (ETN), including nine chromosomes with 28S rDNA loci and five with 5S rDNA loci; those located on biarmed chromosomes identified *C. elongatoides* whereas the rest resembled *C. taenia*, but also exhibited contributions from *C. tanaitica*. Colocalization of both rDNA sequences was observed in two acrocentric and submetacentric chromosomes. Four *Cobitis* females had 3n=75 chromosomes (EEN) with five out of eight 28S rDNA loci in biarmed chromosomes clearly indicating the genome of *C. elongatoides*. The remaining loci in subtelocentrics have to come from *C. tanaitica*.

The observed number of both 28S and 5S rDNAs sites in *Cobitis* allotriploids was disproportionally inherited from the two or three parental species. The obtained data indirectly indicated previously unknown patterns of rDNAs distribution in the karyotype of *C. tanaitica*, very similar to those in *C. taenia*. Thus, the current study provides insight into the heritability of rDNA in *Cobitis* and their cytogenetic features.

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EXPRESSION AND LOCALIZATION PATTERN OF MYOSIN VI DURING DEVELOPMENT OF THE *DROSOPHILA* BRAIN

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The main goal was to determine the cellular localization of myosin VI (M6) in the *Drosophila* brain from the larval stage to the imago stage, using the Gal4/UAS binary system and Flp/frt-mediated DNA recombination technique, which are powerful tools for controlling the temporal and spatial gene activity and neural circuitry manipulation in *Drosophila* (Fore et al., 2011). M6 is the only actin-based motor protein that moves towards the minus end of actin filaments. Although M6 is involved in numerous cellular processes as an anchor and a cargo transporter, little is known about its role in the development and function of the nervous system. So far, it has been shown that mice with a mutation in the M6 gene show a reduction in the number of synapses, abnormally short dendritic spines, and astrogliosis (Osterweil et al., 2005). Study of basic aspects of neurobiology, including bioimaging of neuron and glial cell interactions, is much easier using models of neuronal networks in invertebrates. Therefore, in our research we decided to use *Drosophila* – an excellent model organism for behavioral, neurobiological, and genetic research. We demonstrate the bioimaging of different cell populations in the *Drosophila* brain, including neuron-specific GFP fluorescence and immunofluorescence for glial cells or M6. Our results may be a starting point for research on the potential role of this unique motor protein in the functioning of neuronal cells and the interaction of neurons with glial cells, which has not been studied so far.

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MYOSIN VI CAN MODULATE THE RECRUITMENT AND EARLY MIGRATION OF BORDER CELLS BUT IS NOT ESSENTIAL FOR DEVELOPMENT OF EGG CHAMBERS IN *DROSOPHILA*

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Oogenesis in *Drosophila* is divided into 14 stages, and its particularly fascinating phase unfolds during stages 8-10, characterized by the collective migration of border cells. These specialized cells undergo a partial epithelial-to-mesenchymal transition and move as a cluster of cells through the maturing egg chambers towards the oocyte. The coordinated movement of border cells is precisely regulated by complex cytoskeletal rearrangements that include the formation of lamellipodia, stabilization of the cell cluster, and guidance of border cells between nurse cells. One of the cytoskeletal proteins that is expected to play a crucial role in border cell migration is myosin VI. This actin-based molecular motor is unique among known myosins for its ability to move towards the minus end of actin filaments and its activity is mediated by various cargo adapter proteins. Myosin VI has been shown to be highly expressed in border cells, and its depletion inhibits their migration (Geisbrech and Montell, 2002). However, since then, no results have been presented that could explain the mechanism of action of myosin VI during border cell migration. We therefore decided to re-eveluate the role of this protein in the collective migration of *Drosophila* border cells, focusing primarily on the potential interaction of myosin VI with GIPC1 – the only myosin VI cargo-binding partner identified in both mammals and *Drosophila*. We demonstrated that although myosin VI may participate in the formation of border cell clusters and their early (but not late) migration, it is not a key protein in the morphogenesis of the egg chamber and, consequently, in the development of a mature egg with functional micropyle.

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We would like to thank Kathryn G. Miller (Washington University in St. Louis, MO, US) for providing the primary antibody against *Drosophila* myosin VI. The project was supported by a grant from the Nicolaus Copernicus University in Toruń (Poland), the "Excellence Initiative – Research University – Grant4NCUStudents – 6th edition" programme.

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Microspore embryogenesis induction in Polish winter bread wheat: Novel approaches and opportunities

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The redirection of development and initiation of microspore embryogenesis (ME) are closely linked to disrupted redox homeostasis and the production of reactive oxygen species (ROS). High levels of ROS impact cell vitality and can induce the process of programmed cell death (PCD). The aim of this study was to enhance the potential of microspores to cope with the stress and to increase the effectiveness of ME in recalcitrant breeding materials of winter wheat (*Triticum aestivum* L.). Various modifications were implemented into the ME inducing procedure, ranging from very simple adjustments altering the growing conditions of the donor plants to more complex modifications of tillers pre-treatment and *in vitro* culture conditions (Dubas et al., 2024). Physiological, cytological and biochemical analyses were conducted to assess the efficiency of ROS scavenging and the management of nutrients (macro- and microelements). Among several modified procedures, the one combining spike pre-treatment with low temperature, selenium salt (SeS), and mannitol (MAN) was selected as the most potential in microspore reprogramming towards embryogenic development. The procedure influenced on the content of various macro– and micro-elements in the anthers, increased non-enzymatic antioxidative defence, and diminished the intensity of oxidative stress (ROS generation). Overall, the *in vitro* procedure developed resulted in higher microspore viability and increased effectiveness of microspore reprogramming.

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EFFECT OF THE PUMPKIN SEED CAKE-ENRICHED DIET ON TESTES OF IMMATURE RABBITS *ORYCTOLAGUS CUNICULUS F. DOMESTICUS* (LEPORIDAE) - PRELIMINARY STUDIES

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Nowadays, many people experience reproductive health issues, for which vitamins and microelements can provide natural support. Pumpkin seeds (*Cucurbita pepo*) are an excellent source of vitamin E, magnesium, iron and many microelements. Therefore, pumpkin is believed to be beneficial for improving reproductive health (Aghaei et al., 2013; Al-Salhie et al., 2017; Bakeer et al., 2021; Dotto and Chacha, 2020). The production of pumpkin oil from pumpkin seeds results in the formation of redundant pumpkin seed cake, which contains all the most valuable substances. The study aimed to investigate whether a diet enriched with pumpkin seed cake affects the reproductive system of rabbits *Oryctolagus cuniculus f. domesticus*. Thirty-five-day-old rabbits were divided into three groups with varying content of pumpkin seed cake in their feed mixture. The study assessed the effect of the diet on various testicular parameters was evaluated through morphological and histological analysis of rabbit testes. The paired testes of all studied immature specimens consisted of numerous seminiferous tubules, in which spermatogonia, spermatocytes and spermatids were distinguished. The analysis revealed slight differences between the studied groups, which were not statistically significant. Further investigation should be conducted and tested in mature specimens.

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THE IMPACT OF ALLOTRIPLOID *COBITIS* (TELEOSTEI, COBITIDAE) FEMALES GENOTYPE ON THEIR PROGENY PLOIDY AND PHENOTYPES

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Fish of the genus *Cobitis* provide a model for studying hybridization and polyploidization processes. The genome of allotriploid *Cobitis* females consists of two or three species: *C. taenia* (TT), *C. elongatoides* (EE), and *C. tanaitica* (NN). Triploid females reproduce mainly gynogenetically, forming successive clonal triploid females, or sexually, forming tetraploids of both sexes. Tetraploid progeny are believed to be less viable compared to triploid ones.

The aim of this study was to investigate the influence of the genomic composition of triploid *Cobitis* females on the ploidy and phenotypic regularity of their progeny (larvae). Seventeen triploid females were collected from three diploid-polyploid populations whereas *C. taenia* males were collected from an exclusively diploid population. Both parental males and females underwent ploidy verification using a flow cytometer, with females additionally undergoing genome composition testing through microsatellite analysis and karyotyping. Subsequently, 10 triploid ETN and 7 triploid EEN females were used to artificial spawning. The obtained progeny were analysed during the initial three days post-hatching (1-3 dph) for ploidy (flow cytometer) and external morphological features, distinguishing between normal and abnormal larvae. One-way analysis of variance (ANOVA) and Tukey's test for unequal N were used to determine the impact of the *Cobitis* female genome composition on the progeny phenotype and ploidy.

