Quaderni del Centro Studi Alpino – IV

UZI

76° CONGRESSO
15-18 settembre 2015
VITERBO

Proceedings of the 76th National Conference of the Unione Zoologica Italiana

A cura di Marzio Zapparoli, Maria Cristina Belardinelli

Università degli Studi della Tuscia
2015

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Università degli Studi della Tuscia
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Viterbo 2015

76th National Conference of the Unione Zoologica Italiana

Università degli Studi della Tuscia
Viterbo, 15-18 September 2015

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The Centro Studi ALPino (CSALP) is an administrative unit of the Università della Tuscia (Italy), whose headquarter is in Tesino plateau (Trento province), with teaching and conference facilities located mainly in Pieve Tesino and research facilities mainly in Cinte Tesino. It was established in 2003 thanks to the efforts of two professors of the Università della Tuscia, Enzo Avanzo and. Ervedo Giordano, the cooperation of the municipality of Pieve Tesino and the financial support of the government of the Trento Province. The Center is run by a management committee, appointed by the rector every three years, which includes representatives of all departments of the university.

The primary goals of CSALP are: (1) to provide logistical support for teaching and research activities in the alpine forest area; (2) to develop cooperation with local communities, national and international universities and research institutions; (3) to promote and host seminars, workshops, technical and scientific courses.

In the last ten years CSALP was involved in some research projects concerning environment monitoring and forest planning: (a) CARBOITALY (sponsor: MIUR - Italian Ministry for University and Research) aiming at the quantification of carbon sinks in forest and agriculture ecosystems; (b) MONFORINN (sponsor: Provincia Autonoma di Trento) with the goal of assess the use of high-resolution satellite images for forest monitoring; (c) PFIT Asiago e Cadore, Longaronese, Zoldano (sponsor: Regione Veneto) with the aim of developing innovative methods for inventory and decision making process in forest planning; (d) C_FORSAT (sponsor MIUR) with the aim of quantifying both gross (GPP) and net primary production (NPP) of Italian forest ecosystems with a spatial resolution of 1 km. CSALP is also part of MOUNTFOR, a Project Center of the European Forest institute, which acts as a network of national and international universities and research institutes to promote research activities in all the aspects of mountain forests.

Luigi Portoghesi
President of the CSALP Management
Committee

Introduction

Dear Colleagues,

on behalf of the Organizing and Scientific committees I would like to thank all participants to the 76th National Congress of the Unione Zoologica Italiana for having come so numerous to our annual meeting. It is a great honour for us that this important event is held here for the first time in Viterbo, at the University of Tuscia, in the prestigious medieval complex of S. Maria in Gradi. This historical building represents a significant example of how history and innovation can be fruitfully combined. History and innovation are the leitmotif of our young University, in which Zoology and Comparative Anatomy have played a remarkable role – both in cultural and scientific terms – ever since its foundation. The 76th National Congress of the Unione Zoologica Italiana intends to offer to the participants the opportunity to experience an amazing intellectual venture in the diversity and quality of the scientific knowledge that make our Society so rich. In this perspective, the 76th National Congress will approach such thought provoking themes as the ones planned for the following three Symposia:

1. Reproductive strategies: behavioral, morpho-functional and evolutionary aspects
3. Physiological aspects and internal defense system: evolutionary adaptations and applications in biotechnology
2. The modern systematics: between structural morphology and molecular approaches

Aim of these Symposia is to review the state-of-the-art of some topics and the innovative aspects that characterize recent research in Animal Biology. We should also emphasize how innovation does depend on our ability to provide scientifically sound and ethically sustainable interpretations, in highlighting the variety of techniques currently employed to unravel key aspects of Life. The 76th Congress will end with a Round Table primarily focusing on the public use of Protected Areas in Italy. This should provide the opportunity to discuss some of the highly debated topics on Nature Conservation and sustainable use of natural resources, allowing to evaluate pros *and* cons *of the present nature/nurture relationship.*

The possibility to compare new ideas on such topics as they freely emerge in the discussion constitutes an essential ingredient for encouraging interest, engagement and participation in the activity of our Society and for providing new guide lines for the disciplines of Zoology and Comparative Anatomy. Special attention has been given to young researchers. Of the 150 scientists attending this Congress, over 30% are PhD students or Research assistants. Among them, ten will be invited to present their work in the "9th UZI Awards for young Researchers" session, and four will receive the prize awarded. Recognition of these excellences is meant to be an incentive for strengthening their passion in Zoology and Comparative Anatomy and to encourage them to remain active in spite of present difficulties.

