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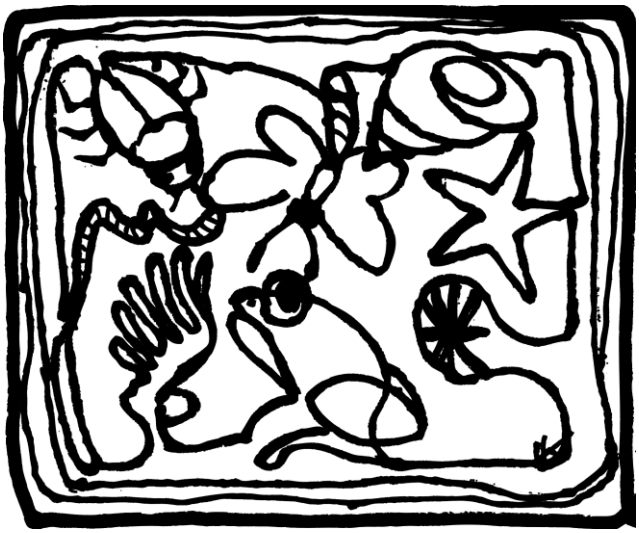
integrative zoologie

DEPARTMENTAL SEMINAR INTEGRATIVE ZOOLOGY Summer Term 2015

Programme and Abstracts

Tuesdays, 10-11:30 hrs
SR 3, UZA1, Althanstraße 14, 1090 Wien



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Breeding Carnolian bees on different comb cell sizes and analyzing the effect of the different cell sizes on the *Varroa* infestation rates.

(Small breeding cells in honeybee - An experimental approach.)

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Worldwide on Apis mellifera the "new" ectoparasite Varroa destructor is considered as a major cause of bee colony-looses.
In 1997, after the arrival of the Varroa mite (*Varroa destructor* Anderson & Trueman, 2000) in Arizona, Ed and Dee Lusby observed a better survival rate of their colonies on honey combs with a small cell size (5,08 mm). Nowadays the western honeybees are commonly kept at the cell size 5,4mm to 5,6 mm. In this study, I analyzed whether small cells (4,9 mm) have a negative effect on the population development of varroa mite compared to the standard cell size (5,5 mm).
First I had to search in my Carnolian (*Apis mellifera carnica* Pollmann, 1879) closed breeding population for colonies who could built small cells. Then I created 2 groups of test colonies consisting of young sister queens and 1,5 kg varroa-infested bees on small cell size (4,9 mm) and large cell size (5,5 mm) respectively. To prevent any contamination with residues from miticides new hives and organic-certified foundation were used. The bottom boards were equipped with mesh-protected drawers to collect the natural mite mortality.
The data of 3 years, analyzing 494 test-colonies, have shown a positive result: the colonies on small cells had a slower varroa-mite population development.
For 2 additional years the *Varroa* reproduction parameters were determined.

Four lines with 80 new colonies on large cells and small cells with small sized bees were created. From each colony a comb with older sealed brood was analyzed for reproductive success of *Varroa*.
The following year 2 lines with small cells queens and bees were used for 20 new test-colonies. Each fitted with 4 small cell and 4 large cell drawn combs alternately positioned. The infested brood cells were analyzed again.
The collected data allowed to calculate the VSH parameter (=*Varroa* Sensitive Hygienic) as the quotient of brood cells with a non-reproductive varroa per total number of infested brood-cells.
Comparing both cell sizes in one colony has shown that there is a higher level of VSH for infested brood-cells on small cells in the selected Carnolian bee population.
The heritable traits "ability to construct small cell size comb" plus "VSH" results in a reduced population growth of Varroa in those colonies where worker bees when both traits are present.
It remains unclear what made the VSH-activity on small cell size combs more intensive as compared to the large cell size infested cells in the same colony.

WE ALL KNOW WHAT A SPECIES IS, OR DO WE…?
Species concepts and the species problem – new insights and developments in a never-ending debate.