Regardless of the female genome, both 3n and 4n progeny as well as mosaic individuals (most frequently 3n-4n) were observed. No statistically significant difference was detected in the occurrence of 3n and 4n progeny based on the female's genomic composition. However, it was shown that significantly more abnormal and mosaic progeny were obtained from ETN females compared to EEN. Surprisingly, 4n offspring did not exhibit significantly more abnormalities than triploid ones. Statistically significantly more abnormal progeny were observed among mosaic individuals.

In summary, triploid *Cobitis* females, regardless of their genomic composition, possess the ability for both clonal and sexual reproduction. Moreover, the ETN genome appears to yield a less favourable outcome due to a greater number of mosaic and abnormal offspring.

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Does bisphenol A affect the cell cycle and midgut stem cells in the freshwater shrimp *Neocaridina davidi* (Crustacea, Malacostraca)?

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Environmental pollution is a fundamental ecological problem, negatively affecting the health of humans and other living organisms. Many reports prove that compounds of plastics (e.g., bisphenol A) can cause numerous diseases and, when present in the environment, can also indirectly affect higher organisms through subsequent links in the food chain (Canesi and Fabbri, 2015). The object of our research on the toxicity of bisphenol A (BPA) is *Neocaridina davidi*, a freshwater shrimp popular among breeders. It is characterized by a simple body structure, high fertility, and ease of breeding, so it has been of interest to researchers for a long time. Its midgut consists of the intestine and the hepatopancreas. The intestinal epithelium comprises digestive and stem cells, while the hepatopancreas is composed of stem, fibrillar, storage, and resorptive cells (Sonakowska et al., 2015). Our study aimed to check ultrastructural changes in the stem cells and changes in the cell cycle caused by BPA. *N. davidi* specimens were divided into control and experimental groups according to exposition to bisphenol A (10 mg/l for 24, 48, and 72h). Measurements using the Cell Cycle Kit showed statistically significant differences in the number of cells in the M phase of the cell cycle. Both in the case of the intestine and the hepatopancreas, the number of cells in the division phase was higher in individuals from experimental groups than in the control group, with the highest number of cells in the M phase being noted in the 48-hour groups. Intestinal cells treated with bisphenol A for 48 hours had the highest division activity. No changes in the ultrastructure of midgut stem cells were observed in any group. The results indicate intensive, regenerative processes activated by the action of BPA.

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rDNA markers in parentage testing of two *Hieracium* species

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The genus *Hieracium* comprises easily identifiable diploids that reproduce sexually and a vast number of polyploids which reproduce apomictically. They are classified as near-obligatory apomicts, meaning they produce seeds asexually but can still generate some fertile pollen. Occasionally, they can release unreduced (3x) pollen grains that may fertilize sexual diploid plants. It can result in offspring possessing a complete genome set from the apomictic parent along with one genome from the diploid mother plant, leading to the emergence of new microspecies at a higher ploidy level.

After rDNA-FISH analysis the karyotype of triploid *H. alpinum* revealed a unique small chromosome with terminally located 5S rDNA, a feature absent in the diploid form of this species and not observed in other *Hieracium* plants (Grabowska-Joachimiak et al., 2023). Surprisingly, further investigation of higher polyploids (4x, 5x) within the H. section Alpina also exhibited this distinctive chromosome. These findings suggest evolutionary pathway where the higher polyploids likely originated from the triploid *H. alpinum*.

In *Hieracium*, numerous polyploid forms with uncertain origins are thought to be hybrids of *H. alpinum*. Utilizing the rDNA-FISH method could help in confirming the involvement of *H. alpinum* in their ancestry. This study focused on analyzing two endemic taxa, *H. amaurocranum* and *H. jasiewiczii*, which are considered descendants of *H. alpinum*.

The involvement of *H. alpinum* as a parent was verified only in *H. amaurocranum*, where the 5S chromosome marker was identified. Since this chromosome is characteristic of polyploid forms of *H. alpinum*, it is reasonable to infer that a tri- or tetraploid plant from this group served as the pollen donor. Although no 5S chromosome was found in *H. jasiewiczii*, the potential contribution of *H. alpinum* to its origin cannot be dismissed, possibly as the diploid maternal plant.

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Does temperature affect the body length of the allotriploid *Cobitis* offspring (Teleostei, Cobitidae)?

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In the face of global environmental changes, understanding the adaptive responses of organisms is paramount for predicting and mitigating their implications. The study aimed to assess the impact of temperature on progeny size of triploid *Cobitis* females reared under varying thermal conditions: 18, 22 and 28°C. Triploid *Cobitis* females were caught from the Pilica River, while diploid *C. taenia* males were collected from Lake Legińskie or the Łyna River. Polyploid offspring were produced through crosses between triploid *Cobitis* females and *C. taenia* males. The ploidy status of all individuals was determined either karyologically or via flow cytometry. Eggs obtained from each female were divided into three groups, fertilized, and then incubated at 18, 22 and 28°C, respectively. Body length measurements were conducted on approximately 30 larvae from each cross and temperature condition at six different developmental points: 1, 2, and 3 days post-hatching (dph), at 14 dph, and after 2 and 6 months post-hatching. Statistical analyses were performed using ANOVA followed by post hoc Tukey tests in Statistica 13.

It was shown that during the first three days and 14 days after hatching, the offspring reared at different temperatures did not differ statistically significantly in size. However, after 2 months, progeny reared at 28°C exhibited significantly greater body length compared to those reared at 18°C, while no differences were evident between offspring raised at 18°C and 22°C or between 22°C and 28°C. Furthermore, after 6 months, progeny reared at 22°C and 28°C displayed significantly greater body length compared to those reared at 18°C, with no significant differences observed between individuals raised at 22°C and 28°C.

The results demonstrate that temperature influences the size of allopolyploid *Cobitis* offspring. However, statistically significant differences in size were only evident during the second and sixth months of life, particularly between the highest and lowest temperatures analyzed.

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THE TOPOGRAPHY OF ORGANS IN THE THORACIC CAVITY OF THE SHORTHAIR CAT DURING PRENATAL DEVELOPMENT.

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During prenatal development, the spatial arrangement of organs in mammalian thoracic organs undergoes significant changes due to the growth of the heart, lungs, and thymus. This study aims to evaluate topographical changes in the developing thoracic organs of the domestic cat. The research material was formalin-fixed feline fetuses aged 24 - 62 days p.c. Dissection of the thoracic cavity was performed, and macroscopic observations were documented using a digital microscope and morphometric analysis using Multiscan software. Some fetuses were analysed by SEM, magnetic resonance scanner type Brucker BIOSPEC 70/30USR, and 3D reconstruction od tissues was performed in AMIRA software. According to Polish law and EU directives, the research did not require the approval of the Local Ethical Committee. From 24 days p.c., the heart is the most prominent organ in the middle mediastinum and extends on a level between the 3rd to the 8th cartilaginous primordia of ribs. The apex of the heart is directed ventrally to the left of the median plane of the thoracic cavity. The thymus has an elongated cervical lobe near the caudal part of the trachea and a small oval thoracic lobe ventrally at the thoracic inlet. The narrow lobes of the lungs extend dorsally in the direction of the diaphragm. Between 35 and 37 days p.c., the heart is directed more oblique and left ventrocaudally toward the diaphragm. The narrow cervical part of the thymus reaches the mid-height of the trachea. In contrast, the thymus's oval, undivided thoracic lobe is positioned ventrally on the level of the 1st and 4th ribs. The growing in-size right and left lobes of lungs with well-visible interlobular fissures extend in space between the 1st and 9th ribs. Between 40-45 days, the heart takes position between the 4th intercostal space and the 8th rib. The base of the lungs fully reaches the diaphragm and, on both sides, rolls up ventrally around the heart. Extensive growth of the left thoracic lobe of the thymus is noted. Between 50 to 60 days p.c., the well-lobulated thoracic part of the thymus gets wider and extends behind the sternum and in front of the pericardium and great vessels of the heart.

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IMMUNODETECTION OF GMCSF IN *GALLERIA MELLONELLA* HEMOCYTES AFTER INFECTION WITH FUNGUS *CONIDIOBOLUS CORONATUS* AND APPLICATION OF FUNGAL METABOLITES

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Insect models share many signaling and energy metabolism pathways with mammals, as well as some structural and innate immunity components, and are hence considered novel tools for researching human diseases and establishing drug toxicity. Furthermore, such models represent cost-effective, easy to rear and time-efficient methods for investigating physiological processes and supporting the early steps of drug discovery. However, further studies are needed to better understand the immunological similarities between vertebrates and invertebrates.

