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MUNICH GRADUATE SCHOOL FOR
EVOLUTION, ECOLOGY AND SYSTEMATICS

EES LMU

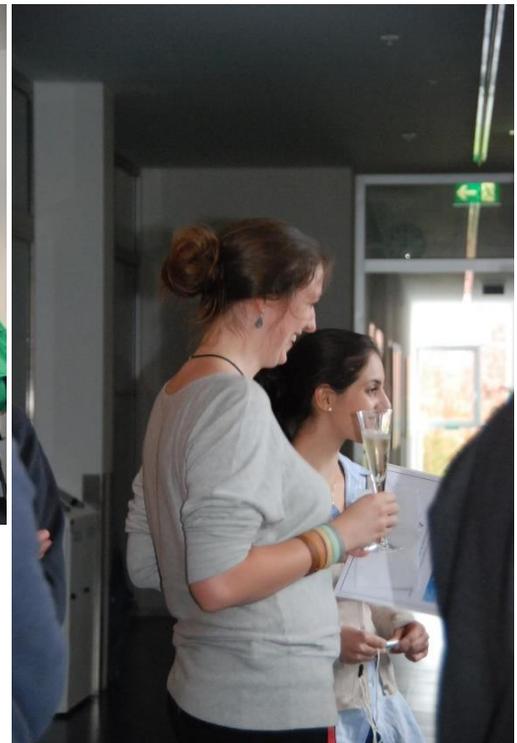
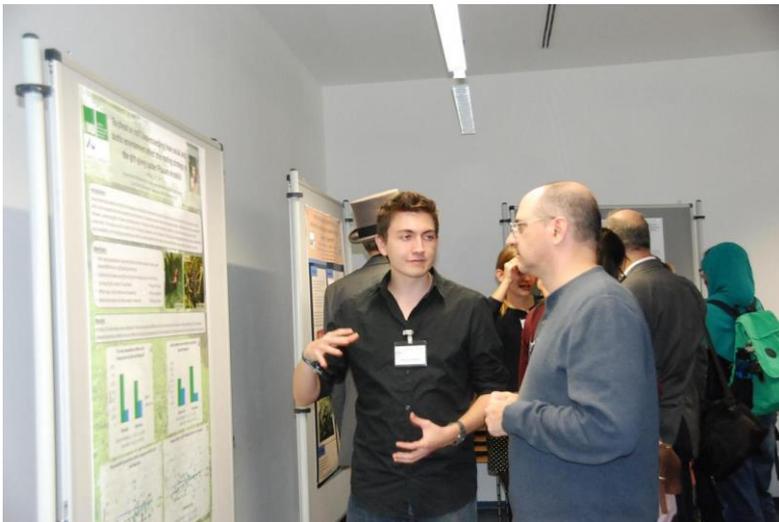
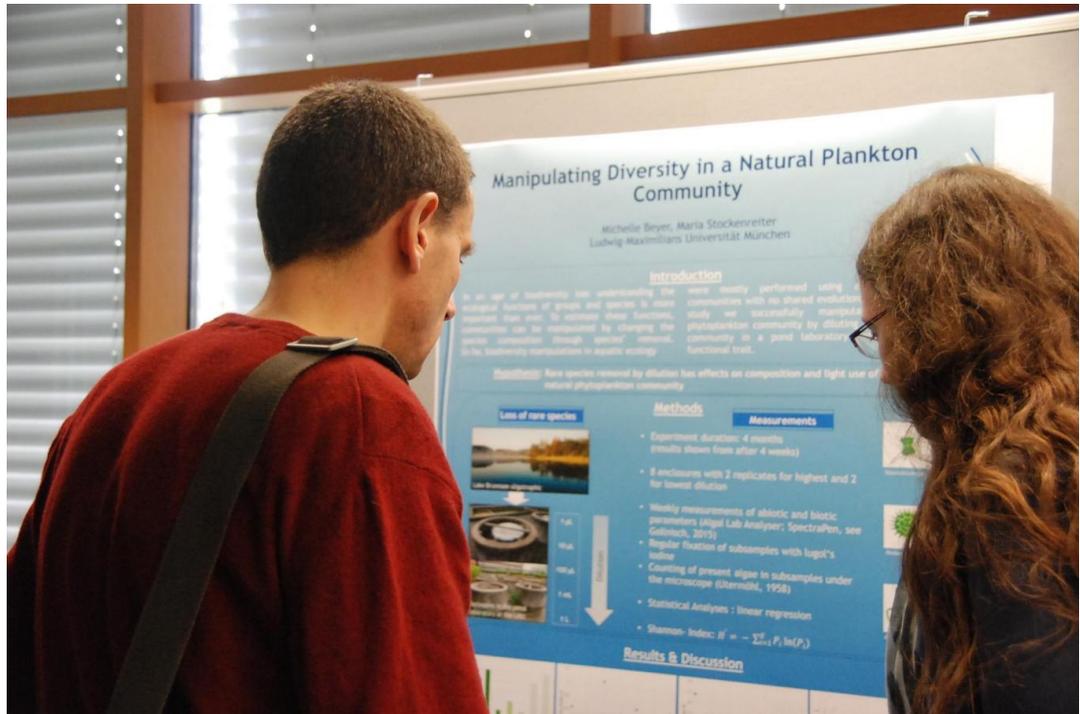
WELCOME TO THE 9th EES CONFERENCE



