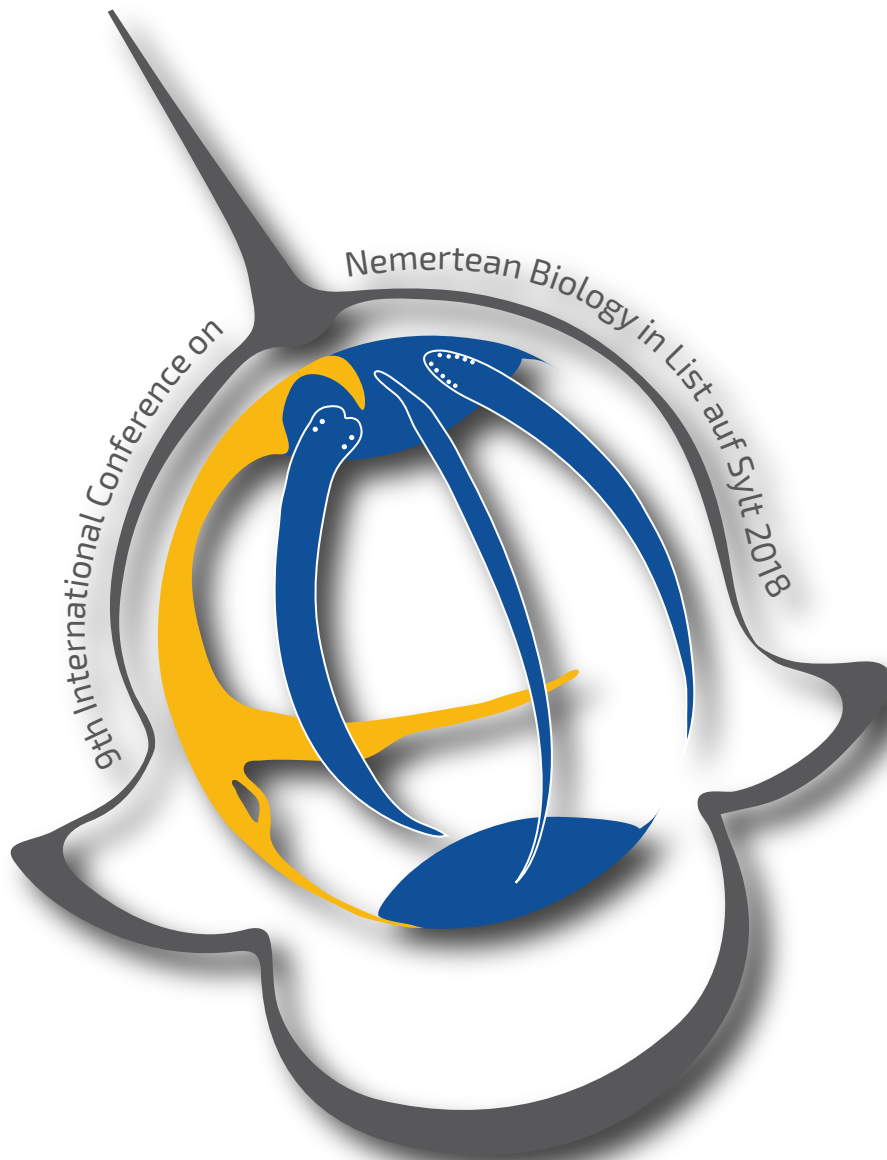


9th International Conference on Nemertean Biology in List auf Sylt

13th-17th August 2018





Schedule

Monday, 13 August 2018,

19:00 Ice Breaker @ Erlebniszentrum Naturgewalten

22:00 Nocturnal Nemertean Hunt

Tuesday, 14 August 2018

7:00-

8:30 Breakfast

9:30 Welcome Note

10:00 Thomas Bartolomaeus – World Natural Heritage Wadden Sea

10:30 Coffee break

11:00 Jörn von Döhren – small larvae – big difference? What comparative developmental morphology can tell us about the evolution of larvae in Nemertea – and others

11:30 Cong-Mei Xu – Expression patterns of hox genes in *Lineus sanguineus* during regeneration

12:00 Hiroshi Kajihara & Natsumi Hookabe – Why do certain species of heteronemerteans get so long?

12:30- Lunch break

14:00

14:00	José Alfaya – Larval dispersion of the commensal nemertean <i>Malacobdella</i>
14:30	Shi-Chun Sun – Nemerteans of the genus <i>Prosadenoporus</i> (Hoplonemertea: Monostilifera) from China seas
15:00	Alexej Chernishev – Deep-sea Benthic nemerteans: What do we know of them so far?
15:30	Coffee break
16:00	Christina Sagorny – Diversity and Distribution of <i>Cephalothrix</i> species (Nemertea: Palaeonemertea) in European Waters
16:30	Timur Magarlamov – Pseudocnidae of ribbon worms (Nemertea): morphology, maturation and potential utility for nemertean systematics
17:00- 17:30	Thomas Bartolomaeus – Is there any phylogenetic signal in spermatozoa – a case study from nemerteans

18:30 Dinner @ Gosch Lister Fischhaus

Wednesday, 15 August

7:00- Breakfast
8:30

9:30 Rachel DuBose – Histologic analysis of nemertean osmotic stress

10:00 Arusa Ashfaq – Examination of Invertebrate Tachykinin Receptor genes

10:30 Coffee break

11:00 Augustus Malan – Role of Ca⁺⁺ and cAMP on nemertean heat shock gene expression following environmental stress

11:30 Håkan Andersson – Alpha-nemertides – a novel family of nemertean peptide neurotoxins

12:00 Poster teaser

12.30- Lunch break
13:45

14:00- Research vessel trip with the MS Mya
16:30

Old Frisian Village tour in Keitum

17:00- Poster session with beer, brezels and Wine
19:00

19:30 Dinner @ Bootshalle

Thursday, 16 August

7:00- Breakfast

8:30

9:30 Daria Krämer – Species Identification and delimitation in nemerteans – Meet me half way between morphology and molecular based taxonomy

10:00 Patrick Beckers– Next generation histology in nemertean morphology

10:30 Coffee break

11:00 Sonia Andrade – Genetic diversity in South American Nemerteans: new approaches and perspectives

11:30 Irina Cherneva - Worms of doubt: nemerteans of the genus *Lineus* from the White Sea tidal zone

12:00 Malin Strand – Nemertean taxonomy in WoRMS

12.30- Lunch break

14:00

14:00- Workshop on Nemertean biology and systematics

18:00

19:30 Dinner

Friday 17 August

7:30- 8:00	Farewell Breakfast
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10:00	End of conference
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Map of List

Appartement
Alleena List/Sylt
Gbr Berntje +
Simone Fügner

BBQ-Dinner - 15th August
AWI-Guesthouse Möbius
Listlandstr. 18b
25992 List/Sylt



Conference
Gästehaus Diedrichsen
AWI Wattenmeerstation
Sylt
Hafenstr. 43
25992 List/Sylt

Breakfast
Hotel Easy Living
Hafenstr. 2A
25992 List/Sylt



Ice Breaker - 13th August
Erlebniszentrum Naturgewalten
Hafenstr. 37
25992 List/Sylt