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Mammal Collection,
Natural History Museum Vienna

Species are the most fundamental and most important units in evolutionary biology and systematics. The talk aims at giving an accessible overview of the main topics and difficulties of the species

problem – what is a species, and why are there so many seemingly incompatible species concepts? Somewhat surprisingly, the question what a species is (its ontology) is not so contentious at all; it is species delimitation that is much more problematic. I will present a short summary of the following issues: history of species thinking; species taxa vs. the species category; (philosophical) classes and individuals in taxonomy; the presently accepted hierarchy of species concepts that distinguishes ontological and operational concepts; recent trends in biological systematics involving the growing popularity of the Phylogenetic Species Concept (PSC) and its serious negative consequences

One hundred meters below sea level - a collection of fish.

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Flower handling and suction feeding: Adaptations for nectar-feeding in long proboscid flies of South Africa.

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Several South African fly species of Nemestrinidae and Tabanidae have proboscides that are highly variable in length and up to twice as long as the body in some individuals. Adapted to long-spurred flowers, they represent model organisms for reciprocal adaptation and are considered as important specialised pollinators in the Cape Floristic Region. Morphological investigations of the feeding apparatus of Prosoeca (Nemestrinidae) revealed a proximal proboscis composed of several interlocked components. The elongated distal part is formed by a single component, making this proboscis structure unique amongst nectar-feeding insects. Field video analyses of flower handling time verified that long-proboscid individuals spent more time on flowers then shorter-proboscid individuals. The two-part suction pump in the head, however, showed a positive allometric relationship with proboscis length, suggesting, that long-proboscid individuals are able to take up more nectar in a single visit. Proboscis and suction pump morphology of blood- and nectar-feeding females of Philoliche (Tabanidae) are similar to Prosoeca, i.e. the proboscis relies on a pressure gradient provided by a two-part suction pump, and which showed a positive allometric relationship with proboscis length. The proboscis differed, however, as it is divided into two functional units – a short, proximal piercing and an elongated single piece nectar up-taking part. Preliminary results of morphometric measurements taken from Prosoeca revealed a significant temporal variation in body and proboscis length, leading to the putative conclusion that allometric relationships rather than coevolution between flowers and flies might have led to the formation of an elongated proboscis in nemestrinid flies.

In conclusion, I revealed the structural adaptations of proboscis and suction pumps associated with an elongated proboscis in context with the coevolution of nectar feeding in Brachycera. The interdisciplinary approach allowed new insights into the adaptations to nectar feeding in these remarkable insects.

NanoSIMS, light and electron microscopy, a correlative approach to study carbon transfer in the giant ciliate symbiosis.

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Evolutionary theory of cooperation defines inter-species cooperation as mutual beneficial association, in which benefits received from the partner exceed the costs incurred. However, empirical data are extremely scarce. They require model systems, which can be cultivated and experimentally manipulated. Among the thiototrophic (sulfur-oxidizing, chemolithoautotrophic) symbioses only the colonial ciliate Zoothamnium niveum and its ectosymbiont Candidatus Thiobios zoothamnicoli has been successfully cultivated so far. Therefore this system is especially suited for testing what are the benefits provided by the partners and under which conditions mutualism is maintained. This symbiosis colonizes shallow-water whale and wood falls and plant debris. It grows extremely fast and reproduces rapidly under controlled conditions in flow-through aquaria. We performed 14C and 13C pulse chase experiments under various chemical conditions and tissue autoradiography as well as NanoSIMS analysis to follow carbon fixation, -incorporation, and translocation. We confirmed that the symbionts fix inorganic carbon in presence of sulfide. In the absence of external sulfide they utilized the internal sulfur storage. Translocation of fixed organic carbon from the symbiont to the host was evident as label over host tissue after a short pulse of 25 min indicating fast release and uptake and incorporation into

host tissue. In addition translocation of fixed organic carbon through digestion of the symbiont was also highlighted as label over host tissue increased over time during the chases. Digestion of symbionts was confirmed by the observation of digestive vacuoles under the TEM after cytochemical detection of the lysosomal enzyme acid phosphatase. Fluorescent in situ hybridization also confirmed that the bacteria observed in such digestive vacuoles are the symbionts. While both types of translocation (release of organic compounds and digestion) have been already shown in some thiotrophic endosymbiosis, this is the first time we can demonstrate these processes in an ectosymbiosis. We also performed a set of cultures and estimated host and symbiont fitness under various abiotic and biotic conditions. We conclude that host nourishment comes from symbionts and free-living microbes in about equal shares.

