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integrative  
**zoologie**

**DEPARTMENTAL SEMINAR  
INTEGRATIVE ZOOLOGY  
WS 2012**

**Programme and Abstracts**

Tuesdays, 10-11:30 hrs

SR 3, UZA1, Althanstraße 14, 1090 Wien



**DEPARTMENTAL SEMINAR - INTEGRATIVE ZOOLOGY**  
**Abstracts WS 2012**

**Liebe AbteilungsmitgliederInnen, liebe der Integrativen  
Zoologie verbundene externe KollegInnen,**

Es ist mir eine große Freude, Ihnen die erste Ausgabe des Abstractbandes zum Seminar unseres gemeinsamen Departments für Integrative Zoologie zu präsentieren. Die Idee zu einer solchen Zusammenstellung stammt aus dem ehemaligen Department für Evolutionsbiologie, und ihre konsequente Weiterführung soll auch künftig Zeugnis der eindrucksvollen methodischen und organismischen Bandbreite, mit der zoologische Forschung an unserem Department betrieben wird, ablegen. Ergänzt und bereichert werden die hier präsentierten Arbeiten aus dem Department durch solche hochrangiger geladener externer Seminarredner. Ich bedanke mich bei allen Vortragenden für ihre Beiträge und bei Frau Mag. Sonja Matuš für die mühevollen Zusammenstellung der Beiträge, den Entwurf und das Layout dieses Bandes, sowie bei Prof. Harald Krenn für die Gestaltung des Umschlages. Möge das Seminar des Departments für Zoologie auch in Zukunft eine breite, stimulierende Plattform zur Präsentation und Diskussion moderner zoologischer Forschung dienen.

Mit kollegialen Grüßen,



Andreas Wanninger, im Mai 2013

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## Neurogenesis in Solenogastres (Mollusca)

**Emanuel Redl**

emanuel.redl@univie.ac.at

*PhD Thesis*

*FWF Project P24276: Integrative developmental studies on basal molluscs*

*Supervisor: Andreas Wanninger*

The Mollusca constitutes the most species-rich metazoan phylum after the arthropods, exhibits a large variation of body plan morphologies, and its representatives can be found in most ecosystems worldwide. However, despite a long history of molluscan research, their evolutionary emergence remains obscure. The discussion about their sister group is still ongoing and even the old question whether molluscs are derived from a segmented or unsegmented ancestor has not been resolved yet.

In order to contribute to this issue, we investigated the development of the nervous system in *Wirenia argentea* and *Gymnomenia* spec. nov., two species of Solenogastres, the extant molluscan group that is often thought to bear important similarities with the last common ancestor to all molluscs. Our research was mainly focused on the following two questions:

1. Are there any traits of ancestral segmentation, e. g., a formation of a series of neural components in an anterior to posterior progression or a posterior growth zone, discernible during the development of Solenogastres?
2. Is the apical organ of the solenogaster larva of the complex “Kamptozoa-Polyplacophora-type“, which is one of the proposed synapomorphies that suggest a sister group relationship of Entoprocta (Kamptozoa) and Mollusca?

We applied immunocytochemical staining in conjunction with confocal laser scanning microscopy (CLSM) and 3D-reconstruction, as well as transmission electron microscopy (TEM) in order to answer our research questions and to provide a model for the supposedly basal condition in Mollusca.

Our data reveal some interesting new features of neurogenesis in Solenogastres. First, no signs of segmentation are discernible during the development of the nervous system in both species. Instead, neurogenesis starts from an apical as well as an abapical proliferation center and the two – more dorsally situated – lateral nerve cords develop earlier than the pair of ventral ones. Furthermore, a fifth longitudinal concentration of serotonergic cells, which occupies a ventromedial position, is at least transitorily present. A complex apical organ of the “Kamptozoa-Polyplacophora-type“ was not found in any of the two species.

## **Myogenesis in *Wirenia argentea* (Solenogastres, Mollusca)**

**Maik Scherholz**

maik.scherholz@univie.ac.at

*PhD Thesis*

*FWF Project P24276: Integrative developmental studies on basal molluscs*

*Supervisor: Andreas Wanninger*

Mollusca is an extraordinarily rich metazoan phylum, second only to Arthropoda. Approximately 130.000 described extant species and a wide range of morphologically distinct taxa including Polyplacophora, Solenogastres, Caudofoveata, Monoplacophora, Bivalvia, Gastropoda, Scaphopoda and Cephalopoda have evolved within the phylum. The vermiform and spicule-bearing aplacophoran Solenogastres (=Neomeniomorpha) and Caudofoveata (=Chaetodermomorpha) are often considered as basal groups, which supposedly share several characters with the hypothetical last common molluscan ancestor. However, despite intense research, the evolutionary origin of molluscs is still highly controversial. Amongst others, the question whether or not the “urmollusc” had a segmented bodyplan, is still unresolved. In order to contribute to these issues, we use an “evo-devo-approach”. We focus on myogenesis in the model *Wirenia argentea* (Solenogastres) to test for recapitulation of ancestral segmentation traits during development. In contrast to segmented phyla such as annelids and arthropods, preliminary results show no evidence of teloblastic growth, but rather a simultaneous appearance of seven paired dorso-ventral muscles as well as a distinct ring musculature. Furthermore, we found a complex muscle system in premetamorphic larval stages of the solenogaster *Wirenia argentea* that shows striking similarities to polyplacophoran larvae. Several characters such as an enrolling muscle, a ventro-lateral muscle and a rectus muscle were formerly

considered as apomorphies of Polyplacophora and must now be considered shared characters of Solenogastres and Polyplacophora. We also discovered a hitherto unknown muscle, which we term “ventro-median muscle”, in both solenogaster and polyplacophoran larvae. Most of the shared larval characters are transitory elements during myogenesis of *Wirenia* and are not present in adult specimens. The new findings support a close relationship of Solenogastres and Polyplacophora and, in the light of recent phylogenomic studies and fossil data, support the Aculifera hypothesis.