The pro-inflammatory cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) acts as a growth and differentiation factor for granulocyte and macrophage populations in mammals. While such cytokines are known to be evolutionarily conserved, little is known of the insect analogues to mammalian cytokines and cytokine receptors.

The mammalian pathogen *Conidiobolus coronatus* demonstrates strong entomopathogenic potential against larvae. Previous research indicated the presence of two β-carboline alkaloids, harman and norhaman in fungal medium; these two metabolites affect the hemocytes, i.e. the immunocompetent *Galleria mellonella* cells, of *G. mellonella*. The main goal of the present study was to determine the effect of fungal infection and administration of harman and norharman on the level of GM-CSF in *G. mellonella* hemocytes.

G. mellonella larvae were exposed for 24 hours to sporulating *C. coronatus* colonies. Following this, one group of insects was collected for examination (F24) while the rest were left for another 24 hours before collection (F48). Additionally, larvae were exposed to harman and norharman (750, 1000, 1250 ppm), topically and by mixing with food. The hemocytes were tested for GMCSF using ELISA and immunocytochemistry (fluorescence microscopy and flow cytometry). Additionally, the impact of GM-CSF on cell viability was examined by direct application to cultured hemocytes (20 and 40 pg/ml in well).

Infection with *C. coronatus* increased the level of GMCSF in *G. mellonella* hemocytes, as did topical and food administration of both alkaloids. The *in vitro* application of GMCSF had no impact on cell viability. Our results may have a considerable impact on research concerning innate immunology, insect physiology and mycology.

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Comparative study of lung differentiation in two lizard species

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A simple saccular structure generally characterizes reptilian lungs. The differences between them are mainly limited to the internal structure. Lung differentiation in reptile embryos is poorly understood, so this study aimed to compare the differentiation of the lung structure in the brown anole (*Anolis sagrei*) and mourning gecko (*Lepidodactylus lugubris*) in particular developmental stages. Embryos of both species were derived from breeding at the Institute of Biology, Biotechnology and Environmental Protection. The embryos of the lizard species were isolated at regular intervals until hatching, and the age of the embryos was calculated using a species-specific developmental table (Sanger et al., 2008; Griffing et al., 2019). The embryonic tissues, including lungs, were fixed in Bouin solution and, after dehydration, embedded in paraffin. Paraffin blocks were cut on the 7µm thick serial sections. Histological sections were stained using hematoxylin-eosin and Azan Heidenhan's methods. Embryonic tissues were analyzed under light microscopy. Based on serial paraffin sections 3D reconstructions of developing lungs and muscle systems were made. The results of these studies indicated that the process of lung formation in both lizard species can be divided into three phases. Towards the end of embryonic development, the trabeculae in the lung wall of the brown anole form a characteristic pattern with large meshes. In contrast, the smooth muscle network forming during lung differentiation in the mourning gecko is irregular, with smaller meshes, not as distinct as that of the brown anole.

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Regular course of embryological processes in natural and artificial autopolyploids of *Arabidopsis arenosa*

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A pseudometallophyte *Arabidopsis arenosa* (Brassicaceae), a metal- and drought-tolerant indicator of calamine flora in Western Europe is a good candidate for phytoremediation due to its ability to accumulate (hyperaccumulate) Zn and Cd. Several attempts have been made to increase plant size (biomass) to improve phytoextraction efficiency. Artificial autooctaploids (2n = 8x = 64, 2C ≈ 1.6 pg) for this study were derived from the natural metalliferous population of tetraploids (2n = 4x = 32, 2C ≈ 0.8 pg) *via* indirect organogenesis, without using common antimitotic agents (Kurdziel et al., 2023). Embryological processes were investigated in maternal plants growing in the field and in hydroponic culture (offspring of both 4x maternal plants and 8x regenerants). The sexual reproduction in 4x and 8x plants was occurring properly. The 7-celled embryo sac (female gametophyte) developed according to the *Polygonum* type. There were no signs of developmental disturbances and degeneration typical for autopolyploids in plants cultivated in hydroponics whereas in plants from the field disturbances in the female germline occurred at the frequency reaching up to 28% (Kwiatkowska and Izmaiłow, 2014). Pollen viability was high (90-100% stainability by Alexander dye). Seeds of tetraploids collected from the field and obtained from octaploids showed high germination capacity and the seedlings developed properly.

Such a stable polyploid lineage manifesting normal course of embryological processes could further serve practical applications in plant engineering (including phytoremediation). Disturbances in the female germline in plants from the field are the result of the heavy metal influence on *A. arenosa* sexual reproduction.

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The influence of titanium compounds on the reproductive processes of *Solanum lycopersicum* L. (Solanaceae)

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Tomato is an important and widely cultivated crop with high nutritional value. The effects of titanium on growth, yield, leaf number, nutrient content or stress response have been extensively studied (e.g. Lyu et al., 2017). However, detailed information on the influence of titanium on embryological processes is lacking.

We investigated the effect of titanium (in the form of Tytanit® and titanium oxide) on the reproduction of *Solanum lycopersicum* L. cv. 'Bajaja'. Flowers and fruits were fixed in FAA solution and cleared with methyl salicylate (Kwiatkowska et al., 2019, modified). The number of ovules in the ovaries and the subsequent stages of embryogenesis were examined in control and titanium-treated plants. A total of 450 flowers/fruits were analyzed from 15 plants (five per each of the three treatments). The results showed a positive effect of Tytanit® on the number of ovules and seeds in comparison with the control and the plants treated with titanium oxide (statistically significant difference according to the F-test). A very interesting phenomenon, such as the acceleration of the development of the reproductive organs, anthers and ovules, as well as self-pollination in flower buds, was observed in flowers after Tytanit® treatment. In addition, in the post-anthesis stages, faster embryo development was observed after Tytanit® treatment compared to embryogenesis in control and titanium oxide-treated developing fruits.

Our research into the influence of biostimulants on the generative reproduction of plants is part of the current trend of combining scientific research with industrial and manufacturing activities.

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SENSITIVITY OF SOLITARY BEE *OSMIA BICORNIS* LARVAE TO ROAD DUST

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Wild pollinators play a crucial role in maintaining the health of ecosystems. However, they are at risk of exposure to pollutants while foraging, making it essential to understand and address these threats immediately.

One such threat is road dust, which contains tire and road wear particles (TRWP) that can have direct and indirect toxic effects on pollinators. The direct effects are caused by the harmful effects of rubber microparticles and the abrasion of car parts and road surfaces, while the indirect effects result from pollutants like pesticides and heavy metals that can be absorbed by tire dust particles. Since many European countries have extensive areas of pollinator-dependent crops located near heavily trafficked roads, pollinators exposed to these pollutants while foraging may experience stress reactions, immune disorders, and changes in energy allocation.

This project objective is to explore the effects of road dust on the condition of solitary bees in ecosystems that are in close proximity to high-traffic roads. Traffic-generated TRWP discharge pollutants, including microplastics (MP), into the air, which can be hazardous to the health of pollinators due to their potentially toxic properties and ease of ingestion.

To conduct the research, the study will use *Osmia bicornis*, a common European species, as a model. This species is well-studied in terms of its general biology, nesting, and development. The research site will be set up to create ideal conditions for the development of *Osmia* species, including nest tube setups and a contaminated pollen source. Sensitivity of larvae to road dust exposure through oral ingestion will be researched by examining life history parameters such as survival and growth of larvae and biomarkers of stress and non-specific immunity. The study will evaluate the impact of contaminated pollen on the studied organisms by analyzing the functional traits of their larvae.

This research is significant for understanding the impact of road dust on wild pollinators and developing effective strategies to protect their health and biodiversity.

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THREE-DIMENSIONAL ULTRASTRUCTURE OF FEMALE GERM CELLS OF SELECTED EARTHWORMS

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Serial block-face imaging is a sophisticated microscopy technique utilized to create three-dimensional reconstructions of biological samples. The process involves imaging the surface of a sample block, removing thin layers of material, and capturing images of the newly exposed surface. This process continues, capturing images at progressively deeper layers to compile a 3-D representation of the sample.

While commonly employed for neural tissue studies, our research highlights its significant potential in investigating invertebrate gonads (Świątek et al., 2023; Urbisz et al., 2022; Urbisz et al., 2020). Despite earthworms' well-understood morphology and anatomy, data on ovarian structure and oogenesis in Crassiclitellata remain limited.