Extremely long mouthparts in butterflies (Lepidoptera): Form, function and evolution.

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Extremely long mouthparts of insect flower visitors evolved by natural selection to gain access to highly rewarding deep-tubed flowers. Such morphological specializations have been regarded as the outcome of a mutualistic coevolutionary arms race, assuming that these insects act as efficient pollinators for their nectar host plants. This study presents an integrative approach combining morphological, biometrical and behavioral data obtained from various butterfly species from a Costa Rican lowland rainforest area differing substantially in proboscis length to shed light on the form, function and (limits to the) evolution of

extremely long butterfly proboscides. Butterfly proboscides that greatly exceed the length of the body are rare, occur only in some species of Neotropical Riodinidae and Hesperiidae and result from an allometric scaling relationship with body size. Constraints on the evolution of increasingly long butterfly proboscides may come from these scaling relationships combined with the butterfly's flight style and flower-visiting behaviour and/or developmental processes during the pupal phase. Disproportionally long proboscides evolved once within Riodinidae and at least three times convergently within Hesperiidae. Long-proboscid butterflies experience relatively low extra expense on the proboscis musculature and sensilla equipment, but significant anatomical costs in terms of reinforced haemolymph muscles and enlarged suction pump musculature, as well as thick cuticular proboscis walls. Long-proboscid butterflies do not suffer from reduced intake rates due to a combination of morphological adaptations, such as large body size and an enlarged food canal. The time spent to take up nectar increases with proboscis length, suggesting that long-proboscid butterflies drink larger amounts of nectar from deep-tubed flowers. Despite these advantages, functional costs of exaggerated mouthparts exist in terms of longer manipulation times per flower. Adult butterflies steal nectar from flowers and the larvae of some long-proboscid butterflies feed on the flowers of their nectar host plants. These findings indicate an antagonistic relationship between long-proboscid butterflies and their host plants and challenge the traditional view on the mutualistic coevolution of long-proboscid flower visitors and deep-tubed flowers.

CITES - the Washington Convention.

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CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) is an international agreement between governments. Its aim is to ensure that international trade in specimens

of wild animals and plants does not threaten their survival.
The trade is diverse, ranging from live animals (e.g. corals, reptiles) and plants to a vast array of wildlife products derived from them, including food products, exotic leather goods, wooden musical instruments, hunting trophies and medicines. Today, CITES protects more than 35,000 species of animals and plants. Because the trade in wild animals and plants crosses borders between countries, the effort to regulate it requires international cooperation to safeguard certain species from over-exploitation. The text of the Convention was finally agreed at a meeting of representatives of 80 countries in Washington, D.C., the United States of America, on 3 March 1973, and on 1 July 1975 CITES entered in force. Although CITES is legally binding on the Parties it does not take the place of national laws. Therfore, each Party has to adopt its own domestic legislation to ensure that CITES is implemented at the national level. Due to the European Single Market and the absence of systematic border controls within the EU, the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) have to be implemented uniformly in all EU Member States. CITES is implemented in the EU through a set of Regulations known as the EU Wildlife Trade Regulations. All import, export, re-export and introduction from the sea of species covered by the Convention has to be authorized through a licensing system. Each Party to the Convention / EU member state must designate one or more Management Authorities in charge of administering that licensing system and one or more Scientific Authorities to advise them on the effects of trade on the status of the species. The European Commission is assisted in its work by the Committee on Trade in Wild Fauna and Flora (COM) composed of representatives from Member State Management Authorities and chaired by the Commission Council Regulation (EC) No 338/97 also establishes a Scientific Review Group (SRG) consisting of representatives from the Member States' Scientific Authorities. SRG and COM meets four times a year in Brussels. The species covered by CITES are listed in Appendices, according to the degree of protection they need. The Parties (member States) to CITES are collectively referred to as the Conference of the Parties (CoP). Every three years, the CoP meets to review the implementation of the Convention. Furthermore, an Animal and a Plant committee of experts

was established at CoP6 to fill gaps in biological and other specialized knowledge regarding species of animals and plants that are (or might become) subject to CITES trade controls. Their role is to provide technical support to decision-making about these species. The Animals and Plants Committees meet twice between meetings of the Conference of the Parties.