Given these premises, the annual Congress of our Society is not only an important platform for strengthening our collaborations, as usually, but also the scenario where new relationships and new developmental strategies could be planned and pursued. It is therefore with great pleasure and enthusiasm that we wish you all a fruitful and interesting stay at 76th National Congress of the Unione Zoologica Italiana.

Viterbo, September 15-18, 2015

Anna Maria Fausto
President of the Organizing Committee
76th National Congress
of the Unione Zoologica Italiana

Opening lecture

AXEL MEYER

Chair in Zoology and Evolutionary Biology, University of Konstanz, Germany

GENOMICS OF PARALLEL ADAPTATIONS IN THE REPEATED ADAPTIVE RADIATIONS OF CICHLID FISHES

Cichlid fishes are a textbook example for explosive rates of speciation and the formation of hyper-diverse adaptive radiations. Hundreds of endemic species each comprise the adaptive radiations in the East African Rift Lakes Victoria, Malawi and Tanganyika. In the case of Lake Victoria >500 endemic species evolved within less than 100,000 years. Despite this huge diversity of cichlids these adaptive radiations also famously evolved particular morphotypes and ecological guilds repeatedly in parallel. Also in a chain of crater lakes in Nicaragua small endemic adaptive radiations live in each of these lakes. In these crater as well, some of which are less than 2,000 years old, certain types of ecological types evolved repeated. The recent publication of five genomes of cichlid fishes (BRAWAND *et al.*, 2014, Nature) now provides reference genomes for further analyses. The initial research focused on finding reasons for why there are so many cichlids - several genomic features were found that might have contributed to the biodiversity of cichlids. Now we can begin to ask whether *de novo* mutations repeatedly resulted in the evolution of parallel phenotypes or whether standing genetic variation forms the basis of parallel evolution in the adaptive radiations in Africa and Nicaragua.

Symposium I

Reproductive strategies: behavioral, morpho-functional and evolutionary aspects

Coordinators:
Anna Maria Fausto, Gabriella Chieffi Baccari

Invited lectures

MARCO APOLLONIO[1], FRANCESCA BRIVIO[1], ROBERTA CHIRICHELLA[1], SIMONE CIUTI[2]

[1] Department of Science for Nature and Environmental Resources, University of Sassari, Sassari, Italy
[2] Department of Biometry and Environmental System Analysis, University of Freiburg, Freiburg, Germany

THE REPRODUCTIVE STRATEGY IN UNGULATES

The reproductive systems represent a good topic to investigate animal behavioural plasticity in response to environmental factors. Here we considered three main issues: the intra-specific plasticity of mating systems, the inter-individual plasticity in the adoption of mating strategies among the same population and the increase of knowledges on the lek, one of the more complex reproductive system.

Ungulates are suitable candidates to achieve these aims as they includes species with a wide distribution, living a vast array of ecological conditions and adopting all known mammalian reproductive systems.

Animal mating systems show a remarkable variation among species, depending on the species characteristics as well as on ecological factors. Plasticity in reproductive behaviours was reported among and within populations, also on account of distinct male alternative mating tactics (AMTs). AMTs occur when individual of the same sex find different solutions to face reproductive competition. AMTs commonly change according to individual factors (age, body size, physical condition) and external variables (resources, predation, animal density).

In polygynous species, males' reproductive success depends mostly on their fighting with other males so as to gain access to potential mates. Consequently, depending on the body growth characteristic of each species, it has evolved AMTs age-dependent or age-independent.

It has been shown that also environmental conditions are important in shaping male reproductive behaviour. In particular, weather may affect the AMTs in species whose rutting period occurs late in fall or in winter, when environmental conditions and the snow cover in particular may vary considerably.

