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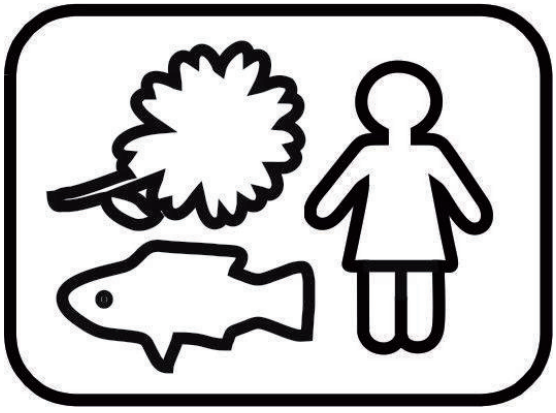

ABSTRACTS

XXXIII Conference on Embryology
Plants • Animals • Humans

May 23-26, 2018
Olsztyn, Poland

Polish Academy of Sciences – Krakow Branch
Polish Academy of Arts and Sciences

ACTA BIOLOGICA CRACOVIENSIA
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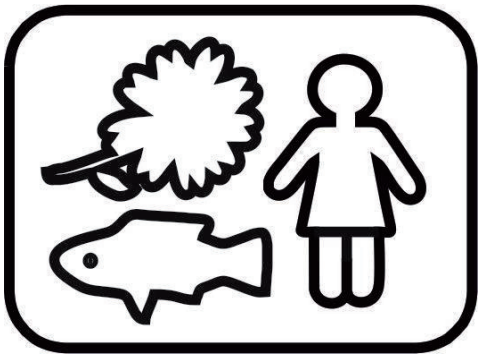

XXXIII Conference on Embryology
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COMMEMORATIVE LECTURES

In memory of Professor Maciej Zenkteler a initiator of plant tissue and organ *in vitro* culture and experimental embryology in Poland

Elżbieta Kuta*

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Professor Maciej Zenkteler, a native of Poznań, was an outstanding scientist, an expert in plant tissue and organ cultures and experimental embryology. He developed a number of useful *in vitro* techniques, now widely applied. His scientific life was dedicated to experimental botany and embryology and scientific interest evolved from the processes of re- and differentiation during *de novo* plant organogenesis, anther cultures for androgenic embryos formation, to *in vitro* angiosperm ovule pollination in interaspecific and intergeneric crosses and hybrid embryos cultures. His research were very important not only for plant embryologists but also for plant breeders having an application aspects.

Professor Zenkteler graduated from the Adam Mickiewicz University in Poznań with a master's degree and then with a Ph.D, tenure and professor in life sciences. He was for twenty five years (1978–2002) head of the Department of General Botany at the Faculty of Biology, vice-director of the Institute of Experimental Biology (1981–1984), the President of Polish Botanical Society, Poznań branch (1992–2001), the member of several Polish Academy of Sciences Divisions. Prof. Zenkteler was a mentor of graduate students and junior faculty alike, taught multiple courses in botany, led workshops, practical *in vitro* courses for students and researchers from Polish and foreign academic centers, and supervised numerous master and doctoral dissertations. From 2002 was Professor Emeritus in the Department of General Biology at the Adam Mickiewicz University and was active to the very end visiting and working in laboratory, writing papers, attending conferences. In 2012 the Adam Mickiewicz University celebrated Professor Zenkteler doctorate reneval after 50 years. He was bestowed several important awards for excellent achievements.

Professor Maciej Zenkteler passed away on August 6, 2017 in Poznań in his 86th year. The devotion to the science and personality won him the admiration of all who knew him. We will missed Him sorely.

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ZENKTELER M, ZENKTELER E. 65 years of *in vitro* culture in Poland. *Acta Societatis Botanicoruum Poloniae* 82(3):183–192.

Prof. dr hab., dr h. c. Zofia Bielańska-Osuchowska, Nestor of Embryology (1919-2017)

Paweł S. Sysa

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Professor Zofia Bielańska-Osuchowska began her biological studies in Krakow at the Jagiellonian University (JU) in 1937. She continued her studies after the war at Zoology Department of the JU, obtained her Master's degree in Zoology in 1951. She became an assistant at the Division of Histology and Embryology of Animal Anatomy Department at the Veterinary Faculty of the Warsaw Agricultural University in 1953. She was associated with the Department even after retirement in 1991. She obtained the degree of Candidate of Biological Sciences at the University of Warsaw in 1958 based on a dissertation, devoted to the study of insect ovaries. Her habilitation thesis (1961) was devoted to the differentiation of gonads of domestic pigs. The titles of Associate Professor (1972) and Full Professor (1980) were obtained due to the highly estimated original scientific achievements focusing on embryological issues and, first of all, model researches on the embryonic and fetal development of pigs. She carried out multidirectional researches on the development of the endocrine system, digestive system, structure and functioning of the pig's placenta as well as its regulatory role in forming the endocrine glands.

Professor Z. Bielańska-Osuchowska always paid special attention to didactic work. She edited the highly appreciated edition "Repetition in Histology" and "Structures and Functions of Cells and Tissues". She has developed scripts "Animal Embryology", but especially valuable work was the textbook "Embryology" (1977), supplemented in 1983, 1994 and 2001. In 2004, she published an "Outline of Organogenesis. Differentiation of Cells in Organs". Her *magnum opus* was the textbook "The Mammalian Sperm, an Extraordinary Cell" (2018), presenting current knowledge on morphology, ultrastructure, histochemistry, genetic mechanisms, regulatory proteins and participation of that cell in the fertilization mechanisms and early stages of embryogenesis. She was an active promoter of modern achievements of biology through editorial work in "Advances in Cell Biology", "Folia Morphologica", "Folia Histochemica et Cytobiologica" and "Reproductive Biology". Over 30 years, she was co-organizer of instructional conferences for young scientists, devoted to "cell biology".

She was a member of many scientific societies and she was granted many honorary distinctions, including the title of Doctor *Honoris causa*.

PLENARY LECTURES

Viviparity and matrotrophy in two closely related dermapteran species, *Hemimerus talpoides* and *Arixenia esau* rely on disparate modifications of their female reproductive organs

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Three main reproductive strategies have been described among insects: most common oviparity, ovoviviparity and viviparity. In viviparous species, the embryos develop inside the female reproductive system of the mother which provides gas exchange and all the nutrients necessary for development. Such a strategy of maternal care is also termed matrotrophy (Ostrovsky et al., 2016). Here I summarize results of histochemical, ultrastructural and biochemical analyses of reproductive systems as well as developing embryos of two closely related epizoic species of earwigs (Dermaptera), *Hemimerus talpoides* and *Arixenia esau*. These analyses clearly indicate that morphological as well as physiological modifications (adaptations) supporting viviparity and matrotrophy in *Hemimerus* and *Arixenia* are entirely different. Most importantly, the *Hemimerus* embryos complete their development inside terminal (largest) ovarian follicles, whereas those of *Arixenia*, after initial developmental stages, are transferred to highly dilated lateral oviducts, *i.e.* the uteri, where they develop until hatching. Obtained results suggest that viviparity in hemimerids and arixeniids had evolved independently and that different organs/tissues had been employed in embryo nourishment during the evolvement of the matrotrophy in these dermapteran subgroups.

ACKNOWLEDGEMENTS:
This study was supported by a research grant OPUS 11 (UMO-2016/21/B/NZ8/00560) from the National Science Centre, Poland

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Reproductive ability and embryonic development of *Cobitis* hybrids (Pisces, Cobitidae) with different ploidy levels

Dorota Juchno*, Alicja Boroń, Anna Przybył, Karolina Kowalewska

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The diploid-polyploid populations of *Cobitis* distributed in Poland are dominated by triploid (3n) hybrid females and usually composed also of one diploid (2n) parental species, *C. taenia* or *C. elongatoides* and low number of diploid hybrid females, and tetraploid (4n) hybrids of both sexes. Hybrid females (2n, 3n) of *Cobitis* as a rule produce unreduced eggs which mainly develop gynogenetically, but some of the eggs incorporate sperm genome and develop into triploids and tetraploids (Choleva et al., 2012). We examined 2n, 3n and 4n *Cobitis* hybrids of both sexes by determining their ability to produce gametes. We also present the comparative studies of the embryonic and larval development of these fish. The diploid hybrid progeny were obtained in the reciprocal crosses between females and males of *C. taenia* and *C. elongatoides* whereas polyploid offspring in crossing between triploid *Cobitis* females and *C. taenia* males. The ploidy level of all investigated fish was identified karyologically or using flow cytometry. Diploid, hybrid F1 progeny showed the same proper pattern of embryonic and larval development. Two-year-old F1 hybrid females were mature and these females back-crossed with *C. taenia* males had properly developing progeny. In testes of two-year-old F1 hybrids, no spermatids and spermatozoa, only early stages of spermatogenesis and pyknotic cells indicating the degeneration process were observed. The crosses between 3n *Cobitis* females and *C. taenia* males lead to triploid (55%) and tetraploid (45%) progeny. However, the number of 4n decreased rapidly after hatching due to significantly lower survival rate in comparison to 3n. Tetraploid females attain maturity and produce mature eggs whereas tetraploid males are sterile. The obtained data extend knowledge about reproduction and functioning of mixed *Cobitis* populations.

ACKNOWLEDGEMENTS:

This work was supported by The National Science Centre, Grants number N N303 068834 and 2011/03/B/NZ8 /02982 carried out at the University of Warmia and Mazury.

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Myosin VI in actin organization and dynamics during spermiogenesis

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The actin cytoskeleton, including a number of actin-binding/regulating proteins (ABPs), has been implicated in various aspects of spermiogenesis, a complex developmental process of formation of fully differentiated male gametes – spermatozoa. Stable actin structures, called actin cones, mediate spermatid individualization during the final step of *Drosophila* spermatogenesis when syncytial spermatids are reorganized into individual mature sperm. One of the proteins that play a key role in this remodelling process is myosin VI (MYO6), a versatile actin-based molecular motor that has been implicated in a variety of different cellular processes, including endo- and exocytic vesicle trafficking, Golgi morphology, and actin structure stabilization. Specific localization of MYO6 in actin cones is strictly required for their proper formation and function during spermatid individualization (Noguchi et al., 2006, 2008; Isaji et al., 2011; Lenartowska et al., 2012). Because MYO6 mutant males are sterile, a role of MYO6 in *Drosophila* spermiogenesis is crucial.The fundamental mechanisms that control spermatogenesis are conserved between evolutionary distinct animal species, thus we hypothesized that MYO6 may also have an important role in sperm maturation in mammals. Despite that MYO6 knockout (KO) mice display several defects in different cell types and the males exhibit somewhat reduced fertility, no studies have been published that address the possible function for MYO6 in mouse spermiogenesis. To test this, we examined MYO6 expression in mice testes and found that two out of four MYO6 splice variants are expressed in the male gonads. Further, we showed that MYO6 is associated with Golgi complex and highly specialized actin-rich structures involved in acrosome biogenesis and nuclear shaping, such as the acroplaxone, manchette, and Sertoli cell actin hoops (Zakrzewski et al., 2017). The ultrastructural analysis of MYO6 KO developing spermatids revealed a number of morphological disruptions, especially of the Golgi complex, the endoplasmic reticulum (ER) as well as the acrosome. Finally, we compared distribution of marker proteins for the Golgi and the ER, and of a few ABPs in developing spermatids of wild type (WT) and MYO6 KO mice males. The obtained results showed significant changes in localization of all the proteins tested when MYO6 was not expressed. This is the first evidence that MYO6 may be involved in some key events of spermiogenesis in mammals.

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The synthetic analogs of *Neb*-colloostatin as potential bioinsecticides that impair the insect reproduction

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Among about million insect species living on earth, a lot of them is harmful to man from medical or agri- and horticultural points of view. Currently used insecticides are not enough selective, potent against insects and safe for human. Consequently, the attention of scientists is focused on the isolation of various random plant or bacterial compounds and then testing their potential insecticidal activity. We propose a use the analogs of *Neb*-colloostatin – a natural insect's peptide hormone with well confirmed gonadoinhibitory and hemocytotoxic properties, in pest control (Bylemans et al., 1995; Czarniewska et al., 2012; Czarniewska et al., 2014). Among *Neb*-colloostatin analogs investigated in previous studies (Kuczer et al., 2013), we chose to study a more active molecules than the native peptide. We treated adult *Tenebrio molitor* beetles with nanomolar doses of these compounds. All tested analogs caused degenerative changes in ovary, a significant decrease in eggs laying and hatchability of larvae. The analogs were more potent than *Neb*-colloostatin. The potent gonadoinhibitory action of tested analogs in conjunction with their strong hemocytotoxic activity, suggest undertaking further studies aimed at using these synthetic peptides as agents limiting the number of pest.

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Circadian synaptic plasticity

Elżbieta Pyza

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In both vertebrates and invertebrates synapses in the brain oscillate during the day and this rhythm is maintained in constant darkness (DD), indicating that it is generated by the internal circadian clock. In the visual system of the fruit fly *Drosophila melanogaster* we found that the number of tetrad synapses, formed between photoreceptors and the first order interneurons, increases twice during the day, in the morning and in the evening while feedback synapses contacting L2 interneurons back to the photoreceptor terminals are most numerous at night (Woznicka et al., 2015). It means that different types of synapses show specific daily patterns in the frequency changes. Tetrad synapses are also sensitive to light and their number increases any time of the day after a short light pulse. In contrast feedback synapses are not sensitive to light. In addition to synapse numbers, synaptic proteins also oscillate in the abundance during the day. The number of synapses and levels of synaptic proteins do not oscillate in mutants of clock genes and mechanisms responsible for daily remodeling of synapses include circadian expression of genes and proteins involved in TOR kinase signaling and autophagy. Similar cyclic changes in the number of synaptic contacts were also found in the mouse brain, in somatosensory cortex. In the layer IV (barrel cortex) where there are cortical representations of vibrissae, excitatory synapses peak during the day when mice sleep while inhibitory synapses increase in the frequency at the beginning of night when mice are highly active in locomotor activity (Jasinska et al., 2015). While excitatory synapses oscillate in the number only in light/dark conditions (LD), inhibitory synapses oscillate not only in LD but also in DD, which confirms that their number is controlled by the circadian clock. Moreover, dendritic spines, which carry postsynaptic elements of synapses change their morphology and during the day there is more one-synapse dendritic spines but at night more double-synapse spines. The results, which have been obtained so far in both *Drosophila* and mice, clearly indicate that brain is a plastic organ not only during development but also in adult animals. The brain structure changes during the day and night, and these changes are correlated with sleep and activity cycles of animals.

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ORAL PRESENTATIONS

Formation and degradation of micronuclei during gametogenesis of the hybridogenetic frog *Pelophylax esculentus*

Magdalena Chmielewska[1]*, Dmitry Dedukh[2], Katarzyna Haczkiewicz[3], Beata Rozenblut-Kościsty[1], Mikołaj Kaźmierczak[1], Krzysztof Kolenda[1], Ewa Serwa[1], Agnieszka Pietras-Lebioda[1,4], Alla Krasikova[2], Maria Ogielska[1]

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Micronuclei are small extranuclear chromatin structures, arising in cancer cells due to chromosomal instability or in some plant hybrids, lampreys and birds during programmed genome elimination. They were found in gonocytes of edible frog *Pelophylax esculentus,* a natural hybrid between *Pelophylax lessonae* and *Pelophylax ridibundus*, reproducing by hybridogenesis and removing one of parental genomes from the germ line. Because the cellular mechanism of micronuclei formation and degradation remains unknown in this hybrid animal, we aimed to examine the process by means of TEM and immunofluorescence microcopy studies on gonads from diploid and triploid hybrid tadpoles.

In the cytoplasm of gonocytes we observed 1-5 micronuclei per one cell, ranging in size between 0.8-4μm. When examining gonocytes labelled for nuclear pore complex (NPC) proteins we have found chromatin protrusions of the main nuclei, similar to micronuclei in shape and size, which we interpreted as an early stage of micronuclei budding from interphase cells. The significant portion of micronuclei showed higher chromatin condensation level together with epigenetic chromatin modification, evidenced by a decreased level of histone H4 acetylation and an increased histone H3 tri-methylation at lysine 9, indicating DNA inactivation. Micronuclei presented various signs of degeneration, depletion of NPC, fragmentation of nuclear envelope and encapsulation by double lipid membranes. Furthermore, the presence of autophagosome marker protein, microtubule-associated protein light chain 3, revealed that the micronucleated chromatin was degraded due to nucleophagy while gonocytes remained intact. Evidence provided led us to the conclusion that micronuclei appearance in *P. esculentus* gonocytes is a physiological process of programmed DNA elimination.

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Structural basis of intercellular communication during Arabidopsis somatic embryogenesis

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Cell-to-cell communication is the basic parameter coordinating the development of a multicellular organism, both animal and plant. Formation of spatial patterns and the specification of the developmental programs of individual cells requires such information exchange, which in plants is of particular importance, as a part of a positional information. Cell-to-cell exchange of information take place through plasmodesmata (PD) which are the structural basis of symplasmic communication in plants.

Somatic embryogenesis (SE) is a model system in the study of mechanisms underlying changes in the cell fate including the role of symplasmic communication and the structure and distribution of PD. In presented studies Arabidopsis was choose because: 1/so far embryogenesis is best described for this plant at morphological, histological, molecular levels and symplasmic communication, 2/it has been sequenced and most recognized genome in terms of structure and function among the higher plants, 3/there are genomic databases and a huge collection of mutants and transgenic lines.

Thus, the aim of the study was to analyze quantitatively and qualitatively PD during the SE process. For the studies different transgenic lines of *Arabidopsis thaliana* were used and analyzed on the histological and ultrastructural level. Moreover, the movement of symplasmic transport tracers was monitored during the SE with the use of fluorescence microscopy including confocal technique. Performed studies showed spatio-temporal differences in PD distribution between cells realizing different developmental programs including somatic embryos.

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Structure and development of squamate egg teeth

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The development of most animals occurs inside capsules, shells or barriers of other types. When the development ends these barriers have to be broken during the process of hatching. In the course of evolution different adaptations arose to facilitate that process. Embryos of squamates use egg teeth to break their hard egg shells. The egg tooth is a transient structure present in both viviparous and oviparous species of snakes and lizards (Hill and De Beer, 1950). In most species belonging to Unidentata clade only one egg tooth is present while geckos and dibamids possess two egg teeth (Vidal and Hedges, 2009). Knowledge regarding the development of the egg teeth in reptiles is currently limited in comparison to the knowledge about similar processes in teeth of other vertebrates. Our studies were based mainly on light and transmission electron microscopy analyses of egg teeth structure and development in five Squamate species – *Natrix natrix, Lacerta agilis, Anolis sagrei, Eublepharis macularius* and *Lepidodactylus lugubris*. We found that egg teeth exhibit traits which are characteristic for given species. General structure of the egg tooth is similar to structure of typical tooth characteristic for all vertebrates. However, we discovered some interspecific differences in its development and differences in its structure (shape, angle, presence of accessory cones, way of attachment to premaxilla and patterns of dentine affinity for stain which might suggest different patterns of hard tissues mineralization) which might be proven useful in future studies concerning phylogenesis of Squamata. Additionally we have shown that spatial orientation of egg teeth in two investigated species is different. Egg teeth of *Eublepharis macularius* is facing inward while egg teeth of *Lepidodactylus lugubris* is facing outward. Differences in orientation of egg teeth might be result of differences in the hardness of the egg shells between these two species.

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Embryological processes in young ovules of selected diploid and tetraploid *Hieracium* and *Pilosella* species (Asteraceae)

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The genus *Hieracium* forms polyploid series (basic chromosome number x=9) and is a typical example of a taxonomically complicated agamic complex. In Europe, it was divided into the subgenera *Hieracium* s. str. and *Pilosella* that are currently accepted as separate genera. *Hieracium* and *Pilosella* comprise both sexual species (diploids, some polyploids) and apomictic taxa (mostly polyploids) but differ in the type of gametophytic apomixis. Mitotic diplospory occurs in *Hieracium* and obligate apomixis is usually considered as the only mode of polyploids reproduction within this genus (Mráz and Paule, 2006). *Pilosella* is a model system for the investigation of aposporous seed formation mechanisms, and polyploid species show facultative apomixis (Koltunow at al., 2011). The aim of this study was to analyze early reproductive processes in the ovules of diploids *Hieracium transylvanicum* and *H. pavichii* as well as in tetraploid *H. brzovecense* that does not produce any viable seeds. The latter two taxa represent the *Pilosella* genus. In the analysed diploid species, sexual development path was the main reproductive strategy, however, both species showed a tendency to apospory. In some ovules, drop-shaped aposporous initials have been observed in the immediate vicinity of the megaspore mother cell. Thus, these results suggest that polyploidy is not an obligate requirement for the expression of apomixis. It was also unusual that the walls of aposporous initials contained callose, while this cell wall component is considered a marker of only germline cells in the ovules. Likewise, callose was present in the walls of aposporous initial cells in young ovules of *H. brzovecense*. At this stage of the research, determining the function of callose surrounding aposporous initials seems difficult, but in the case of the analysed species it cannot be ruled out that callose function is to isolate aposporous initial cells and suppress apomictic development. This may be one of the reasons why *H. brzovecense* produces empty seeds.