Three-dimensional analysis using serial block-face imaging provides comprehensive insights into ovarian ultrastructure and processes. This facilitates better comprehension of ovarian architecture and oocyte development processes. It also allows accurate measurements of organelle and cell volumes during oogenesis, enhancing understanding of ovarian component functions and interactions. Additionally, it aids in detecting subtle changes in ovarian structure, challenging to identify in conventional two-dimensional electron microscopy analyses.

Our findings underscore the benefits of employing three-dimensional methods to analyze earthworm ovaries. For example, they enable precise visualization and volumetric calculation of the cytophore – i.e., the central cytoplasmic core interconnecting germ cells into a cyst. Furthermore, advanced morphometry of heterochromatin and its physical interactions with the nuclear envelope are possible due to the applied methods. However, the presented data only scratches the surface of the capabilities offered by three-dimensional analysis of ultrastructure.

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THE IMPACT OF WATER-SOLUBLE AMINO-FULLERENES ON THE DEVELOPMENT OF *D. MELANOGASTER* LARVAE

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Photodynamic therapy combines non-toxic photosensitizers (WSAF) with light to generate reactive oxygen species (ROS), leading to cell death. We chose the fruit fly *Drosophila melanogaster* as our model species because it is among the most economically important pest insects worldwide. Different species from this taxa are responsible for destroying many fruits and fleshy vegetables globally. The damages caused by these species are so critical that broad-spectrum eradication programs have been developed worldwide. *D. melanogaster* is one of the most commonly used model organisms for various biomedical sciences, including embryology, genetics, cell biology, and toxicology. Utilizing D. melanogaster allows us to thoroughly investigate WSAF's potential as a new anti-pest agent at physiological, histological, and molecular levels. Our data from previous studies show that WSAF, without the photodynamic effect, has no toxic effects on adult specimens of *D. melanogaster*. The presented results are part of a project aimed at conducting multi-directional studies on the photodynamic effects of water-soluble aminofullerenes (WSAF) on *D. melanogaster* larvae. This approach explores a novel method for controlling food pests during transportation and storage. Thus, our goal is to investigate the potential of various fullerenes (C60, C70) to produce ROS in the intestine of *D. melanogaster* larvae and study the processes related to changes in ROS concentration. Using the MUSE cytometer (Muse Oxidative Stress Kit), we analyzed the level of ROS-positive cells in three larval stages: L1, L2, and L3. Muse Multi-Color DNA Damage Kit enabled to detect activation of ATM (a member of the phospho inositide 3-kinase (PI3K)-related Ser/Thr protein kinase family) and histon H2A.X activated cells. Thus, it is used to indicate the level of DNA damage within cells. Moreover transmission electron microscope was used to study ultrastructure changes in intestine at all larval stages. The results will be presented using statistical methods.

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THE OVARY STRUCTURE OF *MILNESIUM INCEPTUM* (TARDIGRADA: APOCHELA) – PRELIMINARY RESEARCH

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Milnesium inceptum is a representative of the order Apochela. Individuals of this species, similar to other species in this genus (Poprawa and Janelt, 2019), usually lay smooth eggs to the exuvium. The aim of our study was the analysis of the gonad structure of *M. inceptum*.

The ovary of *M. inceptum*, similarly to the ovary of other members of Eutardigrada (Poprawa and Janelt, 2019), is an unpaired structure located on the dorsal side of the body. Its size depends on the phase of oogenesis. During previtellogenesis, the gonad is small, but just before oviposition, it occupies most of the animal's body, pressing on the midgut. The ovary is attached to the body wall by a terminal filament. The ovarian wall is composed of a single-layer flat epithelium. The gonad is divided into the germarium (located in the top part of the ovary) and the vitellarium (occupying most of the ovary). The germarium contains oogonia, which undergo numerous cell divisions. As a result of incomplete divisions, clusters of germ cells are formed, which move to the vitellarium. Further phases of oogenesis, such as late previtellogenesis, vitellogenesis and choriogenesis, occur in the vitellarium. The germ cell cluster of *M. inceptum* has a completely different structure than those described in Eutardigrada (Poprawa and Janelt, 2019). It consists of several interconnected multinuclear cells constituting the central part of the cluster. Numerous mononuclear cells are attached to multinuclear cells by cytoplasmic bridges. During the oogenesis, some of the mononuclear cells of the cluster differentiate into oocytes. Multinuclear cells play the role of trophocytes which support oocytes. The material for the yolk is synthesized in all cells of the cluster and transported to the oocytes via cytoplasmatic bridges.

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The effect of plastic consumption on the cell cycle of *Galleria mellonella* (Insecta, Lepidoptera) larvae

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Plastic, used in the packaging industry for everyday products, is prevalent today. It is made of synthetic polymers such as polypropylene. The decomposition process of plastics can take up to 100 to 1000 years. The consequence of this is not only his presence in urban agglomerations but also in natural ecosystems. Scientists have been looking for a way to quickly and effectively degrade plastics for many years, looking for the possibility of using living organisms capable of carrying out this process. The world of science is looking for a solution in bacteria capable of digesting, for example, polypropylene. Many such species occur in the microflora of some insects. A few years ago, it was proven that the greater wax *Galleria mellonella* larvae can digest polypropylene. The digestive tract of caterpillars contains bacteria that enable them to carry out this process. However, there is still no comprehensive data on the impact that consuming plastic may have on larvae. It is difficult to assess whether such a procedure will affect the structure and functioning of internal organs. This study aimed to check whether polypropylene in the larvae of *G. mellonella* diet does not affect cell cycle disorders in selected organs. The experimental groups (GC, GS, G24, G48) were subjected to starvation, and then the G24 and G48 groups were fed with polypropylene for 24 or 48 hours. The larvae were then anesthetized with chloroform and decapitated. The silk gland, midgut, and fat body were isolated and analyzed. The tests were performed using a Muse cytometer and Cell Cycle Kit. The results shed a positive light on using *G. mellonella* larvae to biodegrade plastic. A polypropylene diet does not disrupt the cell cycle in selected caterpillar organs, and the differences are not statistically significant. It can, therefore be concluded that the lack of an increase in the number of cells undergoing mitosis suggests no need for regeneration

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A COMPARATIVE STRUCTURAL ANALYSIS OF EGGS IN THREE SPECIES OF THE GENUS *PARAMACROBIOTUS* (TARDIGRADA: EUTARDIGRADA)

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Tardigrades are tiny invertebrates famous for their resilience to various stressors. Eggs serve as taxonomic markers among tardigrade species by both morphology and laying methods (freely in the environment or in exuvium). These differences not only help with the identification of species but also offer valuable understandings of evolutionary adaptations.

The aim of our research was to describe the structure of the eggs of three species from the genus *Paramacrobiotus.* The research was conducted using Light Microscopy (LM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) techniques. The eggs of *Paramacrobiotus fairbanksi, Paramacrobiotus experimentalis* and *Paramacrobiotus gadabouti* are laid straight into the environment, not within the exuvium (Schill et al., 2010; Kaczmarek et al., 2020; Kayastha et al., 2023). However, the internal surface of the areolae in *P. experimentalis* exhibits a corrugated structure, while *P. gadabouti* a surface with pores. Interestingly, *P. fairbanski* presents a combination of both these features. *P. gadabouti* having ornamentation of the *richtersi* type, contrasting *P. experimentalis*, which shows ornamentations of the *areolatus* type (Kaczmarek et al., 2020; Kayastha et al., 2023). Additionally, there is a notable difference in the morphology of the top endings of the processes, and the number of areolae surrounding each process. Understanding the differences in egg morphology and laying methods between species is crucial for accurate taxonomic classification and evolutionary studies.

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Immunohistochemical analysis of cell wall components during the development of *Fagopyrum esculentum* and *F. tataricum* zygotic embryos

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Buckwheat is a genus that includes 23 species, including *F. esculentum* and *F. tataricum*, the most commonly cultivated. Buckwheat is rich in gluten-free proteins, amino acids, fatty acids, vitamins, and bioactive compounds such as rutin and other elements. *F. esculentum* is more widely distributed; however, its seed yield is often relatively low and unstable. It is the result of *F. esculentum* self-incompatible as it develops two types of flowers: Pin (pistils longer than stamens) and Thrum (pistils shorter than stamens). *F. tataricum* produces only homostylous flowers capable of self-fertilisation (Tomasiak et al., 2023).