One of the most complex reproductive systems is the lek strategy, especially among ungulate species. Here we focus on this reproductive system showing how a multiple-tasking approach (i.e., individual and population level) may better explain the existence and persistence of this reproductive system in the ungulate populations. We used fallow deer as model species as its reproductive behaviour include the adoption of all mammalian mating systems including lekking.

CLAUDIO SETTE

Dipartimento di Biomedicina e Prevenzione, Università di Roma Tor Vergata

GENE EXPRESSION REGULATION DURING SPERMATOGENESIS: THE ROLE OF RNA BINDING PROTEIN SAM68

The production of a male gamete requires the expression of many proteins and RNA specific so temporally controlled. This fine regulation of gene expression is essential for the spermatogenesis progression. Since in some stages of this differentiation process the transcription of new RNA is not possible, as during the meiotic homologous recombination or spermiogenesis, a key role is played by the RNA post-transcriptional regulation. In this regard, many proteins binding to the RNA are highly expressed in male germ cells and play an essential role in their differentiation. For example, the protein SAM68 is expressed during the meiotic prophase and the early stages of spermatogenesis, when transcription is still very active. SAM68 binds a subgroup of RNA coding for key functions of germ cell, and modulates the expression, intron splicing and translation in the cytoplasm. The ablation of Sam68 gene causes male infertility in mice, due to meiotic and post-meiotic defects leading to a massive apoptosis of round spermatids and formation of a few mature sperm. In addition, spermatozoa are not very mobile, and in vitro they are unable to fertilize oocytes. Transcriptome analysis has identified several specific genes in spermatogenesis whose expression was altered in spermatocytes and the spermatids lacking SAM68. In conclusion, our studies demonstrate that SAM68 protein is essential for the correct progression of spermatogenesis and highlight the molecular mechanisms controlled by this protein in differentiating germ cells.

Symposium I

Reproductive strategies: behavioral, morpho-functional and evolutionary aspects

Coordinators:
Anna Maria Fausto, Gabriella Chieffi Baccari

Oral presentations

CRISTINA GIACOMA[1], VALERIA TORTI[1], ROSE MARIE RANDRIANARISON[2],
GIOVANNA BONADONNA[1], MARCO GAMBA[1]

[1] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Via Accademia Albertina 13, 10123 Torino, Italia
[2] Département de Paléontologie et d'Anthropologie Biologique, Faculté des Sciences, Université d'Antananarivo, Madagascar

MECHANISMS UNDERLYING PAIR BONDING IN *INDRI INDRI*

Indri indri is a socially monogamous primate inhabiting the mountain rainforests of eastern Madagascar. Indris live in territorial family groups, usually a breeding pair and their offspring. Indris' mating system is strongly influenced by their territorial behavior and their singing activity. The extra-pair copulation documented was contextual with territorial instability and unusual singing behavior. The indris' song is a long sequence of vocal utterances that serve regulating spacing between neighboring groups and maintaining group cohesion. Starting from the fact that conspecific group encounters in indris are very rare and there is a strong vocal sex dimorphism, it was suggested that the song might play a role in indris' mating behavior and facilitate the formation of new groups. The mating season lasts from late November to February, but the breeding behavior is more often observed in December and January when territorial behaviors and social interactions also increase. The main objectives were to analyze factors favoring and challenging pair bonding in indri. Data were collected across three different sites: Analamazaotra Special Reserve (Andasibe-Mantadia National Park, 18° 56' S, 48° 25' E), 'Station Forestière' of Mitsinjo (18° 56' S, 48° 24' E) and Maromizaha Forest (18° 56' 49" S, 48° 27' 53" E), all in CE Madagascar. In our nine-year-long study, we found that indris move throughout their territories (DPLs range: 125.12 – 514.43 m), showing exclusive home ranges (intra-group overlap over years: 59% - 99%). We observed that territorial boundaries are stable over time, and that social groups maintain a persistent spatial position over years (annual shift of MPC100 centroids: 33.88 + 29.22 m). The general pattern of ranging and spatial behavior that we observed offers insights about monogamy prerequisites: indris use relatively small non-overlapping territories, which allow effective mate guarding. They invest in relatively infrequent but highly detectable advertisement call thereby avoiding territorial disputes. Only when necessary they start threatening each other with very prominent territorial displays, which seldom degenerate in physical confrontations. Inter-group encounters were located near the territorial boundaries and occurring at very low rate, supporting the hypothesis that indris' advertisement songs regulate intergroup spacing.