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Germ cell clusters organization and their fate during oogenesis in *Thulinius ruffoi* (Tardigrada, Eutardigrada, Parachela)

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Tardigrades are a group of microinvertebrates among which gonochorism, hermaphroditism and an ability to parthenogenetic reproduction occur. Parthenogenesis in tardigrades can be meiotic (automictic) or ameiotic (apomictic), which is more common in tardigrades (Bertolani, 1982). *Thulinius ruffoi* is a freshwater species with a typical for tardigrades female reproductive system and able to parthenogenesis. An ovary is divided into germarium and vitellarium. Within the germarium, complete and incomplete cytokinesis take place. The potential stem cell in the tip of the germarium divides completely, and the daughter cell of this division is a cystoblast which divides incompletely. As a result of incomplete cytokinesis, the cluster of cystocytes is formed. The cells in the cluster are interconnected by the cytoplasmic bridges. From the germarium, germ cell cluster is going to the vitellarium. In the vitellarium, differentiation of the cystocytes, synthesis and accumulation of yolk, formation of the egg capsule, as well as cell death occur. Female germ cell cluster has a branched structure. Among cells forming the cluster only one cell develops into the oocyte, while the remaining cells become the trophocytes (nurse cells). The trophocytes synthesize macromolecules (rRNAs), the yolk material, ribosomes and organelles which are transported to the oocyte. The trophocyte may be connected with a different number of other trophocytes by the cytoplasmic bridges. In our studies, we used various methods such as: a light microscopy, a transmission electron microscopy, a confocal microscopy, SBEM method and open-source software for creating the 3D reconstructions (Schindelin, et al., 2012).

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Embryonic development of the vomeronasal organ and associated structures in the brown anole, *Anolis sagrei* (Squamata: Iguania)

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The vomeronasal organ (VNO), otherwise known as the Jacobson's organ, is a part of accessory olfactory system which tends to be sensitive to less-volatile molecules like pheromones and some prey odours. The VNO of lizards and snakes (Squamata) is generally well separated from the nasal cavity and contains the sensory epithelium of the dorsal dome and the non-sensory epithelium of the ventral concha (mushroom body). The VNO communicates with the oral cavity through its duct which opens on the palate anteriorly to the choana (Bellairs and Boyd, 1950). In contrast to such vomeronasal specialists like snakes, monitor lizards or lacertid lizards, members of Iguania (iguanas, chameleons, agamids) rely mainly on visual cues. The brown anole (*Anolis sagrei*) as a member of Iguania is considered to be a visually oriented predator. Moreover, it represents trunk-ground ectomorphs (Pianka and Vitt, 2003). The phylogenetic position and arboreal lifestyle may imply a reduction of the VNO in the brown anole (Pratt, 1948). However some studies suggests that vomeronasal chemoreception may still be important in this species (Baeckens et al., 2016). Our study aims to analyse embryonic development of the VNO and associated structures (such as the nasal cavity, choanal groove, nasal gland, Harderian gland and vomeronasal nerve) of the brown anole utilizing: micro CT imaging, light and transmission electron microscopy. Our study indicates that the VNO is the functional structure in the brown anole. Moreover, on the basis of comparison with the previous studies, we were able to detect some conservative events during development of the VNO and associated structures in Squamata and find some unexpected similarities between anoles and such vomeronasal specialists like lacertids lizards.

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Genomic composition of the male germ line cells in diploid and triploid water frog hybrids *Pelophylax esculentus* based on genomic *in situ* hybridization (GISH)

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Edible frogs *Pelophylax esculentus* are interspecies hybrids that reproduce by hybridogenesis. A model concerning this type of gametogenesis has assumed that in males elimination and endoreduplication of chromosomes take place in prespermatogenesis during mitotic divisions of gonocytes. After the last series of divisions, which coincide with the completion of metamorphosis, gonocytes transform into spermatogonial stem cells (SSCs) possessing clonal genomic constituents derived from one of the parental species, *Pelophylax lessonae* or *Pelophylax ridibundus*.

Our analyses based on GISH revealed that the majority of SSCs in diploid and triploid males followed a typical scenario of hybridogenesis with diploid metaphase chromosome sets presenting a unified genomic content. Nevertheless, in a portion of metaphase plates we observed varying numbers of chromosomes ranging from haploid to tetraploid sets, frequently aneuploid. We interpreted this findings as inconsistencies in chromosome elimination and reduplication processes that could result in gametes with variable ploidy level or aneuploid ones.

Apart from homozygous SSCs, we found heterozygous chromosome sets in 2 diploid juvenile males, which may suggest that either hybridogenesis is prolonged and some gonocytes have not completed chromosomal rearrangements, or individual cells do not undergo genome elimination resulting in unreduced gametes, or finally that such specimens are not hybridogenetic. All triploid individuals possessed simultaneously homozygous and mixed chromosome sets with a prevalence of the homozygous ones.

Observed diversity in genomic composition within one gonad may indicate that each gonadal stem cell could supposedly be programmed in a different manner. In turn, their daughter cells could follow different scenarios compared to descendants of other stem cells.

Whether all genomic composition variants develop into spermatozoa is very unlikely, considering the fact that histological examination of all studied testes showed many apoptotic cells in the seminiferous tubules, probably arising as a result of chromosomal aberrations.

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Embryonic diapause as unique process during pregnancy among animals

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Embryonic diapause is a period of delay occurring after a blastocyst has formed, when cell division stops and the blastocyst remains unattached for a prolonged period of time (Daniel, 1970). It occurs in 130 animal species (Fenelon et al., 2016), but it is not known whether it is a mandatory or incidental process. Suggested reasons of diapause are unfavorable climatic conditions, e.g. temperature (Markofsky and Matias 1977), fotoperiod (Fenelon et al., 2016), lack of food and stress (Kondoh et al., 2009) and protection against predators (Aitken 1974). Temporal changes in concentrations and synthesis of ovarian steroids and prolactin on reactivation of diapausing blastocysts have been showed (Lambert et al., 2001, Ptak et al., 2012). An exemplary species in which embryonic diapause take place is roe deer. Roe deer's pregnancy might vary it's length depending on if the fertilization did take place in summer or late autumn, owing to the fact that female roe deer can occur the diapause stage (Pielowski 1984).

We focused on studies of mRNA and protein expression of and enzymes which synthetize progesterone and estradiol, its receptors in uterine tissues and measurement of these steroids concentration in peripheral blood in roe deer females.

Material was collected *post mortem* during hunting season and divided into groups: pregnant (I and III month of pregnancy) and non-pregnant. The concentration of P4 was measured by RIA. mRNA expression of 3-β-hydroxysteroid dehydrogenase (3βHSD), 20-alpha steroid dehydrogenase (20αHSD) and P4 nuclear receptors type A and B was evaluated by Real-Time PCR and protein expression by Western Blotting respectively.

We showed that progesterone plasma concentration increased during I and III month of pregnancy compared to oestrous cycle ($P < 0.05$). In endometrium and myometrium during pregnancy development process P4 synthesis with varying intensity and inactivation of this steroid take place. During diapause process receptivity of the uterus is changing in reference to P4 in females of roe deer.

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Callose in the ovules of apomictic and amphimictic angiosperms – new data

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In the ovules of the majority of sexually reproducing flowering plants, except for tetrasporic species, callose is a cytological marker of the megaspore mother cell wall and acts as a selective molecular filter providing genetic autonomy during the shift from the diploid to the haploid phase. In the case of apomictic species, the available data indicated total absence of callose in the ovules of taxa with gametophytic apomixes. Lack of callose in the cell wall of the cell entering apospory and diplospory was considered to be a consequence of the expression of apomixis or a factor that causes it (Carman et al., 1991). However, our latest results clearly prove that meiotic diplospory of the *Taraxacum* type is accompanied by callose deposition (Musiał et al., 2015; Musiał and Kościńska-Pająk, 2017). Moreover, we have recently discovered the presence of callose also in the course of mitotic diplospory in *Erigeron annuus*. Thus, the newest research showed that lack of callose deposition during early apomictic processes is not obligatory for apomicts and callose can play a similar regulatory function in cell-cell communication between somatic tissues and reproductive precursor cell in the ovules of both sexual and diplosporous taxa.

In addition, our observations indicate that a re-examination of taxa with tetrasporic female gametophyte development would also be desirable. Recently, we detected the presence of callose also during coenomegaspore formation in the ovules of tetrasporic *Rudbeckia* species, however, in this case callose generally showed an unusual pattern of distribution.

Thus, it should be emphasized that callose events in the ovules of both apomictic and amphimictic angiosperms is still not fully explored, and so far the molecular mechanisms responsible for synthesis and degradation of callose during female sporogenesis remain unknown.

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Outer structures of the egg cell envelope in freshwater fish

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Fish egg cells consist of the egg envelope, plasma, nucelus and yolk. In most fish species, ova are spherical in shape and enveloped in a smooth cellular membrane. The egg membranes are synthetised by an ovary, while the shells (characteristic for the elasmobranchii) are produced in the oviduct from secrections of appropriate glands. Cyprinid fish eggs are covered with a radial membrane (primary membrane – produced by the oocyte) and a thin membrane over it (secondary membrane – produced by the ovary) (Bieniarz and Epler, 1991).The outer layer contains glycoproteins (compounds of sugars and proteins), which help oocytes stick to the substrate under natural conditions. Immature egg cells do not have such a layer. The membrane of an egg cell just deposited is soft and slightly corrugated, but once it enters into contact with water, it swells and stiffens, which makes it more resistant to deforming forces (Brysiewicz et al., 2011). The membranes swell, differentiate into layers and become several-fold thicker than immediately after the deposition. After some time, the adhesive substance solidifies and attaches the egg firmly to the substrate. Eggs of phytophilous and litophytophilous fish adhere most strongly to the substrate (Balon, 1975; Mann, 1996). Other fish species lay spawn equipped with various projections. The spawn of *Osmerus eperlanus* has saucers structures, which decelarate its downward fall, while the spawn of some species of gobies (the genus Gobius) is elongated in shape and has a bunch of filamentous appendages at one end, with which it attaches itself to submerged plants. Sarasins buntingi (*Xenopoecilus sarasinorum*) lay sereval grains of spawn, which throughout the whole embryonic development remain attached to the female and are located above her ventral fins. Individual grains stay attached to the female owing to several flexible, very tightly wrapped threads. The spawn of *Oryzias woworae* possesses short projections over the whole surface of the egg membrane, which attach to water plants. The above structures of the egg membrane show a wide range of adaptations to particular habitat conditions.

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Reproduction of *Sinanodonta woodiana* (Bivalvia: Unionidae) – an invasive mussel species for the fauna of Poland

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Sinanodonta woodiana, the Chinese pond mussel, is an invasive species indigenous in Asia, which naturally spreads to new locations as a glochidium parasitizing fish. Human activity has facilitated a continental-scale spread of *S. woodiana*, and now the species occurs in European artificial reservoirs and is beginning to invade natural environments.

This study aimed to investigate the reproductive activity of *S. woodiana* that colonised a riverine channel of a power plant with a cooling water system. We used histological and stereological methods to determine gonad structure, changes in reproductive follicles (acini) during gametogenesis, and brooding periods.

Water in the channel of the "Dolna Odra" power plant (Poland) did not freeze during the winter, and its mean annual temperature was 18.4°C. The population sex ratio was female biased ($\chi^2 = 25.70$, $df = 1$, $P < 0.0001$). Ovaries and testes in mussels were formed by reproductive follicles (acini). Previtellogenic and vitellogenic oocytes were attached to the follicle wall via the cytoplasmic stalk, and mature ovulated oocytes were present in the follicle lumen. Females incubated the offspring (glochidia) in gill marsupia of outer demibranchs and were characterized by multiple tachytictic brooding periods. In males typical and atypical spermatogenic pathways were identified. The atypical spermatozoa were released from multinucleated cysts and had significantly shorter heads than typical spermatozoa. Individuals with mature gonads were present over the whole two-year study period, which indicates the continuous activity of gonads. The research provides the first direct information about the reproductive dynamics of this invasive species outside its original Asiatic range.

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Symbiotic microorganisms of scale insects of Phenacoccinae subfamily (Hemiptera, Coccomorpha, Pseudococcidae): distribution, ultrastructure and transovarial transmission

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Scale insects, like other sap-sucking hemipterans, are associated with symbiotic microorganisms which provide them essential nutrients lacking in the plant sap. In comparison to other hemipterans, scale insects are characterized by a large diversity of symbionts. We investigated the symbiotic systems of seven species of scale insects of the Phenacoccinae subfamily: *Phenacoccus aceris, Phenacoccus piceae, Rhodania porifera, Coccura comari, Longicoccus psamophilus, Spinococcus calluneti, Ceroputo pilosellae*. Molecular analyses based on bacterial 16S rRNA genes have revealed that all the investigated species of Phenacoccinae are host to only one type of symbiotic bacteria – large pleomorphic betaproteobacteria - *Tremblaya phenacola*. In all the examined species bacteria are localized in the specialized cells of the host-insect termed bacteriocytes and are transovarially transmitted between generations. The mode of transovarial transmission is similar in all species investigated. Infection takes place in the neck region of the ovariole, between the tropharium and vitellarium. Symbionts leave the bacteriocytes and move towards the ovaries. They then migrate to the space between the follicular epithelium and nutritive cord via the cytoplasm of the follicular cells or through the spaces between neighboring cells. Next, the symbionts move along the nutritive cord to the perivitelline space and gather in the deep depression of the oolemma where they create a characteristic structure termed the "symbiont ball".

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Is pollen heteromorphism in *Viola* L. correlated with species ploidy? – the current hypotheses re-examined

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Pollen heteromorphism, defined as the production of several pollen morphs with different aperture numbers in all flowers of a plant, is common in *Viola* L. (Dajoz 1999; Nadot et al., 2000). Pollen is heteromorphic when the frequency of one morph is < 95%. As pollen tubes are growing through apertures, it was postulated that the occurrence of pollen heteromorphism has to evolve under selection pressure. The comparative studies on *Viola* phylogeny based on ITS markers, pollen heteromorphism, and polyploidy indicated that 3-aperturate pollen grain is considered an ancestral character while more-aperturate (4- 5-, 6-) as derived and that pollen heteromorphism is correlated with polyploidy (diploidy *vs* polyploidy) in all sections of *Viola,* except of highly (74% of species) pollen-heteromorphic *Melanium* (Nadot et al., 2000). We re-examined pollen heteromorphism in *Viola* taking into consideration a new genus phylogeny and ploidy of 16 sections (Marcussen et al., 2015). Despite two diploid (2*x*) South African sections *Andinium* and *Rubellum* and one North American *Chamaemelanium*, the remaining 13 sections are polyploid (4*x*–12*x*). Pollen aperture number was analyzed in 41 species of six *Viola* sections (*Chamaemelanium*, *Erpetion*, *Melanium*, *Nosphinium, Plagiostigma*, *Viola*) in aspect of pollen heteromorphism (presence /absence) and its correlation with different ploidy levels of the polyploids and genome size. Such approach was not included into studies of Nadot et al. (2000), moreover, the estimated ploidy of species in this paper has to be re-evaluate (diploids should be treated as tetraploids according to new *Viola* phylogeny). We concluded that: 1) there is a correlation between pollen heteromorphism and ploidy level in some sections (e.g. *Viola*) but it is not a rule in the remaining sections; 2) genome size correlates with the number of apertures and rapid diploidisation of polyploids (a few Ma) can explain the lack of pollen heteromorphism even in highly polyploid lineages; 3) in *Melanium* section pollen heteromorphism does not correlate with chromosome number and ecological factors (Słomka et al., 2018).

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Antibacterial peptides in embryos of the spider *Parasteatoda tepidariorum* (Theridiidae, Araneae)

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The current knowledge about immune system of spiders is based only on studies on adult individuals (Nentwig, Kuhn-Nentwig, 2012). There is no data about the defensive system of spider embryos. During the embryonic development, spiders are protected by the egg cocoon (Hieber, 1985). However, after hatching from the cocoon, the nymphs rely only on their own defense mechanisms. Consequently, it was assumed that the embryos produce antibacterial peptides early enough to be ready for contact with pathogens after emergence. In order to determine the presence of these mechanisms, ELISA test and SDS-page electrophoresis were used for protein detection. The embryos of *Parasteatoda tepidariorum* were collected from the laboratory-bred strains led in the Department of Animal Physiology and Ecotoxicology, University of Silesia in Katowice. The following experimental groups were selected: 24h, 48h, 72h, 96h, 144h, and 168 h old embryos and 24h old nymphs. Each of the group was divided into two subgroups: embryos left in untouched cocoons and embryos deprived of the cocoons for 24 hours. The aim of the research was to show possible presence of antibacterial proteins – lysozyme and defensins, as well as to check if their level changes with the age of embryos and also verifying if removing of cocoon affects the level of antibacterial peptides. The presence of both proteins was confirmed. The highest level of lysozyme was found in the 144-168h old embryos. The study also shown that the highest level of defensins was in the 72h group. Furthermore, there were statistically significant differences in groups 168h in lysozyme and 72h in defensins between the experimental groups with or without the cocoon. According to the results, during embryonic development, *P. tepidariorum* spiders begin to rely not only on efficient protective role of egg sac, but also on their own defense mechanisms.

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Neb-colloostatin and its analogs interfere with cellular immune response during the development of the *Tenebrio molitor* beetle

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High reproductive potential and effective resistance mechanisms against microorganisms or insecticides, make insects the largest group of invertebrates with potent adaptations to unfavorable environmental conditions. Cellular response in insects is directly dependent on hemocytes that are involved in immune processes: phagocytosis and nodulation. All aspects of insects life including reproductive cycle and immunity are under strict hormonal control. Therefore, by introducing synthetic peptides of known effects into the insects body, it is possible to control their life parameters such as reproduction and viability. We propose a use the analogs of *Neb*-colloostatin – an ovarian insect's peptide hormone with the gonadoinhibitory and hemocytotoxic (Czarniewska et al., 2012; Kuczer et al., 2013; Czarniewska et al., 2014) properties as agents interfering with cellular immunity of various developmental stage in insects. We investigated an impact of the *Neb*-colloostatin analogs on cellular immune response of larvae and adult of the mealworm. Our studies showed that all tested analogs significantly decrease the cellular immune response compared to the controls, as a consequence of the hemocytotoxic action of these analogs in mealworm. Moreover, we observed increased mortality of *Tenebrio molitor* after analogs injection, especially in the case of male beetles. This immunoinhibitory action of the *Neb*-colloostatin analogs was stronger than effect caused by the native peptide. The immunotropic activity of synthetic analogs of *Neb*-colloostatin suggest the need to continue research to assess the insecticidal activity of these peptides.

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Application of induced gynogenesis for generation of rainbow trout *Oncorhynchus mykiss* (Teleostei, Salmonidae) clonal lines

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Genetically uniform fish have great potential in the biomedical research. However, production of fully homozygous specimens using traditional inbreeding programs is time consuming and requires continuous mating of siblings for at least 20 generations. Application of gynogenesis that results in production of specimens with only maternal DNA accelerates generation of the homozygous clones in fish. Induced gynogenesis includes activation of eggs with UV-irradiated homologous or heterologous sperm and diploidization of embryos using physical shock that applied after insemination blocks extrusion of the second polar body (early shock, meiogynogenesis) or inhibits the first cell cleavage (late shock, mitogynogenesis). Individuals provided in the course of mitogynogenesis are fully homozygous Doubled Haploids (DHs). Eggs from DH females used for another round of gynogenesis should develop into clonal specimens. Using such approach, genetically uniform individuals are provided within two generations (Komen and Thorgaard, 2007). In April of 2012, successful development of gynogenetic DH rainbow trout (*Oncorhynchus mykiss*) was induced using UV-irradiated homologous and grayling (*Thymallus thymallus*) spermatozoa and high hydrostatic pressure (HHP) shock (7500 psi/4 min.) applied 350 min. after egg activation. Only few of the gynogenetic DH females survived till sexual maturation reached within four years of rearing. To generate rainbow trout clonal lines, eggs from 4-year-old DH females were activated by the UV-irradiated grayling spermatozoa and subjected to HHP treatment (9500 psi/3min.) applied 35 min. after insemination. About 20% of the activated and HHP treated eggs developed into gynogenetic embryos. Most of them hatched and survived till further developmental stages. Analysis of ten polymorphic microsatellite DNA markers from different linkage groups confirmed genetic identity of the DH egg donors and their gynogenetic progenies, the clones.