## **3D micromorphological methods for quantifying form and function of the baculum in cryptic bat species**

**Nele Herdina**

annanele.herdina@univie.ac.at

*PhD Thesis*

*Supervisors: Helge Hilgers and Barbara Herzig*

*Department of Theoretical Biology & Department of Integrative Zoology, University of Vienna; Mammal Collection, Natural History Museum Vienna*

Although the baculum (os penis) has long been used successfully in species discrimination and many authors have suggested that the baculum plays a mechanical role in copulation for bats and other mammals, its specific function is still unknown. The aims of my PhD project are 1) Quantifying intra- and interspecific variation of baculum morphology in pipistrelle bats. 2) Describing the precise mechanical function of the baculum. 3) Revising the “wing veins” taxonomic character. 4) Describing baculum ontogeny histologically. The bacula of museum specimens (Národní Muzeum, Prague) of *Pipistrellus pipistrellus*, *P. pygmaeus*, and *P. hanaki*, which had already been identified by molecular genetic methods, were imaged with high-resolution microCT and measured. The age of the bats was estimated by tooth wear and ossification of the metacarpal/finger joints. Wing bones were measured and “wing veins” patterns drawn. Geometric morphometrics will be used to quantify and locate variations in baculum shape. The function of the baculum was tested using finite element (FE) models of flying fox penes (of all seven species of *Cynopterus*; Museum Zoologicum Bogoriense). For each species, a resected baculum and an iodine-stained penis were microCT imaged. The microCT stacks were reconstructed and transformed into 3D FE-models using Amira, Mimics, and Geomagic Studio, and then analyzed using Strand7.

The material properties for bone (Young's modulus: 17300MPa, Poisson's ratio: 0.3), corpora cavernosa (Young's modulus: 17MPa, Poisson's ratio: 0.4), and the other soft tissue of the penis (Young's modulus: 0.035MPa, Poisson's ratio: 0.4) were taken from published sources. With a pressure of 0.004MPa applied normal to the surface of each penis, the deformation of the glans penis clearly reflects the shape of the baculum. Therefore, females may be able to perceive the shape and size of the baculum during copulation, and the baculum may allow males to expand the female cervix to optimize sperm deposition. The models also support the hypotheses that the baculum protects the distal part of the urethra during copulation and serves as a stiffening element within the soft glans penis tissue.

## Evolution and development of the cephalic region in conchiferan mollusks

**Tim Wollesen**

tim.wollesen@univie.ac.at

*Post Doc*

*Group Leader: Andreas Wanninger*

Conchiferan mollusks such as gastropods, scaphopods, cephalopods, bivalves, and monoplacophorans exhibit diverse body plans with heads differentiated to various degrees. Traditionally, the arrangement of ganglia or brain lobes in the cephalic region has been used to infer the evolution of the central nervous system (CNS). Among certain bilaterians distinct neurotransmitter expression domains have been assumed to be evolutionarily conserved. In my phd-project the CNS development of coleoid cephalopods, i.e. all recent cephalopods but nautilus, was investigated by means of immunohistochemistry and confocal laserscanning microscopy. Fluorescence-coupled phalloidin was used as a neuropil marker to reconstruct the developing brains. As early neuronal elements, FMRFamide-related neuropeptides and their encoding gene *InFMRF* are expressed in cell somata of the palliovisceral lobes of the pygmy squid *Idiosepius notoides*. Other neurotransmitters and peptides such as serotonin or the alpha-neuropeptide of the VD1/ RPD2-system are expressed in distinct domain significantly later during the development of several cephalopod species. Serotonin as well as FMRFamide-related peptides are expressed in similar neuronal subsets in corresponding developmental stages of *I. notoides* and *O. vulgaris*. This might serve as an indication for the homology of these neuronal subsets between decapod and octopod cephalopods.

In my postdoctoral project, expression patterns of selected homeobox genes that are involved in the establishment of the cephalic region are analyzed. Preliminary results indicate that

homeobox genes such as *hedgehog*, *gsx*, or *otx* are expressed in the CNS of *I. notoides* and appear to have a similar function as their orthologs in other bilaterians. More gene expression analyses are currently carried out on a number of other conchiferan homeobox gene orthologs and will provide deeper insights into the development and evolution of the molluscan cephalic region.