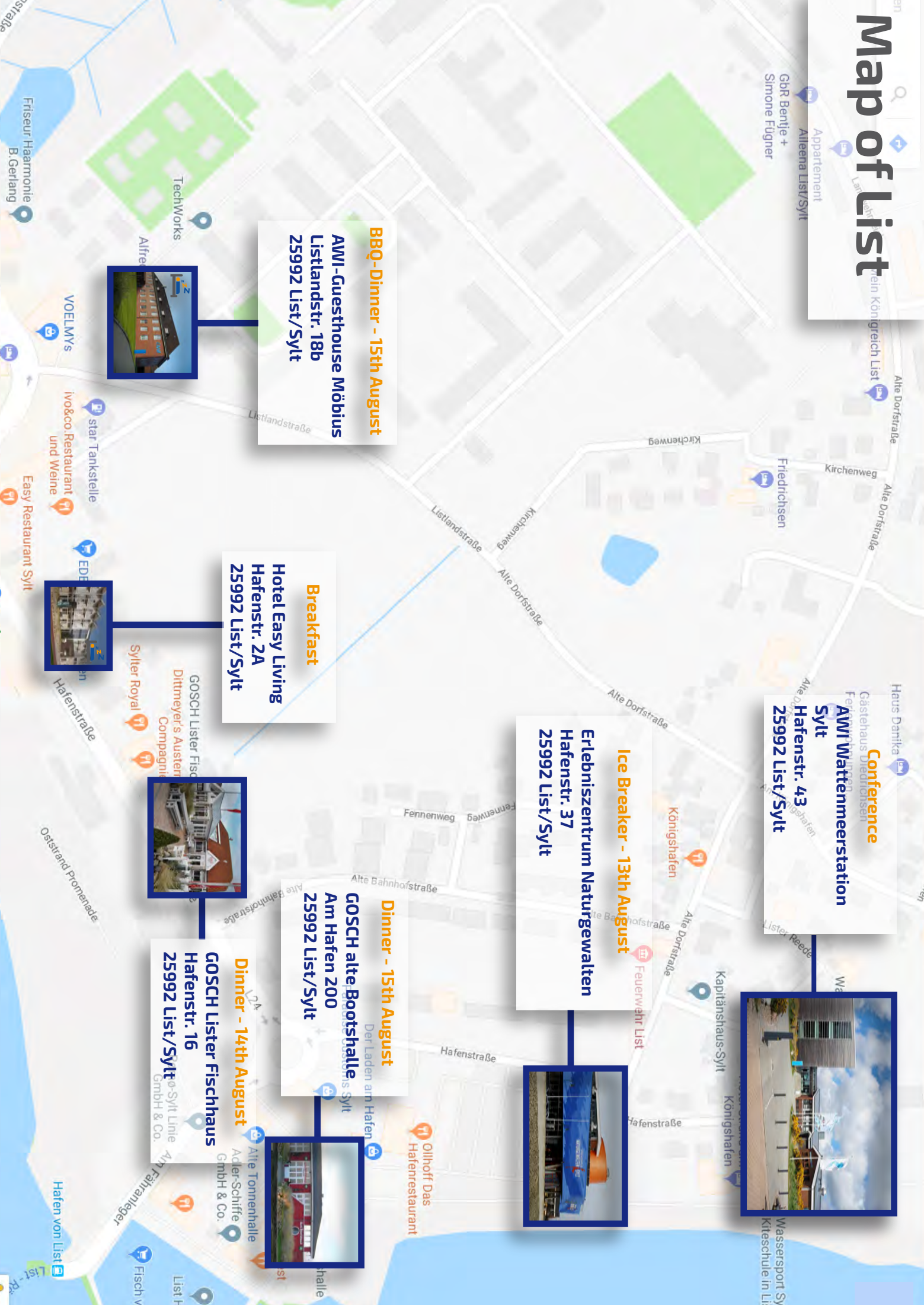


Dinner - 15th August
GOSCH alte Bootshalle
Am Hafen 200
25992 List/Sylt

Dinner - 14th August
GOSCH Lister Fischhaus
Hafenstr. 16
25992 List/Sylt



Dinner - 15th August
GOSCH Lister Fischhaus
Hafenstr. 16
25992 List/Sylt



Nemertean talks



Tuesday, 14th August

Small larvae – big difference? What comparative developmental morphology can tell us about the evolution of larvae in Nemertea – and others

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Nemertea is a taxon of unsegmented spiralian worms. With respect to their morphology, the animals allegedly show the ancestral spiralian condition in almost all organ systems. Traditionally regarded as closely related to Platyhelminthes (flatworms), Nemertea are presently considered as part of the trochozoan radiation. Like many other spiralian clades, the various nemertean lineages develop through different types of pelagic larvae. Due to its characteristic shape and the spectacular metamorphosis, the pilidium represents the most well-known larval type of Nemertea. The classical hypothesis that the pilidium represents a primary larva of the trochophore-type has recently been disproved. The pilidium is a derived larval type having evolved in the stem lineage of Pilidiophora. The remaining larval types in Nemertea are less well characterized. Comparative study of development of the various larval types yields insights into the evolution of several organ systems in Nemertea. From this data, the ancestral nemertean larval type can be reconstructed. Furthermore, the findings indicate that the ancestral larval type of Nemertea is not a trochophore-type larva.

Expression patterns of hox genes during regeneration in *Lineus sanguineus*

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Hox genes, a subfamily of homeobox genes, are highly conserved patterning genes to specify the body plan of multicellular organisms, by assigning different identities to cells along the A-P axis of the developing organisms. To better understand the functions of hox genes in nemertean, we examined their expression in normal and regenerating *Lineus sanguineus*, which has high regeneration capacity and usually reproduces asexually, using whole-mount in situ hybridization and real time quantitative PCR. In addition to the 6 genes determined by previous studies, 2 genes, *Scr* and *Lox4*, were identified through transcriptome analysis. During anterior regeneration, the expression of *Hox4*, *Lox4* and *Hox9* was upregulated, while the other genes showed an expression pattern of downregulation followed by upregulation. During posterior regeneration, expression level of all hox genes but *Hox1*, *Hox3* and *Scr* was more than 4 times higher than that of each control. Whole-mount in situ hybridization results showed that in normal worms, all the 8 hox genes expressed weakly in the blood lacuna bathing the rear portions of cerebral sensory organs (relatively strong in *Hox3*) and strongly in intestine. All but *Scr*, *Hox7* and *Hox9* expressed in cephalic blood vessels. *Hox1*, *Hox4*, *Hox6*, *Hox7* and *lox4* also expressed weakly in stomach region. During anterior regeneration, all hox genes expressed in stomach region. During posterior regeneration, the expression of all hox genes except *Hox 1*, *Hox 3* and *Scr* in blood lacuna was stronger than that in intact worms. In addition, *Hox7*, *Lox4* and *Hox9* expressed also in blastema, and *Hox4*, *Hox6*, *Hox7* and *Lox4* also expressed in mouth tissues. These results suggest that all hox genes except *Lox4* play positive roles in the reconstruction of anterior body part, while *Hox7*, *Lox4* and *Hox9* playing crucial roles in the formation of posterior body region. *Hox1*, *Hox3* and *Scr* seems not take part in the posterior regeneration.

Why do certain species of heteronemerteans get so long?

Hiroshi Kajihara & [Natsumi Hookabe](#)

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Some heteronemerteans grow up very long. For instance, *Baseodiscus hemprichii* is recorded to have reached 8 m (Iwata 1954) and *Lineus longissimus* 55 m (McIntosh 1873–1874). Why do they get so long? Potential proximate explanations in terms of mechanism and ontogeny include that *i*) there should be a set of 'body-elongation genes' and relevant transcription regulatory system, which behave more actively than in other species; *ii*) these species must be an indeterminate grower, in which growth is not terminated after reaching adulthood (Geoffrey et al. 2001); and *iii*) these species may live longer than other species, and the longevity may be achieved by a putative low mortality in adult, which, again, may be achieved by toxins they produce (e.g., Jacobsson et al. 2018). What about ultimate explanations as to evolution and adaptation? In terms of body plan, heteronemerteans are distinct from palaeo- and hoplonemerteans in that they have additional body-wall components (such as the cutis and inner longitudinal muscle layers) as well as pseudo-metameric transverse blood vascular connectives in the intestinal region. These extra features might have caused a phylogenetic pattern where heteronemerteans were longer than palaeo- and hoplonemerteans. We compiled a body-size dataset from literature and compared the body length between Palaeo-, Hetero-, and Hoplonemerteans. While there was a statistically significant difference in body length between Hetero- and Hoplonemerteans (and also between Palaeo- and Hoplonemerteans), there was no significant difference between Palaeo- and Heteronemerteans (the longest palaeonemertean being '*Tubulanus polymorphus*' sensu Coe (1901) from Alaska, reaching 3 m), suggesting that anatomical complexity is not likely to be relevant to body length. We found that *Lineus longissimus* can possess more than 700 eggs (oocytes, precisely) per ovary; this number is outstanding within the entire phylum. From this observation, we hypothesized the following scenario. Longer period of planktonic duration evolved for greater dispersal potential in favour of lesser chance of extinction by local habitat destruction where the adult lived. This has evolved in compensation for higher mortality during the planktonic phase, compelling higher fecundity, which can be achieved by increasing *i*) the number of eggs per ovary and *ii*) the number of ovaries by extended body length. While the actual causality can be in opposite direction, there should be a correlation between *i*) planktonic larval duration, *ii*) body size, and *iii*) fecundity (number of oocytes per ovary). We conjecture that *L. longissimus* has a longer period of planktonic larvae.