FRANCESCA FRATI[1], SILVANA PIERSANTI[2], ERIC CONTI[1],
MANUELA REBORA[2], GIANANDREA SALERNO[1]

[1] Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università di Perugia
[2] Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia

OLFACTORY CUES AND SEX RECOGNITION IN A POLYMORPHIC DAMSELFLY

In polymorphic damselflies discrimination of females is complex owing to the presence of andromorph and gynomorph females. To date there is no evidence that damselflies use modes of communication other than vision in mate searching and remote sex recognition and it is still controversial which precise cues are relevant to males for discriminating among potential mates. The behavioural (one-way olfactometer) and electrophysiological (EAG) investigations performed in the present study on *Ischnura elegans,* a polymorphic damselfly belonging to Coenagrionidae, suggest for the first time the involvement of chemical cues in Odonata sex recognition. In the bioassays males preferred female to male odour, with no significant difference between the stimuli from males and the control. The bioassays also suggest some ability of males to distinguish between chemical cues of the two female morphs. The ability of males antennae to perceive odours from females has been confirmed by electrophysiological recordings. These findings are important to get insight into the chemical ecology of Odonata, a field so far uninvestigated, owing to the remarkable power of vision of these insects. The present data can also shed light into the problem of olfaction in Paleoptera, considered anosmic for a long time. Moreover, investigating the role of olfaction in *Ischnura* female recognition could be useful to clarify controversial aspects of the mating behavior of polymorphic damselflies. This research is part of a FIRB Futuro in Ricerca project entitled “A new insight in chemical ecology of insects: role of chemical cues in visual dependent insects” (RBFR10Z196).

GIANLUCA POLESE, CARLA BERTAPELLE, ANNA DI COSMO

Dipartimento di Biologia, Università degli Studi di Napoli "Federico II"

CENTRAL AND PERIPHERAL CONTROL OF REPRODUCTION IN *OCTOPUS VULGARIS* AND INVOLVEMENT OF CHEMICALS PERCEPTION

Among invertebrates reproductive strategies are much more diversified than vertebrates. However, the mechanisms that underlie the neural and neuroendocrine control of reproduction are conserved. In *Octopus vulgaris* mainly two lobes, subpedunculate lobe and olfactory lobe (supraoesophageal mass), and a neuroendocrine gland (the optic gland) are the neuroanatomical areas involved in the central control of reproduction. In those, several neuropeptides, NMDA receptors, an oestrogen receptor, oct-ER work in synergy to modulate optic gland activity, in turn involved in the production of a gonadotropin still unknown. The olfactory lobe plays a crucial role in mediating sexual behaviour, functioning as an integrative centre in which converges chemosensory information intercepted by olfactory organ via the olfactory nerve. Recently we have found for the first time in *Octopus vulgaris* that olfactory sensory neurons (OSNs) are immunereactive to several peptides involved in reproduction and feed intake such as GnRH and NPY (POLESE *et al.*, 2015). Interestingly we observe that OSNs are surrounded by a net of GnRH-ir terminations coming from posterior olfactory lobe neurons. This suggests a context-dependent modulation of olfactory epithelial activity by olfactory lobe making the octopus female more sensitive to a sex related stimulus rather than a food one during reproduction. The control of reproduction at peripheral level is mediated by fusiform ganglion that innervates the reproductive tracts in both male and female. Neuropeptides, sex steroid hormones, optic gland hormone and a recently discovered chemoattractant factor octo-SAP are the peripheral physiological players responsible of reproductive behaviour. Despite the advanced state of knowledge about the mechanisms that regulate reproduction in *Octopus vulgaris* and cephalopods in general, many questions remain unanswered. However the CNS transcriptome data together with the behavioural data may answer to some of them.

DIEGO FONTANETO

Consiglio Nazionale delle Ricerche, Istituto per lo Studio degli Ecosistemi (CNR-ISE), Largo Tonolli 50,
28922 Verbania Pallanza, Italia

REPRODUCTIVE STRATEGIES INFLUENCING THE SPECIATION PROCESS: DO 'SPECIES' EXIST IN ASEXUAL ANIMALS?

