Charles University in Prague
Faculty of Science
Department of Zoology

Doctoral study programme: Zoology
Summary of the Doctoral thesis

Comparative analyses of cranial skeletogenesis and odontogenesis in basal Ray-finned fishes

Mgr. Anna Pospíšilová

Supervisor: Mgr. Robert Černý, Ph.D.

Praha
2020

Publications attached to this thesis

I. **Pospisilova A**, Brejcha J, Miller V, Holcman R, Šanda R & Stundl J (2019) Embryonic and larval development of the Northern pike: a new emerging fish model system for evo-devo research. *Journal of Morphology* 280, 1118–1140.

IF (2019) = 1,563

II. **Pospisilova A**, Stundl J, Metscher BD, Brejcha J, Psenicka M & Cerny R (in prep) Comparative cranial skeletogenesis in fishes: towards understanding of developmental strategies of fish craniofacial diversity.

III. Rizzato PP*, **Pospisilova A***, Hilton EJ & Bockmann FA (2020) Ontogeny and homology of cranial bones associated with lateral-line canals of the Senegal Bichir, Polypterus senegalus (Actinopterygii: Cladistii: Polypteriformes), with a discussion on the formation of lateral-line canal bones in fishes. *Journal of Anatomy* 00, 1–29.

* These authors contributed equally to this work.

IF (2019) = 2,013

IV. Stundl J, **Pospisilova A**, Jandzik D, Fabian P, Dobiasova B, Minarik M, Metscher BD, Soukup V & Cerny R (2019) Bichir external gills arise via a heterochronic shift that accelerates hyoid arch development. *eLife* 8, e43531.

IF (2019) = 7,080

V. Stundl J, **Pospisilova A**, Matějková T, Psenicka M, Bronner ME & Cerny R (2020) Migratory patterns and evolutionary plasticity of cranial neural crest cells in ray-finned fishes. *Developmental Biology* (forthcoming)

IF (2019) = 2,895

VI. Soukup V, Tazaki A, Yamazaki Y, **Pospisilova A**, Epperlein H-H, Tanaka EM & Cerny R (in prep) Oral and palatal dentition of axolotl arises from a common tooth-competent zone and co-localizes with the ecto-endoderm boundary.

VII. **Pospisilova A**, Stundl J, Brejcha J, Metscher BD, Psenicka M, Cerny R & Soukup V (in prep) The dentition is a highly dynamic organ system during the sterlet sturgeon ontogeny (*Acipenser ruthenus*).

Abstract

Skeletal (cartilaginous, bony, and dental) tissues undoubtedly exemplify the key innovation of vertebrates. Among all recent vertebrates, the most numerous and successful lineage is represented by the Ray-finned fishes that, accordingly, exhibit an amazing variety of skeletal architectures and phenotypic adaptations. In order to depict fundamental principles of fish cranial skeletogenesis the developmental formation of skeletal architectures was described, compared and analyzed using members of early branching fish lineages, that exemplify very different strategies of skeletogenesis. While the Senegal bichirs and the tropical gars are heavily armored forms with a massive exoskeleton and hyperossified dental structures covering the whole oropharyngeal region, the sterlet sturgeon, on the contrary, possess mostly cartilaginous skeleton and reduce their dental structures during early development. Whole analysis is underpinned by the northen pike, teleostean species with lightened skeletal architecture with a comparable number of cranial elements. The present study represents the first complex comparative analysis of their skeletogenesis and odontogenesis. This allowed us to define developmental strategies founding different lineage-specific skeletal architecture of vertebrates.

A comparative description of the developmental dynamics of cartilaginous and bony tissues revealed the distribution of heterochrony at various levels. We identified differences in (i) sequence of cranial cartilaginous and bony regions development, (ii) relative timing of cartilage and bone formation, and (iii) duration (overal rate of development) of these processes. The quantitative analysis uncovered a surprisingly different interspecies dynamic of skull density development with the most progressive beginning of ossification in sterlet. Moreover, different timing of key skeletel markers expression obviously represents an important factor regulating the process of ossification. Developmental heterochrony was also identified during the early stages of head tissue differentiation with accelerated morphogenesis of the hyoid stream of cranial neural crest. This developmental heterochrony is associated with the early formation of hyoid structures during larval development.