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Adaptations in the gonad structure in *Pantodon buchholzi* (Teleostei: Osteoglossomorpha) practicing insemination.

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Teleostei, the largest vertebrate group, demonstrate diversity in reproductive modes from external to internal fertilization and from oviparity to viviparity (Breder and Rosen, 1966). Insemination, present in only about 500 species of Osteoglossiformes, Characiformes, Siluriformes, Osmeriformes, Ophidiiformes, Perciformes, Atheriniformes, Beloniformes, Cyprinodontiformes and Scorpeniformes evolved independently and many times in representatives of both primitive and advanced taxa (Pecio 2010).

Osteoglossomorpha, primitive teleosts group is very unique because of appearance of different types of sperm: aflagellate aquasperm (*Gymnarchus niloticus*), uniflagellate aquasperm (*Papyocranius afer*) and complex (intro)sperm (*Pantodon buchholzi*). The latest type of sperm described by van Deurs & Lastein (1973) suggested the occurrence of insemination.

Our studies of testis and ovary structure in *P. buchholzi* using LM and TEM techniques give evidence that both sexes exhibit features described in literature only in inseminating species (e.g. in males testis structure divided into spermato- and aspermatogenic part, modification of sperm structure, formation of sperm bundles; in females adaptations in ovary structure including presence of area for sperm storage). Histological examination of testis show that testis is divided into anterior, spermatogenic part and posterior, functioning as testicular gland. In the anterior part, at the end of spermiogenesis are formed sperm bundles, which are released into the lumen of seminiferous tubules, filled by secretion of the Sertoli cells. The seminiferous tubules in the distal region of spermatogenic part coalesce and form reduced testicular gland, where Sertoli cells transform into the epithelial, secretory cells. This part is functioning as a reservoir of sperm packets. In females, the ovary cavity is lined by deeply folded mucosa, and epithelium cells showing secretory activity of AB-positive substances, and modifications of the apical surface creating microvilli and cilia.

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Histogenesis of the uterine horns in domestic cat (*Felis silvestris catus*); LM and SEM vascular microcorrosion cast study

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The uterus derived from the paramesonephric ducts (PM), which develop as an invaginations of a coelomic epithelium into cranial parts of urogenital ridges and differentiate in pursuance of a pattern assigned to uterine tubes, uterus and also upper vagina. The purpose of the study was to describe the histogenesis of uterine horns in domestic cat in two stages of prenatal period, i.e. during the development of uterine segments of PM, and during the differentiation of PM into the uterus. The particular emphasis put on describing the moment of differentiation of mucosa and muscularis and changes in the uterine intramural angioarchitecture. The research material was fetuses of domestic cat, aged 27 – 63 day p.c. In the study used LM observations of histological slides and SEM observations of the vascular microcorrosion casts.

The histological analyses revealed that the wall of uterine segment of PM consists of the pseudostratified epithelium, surrounded by the mesenchyme. Until 42nd day p.c. mesenchyme differentiate into loose connective tissue. The vascular system of PM responds to the circular arrangement of the loose connective tissue and consists of the circumferential arterioles, which branch into capillaries. The capillaries form a simple intramural network and join to the superficial collecting venules.

Differentiation of the wall of uterus in cat occur between 44th – 46th day p.c., when mucosa and muscular layer became distinguishable. The myocytes in muscular layer are arranged circumferentially, while the subepithelial fibryocytes in mucosa layer are characterized by a row layout. Between 55th – 63rd day p.c. the lamina propria of mucosa is slightly undulated and lined with a pseudostratified epithelium with variable height, whereas the muscularis is a single circumferential layer. The differentiation of wall of uterus follows the extension of the intramural vascular network, dividing into subserosal and mucosal network. The SEM observations revealed that the circumferential arterioles of subserosal vascular network ramify, supply the muscularis and penetrate the basal zone of the lamina propria of mucosa. The mucosal arterioles subsequently divide into the subepithelial capillaries, which run vertically and correspond to the row layout of the fibrocytes. The blood from the mucosa is drained by venules, which pass through the muscularis and join to the superficial venules.

The influence of trenbolone (xenohormone) on the differentiation and development of gonads in *Xenopus laevis*, *Bufo viridis*, and *Hyla arborea*

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Natural and synthetic endocrine disrupting compounds (e.g. xerohormones) are increasingly detected in the aquatic environment. These substances interfere with the endocrine system and can affect animal development. Trenbolone is an androgen anabolic steroid used in veterinary medicine in livestock to increase muscle growth. In this study, we tested the influence of trenbolone on sexual differentiation and development of male and female gonads in three deeply divergent anurans: the model-species *Xenopus laevis* (Pipidae) and two non-models, *Hyla arborea* (Hylidae) and *Bufo viridis* (Bufonidae). We used a high-standard flow-through-system to simultaneous exposure of tadpoles to three concentrations of 17b-trenbolone (10^{-10}M, 10^{-9}M, 10^{-8}M) (Tamschick *et al*. 2016 and Tamschick *et al*. 2016). The genetic sex was established by molecular methods and the phenotypic sex and morphology of gonads were analyzed by histology. Trenbolone exposure resulted in increased mortality of froglets after metamorphic climax, especially in *Hyla arborea*. We have not found other species-specific differences in the vulnerability to trenbolone. We detected gonadal impairments as partial or total sterility, underdevelopment, and fragmentation or shortening in both testes and ovaries.

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Transovarial transmission of symbiotic microorganisms in *Elymana kozhevnikovi* and *Elymana sulphurella* (Insecta, Hemiptera, Cicadomorpha, Cicadellidae: Deltocephalinae)

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Ovaries of *Elymana kozhevnikovi* and *Elymana sulphurella* are accompanied by large organs termed bacteriomes. The bacteriomes are composed of giant cells termed bacteriocytes which are tightly packed with symbiotic microorganisms. In the peripheral region of the bacteriome bacteriocytes containing *Sulcia muelleri* bacteria are present, whereas in its central region bacteriocytes contain *Nasuia deltocephalinicola* bacteria. Ultrastructural and molecular analyses have revealed that bacteria *Sulcia* are accompanied by *Sodalis*-like bacteria, while *Nasuia* by *Arsenophonus* bacteria. Apart from bacteriocytes, *Sodalis*-like bacteria are also present inside cells of the bacteriome sheath, whereas *Arsenophonus* are distributed in fat body cells. Symbiotic bacteria are transovarially transmitted between generations. In the reproductive females, these symbionts leave the bacteriocytes and begin to invade the ovaries. Symbiotic microorganisms enter the cytoplasm of follicular cells surrounding the terminal oocytes. Then, the symbionts leave the follicular cells and gather in the perivitelline space (i.e. space between the oocyte and follicular epithelium) in the deep invagination of the oolemma. The bacteria residing in the perivitelline space closely adhere to one another. In the studied species the symbiotic bacteria do not enter the ooplasm till the end of the oocyte growth.

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Apical cell in leech ovaries – a putative niche for stem cells. Its ultrastructure and 3D morphology

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The leech ovary is composed of a coelomic sac (ovisac), which usually houses several so-called ovary cords (ovary strings). The ovary cords, in turn, are composed of syncytial germ-line cysts that are enveloped by somatic cells. Oocytes and nurse cells develop within the syncytial cysts, whereas somatic cells appear to support the germ cells mechanically. In one group of leeches, Arhynchobdellida, however, a single and relatively large (up to 50 µm in length) somatic cell attracted our attention. This cell, which is called an apical cell (AC), is always located at the anterior tip of the ovary cord (close to the oogonia) and bears several characteristic morphological features:

1) The AC forms numerous long and short cytoplasmic protrusions that penetrate the spaces between neighbouring germ and somatic cells;
2) its perinucelar cytoplasm is loaded with a large amount of mitochondria, Golgi complexes, dense vesicles and microtubules;
3) the nuclear envelope is lined with a thick layer of nuclear lamina;
4) the cell periphery is filled with cytoskeletal filaments that resemble the intermediate filaments morphologically;
5) there are numerous cell-to-cell junctions between the AC and the neighbouring germ and somatic cells, and these junctions are similar to the hemidesmosomes and adherens junctions.

Although the function of the AC is still unknown due to lack of molecular studies, it has been suggested that it may create a niche (microenvironment) for maintaining the germ and/or somatic stem cells (Świątek et al., 2010).

During our studies, we have compared the ultrastructure and micromorphology of the ACs from several blood-feeding leech species such as *Limnatis nilotica*, *Hirudo medicinalis*, *H. verbana*, *H. orientalis* and *Haemopis sanguisuga*. To fully visualise the morphology of the ACs, we prepared 3D reconstructions of the ACs in three species (*H. verbana*, *H. orientalis* and *H. sanguisuga*), which were based on serial semi-thin sections. We found that in all of the species that were studied, the ultrastructural properties and morphology of the ACs are broadly similar. The 3D reconstructions confirmed that the ACs form numerous short and long processes that penetrate the spaces between neighbouring cells.

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New data on the ovary organisation in oligochaetous Clitellata

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In clitellate annelids, the ovary organisation and course of oogenesis have been intensively studied in recent years. Our observations have revealed the great variability in ovary morphology and several different types of ovaries have been distinguished. We have also suggested a possible scenario of female gonad evolution due to the fact that the ovary organisation seems to be conserved on the family/subfamily level.

A common feature of ovary organisation in Clitellata is the formation of syncytial germ-line cysts at the beginning of oogenesis. The cyst architecture is common for all clitellates that have been studied to date – germ cells are located at the cyst periphery and each one is connected to a common, anuclear mass of cytoplasm (cytophore) via one cytoplasmic bridge. During oogenesis, the germ cells differentiate into one/several oocyte(s) and numerous nurse cells; thus it is the meroistic mode of oogenesis.

Our recent studies have been mainly focused on several representatives of non-leech Clitellata, i.e. oligochaetous clitellates, in which the structure of the ovaries and oogenesis remain unknown. Morphological, histological and ultrastructural observations have allowed us to add new data for the following species:

1) *Grania postclitellochaeta* (Enchytraeidae), a marine species that is morphologically well separated from other enchytraeids, the ovaries have the same structure as in *Enchytraeus albidus*, i.e. they have a grape-like shape and are composed of 16-celled cysts with one oocyte and 15 nurse cells; this suggests that the ovary organisation has a conservative character among this taxon;

2) *Insulodrilus bifidus* (Phreodrilidae – a small family of minute oligochaetes that inhabit freshwaters in the Southern Hemisphere), whose ovaries are composed of several separate cysts, which detach from ovaries early, before the morphological differentiation of the oocyte and nurse cells;

3) *Thalassodrilides* cf. *briani* (Limnodriloidinae, Naididae) and *Haplotaxis* sp. (Haplotaxidae), whose ovaries are conically elongated structures in which the developmental gradient of the germ cells occurs along the long axis; such an ovary organisation seems to be the most widespread among clitellate annelids. Moreover, a 3D reconstruction of *T.* cf *briani* ovary revealed that the entire ovary is built from one huge germ-line cyst that is composed of hundreds of nurse cells and one growing oocyte.

A new pollination strategy in *Viola* - nyctinastic, entomophilous chasmogamous flowers which function changes with circadian rhythm

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Viola banksii K.R. Thiele & Prober of the eastern Australian and Tasmanian sect. *Erpetion* of the genus *Viola* is non-cleistogamous species, developing exclusively open, entomophilous, chasmogamous flowers (Thiele and Prober, 2003). We documented nyctinastic petal movements in *V. banksii*. Flowers are opened during the day and closed at the night. Nyctinasty has not been observed in other *Viola* sections.The degree of nyctinasty during the floral phenological cycle is correlated with stigma receptivity and self-pollination. Overall floral traits as nice fragrance, petal color, anterior petal venation, indurated green area at the base of the anterior petal are attractive to insects. Unlike most other violets, the protuberances of two anthers do not function as nectaries therefore the flowers offer no nectar to insect visitors reward (Little and Leiper, 2013), representing most likely deceit strategy. The short flowering time (5-6 days) and stigma receptivity (2-3 days) of individual flowers limit opportunities for insect visitation and cross-pollination. Night-closed flowers of *V. banksii* appear to facilitate self-pollination. Self-compatibility was confirmed by pollen tube growth tracking from the stigma to the ovule in spontaneous and hand self-pollinated flowers. We hypothesize that flowers of *V. banksii* function as chasmogamous, entomophilous adapted to cross pollination during the day, and act as cleistogamous adapted to self-pollination at night. Such a system has not been described elsewhere in *Viola*. Low genetic intra- and inter-populational differentiation detected by ISSR (Inter Simple Sequence Repeat) molecular markers confirms strong influence of selfing (enhanced by clonal propagation) in Australian *V. banksii* populations with rather weak impact of cross pollination.

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A new type of Teleostei olfactory organ morphological structure in *Macrognathus aculeatus* (Mastacembelidae)

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Diversification of the fish olfactory organs is an adaptation to inhabited environment. Current studies of the lesser spiny eel *Macrognathus aculeatus* are yet another example. That freshawater species are crepuscular and nocturnal burrowers, which may pray on other fishes.

For the study we used 6 specimens of *M. aculeatus* of 8,3 cm – 14,8 cm total body length (TL). Observations were being conducted using the light and electron microscopy techniques (SEM, TEM). That species has two symetrical olfactory chambers elongated into the narrow canals with diversified diameter. Behind the tube-like inlet nostril with four short insets, olfactory channel is covered with non-sensory epithelium. In the half of its lenght, canal is widening gradually in direction to the slim-line outlet nostril. Inside the canal, epithelium forms leaf-like elongated olfactory rosette (in the midway). Narrow, poorly marked median raphe of olfactory rosette is localized in the centripetal wall of the canal. On the longitudinal cross section of canal there is noticeable relocation of the olfactory lamellae rows towards each other. At the outlet nostril area, from the centripetal side of the canal, an extensive, thin – walled accessory nasal sacs are formed and in the middle of the snout, they lay very close to each other, but do not merge. According to TEM observations, olfactory sensory neurons (OSNs) are present in the olfactory lamellae epithelium (among numerous mucous and ciliated supporting cells) and on mucous membrane surrounding rosette in the olfactory canal. We observed two types of the olfactory cells: ciliated OSNs and microvillus OSNs.

We believe that location of the sensory epithelium deep into the canal might protect against a mechanic damages causes by a particles which may flow with water. Regarding the peculiar structure of the olfactory organ and interesting behavior of *M. aculeatus* it would be worthwhile to conduct more research referring to this subject.

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POSTERS

Morphology of ovaries and the mode of oogenesis in viviparous earwig, *Hemimerus talpoides*

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Earwigs (Dermaptera) were usually classified in three taxa: the free living Forficulina and two viviparous groups, the Hemimerina and Arixeniina. Recent molecular and histological analyses suggest that both viviparous groups should be included into the most derived taxon of the Forficulina, the Eudermaptera (Jarvis et al., 2005; Tworzydło et al., 2013). We present results of ultrastructural analyses of the ovary morphology and the mode of oogenesis in a representative of the Hemimerina, *Hemimerus talpoides*. Our results support the idea that the Hemimerina should be classified within the Eudermaptera. We additionally show that the ovaries of the studied species are characterized by two peculiar modifications, i.e. the presence of numerous tracheoles in contact with the basement lamina covering the ovarioles, and an unusual development of the ovariole stalks. We believe that both characters are related to viviparity and unconventional "intra-ovariolar" embryo development. Finally, our study also indicates that the oocytes of *Hemimerus* reveal characters associated with a matrotrophic type of embryo nourishment. They are completely yolkless and devoid of the typical, multilayered egg envelopes; instead, they comprise unconventional organelles, i.e. para-crystalline stacks of endoplasmic reticulum cisternae and translucent vacuoles. It seems that those organelles start to function after initiation of the embryonic development.

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Early ontogenetic development of Chinese sleeper *Perccottus glenii* Dybowski, 1877

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The aim of this study was an analysis of embryogenesis and evaluation of morphological changes in embryos of Chinese sleeper *Perccottus glenii* Dybowski, 1877, a representative of an alien invasive ichthyofauna in Polish freshwaters. The mature specimens of Chinese sleeper caught by electrofishing in spring 2017, in limnetic middle-part of Wloclawek Reservoir (Central Poland), have been transported to the Center of Aquaculture and Environmental Engineering University of Warmia and Mazury in Olsztyn. As a results of semi-natural spawning (using photothermal stimulation of spawners), it was obtained a few thousands of eggs deposited on artificial substratum. Incubation of eggs lasting about 6 days was carried out at a constant water temperature of 19(±0.2)°C. Samples for embryological analysis was collected daily in 24-hours interval, preserved each time in 2% glutaraldehyde solution. Photographic documentation and measurements were made using the image analysis program JENOPTIC ProgRes C3 coupled with binocular microscope LEICA MDG33 type. It was confirm that mature oocyte of Chinese sleeper are characterized by elongated, symmetrical-ellipsoidal shape with an average length 3.53 (± 0.23) mm, and mean width 1.34 (± 0.16) mm. On one of the eggs poles a cone-shaped edging occurred about 1 mm high and wide, used to sticked deposited eggs to substratum through mature female was observed. Yolc-sac characterized by a homogeneous structure and dark-yellow colour had a mean diameter 1.05 (± 0.21) mm which proves a large perivitelar space. Gastrulation began about 38°D of incubation, and ended 72-hours after fertilization (57°D), when the elongated embryo's body was clearly divided into the head, trunk and tail parts. Eyed egg stage correlated with the phase of embryo withdrawal from yolk-sac and occurred in 96-hour of embryos development (76°D). Hatching of embryos occurred between 95 to 115°D followed by the head part and the mean total length of larvae was 2.8 (± 0.15) mm. Analysis of the specificity of early ontogenetic development of Chinese sleeper and description of its vulnerable stages, can contribute the knowledge of reproductive success and the rapid spread of this alien fish species in Polish waters. However these data in the future may permit effective action for eradication or inhibition of its expansion, particularly in isolated, small freshwater ecosystems, where Chinese sleeper is an important element of ichthyofauna structure.

Development of the female gametophyte in *Sedum sediforme* (Jacq.) Pau (Crassulaceae)

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Sedum sediforme (Jacq.) Pau belongs to the genus *Sedum* series *Rupestria.* It is the most species-rich genus in Crassulaceae, which comprises approximate 420 species. The aim of the work was to describe the type of the female gametophyte development in *S. sediforme*. The ovules were analysed using light, Nomarski differential interference contrast (DIC) microscopy and transmission electron microscopy.
The female gametophyte of *S. sediforme* develops in an anatropous and bitegmic ovule. The nucellus of study species is *Sedum* type (crassinucellate ovule). Cyto-embryological studies and observations of cleared ovules reveal the triad formation during megasporogenesis. The embryo sac mother cell is localized chalazally. The female gametophyte develops from one-nucleate functional megaspore and it formation conforms to the *Polygonum* type. Egg cell, two synergids, central cell and three antipodal cells form the embryo sac of *S. sediforme*. The ultrastructural observations show the wall ingrowths formation in both synergids (filiform apparatus) and antipodal cells. Moreover, the plasmodesmata with electron-dense material were observed in outer walls of ephemeral antipodal cells. The presence of simple, branched and with adjacent electron-dense dome plasmodesmata was noticed during the female gametophyte development. The ovule and embryo sac elongate during the development. Finally, the mature female gametophyte is built of an egg apparatus (two synergids and egg cell) and central cell. Data obtained during this study reveal new features of the female gametophyte cells in *Sedum*.