## **Unravelling deuterostome evolution: insights from the development of the hemichordate *Saccoglossus kowalevskii***

**Sabrina Kaul-Strehlow**

sabrina.kaul-strehlow@univie.ac.at

*Post Doc*

*Group Leader: Andreas Wanninger*

Hemichordate, together with Echinodermata and Chordata belong to the major taxon Deuterostomia. The supposedly derived chordates are known by a certain characters, such as notochord, neural tube, pharyngeal gill slits, post-anal tail, or characteristic left-right (*LR*) *asymmetries* in the organization of particular structures. However, the evolutionary origin of each of the aforementioned features is still unsolved. This is aggravated by the fact that still no consensus has been reached regarding phylogenetic relationships among deuterostome taxa, especially concerning non-chordate deuterostomes. While molecular phylogenetic analyses generally support a taxon Ambulacraria consisting of Hemichordata and Echinodermata, most morphological studies place Enteropneusta (or Hemichordata) in a closer relationship with Chordata. These arguments include the presence of a dorsal hollow neural tube, pharyngeal gill slits and a trimeric organization of the coelomic cavities. Although most of these morphological similarities between Hemichordata and Chordata have long been acknowledged, the development and ultrastructure of these organ systems remain largely understudied in hemichordates. For that reason, I investigated the development of the mesoderm and nervous system in *Saccoglossus kowalevskii* (Enteropneusta) using TEM, SEM, histology and computer aided 3D-reconstruction.

The data from this study show that the collar cord within the mesosomal region in *S. kowalevskii* develops by invagination of a middorsal plate that is closed in a posterior to anterior direction by

enclosing a central fluid-filled canal. The final collar cord is surrounded by a sheath of *extracellular matrix* and lies subepidermal in position. According to further ultrastructural details as synaptic vesicles, different neuronal cell types and a separation into somata and neuropil, the collar cord of enteropneusts is suggested to represent an integrative centre of the nervous system.

During early development of *S. kowalevskii* the five main coelomic cavities (single protoel, paired mesocoel and metacoel) derive as separate outpocketings from the endoderm via enterocoely. In contrast, the pericardium, a small sixth coelomic cavity within the anterior protoel derives from the ectoderm via schizocoely. Later on, pharyngeal gill slits develop as endodermal pouches that fuse with the ectoderm to form dorsolateral openings within the trunk region of *S. kowalevskii*. Moreover, I documented a left-biased development of the gill slits and skeletal rods, being reminiscent to the situation found in cephalochordates.

Concluding, the detailed analysis of the development of the collar cord, pharyngeal gill slits and formation of the coelomic cavities in enteropneusts altogether support previously acknowledged similarities with the corresponding structures found in chordates, i.e. neural tube, gill slits, and coelom formation. Furthermore, the data presented here lead to suggest homology between these structures. Following this, morphological data from this study support a closer relationship between Enteropneusta and Chordata.

## **A modeling approach to understand fluid dynamics and functional morphology in leucon-type sponge canal systems**

**Jörg U. Hammel**

joerg.hammel@uni-jena.de

*Institut für Spezielle Zoologie und Evolutionsbiologie, Friedrich-Schiller-Universität Jena, Germany*  
*Section Computational Science, Universiteit van Amsterdam, The Netherlands*

As sessile filter feeding animals sponges (Porifera) rely on highly efficient fluid transport systems. Due to the high complexity of the 3D network of incurrent and excurrent canals in the leucon-type aquiferous system only limited data on flow velocities and transport rates are available to date and are mainly based on theoretical assumptions. This is mainly due to the limited availability of morphometric and quantitative data on canal system architecture. The fresh water sponge *Spongilla lacustris* is an ideal model system to study flow in sponge. Due to its transparency flow velocities can be determined experimentally inside canals near the surface and at the osculum. The limited architectural complexity and small body size enables the extraction of a 3D canal system model from a single specimen. Reconstructions of the canal system were based on SR- $\mu$ CT data. Flow through the canal system was studied using a finite element modeling approach. In order to verify the results of our modeling approach we conducted flow velocity measurements by particle tracking velocimetry at the osculum. Outflow velocities were between 0.6 – 07 mm/s in our experiments and simulations. By quantitative morphometrics we determined the size and number of choanocyte chambers from a single specimen. Observed flow velocities in canal segments of different hierarchical orders were lower in our simulations than predicted based on calculations from the current model of flow for sponges. Results from our modeling

approach in combination with experimental observations lead to a new mathematical description for a more general flow model in sponges taking functional morphological features and the observed higher architectural complexity into account.

## **Multimodal Signaling in African Reed Frogs: Attraction through calls, colors and chemical cues?**

**Iris Starnberger**

iris.starnberger@univie.ac.at

*PhD Thesis*

*Supervisor: Walter Hödl*

During calling the vocal sac serves anurans as an air reservoir, in minimizing the loss of sound energy and in distributing calls omnidirectionally. The vocal sac's role beyond acoustics has been largely neglected in the past. The diversity in vocal sac coloration and shape found in different anuran species is striking to the human observer and recently the vocal sac's role as visual cue has gained vast attention. Males of many reed frog species (Anura: Hyperoliidae) have a prominent colorful patch on their vocal sac, which gains in conspicuousness once the vocal sac is inflated. To date, the function of this gular patch remains unknown.

Histological analysis indicates that the gular patch is a gland extending into the vocal sac cavity. We suggest that this gland, which exceeds 10 times the thickness of the vocal sac skin, produces volatiles emitted while calling. Furthermore, the coloration of vocal sac and gular patch might be species specific and serve as visual signal component in calling males. First results of an explorative study integrating histological and biochemical analyses, spectrometry and behavioral experiments to investigate signal modalities and their role in intra- and intersexual communication will be presented.

We propose that reed frogs use a complex combination of acoustic, visual and chemical signals in species recognition and mate choice.