Program & Abstracts

October 8th, 2015 Biocenter, LMU in B.01.019





9th EES Conference October 8th 2015

Biozentrum, Kleiner Hörsaal 1 (room B.01.019)



PROGRAM

- 9:00 Opening words**
Professor Dr. John Parsch
- 9:10 EES PhD talks (chair Prof. Dr. Parsch)**
- 9:10 **Ann Kathrin Huylmans** - "Evolution and Regulation of Sex-Biased Genes in Two Arthropod Species"
- 9:30 **Korbinian von Heckel** - "The transcriptional basis of cold adaptation in *D. melanogaster*"
- 9:50 **Oscar Alejandro Pérez** - „How to assess divergent evolution between the nuclear host and chloroplast endosymbionts"
- 10:10 Coffee break**
- 10:30 EES Master talks (chair Prof. Dr. Haszprunar)**
- 10:30 **Jessica Huang** - "Resource use in mixotroph marine jellyfish"
- 10:45 **Raúl González Pech** - "Individual plasticity vs. stress response: lessons from a transcriptome survey of an adult coral exposed to low pH"
- 11:00 **Raphael Gollnisch** - "Diversity dependence of light use efficiency in phytoplankton communities"
- 11:15 **Mark Scherz**- "Disentangling cryptic diversity of Madagascar narrow-mouthed frogs (*Anura: Microhylidae: Cophylinae*) with an integrative dataset"
- 11:30 Poster presentations** - Why look at the posters? 3rd semester EES students
- 11:50 Lunch on your own**
- 12:50 - 14:15 Poster session in C.00.13**
- 13:30 - 14:30 EES Faculty meeting (B.01.015)**
- 14:30 EES Master talks continue (chair Dr. Schultes)**
- 14:30 **Armin Scheben** - "Neotropical origin, transoceanic dispersal, and cryptic speciation in the leafy liverwort *Ceratolejeunea (Lejeuneaceae, Porellales)*"
- 14:45 **Laura Hardulak** - "The transcriptomics of environmental sex determination in *Daphnia*"
- 15:00 **Cen Zeng** - "The Evolution of FtsX-Domain Proteins in *Firmicutes Bacteria*"
- 15:15 **Rosa Elena Andrade Aguirre** - "Regulation of *Lotus japonicus* root nodule symbiosis by nitrate via inhibition of *NIN* expression"
- 15:30 **Ella Lattenkamp** - "Passive listening in *Rhinolophus ferrumequinum* - Do horseshoe bats complement their echolocation with listening for prey-generated sounds?"
- 15:45 Coffee break**
- 15:45 Prize committee meeting (room B.01.015)**
- 16:15 Awards and graduation ceremony**

EES PhD Talks

Korbinian von Heckel

Evolutionary Biology, Biozentrum, Planegg-Martinsried, Ludwig-Maximilians University, Munich, Germany

The transcriptional basis of cold adaptation in *D. melanogaster*

genomewide transcriptional response towards a cold shock in ancestral tropical and derived temperate fly strains via RNAseq *Drosophila melanogaster* - nowadays a cosmopolitan human commensal - is of afro-tropical origin and colonized temperate habitats only after the last glaciation event about 15,000 years ago. An increase in cold resistance has likely been one of the major adaptations facilitating this range expansion. My PhD project aims at uncovering genes that are responsible for the increased cold tolerance of European flies, with particular regard to adaptive changes in gene expression. To this end, I have measured the genomewide transcriptional response towards a cold shock in ancestral tropical and derived temperate fly strains via RNAseq. I will present general characteristics of the cold shock response, highlight population differences and discuss the role of canalization and phenotypic plasticity.