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Research perspectives using house cricket (*Acheta domesticus*) mutants with different eye colours (yellow, white)

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Lines of eye-colour mutants (yellow and white) of the house cricket *Acheta domesticus* have been bred in the Department of Animal Physiology and Ecotoxicology since 2015. Besides their sex, the specimens of the yellow line have yellow eyes and the specimens of the white line have white eyes. These lines are characterised by the lack of visual pigments from the pteridines and ommochromes groups. These deficits indicate disturbances in the tryptophan metabolism pathway. Studies on other animal models have indicated a relationship between this amino acid and the various processes that occur in the body. These organisms are often research models for human diseases such as depression, Parkinson's disease, diabetes and Alzheimer's disease.
As a result of the research that is conducted on cricket lines, various disorders have also been detected at the physiological and developmental levels, such as prolonged development, reduced survival, aggressive behaviour disorders, reduced metabolic rate and reduced fertility. For this reason, these lines appear to be a new research model in many aspects of insect biology. One of these is the reduced egg quality in the females from the white line, which is as much as 50% lower than in the wild line. On the other hand, such changes have not been observed in the yellow line individuals. The aim of the study was to determine the causes of decreased fertility in this line at the level of the gonadal structure and during the course of spermatogenesis and oogenesis processes. An ultrastructural analysis of ovaries and testis of both lines (wild and white), the structure of spermatophores that are produced by the males and the effectiveness of the spermatogenesis process in the form of the number of sperm that are present in the seminal tubules and in spermatophores was carried out.

Ovarian structure in some species of bonytongue fishes (Teleostei, Osteoglossiformes)

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Teleosts ovaries are mostly paired structures, but in some species during embryological development occurs fusion of gonads resulting single ovary (e.g. Poeciliidae) (Wourms, 1981; Lombardi, 1998; Uribe at al., 2005). Ovary is a saccular structure with central lumen lined with folded mucosa, which in inseminating species functions as sperm storage organ. Caudal part of the ovary (=gonoduct) opens to the exterior at the genital pore.
In the Osteoglossiformes, demonstrating various reproductive strategies, ovary structure differs between families. All species of the families Osteoglossidae, Arapaimidae, Notopteridae, Gymnarchidae and Mormyridae practicing external fertilization possess the ovary single. The only living member of Pantodontidae, *Pantodon buchholzi*, is unique species in Osteoglossiformes practicing insemination, which possess paired gonads with adaptations to insemination.
All of the studied species from Mormyridae (*Gnathonemus niger, G. tamandua, Mormyrus rume*) and Osteoglossidae (*Osteoglossum bicirrhosum*) possess single ovary with cavity lined with slightly folded mucosa. Opposite to externally fertilizing species, in *P. buchholzi* mucosa bordering the ovarian cavity is deeply folded and forms numerous crypts probably storage sperm. Apical part of epithelial cells of mucosa possess both cilia and microvilli.
As well as in *P. buchholzi* and *O. bicirrhosum* gonoduct is a short part of the entire gonad, in Mormyridae species gonoduct is relatively large and occupies about 50-60% of total gonad length.
In all studied ovaries the germinal epithelium contains prefollicular cells, nests of oogonia and oocytes beginning primary growth, whereas in stroma vitellogenic oocytes are placed. All studied females were before ovulation, because none of the ovary contain ovulating eggs.

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Histological study of the tongue of neonate Pygmy hippopotami (*Choeropsis liberiensis*, Cetartiodactyla: Hippopotamidae)

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According to the IUNC Red List, the pygmy hippopotamus (*Choeropsis liberiensis*) is an endangered species. To date, there have been reports of the structure of the tongue of the common hippopotamus (*Hippopotamus amphibious amphibious) (Yoshimura et al., 2009), but the lingual structure of the* Pygmy hippopotamus has not been described. The study material was obtained from two neonate Pygmy hippopotami from the Wrocław Zoological Garden, who died due to delivery complications. A macroscopic analysis was carried out and samples were collected for a histological (hematoxylin&eosin, Masson-Goldner trichrome, Movat pentachrome stainings) and histochemical (Alcian blue pH 2.5, Hale`s dialysed iron, periodic acid Schiff stainings) analysis. The tongue of the neonate pygmy hippopotami was 11 cm long. It had a rectangular shape and did not have a median groove. Mechanical papillae in the form of filiform papillae were present on the apex and body of the tongue, while the conical papillae were present on the surface of the root of the tongue. In addition, two types of gustatory papillae – the fungiform papillae and the foliate papillae – were present. There were approximately 25 round fungiform papillae/cm^2 on the apex of the tongue, which were also the smallest papillae. There were approximately 16 fungiform papillae/cm^2 on the body of the tongue, which were up to 2 mm large on the lateral margin of the tongue. The foliate papillae were formed from 8-9 slit-like parallel grooves of the tunica mucosa and some grooves were divided into two parts. The foliate papillae were approximately 5 mm wide and 8 mm long. The histological examination showed that a keratin layer covered the mechanical papillae and that clearly visible keratohyaline granules were present in the anterior part of each filiform papilla. Some taste pores were present on the surface of the fungiform papillae, which were not covered by a keratin layer. Elongated taste buds were present in the dorsal and lateral epithelium of the foliate papillae. Serous von Ebner`s glands were present below the foliate papillae. Mixed glands were found deeper, in the area of the root of the tongue. The lingual prominence, which is well-defined in the common hippopotamus, was not pronounced in the neonate pygmy hippopotami.

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Reproductive system in *Epipactis helleborine* (L.) Crantz (Orchidaceae)

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E. helleborine is a wide distributed species in Eurasia and North America, occurs in both natural and disturbed habitats and it's reproductive success may result from undergoing both auto- and allogamy (e.g. Fredrickon 1992; Tałałaj and Brzosko 2008; Rewicz et al., 2017). The present work focused on embryology of Pomeranian *E. helleborine* for investigating allo- / autogamous or apomictic potential of this population. As expected, the single archesporial cell in the ovule became the megaspore mother cell which then undergone monosporic sporogenesis to form T-shape tetrads of megaspores. The innermost megaspore gave rise to the 7-celled embryo sac. Double fertilization resulted in the zygote followed by embryo formation, and few nuclear endosperm that disappeared till the 8-celled embryo stage. Similar developmental time-table and reproductive success were assigned to open-pollination, induced allo-, auto- and geitonogamy. A significant decrease and delay in the embryo formation was the feature of spontaneously self-pollinated flowers. Despite the absence of apomictic events, pollination was not necessary to achieve successive stages of the ovule, megaspore and megagametophyte development till maturity, in emasculated flower buds. In the context of autogamous capability, *E. helleborine* has no prezygotic barriers for selfing, as we have evidenced embryologically.

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Ontogenesis of melatonin synthesis pathway in the goose pineal organ

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The profile of melatonin synthesis-related indoles in the embryonic pineal organ has been studied exclusively in the turkey. To provide more data for comparative analyses, in the present research we investigated the ontogenesis of pineal indole metabolism in the goose.

The study was performed on 14-, 16-, 18-, 20-, 22-, 24-, 26- and 28-day-old embryos (14ED - 28ED) of the domestic goose, incubated under 12L:12D cycle. The embryos were killed at 14.00 and 02.00, the pineal organs were immediately removed and frozen at -75ºC. The indole contents were measured by HPLC.

The content of tryptophan showed no significant changes during development and no day-night variations. The level of 5-hydroxytryptophan was significantly higher during scotophase than during photophase starting from 16ED. Both day-time and night-time contents of this compound increased stepwise up to 26ED and then remained constant. The serotonin level also showed day-night differences, however they were less prominent comparing to those noted in a case of 5-hydroxytryptophan. The serotonin content was low at early stages of development and rapidly rose between 22ED and 26ED. N-acetylserotonin remained at low level up to 22ED, then markedly increased, and from this stage showed significant day-night variations. The developmental and diurnal changes in melatonin level were parallel to the changes in N-acetylserotonin level, however the content of melatonin was lower by about 30 % at a day-time and by about 50 % at a nighttime than the content of its immediate precursor. The level of 5-hydroxyindole acetic acid increased rapidly between 20ED and 22ED. Like serotonin, 5-hydroxyindole acetic acid showed diurnal variation of its level, however, the time-courses of developmental changes of both indoles were not parallel. The content of 5-hydroxytryptophol was much lower than 5-hydroxyindole acetic acid. 5-Methoxyindole acetic acid and 5-methoxytryptophol (5-MTOL) occurred at measurable levels from 18ED and 26ED, respectively, and from these stages their levels increased.

The obtained data showed that the embryonic development of metabolic pathways related to the melatonin synthesis differs significantly between the turkey and the goose. The synthesis of 5-methoxyindoles starts much earlier in the goose than in turkey, and it is much higher. Moreover, in contrast to the turkey, in the goose, the levels of 5-hydroxytryptophan and serotonin show day-night variations from early stages of the pineal embryonic development.

Viviparity in the epizoic dermapteran, *Arixenia esau*: modifications of the larval excretory organs

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Insects are predominantly oviparous and viviparity is restricted to a few groups only. In earwigs (Dermaptera), viviparous species occur in two epizoic families: Arixeniidae and Hemimeridae (Hagan, 1951). Both morphological and physiological aspects of viviparity in dermapterans are rather poorly characterized. Previous studies revealed that in viviparous dermapteran *Arixenia esau*, embryonic development takes place both in the ovary and lateral oviducts or uteri, where embryos are nourished by specialized maternal tissue (Tworzydlo et al., 2013). Here, we explore how the metabolic waste produced by the developing offspring are eliminated from the mother's reproductive system. We analyze morphological changes accompanying development of the excretory organs in the *Arixenia* larvae and how they relate to metabolic waste elimination during development inside mother's body. Our comparative analyses of the early and late first instar larvae revealed characteristic modifications in the cellular architecture of the Malpighian tubules**,** indicating that these organs are functional. In addition, the results of the electron probe microanalyses suggest that the larval Malpighian tubules are mainly involved in maintaining ion homeostasis. We also found that the lumen of the larval alimentary track is occluded by a cellular diaphragm at the midgut-hindgut junction and that cells of the diaphragm and midgut accumulate metabolic compounds. Such an organization of the larval gut apparently prevents fouling of the mother's organism with the offspring metabolic waste and therefore can be regarded as an adaptation for viviparity.

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Structure of the germarium in *Thulinius ruffoi* (Tardigrada, Eutardigrada, Parachela)

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The ovary of gonochoric and parthenogenic species of tardigrades is divided into germarium and vitellarium that are varied in structure and function. The germarium is a small region located in the anterior part of the ovary, while the rest is the vitellarium (Węglarska, 1979). *Thulinius ruffoi* is able to reproduce by parthenogenesis. We showed, using SBEM method and open-source software for 3D reconstructions (Schindelin, et al., 2012), that the most apical region of germarium is created by two somatic cells of the ovary wall which form the tip of the ovary and surround the germarian cells like a cap (cap cells - CCs). Each of these cells possesses one cytoplasmic ligament, by which the ovary is attached to the dorsal body wall. Directly behind the CCs, two cells which probably are the female germline stem cells (GSCs) have been detected. In the posterior part of the germarium young, developing clusters of the female germ cells (DC) may be present. Within the germarium, complete and incomplete divisions occur. The result of incomplete divisions is the presence of the intercellular bridges between the cells of the DC. The germarian cells can be clearly different in morphology, which also affects the cell nuclei. Morphological variability is demonstrated by young cystocytes of the DC, GSCs, and CCs. Such factors as: ovary tension, physiological condition, the proximity of the other anatomical structures (muscles, storage cells) or organs (midgut) may have an impact on the shape of the germarian cells.

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Male germ-line cysts of the medicinal leech *Hirudo verbana* (Annelida, Hirudinida)

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Hirudo verbana is one of five species that belong to the medicinal leeches from the genus *Hirudo*, which belongs to the so-called European medicinal leeches. *H. verbana* occurs from Western Europe to Turkey and Uzbekistan. Our knowledge about the spermatogenesis of medicinal leeches is still not sufficient. To date, nothing is known about spermatogenesis and the functioning of male germ-line cysts in *H. verbana*.

Hirudo verbana specimens, similar to other clitellate annelids, are hermaphrodites. The male reproductive system of *H. verbana* is composed of nine pairs of testes, which are located in segments XII-XX. Each testis contains numerous syncytial groups (cysts, clusters) of developing germ cells. All of the interconnected germ cells in a given cyst develop in complete synchrony and are at the same stage of spermatogenesis, thus there are speramatogonial, spermatocytic and spermatids cysts. However, there is no synchrony between the cysts, and therefore different developmental stages can occur within a testis cyst. The organisation and shape of germ-line cyst, as well as the number of interconnected cells, differ and depend on the stage of spermatogenesis. The germ-line cysts are formed as a result of cell divisions that are not followed by complete cytokineses. As a rule, all germ cells in a given cyst are attached to a central anuclear cytoplasmic mass, the cytophore. The function of the cytophore is to interconnect all of the germ cells into a cyst and to mediate the sharing of the cytoplasm and macromolecules between sister cells. Each germ cell is connected to the cytophore by only one specific cellular junction, which is called a stable intercellular bridge, cytoplasmic bridge or ring canal. The intercellular bridges are formed during late cytokinesis, which has not yet been completed. In fact, they are contractile rings that do not close during late cytokinesis. Intercellular bridges are rich in mitochondria and are enriched with F-actin.

The material was analysed using light, fluorescent and transmission electron microscopy. These techniques were used to analyse the general morphology of the cysts, their structure and ultrastructure. Special attention was paid to the changes in the amount of F-actin and the organisation of the microtubular cytoskeleton at the consecutive stages of spermatogenesis.

Chelicerate ovary in a South African camel spider (Chelicerata, Solifugae) – universal and unique structural features

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Solifugae (camel spiders) is a poorly studied Chelicerate order of a controversial phylogeny (Regier, et al., 2010). Among tracheate arachnids Solifugae are distinguishable due to their highly active lifestyle (Punzo, 1998). The structure of the ovaries in this chelicerate group is hardly known. We show that in *Solpugema* sp., the African camel spider, the ovaries are paired and show general structure typical of chelicerates (Makioka, 1988). The oocytes grow exposed to the body cavity on the ovary surface and the mature ovaries take a grape-shape. Growth of the oocytes is asynchronous, and so within the ovary there are the oocytes at successive stages of previtellogenesis and vitellogenesis. The externally positioned oocytes maintain connection with the ovarian wall by means of the oocyte stalks built of somatic epithelial stalk cells. In the ovaries of *Solpugema* the oocyte stalks are very small. Similar to other chelicerates, the ovarian lumen is lined with simple columnar epithelium. Our morphological and ultrastructural observations revealed that the ovaries in the investigated camel spider exhibit some unique features. One of them is complex structure of the ovarian wall. An external layer of the ovarian wall comprises striated muscles and an extremely well developed tracheal system immersed within the extracellular matrix. Moreover, the ovarian epithelium is highly folded and forms regular and deep invaginations (crypts). The potential role of structural modifications found in the ovary of *Solpugema* is considered.

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Morphology and ultrastructure of overwintering eggs of the pea aphid *Acyrthosiphon pisum* (Insecta: Hemiptera: Aphididae)

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Aphids (Hemiptera Aphididae) are sap-feeding insects with more than 5000 known species. Many of them are considered as important economic pests. Aphids can have different life cycles, patterns of host alternation and different morphs depending on seasonal or climatic parameters. Their morphology and general appearance can be also modified by their relationships with other organisms, most notably with their host plant. Polymorphism in aphids is with their complicated life cycles one of the most important reasons for their such big evolutionary success also as plant pests.
For most of the year aphids form large colonies formed by apterous and alate viviparous females which reproduce parthenogenetically. Once in the year, mostly in the autumn, in their life cycle a bisexual generation occurs. Sexual morphs – oviparous female and male copulate and the female lays so called overwintering eggs and the next year generation will hatch from those in the spring. Winter eggs are, together with the sexual generation, one of the poorly examined life stages in aphids.
Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used for the first time to elucidate the general and fine morphology and ultrastructure of the overwintering eggs of the model aphid species – the pea aphid (*Acyrthosiphon pisum*). Moreover, we examined the influence of biogenic amines (putrescine, spermine, tyramine, tryptamine and cadaverine) of the eggs shells in a comparative approach. The results of the work show the general shape and size of the eggs, surface and structure of the chorion of treated and control eggs.

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Spatial and temporal organization of F-actin in mature pollen grain of *Convallaria majalis* (L.)

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In mature pollen grain, among all Angiosperms, at the beginning of pollen hydration microtubules (MTs) are organized in a basket-like configuration surrounding generative nuclei (GN) and lacking MTs in vegetative cytoplasm. In contrast, microfilaments' (MFs) network show numerous randomly distributed circular profiles and coarsely granular associated with the vegetative nucleus which subsequently transform into fusiform patterns in whole pollen cytoplasm (Pierson and Li, 1992). In hydrating pollen grain of *Convallaria* shortly after 15 minutes incubation in the culture medium unique F-actin organization is observed. 3D reconstructions reveal thick MFs, like inter-apertural strands, tightly enclasping generative cell and formed in the shape of lens with long tails on both ends which sometimes accumulate and mark the apertural pole. The thickest bundles of MFs locate under sporoderm, where these MFs are in close location to generative cell, in some cases nearly entwining highly folded GN with thin layer of generative cell (GC) cytoplasm. Phalloidin conjugated with fluorochrome staining, antibodies detection against actin (at light and electron microscopy level) and negative controls with actin inhibitors seems to support the thesis that mature pollen grain contain generative cell lacking MFs. It is interesting if such unique F-actin organization in this species is associated with striped projections (SP) at the cytoplasmic face of the protruding lobes outer membrane of the GC, arranged in groups, parallel to each other and equally spaced (Bohdanowicz et al. 1995). Immunogold labelling may suggest that these SP of GC contain myosin-like protein and further ultraimmunolabeling investigation may unravel the nature of this potential actomyosin arrangement.

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The second case of Haldane's rule in plants

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The preferential sterility and/or inviability of heterogametic sex in hybrids (Haldane 1922) was commonly observed in animal taxa. The most likely cause of this phenomenon are incompatibilities involving sex chromosomes (Laurie 1997). This mechanism is considered very important in speciation genetics because it determines the early formation of reproductive barriers between closely related forms. However, the conformity with Haldane's rule has been so far tested almost exclusively in animals. In this work, we analysed male fertility and male viability, two phenomena associated with Haldane's rule in experimental reciprocal hybrids between two races of *Rumex hastatulus* possessing different sex chromosome systems (XX/XY vs. XX/XY1Y2). In addition, the chromosomes and DNA of original *R. hastatulus* races and obtained hybrids were analysed in order to find markers useful in further research.
It was shown that in one of obtained *R. hastatulus* hybrid both male fertility and male viability were affected, whereas in the hybrid resulted from the opposite cross they were unaffected and similar to the observed in the pure parents. The *R. hastatulus* is the second plant in which Haldane's rule was evidenced. The observed cross asymmetry in Haldane's rule for both male sterility and male rarity (so named "Darwin's corollary", Turelli and Moyle 2007), widespread in animals, was not observed in any non-animal species so far.

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Changes in the ultrastructure of digestive cells in the midgut epithelium of *Lithobius forficatus* (Myriapoda, Chilopoda) according to starvation and re-feeding

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Lithobius forficatus (Myriapoda, Chilopoda) is a representative of centipedes, and is commonly found in Poland and all over the Europe. It plays an important role in ecosystems because it supports the processes of aeration, humification, and soil formation. Therefore, it is considered as an important bioindicator. It hunts for smaller invertebrates, among others insects, as well as earthworms and naked snails. In the environment, this species may be exposed to numerous dangerous factors, as well as to situations commonly occurring in the environment, such as starvation. Depending on the place of living and the season, individuals may be exposed to short as well as long periods without the possibility of eating food. The digestive system is treated as a very good research model, due to its structure and ultrastructure connected with digestive processes (synthesis, secretion, absorption, etc.), but it also takes part in the storage of substances and detoxification of toxic substances that can be taken with food. The midgut of *L. forficatus* is made up of three cell types: secretory cells, regenerative cells and digestive cells. The species were divided into 4 groups: starved for 14 days, re-fed for 7 days after 14 days of starvation, re-fed for 14 days after 14 days of starvation and re-fed for 21 days after 14 days of starvation. We used methods such as the light microscopy, transmission electron microscopy (TEM) and histochemical methods (Bonhag's method, Schiff's (PAS) method and Sudan black B). Based on the research, it can be concluded that starvation and re-feeding affect the midgut epithelium. Therefore, activation of autophagy and necrosis together with the changes in the amount of reserve materials were observed.