The possibility for independently evolving entities to form and persist in the absence of sexual recombination in eukaryotes has been questioned; nevertheless, there are organisms that are notorious to be asexual and to have apparently diversified into multiple species as identified by taxonomists. These organisms have therefore been named as an evolutionary paradox. We explore three alternative hypotheses attempting to solve the apparent paradox, focusing on bdelloid rotifers, the most studied group of organisms in which all species are considered asexual: (1) they may have some hidden form of sex; (2) species do not represent biological entities but simply convenient names; (3) sex may not be a necessary requirement for speciation. We provide ample evidence against the first two hypotheses; then, we explore the role of sex in speciation comparing bdelloid and monogonont rotifers, and conclude with some caveats (e.g. the recently discovered role of horizontal gene transfer) that could still change our understanding of speciation in asexual animals.

ANDREA LUCHETTI, BARBARA MANTOVANI

Dipartimento Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Via Selmi 3, 40126 Bologna

PARTHENOGENESIS: ITS IMPACT ON GENETIC VARIABILITY IN DIFFERENT REPRODUCTIVE SYSTEMS

Parthenogenesis is an unisexual reproduction consisting in the virginal egg development. At the genetic level, irrespective of its mechanisms, parthenogenesis produces clonal lines. The adaptive capacity of parthenogenetic organisms has been explained by several hypotheses, including the General Purpose Genotype (GPG) and the Frozen Niche Variation (FNV); these suggest unisexuals as ecologically generalist or specialist, respectively. A critical issue for adaptation of unisexuals (thus linked to their being either GPG or FNV) is their genetic variability and, therefore, how they face the accumulation of deleterious mutations (Muller's ratchet hypothesis). Transposable elements, i.e. DNA sequences able to jump from one genomic location to another, are a proxy for the study of potentially deleterious mutations and show evolutionary/genomic dynamics affected by the reproductive strategies of the host species. To understand the role of unisexuality in the management of transposon insertions, we are currently analyzing three animal models with parthenogenetic strategies different by origin, mechanisms and genetic impact. Stick insects of the genus *Bacillus* show taxa with facultative and obligatory parthenogenesis (either of hybrid or non-hybrid origin); results obtained with different methods and on different transposable elements, support the link between reproductive strategies (unisexuality/bisexuality) and transposon insertions dynamics. In particular, simulations based on empirical data indicate that, in facultative unisexuals, recombination plays a major role, while in obligatory unisexuals possible endogenous mechanisms of transposon silencing could have reduced/interrupted transposon insertion activity. *Reticulitermes* termites are characterized by eusociality with potential inbreeding and, in some species, by facultative parthenogenesis. Here transposon dynamics does not appear linked to presence/absence of parthenogenesis, which in this case acts for the maintenance of genetic variability, at variance of what is usually observed in other systems. Recently, we started the genome sequencing of the "living fossil" *Triops cancriformis*, a crustacean branchiopod species that shows parthenogenetic, hermaphroditic (with possible androdioecy) and bisexual populations, geographically partitioned; data are still under interpretation.

LILIANA MILANI, MARIA GABRIELLA MAURIZII, FABRIZIO GHISELLI,
ANDREA PECCI, MARCO PASSAMONTI

Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna

EXPRESSION OF MITOCHONDRIAL AND NUCLEAR ELEMENTS DURING PRIMORDIAL GERM CELL PROLIFERATION AND DIFFERENTIATION IN THE BIVALVE SPECIES *RUDITAPES PHILIPPINARUM* (BIVALVIA VENERIDAE)