Ann Kathrin Huylmans

Evolutionary Biology, Biozentrum, Planegg-Martinsried, Ludwig-Maximilians University, Munich, Germany

Evolution and Regulation of Sex-Biased Genes in Two Arthropod Species

Males and females of most species differ strongly in their morphology, metabolism, and behaviour. However, depending on their mode of sex determination, both sexes usually share a large part of their complete genome. Hence, most of the differences can be attributed to differential gene expression. The sex determination system can have a strong influence on how these sex-biased genes evolve and how they are distributed in the genome. We study different aspects of this in two species of arthropods, *Daphnia galeata* with environmental sex determination on the one hand and *Drosophila melanogaster* with genetic sex determination on the other. In *D. melanogaster*, we find that dosage compensation shapes sex-biased gene expression on the X chromosome and differs between tissues. *D. galeata*, a non-model organism, shows very low sex bias that may be attributable to the switch between sexual and asexual reproduction.

Oscar Alejandro Pérez-Escobar

Department of Biology, Systematic Botany and Mycology, Ludwig-Maximilians University, Munich, Germany

How to assess divergent evolution between the nuclear host and chloroplast endosymbionts

Phylogenetic relationships inferred from multilocus organelle and nuclear DNA are often difficult to resolve because of evolutionary conflicts among gene trees. However, conflicting or outlier associations (linked terminals in two phylogenies) among these data sets often provide valuable information on evolutionary processes such as hybridization, incomplete lineage sorting and Horizontal Gene Transfer. Statistical tools used in cophylogenetic studies have the potential to test for concerted evolution between organelle and nuclear data sets and reliably detect outlier associations. Two distance-based methods, PACo (Procrustean Approach to Cophylogeny) and ParaFit, were used in conjunction to detect those outliers contributing to conflicting phylogenies. We explored their efficiency retrieving outlier associations, and the impact of input data (unit branch length and additive trees) between data sets, by using several simulation approaches. Additionally, in order to test their performance using real data sets, we inferred the phylogenetic relationships within *Neotropical Catasetinae* (*Epidendroideae*, *Orchidaceae*), which is a suitable group to investigate phylogenetic incongruence due to hybridization processes between some of its species. A comparison between trees derived from chloroplast and nuclear sequence data reflected strong, well supported incongruence within *Catasetum*, *Cynoches* and *Mormodes*. As a result, outliers among chloroplast and nuclear data, and in experimental simulations,

were successfully detected by PACo when using patristic distance matrices obtained from phylograms, but not from unit branch length trees. The performance of ParaFit was overall inferior compared to PACo, using either phylograms or unit branch lengths as input data. Because workflows for applying cophylogenetic analyses are not standardized yet, we provide a pipeline for executing PACo and ParaFit as well as displaying outlier associations in plots and trees using the software R. The pipeline renders a method to identify outliers with high reliability and assess the combinability of the independently derived data sets by means of statistical analyses.

Master Talks

Rosa Elena Andrade Aguirre

Regulation of *Lotus japonicus* root nodule symbiosis by nitrate via inhibition of *NIN* expression

Legumes establish root nodule symbiosis (RNS) with nitrogen-fixing soil bacteria (rhizobia). Nitrogen fixation by rhizobia inside root nodules requires carbon sources delivered by the host plant. Since nitrogen fertilizers provide nitrogen at lower carbon investment, nodule formation is repressed when nitrate is available. In *Arabidopsis thaliana* Nodule Inception like proteins (NLPs) have a role in nitrate sensing, acting as transcription factors that regulate the expression of primary nitrate-responsive genes. NLPs are therefore candidates for coordinating nitrate availability with RNS. We aimed to analyze the role of nitrate and NLP's in the RNS of the model legume *Lotus japonicus*. We confirmed that nitrate inhibits nodulation and that nodule number is correlated with *NIN* (*NODULE INCEPTION*) expression. CYCLOPS activates *NIN* transcription, and *NIN* inhibits its own expression. We observed that nitrate interferes with the transactivation of the *NIN* promoter by phosphomimetic CYCLOPS-DD, and the inhibition is independent of the CYCLOPS-DD binding site (*CYC-RE*) previously identified. We aimed to investigate whether NLPs inhibit *NIN* expression as *NIN* does, because of their conserved DNA-binding domain. Transient expression assays in *Nicotiana benthamiana* showed that NLP4 was localized in the nucleus and repressed CYCLOPS-DD mediated transcriptional activation of *NIN* as *NIN*. To test the binding capacity of NLP4 to the *NIN* promoter the C terminal part of NLP4 (NLP4-C) was used in electrophoretic mobility shift assays. However, this truncated protein did not bind *in vitro* to the region where *NIN* does. These results open a new perspective for the inhibition of nodulation by nitrate mediated by NLP4.