A detailed description of odontogenesis based on unique developmental series of basal Ray-finned fishes revealed substantial variation in dental distribution and early patterning of their dentition. The expression of key dental markers depicting the early process of dentate field differentiation was thoroughly described for the first time. Analysis of dental mineralization patterning allowed to trace the dynamics of teeth replacement among studied lineages. These data revealed the ancestral state of dentition development in bichir contrasting with exceptionally derived sterlet dentition. Moreover, aspects of teeth formation and development of complex dentition of gar were described in detail for the first time.

Key words: cranial skeletogenesis, odontogenesis, heterochrony, Ray-finned fishes, cartilage, bone, dentition, neural crest

Abstrakt

Skeletální, tzn. chrupavčité, kostěné a zubní tkáně patří ke klíčovým inovacím nás obratlovců. Jednu z druhově nejpočetnějších skupin obratlovců vůbec reprezentují paprskoploutvé ryby (Actinopterygii), což se odráží i v mimořádné diverzitě jejich skeletálních systémů. V této práci se zabývám překvapivě odlišnou kraniální i dentální architekturou zastoupenou již u členů bazálních linií paprskoploutvých ryb. Zatímco bichiři (*Polypterus senegalus*) a kostlíni (*Atractosteus tropicus*) svým masivním exoskeletem hlavy i trupu představují doslova obrněné formy se zuby několika typů pokrývajícími celou orofaryngeální oblast, u jeseterů (*Acipenser ruthenus*) došlo k rozsáhlé redukci a restrukturalizaci skeletu včetně dentice, takže dospělci jsou bezzubí. Celou analýzu zastřešuje štika (*Esox lucius*), tedy zástupce kostnatých ryb s odlehčenou skeletální architekturou při zhruba srovnatelném počtu kraniálních elementů. Předkládaná práce nabízí první komplexní srovnávací analýzu jejich skeletogeneze a odontogeneze, která nám umožní porozumět tomu, co vývojově zakládá odlišnou kraniální architekturu obratlovců.

Komparativní deskripce růstové dynamiky chrupavčitých a kostěných tkání odhalila distribuci heterochronií na několika úrovních. Rozdílné bylo tedy (i) pořadí (sekvence) formování chrupavčitých i kostěných kraniálních regionů, (ii) relativní načasování počátku samotné chondrogeneze a osteogeneze, stejně jako (iii) doba trvání těchto procesů. Překvapivě odlišná pak byla mezidruhová dynamika vývoje denzity kostí s nečekaným nejprogresivnějším počátkem osifikace u jesetera. Odlišné načasování exprese klíčových skeletálních markerů raných stádií vývoje chrupavky (*COLII*) a kosti (*Col10a1*) zjevně představuje důležitý faktor regulující následný proces mineralizace. Významná vývojová heterochronie pak byla identifikována během raných stádií diferenciace hlavových tkání se zrychlenou morfogenezí hyoidního proudu hlavové neurální lišty zakládající klíčové hyoidní struktury larválních stádií.

Již základní deskripce odontogeneze unikátních vývojových řad bazálních paprskoploutvých ryb prokázala výraznou variabilitu v distribuci a v raném patternování dentice. Zcela poprvé byla detailně popsána exprese klíčových zubních genů ilustrující proces zakládání zubních polí u těchto linií. Vizualizace mineralizace zubů pak umožnila vysledovat dynamiku jejich nahrazování. Získaná data poukázala na zachovalé ancestrální prvky ve vývoji dentice bichira kontrastující s výjimečně odvozenou denticí jesetera. Zcela poprvé byly popsány aspekty zakládání a vývoje komplexní zubní dentice kostlína.