## **Determinants of fitness in species with complex life cycles – an island experiment in the Neotropical frog *Allobates femoralis***

**Eva Ringler, Max Ringler**

eva.ringler@univie.ac.at, max.ringler@univie.ac.at

*Post Docs*

*FWF Projekt P24788: Determinants of fitness in species with complex life cycles: an island experiment in the Neotropical frog *Allobates femoralis**

*Project Leader: Eva Ringler*

Amphibians hold a unique position as the only vertebrate group where basically all members feature complex life cycles with two distinct free-living stages that have to face entirely different challenges in their respective environment. Amphibians are often portrayed as generally congregating at confined aquatic sites to deposit rather large egg clutches during short reproductive bouts, but they actually show a remarkably wide array of different reproductive strategies. Neotropical poison frogs (Dendrobatidae), for example, hold an extraordinary position within amphibians due to their elaborate reproductive behaviour which is found across the whole taxon. Dendrobatid frogs are characterised by prolonged breeding, territoriality of at least one sex, and uni- or biparental care.

The pan-Amazonian frog *Allobates femoralis* (Dendrobatidae) is a highly suitable species for studies at the individual and also population level. Throughout the prolonged breeding period, which corresponds to the rainy season, males are highly territorial. Females are iteroparous and show strong site fidelity. Courtship and mating takes place inside the male territories. The *A. femoralis* population which has been studied from 2008-2012 is located near the research station ‘Saut Pararé’ in the nature reserve ‘Les

Nouragues', French Guiana. Through molecular parentage analyses of two successive generations of adult individuals a highly polygynandrous mating system was identified. Despite significant differences in the reproductive behaviour of males and females, successful individuals of both sexes had similar numbers of mating partners and produced similar numbers of adult progeny. Furthermore, a high percentage of the males and females in the population produced progeny which survived until adulthood. Reproductive success was significantly higher in territorial males, but not related to territory size in males or body size in both sexes. Parentage assignments of one offspring generation at two different life history stages were used to identify patterns of parental relatedness on reproductive success in *Allobates femoralis*. There was no correlation between genetic and spatial distance in our population under study. Through a stepwise spatial restriction of candidate males we were able to discern between spatial and genetic effects of mate choice. Females mainly (in 72%) chose their mating partners within a radius of 20 m. Our results show no evidence of behavioral inbreeding avoidance in *A. femoralis* but indicate a selective benefit of matings between partners of intermediate genetic divergence. Territory occupancy, but not territory size, was identified as a significant determinant of reproductive success in *A. femoralis* males. Given the distinct difference between the core area that is occupied during calling and the area a male is willing to defend, results in two possible definitions of male territory size. Our finding that the extension of the defended area did not correlate with the area occupied during calling is of particular relevance to studies that investigate the effect of territory size on reproductive success. In a current FWF project we will investigate the determinants of fitness in the Neotropical frog *Allobates femoralis*, by exploring the effects of parental relatedness and parental investment on individual reproductive success at various life cycle stages and the associated patterns of space use. To this end we have installed an experimental population of *A. femoralis* on a river island of ~5 ha in size. The island is situated in close distance to the population on the mainland

where we have been conducting research since 2008. Additionally, we perform specific breeding and behavioural experiments with an ex-situ breeding population at the University of Vienna. The combined approach of controlled and natural field studies, and laboratory experiments will allow us to address questions on the effects of parental relatedness on fertilization success, larval survival in the clutch and survival of post-metamorphic juveniles. At the same time the island population and experiments in captivity allow us to answer questions on patterns of juvenile dispersal and adult spatial behaviour, as well as the use of spatial, visual, and acoustic information for parental behaviour and space use in males and females.

## **Brain anatomy in Diplura**

**Alexander Böhm**

alexander.boehm@univie.ac.at

*Diploma Thesis*

*FWF Project 20497 "Are the Hexapoda monophyletic?"*

*Supervisor: Günther Pass*

Since the best descriptions of dipluran brain anatomy go far back to Hanström (1940) the brains of *Campodea augens* and *Catajapyx aquilonaris* were re-investigated in detail. The analyses are based on 3D reconstruction of the major neuropils and tracts from semithin section series and on antibody staining. Remarkable are the large central body with 9 subdivisions and the well developed mushroom bodies. Antibody staining against a catalytic subunit of protein kinase A (DC0) was used to further characterize the mushroom bodies. In both investigated dipluran species the mushroom bodies glomerular calyces the lateral protocerebrum that receive input from the olfactory lobes. *Catajapyx* possesses mushroom bodies that are connected across the midline, a unique condition within hexapods. While mushroom- and central body structure in *Diplura* clearly indicate a common origin, they are more difficult to interpret in a wider phylogenetic context.

## **Bumblebees (*Bombus*) in Vorarlberg (Western Austria) - faunistics, flower visiting habits, habitat usage and association**