Raphael Gollnisch

Diversity of light use efficiency in phytoplankton communities

In the context of global biodiversity loss, understanding and quantifying the consequences of diversity in the phytoplankton--zooplankton interface is a major topic in ecology. Loss of species connected with a loss of certain species connected traits, and therefore affects ecosystem functioning. Differences in absorbance patterns along diversity gradients of assembled polycultures from microalgal laboratory strains and in natural phytoplankton communities from experimental mesocosm studies were analyzed to investigate the impact of a functional--trait loss in both artificially composed phytoplankton polycultures and natural phytoplankton communities. Results from both experiments underline the crucial role of functional diversity on absorbance of light over the PAR (photosynthetic active radiation) spectral range. This characterizes the relationship of species reduced natural phytoplankton communities with their light use efficiency in general for the first time. Pigments as key traits for primary production are connected to other life history relevant traits like fatty acids which determine food quality and might therefore influence trophic transfer efficiency.

Raúl A. González Pech

Individual Plasticity vs. Stress Response: Lessons from a Transcriptome Survey of an Adult Coral Exposed to Low pH

Ocean acidification is one of the major threats for coral reefs at a global scale. The predicted decrease of the surface water pH by the end of the century could imply a rough future for organisms with carbonate skeletons, such as stony corals. However, the severity of the impacts on coral reefs remains as matter of controversy. In spite of the several studies on the physiological response of stony corals to changes in pH, the response of the holobiont (*i.e.* the coral itself plus all its symbionts) remains largely unexplored. In the present study, I assessed the changes in overall gene expression of *Montipora digitata* and its micro-algal symbionts after exposing them for 3 days to a low pH value (~7.6). Differences in gene expression could be not only attributed to the treatment but also considerably to individual plasticity and stochastic gene expression. Still, the effect of low pH on the coral gene expression was rather small, impacting mostly the transcription levels of proteins potentially involved in extracellular matrix composition, organic skeleton organization and calcium precipitation. Coral calcification rate was decreased after the 3-days exposure to low pH, supporting the gene expression findings. No significant modification in gene expression of the symbionts suggesting that the host represents a stable environment by protecting them of severe environmental conditions. Additionally, the present work contributes with transcriptome data for Scleractinia and *Symbiodinium* that can be used in future research

Laura A. Hardulak

Freshwater crustaceans from the genus *Daphnia* are emerging model organisms in the field of environmental genomics. *Daphnia* are cyclic parthenogens and their sex is determined by the environment. In this process, methyl farnesoate (MF), the crustacean analog of insect juvenile hormone, is the primary mediator of the switch to sexual reproduction. When exogenous MF was added to non-male-producing (NMP) clones of *D. pulex*, they exhibited very different transcriptomic responses compared to regular clones. We hypothesized that a significant proportion of the genes affected by MF would be sex-biased, because physiological changes that are mediated by this hormone are primarily related to reproduction and sex differentiation. Accordingly, we found that significantly more genes affected by MF in NMP *D. pulex* were female-biased genes. We further compared sex-biased gene expression between *D. pulex* and *D. galeata*, a novel *Daphnia* model species whose transcriptome was recently sequenced in our lab. We found that they followed similar patterns. What is more, female-biased genes tended to be more conserved than male-biased genes, as revealed by orthology analysis of these two species. Finally, a gene ontology analysis in the NMP treated with MF revealed enrichments of terms associated with regulation of response to stimulus, signal transduction, and cell communication. At the same time, in the facultatively sexual clone, terms related to general metabolic and protein processes were found to be only marginally significant. The limited extent of gene expression perturbations uncovered by the GO analysis indicates that artificially adding MF for the purpose of producing male daphniids for laboratory research should not produce extraneous effects on the transcriptome.