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Protoplast cultures of cabbage (*Brassica oleracea* L.) – histological evaluation of callus and regenerants

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Protoplasts were isolated from leaf mesophyll tissue of *in vitro* cultured plants of two haploid and two diploid accessions. Isolation was done according to protocol of Kiełkowska and Adamus (2012). Then protoplasts were immobilized in a calcium alginate layers according to Kiełkowska and Adamus (2014). Embedded protoplasts were cultured in modified Kao and Michayluk (1975) medium with 0.1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 mg l^{-1} zeatin, pH 5.6. After approximately 2 months of culture callus colonies were obtained. Prior to further development calli colonies were freed from alginate layers according to Kiełkowska and Adamus (2014) and subjected to regeneration. Obtained callus and regenerants of different ploidy (1n, 2n, 4x) were subjected to histological analysis using Technovit resin. Obtained results revealed differences in morphology between embryogenic and non-embryogenic callus. Performed analyze allowed for characterization of early stages of organogenesis from callus. We found also differences in leaf anatomy in dependency from the ploidy level of regenerants. In general haploid regenerants had thinner epidermis and smaller mesophyll cells, compare to diploid control and regenerants of higher ploidy (2n, 4x).

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Histological and histochemical study of the Harderian gland of neonate Pygmy hippopotami (*Choeropsis liberiensis*)

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In the present study, the histological and histochemical analysis of the Harderian gland in the two newborn pigmy hippopotamus (*Choeropsis liberiensis* or *Hexaprotodon liberiensis,* Morton, 1849) was performed. The study samples were collected from 2015 to 2017. The animals were obtained by the Wroclaw Zoological Garden (Poland). The study was carried out with the permission of the District Veterinary Officer (Wroclaw, Poland; No. PIW Wroc. UT-45/5/16, No. PIW Wroc. UT- 45/6/16, No. PIW Wroc. UT-45/8/16). The animals were not killed for the purpose of this study and were obtained *post-mortem*. The Harderian gland were examined using light microscopy (H&E, picro-Mallory trichrome, Masson-Goldner trichrome, Movat pentachrome (modified Russell Movat), MGP Y, PAS, alcian blue pH 2.5 and HDI staining). The Harderian gland was a multilobar tubuloacinar gland covered by a thin connective tissue capsule consisting of collagen and elastic fibers. The interlobar septa divided the gland into numerous large lobes and single smaller lobes. Numerous blood vessels were present within the interlobar septa. The acini were composed of a small lumen made of tall conical cells with a basophilic cytoplasm. The Movat-pentachrome staining showed the presence of mucous acini in this gland. The tubules that had a large lumen were composed of a single cubic cell layer. Single, poorly branched main ducts were visible in the connective tissue stroma, which were composed of a simple cuboid epithelium with goblet cells. The MGP Y staining presence of single plasma cells. Based on histochemical study the Harderian gland of Pygmy hippopotamus has mucous nature. The goblet cells of the main ducts in Harderian gland in pygmy hippopotamus was strongly PAS and alcian blue pH 2.5 stain and HDI middle positive reaction.

Comparative micromorphology and anatomy of generative structures among three *Colobanthus* species

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Micromorphology and anatomy of flowers, fruits and seeds of the three species of the genus *Colobanthus* (Caryophyllaceae) growing in natural conditions in the Antarctic, Subantarctic and in a greenhouse in Olsztyn has been studied. Our macroscopic observations and microscopic studies of generative structures among *Colobanthus apetalus* (Tierra del Fuego National Park, Argentina), and *C. quitensis* (King George Island, Antarctica), showed that these species develops two types of bisexual flowers: opening, chasmogamous flowers and closed, cleistogamous ones. In *C. apetalus* and *C. quitensis* cleistogamy was caused by a low temperature, high air humidity and strong wind. A small number of microspores differentiated in the microsporangia of *C. apetalus* and *C. quitensis*, which is typical of cleistogamous species. Microsporocytes formed very thick callose walls. More than twenty spheroidal, polypantoporate pollen grains differentiated in the microsporangium. In the *C. apetalus* and *C. quitensis*, pollen grains germinated on the surface of receptive cells on the dry stigma of the pistil or inside the microsporangium. Transmission tissue in a two-chamber ovary of *C. lycopodioides* pistils, as in the other two of *Colobanthus* species with five-chamber pistils, contain large amounts of proteins and lipids, and small polysaccharides. In *C. apetalus* and *C. quitensis* a monosporic embryo sac of the Polygonum type arises in the crassinucellar ovule. In the nucellus tissue formed and stored large amounts reserve materials. Almost 85% - 90% of *C. apetalus* and *C. quitensis* ovules developed and formed perispermic seeds with a completely differentiated embryo under both, natural and in a greenhouse conditions. In an incubator at 20°C and air humidity of approx. 80-85%, seeds of *C. apetalus* germinated in capsules (bags) within a short time after formation. Resulting seedlings planted in the horticultural substrate grew and developed. We have not observed the phenomenon of vivipary in *C. quitensis*, neither in natural conditions in Antarctica, and during several years of cultivation in greenhouse conditions.

Global DNA methylation during early development of *Cobitis* diploids - preliminary studies

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The spined loach *Cobitis taenia* and the Danube loach *C. elongatoides* distributed in Poland hybridized and occur mainly in mixed, diploid-tetraploid populations (Boroń 2003). Epigenetic gene silencing mechanism apart others revealed as methylation of DNA may play an important role during the formation of hybrids (Comai 2000). Methylation DNA pattern dynamically change during embryonic development.

In mammals and *Danio rerio* global methylation of spermatozoa DNA was high, whereas in oocytes and early embryonic stages was low. During the next stages of embryonic development occurs remethylation DNA (Fang et al. 2013; Labbé et al. 2017). In this study a preliminary data on global DNA methylation of embryonic developmental stages of *C. taenia* and *C. elongatoides* as well as of their diploid hybrids is presented. Samples were collected at two blastomeres, the blastula, the gastrula and post hatching (larvae) stages. Additionally tissues from ovaries and testes of parental individuals were analysed. Level of global DNA methylation was appointed by density of 5 methylcytosine (5mC) with use enzyme-linked immunosorbent assay method (ELISA). The results indicated patterns of global DNA methylation during *Cobitis* development. Different level of 5mC between *C. taenia* and diploid hybrids of both species has been noticed; statistically significant differences ($p < 0,05$) concerned the gastrula and the larvae stages. The lack of such differences in the level of general DNA methylation in early embryonic stages of analysed species may correspond to their close relationship. Further studies will be conducted on a larger number of individuals to verify this data.

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Differentiation of the head structures in the brown anole *Anolis sagrei* (Squamata, Iguania)

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The brown anole (*Anolis sagrei*) is a common lizard in North America. This species is a member of large clade Iguania and family Dactyloidae. Representatives of this family are characterized by many adaptations to arboreal lifestyle. Moreover, phylogenetic position of Iguania within Squamata is unclear in the light of discordance of morphological and molecular data (Vidal and Hedges, 2009). Given these facts the anole is an excellent model organism to study developmental bases of evolutionary and morphological diversification. The heads of reptiles are complex structures containing brain and many other sense organs. The purpose of this study was to examine external morphology and histological features of developing brown anole *Anolis sagrei*. This species is maintain and breed in our laboratory. The eggs of anole were incubated in the laboratory in constant temperature at 30°C and the embryos were isolated at regular intervals of time from egg lying till hatching. The material was fixed in Karnovsky solution, dehydrated and stored in 70% alcohol. The model collection included 90 embryos isolated at each day of incubation from hatching. Isolated heads of embryos were fixed in Bouin's solution embedded in paraffin and cut with using rotary microtome. Sets of H&E stained histological serial section allowed us to follow differentiation of chosen craniofacial and neural structures (such as: parietal eye, lateral eyes, eyelids, nasal pits, pattern of scales on the pileus, egg tooth). On the base of this study we prepared the list of diagnostic characters useful for our future studies of circadian clock centers formation.

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Accumulation of raffinose family oligosaccharides in maturing seeds of pea (*Pisum sativum* L.)

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The aim of the study was to compare the dynamics of the accumulation of raffinose family oligosaccharides (RFOs) during the maturation of 4 cultivars of pea seeds (*Pisum sativum* L.). Analyses of sugars in whole seeds were carried out using the GC-FID method from the beginning to the end of the accumulation of storage materials (from 18-20 to 38-44 day after flowering, DAF). The changes in the level of the expression of essential genes for the biosynthesis of RFOs - galactinol synthase (*PsGolS1* and *PsGolS2*) and rafinose synthase (*PsRS*) in the seeds of cv. Venus and Kelvedon Wonder were compared.
The gradual decrease in the initially high content of sucrose and *myo-inositol*, with the simultaneous production of galactinol and subsequently: raffinose, stachyose and verbascose, were the common changes in the composition and the content of soluble carbohydrates. The process of RFOs accumulation intensified with the maturation of seeds, however, it was most intensive during their natural desiccation. The increased concentration of RFOs may have been influenced by the activity of raffinose synthase - the level of expression of *PsRS* in seeds accumulating higher amounts of RFOs (Kelvedon Wonder) was higher than in seeds accumulating less RFOs (Venus). On the other hand, the accumulation of RFOs in seeds of cv. Kelvedon Wonder and Telefon could be the result of substitute storage of carbon in the form of non-reducing oligosaccharides, when the accumulation of the main storage material of pea seeds (starch) is disturbed. The gene mutation of the starch biosynthetic pathway in the seeds of cv. Kelwedon Wonder and Telefon is determined by their phenotype – mature seeds are wrinkled and irregular, while the seeds of cv. Venus - round and smooth.

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Accumulation of cyclitols and low-molecular weight sugars in maturing seeds of fenugreek and buckwheat

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Myo-inositol, common in plant tissues, and its rare isomers – *D-chiro-inositol* or methyl ether derivatives – D-pinitol and D-ononitol, have a number of health-promoting properties such as: acting antioxidant, anti-inflammatory, they lower the level of glucose in the blood, regulate the accumulation of adipose tissue and have and anti-cancer properties (Croze et al. 2013). For these reasons, natural plant sources rich in cyclitols are search for potential use for both – isolation and direct use as pro-health food.
The aim of the research was to determine the changes in the composition and the content of cyclitols and its α-D-galactosides, in the context of the accumulation of other sugars in maturing seeds of fenugreek (*Trigonella foenum-graecum* L.) and buckwheat (*Fagopyrum esculentum Moench*). D-pinitol occurs in fenugreek (Yasui i Ohashi 1990) and *D-chiro-inositol* in buckwheat (Obendorf i Górecki 2012).
The changes in the composition and the content of soluble carbohydrates in seeds of fenugreek consisted of the initial accumulation of significant amounts of monosaccharides (glucose, fructose) and sucrose, while during the maturation of seeds, the content of these sugars gradually decreased, and the seeds accumulated raffinose family oligosaccharides (mainly stachyose and raffinose). At the same time, α-D-galactosides of D-pinitol were formed in the seeds - mono- and di-galactopinitols and ciceritol. At the end of maturation, verbascose and tri-galactopinitol A appeared in the drying tissues. The predominant cyclitol was D-pinitol and its concentration was higher than *myo-inositol* throughout the maturation of seed.
In the seeds of buckwheat, unlike in fenugreek, the accumulation of RFOs (raffinose and stachyose) occurred only temporarily, while the dominant direction of changes was the accumulation of α-D-galactosides of *D-chiro-inositol* - phagopyritols (mainly mono-galactosides – phagopyritol B1 and A1). The dominant cyclitol was D-*chiro-inositol* and its concentration decreased during the maturation of seeds (from 2.07 to 0.55 mg g^{-1} DW).
In the mature seeds the concentration of specific cyclitols – D-pinitol in fenugreek and *D-chiro-inositol* in buckwheat was 4.44 and 0.55 mg g^{-1} DW, respectively.

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Unique class of slow muscle fibers during grass snake (*Natrix natrix* L.) myogenesis

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Our studies of the grass snake (*Natrix natrix*) trunk muscles differentiation revealed that during myogenesis two classes of muscle fibers were developed. The first class (C1) was composed of typical muscle fibers. The myofibrils (unites building contractile apparatus) were regularly distributed in C1 fibers. The second class (C2) of muscle fibers was characterized by significantly different features. In C2 fibers we observed myofibrils located centrally and the absence of contractile apparatus in subsarcolemmal sarcoplasm in light microscope and TEM. The ultrastructural analysis showed the presence of lipid droplets (LDs) surrounding centrally located nuclei. The mentioned observation was confirmed by BODIPY® staining. Furthermore, our immunocytochemical analysis showed that LDs-rich fibers were expressed slow myosin heavy chains (SlowMyHC). In contrast to C1, we did not observe the presence of satellite cells closely adhering to C2 muscle fibers.
The phenomenon of LDs in C2 muscle fibers was detected for the first time during Egyptian cobra (*Naja haje*) and grass snake myotomal myogenesis (Khannoon et al., 2016; Lewandowski et al., 2017). In agreement with previous studies, we strongly believe that the storage of LDs in some muscles is the most economic form of energy storing. The presence of LDs in SlowMyHC muscle fibers is a characteristic feature of snakes myogenesis, not observed in other Reptilia and vertebrates.

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Division of autonomous organelles during the development of the male gametophyte in *Tinantia erecta* Jacq. (Fenzl.)

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During meiosis in microsporogenesis, autonomous cellular organelles. i.e. plastids and mitochondria, move and separate into daughter cells according to a specific pattern. This process called chondriokinesis is characteristic for a given plant species. Chondriokinesis was observed at the end of the 19th century, and in 1938 it was systematized by Bąkowski (1938). The proposed classification comprised four main types of chondriokinesis: neutral, capsular, polar and equatorial. Additionally, intermediate types, e.g. capsular-polar chondriokinesis, and more complex types, e.g. neutral chondriokinesis equatorial during telophase have also been described. The key criterion for classification of the chondriokinesis types was the arrangement of cell organelles during two meiosis phases: metaphase I and telophase I. Currently, the process of chondriokinesis in plants has been completed and verified by Tchórzewska (2017). The autonomous organelles participate in the cytoplasmic inheritance; therefore, their precise distribution to daughter cells determines formation of identical, viable microspores. Furthermore, disturbances in the distribution of these organelles often cause cytoplasmic male sterility (Majewska-Sawka et al., 1993; Chase, 2006; Tchórzewska, 2017). The cytoplasmic skeleton of the cell actively participates in the process of chondriokinesis in microsporogenesis. To date, many microtubule and microfilament configurations that occur during the division of the meiotic cell have been described. Disorders in the formation of the cytoskeleton configuration in microsporogenesis result in disturbances in chondriokinesis, which in turn may lead to the formation of non-identical pollen grains. In this study, the course of chondriokinesis during the development of the male gametophyte in *T. erecta* was analyzed, with particular emphasis on the dynamically changing microtubular cytoskeleton. The study was conducted using optical, fluorescence, and transmission electron microscopes. The analyzed species were found to have a neutral-equatorial type of chondriokinesis, accompanied by appropriately changing microtubule configurations.

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Development of the olfactory system in the brook lamprey (*Lampetra planeri*)

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The lamprey development is of interest for a number of reasons, among them the most important is the phylogenetic context. Despite may similarities, lampreys differ in many aspects, including their life style (nonparasitic, freshwater parasitic, anadromous parasitic) and time-course of ontogenesis. Several aspects of lamprey biology are poorly recognized and requires further studies. The developing lamprey is termed an embryo before it hatches, a prolarva just after hatching and then a larva or ammocoete. The lamprey larva undergoes radical modifications of their morphology during metamorphosis into the adult form. The aim of study was to analyze the developmental changes occurring in the olfactory system during the larval stage of life and the metamorphosis of the brook lamprey.

Larvae at the ages estimated as one, two, three and four years, metamorphosis larvae and adults of the brook lampreys were collected from the natural environment during the conservations of canals connecting the fish ponds with river. The collection of animals and their euthanasia have been approved by the Minister of Environment and the Local Ethical Commission. The lampreys were anesthetized with 2-phenoxyethanol and killed by decapitation. The heads were fixed in 4 % formalin, embedded in paraffin and cut into serial sections, which were stained with hematoxylin and eosin.

The olfactory system of larvae, independent of their age was poorly developed. It consisted exclusively of the small nasal sac connected by a short, wide tube with the nasal opening. The nasal tube did not contain the closing valve. The nasal sac had a form of simple cavity and was not divided by the nasal lamellae. The caudal and later surfaces of the caudal sac were covered by the olfactory epithelium. The primordium of the accessory olfactory organ occurred in a form of short tubules connected with the epithelium of the nasal sac. The olfactory system underwent deep reorganization during metamorphosis. In adult lampreys, the nasal sac was divided into cavities by well-developed lamellae covered by olfactory epithelium. Numerous bundles of nerve fibers connected the nasal sac with the brain. The entrance to the nasal sac was covered by the valve. The accessory olfactory organ was well-developed. The olfactory organ system of the brook lamprey should be considered an interesting model to study on development of sensory epithelia and neurogenesis.

Ultrastructure, distribution and vertical transfer of symbionts in planthoppers (Insecta, Hemiptera, Fulgoromorpha)

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The aim of our research was to describe by means of molecular, histological and ultrastructural techniques the symbiotic systems in three representatives of planthoppers: *Ommatidiotus dissimilis* (Caliscelidae), *Dictyophara europaea* (Dictyopharidae) and *Ranissus edirneus* (Dictyopharidae) with particular emphasis on the vertical transmission of symbionts from one generation to the next. The results of molecular investigations have revealed that all the species examined are associated with three types of obligate symbiotic bacteria. Apart from bacteria *Sulcia* and *Vidania*, which are regarded as ancestral symbionts of Fulgoromorpha, elongated gammaproteobacteria are present in all the species examined. Histological observations have shown that these bacteria are harbored in separate bacteriomes which are localized in the abdomen of the host insect. The FISH assay, with symbiont specific probes, allowed us to identify the large, lobated bacteria as *Vidania*, the pleomorphic ones as *Sulcia* and the elongated bacteria as gammaproteobacteria. Analyses of semithin sections have shown that all of the bacteria are transmitted between generations via female germ cells. All types of symbionts simultaneously infect the posterior pole of the terminal oocyte. What is of special interest is that bacteria *Sulcia* and *Vidania* transform before migration. Bacteria *Sulcia,* which begin to escape from the bacteriocytes, stain more intensely with methylene blue. In turn, *Vidania* symbionts change shape and become almost spherical. Similarly to the situation in other representatives of Auchenorrhyncha, symbionts migrate to the perivitelline space through the cytoplasm of follicular cells which surround the oocytes. After passing through the follicular epithelium, symbionts create the "symbiont ball" in the deep depression of the oolemma.

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Glycogen distribution in zebrafish muscles after glycogen phosphorylase (*pygm*) knockdown

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The skeletal muscles requires constant access to the source of energy. Depending on the intensity of exercise, the muscles use energy obtained from fatty acids or glycogen. One of the key enzymes of glycogenolysis is myophosphorylase.
The mutations in the human muscle form of glycogen phosphorylase (*PYGM*) lead to an inherited metabolic disorder called McArdle's disease. Affected people suffer from exercise intolerance with premature fatigue, muscle stiffness and cramps and myalgia. One of the symptoms observed usually in adults is an extensive glycogen accumulation in muscles leading to muscle fibers disruption (Migocka-Patrzalek et al. 2015).
Zebrafish (*Danio rerio*) is a good animal model to investigate human myopathies (Plantie et al. 2015). Our goal is to determine if this animal could be also used as a useful model of McArdle's disease. Therefore, we examined the effect of muscle glycogen phosphorylase (*pygm*) knockdown on glycogen distribution in the larval zebrafish muscles.
We used the morpholino technique to knockdown the zebrafish orthologs of human *PYGM*. The morpholino oligonucleotides were injected into one-cell stage embryos. Glycogen presence in morphant's muscles was analyzed by the use PAS staining with an additional step in which tissues were incubated with dimedone. This modification allows us to observe exclusively glycogen distribution (without other carbohydrates). Additionally, we stained analyzed tissues with hematoxylin and eosin to show the cells shape and structure.
Obtained data showed that in zebrafish glycogen was present both in slow and fast muscles. In zebrafish with *pygm* knockdown the glycogen distribution remained unchanged, but the structure of muscles was altered. The lack of changes in glycogen distribution may be due to the young age of analyzed zebrafish and may change during further development. It is possible, that the glycogen accumulation in the muscles will be visible in the adult animals, as it is observed in humans.