**Bernhard Schneller**

bernhard.schneller@univie.ac.at

*Diploma Thesis*

*Supervisor: Harald Krenn*

Large areas of Vorarlberg (Austria) are underrepresented with reference to research on bumblebees. About 5000 individuals belonging to 34 *Bombus* species (of 46 species all over Austria) had been collected in Vorarlberg at 322 localities, mainly below 2000 m a.s.l.. The aim of this study was a mapping of the bumblebees of Vorarlberg, focussing mainly on uninvestigated areas. In addition, a survey of different ecological factors was conducted. Within the period from 1 July to 30 August 2012 more than 2700 individuals belonging to at least 24 species of the genus *Bombus* were documented in Vorarlberg (Austria). The individuals were collected at 407 localities at altitudes ranging from 400 to 2780 m a.s.l.. Based on the collected data, an analysis of habitat preferences, specific flower visiting behaviour and associations of the different *Bombus* species will be conducted. The collected data shows that *B. pascuorum* and *B. hortorum* are the most frequent species at lower altitudes (< 1500 m a.s.l.). In contrast, at higher altitudes (> 1500 m a.s.l.) most individuals belong to *B. pyrenaicus*, *B. wurflenii* and *B. monticola*. Furthermore, the most frequently visited flowering plants can be found within the genera *Trifolium*, *Cirsium*, *Carduus*, *Rhododendron*, *Impatiens* and the families Lamiaceae and Campanulaceae. Moreover, the highest abundance of bumblebees is expected to appear at altitudes between 1300 and 2300 m a.s.l.. In addition, nectar robbery was proven for short-tongued species (e.g. *B. wurflenii*, *Bombus terrestris*, *B. lucorum* complex). The first record of *Bombus alpinus* in Vorarlberg since 1931 is remarkable.

## **Insights in the evolution of larval development in Branchiopoda (Crustacea) using an Event-Pairing approach: First indications of the evolutionary transformation towards Cladocera**

**Martin Fritsch**

[martin.fritsch@uni-rostock.de](mailto:martin.fritsch@uni-rostock.de)

*Zoological Institute, University of Rostock, Germany*

Cladocera, also known as water-fleas, are the ecologically most important group within the Branchiopoda (Crustacea). They are unquestionably branchiopods but their evolutionary origin remains unclear. One favored explanation of their origin is that they evolved from a reproductive larva of a “clam shrimp-like” ancestor. In order to reveal a transformation and to identify (potential) changes in chronology (heterochrony), we investigated and compared the development of representatives of all six recent branchiopod taxa. One of the Anostraca, (*Branchinella* sp.), one of the Notostraca (*Triops cancriformis*), one of the three clam shrimp taxa: Laevicaudata (*Lynceus biformis*), Spinicaudata (*Leptestheria dahalacensis*) and Cyclestherida (*Cyclestheria hislopi*); and one of the Cladocera (*Penilia avirostris*). All six taxa develop indirectly, although the exact modes of the development are quite different, especially those of the Cyclestherida and Cladocera in comparison to all the others. The development of the nervous system, labeled and analyzed using immunohistochemical techniques was investigated with a confocal microscope. That of the external morphology was scanned either with an electron microscope or the autofluorescence of the external cuticle was detected with a confocal microscope. *Branchinella* sp., *T. cancriformis*, *L. biformis* and *L. dahalacensis* hatch as a free-swimming nauplius and the nervous system and external morphology develops gradually. In *C. hislopi* and *P. avirostris*, on the other hand, several internal and external structures develop prior to the hatching of non-swimming

embryo-like larva which is still carried in a dorsal brood pouch. The development in *Branchinella* sp., *T. cancriformis*, *L. biformis* and *L. dahalacensis* is directed from anterior to posterior, whereas in *C. hislopi* and *P. avirotris* a more synchronous anterior and posterior differentiation is present. A comparative analysis of all the developmental sequences using a Parsimov Event-Pairing approach gives us the first indications of the evolutionary transformation which the Cladocera may have undergone from a clam shrimp-like ancestor.

## **Neurogenesis in *Lyrodus pedicellatus* (Bivalvia) as inferred by immunocytochemistry and confocal microscopy**

**Tabea Gianordoli**

tabea.gian@yahoo.de

*Diploma Thesis*

*Supervisor: Andreas Wanninger*

*Lyrodus pedicellatus* is a marine bivalve mollusc that belongs to the Teredinidae, the so-called shipworms. They have a worm-like body and are wood-boring. The larvae of *Lyrodus pedicellatus* develop in the gills of the adult shipworms. Although some data concerning neurogenesis of mollusks are already available, bivalve neurogenesis is only little investigated. In this work the development of serotonin-, FMRFamide- and tubulin-immunoreactive components are examined using immunocytochemistry and confocal microscopy.

The earliest larval stage found has already a velum with an apical tuft and well-developed serotonin-containing apical cells (apical organ). During development these apical cells seem to disappear and at the same time serotonergic cells of the pedal and cerebral ganglion as well as the visceral loop appear. By this time the larvae are already in a late veliger stage with a well-developed velum. In this stage the serotonin innervation of the foot is already well developed. With subsequent growth, in pediveliger stages, the serotonergic cells of the cerebral and pedal ganglia, the visceral loop develop further, as does the serotonin innervation of the foot. In the late pediveliger stage the velum is nearly reduced. The expression with the neuronal marker FMRFamide is so far inconclusive and needs further attention.

## **Myogenesis in the wood boring bivalve *Lyrodus pedicellatus***

**Andrea Wurzinger-Mayer**  
amawu44@gmail.com

*Diploma Thesis*  
*Supervisor: Andreas Wanninger*

Among bivalves *Lyrodus pedicellatus* has a very unusual worm-shaped body. The larval stages, however, seem to follow the traditional bivalve way of development and undergo a trochophore, veliger and pediveliger stage with transitory larval musculature, adult muscles and a hinged round shell. Our research focuses on larval and early juvenile stages of this mollusc and the remodelling of the muscular bodyplan during metamorphosis. To visualize the muscle cells, fluorescence staining of filamentous actin (F-Actin) in combination with confocal laser scanning microscopy (CLSM) and 3D reconstruction software was applied. Myogenesis starts with a pair of dorsal and median velum retractors. Simultaneously with the larval muscles, the first anlagen of the adult anterior adductor muscle form. Slightly later the paired anlage of the foot retractors develops, which soon fuses to a median structure that represents the converging part of the retractors. Presumptive foot rotators connect to the muscular grid of the foot. A massive posterior adductor co-occurs with a well-defined accessory adductor. Prior to metamorphosis the degradation of the velum and its retractors starts. Both structures are eventually lost during metamorphosis. Further investigations of myogenesis between the early trochophore and metamorphosis will assess, as to what degree larval muscles are incorporated into the adult bodyplan.