Molecular evolution of reptiles in Austria: Intraspecific genetic diversity, Würm glacial refuges and postglacial colonization routes of selected species.

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Key words: reptiles, Austria, intraspecific diversity, mitochondrial genes, glacial refuges

Due to the climatic conditions during the last pleistocene glaciation, it is highly improbable that amphibians and reptiles could have survived this period in most parts of Austria. The present distribution of reptiles in Austria is generally considered to be the result of postglacial and thus relatively young colonisations from glacial refuges. In the course of the present study genetic analyses of three species were performed including samples from the whole territory of Austria, as well as from neighbouring regions to locate glacial refuges and reconstruct postglacial colonisation routes.

Neuromuscular development in Patellogastropoda (Gastropoda: Mollusca) and its importance in the reconstruction of ancestral gastropod bodyplan features.

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Within Gastropoda, limpets (Patellogastropoda) are considered the most basal branching taxon and its representatives are thus crucial for research into evolutionary questions. Here, we describe the development of the neuromuscular system in Lottia kogamogai. In trochophore larvae, first serotonin-like immunoreactivity (lir) appears in the apical organ and in the prototroch nerve ring. The arrangement and number of the serotonin-lir cells in the apical organ (three flask-shaped, two round cells) is strikingly similar to those in putatively derived gastropods. First FMRFamide-lir appears in veliger larvae in the anlagen of the future adult nervous system including the cerebral- and pedal ganglia. As in other gastropods, the larvae of this limpet shows one main and one accessory retractor as well as a pedal retractor and a prototroch muscle ring. Of these, only the pedal retractor persists until after metamorphosis and contributes to the adult shell musculature. We found a hitherto undescribed, paired muscular system that inserts at the base of the foot and runs towards the base of the tentacles. An apical organ with flask-shaped cells as well as one main and one accessory retractor muscle is commonly found among gastropod larvae, and thereby might have been part of their common ancestor.

The nervous system of *Hyalinella punctata* (Bryozoa, Phylactolaemata).

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The Bryozoa are filter feeders inhabiting freshwater and marine environments. The Phylactolaemata are the most basal group and thus represent a relevant taxon to illuminate the morphological ground pattern of bryozoans. This investigation aims to characterize the nervous system of the phylactolaemate *Hyalinella punctata* with techniques previously not applied to phylactolaemate bryozoans. For the investigation of the nervous system of *Hyalinella punctata* immunocytochemical stainings using anti alpha tubulin antibodies combined with confocal laser scanning microscopy as well as histological sections are used. The cerebral ganglion is located between the anus and the pharynx and has a fluid filled lumen. Two ganglionic horns and the circum oral nerve ring emanate thereof. The pharynx is innervated by a diffuse nerve net which terminates at the cardiac valve. A nerve plexus innervate the epistome. The tentacle sheath is innervated by several nerve fibres which extend distally into the thin nerves innervating the body wall. In *Hyalinella punctata*, six tentacle nerves were found with two lateral and one median nerve on the frontal side and the same number on the abfrontal side. The medio-abfrontal nerve fibres branch off more distally. All tentacle neurite bundles have an intertentacular origin. The fluid filled lumen in the cerebral ganglion and the ganglionic horns represent a typical situation for Phylactolaemata. The visceral innervation of *Hyalinella punctata* seems to be more diffuse than in derived bryozoan species. The tentacle nerve number found in *Hyalinella punctata* is supported by a recent paper about a freshwater Bryozoa. However, there are contradictory results concerning the tentacle nerve number in Phylactolaemata. In contrast to the Gymnolaemata, the

Phylactolaemata have a higher number of tentacle nerves. Since the Phylactolaemata are considered as the basal group of Bryozoa, it can be speculated that the tentacle nerve number was reduced in the course of evolution in gymnolaemates.