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Effect of phytosulfokine and putrescine on regeneration capacity in protoplast cultures of coriander (*Coriandrum sativum* L.) and cumin (*Cuminum cyminum* L.)

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Coriander (*Coriandrum sativum* L.) and cumin (*Cuminum cyminum* L.) are members of Apiaceae family, one of the most important families in agricultural production. Protoplasts of these species are considered to be used in complementary fusion with carrot protoplasts as an alternative source of cytoplasmic male sterility.
In the present research, protoplasts of botanical form of *C. sativum* (PlantiCo, Poland) and two accessions of *C. cyminum* (botanical form - Heirloom, Lake Valey Seed, USA and CUMI 27 - Gatersleben Gen Bank, Germany), isolated from 3-week-old shoot cultures, were immobilized in alginate layers and cultured for 8 weeks in CPP medium (Grzebelus et al., 2012). The effect of phytosulfokine (100 nM), putrescine (8 mg/l) or both of them on protoplast regeneration capacity was examined. Then protoplast-derived callus/proembryogenic mass was used in regeneration process and cultured on hormone-free solid medium (Grzebelus et al., 2012). To determine regeneration ability of protoplasts (1) plating efficiency, (2) callus/proembryogenic mass formation and (3) somatic embryo development were assessed.
Supplementation of CPP medium with phytosulfokine or phytosulfokine and putrescine increased mitotic activity of coriander and cumin cells. Moreover, in these conditions intensity of callus formation was significantly higher in cumin protoplast cultures. However, among all examined accessions only few embryos from protoplast-derived tissue of *C. cyminum* were developed.

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Cyto-embryological approach to the problem of low seed set in *Medicago sativa* L.

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Low seed production is one of the crucial limiting factors in alfalfa cultivation in the climatic conditions of Poland. Direct selection towards increased yield of seeds is not effective due to polygenic and quantitative character of traits affecting yield and their dependence on environmental conditions such as temperature, insolation and precipitation. As a consequence, the slow selection response appears in breeding programmes. One of the approaches to overcome this problem is including of inflorescence mutants to breeding of *M. sativa* L. We used three spontaneous inflorescence mutants: *lp* (long peduncle), *br* (branched raceme) and *tf* (top flowering), and the Radius cultivar as a reference. Only *lp* form had on average 22% higher yield of seeds than the reference cultivar. Seed yield per *lp* plant was 18,7-27,0 g depending on weather conditions. The average seed yield per plant in *tf* and *br* mutants was 12 g and 16 g, respectively. When compared to cv. Radius, increased flower numbers per inflorescence appeared in all mutations but only *lp* plants improved the number of seeds per inflorescence and strongly increased the seed yield per plant. Therefore the final effect of inflorescence morphology on seed production was lower than expected. To explain that, we analysed embryo sac formation, callose deposition in the ovules, pollen tube growth, and early embryogenesis. All *lp*, *br*, and *tf* mutants showed higher frequency of ovule degeneration when compared to the reference cultivar. The ovaries contained 8 to 11 ovules but 9-45% (24% on average) of the embryo sacs showed various signs of degeneration before anthesis. We observed narrow structures without proper cells or short embryo sacs with the small egg apparatus. Moreover, callose deposits of various sizes were found at early stages of ovule development, and large deposits appeared in the older ovules. No differences in pollen tube growth were found in the inflorescence mutants after pollination. Callose deposition in the ovules increased shortly after pollination, and 4 days after pollination the callose widely appeared in the nucellus of many ovules. Fertilized ovules were 3-4 times less numerous, and embryogenesis was delayed in the *lp*, *br*, and *tf* mutants when compared to cv. Radius. However, most of the ovules were malformed both in the cv. Radius (62%) and in the inflorescence mutants (69-86%). Finally only 18-30% of ovules developed into young seeds 16 days after pollination.
In conclusion, the effect of higher flower number per inflorescence was reduced in the mutants by higher frequencies of ovule degeneration. Poor seed setting in the genotypes studied here was the result of (1) disturbed embryo sac development, (2) callose deposition in the ovules before and after pollination, and (3) poor fertilization effectiveness and young seed abortion.

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Differences in epigenetic modifications in the egg cell and central cell of *Hyacinthus orientalis* L. mature embryo sac

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During the sexual reproduction of flowering plants, epigenetic control of gene expression and genome integrity by DNA methylation and histone modifications plays an important role in female gametophyte gametogenesis. Epigenetic modifications regulate the structure and activity of the chromatin. Our previous reports of nuclear metabolism and changes in the chromatin organization and total transcriptional activity in *Hyacinthus orientalis* mature embryo sac cells indicate that in the egg cell and central cell, whose activity is silenced, the chromatin is largely dispersed compared to the somatic cell (Niedojadło et al., 2011a, b). Therefore, in this study we have focused on the distribution pattern of different epigenetic marks in the target cells for the male gamete. Using immunofluorescence techniques, we localized heterochromatin (5-methylcytosine, H3K9me^2, H3K27me^2) and euchromatin (acetylation of lysine residues, H3K4me^3) markers.
Our data indicate that, in the transcriptional silenced egg cell and central cell, the different levels (but lower that observed in the somatic cells) of both eu- and heterochromatin marks were present. Interestingly, we observed unique localization of the analyzed chromatin modifications in the nucleolus, indicating the precise mechanisms of the regulation of rDNA expression. We conclude that a balance between DNA methylation and composition of various histone modifications determines and regulates the structure and activity of chromatin regions in hyacinth female gametophyte cells. We also, propose that the differences in the specific epigenetic state of the egg cell and central cell is related to the different acquired fates and biological functions of these cells.

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Androgenic haploid as a source of apomictic diploids in *Capsicum annuum* L.

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The doubled haploid technology gives an opportunity for the rapid genetic stabilization of *Capsicum annuum* L. postmeiotic recombinants. *In vitro* induced androgenesis is the most important tool for the haploids production. On the other hand a number of diploid plants are observed among the regenerates (Nowaczyk *et al*. 2016). Spontaneous diploids are the best material in breeding because the difficulties and unexpected mutations inherent in treatment with colchicine would be circumvented. In the research the analysis of diploid progeny of anther-derived haploid has been presented. The fruits of haploid plant comprised eight diploid seed. The plants from these seed were the subject of phenotypic and molecular analysis in the investigation. They were different in relation to fruit phenotype and molecular characteristics. RAPD analysis let to divide the considered population in two groups. One of them has been constituted by three plants differed from the rest population with regard to the products of RAPD reactions for OPAE10, OPAE11 and OPB10 primers. The relation between the two fruit traits (mean weight and length) and the effects of activity of primers mentioned above let to suggest that they could be used as the molecular markers. The most important conclusion of the results obtained is as follows: haploid plants may be the source of diploid, apomictic seed. Additionally, the morphological and molecular differences among these diploids can increase the genetic variability of population. In the investigations the low effectiveness of examined genotype for production of androgenic plants has been observed. This inconvenience has been reduced by interesting, apomictic offspring of one haploid plant. Concluding, the anther-derived haploid was able for the diploid seed production. The plants grown up from these seed were different with regard to phenotype as well as to some sites of their genome.

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Effectiveness of embryogenesis in the progenies of diploid plants derived from *in vitro* anther culture of *Capsicum annuum* L.

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Among the factors that affect the effectiveness of the induced androgenesis of anthers- or microspores-*in vitro* culture, the genotype of the donor plant plays a particular role. In *Capsicum* genus, the differences are observed for species, cultivars and line. Sweet-fruited cultivars show great differentiation in respect to the androgenic response. Great differences between *C. annum* L. F_2 recombinants were also observed The genotype has a significant effect on the induction of androgenesis and on the subsequent development of embryoids to plantlets, but it also affects on the frequency of spontaneous diploidization (Nowaczyk et al., 2009, 2015). The hybrids between breeding line ATZ and cultivars `Portos` and `Corno di toro` were the initial plant material. The plants of F_3, F_4 and F_5 progeny of three F_2 diploids derived from *in vitro* anther culture have been used as the research material. The anther culture-derived diploids mentioned above (marked A, B and C), distinguished themselves as ones with relatively good androgenic response and originality of fruit characters. The F_3 progeny of anther culture-derived diploid marked C was phenotypically uniform. For the confirmation of molecular similarity of the progeny mentioned above the RAPD analysis for ten plants has been done. Ten primers used (A06, A10, A11, A14, A17, A19, OPB19, OPAE10, OPAE11, OPAE190) have generated 78 monomorphic and 5 polymorphic products. In each of the populations, the plants with non-androgenic response were observed. On the other hand, some of the individuals produced a large number of embryos. The highest-responded plants were noted in the populations of "A" progeny in each year of the investigation. Among regenerates the haploid and diploid plantlets have been observed. Additionally two mixploid forms (haploid/diploid) were found. The percentage of diploids in the plantlets populations ranged between 14% and 67%. Particularly high androgenic response of one donor plant in F_5 progeny of one anther-derived diploid pointed out the chance for increasing the effectiveness of androgenesis by selection.

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The effect of nanodiamonds on cellular immune response and development of the *Tenebrio molitor* beetle

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Recent years have witnessed rapid development of research and applications in the area of nanosciences and nanotechnology. Currently, nanotechnology applications focus on the use of nanoparticles as carriers of biologically active molecules such as nucleic acids and peptides to cells for therapeutic purposes (Purtov et al., 2010). Nanodiamonds as ultra-fine particles with dimensions between 1-100 nanometers and excellent mechanical and optical properties, as well as with large surface area are an attractive material for adsorbtion of various biomolecules. Moreover, the current reports reveal that scientists are focused as well on the application of nanodiamonds in nanocomposites, tissue scaffolds and surgical implants (Brady et al., 2015). Current bibliographic data is not clear and often contradictory, when it comes to the toxicity and safety use of these nanoparticles (Zhang et al., 2010). For this reason, the effects of nanodiamons on organisms before their application should be carefully investigated not only in cell cultures *in vitro*, but mainly *in vivo*. In order to evaluate *in vivo* effects of nanodiamonds on cellular immune response and development, we used a model organism – the *Tenebrio molitor* beetle. Larvae and adult mealworms were treated of nanodiamonds solution in nanomolar dose by topical application. Our studies show that nanodiamonds do not affect the processes of cellular immune response. Moreover nanoparticles of diamond do not interfere with the proper development and metamorphosis of insects.

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Post-hatching development of rudimentary-receptor pinealocytes in the domestic turkey

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The avian pineal organ comprised three types of non-stromal cells: pinealocytes, supporting cells and nerve cells. Receptor cells, rudimentary-receptor cells and secretory cells are distinguished among pinealocytes. The rudimentary receptor cells are the most abundant and change during post-hatching development. However, the knowledge dealing process of these cells transformation is scare and based mainly on study of the chicken pineal organ.

In the present study, we investigated the changes in ultrastructure of the rudimentary-receptor pinealocytes in the domestic turkey during post-hatching period. The study was performed on birds at the age of 2 days and 1, 2, 4, 10, 20, 30, 40 weeks, kept under a cycle of 12 hours light : 12 hours dark. Pineals were collected immediately after euthanasia and prepared according to standard procedures to study using transmission electron microscopy.

The significant changes in the structure of rudimentary-receptor pinealocytes closely related to age were observed. Two main periods of modifications of their morphology were distinguished. The first one lasted from the 2nd day to the 20th week of life and included the prolongation of cells and the formation of stratified organization of cytoplasm. In turkeys at the age of 4 and 10 weeks, the rudimentary-receptor pinealocytes consisted of a strongly elongated cell body with nucleus located in its basal part and bulbous apical protrusion. The supra-nuclear region of the cell showed presence of three clearly delimited zones comprising rough endoplasmic reticulum, Golgi complex and mitochondria.

In 20-week-old turkeys, pinealocytes were very long, columnar cells with prominent apical protrusion projecting into follicular lumen and basal processes reaching to the basement membrane. They contained numerous, long microtubules.

The second period of modifications lasted from 20 to 40 weeks of life and comprised the regressive changes of pinealocytes. The pinealocytes became much shorter and the number of organella was reduced. Pinealocytes possessed a very short, diminish apical protrusion, which was frequently filled up by the cellular debris with variable electron density.

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The developmental abnormalities of embryos resulted after crossing of *Solanum lycopersicum* L. with *Solanum sisymbriifolium* Lam.

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The *S. lycopersicum* L. species, edible vegetable crop, has a significant economic value. For the consumption purposes, many cultivated varieties were developed from the tomato. One of the most important difficulties of *S. lycopersicum* breeding is a low resistance to the pathogens which can cause significant loses every year. *S. sisymbriifolium* Lam. is recognized as a species, resistant to numerous pathogens like nematodes, fungi or bacteria. Due to this, *S. sisymbriifolium* could be an important source of the resistance genes for the tomato cultivars. In our studies we conduct the *in vivo* cross-pollination, to check the possibilities of obtaining the hybrid progeny.

Male-sterile plants of *S. lycopersicum* were pollinated *in vivo* with pollen grains collected from the *S. sisymbriifolium* MM 568 ones (sourced from INRA UR1052). Both species were delivered by the Rijk Zwaan R&D Company. Embryological analysis were performed on ovules isolated from the enlarged fruits. The development of embryos resulted after crossing were compared with the control ones, obtained after selfing.

The embryological analysis revealed the serious developmental abnormalities of embryos obtained after crossing *S. lycopersicum* x *S. sisymbriifolium*:

1. Growth inhibition of embryos at several-celled and globular stage despite the high mitotic activity,
2. The endosperm structure comprised of flattened cells with dense cytoplasm,
3. Significant enlargement of the globular embryos instead of the proper development to the heart stage,
4. Degeneration of oversized, globular embryos usually 4-5 weeks following pollination.

These abnormalities can be related with the post-zygotic incompatibility. More details concerning the structure of hybrid embryos in comparison with the control ones, will be demonstrated on the poster.

3D-reconstruction of the mesonephric and paramesonephric ducts during the prenatal development of female domestic cat (*Felis silvestris catus*)

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During the morphogenesis of the genital tract in female develop a pair of male-specific mesonephric dusts (MD) and a pair of female-specific paramesonephric ducts (PD). The mesonephric ducts are necessary for the proper development of paramesonephric ducts, because they stimulate their growth. After that the MD gradually regress, while the tubal and uterine segments of PD differentiate into uterine tubes and uterus.

The study aimed to describe the developmental changes of the mesonephric and paramesonephric ducts in female fetuses of domestic cat, aged 23rd – 63rd day p.c. The Amira FEI software was used to prepare the three-dimensional reconstructions of the genital tract and thereby to reveal the arrangement of the mesonephric and paramesonephric ducts in a common sheath of mesenchyme. The results supplemented with the observations of histological slides and morphometric analysis.

The mesonephric ducts in domestic cat develop c.a. 23rd day p.c, while the paramesonephric ducts appear in the cranial segments of mesonephroi about 2 days later. The MD are lined with a pseudostratified epithelium and are characterized by a wide lumen and the PD have a simple cuboidal epithelium and narrow lumen. Based on the 3D reconstructions of developing genital tract estimated that until 33rd day p.c. the mesonephric ducts reach up to 3/4 length of ovaries and end caudally singly in the urogenital sinus. The paramesonephric ducts cranially extend above the ovaries and open to the abdominal cavity, and caudally they fuse to form a single utero-vaginal canal. The morphometric analysis revealed that until 34th day p.c. the diameter of both of the dusts increase.

On 43rd day p.c. the MD reach up to 1/2 length of ovaries and occur in the mesenchyme along the entire length of the uterine segments of PD and also in utero-vaginal canal. After that the PD continue to grow and differentiate into uterine tubes and uterus, while the MD regress. Until 46th day p.c. the diameter of MD is almost 4-time smaller than the diameter of PD. From 50th day p.c. to the moment of birth the MD occur only as a residual ducts in a lower part of body of uterus.

Release of N-acetylserotonin and melatonin from the embryonic pineal organs of the domestic turkey in the superfusion culture

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Our previous *in vivo* study showed that the synthesis of melatonin (MLT) begins in the turkey pineal organ during the last trimester of the embryonic life, much later than in the chicken. Moreover, the level of MLT is very low up to 24th day of incubation of turkey eggs and rises markedly just before and after hatching. In contrast, the direct precursor of MLT, N-acetylserotonin (NAS) is synthetized in the turkey pineal organ in considerable quantities from the middle of the second trimester of the embryonic period. In the present study we investigated the release of NAS and MLT from the embryonic turkey pineal organs incubated in the superfusion culture under different light conditions. The aims of study were to compare the release of both compounds at three stages of development and to determine the presence of diurnal and circadian changes in the release of NAS and MLT at these stages.

The study was performed on 22-, 24- and 26-day-old turkey embryos (22ED - 26ED). The embryos were killed at 12.00, the pineal organs were immediately removed and incubated in the superfusion culture under 12L:12D, 12D:12L, 0L:24D and 24L:0D light conditions. The indole contents in medium samples were measured by HPLC.

The release of NAS was significantly higher during scotophase than photophase at all examined developmental stages under 12L:12D and 12D:12L cycles. Moreover, circadian variations in release of NAS were found under continuous darkness and continuous illumination. The amplitude of diurnal rhythm of NAS release increased with the age of embryos. The level of MLT was below the limit of quantification or very low in the medium samples from the cultures of the pineal organs taken at 22ED and 24ED. In contrast, the secretion of MLT was high (considerably higher than the release of NAS) in the cultures of the pineal organs taken at 26ED and showed a diurnal rhythm.

The present data confirm the results of our previous *in vivo* studies, which showed that the synthesis MLT in the turkey pineal organ is very low up 24ED and the markedly raises. Moreover, they demonstrate that the embryonic turkey pineal organ, at least from 22ED, is photosensitive and possess a functioning circadian oscillator.

The oocytes size of *Carassius gibelio* (Pisces, Cyprinidae) diploid and triploid females

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The Prussian carp *C. gibelio* (Bloch, 1782) is one of the most widespread freshwater invasive species due to high plasticity of adaptations to changing environment and reproductive plasticity, including rapid process via gynogenesis using the sperm of other species for induction of eggs development. This species created the unisexual populations, consisting almost entirely of gynogenetic triploid (3n) females and recently also mixed bisexual-gynogenetic populations, where diploid (2n) females and males coexist with 3n females, and rarely 3n males.

The aim of this study was to compare size of oocytes at various stages of development in 2n and 3n individuals. Females were collected before the spawning (April) from the Siemianowka Dam Reservoir. Ploidy of individuals was verified by flow cytometry analysis. In order to determine the gonadosomatic index (GSI) the ovaries were weighed with 0.01g accuracy and GSI calculated as gonad weight/body weight x 100% for both group. Fragment of the ovaries were fixed in Bouin's solution, dehydrated and embedded in paraffin. Histological sections (7 µm) were stained with hematoxylin and eosin. The stages of oocytes development were adopted after Juchno and Boroń (2018). The diameters of oocytes were measured (whose cross-section contained a nucleus) with 0.01µm accuracy using the Multiscan software.

The weight of ovaries among collected females fluctuated from 62.7 to 187g. The GSI value ranged from 11% to 20% for 2n females and its mean value (16.75%) was significantly higher ($p<0,05$) than those of 3n (10.6%), ranged from 8 to 17. Typically as for multi-spawning species ovaries contained oocytes in all the developmental stages: previtellogenic (B), the cortical alveolus (D) and exogenous vitellogenesis (E). Statistically significant differences ($p<0.05$) between the size of oocytes at various stages of development in 2n and 3n females were found. The size of particular oocyte stages increased with development from stage B to E.

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Calcium-binding proteins immunoreactivity in *Cavia porcellus* (Rodentia) hippocampus 30 days after postconception

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The dentate gyrus (DG) and hippocampus proper (HP) is a complex network critical for learning and memory guided behaviours. In these processes distinct role plays calcium-binding proteins (CaBPs), such calbindin CB-D28k, calretinin and parvalbumin, which are approved anatomical and developmental markers both in rat and human (Enderlin et al., 1987; Abraham et al., 2009). Studies concerning presence of CaBPs in guinea pig are lacking, thus, the aim of the study was to fulfil this gap. Frozen sections were stained by immunohistochemical methods using antibodies against calbindin D28k, calretinin, parvalbumin and neuronal (NeuN) proteins.