The first representatives of germ line to appear in the embryo are primordial germ cells (PGCs), and some conserved proteins play a role in their determination and specificity (e.g.: VASA). Also mitochondria have an active role in germ line development and to further understand this role, it is fundamental to deal with their inheritance dynamics. Metazoa generally experience strictly maternal inheritance (SMI) of mitochondria. The only known exception is represented by some bivalve molluscs that show doubly uniparental inheritance (DUI). Recently, a novel protein (RPHM21) was shown to be encoded by the male-transmitted mtDNA of the DUI species *Ruditapes philippinarum*. *In silico* analyses suggested its viral origin, and we hypothesized that the endogenization of a viral element provided sperm mitochondria with the ability to invade male germ line, thus being transmitted to the progeny. We investigated the dynamics of germ line development in relation to RPHM21 and expression patterns. We used specifically produced antibodies to detect germ cell proliferation and to compare the localization of VASPH (*R. philippinarum* VASA homolog) and RPHM21. Bivalves show a mechanism of seasonal gonad production, in which the gonad is re-adsorbed after spawning, and rebuilt *de novo* at the beginning of the subsequent reproductive season. Based on VASPH staining, we determined that in *R. philippinarum* the proliferation of PGCs begins among the simple columnar epithelium (batiprismatic cells) of the gut. Then PGCs appear to migrate in the connective tissue. Germ cells at initial stage of gametogenesis were also VASPH-stained. Since adult specimens showed an intense proliferation of PGCs in the gut, we deduced that this reservoir of PGCs migrate from the intestinal epithelium and reach the presumptive gonad localization where they rebuild the gonad at every reproductive season. Both VASPH and RPHM21 were localized in male PGCs, at one side of the cell cytoplasm, but while VASPH was detected in all PGCs, RPHM21 appeared to be expressed only in a subpopulation of them. Since RPHM21 was detected in all spermatozoa, we propose that the germ cells expressing it could gain advantage over the others during spermatogenesis. Specifically, RPHM21 might have a role in activation and proliferation of male PGCs, or might be involved in a process of active elimination of germ cells not expressing it, something that would resemble a meiotic drive.

FRANCESCA SCOLARI[1], JOSHUA B. BENOIT[2,3], VERONIKA MICHALKOVA[3, 4], PETER TAKAC[4], ADLY M.M. ABD-ALLA[5], GIULIANO GASPERI[1], ANNA R. MALACRIDA[1], SERAP AKSOY[3], GEOFFREY M. ATTARDO[3]

[1] Dipartimento di Biologia e Biotecnologie 'Lazzaro Spallanzani', Università di Pavia, Pavia, Italy
[2] Department of Biological Sciences, McMicken School of Arts and Sciences, University of Cincinnati, Cincinnati, OH, USA
[3] Department of Epidemiology of Microbial Diseases, Yale School of Public Health, Yale University, New Haven, CT, USA
[4] Section of Molecular and Applied Zoology, Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia
[5] IPC Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria

SEMINAL FLUID PROTEINS AND SPERMATOPHORE ASSEMBLY IN *GLOSSINA M. MORSITANS* (DIPTERA, GLOSSINIDAE): THE MALE CONTRIBUTIONS TO VIVIPAROUS REPRODUCTION

Insect seminal fluid is a mixture of proteins, carbohydrates and lipids produced in the male reproductive tract and transferred to the female along with sperm upon mating. Seminal fluid proteins (SFPs) are known to play key roles in inducing multiple post-mating responses in the female including regulation of sperm storage, sexual receptivity and ovulation. Such modulatory capabilities make SFPs ideal targets for the development of novel methods aimed at controlling insect disease vector populations. While SFPs have been investigated in many insects, little is known about their molecular identities and functional roles in the context of the viviparous biology of tsetse flies (Diptera: Glossinidae), the sole vectors of the human and animal forms of African trypanosomiasis. In tsetse, proteins/substances and sperm produced by the male are assembled in the female uterus into a capsule-like spermatophore structure that is transiently formed post-copulation. Here, we applied RNAseq and proteomics approaches to uncover the composition of the spermatophore in *Glossina morsitans morsitans*, and found that the majority of the identified 287 spermatophore proteins derived from the male, with products from both the testes and the male accessory glands (MAGs) composing this structure. Female-derived materials appear to only partially contribute to spermatophore formation, unlike in the mating plug of the mosquito *Anopheles gambiae*. The MAGs produce a small number of highly abundant tsetse-specific proteins with yet unknown functions, in addition to protein with predicted roles such as enzyme inhibitors, peptidase regulators, and odorant binding proteins. The testes contribute a more diverse array of less abundant proteins associated with binding, oxidoreductase and transferase activities, as well as components of the cytoskeleton and transporters. Of note, more than half the spermatophore proteins displayed no similarity to SFPs characterized in other Diptera, which could reflect both the fast evolutionary pace these proteins typically display as well as the divergent nature of tsetse viviparous reproductive biology. An expanded knowledge of seminal fluid proteins' identity and mode of action will be highly relevant for the development of novel species-specific tools interfering with female fertilization. This will in turn improve vector population control strategies, primary methods to prevent trypanosomes' transmission.