Larval dispersion of the commensal nemertean *Malacobdella arrokeana* in North Patagonics gulfs, Argentina

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The nemertean *Malacobdella arrokeana* is a commensal of the edible giant bivalve *Panopea abbreviata*; both species have a restricted geographic distribution, high specificity and populations separated by natural barriers. This supposes a high genetic structuring among populations and low intra-population variability; nevertheless, a lack of genetic structure was detected previously between *M. arrokeana* populations from Atlantic norpatagonic gulfs using mitochondrial and nuclear markers. Here, we present the first attempt to explain this lack of genetics structure, integrating larval behavior and bio-oceanographical model simulations (ROMS) which corroborate that the population genetic results are in concordance with particle dispersion model simulation. Cultured larvae obtained had a mean planktonic life of 30 days after fertilization. Larval morphology (planuliform) and behavior suggest that the dispersion is influenced by passive transport related to season and hydrodynamic diffusion patterns present in the area. Our results are useful to understand the dispersion and settlement of larvae along the north Patagonic gulfs and provides novel baseline data in larval connectivity and oceanographic circulation patterns.

Nemertean of the genus *Prosadenoporus* (Hoploneurata: Monostilifera) from China seas

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Three species of *Prosadenoporus* have been recorded from Chinese coasts: *Prosadenoporus spectaculum*, *Prosadenoporus mortoni* and *Prosadenoporus fujianensis*. Examination of specimens collected since 1970s' shows that these species are distributed only in tropical and subtropical areas, as the other members of the genus. An undescribed species, which is distributed farther north (39°51'N), was collected in supralittoral clay at estuaries of Yalu River and Changjiang (Yangtze) River. It is up to 405 mm long and 1.5-3.0 mm wide, and dorsoventrally flattened. Color of anterior body region is orange-brown, lighter on the ventral side. Mature male is brownish while mature female is olive or greyish green in the intestinal region. The intestinal region possesses numerous irregularly-shaped white patches, much more abundant on the ventral side. The dark pigmentation along the mid-dorsal line forms a longitudinal stripe, which is not distinct in the anterior region. Proboscis is not used for locomotion. Stylet basis is cylindrical, truncated at posterior end, 203-236 (212.5±15.6; n=4) µm long and 50-58 (53.5±3.6; n=4) µm wide. Central stylet is 129-174 (143.9±20.3; n=4) µm in length. Ratio of stylet: basis is 0.63-0.74 (0.67±0.04; n=4). The species is morphologically different from *P. fujianensis* by having a smaller and slenderer basis (length 203-236 vs 506-595µm; width 50-58 vs 195-206µm), a smaller central stylet (129-174 vs 243-263µm), and a larger stylet:basis ratio (0.63-0.74 vs 0.44-0.48). There is a distinct gap between COI sequences of the two species (intraspecies similarity 98.7-100%; interspecies similarity 90.9-91.7%). In addition, we collected specimens of *Pantionemertes* cf. *daguilarensis* from Shenzhen (close to the type locality of *P. daguilarensis*). Our preliminary molecular analysis supports the previous argument that this species may not belong to genus *Prosadenoporus*.

Deep-sea benthic nemerteans: What do we know about them so far?

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The phylum Nemertea comprises about 1,300 described species, but only 16 benthic nemertean species were known from depths of 1,000–3,000 m prior to the beginning of our study. Thanks to the material collected during four international deep-sea expeditions to the northwestern Pacific and the Vema Fracture Zone, it has become evident that nemerteans are common members of abyssal benthic communities. Our data confirm the high species diversity of deep-sea nemerteans in the northwestern Pacific Ocean. In samples from the abyssal zone, a major part of nemerteans belong to three groups: (1) palaeonemerteans (Carininidae and Tubulanidae); (2) Heteronemertea; and (3) Eumonostilifera. The heteronemerteans are dominated by baseodiscid-like nemerteans (primarily *Sonnenemertes cantelli*), whereas lineids occur rarely. Of the 20 species of abyssal eumonostiliferous nemerteans, only *Abyssonemertes kajiharai* from the Vema Fracture Zone has been described. In the nearest future, we are going to describe two new *Gononemertes*-like nemerteans obtained from carnivorous ascidians of the genus *Culeolus*. According to our phylogenetic analysis, these species don't form a common clade with the shallow-water *Gononemertes parasita*. One cephalotrichid species (*Cephalothrix iwatai*) was found in the pseudoabyssal zone of the Sea of Japan. In the abyssal of the Sea of Okhotsk, two species of reptantian nemerteans were collected: *Uniporus alisae* and an undescribed species from a new family. A phylogenetic analysis has shown a highly supported relationship of *U. alisae* with the undescribed species of Cratenemertea from the Sea of Okhotsk. In the hadal zone, the nemertean diversity is much lower than in the abyssal zone: a total of about twelve morphospecies of the palaeonemerteans (Carininidae and Tubulanidae), heteronemerteans and eumonostiliferous nemerteans were obtained from depths of 6–9 km. The maximum depth, at which benthic nemerteans were found, is 9,577 m (Kuril-Kamchatka Trench). *Nemertovema hadalis*, collected in the Puerto Rico Trench from a depth of 8,339 m, is the deepest described nemertean species. Of particular interest is the new undescribed nemertean from the Kuril-Kamchatka Trench, associated with the hadal actinian *Galatheaanthemum* sp. The further studies will be aimed at isolating and analyzing DNA sequences from the largest possible number of specimens of abyssal and hadal nemerteans in order to estimate their actual biodiversity in the northwestern Pacific.

Diversity and Distribution of *Cephalothrix* Species (Nemertea: Palaeonemertea) in European Waters

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Many nemertean genera often lack clear diagnostic morphological characters, rendering accurate species delimitation based on morphological traits difficult. Therefore, recent studies often concentrate on molecular methods to solve taxonomic issues and uncover cryptic species. For this, several tree-based and non-tree-based methods as well as phylogenetic analyses can be employed. A particularly problematic nemertean genus when it comes to morphology is the palaeonemertean genus *Cephalothrix*. Within the last 150 years 15 to 20 *Cephalothrix* species have been described from Europe. However, little is known about the actual diversity of the genus *Cephalothrix* in European coastal waters. In order to gain information on the number and distribution of European cephalotrichids, we collected specimens in six different locations in Europe. We analyzed a dataset comprising the barcoding region of the mitochondrial cytochrome c oxidase subunit I (COI) of European *Cephalothrix* specimens with tree-based and non-tree-based delimitation methods. In the course of this study, the usefulness of the applied delimitation methods as well as the suitability of the COI sequence was tested. Our results show the presence of 12 to 13 distinct clades of which several can be assigned to well-known European species. Furthermore, one clade seems to represent a non-native introduced *Cephalothrix* species, while another has never been recorded from Europe before. As our analyses resulted in more species than currently recognized, especially when further worldwide data is included, cryptic speciation within the genus *Cephalothrix* seems likely. The applied delimitation methods based on the COI gene in some cases proved to be unsuitable for assessing the actual species diversity. In order to fully resolve European cephalotrichid diversity, additional molecular markers have to be included in future analyses.