## **Expression of the VD<sub>1</sub>/RPD<sub>2</sub> $\alpha$ 1-neuropeptide during development of a basal gastropod**

**Melanie Schreiner**

melanie-schreiner@gmx.de

*Master Thesis*

*Supervisor: Andreas Wanninger & Martin Glösmann (VetMed)*

Several molluscs express the VD<sub>1</sub>/RPD<sub>2</sub>  $\alpha$ 1-neuropeptide in their central nervous system (CNS). It was first detected in the pulmonate gastropod *Lymnea stagnalis* where it is predominantly expressed in the visceral and right parietal ganglion. Further examinations revealed the expression of that neuroactive compound in the CNS of other adult gastropods, bivalves and cephalopods. The present study examines the expression of the VD<sub>1</sub>/RPD<sub>2</sub>  $\alpha$ 1-neuropeptide during development of the patellogastropod *Lottia kogamogai*, which is considered as a basal gastropod. With the use of immunocytochemistry and confocal laser scanning microscopy (CLSM) the distribution of the VD<sub>1</sub>/RPD<sub>2</sub>  $\alpha$ 1-neuropeptide was examined during larval development and in adults of *L. kogamogai*. Trochophore and pretorsional veliger larvae were devoid of signal. The VD<sub>1</sub>/RPD<sub>2</sub>  $\alpha$ 1 neuropeptide first appears in the posttorsional veliger. The nervous system of veliger larvae prior to metamorphosis shows prominent tentacle nerves, anlagen for the future cerebral ganglia, a visceral loop and anlagen for the pedal ganglia with foot nerves branching into the anterior and posterior side of the foot. VD<sub>1</sub>/RPD<sub>2</sub>  $\alpha$ 1-neuropeptide expression is restricted to the anlage of the future cerebral ganglia, the visceral loop and the anlage of the pedal ganglia. In the adult animal, the cerebral ganglia have a high expression of that neuroactive compound. The results obtained herein show that the VD<sub>1</sub>/RPD<sub>2</sub>  $\alpha$ 1-neuropeptide is not present in larval neural components that are lost during metamorphosis, such as the apical organ. By contrast, the

expression is restricted to the ganglionic anlagen of the future adult CNS. This strongly suggests that the  $VD_1/RPD_2$   $\alpha 1$ -neuropeptide is primarily important for postmetamorphic life cycle stages of *Lottia kogamogai*. Whether this holds true for other gastropods (and molluscs) as well needs to be assessed by further comparative neurodevelopmental studies.

## Ten year of research and teaching in Red Sea coral reefs

**Jürgen Herler**

juergen.herler@univie.ac.at

***Project:** Adaptations and mutualism between corals and fishes*

*Project Leader: Jürgen Herler*

Abstract: At the end of my current FWF-project, I want to use the opportunity to present a short summary of my main research findings and university teaching in the northern Red Sea from 2003 to 2012. The northern Red Sea is a perfect region for doing research in shallow water coral reefs. Reefs are generally in very good condition and abundant there. They are species-rich and can be easily accessed from land. Diverse habitats (coral reef, seagrass meadows, mangroves) are available within a one-hour-car-ride. Therefore, and because of its close distance to Europe, it is not only a good destination for student courses, but also for doing field research. During my last research projects, I focussed on the habitat associations and morphological adaptations of highly specialized reef fishes. This involved mainly gobiids of various genera but in particular the coral-associated genus *Gobiodon*. These gobies are a highly interesting model group of ecological and morphological specialisation and my students and I discovered several important traits in these fishes. Apart from social-status-dependent migration rates, we discovered that fishes decide about coral condition when choosing host corals and might therefore serve as reef health indicators. In addition, their toxic skin was found to be an effective predator repellent and may additionally serve as a repellent for fishes which feed on their host corals, as field and laboratory experiments have revealed. Therefore we can assume a mutualistic relationship in that these fishes may reduce the impact of corallivory on host corals. Geometric morphometric measurements and displacement experiments have found a very strict phenotype-

habitat match and confirmed a specific relationship between fish size and shape and coral geometry. The importance of specific characters of coral geometry (branch length, interbranch distance) was also reflected in their correlation with coral colony size. The dependence of fishes on large corals with specific geometry may have fundamental impact on the fitness of associated fishes when reef deterioration changes not only coral abundance but also average colony size. Such changes may favor selection of smaller species and/or earlier maturation and may change competition patterns between species. Other, more fundamental ecological consequences may include an increased extinction risk of extreme habitat specialists.