Jessica Huang

As global change affects upper-water stratification in oceans, nutrient cycling becomes more difficult and the upper water column becomes more oligotrophic. Such an environment favors organisms that employ a generalist life history strategy in terms of nutrition such as mixotrophy by combining heterotrophic (predation) and autotrophic (photosynthesis) pathways. Such a life history strategy can often be found in algae but also in animals such as those within the group of Cnidaria. Two mixotrophic jellyfish species, *Mastigias spp.* (golden jellyfish) and *Cassiopea spp.* (upside-down jellyfish) hosts the

photosynthetic endosymbiont dinoflagellate, *Symbiodinium spp.*, and are both found in Palau, Micronesia.

I investigated resource use and niche differentiation among these mixotroph jellyfish species, as they could be potential profiteers in future ocean environments. I analyzed light use efficiency measurements from previous years' and the current year. Additionally I analyzed stable carbon and nitrogen isotopes to determine the trophic position of *Mastigias spp.* and *Cassiopea spp.* in relation to each other and among other organisms found in their environment. I also conducted an experiment investigating the presence of nitrogen-fixing bacteria (NFB) in *Cassiopea spp.* Previous years' data had indicated a stable nitrogen signature pointing towards an endosymbiotic NFB presence that would allow *Cassiopea spp.* to utilize airborne nitrogen as a potential nitrogen source.

My results show both mixotroph jellyfish species *Mastigias spp.* and *Cassiopea spp.* and their endosymbionts are using resources very similarly. It also seems that endosymbionts of the genus, *Symbiodinium*, are not perfectly well adapted to the underwater light climate of marine lakes. However, both jellyfish species seem to rely mainly on endosymbionts for energy and matter for growth independent of the habitat they inhabit (lakes versus coves/lagoons). *Mastigias spp.* was more linked to the pelagic food web and seemed to have a higher contribution of zooplankton to its nutrition compared to *Cassiopea spp.* There was experimental and observational support for NFB presence in *Cassiopea spp.* in lake environments. This observation is the first stable isotope and experimental support that jellyfish can host nitrogen-fixing bacteria as symbionts.

Ella Z. Lattenkamp

Passive listening in *Rhinolophus ferrumequinum*

Echolocation is a highly sophisticated sensory system for actively probing light- deficient environments. However, due to the stroboscopic and directional emission of the calls and the strong attenuation of ultrasonic frequencies, the space that can be probed by biosonar is limited both temporally and spatially. This limitation is likely to favour the opportunistic use of additional information such as prey-generated rustling sounds for prey detection. Thus, bats may exploit a much wider range of environmental information than provided by their biosonar.

In the present study I tested this hypothesis in greater horseshoe bats (*Rhinolophus ferrumequinum*), whose echolocation is specialized for the detection of fluttering insects, but spatially strongly limited due to high call frequencies. I predicted that bats would react to prey- generated sounds by steering their sonar beam towards the position of the sound source for further biosonar-based evaluation. To present both rustling and control sounds and monitor bat echolocation, I used a self-developed spherical array of three loudspeakers and eight microphones. Bats were trained to perch in the centre of the spherical array. To verify the bat's sensitivity to prey-generated sounds phase-scrambled and amplitude-inverted modifications of the recorded rustling sounds were used as controls. The bat's echolocation behavior was recorded simultaneously with all eight microphones for offline analysis of relative call intensity and thus call direction.

Here, I show that greater horseshoe bats react to prey-generated sounds. Beam movement and direction are also guided by the extended perception of sound sources via passive listening. Although the temporal reaction patterns and the strength of reaction vary between individuals and stimuli, I demonstrate that passive listening is used in addition to echolocation and that the biosonar is directed towards the sound source in reaction to the presented stimuli.