Pseudocnidae of ribbon worms (Nemertea): morphology, maturation and potential utility for nemertean systematics

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The eversible nemertean proboscis is a derived character of the taxon used for prey capture. The glandular epithelium of this organ contains a variety gland cells as well as support and sensory cells. In anoplan nemerteans specific gland cells produce structures referred to as pseudocnidae because of their superficial resemblance to cnidae of cnidarians. The structure of pseudocnidae of 34 species of Palaeo- and Heteronemertea were investigated with confocal laser, scanning- and transmission electron microscopy. Pseudocnidae were not observed in species of the genera *Carinina*, *Baseodiscus*, and *Sonnenemertes*. In the species examined mature pseudocnidae are released on the proboscis epithelium surface forming monolayered clusters with the bases of all pseudocnidae in the cluster situated proximally. Most of the mature pseudocnidae observed were situated on the surface of bacillary gland cells. In *Carinoma mutabilis*, all species of the genus *Cephalothrix* and some lineid species, two types of pseudocnidae were consistently differentiated based on size (small and large pseudocnidae). These are always produced in separate pseudocnid-forming cells. Initially, immature pseudocnidae have three layers: an outer cortex, a precore-layer, and a centrally situated filament core. Later a fourth layer, the medulla, develops between the cortex and precore-layers. In large and small pseudocnidae of *Cephalothrix* species and small pseudocnidae of *Carinona mutabilis* the core has a simple organization, consisting only of a tube-like filament. The core structure of other species is more complex: in most species of Tubulanidae the pseudocnida core consists of the outer core layer and a centrally situated rod-shaped filament, whereas for most species of Heteronemertea, the core possesses the outer core layer and a centrally situated tube-like filament. Pseudocnida extrusion involves discharge of the outer core and results in the destruction of the precore and medulla layers leading to the homogenization of the inner content. Ultrastructural data obtained suggest that variation in pseudocnida morphology may be useful in nemertean systematics. For example, unique features of pseudocnida structure shared by *Cephalotrichella*, and *Balionemertes* are consistent with other characters supporting a separate clade (family Cephalotrichellidae) for these genera.

Is there any phylogenetic signal in spermatozoa – a case study from nemerteans

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Ultrastructure of spermatozoa is widely used as source for phylogenetic inference, since sperm cells are likewise easy to obtain and to analyze ultrastructurally. Spermatozoa on the other hand are highly adapted to the mode of reproduction and thus subjected to sexual selection. Due to these strong functional constraints they should be prone to convergent evolution, given especially that sperm cells are rather poor in characters. If so, sperm cells should not contain much phylogenetic signal and functional constraints shaping their ultrastructure should outperform any influence of historical constraints (evolutionary heritage). Accordingly their inclusion into phylogenetic analyses should increase noise over signal and weaken the quality of such analyses. In order to test whether there is any signal in sperm ultrastructure and to find out whether historical constraints that must underlie functional constraints still influence sperm ultrastructure, we analyzed sperm ultrastructure in 14 nemertean species and added these to our existing matrix. The matrix represents closely related groups and major nemertean lineages. Our study shows that sperm cells possess phylogenetic signal, but this erodes with increasing depth of branching. Although certain nemertean subtaxa can clearly be characterized by specifics in their sperm ultrastructure, this is impossible for the taxon Nemertea or for high ranking nemertean subgroups. Although this result might have been expected, we can show that functional constraints posed upon spermatozoa by sexual selection or the mode of reproduction do not outperform the influence of historical constraints on sperm ultrastructure – at least at lower systematic levels.

Wednesday, 15th August

Histologic analysis of nemertean osmotic stress

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The ribbon worm *Paranemertes* sp. is able to endure cyclic changes in osmolarity of the intertidal zone during high and low tides. We hypothesized that *Paranemertes* sp. tolerated these cyclic osmotic changes by regulating the amount and chemistry of mucus secreted during these osmotic stresses. Based on our hypothesis, we expected that *Paranemertes* sp. would release copious amounts of mucus resulting in smaller or empty mucous cells following osmotic stress. Additionally, since mucin secretion is an active process, we sought to examine the role of two keystone cell products for cellular activity: Ca^{++} and cyclic adenosine monophosphate (cAMP). *Paranemertes* sp. were collected from the intertidal mud flats of the reserve of University of California-Davis, Bodega Marine Laboratory, subjected to osmotic stress (hypotonic= 24 ppt, isotonic = 34 ppt, hypertonic = 44 ppt sea water) for 2 hrs, and preserved for microscopic analysis. To assess the potential role of intracellular Ca^{++} , separate animals were pre-incubated with the calcium channel blocker Nifedipene; whereas the role of cAMP was examined by pre-incubation with the adenylate cyclase agonist Forskolin prior to osmotic stress. Paraffin-embedded sections of *Paranemertes* sp. were stained by Alcian blue-periodic acid shift technique to determine mucin chemistry and volume remaining within the goblet cells in the epidermis of these animals as a reflection of the amount and quality of mucus remaining in the goblet cells. *Paranemertes* sp. stressed by hypotonic conditions demonstrated significant weight gain (0.098 ± 0.024 g; $p < 0.01$) compared to isotonic animals (0.024 ± 0.008 g), whereas those stressed in a hypertonic condition demonstrated significant weight loss (-0.022 ± 0.005 g; $p < 0.01$). No significant change in weight was observed with animals pre-treated with Nifedipene or Forskolin ($p = 0.21$) in these stress conditions. In response to hypertonic stress, *Paranemertes* sp. secreted predominantly acidic mucin proteins; whereas hypotonic-stressed nemerteans were stimulated to secrete mucins that were mostly of neutral chemistry. Incubation in the adenylate cyclase agonist Forskolin appeared to moderate the mucin secretory response, particularly in the hypertonic environment where the remnant epithelial mucus was similar to that seen in the isotonic group. In contrast, incubation with calcium channel antagonist Nifedipene exaggerated the hypertonic response where the mucus cells appeared to only contain acidic mucins. In summary, *Paranemertes* sp. appear to modulate their quality of mucins secreted in response to osmotic stress through a mechanism in which elevated cyclic AMP and cellular Ca^{++} fluctuations.

Examination of Invertebrate Tachykinin Receptor genes

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Pain is the biochemical mechanism through which distress is conveyed to an organism. Damage to a cell prompts the cell to release a neurotransmitter, referred to as *Substance-P* - a chemical that interacts with a receptor, before eventually generating a response. This receptor - the *Substance-P* receptor - falls under a broad umbrella of important signaling proteins, known as tachykinin receptors, or TKRs for short. TKRs are genetically similar and account for a wide range of vital biochemical mechanisms not only for pain sensation but also blood pressure regulation and intestinal peristalsis. Most of the knowledge accumulated on TKRS are derived from their role in pain and stress response in vertebrates yet, little is known of their evolutionary lineage, or if these receptors are found in other living phyla, such as invertebrates. In 2015, our laboratory identified a short segment of a gene coding for an invertebrate TKR receptor, and found to be homologous to vertebrate TKR receptors. The goal of this project is to further clarify and shed light on the *Paranemertes* sp. genome. *Paranemertes* were collected during the low tide season at the mud flats of the Bodega Marine Laboratory of the University of California-Davis. Animals were harvested for RNA and genomic DNA using Trizol reagent. Extracted RNA was then subjected to reverse-transcription to create cDNA. Genomic DNA and cDNA were then used as templates on which we attempted to screen a number of polymerase chain reaction (PCR) primers. These PCR primers were designed using a multitude of invertebrate TKR sequences. Using this system, we have recently identified four potential TKR amplicons from *Paranemertes* sp. using primers that were designed against the published TKR nucleotide sequence of *Drosophila melanogaster*, which were 300, 1000, 1400 and 2000 nucleotide base pairs in length. We have also identified five potential amplicons using primers that were designed against the ascidian tunicate *Ciona intestinalis* TKR nucleotide sequence, which were 200, 300 and 600 base pairs in length. Additionally, we have also identified a single amplicon of 1000 base pairs using primers directed against the nematode *Caenorhabditis elegans* TKR nucleotide sequence. We are currently awaiting sequence analysis to determine the nucleotide sequence of these amplicons to determine if indeed they are the *Paranemertes* sp. TKR gene. Sequence analysis will open the door for additional knowledge about the location and function of TKR in *Paranemertes* sp., and provide valuable evidence for the evolutionary lineage of this important receptor.