## **Feeding strategies in a territorial corallivorous butterflyfish with respect to coral identity, size and occupation by coral gobies**

**Ulrike Harant**

uharant@gmx.at

*Master Thesis*

*Supervisor: Jürgen Herler & Andreas Wanninger*

Corallivory is an equivalent to terrestrial plant-herbivory and therefore important for the successful functioning of marine ecosystems. Nonetheless, the role of coral feeding butterflyfishes has long been underestimated. In this study the food preferences of an obligate corallivorous butterflyfish *Chaetodon austriacus* were determined on a fringing reef near Dahab, in the Gulf of Aqaba, northern Red Sea. The main prey corals of *Chaetodon austriacus* were *Acropora*, *Stylophora* and *Pocillopora*. Ivlev's electivity index revealed that only *Acropora selago* and *Pocillopora* were consumed more than they were available whereas *Stylophora* was consumed according to its availability. The density of *Acropora selago* was found to be an important determinant for territory size in *Chaetodon austriacus* as high density of this coral species lead to smaller territories along with higher feeding rates of the fish. Thus, it can be assumed that *Acropora selago* is a high quality prey coral for *Chaetodon austriacus*. Predation on the coral colony level was investigated by tagging corals and collecting data on the frequency of visits and the bites taken per visit and fish. Predation frequency was very high on large colonies but predation intensity, as it is size-related, decreased with colony size. Furthermore, larger colonies were preferred over medium and especially over small-sized coral colonies.

The hypothesis that mutualistic gobies of the genus *Gobiodon* are able to defend their host colony from high predation by butterflyfishes could be confirmed and the size in which this effect

is present could be determined. Colonies of *A. selago* with occupation of *Gobiodon* sp.3 in a size range of 250-500 cm<sup>2</sup> received significantly less bites and visits per hour and less time was spent feeding on occupied colonies than on unoccupied. Predation intensity was highest in small and medium-sized colonies as it is size-related. Due to ongoing climate change along with frequent bleaching events, future acroporid coral communities are expected to consist mainly of juvenile small or medium-sized colonies. Thus, the effect of resource defense of mutualistic gobies will become even more crucial, as they can direct predation of butterflyfish towards unoccupied coral colonies.

## **Origin and early splits in Hexapoda: A multidisciplinary approach within the 1 kite project**

**Daniela Bartel, Nikola U. Szucsich**

daniela.bartel@univie.ac.at, nikola.szucsich@univie.ac.at

*PhD Thesis (D.B), Post Doc (N.U.S)*

*FWF Project 23251 "The most ancient splits in insect phylogeny"*

*Project Leader: Günther Pass*

Starting more than 400 million years ago, the hexapods evolved to the most successive groups of organisms in terms of species richness, diversification and adaptive radiation. While generally highly diverse, differences among groups become puzzling when comparing the oldest lineages of hexapods, the primarily wingless groups, with pterygotes. The resulting disparity is even more perplexing when adding crustacean subgroups, discussed as the potential sister group of Hexapoda, to the comparison. Allowing for no clear polarization of phylogenetic characters, these factors impede reconstructing the earliest splits and consequently the early evolution of hexapods. Five primarily wingless groups are traditionally subsumed in an assemblage denominated "apterygotes" or "basal hexapods": Collembola, Protura, Diplura, Archaeognatha, and Zygentoma. The evolutionary period in which their ancestors splitted from the stem lineage of pterygotes is marked by two major transitions, and thus is of special interest regarding character evolution: (i) the conquest of land, or terrestrialization, and (ii) the conquest of air, with the evolutionary acquisition of wings.

The first phylogenomic studies could not unambiguously resolve the relationships among primarily wingless insects. Thus new methods have to be used to specifically test for conflicting signal, and to look for explanations of the causes of these conflicts. The advent of phylogenomics, is characterized by (i) an underrepresentation of basal hexapods and (ii) a high amount of

missing data. Embedded into the recently started 1KITE project which aims to sequence and analyze 1000 insect transcriptomes, working groups of 3 universities (Austria, Germany, and Japan) focus on the relationships and evolution of primarily wingless insects. With about 100 taxa final aim of this project is to analyze the largest taxon sampling concerning the basal hexapod lineages. Phylogenetic questions to be tested encompass: (i) monophyly of Hexapoda and Entognatha, (ii) sister group relationships among the entognathous groups (Collembola, Protura, Diplura) (iii) the sister group of the Hexapoda, and (iv) the phylogenetic position of the living fossil *Tricholepidion gertschi*. We will analyze not only molecular data sets but also investigate the reliability of these results with new data from developmental, embryological and morphological studies. First results will be presented along with focus, aims and workflow. The high coverage of the preliminary data matrix is perfectly suited to apply the described methods of specific testing.

## **Open the gates to the diversity of Protura: Establishing DNA barcoding in a taxonomically challenging group**

**Carol Resch**

carol.resch@univie.ac.at

*Diploma Thesis*

*FWF Project 20497“Are the Hexapoda monophyletic?”*

*Supervisor: Günther Pass*

Protura are tiny entognathous hexapods living in the soil. Due to the lack of conspicuous morphological characters, species determination is extremely challenging and only a handful of scientists are able to accomplish this task.

The present study was triggered by the close spatial vicinity of our university to the worlds' biodiversity hotspot of Protura where 23 syntopic species could be found (Christian and Szeptycki 2004). To test the species diversity recorded by traditional morphological determination, the molecular range of 171 specimen of Protura occurring in Austria was examined with two markers, the DNA barcoding region of the mitochondrial CO1 gene and a fragment of the nuclear 28S rDNA. Aim of this pilot project was the establishment of DNA barcoding in Protura applying a non-destructive DNA-extraction method.