ROMANO DALLAI[1], FRANCESCO PAOLI[2], PIO FEDERICO ROVERSI[2],
MARCO GOTTARDO[1], DAVID MERCATI[1]

[1] Dipartimento di Scienze della Vita, Università di Siena
[2] Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di ricerca per l'Agricoltura e la Pedologia (CRA-ABP), Firenze

THE PECULIAR SPERM FLAGELLUM OF COCCOIDEA (HOMOPTERA, INSECTA): ORIGIN AND STRUCTURAL ORGANIZATION

Coccoidea sperm are characterized by a flagellum consisting of a bundle of microtubules, regularly organized in circles or spirals, surrounding an axial cylindrical nucleus. Acrosome, mitochondria and centrioles are missing, but sperm are motile thanks to the presence of dynein arms between adjacent flagellar microtubules. In the past, the origin of the bundle of microtubules was investigated without results. Studying the spermiogenesis of the Archeococcoidea *Matsucoccus feytaudi* we were able to observe in the spermatid a couple of centrioles, mitochondria and an MTOC (Microtubule Organizing Centre) responsible for the production of the bundle of microtubules forming the peculiar flagellum of this species. The presence of γ-tubulin at the MTOC was ascertained by immunolocalization using an anti-γ-tubulin antibody. During the spermiogenesis a cylindrical nucleus is formed, which is adapted in the axial region of the microtubule bundle. At the end of spermiogenesis, centrioles, mitochondria and the MTOC were eliminated. This study was extended to several species of Neococcoidea. During spermiogenesis, no centrioles and MTOC were found; however, the proximal regions of microtubules in the sperm flagellar bundle were thicker, suggesting the presence of γ-tubulin at these levels. A progressive involution of the basal body functionality is hypothesized, starting from Aphidoidea, through Psylloidea and Aleyrodoidea, up to Coccoidea. The loss of a conventional axoneme and of the consequent sperm motility was regained in the Coccoidea by a peculiar axonemal structure generated by an MTOC or by simpler microtubular organizing centres.

MARCELLA CARCUPINO[1], FRANCESCA BIAGI[1], ANNA RITA TADDEI[2], ANNA MARIA FAUSTO[3]

[1] Dipartimento della Natura e del Territorio, Università di Sassari
[2] Centro grandi attrezzature, Sezione di Microscopia Elettronica, Università della Tuscia, Viterbo
[3] Dipartimento per l'Innovazione nei sistemi Biologici, Agroalimentari e Forestali, Università della Tuscia, Viterbo

ARE THE MALE GONADAL MORPHOLOGY AND THE SPERMATOGENETIC PROCESS RELATED TO THE MATING PATTERNS IN SYNGNATHIDS?

The elaborate courtship behaviour of Syngnathidae involves mating patterns with only one partner (monogamy) or multiple partners (polygamy, varying from the classic case of polyandry topolygynandry). A close link among the ovarian structure, mode of egg production and mating pattern has been suggested for this teleost family. The mode of egg production seems to be determined by the ovarian structure which, more or less, limits the range of the mating pattern by affecting the timing and length of the females' mating period. The aim of this work was to evaluate if even the testis structure and the spermatogenetic process were similarly related to the mating pattern. Testes of mature males of the *Nerophis ophidion* were analysed using both light and transmission electron microscopic techniques. Results, compared to those obtained in other syngnathids, have shown the existence of two different testis types. *N. ophidion* shares with another gastrophorine species, an organization of the male gonad characterized by two distinct regions, whereas all urophorine species have testes of uniform morphology. The testis structure seems therefore only related to the phylogenetic relatedness. On the other hand, *N. ophidion*, like all other synagnathids species previously analysed, has spermatogenesis of the semicystic type, which has been interpreted as one of the possible mechanisms evolved to reduce the cost of sperm production. However, both gastrophorine and urophorine species, with polyandrous or monogamous mating pattern, have a typical semicystic spermatogenesis where germ cells advance individually through spermiogenesis inside the lumen. On the contrary, polygynandrous species, such as those belonging to the genus *Syngnathus,* have an unusual type of semicystic spermatogenesis, here named "group-synchronous type", in which developing germ cells inside the testis lumen are polynucleate and polyflagellate cells. Therefore, the two types of spermatogenesis seem to be constrained more or less to the mating type. In particular, the "group-synchronous type" may represent a modality able to reduce the loss of sperm, which could seriously affect the reproductive success of polygynandrous species, characterized by multiple mates within a short time span. In contrast, the typical semicystic type occurs in those monogamous or polyandrous species in which males, like females, mate much less frequently and do not release sperm for a long period of time.