Role of Ca⁺⁺ and cAMP on nemertean heat shock gene expression following environmental stress

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Transcription, occurring in all organisms, is the process of forming a copy of the cell's genetic code (DNA) into messenger RNA (mRNA) which is then translated by ribosomes into the preliminary protein. The final shape of the protein is then achieved through folding into three-dimensional structures to ensure their specific biological functions and is assisted by heat shock proteins (Hsp). Our lab has previously found that Hsp expressions in nemerteans vary depending on the environmental stress, e.g. temperature (Okazaki et al., 2001) and salinity (Okazaki et al., 2006). We hypothesized that the regulation of cellular Ca⁺⁺ or cyclic adenosine monophosphate (cAMP) concentrations are involved the transcriptional regulation of Hsp70 following osmotic stress. Eukaryotic cells tightly regulate both Ca⁺⁺ concentrations intra- and extracellularly, as well as cAMP; changes to either intracellular Ca⁺⁺ or cAMP concentrations reflect significant metabolic activity. We utilized the intertidal ribbon worm, *Paranemertes sp.*, which is subjected to daily environmental stresses, such as salinity changes during tidal fluctuations. Nemerteans were exposed to: 1) 24 ppt, 34 ppt (control), and 44 ppt; 2) the same three salinities with a Ca⁺⁺ voltage-gated channel blocker Nifedipine; and 3) the same three salinities with adenylate cyclase activator Forskolin for 2 hours. Nemerteans were weighed before and after the experiments. Experiments 1 and 2 revealed significant weight changes ($p < 0.01$): weight gain and loss were observed in 24 ppt (ranging 0.072-0.098 g) and 44 ppt (ranging -0.022 to -0.014 g), respectively. No significant weight changes ($p = 0.21$) were observed in Experiment 3. The mRNA of the nemerteans from all three experiments were extracted and processed for 18S:Hsp70 transcriptional activity using Reverse Transcription-Polymerase Chain Reaction. For Experiment 1, 18s:Hsp70 ratios showed significant changes ($p < 0.0001$); the highest 18s:Hsp70 ratio (1.24 + 0.4) was observed in the nemerteans from the 44 ppt treatment while the ratios from the 24 ppt (0.25 + 0.06) and 34 ppt (0.28 + 0.17) treatments were nonsignificant. For Experiment 2, results were inconclusive due to analytical difficulties. For Experiment 3, 18s:Hsp70 ratios showed no significant differences ($p = 0.41$). Our preliminary results indicate increased Hsp70 transcriptional activity under hyperosmotic conditions (44 ppt). While the role of Ca⁺⁺ flux during osmotic regulation is still unresolved, elevating intracellular cAMP blocked the change in Hsp70 transcription under hyperosmotic conditions. The regulation of cAMP could therefore play a role in the transcriptional regulation of Hsp70 following osmotic stress.

Alpha-nemertides – a novel family of nemertean peptide neurotoxins

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We recently discovered a novel family of neuroactive peptides in nemerteans, which we have named alpha-nemertides (1). One of these peptides, nemertide alpha-1, has been the subject of detailed studies with regard to structure and effects. The peptide exhibits exceptional potency against a number of arthropod species. Moreover, *in vitro* experiments suggest that alpha-1 acts primarily on voltage-gated sodium channels, and that this action is selective for arthropods by two orders of magnitude over vertebrate species. Using transcriptomic and proteomic approaches, we have identified 10 alpha-nemertides, but this number is likely to increase. These peptides alongside with a series of mutants are currently under evaluation by our group, with the goal to improve our understanding of structure-function relationships. In addition, we are considering potential practical uses of alpha-nemertides. In this talk, I will describe the current status of this research project.

1. E. Jacobsson *et al.*, Peptide ion channel toxins from the bootlace worm, the longest animal on Earth. *Scientific reports* **8**, 4596 (2018).

Poster teaser

15th August 12:00-12.30


Håkan Andersson	Mapping the diversity of nemertean peptide toxins
Thomas Bartolomaeus	Radial glia mechanically protects the intrapidermal nervous system in <i>Carinina ochracea</i> (Carininidae, Nemertea)
Fernando Ángel Fernández-Álvarez	New information on the alien terrestrial nemerteans of the Iberian Peninsula
Natsumi Hookabe	Molecular systematics of the heteronemertean genus <i>Dushia</i> (Nemertea, Pilidiophora)
Cecili Mendes	Beyond Stranger Stripes: Morphological polymorphism and genetic diversity in <i>Nemertopsis</i> cf. <i>bivittata</i> along the Brazilian coast
Daria Krämer	A new monostiliferan species (Nemertea:Hoploneurata) from the Mediterranean Sea in Italy
Jon Norenburg	An Anchialine Cave-Adapted Nemertean is a New Species of Reptantia
Shi-Chun Sun	A <i>Carcinonemertes</i> nemertean transferring sperms by spermatophore
Anna Vlasenko	Search for tetrodotoxin and its analogues in some nemertean species from the Sea of Japan, Russia
Jörn von Döhren	Open up for the big stuff! –Larval feeding without a prototroch

Thursday, 16th August

Species Identification and Delimitation in Nemerteans - Meet me half way between morphology and molecular-based taxonomy

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Studying ribbon worms involves the exploration of species diversity and the phylogenetic relationships among species. However, working on nemertean taxonomy is challenging, since external and internal morphology yield only relatively few diagnostic characters. As observed in many other taxa, identification and delimitation of nemertean species has shifted from morphology-based to DNA-based methods. In DNA-based taxonomy, a barcode sequence for species identification or a broad set of sequences for species delimitation are used. Compared to the traditional, morphological approach, this allows for assessing the diversity of this taxon in a faster and more efficient manner, especially when dealing with cryptic species. Nemertean species descriptions and re-descriptions are considered valid if they are based on the barcode sequence, a description of external characters in combination with color photographs, and voucher specimens in ethanol. However, at this point, our knowledge about the internal morphology of nemerteans represents an amount of information that is not yet matched by molecular data. We therefore consider it as crucial to provide additional data on the internal morphology as supplement to species description whenever this information is available. We collected data on nemerteans sampled from the European Atlantic and North Sea Coasts of Europe. We compile a species catalogue that includes voucher material, sequence data, color photographs, descriptions on external characters, and links to histological section series/ μ CT scans, which are uploaded in public data repositories (MorphDbase). We aim at clarifying the status of some type species and species that are overall difficult to delimit from congeners. In the future, this will provide a backbone for the revision of ambiguous sequence data in Genbank and will enable the successful application of DNA-based taxonomy.

Next generation histology in nemertean morphology

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Modern microscopical methods like μ CT, FIB, and cLSM have almost replaced traditional histology in the comparative study of animal anatomy. The main advantage of these modern methods is the provision of volume data that represent anatomical structures in their original arrangement and thus enable automated image processing for anatomical 3D reconstruction. Histology on the other hand provides a higher resolution at cellular level and profoundly eases unambiguous interpretation of the data due to differential, tissue specific stains. Yet, the use of serial section histology for 3D reconstruction underlay three drawbacks: 1) Image data must be acquired section by section and transformed into an aligned image stack. 2) Structure labeling in terms of segmentation of images cannot be automated. 3) High resolution image stacks comprise several gigabytes of data and thus are difficult to handle by software for processing and publication. The latter impedes sharing of original data sets and enforces researchers to rely on morphological descriptions and diagnoses provided by others. Here we present a standardized work flow for serial section histology to circumvent these difficulties. In short, we use a semi-automated microscope to acquire high resolution image data. The images are collected into a stack and aligned semi-automatically. The subsequent conversion of the image data into small tiles allows manual segmentation at any level of resolution. The data are finally deposited in the database *MorphDBase* where they can be accessed freely by other researchers. A survey in brain morphology of diverse bilaterian taxa demonstrates that this work flow fastens serial section histology dramatically and results in comparable, openly accessible and well-documented research data.