Klíčová slova: kraniální skeletogeneze, odontogeneze, heterochronie, paprskoploutvé ryby, chrupavka, kost, dentice, hlavová neurální lišta

Introduction

Skeletal tissues (bone, cartilage, dentine, and enamel) are widely considered as crucial vertebrate innovations that have facilitated their evolutionary radiation and phenotypic adaptation (Gans & Northcutt, 1983; Northcutt & Gans, 1983; Northcutt, 2005; Green et al., 2015; Abzhanov et al., 2004; Albertson et al., 2010). Among recent vertebrates, the ray-finned fishes represent the most numerous and successful lineage and they also exhibit an amazing variety of their skeletal and dental architectures (Fricke et al., 2020; Sire & Hyusseune, 2003; de Beer, 1937; Berkovitz & Shellis, 2017). Though the fossil record suggests this diversity arose very early in the vertebrate lineage, it tells us nothing about the developmental factors or genetic bases enabling so extensive morphological and functional diversity of the fish skulls and teeth.

It has been shown that most of the skeletal tissues supporting head are derived from the cranial neural crest cells (Couly et al., 1993; Santagati & Rijli, 2003). Modulation of this vertebrate-specific cell population, together with interacting endoderm results in modification of head structures (Štundl, 2019). Thus, heterochronic events during migratory patterns of cranial neural crest cells might constitute a substantial source of craniofacial diversity in vertebrates. Moreover, species-specific differences in cranial skeletogenesis of Ray-finned fishes might be based on heterochrony at the level of developmental expression of genes involved in a skeletal matrix formation. Further, variation in relative developmental growth dynamics of skeletal structures can play an important role in the origin and evolutionary diversification of complex skeletal phenotypes (Albertson et al., 2010; Gunter et al., 2014; Powder et al., 2015).

Teeth represent other key innovation of vertebrates that promoted the evolutionary success and diversification of vertebrate lineages. The diverse range of dental phenotypes offered by fish reflects their specialization to diet (Sire & Huysseune, 2003). Despite the differences in dental shape and dentition patterning, amazing conservation has been described at the level of genetic mechanisms underlying tooth development and regeneration (Debiais-Thibaud et al., 2015; Rasch et al., 2016). Thus, teeth development is considered to be a highly stable biological process, and a fundamental question is which developmental mechanisms might establish different dental and skeletal characters observed among studied species.

Aims of the study

This thesis, entitled „*Comparative analyses of cranial skeletogenesis and odontogenesis in basal Ray-finned fishes*“, aims to understand the core principles responsible for different skeletal strategies in fishes.

The aims of this thesis are:

- To characterize cranial skeletogenesis and odontogenesis using whole-mount staining of cartilaginous and bony tissues
- To describe the early stages of skeletogenesis and odontogenesis using gene expression in the mesenchyme of studied lineages
- To analyse the process of skeletogenesis using 3D computed microtomography (micro-CT) enabling detailed resolution and accurate detection of skeletal mass
- To compare the dynamics of cranial structures development and defined developmental modules among species
- To assess obtained results in the context of contemporary knowledge about vertebrate skeletogenesis

Materials & methods

The analyzed material includes a developmental series of (i) the Senegal bichir (*Polypterus senegalus* Cuvier, 1829) obtained from the colony at the Department of Zoology (Charles University in Prague), (ii) the sterlet sturgeon (*Acipenser ruthenus* Linnaeus, 1758) obtained from the hatcheries of the Research Institute of Fish Culture and Hydrobiology in Vodnany (the University of South Bohemia in České Budějovice, (iii) the tropical gar (*Atractosteus tropicus* Gill, 1863) obtained from the Arias Rodriguez's lab in Villahermosa (Mexico), and (iv) the northern pike (*Esox lucius* Linnaeus, 1758) from the commercial hatchery in Litomysl. All fish were maintained and exploited according to institutional and international guidelines for the protection of animal welfare (Directive 2010/63/EU) in the animal facility of the Department of Zoology, Charles University, Prague. This embryonic material was reared and staged as previously described (Štundl, 2019).