The main findings are following. Neurogenesis of the HP is more advanced than DG in which only ectal blade was discernible. Strong NeuN immunoreactivity was observed in HP at the deepest cells (PLd) of pyramidal layer arguing that these cells are first generated. In PLd and PLs (superficial) cells of HP both calbindin and calretinin positive cells were observed. Between these two immunoreactive layers, arising apical dendrites of PLd cells were present. In general, calbindin immunoreaction intensity was greater in HP while CR in the DG. Immunoreaction of parvalbumin was very weak. Taking into account that (i) place cells reside in PLd, (ii) CA3 pyramidal cells carried more spatial information per spike than those of CA1, (iii) afferents to CA2/CA1 region provide spatial information (Masurkar et al., 2017), it can be concluded that calbindin is involved in the Ca2+-dependent cell processing information related to spatial memory formation, prior to eye opening.

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Reproduction in *Capsella rubella*, a close relative of *Arabidopsis thaliana*

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C. rubella is close related to *A. thaliana*, arguably the most important reference plant, and *Boechera* where diploid apomixis is found. A comparative analysis of closely related species allows find and explain changes in the reproductive system (from outcrossing to self-crossing, from sexual to apomictic reproduction) in the background of hybridization and polyploidization (Slotte et al., 2013; Douglas et al., 2015).
Here we presented primarily the cytoembryological study of diploid *C. rubella* which in contrast to its tetraploid sister species - *C. bursa-pastoris* (Shulz and Jensen, 1985), a globally successful invasive weed, has exclusively a central European distribution. We revealed that *C. rubella* reproductive development were largely congruent with those identified for *C. bursa-pastoris* and genotype independent. The differences between plants growing at low (4ºC) or room temperature conditions occurred in the time of each stage achievement and starch amount. The developmental delay and higher starch accumulation in female gametophyte were attributed to the cold.
Megasporogenesis proceeded according to the monosporic type and finished in the formation of tetrads or triads, due to disturbances in the cell wall formation between the micropylar megaspores. At the end of megasporogenesis, only the chalazal megaspore remained enlarged and destined for further development. The female gametophyte developed as the Polygonum type. The formation of microspores and pollen grains proceeded without disturbances and typically for meiotic pathway. After self-fertilization, divisions in the central cell occurred earlier than in the zygote. The Onagrad-type embryo possessed the enlarged basal cell of the suspensor which is characteristic for the *Capsella*. At heart-shape embryo, the endosperm consisted of three regions: cellularised micropylar around the embryo, free-nuclear around the central vacuole and the chalazal cyst; the cytoplasm was rich in thylacoid-filled plastids. The degradation of the endosperm occurred in the further stages of seed development.
Our results of *Capsella* reproduction provides valuable complement to the genomic data of selfing.

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Embryonic development of the postcranial skeleton in a parthenogenetic, pad-bearing gecko, *Lepidodactylus lugubris* (Squamata: Gekkota: Gekkonidae)

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Gekkotan lizards exhibit great morphological variation, particularly in their limbs. This clade contains limbless pygopodids, terrestrial taxa with claws, as well as numerous species with adhesive toepads, which enable them to climb flat surfaces. Adhesive toepads evolved independently multiple times in gekkotans but developmental changes associated with these transformations remain poorly understood. We studied development of the postcranial skeleton in a pad-bearing lizard – the mourning gecko (*Lepidodactylus lugubris*). Embryos were staged according to a developmental table for a related gekkonid – *Paroedura picta* (Noro et al., 2009) – and stained for the presence of cartilage with alcian blue and for the presence of bone with alizarin red S, following Dingerkus and Uhler (1977). The skeleton of a stage 28 embryo (about the middle of the embryonic development) was almost entirely cartilaginous, though shafts of the long bones seemed to start ossifying. First calcifications occurred in a stage 30 embryo, in which stylopodial bones (humerus and femur) were ossified. In the pectoral girdle, clavicles were present but no ossifications were yet visible in the pelvic girdle. A stage 35 embryo had well developed stylopodial and zeugopodial (radius, ulna, tibia and fibula) bones, as well as vertebrae. We observed an anteroposterior direction of ossification in the axial skeleton, as in most other squamates. In a stage 50 embryo (several days before hatching) vertebral dorsal fissures were still present but first ossifications in the pelvic girdle and ribs appeared. However, more distal limb bones seemed to be still unossified.

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Potential implications of a high morphological variation within clutches of the common slow worm (*Anguis fragilis*)

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Normal developmental tables play an enormous role in numerous aspects of embryology – from staging embryos of a single species to different comparative approaches, evolutionary reconstructions and so on. Thus, developmental tables should be as universal (within a given species) as possible and include characters that show little variation. However, many characters frequently used in defining developmental stages are known to exhibit such variation. We constructed a partial developmental table for a limbless, viviparous lizard, the common slow worm (*Anguis fragilis*). We observed significant differences within clutches in several characters, such as degree of mandible development (reaching roughly the level of the anterior margin of the eye in some individuals, but contacting the tip of the maxilla in others), visibility of external nares or degree of scale development. These differences would probably lead to classifying some of those embryos as belonging to different stages than their siblings (which are, presumably, the same age). Potential implications of these observations are discussed.

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A novel approach for studying 3D embryo development of crustaceans (freshwater shrimp *Neocaridina heteropoda*) using the X-ray Microtomography

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Embryogenesis is an important stage during animal development, because the embryo's body undergoes significant transformation which can prepare the entire organism for proper functioning during adult stages. Despite the fact, that studies on embryogenesis and postembryonic development are very interesting, they are also very difficult to conduct in practical terms due to relatively small specimen sizes and high sensitivity of the material. Therefore, a carefully chosen set of non-destructive microscopy techniques has to be implemented in such studies in order to maximize the imaging results without the necessity of using complex sample preparation procedures. Up to now, mainly the light and fluorescence microscopes have been used in order to present the anatomy of embryos, while scanning electron microscopy (SEM) has been used for preparing images of embryos morphology. However, these methods are very time-consuming and invasive – the embryos must be cut and completely destroyed. One such non-destructive technique is the microtomography technique which has only recently begun to be used for studying this kind of material. The project, as the basic one, is connected with the alterations which appear during the embryo development in freshwater shrimp *N. heteropoda* (Crustacea, Malacostraca). It is one of the most-bred freshwater species and the species which is nowadays commonly analyzed. Obtained information gave us much more precise understanding of body plan development than standard techniques like e.g. light microscope. Moreover, the collected data allowed for preparation the detailed 3D reconstructions which can be subjected to interactive manipulation. Up to now, studies of the crustaceans embryo development using X-ray Microtomography have not been conducted.

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Comparison of two visualization methods of embryos of freshwater shrimp *Neocaridina heteropda* (Crustacea, Malacostraca)

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X-ray Microtomography (XMT) is a non-destructive, computed-aid visualization technique widely used in many science areas. This method employs electron X-ray conical beam to visualize structure of analyzed objects. The quality of obtained image depends on many variables. Important are scanner characteristics, like e.g. spot size, maximum sample dimensions. The features of the material/sample are also very important. Two main issues are critical is this case, size and structure of an object. Homogenous objects, like soft tissues, are difficult to analyze because there are no significant differences in attenuation inside them. Staining is necessary but can also disturb the sample preparation and could case artefacts formation on image. Depending on image properties, the particular developmental stages of freshwater shrimp were analyzed using different software. Two applications were used, Mimics 15.0 and Drishti 2.4. First software is developed by Materialise, especially for medical image processing. Mimics relies on differences between particular voxels grey level value. Histogram of values corresponds to the difference in density of the analyzed object. No manipulation of histogram are possible. Drishti is free-license and open-source software. It allows to process large data sets by allowing visualization of smaller sub-volumes. Manipulation of histogram are possible during image processing. Because of yolk content (the yolk covers the major part of embryos body), embryos were processed in Mimics 15.0 and Drishti 2.4 program, in manual way. For each part of body which was segmented (head, abdomen, pleopods, eyes, intestine), a separate mask was created. Due to the fact that automatic analysis was impossible, on each slice, area of particular organ was marked. Finally, for each mask 3D model was generated to visualize the entire embryo organ structure.

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Gonadal sex differentiation in Eurasian perch, *Perca fluviatilis* L.

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The aim of this study was to evaluate time and mode of gonadal differentiation in Eurasian perch (*Perca fluviatilis*). For the study Eurasian perch larvae, obtained following controlled reproduction of wild spawners (three females and six males) obtained from Lake Mamry, with an average initial body weight of 0.6 (± 0.1) mg and an average total length of 6.2 (± 0.3) mm were used. Larvae and subsequently juveniles were reared for three months in recirculating aquaculture system at 20 (± 0.5) °C. Fish were fed *ad libitum* until apparent satiation throughout the experiment. Initially larvae were fed with live nauplii of *Artemia* sp. (AF origin with size of about 430 μm, INVE, Belgium), followed by commercial feed (Aller Aqua, Poland), i.e. Aller ArtEX 2 and Aller Futura. Samples for histological examinations (standard procedure: fixation in Bouin's solution followed by staining using hematoxylin-eosin method) were taken every 5 days. The first primary germ cells (PGC) were observed in the gonads of fish on 31 day post hatching (DPH) and first signs of morphological differentiation of gonads on 35 DPH, where two different types of gonads were observed on histological cross-sections. They were two gonadal ridge growing into one, pear-shaped, suspended with a single string to the peritoneum, which is characteristic of the pouchy type of sack (typical for female gonads of Eurasian perch characterized by only one ovary), or two gonadal ridges clearly separated from one another (male gonads). On 51 DPH, ovaries with single oogonium formed between the walls of the glands and intracranial space and testes with single spermatogonia were noted. Cytological differentiation in the perch occurred faster in the female direction (about 66 DPH), when the fish reached an average body weight of 953 (± 242) mg and on the histological sections of the ovaries single previtellogenic oocytes were visible. Obtained results indicate that Eurasian perch is characterized by differentiated gonochorism, which is important from the point of view of implemented breeding programs, as well as projects aimed at effective production of monosex populations of this species, currently being the object of interest of the aquaculture sector.

Does evolution at different thermal regimes affect cell size and body size? A case study on *Drosophila melanogaster* flies

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Typically, ectotherms decrease their adult size during evolution or development in warmer environments (Atkinson, 1994). Mechanistically, a change in body size can be driven by changes in cell size, cell number or both. There are a number of candidates for proximate mechanisms underlying changes in cell size and body size during development such as biochemical pathways that control cell division, growth and differentiation. The evolutionary significance of the thermal sensitivity of body size and cell size remains unclear, but the theory of optimal cell size (TOCS; e.g. Czarnoleski et al., 2013) predicts that smaller and more costly to maintain cells are adaptive in warm and thermally fluctuating environments to facilitate transport of oxygen and other resources to cells, whereas larger and less expensive cells are adaptive in cold. Addressing this perspective, we report differences in adult size and cell size in *Drosophila melanogaster* flies that underwent experimental evolution in three selective environments: warm and constant (25°C), cold and constant (16°C) and thermally fluctuating between generations (16/25°C). We focused on males and measured thorax length (a proxy of body size) and cell size in Malpighian tubules (measurements on contrast-phase microscopy photos). All flies used for the measurements were developed under common conditions (20.5°C).

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The effect of PTEN inhibitors on mRNA expression of selected factors involved in ovarian follicle maturation in red deer (*Cervus elaphus*)

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In mammals, ovarian follicular growth, ovulation and luteinization are tightly regulated by FSH. FSH promotes rapid activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway, resulting in the phosphorylation of the downstream kinase Akt (acute transforming retrovirus thymoma protein kinase) (Gonzalez-Robayna et al., 2000; Alam H. et al., 2004). The PTEN gene (phosphatase and tensin homolog) encodes a phosphatase enzyme that negatively regulates the PI3K-Akt signaling pathway. Deletion of PTEN in the oocyte increases protein kinase B (Akt) phosphorylation and nuclear export of downstream Foxo3 (Forkhead winged helix box 03) proteins (John et al., 2008). Indeed, Foxo3 gene deletion also activates all dormant primordial follicles in mice (Castrillon et al., 2003). Activated PI3K converts phosphatidylinositol (4, 5)-bisphosphate (PIP2) to phosphatidylinositol (3-5)-trisphosphate (PIP3), whereas the PTEN inhibitor prevents the conversion of PIP3 back to PIP2. Accumulated PIP3 stimulates the phosphorylation of Akt and increases the nuclear exclusion of the transcriptional factor Foxo3. Modifying PTEN-PI3K-Akt-Foxo3 pathway can activate dormant follicles in mice (Li et al., 2010).

The aim of the study was to examine the influence of PTEN inhibition and PI3K stimulation on PTEN, PIK3CA, Akt1, Foxo3 mRNA expression in red deer ovaries.

Material was collected *post mortem* during hunting season. Red deer ovary explants were incubated (24h) with PMSG (FSH analog), and PTEN inhibitors: bpV(pic) or bpV(hopic). The PMSG, bpv(pic) and bpV(hopic) treatment downregulated Akt1 mRNA expression. PMSG and bpV(pic) treatment upregulated Foxo3 mRNA expression. PTEN and PIK3CA mRNA expression was not affected by treatment.

Our study concerning follicle maturation process will be continued towards interaction of PTEN inhibitors and expression of functional proteins.

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Analysis of the causes of reduction of *Iris aphylla* L. populations based on embryological studies

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Iris aphylla (leafless iris) is under strict species protection in Poland. Previous literature data indicate that successive transformation of the communities of the species is the major determinant of the reduction of its populations (Wróblewska et al, 2003; Wróblewska and Brzosko, 2006).

This study presents the development of the male and female gametophyte in the leafless iris. The embryo sac develops in a campylotropous, thin-nucellus ovule developing in the Polygonum mode. The male gametophyte consists of large heteropolar pollen grains with a wide furrow and a smooth surface. The transmission electron microscope analyses demonstrated a normal cytological structure of the pollen grains with numerous starch granules and fat droplets. In the iris, fertilisation takes place and numerous seeds are formed. A single capsule contains approximately 40 healthy albuminous seeds. A tetrazolium assay applied to the seeds has shown their viability at a level of 72%. Despite the well-developed embryo, the seeds did not germinate even after application of stratification, scarification, and pre-soaking treatments. Normally developed embryos dissected from the seeds were inoculated onto an agar solid medium without addition of growth regulators; all of them were found to germinate. Hence, it can be concluded that the barriers preventing iris seed germination are located in the endosperm. Therefore, biochemical analysis of this tissue will be the next stage of the research.

During the investigations, the presence of the iris weevil *Mononychus pumctumalbum* was observed on the plants. The pest caused damage mainly to flowers and seed capsules (Skuhrovec et al., 2017).

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Ovary organization in *Insulodrilus bifidus* (Clitellata, Phreodrilidae)

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Recent studies have shown that among clitellate annelids (annelids with a saddle) a meroistic mode of oogenesis occurs with only one known exception (Urbisz et al., 2017). It means that during early oogenesis syncytial germ-line cysts are formed and within cysts two cell categories emerge: oocytes which continue meiosis and gather reserve material and the so-called nurse cells which do not gather yolk, instead they support oocytes with macromolecules (e.g. RNPs) and cell organelles. In clitellates within syncytial cysts female germ cells are not connected directly one to another, instead of this each cell joins via one intercellular bridge to a common, central cytoplasmic mass, the cytophore. Despite meroism the ovary architecture (shape and content) differs substantially between clitellate taxa. One of the reasons for this diversity is high plasticity in female cyst structure. For example, in the white worm *Enchytraeus albidus* cysts are composed of 16 germ cells and the cytophore is spherical, whereas in the sludge worm *Tubifex tubifex* more than 2 000 cells are interconnected and the cytophore is elongated and branched (Urbisz et al., 2017). Numerous studies have suggested that the ovary structure in Clitellata is conserved at the family/subfamily level.

Phreodrilidae comprises about 50 described species of minute aquatic clitellates mainly inhabiting freshwaters in the Southern Hemisphere. In phreodrilids, gential organs are usually located in segments XI-XII; testes in XI and ovaries in segment XII. Classical descriptions of clitellate annelid anatomy (e.g. Michaelsen, 1928) suggested that in pherodrilids separate groups of female germ cells develop within the body cavity. To shed more light on ovary architecture and oogenesis we sectioned (semi- and ultra-thin sections) four specimens of *Insulodrilus bifidus* – a freshwater species endemic to south-western Australia. We found tiny paired ovaries connected to the intersegmental septum. Each ovary is composed of several separate germ-line cysts enveloped by elongated somatic cells. Cysts are arranged in more or less linear order, and house germ-cells (cystocytes) in the early stages of oogenesis (pre meiotic and meiosis prophase I) only. On the other hand, within the coelom we found previtelogenic and vitelogenic oocytes with its own group of nurse cells. Analysis of serial semi-thin sections revealed that such cysts unite 32 cells – oocyte and 31 nurse cells. It seems that in *I. bifidus* female cysts detach early, before vitelogenesis, from ovaries and continue oogenesis freely floating in the coelom.

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Early embryological processes in the ovules of *Erigeron annuus* (Asteraceae)

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Erigeron annuus (2n=3x=27), a native of North America, is a very common and dangerous invasive species in Poland (Zając and Zając, 2015). *E. annuus* is known to be an apomict with diplospory of the *Antennaria* type (Gustafsson, 1946), however, Polish representatives of this species have not been the subject of an intensive embryological analysis.

An important, but not fully understood, aspect of angiosperms reproduction is the issue of callose deposition during early female reproductive processes in sexual and apomictic taxa. Until now it was thought that callose may be a useful marker for an early identification of the reproduction mode because, unlike in the majority of sexual taxa, accessible data indicated lack of callose in the wall of the cell entering apospory and diplospory. However, callose deposition has been recently documented during meiotic diplospory in species from the Asteraceae family (Musiał and Kościńska-Pająk, 2017). In light of this, we have analyzed *E. annuus* to verify whether callose deposition is also related to mitotic diplospory.

Florets were cleared in methyl salicylate and visualized with DIC optics and aniline blue staining was performed to detect callose.

In the analysed ovule, a single hypodermal archesporial cell functioned directly as a diplosporous megaspore mother cell (DMMC). The DMMC omitted meiosis and after a long interphase it elongated considerably. At the same time, large vacuoles formed at the micropylar and chalazal pole of the DMMC that became one nucleate female gametophyte. In all investigated ovules, callose was deposited at the micropylar pole of the DMMC and persisted to the binucleate female gametophyte stage. *E. annuus* is another diplosporous species of the Asteraceae family, in which callose was revealed during apomictic processes. These findings clearly indicate that lack of callose is not a common feature among apomicts and it can not be a reliable marker for early identification of the repoduction mode, which was previously suggested.

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Ovary structure and course of oogenesis in two species of earwigs from the family Chelisochodae

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The Dermaptera is a small insect group which comprises over 2200 species classified in 11 families. Here we present the results of morphological analyses of the ovary structure and the mode of oogenesis in two species belonging to the Chelisochidae family: *Hamaxas varicornis* and *Hamaxas nigrorufus*.

In both species, the female reproductive system is composed similarly. It consists of a pair of ovaries, paired lateral oviducts and single common oviduct. Ovaries are composed of 14 - 15 short ovarioles of a meroistic-polytrophic type. In a single ovariole a terminal filament, germarium, vitellarium and ovariolar pedicel can be easily distinguished. The germarium is relatively short and it contains differentiating germline cells. In the vitellarium there are usually only 2 - 3 ovarian follicles. Each follicle consists of a single oocyte and one nurse cell surrounded by follicular epithelium. In the oldest ovarian follicles, the trophocyte nucleus attains characteristic complicated shape of a hollow sphere. The oocyte nucleus translocates to the peripheral part of the ooplasm. At the same time, the follicular epithelium becomes diversified into three distinct subpopulations.

In the conclusion, out results showed that (1) ovarian structure in Chelisochidae suggests that this family belongs to the derived earwigs, the Eudermaptera; (2) oocyte-nurse cell complex in studied species arises as a result of one mitotic division of a founder germline cell, the cystoblast, and (3) a characteristic shape of the nurse cell nucleus, arising during later stages of oogenesis is implicated in the transport of the trophocyte components to the oocyte.