Genetic diversity in South American Nemerteans: new approaches and perspectives

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The mechanisms underlying the maintenance and distribution of genetic diversity of nemertean populations are still poorly known. This seems to be especially noticeable in the Tropical and Neotropical regions. In South America, pioneering studies on nemertean taxonomy and distribution were conducted by Diva Côrrea about 60 years ago. Her work provided essential background for the current biogeographic and genetic analyses, especially on the Brazilian coast. Nowadays, the studies on South America are focused mostly on phylogeographic and species delimitation analyses, especially on the interstitial hoplonemerteans *Ototyphlonemertes*. These approaches allowed to uncover co-occurring cryptic species on *Ototyphlonemertes* along the Brazilian and Chilean coasts. Demographic genetic analyses showed that these species have a high rate of gene flow, unexpected for meiofauna species, which can be explained by a metapopulation patchy distribution maintained by frequent colonization/extinction processes. However, the kind of collected data provides a curbed understanding of the historical relationships among the populations as well as the processes their evolutionary history. Natural population studies considering the effect of environment and geography over the organisms distribution and dispersal capability is the core of landscape genetics. Inferring how the historical relationships between populations are reflected on the genetic variability is one of the main challenges on the landscape genetics field. This approach allows the understanding of the relationships between geographic distribution and gene flow with the cumulative environmental and bionomics data. The seascape genetics is the field responsible for the understanding of marine communities and their distribution patterns, usually limited by “invisible” or unexpected boundaries or geographic barriers. *Nemertopsis bivittata* is a direct developer found mostly associated to oyster banks in Brazil. This species has a cosmopolitan distribution, and has been also reported in the Mediterranean Sea, Chile and United States. In order to evaluate the if *Nemertopsis* populations are connected along the Brazilian coast, the Genotyping-by-Sequencing (GBS) tool was applied, which provided thousands of SNPs loci. This allows us to use the seascape genetics approach to infer the historical relationships using the spatial population structure from geo-referenced genetic samples in order to estimate the effective migration surface along the coast.

Worms of doubt: nemerteans of the genus *Lineus* from the White Sea tidal zone

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Nemerteans of the "*Lineus ruber/viridis*" complex have been bothering biologists for a long time. We analyzed the species composition of this complex in the White Sea. Molecular data, based on partial cytochrome c oxidase subunit I and 16S rRNA analysis, indicate the presence of *L. ruber*, *L. viridis* and *L. clandestinus* in the studied area. We found the distinctive morphological feature for each species. Specimens of *L. ruber* in the terminal part of the head have a light margin which grows thicker, forming wide pigment-free areas, due to which the pigment of the anterior part of head usually narrows, acquiring a triangular or trapezoidal shape. The body of *L. viridis* nemerteans has permanent integument wrinkles, clearly visible on relaxed specimens. The anterior third of body of *L. clandestinus* is reddish or crimson, and this colour is not associated with the brain visible through the integument; the posterior 2/3 of the body is greenish, brown, or greyish in colour. External morphology of European and Arctic *Lineus* species has some peculiarities: *L. clandestinus* from the White Sea lacks iridescent ventral fold, and an unusual "green" form of *L. ruber* occurs in the White Sea. Furthermore, the recovered genetic structure is different in the three species. The *L. ruber* TCS haplotype network is distinctly separated into European and "northern" haplotypes. The low level of nucleotide diversity combined with typical star-like structure indicates the ancestral northern *L. ruber* population underwent a significant reduction in effective size in the recent historical past and passed through a bottleneck event. The haplotype network of *L. viridis* showed a close interconnection of Russian and European specimens of this species. The haplotype network of *L. clandestinus* included a smaller number of less diverse haplotypes. The different structure of the *L. ruber*, *L. viridis* and *L. clandestinus* networks might be a result of different dispersal scenarios during the last glacial maximum.

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Nemertean taxonomy in WoRMS

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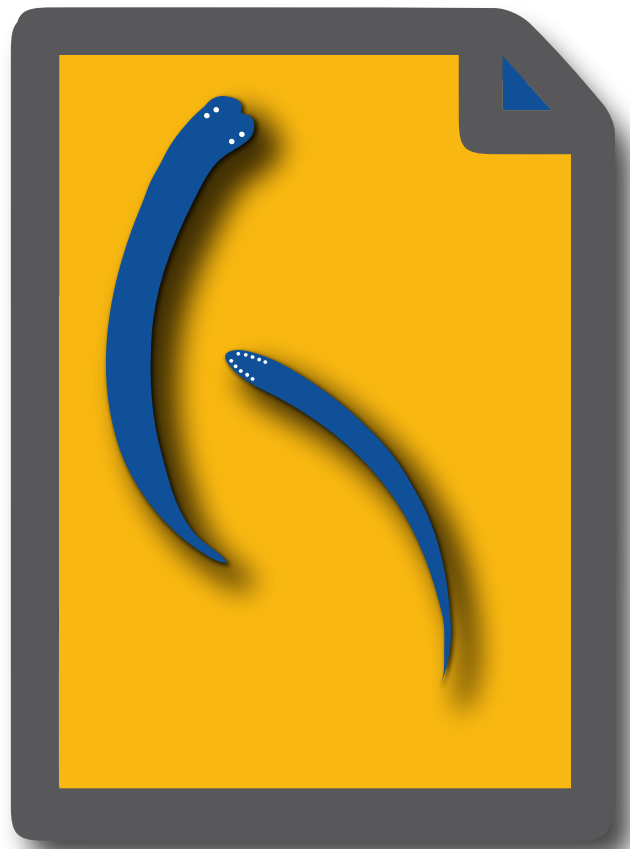
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In April 2017 the editors for Nemertea in World Register of Marine Species, WoRMS, met for three days in Ostende, Netherlands. We had lessons in new functionalities in the system, and we made progress in updating and improving data on nemerteans. We also initiated World Nemertea Database through the system of WoRMS and a proposal for naming of the higher ranks in WoRMS was constructed.

Nemertean posters



Mapping the diversity of nemertean peptide toxins

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Only some 100 research publications involve the discovery or characterization of nemertean toxins. Many involve pyridine alkaloids such as anabaseine and related compounds (1), whereas a series of investigations focus on the occurrence of tetrodotoxin (TTX) (2-4). Moreover, a series of publications by the groups of Kem and Blumenthal from the early 1970's and onward thoroughly report the discovery and characterization of peptide toxins (5); the cytolytic A-toxins and the parborlysins, as well as the neurotoxic B-peptides. The characterization of peptide toxins is cumbersome but advances in both proteomic and genomic methodologies have greatly simplified the procedure. Indeed, a limited number of papers have emerged where such techniques have been employed, and one notable such example involves the discovery of alpha-nemertides (6). However, to date only a minor number of nemertean species have been investigated to any extent with regard to toxin content. In our ongoing project, we attempt to obtain an as complete picture as possible with regard to nemertean peptide toxins. With this poster we aim to provide a brief status report, and to demonstrate the strategies and methods that we employ in this project.

1. W. R. Kem, Pyridine alkaloid distribution in the hoplonemertines. *Hydrobiologia* **156**, 145-151 (1988).
2. K. Miyazawa *et al.*, Tetrodotoxin in two species of ribbon worm (Nemertini), *Lineus fuscoviridis* and *Tubulanus punctatus*. *Toxicon* **26**, 867-874 (1988).
3. M. Asakawa, K. Ito, H. Kajihara, Highly toxic ribbon worm *Cephalothrix simula* containing tetrodotoxin in Hiroshima Bay, Hiroshima Prefecture, Japan. *Toxins* **5**, 376-395 (2013).
4. I. Beleneva, T. Y. Magarlamov, A. Kukhlevsky, Characterization, identification, and screening for tetrodotoxin production by bacteria associated with the ribbon worm (Nemertea) *Cephalothrix simula* (Iwata, 1952). *Microbiology* **83**, 220-226 (2014).
5. W. R. Kem, in *Handbook of Neurotoxicology: Volume I*, E. J. Massaro, Ed. (Humana Press, Totowa, NJ, 2002), pp. 573-593.
6. E. Jacobsson *et al.*, Peptide ion channel toxins from the bootlace worm, the longest animal on Earth. *Scientific reports* **8**, 4596 (2018).