Early skeletal differentiation and determination of tooth competent region before teeth mineralization were identified by whole-mount *in situ* hybridization of skeletal (*Col10a1*) and odontogenic (*Pitx2* & *Shh*) markers. Pre-cartilaginous tissue differentiation was analyzed using whole-mount immunostaining against COLII protein (detailed description of the procedures in Štundl, 2019). The process of cartilage development was visuallized by Alcian Blue staining, skeletal ossification and teeth mineralization was traced by Alizarin Red staining. Application of micro-CT imaging allowed comparative quantitative evaluations of skeletal tissue density development among studied species.

Hard tissue resorption by osteoclasts during tooth shedding was visualized on whole-mount and sectioned samples using staining against TRAP (tartrate-resistant acid phosphatase). For a detailed histological description of tooth replacement and visualization of plicidentine arrangement in gar dentition Mayer’s Hematoxylin & Eosin was applied on sectioned samples embedded in JB4 resin.

Results and Discussion

Using a combination of a number of laboratory techniques such as preparation, classical clearing and staining of cartilaginous and bony tissues, and high-contrast imaging of mineralized cranial structures (by micro-CT), I performed detailed comparative description of cranial skeletogenesis in bichir, sterlet, gar, and pike. These data revealed disproportional patterns of temporal and topographical organization of cranial cartilaginous and bony regions (**Paper II**). Quantitative investigations of mineral tissue development uncovered different developmental dynamics of skeletal tissue density (**Paper II**). Moreover, my data demonstrate that early shifts in the relative timing of early skeletal markers have a significant impact on patterns of fish cranial architectures (**Paper II**). Thus, the distribution of heterochrony at various developmental levels seems to be among the crucial factors responsible for disparate cranial morphotypes. Variations in relative developmental growth rates and timing have been proved to be an important mechanism for evolutionary changes (Alberch et al., 1979).

Moreover, earlier stages of head tissue differentiation revealed significant alternations in the development of cranial neural crest cells. Thus, development of hyoid stream of cranial neural crest is accelerated in bichir, gar, and pike (**Paper IV** & **Paper V**), which is developmentally connected with the early formation of head cartilage in pike and prominent hyoid structures in bichir and gar. In sterlet, middle and posterior streams of cranial neural crest migrate in common hyo-branchial sheet (**Paper V**) which might correspond to highly mobile viscerocranium observed in this species.

Comparison of odontogenesis using staining of mineralized tissues in basal Ray-finned fishes identified teeth of various morphology distributed at the different positions through the oropharyngeal chamber. Analysis of the beginning of dental development revealed surprisingly different pattern of *Pitx2* & *Shh* expression among studied lineages. These two genes defining odontogenic band epithelium are regarded as very conservative markers of the tooth-competent region and variation in their expression pattern might support the observed variation of studied dentitions (Fraser et al., 2004; Rasch et al., 2016). Although the process of tooth replacement is very dynamic especially in bichir and gar, epithelial fold defining the structure of dental lamina wasn‘t previously identified in bichir (Vandenplas et al., 2014) nor in sterlet (**Paper VII**) and gar. Given to their key phylogenetic position I speculate that this condition represents an ancestral way of tooth replacement in Ray-finned fishes.

Conclusion

Comparative skeletal data of this work provides indisputable evidence of a disparate system of skeletogenesis and odontogenesis in bichir, sterlet, gar, and pike. Based on the present study, it is obvious, that each lineage is characterized by exclusive set of skeletal and dental characters emerging during a rather long time period from very early ontogeny. My data suggest that searching for core principles founding different cranial architecture might be a laborious and long-lasting process. However, a number of analyses included in the present work revealed heterochrony distributed at various levels during development as a crucial factor responsible for disparate adult cranial morphotypes.