Tadpole transport trajectories in a neotropical poison frog *Allobates femoralis*.

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Efficient orientation provides many advantages as it reduces costs during movement like risk of predation, energetic expenditure and lost mating opportunities. Navigation mechanisms of free living animals have been mostly studied in long distance migrants, such as birds. Although many amphibians show occasional movements like migrations ranging a few hundred meters or more, in general amphibians are considered to be the most sedentary vertebrates with reduced daily movement. Most studies on movement in amphibians focus on pond breeding temperate-region frogs while comparatively little is known about tropical frogs, which show a variety of complex spatial behavior. Dendrobatidae (dart-poison frogs) exhibit some of the most complex spatial behaviors among amphibians, such as territoriality and tadpole transport from terrestrial clutches to widely distributed larval deposition sites. A recent study in the poison frog *Allobates femoralis* showed that males successfully return to home territories with a very high accuracy after translocation. However, whether the frogs use spatial knowledge about the area to find deposition sites and for homing after the tadpole transport is still unknown. We hypothesized that males rely on spatial learning and therefore on experience for orientating in their local area. If true, highly

directional trajectories to deposition sites as well as back to the territory can be expected. We tested this hypothesis by tracking tadpole carriers using a harmonic direction finder with miniature transponders to (i) the deposition sites and (ii) back to the territory. According to our predictions we found a high accuracy in navigating to deposition sites and a strong homeward orientation after tadpole deposition which emphasize the possible reliance on a learned spatial map.

From Central Amazonian Floating Meadows to the tropical rainforest streams of the Western Ghats: 40 years of frog research in the neotropics.

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The fascinating world of tropical frogs and toads has initiated my metamorphosis from working in an electrophysiological laboratory to becoming an avid herpetologist and behavioral ecologist. Offering insights into the life of a dedicated field biologist I will initially present my work on reproductive biology and communication of frogs from Amazonia, the Atlantic rain forest of Brazil and the highlands of French Guyana. Special attention will be given to both the acoustic strategies used by frogs and toads to attract mates as well as their use of visual signals. Taxon-oriented research widened my interests in a broad range of topics including general life history, reproductive behavior and communication. To make my observations accessible to the public I initiated the production of scientific films on various taxa (frogs, stingless bees, termites, …). With a large team of dedicated students and colleagues my lab has recently been involved in studies on communication in frogs in Europe (Austria), Asia (Sabah, Brunei, India), and Africa (Tanzania, Uganda). Scientifically very rewarding have been our studies on the bioacoustics and population biology of the dart-poison frog *Allobates femoralis*. Due to its stereotypic acoustic and highly specific reproductive behavior, our "handy fellow" has become our main study species and one of the best studied anurans in its natural habitat.

Developmental studies in Solenogastres (Mollusca).

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The Solenogastres (or Neomeniomorpha) are a taxon of aplacophoran molluscs with still contentious phylogenetic placement. Since available developmental data on non-conchiferan (i.e., aculiferan) molluscs mainly stem from polyplacophorans, compiling more data on aplacophorans is crucial to clarify important evolutionary questions concerning the Aculifera and the entire Mollusca. This includes the traditional notion of a segmented ancestry of molluscs, which was revived in the last two decades mainly based on gene expression data. We addressed this issue on the morphogenetic, the cellular, and the gene expression level by investigating the formation of the nervous system, cell proliferation patterns, and expression of the twist ortholog during larval development in two solenogaster species, Wirenia argentea and Gymnomenia pellucida.