Armin Patrick Scheben

Neotropical origin, transoceanic dispersal, and cryptic speciation in the leafy liverwort *Ceratolejeunea* (Lejeuneaceae, Porellales)

Ceratolejeunea is pantropical liverwort genus with some 40 species, occurring in the Americas, Afromadagascar, Asia, and Australia. Currently, the species relationships and historical biogeography of the genus are poorly known. Sequences of two chloroplast regions (*trnL-F*, *rbcL*) and the nuclear ribosomal ITS region of 55 accessions comprising 16 species were obtained to explore the phylogeny of *Ceratolejeunea*. Divergence times were estimated with a Bayesian relaxed clock method and fossil and secondary calibrations. Ancestral areas were reconstructed using the statistical dispersal-vicariance (S-DIVA) and likelihood dispersal-extinction-cladogenesis (DEC) approaches. Here, the first molecular phylogeny of *Ceratolejeunea* is presented, indicating conflict with current subgenus concepts and species circumscriptions based on morphology. Furthermore, the phylogeny suggests the prevalence of cryptic to semicryptic speciation in the genus. Biogeographical analyses provide preliminary evidence for a Neotropical origin of *Ceratolejeunea* and a diversification of the crown group between the Oligocene and Miocene. The observed disjunctions could be explained by four transoceanic dispersal events from the Neotropics to Afromadagascar and one from Afromadagascar to Australasia during the Miocene and Pliocene. In conclusion, the recovered phylogeny suggests the need for a revised classification and can be employed as a framework for morphological re-evaluation of species and subgenus concepts. The inferred prevalence of transoceanic dispersal and cryptic speciation supports the mounting evidence of the general nature of these patterns in liverworts.

Mark D. Scherz

Disentangling cryptic diversity of Madagascan narrow-mouthed frogs (Anura: Microhylidae: Cophylinae) with an integrative dataset

Madagascar's narrow mouthed frogs (Anura: Microhylidae) of the subfamily Cophylinae have repeatedly transitioned among arboreal, terrestrial, and fossorial niches, and consequently exhibit a high degree of homoplasy. This has inhibited progress in resolving the taxonomy of the group. Three-dimensional x-ray reconstruction using micro-Computed Tomography (micro-CT) has opened new opportunities and avenues of investigation in this field, by making skeletal morphology available without damaging specimens. In this thesis, I use external morphology and digitalized skeletal morphology to address three major taxonomic problems in the Cophylinae:

(i) The distinctness of the genera *Stumpffia* and *Rhombophryne*, which has recently been called into question. I show that these are highly distinct genera, and that their synonymization would result in a considerable decrease in diagnosability.

(ii) The differences between deeply divergent lineages of miniaturized frogs, all of which had formerly been considered to be members of the genus *Stumpffia*. I show that, despite overall external morphological similarity, these lineages are distinct in morphology and osteology, and warrant recognition as new genera.

(iii) The absence of any known features that can be used to distinguish between the genera *Rhombophryne* and *Plethodontohyla*. After dividing these genera into morphologically similar, genetically supported species groups, I identify external and skeletal characters that can be used to differentiate among these groups, and consequently the two genera.

I synthesise my findings by outlining the morphological and osteological characters that typify the genera *Rhombophryne*, *Plethodontohyla*, and *Stumpffia*, as well as the genus-level *Stumpffia*-like clades. I produce a key to these groups to aid in future research on this subfamily. I discuss the evolution of the Cophylinae, focusing on the role of the skeleton in homoplasy, niche lability, and size diversity. As our

understanding of their systematics is increasing, it is becoming clearer that the Cophylinae are an ideal system in which to study the interplay between vertebrate morphology and niche occupation.

Cen Zeng

The Evolution of FtsX-Domain Proteins in Firmicutes Bacteria

Antibiotic resistance has increased severely in bacteria, causes global health issues nowadays and alarms researchers worldwide. Up to now, Antimicrobial peptides (AMPs) have always been considered as promising candidates for new antibiotics. However, AMP resistance has been described in many environmental bacteria and even in human pathogens. In this study, we aim to study the so-called bacitracin (Bce)-like resistance systems that are found in many Gram-positive bacteria. These systems consist of a transporter that is presumably responsible for the removal of antibiotics from the cell and a regulatory system controlling the production of the transporter. All these systems share a conserved domain –FtsX- which is also present in other resistance transporters and found in almost all bacteria. By means of bioinformatics analysis, we studied the phylogeny of FtsX-domain and the evolution of FtsX-domain bearing proteins in a large phylum of Gram-positive bacteria, Firmicutes. Initial results showed that among the architectures of FtsX-protein, the one containing a MacB-like periplasmic core domain (PCD) appeared to be the oldest type. Our results also supported the independent origin of the second FtsX-domain in architectures containing two FtsX-domains. Furthermore, we built an open entrance workflow for connection between one research target, FtsX-domain and other genetic markers through this study.