References

Abzhanov A, Protas M, Grant BR, Grant PR & Tabin CJ (2004) Bmp4 and morphological variation of beaks in Darwin's finches. *Science* 305, 1462–1465.

Alberch P, Gould SJ, Oster FG & Wake DB (1979) Size and shape in ontogeny and phylogeny. *Paleobiology* 5, 296–317.

Albertson RC, Yan Y-L, Titus TA, Pisano E, Vacchi M, Yelick PM, Detrich W & Postlethwait JH (2010) Molecular pedomorphism underlies craniofacial skeletal evolution in Artartic notothenoid fishes. *BMC Evolutionary Biology* 10(4), 1–12.

de Beer GR (1937) *The development of the vertebrate skull*. Oxford University Press, Oxford.

Berkovitz B & Shellis P (2017). *The Teeth of Non-Mammalian Vertebrates*. Academic Press.

Couly GF, Coltey PM & Le Douarin NM (1993) The triple origin of skull in higher vertebrates: A study in quail-chick chimeras. *Development* 117, 409–429

Debiais-Thibaud M, Chiori R, Enault S, Oulion S, Germon I, Martinand-Mari C, Casane D & Borday-Birraux V (2015) Tooth and scale morphogenesis in shark: an alternative process to the mammalian enamel knot system. *BMC Evolutionary Biology* 15, 1–17 .

Fraser GJ, Graham A & Smith MM (2004) Conserved deployment of genes during odontogenesis across osteichthyans. *Proceedings. Biological sciences* 271, 2311–2317.

Fricke R, Eschmeyer WN & Van der Laan R (eds.) 2020. Eschmeyer‘s catalog of fishes: genera, species, references. (http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed 03 06 2020.

Gans C & Northcutt RG (1983) Neural crest and the origin of vertebrates – a new head. *Science* 220, 268–274.

Green SA, Simoes-Costa M & Bronner ME (2015) Evolution of vertebrates as viewed from the crest. *Nature* 520, 474–482.

Gunter HM, Koppermann C & Meyer A (2014) Revisiting de Beer’s textbook example of heterochrony and jaw elongation in fish: calmodulin expression reflects heterochronic growth, and underlies morphological innovation in the jaws of belonoid fishes. *EvoDevo* 5(8), 1–13.

Northcutt RG & Gans C (1983) The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Quarterly Review of Biology* 58, 1–28.

Northcutt RG (2005) The New Head Hypothesis Revisited. *Journal of experimental zoology. Part B, Molecular and developmental evolution* 304B, 274–297.

Powder KE, Milch K, Asselin G & Albertson RC (2015) Constraint and diversification of developmental trajectories in cichlid facial morphologies. *EvoDevo* 6(25), 1–14.

Rasch LJ, Martin KJ, Cooper RL, Metscher BD, Underwood CJ & Fraser GJ (2016) An ancient dental gene set governs development and continuous regeneration of teeth in sharks. *Developmental Biology* 415, 347–370.

Santagati F & Rijli FM (2003). Cranial neural crest and the building of the vertebrate head. *Nature reviews. Neuroscience* 4, 806–818.

Sire J-Y & Huysseune A (2003) Formation of dermal skeletal and dental tissues in fish: a comparative and evolutionary approach. *Biological Reviews* 78, 219–249.

Štundl J (2019) *Migration and morphogenesis of neural crest cells in the context of craniofacial development of selected ray-finned fishes*. PhD. Prague: Charles University, pp. 78.

Vandenplas S, De Clercq A & Huysseune A (2014) Tooth replacement without a dental lamina: The search for epithelial stem cells in *Polypterus senegalus*. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 322(5), 281–293.