The plant cell wall is a dynamic network composed of cellulose, hemicelluloses, pectins and, to some extent, proteins and phenolic compounds. The cell wall composition differs among species tissue types, between developmental stages, and under the influence of biotic and abiotic factors. Cell walls can regulate the growth of plant cells and even participate in cell differentiation or morphogenetic processes. However, these processes depend mainly on dynamic changes in cell wall components. Therefore, studying the dynamic changes in the composition and structure of cell wall components during embryogenesis is essential.

Embryogenesis is a critical stage of plant development in which its primary organisation and body plan begin. During this development period, the zygote undergoes a complex series of morphological and cellular changes that form a mature embryo, consisting of an embryonic axis with shoot and root poles and cotyledon.

In this study, we have reported for the first time the chemical composition of the cell walls in *F. esculentum* Pin and Thrum and in *F. tataricum* zygotic embryos of different stages using immunohistochemistry. The results showed spatial-temporal changes in pectin, arabinogalactan proteins and extensin epitopes depending on the developmental stage. Additionally, observations indicate differences in the occurrence of some cell wall components in developing zygotic embryos between the analysed variants.

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MYOSIN VI IN OOGENESIS OF *DROSOPHILA MELANOGASTER*

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Oogenesis of *Drosophila melanogaster* – a convenient model for research in developmental biology – is a complex process involving the differentiation of individual cell populations, intracellular communication, and even cell migration (Hudson and Cooley, 2014). More than 20 years ago, Geisbrecht and Montell (2002) proposed that the unconventional myosin VI, a unique molecular motor with a remarkable ability to move towards the minus end of actin filaments, is an essential protein for border cell migration during *Drosophila* oogenesis. Nevertheless, there is a lack of new studies focusing on the probable mechanism of action of myosin VI in the collective migration of border cells, as well as on the involvement of this protein in other stages of oogenesis. The aim of our research was to identify the localization sites of myosin VI in *Drosophila* egg chambers at various stages of their development in relation to filamentous actin (F-actin) using immunocytochemistry, phalloidin F-actin staining, and confocal microscopy. We demonstrate that myosin VI is abundant in egg chambers at early stages of oogenesis, including oocytes, nurse cells, and follicular epithelial cells. Myosin VI is also present in the border cell cluster during its recruitment and initiation of migration, but is absent from border cells during the later stages of migration. Based on immunocytochemical analysis of myosin VI in specific cells and subcellular domains in the context of actin cytoskeleton architecture, we propose potential roles for this protein in *Drosophila* oogenesis. Moreover, the results of our research enrich the existing knowledge and reopen the discussion on participation of myosin VI in border cell migration.

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THE INFLUENCE OF NICKEL ON STEM CELLS OF MIDGUT OF FRESHWATER SHRIMP *NEOCARIDINA DAVIDI*

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Crustaceans are treated as bioindicators of environmental cleanliness in toxicological tests. Their digestive system is treated as the organ that participates in homeostasis maintenance due to its contact with different stressors transferred with nourishments. Nickel (Ni), due to anthropogenic activity, increased concentrations enter the environment and may cause homeostasis disorders and also cause toxic and lethal effects in high concentrations. The study aimed to check the mitotic activity of midgut stem cells due to the appearance of Ni in the environment. It was also essential to determine whether Ni causes these changes and the body's response to returning to unpolluted water. Thus, the analysis aimed to assess the proliferation potential of midgut stem cells.

Shrimps were exposed to an aqueous nickel solution with a concentration of 8 mg$NiCl_2$/l for one and two weeks. Then, after exposure, they were returned to clean water for another one and two weeks to check whether the changes were reversible. Then, the midgut, which consists of the intestine and the hepatopancreas, was isolated. To achieve the objectives of the study, TEM and flow cytometry were used - tests: Cell Cycle and Ki67 (cell proliferation). The results showed no changes at the ultrastructural level in stem cells, which may indicate the presence of protective mechanisms. However, the presence of Ni in the environment influenced the cell cycle of the analyzed cells and the percentage of proliferating cells, especially in the intestine. In this tissue, in the exposure groups, the rate of cells in the S and G_2/M phases decreased, while in the groups that returned to clean water, a gradual increase in these parameters was observed in proportion to the duration of cleansing. The percentage of proliferating cells increased in the Ni1:0 group and decreased in the Ni1:2 and Ni2:2 groups. Cell cycle analysis showed no significant differences in the hepatopancreas. However, in the Ni1:1 and Ni2:1 groups, a significant increase in the percentage of proliferating cells was observed, and a significant decrease in the Ni1:2 group.

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Prenatal development of the ovarian cortex in the European shorthair cat

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During the formation of the ovary, the mesothelial epithelium of the genital ridges differentiates into an ovarian surface epithelium, which is populated by germ cells and forms cortical cords, representing the ovarian cortex. This study aimed to describe the development of the domestic cat's ovarian cortex.

The material was cat ovaries of embryos and fetuses aged 24 - 63 days p.c. The methods were the LM observations and analysis of 3D models prepared in AMIRA (FEI) software for microscopy image visualization and analysis.

Results show that on 24 - 25 dpc, the ca. 6 µm high simple mesothelial epithelium of the genital ridges passes strands of cells, forming the primary sex cords. The gonocytes are observed beneath the epithelium. By day 28 p.c., the genital ridges become undifferentiated gonads, covered by the ca.11µm high epithelium, connected with the primary sex cords. The sparse gonocytes are observed in the epithelium and the primary sex cords. Between 34 - 36 dpc, in the ovaries, the primary sex cords lose connectivity with the surface epithelium, and the secondary cortical sex cords develop. The oogonia are regularly distributed in the epithelium and sex cords, and their number increases over threefold. By 44 dpc, the stratified 18 µm high ovarian surface epithelium contains double the number of oogonia. The epithelial cells with oogonia are recruited to the cortical sex cords, which elongate and define the cortical layer of the ovary. Between 53 - 63 dpc, 12 µm high, mostly two-layered ovarian epithelium loses contact with the cortical sex cords. The loss of oogonia in the ovarian epithelium is observed, whereas the cortical cords disintegrate and form primary ovarian follicles.

To conclude, during the development of the ovarian cortex, changes in the structure and size of the surface epithelium, the structure of sex cords, and the distribution and number of germ cells are noticed. The most numerous oogonia in the high ovarian epithelium are observed by day 44 p.c., associated with intensive cortical cord growth. Until the end of the prenatal period, from the ovarian surface epithelium, the epithelial cells are recruited to the sex cords, whereas the germ cells populate the cortical cords by day 53 p.c.

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MORPHOLOGY AND HISTOLOGY OF THE OVARIES IN EARTHWORMS BELONGING TO THE GENERA *OCTOCHAETONA* AND *RAMIELLA* – A PRELIMINARY STUDY

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Earthworms (Crassiclitellata) are hermaphrodites and have both female and male reproductive systems. So far, little attention has been paid in the literature to ovaries and the process of oogenesis in these organisms. Therefore, our project involves studying the ovaries of many earthworm families for comparison purposes and to shed light on their evolution. So far, the families Hormogastridae, Megascolecidae, and Lumbricidae have been analyzed and the ovaries have been described at the histological and ultrastructural level. These studies revealed that ovaries may have different morphology, however, in all studied species germline cysts were observed, so this is likely a conservative feature among the Crassiclitellata. Another family chosen for the study is the species-rich and widely distributed family Acanthodrilidae. The results presented here show the morphology and general histology of the ovaries of individuals belonging to the genera *Octochaetona* and *Ramiella*. Already at this stage, it can be concluded that the female gonads in Acanthodrilidae are similar in their organization to those observed in the family Megascolecidae. Studies at the level of gross morphology indicate that ovaries are fan to rosette-shaped with numerous rows of growing oocytes (egg strings), radiating from the ovary center towards the segmental cavity. Further work will focus on the more specific examinations and will be carried out using fluorescence and electron microscopy. This will enable the ultrastructural visualization of female germline cells, including the investigation of the occurrence of germline cysts. Hopefully, it will provide to better understanding of ovary organization and oogenesis in earthworms of the family Acanthodrilidae.