Neurogenesis starts both from the apical and abapical pole of the larva with the formation of the first few cells of the apical organ and an unspecified posterior neurogenic domain. A pair of neurite bundles grows out from both the neuropil of the apical organ and the posterior neurogenic domain. After their fusion in the region of the prototroch, which is innervated by an underlying serotonin-like immunoreactive (–LIR) plexus, the larva exhibits two longitudinal neurite bundles – the future lateral nerve cords. The apical organ has now reached its fully

developed state and exhibits approximately 8-10 flask-shaped cells. The entire ventral nervous system, which includes a pair of longitudinal neurite bundles (the future ventral nerve cords) and a serotonin-LIR ventromedian nerve plexus, appears simultaneously and is established after the lateral nervous system. During metamorphosis the apical organ and the prototrochal nerve plexus are lost.

In younger larvae, cell proliferation occurs mainly in a pair of kidney-shaped areas stretching along almost the whole larval body and in a small domain situated at the abapical end. As soon as the trunk starts to grow in length, the latter domain extends and forms a uniform stripe of proliferating cells along the ventrolateral parts of the animal. All proliferating cells were found to be subepithelial.

Expression of the twist ortholog shows a paired spherical signal in newly hatched larvae. This signal elongates with the outgrowing of the trunk until it forms a pair of uniform stripes along the major part of the length of the animal. In larvae approaching metamorphosis and young postmetamorphic animals the area of twist expression is progressively restricted to a pair of elongated areas posterior to the mouth opening. As with cell proliferation labeling, the signal was found exclusively subepithelial.

Neurogenesis in solenogasters is non-segmental, thus arguing against a segmented molluscan ancestry, but early larvae show distinct similarities to polychaete and other spiralian larvae in, e.g., possessing a pre- and a posttrochal neurogenic domain, which likely constitutes an ancestral spiralian trait. Furthermore, the data currently available suggest a larva with an apical organ including flask-shaped cells, one pair of longitudinal neurite bundles, and a serotonin-LIR innervation of the prototroch as a “phylotypic” larval stage for spiralians.

Data from cell proliferation labeling and twist expression can both be interpreted as showing a pair of mesodermal stripes. Since these stripes are always uniform in signal intensity and spatially homogeneous, both results likewise argue against a segmented molluscan ancestry.

Using muscles and genes to reveal the evolutionary history of Solenogastres (Mollusca).

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Mollusca is an extraordinarily diverse metazoan phylum, second only to Arthropoda. Recent studies suggest a bifurcation at the base of Mollusca, resulting in the primarily single-shelled Conchifera (Bivalvia, Gastropoda, Scaphopoda, Monoplacophora, Cephalopoda) and the spicule-bearing Aculifera (Polyplacophora, Solenogastres = Neomeniomorpha, Caudofoveata = Chaetodermomorpha). However, despite intense research, the evolutionary origin of mollusks is still highly controversial and, e.g., a common aculiferan ancestor still remains difficult to reconstruct due to significant morphological differences between Polyplacophora and Aplacophora (Solenogastres and Caudofoveata).
Our study of solenogaster myogenesis reveals a complex larval musculature exclusively shared by Solenogastres and Polyplacophora, supporting a close relationship of both taxa. The mode of ontogenetic transition from the complex larval to the simple adult solenogaster musculature, which mainly consists of a three-layered body-wall musculature and serially iterated dorsoventral muscles, is an indication for secondary simplification in the aplacophoran lineage. Based on data on myogenesis in Solenogastres and Polyplacophora and the recurrence of a seven-fold seriality, we assume a complex polyplacophoran-like ancestor with presumably seven dorsal shell plates at the base of

Aculifera. This notion is supported by fossil data that revealed an aplacophoran species that bore seven shell plates.
Although our morphological data supply no indication for a segmental mode of formation during solenogaster development, we tested a conceivable scenario of ancestral molluscan segmentation on a molecular level by gene expression analysis in our model Wirenia argentea (Solenogastres). We investigated the gene expression pattern of engrailed and several members of the Pax gene family (pax6, paxβ, pax258), i.e., transcription factors that are either involved in segment formation or are expressed during organogenesis of metamerically arranged organ systems in arthropods or annelids. In congruence with our data on myogenesis, we found no indication of segmentation on gene expression level and, therefore, we conclude that the aculiferans stem from a non-segmented ancestor.

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