Radial glia mechanically protects the intrapidermal nervous system in *Carinina ochracea* (Carininidae, Nemertea)

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There is recent evidence that radial glia cells are highly specialized epidermal cells containing densely packed intermediate filaments. Intermediate filaments (tonofilaments or keratin fibers) are well known from tetrapod vertebrates where they are essential to form the horny layer of their skin. These filaments are a subset of cytoskeletal elements that are used for transmission of mechanical forces from one cell to the other. In contrast to actin filaments, however, intermediate filaments are not dynamic, but form a rigid and inflexible intracellular meshwork. As such they reinforce the integrity of cells and protect them against lethal mechanical stress. An intraepidermal nervous system is potentially subjected to severe mechanical influences from the exterior, a disadvantage that presumably triggered repeated evolutionary dislocation of the nervous systems into deeper body layers. Actually, except for carininid nemertean groups all other nemertean groups possess a subepidermal central nervous system. Based on histology and electron microscopy we revealed that in *Carinina ochracea* the risk of mechanical stress on the integrity of the intraepidermal nervous system is lowered by using radial glia as protective layer. Several layers of these cells form a crescent 'coat' that covers each medullary cord. The cells are characterized by a specific Azan staining. Their ultrastructure shows a dense packing of intermediate filaments as well as intense intercellular connections by large desmosomes. Such a protection also proved to be present in the brain of *Carinina ochracea* where radial glia forms some kind of 'cerebral cranium'.

New information on the alien terrestrial nemerteans of the Iberian Peninsula

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Mainly due to antropogenic translocations, alien species have become one of the highest threats to global biodiversity. Since March 2014, a visual census of the invasive terrestrial flatworms of Sopela (Bizkaia, Euskadi, Spain) consisting in both diurnal and nocturnal samplings have been carried out obtaining more than 900 records (March 28th 2018). During the night of April 5th 2017 a single adult specimen of the terrestrial nemertean *Argonemertes dendyi* (Dakin, 1915) was recorded. Between January 30th and March 28th 2018, 35 specimens were recorded plus two additional ones in Helgueras (Noja, Cantabria, Spain). In Sopela, two *A. dendyi*, 11 *Leptonemertes chalicophora* (Graff, 1879) and 22 nemertean morph C *sensu* Mateos & Giribet (2008) were found. An unidentified terrestrial nemertean egg mass of 3.2 x 2.1 mm was also recorded in this locality, containing ~35 eggs of ~0.4 mm diameter. During this sampling in Sopela, both juveniles and fully mature individuals were recorded, suggesting the presence of stablished populations of the three species in this locality. Many individuals from Sopela and those from Helgueras were sampled in places near beaches and highly exposed to the sea breeze, suggesting these species have tolerance to saline environments exposition. The majority of the individuals were found in highly artificial places, such as gardens or road margins, but also some were found in less antropogenic environments, as grazing lands. Since nemerteans are voracious predators, these species might constitute a threat for the native soil fauna which might be more widespread than previously thought.


Molecular systematics of the heteronemertean genus *Dushia* (Nemertea, Pilidiophora)

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Micrura japonica Iwata, 1952 was originally described from Kyushu, and has been reported from several places along the coasts of Japan from Niigata southward. In southern Japan, *Cerebratulus rubellus* (Stimpson, 1855) is often found sympatrically with *Micrura japonica*; the only difference between the two is the body color (i.e. black '*Micrura japonica*' vs. orange *C. rubellus*). In addition, we obtained a brown form from Kagoshima and Okinawa, which appeared as if they represented a hybrid between the black and orange forms. We inferred the phylogenetic position of '*Micrura japonica*' using five gene markers (16S rRNA, COI, 18S rRNA, 28S rRNA, histone H3 genes) among 42 heteronemertean species, for which sequences of these gene markers were available in GenBank. Our analysis revealed that '*Micrura japonica*' is sister to *Dushia atra* sensu Corrêa, 1963 with full nodal support, suggesting '*M. japonica*' represents a second member of *Dushia* Corrêa, 1963. Our COI haplotype network analysis suggested that specimens from Kagoshima and Okinawa form at least two different biological entities, which involve three color morphs (orange, brown, black), and at least four nominal species (*Meckelia niger* Stimpson, 1855, *Meckelia rubella* Stimpson, 1855, *Micrura formosana* Yamaoka, 1939, *Micrura japonica* Iwata, 1952). Future analyses with topotypes of these nominal species are necessary for objective allocation of these available names to the discovered taxa. If the same taxonomic species turns out to contain the two nominal species *Me. nigra* and *Me. rubella*, nomenclatural precedence should be given to one of them by the act of the First Reviser under Article 24.2.2 of the Code (ICZN 1999), because these two names were published in the same work. Corrêa (1963) fixed *Meckelia atra* Girard, 1851 as the monotypic genus *Dushia*. While *D. atra* sensu Girard (1851) was collected from deep water of Florida, *D. atra* sensu Corrêa (1963) occurs in littoral, shallow waters in the Caribbean. There is little doubt that *Dushia atra* sensu Corrêa (1963) represents an undescribed species, and thus requires a new name. Article 70.3 of the Code (ICZN 1999) stipulates that "if an author discovers that a type species was misidentified, the author may select, and thereby fix as type species, the species that will, in his or her judgment, best serve stability and universality, either 70.3.1. the nominal species previously cited as type species, or 70.3.2. the taxonomic species actually involved in the misidentification". We are inclined to take the latter option.

Beyond Stranger Stripes: Morphological polymorphism and genetic diversity in *Nemertopsis cf. bivittata* along the Brazilian coast

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Nemertopsis bivittata is an Hoplonemertean species with abbreviated larval development and easily recognized by the two parallel black stripes along the body's dorsal region. On the head, the stripes can cross the first pair of eyes, or continue along the head. It is a cosmopolitan species, reported in West Europe, Mediterranean Sea, Red Sea and both coasts of the American continent. In Brazil, *Nemertopsis bivittata* occurs from Ceará to Santa Catarina, 2870km apart, where both morphotypes can co-occur. Multiple studies showed that species without mechanisms of long distance dispersion, e.g. absence of larva, but broad distributions tend to be a complex of morphologically cryptic species. Therefore, we aim to uncover possible cryptic lineages of *Nemertopsis bivittata* in Brazil. Specimens were collected in seven localities covering its distribution in the Brazilian coast. Individuals were photographed and preserved in 99% ethanol, kept at -20°C until DNA extraction. The mitochondrial cytochrome oxidase I region was amplified with standard PCR protocol. Sequences were aligned and a maximum likelihood (ML) tree in RAxML, and an ultrametric tree in BEAST were obtained. Species delimitation analyses were performed to establish the identity of Brazilian populations of *N. bivittata*. The ML tree was used as input to bPTP and the ultrametric tree as input to GMYC. Sequences were also analyzed in TCS, under 95% parsimony, and in the ABGD. Four different color patterns were found along the coast: (1) stripes parallel, beige background (MP1), (2) with gray background (MP2), (3) stripes crossing, beige background (MP3), (4) with gray background (MP4). bPTP and TCS showed similar results, delimitating one group with five individuals: three MP1 and two MP2, all from the South and Southeast coasts. All other individuals are considered as different species individually, in both analyses. ABGD, considering the intraspecific distance between 0.001 and 0.0183, presented a similar pattern of the other two analyses with two more MP1 individuals on the larger clade. GMYC delimitated the same group as bPTP and TCS, along with another group composed by two individuals from Northeast, one MP3 and MP4. These results indicate the presence of several cryptic species in Brazil, especially among individuals with crossed stripes. However, nuclear markers and genomic data still will be analyzed to confirm the number of species in the coast and if there is an association between color patterns and these cryptic species.

A new monostiliferan species (Nemertea:Hoploneurtea) from the Mediterranean Sea in Italy

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The Mediterranean Sea is one of the best documented regions concerning species diversity of Nemertea with about 60 known species. A considerable number have been described based on external characters, however these descriptions do not often include details on their internal morphology. This makes the description and systematic assignment of new species problematic. The last two decades have shown that a combination of molecular barcoding and detailed morphological descriptions can accurately assess systematic affinities of species identified as being new to science. In this study, we are reporting a new monostiliferan hoplonemertean from the Island of Giglio, Italy. So far, no description fits the specimens from Giglio, indicating that they represent a species new to science. We provide a comprehensive data set containing information about habitat, life cycle, outer and inner morphology, and sperm ultrastructure. In combination with molecular markers (COI, 16S, 18S, 28S) we will give a detailed characterization of the species. First results on sperm ultrastructure hint at an affiliation to Amphiporidae (Monostilifera, Hoplonemertea). This study once again shows that even in well studied areas, species diversity in Nemertea is underestimated.