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MYOSIN VI AND ITS CARGO ADAPTOR PROTEINS ARE EXPRESSED IN THE MOUSE EPIDIDYMAL EPITHELIUM

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Unconventional myosin VI (M6) is the only actin-based molecular motor that moves toward the minus end of actin filaments. This unique feature, and the fact that M6 activity in different cells/cellular processes is modulated by different adapter proteins, may explain many of the functions proposed for myosin VI, including endocytosis, secretion, and regulation of actin dynamics. For example, M6 targeting to clathrin-coated pits involves clathrin adaptor disabled-2 (Dab-2) while GIPC1 recruits M6 to APPL1-positive signaling endosomes (Tumbarello et al., 2013). The lack of M6 protein in humans and mice is the cause of wide range of pathologies, such as deafness, hypertrophic cardiomyopathy, hippocampal disorders, problems with food absorption, and pulmonary and cardiac fibrosis. Additionally, we observed reduced fertility in Snell's waltzer mice males (M6 mutants). To date, no studies have been conducted on the involvement of M6 in the structural organization and functioning of the epididymal epithelium, which creates a separate luminal environment for sperm maturation/storage. Most of the epididymal epithelial cells form numerous microvilli in the apical zone, and their functions are based on the exo- and endocytosis. We suspect that M6 is involved not only in maintaining the microvilli organization of the epididymal epithelium but is also in creating the epididimal microenvironment by exchanging cargo between the epithelium and the lumen of the epididymis, where sperm mature. Therefore, the aim of our research was the immunocytochemical localization of M6 and its protein partners involved in endocytosis pathways.

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How does the gonad of the sand lizard (*Lacerta agilis*) differentiate? – Histological and 3D studies

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Squamate reptile's gonad differentiation remains barely described for this reason; the aim of this was the morphology of gonad development in the sand lizard (*Lacerta agilis*) embryos in crucial developmental stages. This study was conducted on the sand lizard (*Lacerta agilis*) embryos. The eggs were obtained from fertilized female individuals and then incubated under laboratory conditions. The age of embryos was calculated using the table of species development (Peter, 1904). The embryo isolation took place in regular time intervals until hatching. The embryonic tissues, including mesonephros and gonads, were fixed in Bouin solution and, after dehydration, embedded in paraffin. Paraffin blocks were cut on the 7µm thick serial sections. Histological sections were stained using hematoxylin-eosin and Azan Heidenhan's methods. Embryonic tissues were analyzed under light microscopy. Three-dimensional reconstructions (3D) of the gonad primordia and mesonephros were made using serial transverse sections. Based on 3D reconstructions, steps of gonad primordia differentiation and spatiotemporal localization of the primordial germ cells within the differentiating gonad were described. Results of this study showed that in the course of sand lizard gonad differentiation, four morphological stages may be distinguished (genital ridge, undifferentiated, bipotential and sexually differentiated gonad). These stages are similar to other vertebrate species. During gonad differentiation, the first primordial germ cells (PGCs) were found within the coelomic epithelium of the genital ridge. Within undifferentiated gonads, the primordial germ cells are located in their central part. After cortex and medulla differentiation (bipotential gonad), the primordial germ cells were placed within both. Just before hatching, the medulla of the studied gonad contained the prominent seminiferous tubules with PGCs.

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In bush cricket oocytes the disintegration of the Balbiani body involves microfilaments and Drp1 protein

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The Balbiani body is an intricate organelle complex found in the oocytes of many vertebrate and invertebrate species, yet its precise morphology and function are not well understood. It has previously been suggested that this complex is involved in the delivery of organelles and macromolecules to the germ plasm, formation of oocyte reserve materials, and transfer of mitochondria to the next generation. In this report we present results of analyses of the Balbiani body morphology and functioning in the oocytes of a bush cricket, *Meconema meridionale* (Orthoptera: Tettigoniidae). A wide combination of molecular and imaging techniques, including computer-aided 3D reconstructions, detection of mitochondrial DNA (mtDNA) synthesis, and immunolocalization studies have been carried out in our studies. We show that the Balbiani body in *Meconema* consists mainly of a network of mitochondria and perinuclear aggregates of electron-dense fibrillo-granular material, termed the nuage. As oogenesis progresses, the mitochondrial network contained within the Balbiani body undergoes profound changes; initially it expands to fill almost the entire ooplasm, then divides into several smaller micro-networks, and finally into individual mitochondria. Our results indicate that dynamin-related protein 1 (Drp1) and microfilaments are involved in this process. In addition, our data show the presence of phagophores surrounding individual mitochondria, indicating that mitophagy occurs within the Balbiani body. Altogether, our results support the idea that the Balbiani body participates in the multiplication and selective elimination of mitochondria and may therefore contribute to the transfer of undamaged (healthy) mitochondria to the next generation. These findings advance our understanding of the processes underlying oocyte development and have wider implications in the fields of developmental biology and reproductive science.

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The Balbiani body in previtellogenic oocytes of two bush-crickets larvae

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The Balbiani body is a membraneless complex of organelles specific to female germline cells in both vertebrate and invertebrate species. It is located in the close vicinity of the oocyte nucleus and always contains numerous mitochondria and granulo-fibrillar material, termed the nuage. Previous studies have demonstrated that the Balbiani body varies in size, shape, and location, even among closely related species (Sekula et al., 2020; 2022). Here, we present the results of our morphological and ultrastructural analyses of early previtellogenic oocytes in two long-horn bush-crickets larvae, *Tettigonia cantans* and *Roeseliana roeselii*. We show that during this phase of development and stage of oogenesis, the Balbiani bodies of analysed species are morphologically similar and relatively compact. They are always located asymmetrically on one side of the oocyte nucleus and comprise two easily discernible layers (zones). The first layer (the one located next to the nuclear envelope, termed perinuclear) is more transparent and comprises small aggregations of the nuage material associated with endoplasmic reticulum elements. The second layer (termed cytoplasmic) consists of significantly larger and varying in size nuage aggregations intermingled with polymorphic mitochondria. Importantly, we found that, at least in some cases, there is a direct contact between mitochondria and the nuage. Furthermore, the nuage material found in the cytoplasmic layer of the Balbiani body can attain at least two forms: more and less condensed. One apparent difference between the analysed species is that the Balbiani body in the oocytes of *T. cantans* contains more expanded and well-developed endoplasmic reticulum cisternae.

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Does paracetamol present in the environment affect the gonad of *Hypsibius exemplaris* (Tardigrada, Eutardigrada)?

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Paracetamol is one of the most commonly used over-the-counter pharmaceuticals in the world. It has analgesic and antipyretic effects similar to nonsteroidal anti-inflammatory drugs (NSAIDs), but unlike them, it does not have anti-inflammatory effects. 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 impacts aquatic organisms (Parolini, 2020). Therefore, our study aimed to analyze the potential effect of paracetamol on the ovary of the tardigrade *Hypsibius exemplaris*. The analyzes were performed for three paracetamol concentrations (0,2 µg/L, 230 µg/L, 1 mg/L) and two incubation times (7 and 28 days). The concentrations of the pharmaceutical for the experiment were selected based on literature data regarding its presence in the aquatic environment (Parolini, 2020). The incubation time was adjusted to the lifespan of the species under study. The research was carried out using a transmission electron microscope.

Paracetamol at a concentration of 0.2 µg/L, regardless of the length of incubation time, did not cause any visible changes in the ultrastructure of both somatic cells of the gonad and germline cells. A similar situation occurred concerning the concentration of 230 µg/L and the incubation time of 7 days. The first changes in the ultrastructure of gonad cells were visible at a paracetamol concentration of 230 µg/L and an incubation time of 28 days. Few mitochondria of both somatic and germline cells lost their mitochondrial crests, and the cisterns of the rough endoplasmic reticulum were slightly distended compared to those observed in the control group. These changes increased with increasing concentration and extending the incubation time. The autophagy process was activated as the number of damaged cell organelles increased.

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MANNITOL-INDUCED ALTERNATION OF ENDOGENOUS HORMONES BALANCE AND ITS EFFECT ON BREAD WHEAT (*TRITICUM AESTIVUM* L.) MICROSPORE REPROGRAMMING TOWARDS EMBRYOGENIC DEVELOPMENT

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The effectiveness of microspore embryogenesis (ME) relies on complex genetic-physiological-environmental interactions and in some cases (e.g. winter wheat), this is far from sufficient for practical use in plant breeding. Our research (Dubas et al., 2024) has revealed that a sequence of low temperature (LT), selenium salt (SeS) and mannitol (MAN) spike treatments can improve the effectiveness of microspore reprogramming towards this alternative developmental pathway. The majority of multicellular structures produced, however, exhibited developmental defects leading to their degeneration.