MSc. Anna Pospíšilová

Date of Birth:	13th April 1989, Zlín, Czech Republic
Email:	pospia@natur.cuni.cz
Phone number:	+420 776 587 113

Affiliation: Laboratory for the study of craniofacial evolution & development (Robert Cerny lab)
Vertebrate zoology research group, Department of Zoology
Faculty of Science, Charles University in Prague

Education:

2015 – present	study **PhD in Zoology:** Charles University in Prague, Faculty of Science, Department of Zoology Thesis: *Characterization of Cranial Skeletogenesis and Odontogenesis in Basal Ray-finned Fishes*
2012 – 2015	**MSc. in Zoology (Mgr.):** Charles University in Prague, Faculty of Science, Department of Zoology Thesis: *Characterization of Cranial Skeletogenesis and Odontogenesis in Basal Ray-finned Fishes*
2009 – 2012	**BSc. in Biology (Bc.):** University of Ostrava, Faculty of Science, Department of Biology and Ecology Thesis: *Euglenids (Euglenophyta) in Ponds of Jistebník (Protected Landscape Area Poodří)*

Employment:

2016 – present	Scientific researcher: Laboratory for the study of craniofacial evolution & development (Robert Cerny's lab), Faculty of Science, Charles University in Prague

Educational stays:

Oct 2016 – Feb 2017: Micro-CT Imaging Lab (Brian Metscher's lab) Department of Theoretical Biology, University of Vienna, Austria
(Supported by OeAD Aktion Österreich-Tschechien scholarship, financed by BMWFW Österreich, ERAMUS+)

Courses:

2013: Time - How Nature Sets the Clock (11th VBC PhD Programme Symposium)
2014: Complexity of Life (12th VBC PhD Programme Symposium, Vienna, Austria)
2014: Light Sheet Microscopy (2014)
2015: Communication (13th VBC PhD Programme Symposium, Vienna, Austria)
2016: 4th ZEISS XEN User Meeting Program, Vienna, Austria
2016: Mind the App (14th VBC PhD Programme Symposium, Vienna, Austria)
2017: The 29th CDB Meeting, Mavericks, new models in developmental biology (Japan)
2017: 8th Edition Introduction to Geometric Morphometrics (Canada, January 9th-13th)

List of Publications

I. **Pospisilova A**, Brejcha J, Miller V, Holcman R, Šanda R & Stundl J (2019) Embryonic and larval development of the Northern pike: a new emerging fish model system for evo-devo research. *Journal of Morphology* 280, 1118–1140.

II. Rizzato PP*, **Pospisilova A***, Hilton EJ & Bockmann FA (2020) Ontogeny and homology of cranial bones associated with lateral-line canals of the Senegal Bichir, Polypterus senegalus (Actinopterygii: Cladistii: Polypteriformes), with a discussion on the formation of lateral-line canal bones in fishes. *Journal of Anatomy* 00, 1–29.

* These authors contributed equally to this work.

III. Stundl J, **Pospisilova A**, Jandzik D, Fabian P, Dobiasova B, Minarik M, Metscher BD, Soukup V & Cerny R (2019) Bichir external gills arise via a heterochronic shift that accelerates hyoid arch development. *eLife* 8, e43531.

IV. Stundl J, **Pospisilova A**, Matějková T, Psenicka M, Bronner ME & Cerny R (2020) Migratory patterns and evolutionary plasticity of cranial neural crest cells in ray-finned fishes. *Developmental Biology* (forthcoming)

Grants:

- **GAUK grant no. 640616** Charles University Grant Agency, 2016 – 2018 (*major investigator)*

Topic: *Comparative analyses of craniofacial mezenchyme and cranial skeletogenesis in vertebrates: a case of basal fishes*

- **Aktion Österreich-Tschechien scholarship** OeAD (ICM-2016-03562), Austria, autumn semester 2016/2017

Topic: *Developmental strategies of fish cranoifacial diversity: comparative micro-CT and quantitative analyses*

- **ERASMUS+ no. 2528026** Austria, autumn semester 2016/2017
- **GAUK grant no. 148514** Charles University Grant Agency, 2014 - 2016 (*co-investigator*)