The commonly used CO1 Folmer primer-set for invertebrate taxa produced no useful results even under various PCR conditions. Therefore primer design and testing became an essential part to establish DNA barcoding in Protura. Due to high intergeneric variation at both ends of the DNA barcoding fragment, the newly designed PCR-primers worked in certain genera only, but performed poorly when applied across our complete taxon sampling. A small set of 28S rDNA primers turned out to work efficiently and almost universally across the investigated proturan genera, but length variation in divergent domains and problems in

sequencing argue against the use of 28S rDNA as an alternative DNA barcode, at least for Protura.

We successfully generated 88 CO1 barcodes (mean length 750 bp) and 90 28S rDNA sequences (approx. 1000 bp). Both markers separate the investigated proturans into 5 major clades and 13 well supported sub-clades. The recorded molecular clusters are exactly corroborated by the morphological determination at both genus and species level. Additionally we discovered a new species to science and the first record of a species for Austria.

Our study demonstrated the great value of DNA barcoding for taxonomically challenging groups. It certainly will help to facilitate species determination in Protura for a broader scientific community. This will be of special importance for many studies in soil ecology where this hexapod group presently remains undetermined in almost all cases.

## **Neuroanatomy of the pilidium larva of *Hubrechtella dubia* (Nemertea)**

**Philipp Maier**

office@imkerei-maier.at

*Master Thesis*

*Supervisor: Andreas Wanninger*

Nemerteans are bilaterally symmetric, dorsoventrally flattened, mainly marine predators and scavengers. An important morphological feature shared by all nemerteans is an eversible proboscis which may be equipped with a stylet to pierce prey. In a recent phylogenetic study nemerteans are divided into the stylet-bearing Nemertea (Hoploneurata) and the non-stylet bearing groups (Paleoneurata, Hubrechtidae and Heteroneurata). Hubrechtidae and Heteroneurata constitute the monophyletic Pilidiophora that share a pilidium larva. The juvenile worm develops from imaginal discs that are located within the larva and in a process known as catastrophic metamorphosis breaks through the larva and devours it.

By use of immunohistochemistry and confocal laser-scanning microscopy the present study aims to describe the nervous system of the pilidium larva of *Hubrechtella dubia* and identify which larval nerve structures are incorporated into the juvenile worm. Preliminary results show the presence of a nerve ring that runs along the margin of the four larval lobes, an oral nerve ring between the vestibule and the stomach, an apical plate with apical tuft and two so far undescribed serotonin-like immunoreactive cell clusters in the posterior region of the larva. In later stages the juvenile and the larva share the same digestive tract. A recent study on *Lineus albocinctus* shows that the oral nerve ring is incorporated into the juvenile worm. Comparative analyses of the data from *Lineus albocinctus* and *Hubrechtella dubia* will allow for assessing similarities and differences concerning neurodevelopmental traits,

including the degree of incorporation of larval neural components into the juvenile nervous system. Thus, our study will contribute reconstructing the neural groundplan of nemertean pilidium larvae.

## **Morphometric and genetic analyses of Mediterranean *Gibbula* species (Gastropoda)**

**Affenzeller Susanne & Haar Nicole**

susanne.affenzeller@aon.at, nicole.haar@hotmail.com

*Master Theses*

*Supervisor: Gerhard Steiner*

*Gibbula* is a genus of snails, first described by Risso in 1826. *Gibbula* belongs to the clade Vetigastropoda, the superfamily Trochoidea and the family Trochidae. *Gibbula* have an atlantic and mediterranean distribution with many species. The shell is shaped like a top, with convex whorls and a high variation in colours and colouration patterns. The only good diagnostic feature for the genus *Gibbula* is the more or less open umbilicus. They can be found abundantly in the shallow subtidal grazing algae on or under gravel. The original descriptions in literature are very vague and the identification literature varies a lot and has imprecise definitions. In addition to these difficulties, type material does not exist or cannot be found or located. Furthermore there are no holotypes or lectotypes, but in some cases many syntypes. Having had a look at all these obstacles we encounter in trying to identify *Gibbula* species, we face the following questions: How many species of *Gibbula* do we have in the Northern Adriatic Sea and around Crete? Can we find the same species composition in Crete and in Croatia? If no, how do they differ? Our sample includes two main locations, namely the northern Adriatic Sea (Rovinj) and Crete. A dual method approach will be used combining geometric morphometric with DNA barcoding, therefore connecting molecular results with morphological characters. The morphometric analysis will be done on shells only, using two standardized photos of each shell, one lateral and one ventral. These will be processed using a Generalized Procrustes Fit standardizing, Principle Components Analysis and Thin Plate Splines. The molecular part of the project will be done

by DNA barcoding procedures, using the standard markers CO I and 16S. Our hypothesis therefore is that genetic clades will correlate with morphological groups. We aim to find out how many clades can be found in our sample. The future steps we aim to take with this project are to identify the *Gibbula* species composition in the Eastern Mediterranean Sea. Furthermore a taxonomic revision of the here identified species will hopefully be done.





**Veranstalter:**

Department für Integrative Zoologie  
Fakultät für Lebenswissenschaften  
Universität Wien  
Althanstrasse 14, 1090 Wien

**Organisation:**

Andreas Wanninger

**Abstractband:**

Sonja Matuš

**Titelbild:**

Harald Krenn

*integrative*  
**zoologie**