Since embryo development is hormonally regulated, we assessed the levels of auxins (AUX) and cytokinins (CK) in the anthers of two winter wheat lines (PO19, K393) and the spring cultivar Pavon using the UHPLC-MS/MS method (Juzoń-Sikora et al., 2023). LT alone or in combination with SeS had little impact on AUX content and composition in wheat anthers. MAN treatment (4 days in 0.7 mol·dm^{-3} MAN) significantly increased the content of all identified AUX (indole-3-acetic acid (IAA), IAA-aspartate, IAA-glutamate and 2-oxindole-3-acetic acid). Conversely, all treatments reduced the content of the most active CK (*trans*-zeatin), while the levels of *cis*-zeatin-*O*-glucoside and *cis*-zeatin-riboside-*O*-glucoside in the studied wheat anthers increased. Such a distinctive alteration of anther hormonal balance could possibly represent a factor inducing developmental abnormalities observed in microspore-derived structures.

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CALNEXIN1 IS CRUCIAL FOR *PETUNIA* POLLEN TUBE GROWTH

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Pollen grain is the male gametophyte of flowering plants. Once transferred to the stigma of the pistil, it germinates and then elongates into a pollen tube that delivers two sperm cells to the embryo sac for double fertilization. The pollen tube is the fastest-growing plant cell. Its rapid elongation is achieved by tip growth, a process that relies on several highly interconnected processes such as: the maintenance of Ca^{2+} gradient and zoned cytoplasm, regulation of the actin cytoskeleton, cytoplasmic streaming, membrane trafficking, cell wall biogenesis, signalling, or protein synthesis and secretion. To achieve their functional form, the newly synthesized secretory proteins must be modified and correctly folded in the endoplasmic reticulum (ER) via enzymes and molecular chaperones. We have previously shown that pollen tube growth is dependent on calreticulin (CRT), the ER luminal Ca^{2+}-binding/buffering chaperone. Post-transcriptional *CRT* gene silencing in cultured *Petunia hybrida* (*Ph*) pollen tubes leads to destabilization of Ca^{2+} gradient, disruption of the actin cytoskeleton organization and function, and consequently inhibition of tube growth (Suwińska et al., 2017; Wasąg et al., 2022). Since CRT closely interacts with the ER transmembrane chaperone - calnexin (CNX) in folding of newly synthesized glycoproteins passing through the ER, we hypothesize that CNX may also be important for pollen tube growth. To address this possibility, we used siRNA to selectively deplete *CNX1* mRNA in *Petunia* (*PhCNX1*) tubes growing in vitro. Using an RNA interference (RNAi) strategy, we found that knockdown of *PhCNX1* gene expression caused pollen tube abnormalities, such as reduced tube length and growth rate, morphological defects, disorganization of the actin cytoskeleton and organelle positioning. Based on our results, we propose that CNX (probably together with CRT) plays an important role in molecular chaperoning during pollen germination and the tube elongation.

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THE POSTERIOR OOCYTE POLE OF DERIVED DERMAPTERANS (EUDERMAPTERA) IS DEVOID OF RESERVE MATERIALS AND COMPRISES A COMPLEMENT OF NON-CANONICAL ORGANELLES

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The Dermaptera (earwigs) is a small insect order with about 2200 described species, usually grouped into 11 families. Karschiellidae, Pygidicranidae, Diplatyidae, Apachyidae, Labiduridae and Anisolabididae are plesiomorphic in many characters and are often assembled together as basal or "lower" dermapterans. The remaining families, *i.e.* Spongiphoridae, Forficulidae, Chelisochidae, Arixeniidae, and Hemimeridae constitute the most derived dermapteran group, referred to as the Eudermaptera.

All dermapterans are characterized by simple ovarian follicles comprising only two germline cells: an oocyte and a single nurse cell (Tworzydlo et al., 2010). Ultrastructural analyses have revealed that in eudermapteran species, *i.e. Forficula auricularia, Apterygida media* and *Opisthocosmia silvestris*, the posterior oocyte pole is devoid of reserve materials (yolk granules and lipid droplets) and apparently differs from the rest of the ooplasm. Due to morphological resemblance of this yolk-free ooplasm to the pole (or germ) plasm of higher insects, we termed this oocyte region, the pseudo-pole plasm. In this report, we show that at the EM level the pseudo-pole plasm contains various non-canonical organelles, *e.g.* dilated vesicles of the rough endoplasmic reticulum filled with fine-granular material, heterogenous dense granules encompassed by smooth (devoid of ribosomes) membrane, accumulations of nuage material associated with endoplasmic reticulum elements as well as mitochondria and symbiotic bacteria. Interestingly, the exact composition of the pseudo-pole plasm apparently differs in investigated species.

In contrast to eudermapteran species, oocytes of basal (lower) earwigs are structurally simple and do not contain any morphologically recognizable region at their posterior pole. The latter observation suggests that the pseudo-pole plasm is characteristic exclusively for the Eudermaptera.

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OVARIAN MORPHOLOGY AND HISTOLOGY IN THE GENUS *DRAWIDA* – A PRELIMINARY STUDY

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Genus *Drawida* Michaelsen, 1900 comprises terrestrial oligochaetes, representing family Moniligastridae. Moniligastrids are regarded as a sister taxon to Crassiclitella ('true' earthworms) and they are the most primitive group among the earthworms. Genus *Drawida* is the most speciose group (around 150 valid species) among Moniligastridae and many *Drawida* species are hard to recognize morphologically. Here, we present the preliminary results on two yet unrecognized *Drawida* species collected in central India. The aim of the study was the ovary micromorphology analysis, as this aspect in *Drawida* is almost entirely missing. The sparse older data only describe the general ovary location within the body and mention the occurrence of characteristic long ovisacs.

Here, we have shown that both studied *Drawida* species possess paired ovaries located in the 11 segments, and two long ovisacs extending from ovaries to the following segments. Ovaries are feathery structures connected to the intersegmental septum on the ventral side and extended laterally and dorsally around the digestive tract. Ovaries are composed of germ cells uniting into syncytial germline cysts. Each cell is connected via one intercellular bridge to the common cytoplasm (cytophore), forming thin and branched cytoplasmic strands. During oogenesis oogonia, germ cells in subsequent stages of meiotic prophase I, and oocytes, are located within the ovaries. Oocytes grow considerably and fill with cell organelles and yolk. As a rule, oocytes in *Drawida* are highly yolky cells with a lot of nutritive material. Large vitellogenic oocytes are deposited into the ovisacs – a bag-like structure that elongates and tightly fills with oocytes.

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PLANT-SPECIFIC CALRETICULIN IS LOCALIZED IN THE NUCLEI OF HIGHLY SPECIALIZED CELLS IN THE PISTIL – NEW OBSERVATIONS FOR AN OLD HYPOTHESIS

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Presently, exactly half a century since its identification, calreticulin (CRT) is known as a ubiquitously expressed protein and evolutionarily conserved among animal and plant kingdoms. Years of research have revealed a significant role of CRT in numerous cellular processes related to calcium signaling and homeostasis, as well as lectin-like chaperoning and quality control of newly synthesized glycoproteins. The diversity of CRT functions is linked to the varying cellular localization of this protein. In plant cells, besides its obvious localization within the endoplasmic reticulum, the presence of CRT has been confirmed for cytosol, dictyosomes and peripheral compartments like the plasma membrane, cell wall and plasmodesmata (Wasąg et al., 2018). Moreover, one of the most intriguing is the localization of CRT in the cell nucleus, described as one of the earliest known locations and supported by the presence of the putative nuclear localization signal (NLS) (Opas et al., 1991). Here, we present immunocytochemical localization of CRT within nuclear compartment of the pistil transmitting tract somatic cells for two selected angiosperm species, *Petunia hybrida* (dicots) and *Haemanthus albiflos* (monocots). Our analysis, covering stages before and after pollination, revealed a similar pattern of the nuclear CRT localization in relation to exchangeable Ca^{2+} in both species despite differences in the anatomical structure of the pistil.

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EMBRYONIC DEVELOPMENT OF THE GIANT ASIAN POND TURTLE, *HEOSEMYS GRANDIS* (TESTUDINES: GEOEMYDIDAE): PRELIMINARY RESULTS

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Turtles have one of the most highly modified body plans among extant reptiles, with the presence of strongly developed body armour (carapace and plastron) and limb girdles located inside the rib cage. Despite many studies on the origin of this body plan, developmental data on many clades of turtles remain scarce. For example, staging tables are available for only two species of geoemydid turtles, *Mauremys japonica* and *M. reevesi*.