An Anchialine Cave-Adapted Nemertean is a New Species of Reptantia

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We present the first report of a nemertean that appears to be an obligate and morphologically adapted anchialine speleobiont. It is a Hoplonemertea Reptantia and characterized by relative reduction of typical reptantic features. The diagnosis is as follows: polystyliferous armature; precerebral cephalic lobe present, small, distinctly narrowed, anterior margin broadly rounded; posterior terminus blunt when swimming or floating, tapered point when anesthetized; body up to 20 mm long, 0.8 mm wide; mouth subterminal, anterior to brain, separate from proboscis pore; cerebral ganglia reddish-orange, dorsal ganglia larger than ventral; cerebral organ posterior to ganglia, with simple pore, duct not forked; eyes and cephalic furrows lacking; epidermis translucent white, bearing numerous elongate (ca. 100 μ m) cirri over entire dorsum; rhynchocoel voluminous, extending to posterior terminus, no diverticula; proboscis white, extending into posterior fifth of body; intestinal diverticula white, contiguous anteriorly, spaced posteriorly, each with 2 terminal shallow, lobate digitations, gaps between diverticula filled with gelatinous extracellular matrix; mid-dorsal blood vessel, vascular cross connections, nephridia not observed; COI sequence for two specimens. The worms appear to lead a primarily pelagic existence, floating passively in a "J" shape, and they resemble the gelatinous appearance common to deep-sea pelagic nemerteans. The worms are capable of vigorous sinusoidal swimming when disturbed. In dishes, they settle to the bottom and glide, unless disturbed. Sanger sequencing of 5 genes (COI, 16S, 18S, 28SrDNA, and Histone 3) reveals substantial genetic divergence from other reptantic nemerteans sequenced to date, which may reflect either or both a) rapid molecular evolution, possibly driven by its speleobiont existence, or b) an ancient origin, which begs the question of its limited distribution and where it was while the caves were dry during the last ice age.

A *Carcinonemertes* nemertean transferring sperms by spermatophore

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Several types of gamete transfer have been reported for nemerteans. The broken-off penis (during copulation) was hypothesized to function as a type of spermatophore in some pelagic nemerteans. In a *Carcinonemertes* nemertean we found on the crab *Charybdis japonica* from the Yellow Sea, China, the male transfers spermatozoa to the female through spermatophores. The adult worm lives in a mucus sheath, which is attached on the egg mass of female crabs and is ornamented with numerous lapilli. The juvenile was found among gill filaments of host and was encysted in a mucus sheath without any ornamentations.

Body color white, yellow, orange or red orange (encysted juvenile). Two eyes, brown, blackish brown or reddish brown in color. Female larger than male, former 2.5-12.5 (6.2±2.7) mm long and 0.19-0.46 (0.30±0.09) mm wide, latter 1.6-8.3 (4.1±1.8) mm long and 0.13-0.32 (0.23±0.06) mm wide. Posterior end of male funnel shaped. Esophagus not ciliated. Body wall sending muscle strands to stomach at junction of stomach and intestine. Anterior region of intestine possessing circular muscles. Proboscis diaphragm wide and short, bulb small. Cephalic glands and submuscular glands well developed. Female stylet basis 22-26 (24.1±1.3) µm long and 6-9 (7.2±0.6) µm wide, stylet 7-10 (8.5±0.8) µm long, stylet:basis ratio 0.308-0.457 (0.353±0.032); male stylet basis 23-28 (24.3±1.3) µm long and 6-9 (7.4±0.8) µm wide, stylet 7-10 µm (8.5±0.9) µm long, stylet:basis ratio 0.292-0.409 (0.352±0.042). Ovaries arranged in one row on each side. Some females bearing 1 to 10+ spermatophores on body surface. Male with numerous testes. Takakura's duct and seminal vesicle surrounded by well-developed blue-staining glands (Mallory), probably serving to form spermatophores. Seminal vesicle possessing well-developed circular muscles. Egg strands transparent. Eggs/embryos 68-81 µm in diameter. Larva with two brown eyes, and anterior and posterior cirri.

Our specimens morphologically resemble *Carcinonemertes mitsukurii*, which was reported originally from the fresh/brackish water crab *Eriocheir japonica* and afterwards from three species of *Charybdis* and one species of *Portunus* crabs in Pacific waters. Present specimens differ from the original description for *C. mitsukurii* by having a smaller body size, a shorter basis, a larger stylet:basis ratio, and the absence of paired anterior caeca. Spermatozoon transfer by means of spermatophores represents a novel mating type in the phylum Nemertea.

Search for tetrodotoxin and its analogues in some nemertean species from the Sea of Japan, Russia

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Many marine ribbon worms Nemertea were shown to possess various toxins, which were used for hunting and protection from predators. The most interesting among the toxins found in these animals is tetrodotoxin (TTX) - a non-protein, low-molecular weight neurotoxin of bacterial origin. TTX was found in both anopla and enopla nemerteans. Some nemertean species have been shown to contain extremely high concentrations of TTX; however, complex studies on the toxin and its numerous analogues presence among nemerteans were not conducted. The purpose of this study is to search for TTX and its analogs in nemerteans from different taxonomic groups.

Samples of Palaeonemertea (*Cephalothrix simula* and *Tubulanus punctatus*), Heteronemertea (*Kulikovia alborostrata*, *Kulikovia manchenkoi*, *Micrura bella*, *Cerebratulus marginatus* and *Nipponomicrura uchidai*) and Hoplonemertea (*Paranemertes* sp., *Quasitetrastemma stimpsoni* and *Collarenemertes bimaculata*) were collected from the Spokojnaya Bay and Vostok Bay of the Sea of Japan, Russia. For the analysis for TTX and its analogues presence in nemertean extracts, the high-performance liquid chromatography-tandem mass spectrometry was used.

The maximum TTX concentration (2.8 mg/g) was found in the *C. simula* extract, the toxin concentration in the extracts of *T. punctatus* and *K. manchenkoi* was significantly lower – 1.2 ng/g and 1.8 ng/g, respectively. In the extracts of *K. alborostrata*, *Q. stimpsoni* and *C. bimaculata* trace amounts of TTX were found. Besides the TTX, four out of the thirty-three its analogues known to date have also been identified in the present research. 5-deoxy-TTX/11-deoxy-TTX was found in extracts of *T. punctatus*, *N. uchidai*, *Paranemertes* sp., *Q. stimpsoni* and *C. bimaculata*. 5,6,11-trideoxy-TTX was found in extracts of *K. alborostrata*, *K. manchenkoi*, *N. uchidai* and *Q. stimpsoni*. Extracts of *T. punctatus*, *N. uchidai* and *Q. stimpsoni* contained 11-norTTX-6(S/R)-ol. 4,9-anhydro-TTX was found only in *C. simula* extract. The obtained data indicate a lack of a correlation between the presence of TTX and the presence of its analogues in nemerteans of different taxonomic groups. For the first time the presence of 5-deoxy-TTX/11-deoxy-TTX and 5,6,11-trideoxy-TTX in nemerteans was shown.

Open up for the big stuff! –Larval feeding without a prototroch

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The pilidium larva of Pilidiophora feeds on unicellular microalgae (microphagous) by means of a band of elongated cilia encircling the mouth opening on the larval lappets. In palaeonemertean larvae, no pre-oral band of elongated cilia (prototroch) as primary feeding structure is present. Although it is known that palaeonemertean larvae feed on larger particles (macrophagous), it is not documented how the food is ingested. Investigation on what and how the larva feeds reveals a feeding mode in which larger particles are captured and ingested by action of the body-wall and buccal musculature. This result implies that elongated ciliary bands are not mandatory for larval feeding. Additionally, the feeding mode shown in the nemertean larva is similar to that shown by many adult worm-like spiralians. In conclusion, the feeding mode observed supports the phylogenetic position of Nemertea as closest related outgroup of Trochozoa.