Topic: *Comparative analyses of migration and morphogenesis of neural crest cells in fishes: understanding developmental events responsible for generation of vertebrate craniofacial diversity*
Investigator: Mgr. Jan Štundl

- **GAUK grant no. 220213** Charles Univesity Grant Agency, 2013 – 2015 (*co-investigator*)

Topic: *Deciphering the evolution of cement organs in ray-finned fishes*
Investigator: Mgr. Martin Minařík

- **GAUK grant no. 337215** Charles University Grant Agency, 2015 – 2016 (*co-investigator*)

Topic: *Deciphering levels of homology between keratinous vs calcified teeth in mouth of vertebrates: structural, developmental and gene expression level analyses*
Investigator: Mgr. Zuzana Karpecká

Selected lectures and posters:

2019: „Cranial skeletogenesis in non-teleost fishes: towards the understanding of developmental strategies of fish craniofacial diversity“ **Pospisilova A***, Stundl J, Metscher B, Gela D & Cerny R (International Congress of Vertebrate Morphology; Prague Czech Republic) *poster presentation*

2019: „Development and Dynamics of the Pharyngeal Dentition in Sturgeon“ Novotna S, **Pospisilova A**, Stundl J, Psenicka M, Gela D, Cerny R & Soukup V (International Congress of Vertebrate Morphology; Prague Czech Republic) *poster presentation*

2018: „Comparative cranial skeletogenesis in non-teleost fishes: towards understanding of developmental strategies of fish craniofacial diversity“ **Pospisilova A***, Stundl J, Minarik M, Metscher B & Cerny R (Interdisciplinary Approaches in Fish Skeletal Biology; Tavira Portugal; Euro Evo Devo; Galway Ireland; V4 Society for Developmental Biology; Brno Czech Republic) *poster presentation*

Best Poster Award IAFSB 2018
Best Poster Award V4 Society for Developmental Biology 2018

2018: „Patterning of teeth at the dichotomy of ray- and lobe-finned fishes“ **Pospisilova A**, Stundl J, Cerny R & Soukup V (Euro Evo Devo; Galway Ireland) *poster presentation*

2018: „Initiation, addition and loss of teeth in a sterlet (*Acipenser ruthenus*)“ **Pospisilova A**, Stundl J, Metscher B, Cerny R & Soukup V (Interdisciplinary Approaches in Fish Skeletal Biology; Tavira Portugal) *poster presentation*

2018**:* „Comparative cranial skeletogenesis in non-teleost fishes: towards understanding of developmental strategies of fish craniofacial diversity“ **Pospisilova A (Evo-Devo Mini Symposium; Prague Czech Republic *oral presentation*

2017: „New fish models for study of craniofacial diversity and modularity“ **Pospisilova A***, Stundl J, Minarik M & Cerny R (Maverick, New Models in Developmental Biology; Kobe Japan); *poster presentation*

2016: „Developmental strategies of skeletogenesis in bichirs and sturgeons: comparative analysis of two disparate cranial architectures“ **Pospisilova A***, Stundl J, Minarik M, Gela D & Cerny R (Euro Evo Devo; Uppsala Sweden); *poster presentation*

2015: „Comparative analysis of skeletogenesis in bichirs and sturgeons, lineages with distinct developmental strategy“ **Pospisilova A***, Stundl J, Minarik M, Gela D & Cerny R (Interdisciplinary Approaches in Fish Skeletal Biology; Tavira Portugal); *poster presentation*

**** marks active presentation***

Professional skills:

Histology (paraffine, plastic resine, vibratome sectioning), TRAP staining, staining Alizarin Red & Alcian Blue, kalcein, immunohistochemistry, RNA *in situ* hybridization, microinjection (CM DiI, CRISPR), microscopy (visible light, fluorescence, SEM, micro-CT), image data processing (Photoshop, Inkscape, Amira, Fiji, Affinity Photo)