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Reproductive events in bog dandelion *Taraxacum mendax* (Asteraceae, Cichorioideae)

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Taraxacum mendax is a pentaploid dandelion (2n=40) belonging to the section *Palustria* (Kula et al., 2013). This section contains of about 131 species occurring mainly in Europe, and in Poland it is represented by 24 taxa (fifteen triploids, six tetraploids, and three pentaploids) that are threatened with extinction due to a progressive degradation of their natural habitats (Marciniuk et al., 2012). So far, only a few bog dandelions from Poland have been subjected to embryological studies. The apomictic mode of reproduction predominates within *T.* sect. *Palustria*, nonetheless the occurrence of reduced female gametophytes has been reported in some triploid and tetraploid taxa within the section (Małecka 1973).

This study focused on early reproductive processes in anthers and ovules of *T. mendax* from the southeastern Poland. A clearing tissue with methyl salicylate has been applied to the examination of non-stained, whole-mount young florets with the use of differential interference contrast microscopy. In addition, aniline blue staining was performed to detect callose. Analysis of anthers revealed an almost regular course of microsporogenesis and formation of highly uniform pollen grains which indicates that this dandelion is a facultative apomict because a strongly disturbed male meiosis and irregular pollen are characteristic of obligatory apomicts. Indeed, though meiotic diplospory of the *Taraxacum*-type was observed in most investigated ovules, the megaspore mother cells may also undergo meiotic division giving rise to tetrads of reduced megaspores. Moreover, additional aposporous embryo sac was visible deep in the chalaza of some ovules containing four or eight-nucleate female gametophyte. Such diversity of reproductive strategy is typical of very young forms and may indicate a hybrid origin of this pentaploid dandelion.

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Mitochondria dynamics during oogenesis in *Enchytraeus albidus* (Annelida: Clitellata)

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Mitochondria are highly dynamic organelles, and their morphology, spatial distribution within a cell and activity are changeable. The dynamism of mitochondria is the result of two opposing processes: fusion – leading to their merging into larger mitochondrial networks and fission – as a result of which individual mitochondrion separate from the network. The morphology of mitochondria depends on the balance between these processes.

The functioning of mitochondria and their importance for the proper course of oogenesis and the development of the future embryo have been intensively studied in recent years. Here, we present the results of an analysis that was focused on the distribution and dynamics of mitochondria (i.e. changes in their occurrence, forming clusters, connecting with the mitochondrial network or disintegrating into a single mitochondrion) in the germ-line cysts in ovaries of *Enchytraeus albidus*. In this species, each cyst consists of 16 cells (the lowest known number of interconnected cells within clitellate annelids). Each germ cell is connected to a small, common and anuclear cytoplasmic mass (cytophore) via a one ring canal. In each cyst, only one cell develops into the future egg; the remaining 15 become nurse cells. Despite the fact that the nurse cells gather cell organelles and store the material that then passes through the ring canals and cytophore towards the growing oocyte, they do not undergo polyploidisation, which indicates that these cells are not highly specialised.

We prepared the three-dimensional ultrastructural reconstructions of the distribution of the mitochondria in the germ cells, ring canals and the cytophore. The 3D reconstructions were created using the SBEM (Serial Block Face Scanning Electron Microscopy) technique which allowed the germ cell polarisation, which is manifested by the extensive mitochondrial network that occurs in the pole of the cell opposite to the ring canal, to be revealed. In the cytophore and ring canals, the mitochondria can also fuse, which indicates that the mitochondrial network seems to extends throughout the entire germ-line cyst. The germ-line mitochondrial network also forms closely adhered aggregates that contain the dispersed nuage material, numerous long cisterns of the endoplasmic reticulum and Golgi complexes.

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Spatiotemporal expression of calreticulin during microsporogenesis in *Petunia* anthers

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The multistage process of pollen grains formation in angiosperms depends on cellular interactions associated with tissue/cell-specific gene expression controlling cell division, chromatin remodeling, cytoskeleton functions, biogenesis of the sporoderm, and Ca^{2+}-dependent cell signaling pathway. We hypothesize that calreticulin (CRT), a prominent Ca^{2+}-binding/buffering endoplasmic reticulum (ER)-resident chaperone in eukaryotic cells, is involved in this process within the anther. CRT has been proposed to have many cellular functions in plants, including Ca^{2+} storage/mobilization and protein folding; its probable role in microsporogenesis may depend on these functions, because a high rate of protein synthesis and intracellular Ca^{2+} homeostasis are strictly required during pollen development and maturation. However, to this time there are very limited data confirming CRT expression during pollen formation. Since the functional role of CRT in microsporogenesis remains unresolved, research on developmental expression pattern of *CRT* gene/s and spatiotemporal distribution of their products in relation to easily releasable Ca^{2+} in developing anther are critical to understand CRT's possible function in this complex process.
To test our hypothesis, we first examined the expression levels/patterns of *CRT* gene belonging to *CRT1/2* subgroup at the successive phases of *Petunia hybrida* (*Ph*) pollen grains formation. Our *in vitro* experiments – northern hybridization with species-specific molecular probe complementary to the *PhCRT1/2* mRNA (Lenartowski et al., 2014) and western blotting with a maize anty-CRT primary antibody (Napier et al., 1995) – showed variable levels of *PhCRT1/2* mRNA as well as CRT protein during different stages of the male gametophyte development. Further, using immunocytochemistry and fluorescent *in situ* hybridization (FISH), we revealed spatiotemporal distributions of CRT and its transcripts in different tissues/cells, including developing pollen and the active tapetum. Finally, we performed cytochemical study by electron microscopy and found that the level of exchangeable Ca^{2+} changes dynamically in both generative and somatic cells during the successive phases of microsporogenesis. Based on our results, we propose that CRT is involved in (1) modulating Ca^{2+} storage/mobilization and (2) molecular chaperoning during the key events within the anther.

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Ontogenetic development of calretinin-containing neurons in the dorsal striatum of the male guinea pig (*Cavia porcellus*)

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The dorsal striatum (StD) is a set of subcortical structure involved in motor learning, habit formation and selection of appropriate psychomotor behavior (Gerfen and Bolam, 2017). Calretinin (CR) was classified as a calcium-buffer protein. It probably plays a role in calcium influx, neuronal excitability as well as neurotransmitter release (Schwaller B., 2010).
The purpose of the study was to examine the distribution of CR in the StD of the male guinea pig during development. Brains from fetal stages (E50, E60 - 50th, 60th day of gestation, respectively), newborn (P0) and P10 (10th day after birth) were used in the study. Frozen sections containing the StD were undergone for immunohistochemistry labelling, using a solution of antibodies raised against CR.
CR-positive perikarya were observed at each of the studied stages, ranging from E50 to P10 in the whole StD. At E50 and E60, CR containing cells were very numerous and they were dispersed in the whole structure. At E50, the aggregation of neurons were also observed dorsally and dorso-medially in the StD, along the lateral ventricle. The number and intensity of labeled neurons violently decreased at P0, where only a few neurons were observed in the StD. The pattern of CR distribution was similar at P10. StD revealed the existence of medium-sized (11-15μm) and small (6-9μm) CR-positive cells vary according to their overall morphological features and immunostaining intensity. Occasionally, single large neurons (about 20 μm) were observed.
The results of the present study show higher CR immunoreactivity in the developing prenatal StD (E50, E60). In guinea pig, this is a period just after an intense formation of synapses, when they get synaptic maturity, which lasts until the end of the gestational period. At birth, the guinea pig brain is almost fully developed (Jones et al., 1974).

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Comparative histochemical analysis of miophilous and sapromiophilous representatives of *Bulbophyllum* Lindl.

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Most of *Bulbophyllum* spp. are pollinated by flies (Diptera). Fly-pollination syndrome is generally described as myophilous or sapromyophilous. Myophilous flowers are relatively small, bright dull coloured (brown or green, often with dark spots or stripes), with the nectar produced in open shallow nectaries. The floral odour can be musky, slightly sweet and fruity or malodorous. Whereas sapromyiophilous flowers imitate brood and food sites of flies (Jürgens et al., 2006; Johnson and Jürgens, 2010). Such flowers present adaptations to pollinators attraction involving morphology and shape, color (brown, purple, greenish, often with great depth), pattern and texture, scent, thermogenesis or motile elements of perianth. Only some of sapromyiophilous species produce nectar. In general, flower-visiting insects receive no reward from the plant (Jürgens et al., 2006).
Bulbophyllum carunculatum Garay, Hamer, & Siegrist (section *Lepidorhiza*) fulfills features that characterize fly-pollinated sapromyophilous flowers, such as presence of motile appendages and see-saw lip and emission of unpleasant scent of rotten waste. In turn, *B. graveolens* J.J. Sm. (section *Cirrhopetalum*) shows features typical for myophilous flowers, including small (up to 5 cm long) bright yellow flowers with dark red, fleshy labellum.
Histochemical results showed great number of similarities. Testing for insoluble proteins (ABB) revealed an abundance of proteins in the epidermal and subepidermal cells of both species, but in *B. carunculatum* also residues of secreted substances stained with ABB. The test with ruthenium red did not reveal the presence of mucilage in *B. graveolens* except for that contained in the idioblasts, whereas in *B. carunculatum* mucilage was detected on the surface of the epidermis. The test for insoluble polysaccharides (PAS) showed an abundance of starch grains in the ground parenchyma of *B. carunculatum*, whereas in *B. graveolens* were far fewer of them. Individual lipid droplets were found mostly in the epidermis and subepidermis of both species. The test for dihydroxyphenols failed to detect any phenolic compounds.
The presence of protein-rich mucilage in *B. carunculatum* is probably associated with the unpleasant scent of the flowers. During the active decay at cadavers, protein sources are broken down into nitrogen, phosphorus and sulfur compounds. Stpiczyńska and Davies (2016) speculates that mucilage, when secreted on the surface, may absorb atmospheric moisture, swell and glisten, causing the floral rewards more attractive to pollinators. Small amount of starch in *B. graveolens* might be caused by its hydrolysis during anthesis, as a source of energy for fragrance and nectar production.

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Immunoreactivity of calbinidin-D28k in the spinal cord of *Cavia porcellus* at E30 stage of fetal development

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Cavia porcellus is a precocial rodent species, which possesses fetal motility like a human (Kan et al., 2009). The very first fetal movement was observed on day 24 and sideway bendings increased rapidly between 24 and 30 days and declined hereafter. The calcium ions play an important role both in muscles and in the development of the nervous system. Their level in cells undergoes constant control, in which intracellular calcium-binding proteins are involved (Zhung et al., 1990; Enderlin et al., 1987). It can be stated that calbindin- D28k (CB) is an early marker for certain neuronal systems in the rat by appearing at day 14 in the diencephalon and spinal cord (Enderlin et al., 1987). The data concerning the ontogenesis of the guinea pig spinal cord is absent and only general macroscopic examinations were conducted (Silva et al., 2009). The results obtained by immunohistochemistry related to CB immunoreactivity in the rat spinal cord are conflicting. The first CB-like immunoreactivity was observed in the ventral horn at E12 by Zhang et al. (1990), while at E14 by Enderlin et al. (1987). The aim of the study was to investigate a presence of immunoreactivity of the calcium-binding protein calbindin in the spinal cord at midgestation of the guinea pig around which general fetal movements reached peak (Kan et al., 2009). The present study revealed that in the posterior horns, small CB-immunoreactive cells with a low level of morphological differentiation preponderated, while more loosely and larger cells with processes were distributed in the anterior horns. The immunoreactivity for CB in differentiating neurons varied from weak to very strong and showed differences between the thoracic and lumbar levels. These characteristics were similar at three different planes (horizontal, transversal and sagittal). The present results suggest that CB in the spinal cord of the guinea pig is involved in discrete processes of cell differentiation and could be related to general movement of the fetus at midgestation whereas the exact role for sensing or buffering calcium ions remains to be elucidated in future studies.

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Apoptosis in the midgut epithelium of schrimp *Neocaridina davidi* (Crustacea, Malacostraca) exposed to starvation and re-feeding

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The aim of experiment was to study changes in mitochondrial activity of the digestive system in freshwater shrimp *Neocaridina davidi* exposed to starvation and re-feeding. A freshwater omnivorous shrimp that originates from Taiwan is 2-3 cm long. Experiment was conducted on adults (males and females) that have been bred in laboratory shrimp tank at constant conditions i.e. T=24ºC, pH=7 and GH=9 ºd. Adult animals were held in separate containers with the same parameters. Shrimps that have been starved for 7, 14 and 21 days were re-fed for the next 4, 7 and 14 days. The midgut is composed of the intestine and hepatopancreas. While the epithelium of the intestine is formed by D- and R-cells, the epithelium of the hepatopancreas has R-, B- and F-cells. Apoptosis in the midgut epithelial cells was detected and analyzed using transmission electron microscopy and immunohistochemical methods (confocal microscope): TUNEL assay and anti-caspase-3 antibodies. The process was only observed in the D-cells of the intestine and the F- and B-cells of the hepatopancreas. The analysis revealed that starvation caused an increase in the apoptotic cells in the intestine and hepatopancreas of *N. davidi*, while the re-feeding caused a decrease in the number of apoptotic cells in both organs of the midgut. There was no difference in the response to starvation and regeneration between starved males and females.

Distant pollination of *Salix* x *Populus* with short storage pollen.

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Establishing of short-rotation coppice plantations are one of the potential strategy for sourcing sustainable biomass for bio-energy. The comparisons among different biomass crops showed that species and hybrids of willow (Salix spp.) and poplar (Populus spp.) are main recommended bio-energy crops for regions with a temperate climate. In order to establish high productive energy biomass plantations the mixture of different willow genotypes has to be cultivated. Broadening the genetic diversity in Salix genus with experimental hybridization methods increase the possibility of creation new plants for biomass production in plantations. In presented study intergeneric crosses between *Salix viminalis* and *Populus tremula* were performed in a cultivation room. One of crucial limitation of distant crosses is lack of synchronous flowering of female and male components of hybridization. In the research, to omit the problem, method of short-term pollen storage was applied. The aim of the study was to assess the effectiveness of hybrid embryo formation after control pollination with stored pollen at 4ºC. The storage time of pollen used in study was range from 1 to 6 days. Freshly collected and stored pollen of *P. tremula* was manually applied to the receptive stigma of *S. viminalis* catkins. After 20 to 25 days of manual pollination, embryos were isolated from swollen pistils. Successful pollination was determined by number of embryo isolated and calculated as a mean number of embryos formed per catkin.

During the study, 1054 *S. viminalis* female inflorescences was pollinated. In total, 2591 putative hybrid embryos were isolated from 4041 swollen pistils. The isolated embryos were at various stage of development. The number of mature embryo isolated was 1935. The immature isolated embryo were at the torpedo (570) or heart (86) stage of development. The percentage (74,68%) of mature embryos was significantly higher than the percentage of immature embryo formed. The mean rate of hybrid embryo formation success, about 6 embryos per catkin, was obtained in all variants of pollination with stored pollen. After control pollination with fresh pollen mean number of embryo formed per catkins was 4.

The results showed that short-term pollen storage can be implemented as an effective method to face the bottleneck of asynchronous flowering of female *Salix* and male *Populus* catkins to improve the effectiveness of hybrid embryo formation.

Ultrastructural and protein localization defects during spermiogenesis in myosin VI-deficient mice

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Myosin VI (MYO6) is the only known pointed-end-directed actin-based motor protein that has been implicated in several cellular processes, including clathrin-mediated endocytosis, Golgi organization/function, basolateral targeting/sorting, epithelial integrity, and nuclear processes. In these seemingly different processes, MYO6 may function as a cargo transporter or protein anchor involved in actin organization/dynamics in specialized cells. In *Drosophila*, MYO6 plays a crucial role in actin-based process during the late phase of spermatogenesis and mutant males lacking MYO6 (*jaguar*, *jar/jar*) are sterile (Noguchi et al., 2006). Because the actin cytoskeleton together with actin-binding proteins (ABPs) have been implicated in various aspects of mammalian spermiogenesis and MYO6 knockout (KO) mice males (*Snell's waltzer*, *sv/sv*) exhibit somewhat reduced fertility (Avraham et al., 1995), we investigated whether MYO6 was associated with actin-mediated events during sperm maturation of mice.
First, we examined MYO6 expression in mouse testis and found that two out of four MYO6 splice variants – with a small insert (SI) and no insert (NoI) – are expressed in the male gonad (Zakrzewski et al., 2017). Further, using immunocytochemistry, we found that MYO6 is present in developing spermatids and the Sertoli cells adjacent to them and linked with actin-rich structures involved in sperm development/maturation, such as the Golgi complex, the acroplaxone, the manchette, and Sertoli cell actin hoops. We next examined MYO6 KO developing spermatids and revealed a number of morphological disruptions, especially of the Golgi complex, the endoplasmic reticulum (ER), and the acrosome. Finally, we compared distribution of marker proteins for the Golgi and the ER, and of a few ABPs in developing spermatids of wild type (WT) and MYO6 KO mice. Our results showed significant changes in localization of all the proteins tested in the absence of MYO6. Thus, we suggest for the very first time an anchoring role for MYO6 in maintaining the morphology of key organelles and F-actin-containing structures involved in acrosome biogenesis, spermatid elongation, and spermiation in mammals.

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The role of innate immunity in the embryonic development of the spider *Parasteatoda tepidariorum*

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Spiders are characterized by the production of spider silk. Spiders use their silk to make a web or other structures for example egg cocoon. The aim of this study was to check the defensive potential of egg cocoon against pathogens. The antibacterial ability of spider embryos was analyzed by infecting cocoons with the potential pathogens: *Micrococcus luteus, Escherichia coli* and selected fungus species. The research material consisted of the spider cocoons of the *Parasteatoda tepidariorum* in two age groups 96h and 144h. In each age group of cocoons, there were research variants: a group of cocoons not treated with a pathogen, with a damaged cocoon cover, pathogen injection into the interior of the cocoon and a group in which a bacterial suspension was applied on to the cocoon surface. Comparing the level of markers of innate immunity after treatment with a potential pathogens let to assess the antibacterial potential of the spider eggs *Parasteatoda tepidariorum.* The level of antibacterial proteins was determined using one of the commonly used immunodetection tests - ELISA. The analysis of results obtained shows that the treatment and age of the cocoons affect the level of antibacterial embryo proteins. We anticipate our results will be an important voice in the discussion on the innate immunity in the world of spiders.

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Ultrastructure and innervation of the dermal glands in the caecilian *Typhlonectes natans* (Amphibia: Gymnophiona)

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The structure of the skin (both at LM and TEM) and the nature of the skin glands secretion are quite well known in Anura, slightly less in Caudata, and almost unknown at Gymnophiona. Maintenence of the proper humidity, pH of the skin surface and passive defense against predators are ensured by the presence of multi-cell mucous and serous glands present in the skin. The proper functioning of the skin depends on discharging of the produced secretion from the glands, which is regulated by the nervous system (Zaccone et al., 2015).There are two types of multicellular glands in the *T. natans* skin: mucous and serous / granular. The diameter of the granular glands is definitely higher (340 um average) than the mucous glands (average 130 mm). Each of the gland is surrounded by a single layer of myoepithelial cells. Secretory cells connect to the surrounding MECs gland through cytoplasmic protrusions. At the cell membrane of MECs supported on the basal membrane, hemidesmosomes were observed. The central part of the MEC cytoplasm is rich in tonofilaments usually arranged parallel to the cell membrane. However, in the cytoplasm neighbouring membranes, numerous small electron-dark granules and elongated mitochondria are present. The cell nucleus, located at one end of the MEC, is truncated and the Golgi appartus cisterns are present in the cytoplasm. The base of the outlet channel in the mucous glands consists the collar-like structure formed of the MECs lying on top of each other, whereas in the granular glands at the base of the outflow channel, no such structures were observed. Both types of glands have been found to have nerve endings contacting MECs cells. Sections through nerve endings were observed at the border: (1) MEC cells and secretory cells consisting the gland wall, (2) between connecting MECs surrounding the gland wall, and (3) between MECs at the base of the gland outflow channel. Quiet often in the MEC cytoplasm, at cross sections at the level of nerve ending, bright synapse-like vesicles were observed.

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Lauriano Er, Żuwała K, Kuciel M, Budzik Ka, Capillo G, Alesci A, Pergolizzi S, Dugo G and Zaccone G. 2016**.** Confocal immunohistochemistry of the preganglionic neurons, innervation of the dermal glands and evolutionary considerations of cutaneous effectors in the caecilian, *Typhlonectes natans* (Amphibia: Gymnophiona). *Acta Zoologica* 97: 154-164.

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