Here, we present preliminary observations on the embryonic development of a critically endangered geoemydid, the giant Asian pond turtle (*Heosemys grandis*). This species is native to southeast Asia and inhabits freshwater-associated ecosystems – flooded forests, wetlands, slow-moving rivers. It is notable for its relatively large size (maximum carapace length can exceed 30 cm) and a long incubation period of up to five months, depending on the temperature.

We examined 36 eggs from the breeding colony at ZOO Wrocław, 12 of which (33.3%) were fertilised. The mean egg length was 57.65 mm (51.6–62.7) and the mean egg width was 39.63 mm (38.6–41.1). In the youngest embryo examined (25 days after oviposition, dpo), the limb buds were evident, but not yet divided into stylo-, zeugo- and autopodium. In the 31 dpo embryo, this division is present, and the autopodium is in the digital plate stage. At this stage, the carapace is visible, but its anterior border is poorly defined. At 51 dpo, the digits are well defined. From 57 dpo, the pigmentation becomes more pronounced and the development slows down.

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Structure of the reproductive system of the sexual generation of the endemic High Arctic species *Sitobion (Metobion) calvulum* (Hemiptera, Aphididae)

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Sitobion (Metobion) calvulum Ossiannilsson is a globally rare Arctic endemic species with highly restricted distribution. It is known only from a few scattered localities adjacent to the inner parts of the Isfjorden – Svalbard's second-longest fjord, on the western coast of Spitsbergen. The species is associated with *Salix polaris* and its taxonomically unrelated root parasite *Pedicularis hirsuta*. *S. (M.) calvulum* is characterized by an extremely adaptive life cycle, with only two generations: the stem mother and sexuales - oviparous females and males, making it a valuable model for reproductive studies.

The reproductive system of the male *S. (M.) calvulum* runs parallel to the longitudinal axis of the body. Testis follicles, four per testis, are elongated and arranged in a rosette. Vasa deferentia run separately. Accessory glands are elongated, with the top part bent at an acute angle to its duct. The outlets of vasa deferentia and accessory glands run separately, opening to the elongated ejaculatory duct. The reproductive system of oviparous females is composed of ovaries (with eight ovarioles each), lateral oviducts, a common oviduct, paired lobate accessory glands, and an unpaired spermatheca. The histological composition and ultrastructure of the reproductive system of sexuales of *S. (M.) calvulum* are broadly similar to the reproductive systems described already in other species of aphids.

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THE OVARY STRUCTURE OF *PARAMACROBIOTUS GADABOUTI* (TARDIGRADA: EUTARDIGRADA)

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Paramacrobiotus gadabouti is a parthenogenetic tardigrade belonging to the family Macrobiotidae that lays ornamented eggs freely into the environment. The species was described based on individuals obtained from a moss sample collected in Ribeiro Frio, Madeira (Kayastha et al., 2023). The aim of our study was the analysis of the gonad structure of *P. gadabouti*.

The ovary of the studied species is an unpaired structure located on the dorsal side of the body above the midgut. The anterior part of the ovary is attached to the body wall by a terminal filament, while the posterior part passes into the oviduct. The ovarian wall is composed of a single-layer epithelium with flattened cells. The interior of the ovary is divided into two zones: a small, top-located germarium and a vitellarium occupying a significant part of the gonad. The germarium is occupied by oogonia, which undergo complete or incomplete cell division. As a result of incomplete cell divisions, groups of female reproductive cells are formed, which move to the vitellarium, where further stages of oogenesis occur. Within the clusters, cells are connected by cytoplasmic bridges, which makes communication between cells possible. Cell organelles and yolk material are transported through intercellular bridges. Within each germ cell cluster, one cell differentiates into an oocyte (future egg cell), and the remaining cells become trophocytes, i.e. oocyte-supporting cells. The research was conducted using light microscopy and transmission electron microscopy.

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GROWTH ANALYSIS OF LEAF EPIDERMAL CELLS IN *ARABIDOPSIS THALIANA DWARF4-102* AT SUBCELLULAR SCALE

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A mutation in the *DWF4* gene, responsible for the biosynthesis of a key enzyme in the brassinosteroid pathway, leads to reduced cell growth and size and plant dwarfism. Mutant leaves have undulated lamina, which may indicate problems with the coordination of growth. Therefore, the aim of our study is to verify a hypothesis that the mutation in the *DWF4* gene affects the spatiotemporal pattern of growth rates and anisotropy. In particular, the mutation effect on the epidermal cell morphogenesis was investigated for *dwf4-102* dwarf mutant. In vivo imaging of leaf epidermal cells was combined with a method to analyze growth at the subcellular scale. To do that, artificial landmarks in the form of fluorescent microbeads were applied to the abaxial leaf side and used to assess outer periclinal cell wall growth. This method facilitates the calculation of the rate and anisotropy of local growth, as well as the determination of the main growth directions for specific regions of the cell.

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EMBRYONIC STEM CELLS DERIVED FROM THE BTBR MOUSE (*MUS MUSCULUS*) MODEL OF IDIOPATHIC AUTISM: VALIDATION OF PLURIPOTENCY AND NEURONAL DIFFERENTIATION POTENTIAL

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The BTBR mouse strain recapitulates multiple behavioral phenotypes relevant to the core symptoms of autism spectrum disorders, thus it is considered a human idiopathic autism model (Meyza KZ et al., 2017). Moreover, the BTBR mice display peculiar neuroanatomy, including the absence of the corpus callosum, and several other axonal network defects (Miller VM et al., 2013). However, the etiopathogenesis leading to the occurrence of the BTBR phenotype remains unclear, and early embryonic developmental windows in this strain have not been investigated.

Here, we report the establishment of two embryonic stem cell lines, one derived from the BTBR strain, and the other from the control strain C57BL/6J, and their validation for *in vitro* and *in vivo* studies intended to understand intrinsic genetic differences in their neuronal differentiation potential. We show that both ES cell lines express pluripotency markers and exhibit clonogenic ability. Moreover, cells were engineered to constitutively express fluorescent proteins (i.e. tau-GFP and Kusabira Orange) to facilitate cell tracing, which allowed to confirm their ability to integrate into the inner cell mass after injection into host mouse embryos. Finally, BTBR and C57BL/6J embryonic stem cells were directed toward the neuroectodermal fates by co-culture with MS5 stromal cells for neuronal induction.

Further experiments will address strain differences in ES cells neural developmental potential and subsequent axonal formation, thus helping in understanding the mechanism underlying the unique neuroanatomy of the BTBR mouse model to study autism etiopathogenesis.

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INVESTIGATING THE EFFECTS OF *TEAD4* SILENCING ON TROPHOBLAST LINEAGE FORMATION IN CHIMERIC MICE (*MUS MUSCULUS*) EMBRYOS

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During early murine embryo development, TEAD transcription factors regulate crucial gene expression for trophoblast (TE) and inner cell mass (ICM) formation in the blastocyst (Hashimoto and Sasaki, 2019). TEAD4 is believed to be pivotal for trophoblast lineage specification and blastocyst formation (Nishioka et al., 2008). However, the precise mechanisms underlying TEAD4's role in early development remain unclear.

This study aimed to examine how silencing the *Tead4* gene affects TE specification and blastocyst formation, and to assess the differentiation potential of *Tead4*-silenced cells in aggregation chimeras.

The siRNA targeting the Tead4 transcript, along with mRNA encoding a GFP reporter, was introduced into mouse embryos via electroporation. Subsequently, silenced embryos were either cultured until reaching the blastocyst stage or aggregated with control embryos the day after electroporation and then cultured further until blastocyst formation. The expression of TEAD4 and CDX2, as well as their co-localization with the GFP reporter, were evaluated through immunostaining and compared with embryos electroporated with a control siRNA. Only 25% of silenced embryos reached the blastocyst stage, although they were retarded and smaller in size, as compared to control embryos. Moreover, in the majority of chimeras created by combining silenced *Tead4* embryos with controls, the silenced *Tead4* cells showed a preference for populating the ICM and its surroundings, rather than the TE.

These preliminary observations confirm TEAD4's essential role in embryonic survival, regulating trophoblast formation, and consequently, blastocyst development. These results will facilitate further exploration of TEAD4's molecular mechanisms in TE development.

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