MARINA PRISCO, LUIGI ROSATI, SALVATORE VALIANTE,
PIERO ANDREUCCETTI, VINCENZA LAFORGIA, MARISA AGNESE

Dipartimento di Biologia, Università di Napoli “Federico II”, Napoli

PACAP AND VIP AND THEIR RECEPTORS IN VERTEBRATE SPERMATOGENESIS

PACAP (Pituitary adenylate cyclase-activating peptide) and VIP (Vasoactive intestinal peptide) are neuropeptides belonging to secretin/glucagon family; they have pleiotropic functions and have been localized in central nervous system and in many peripheral districts (REGLODI *et al.*, 2001; VALIANTE *et al.*, 2007, 2008, 2009; CONLON *et al.*, 2009; VAUDRY *et al.*, 2009), including testis (LACOMBE *et al.*, 2007; MOODY *et al.*, 2012). In recent years we have studied their expression, localization and molecular evolution in non mammalian vertebrate testis (AGNESE *et al.*, 2010, 2012, 2014 a, b; ROSATI *et al.*, 2014) and now we present a study about PACAP/VIP/receptors system involvement in spermatogenesis of vertebrate taxa not evaluated so far. Our immunohistochemical and molecular investigations demonstrate firstly the expression and distribution of both neuropeptides and their receptors (PAC1, VPAC1, VPAC2 for PACAP and VPAC1 and VPAC2 for VIP) in testis of *Coturnix coturnix* and *Mus musculus*; the results suggest that they are implicated in both spermatogenesis and steroidogenesis control. Furthermore, we reassess also the testis situation of *Rattus*, highlighting that the distribution of neuropeptides/receptors system is wider than previously reported in literature, especially regarding VIP localization.

MARIA MADDALENA DI FIORE, ALESSANDRA SANTILLO, SARA FALVO,
GABRIELLA CHIEFFI BACCARI

Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Seconda Università di Napoli, Caserta, Italy

ENDOCRINE ROLE OF D-ASPARTIC ACID IN VERTEBRATE TESTIS: A COMPARATIVE STUDY

D-Aspartic acid (D-Asp) has been detected in the testes of the frog *R. esculenta*, lizard *P. sicula*, duck *A. platyrhynchos*, mouse, rat, pig, boar. D-Asp is prevalently localized in Leydig cells, Sertoli cells and germ cells, particularly in spermatogonia, elongate spermatids and spermatozoa. It has been observed a strong correlation between D-Asp concentration and Testosterone (T) levels in rat testis throughout life. Embryonic rats start synthesizing D-Asp and T in the fetal life, the levels were very low at birth and increased progressively, reaching maximum levels at sexual maturity. When D-Asp was administered to adult rats, it accumulated in the testis. Both i.p. and oral D-Asp administrations to adult rats induced an increase of testis/serum T levels, whereas 17β-estradiol (E2) levels did no change. Furthermore, exogenous D-Asp induced up-regulation of androgen receptor and down-regulation of estrogen receptor expression. *In vitro* D-Asp incubations performed on testicular homogenates of boar demonstrated that D-Asp induced T release as well as E2 synthesis through Aromatase activation. Specific stimulation of T synthesis by D-Asp in purified rat Leydig cells has been demonstrated. D-Asp increased T production by stimulating gene and protein expression of steroidogenic acute regulatory (StAR), a key regulatory factor of cholesterol translocation to the inner mitochondrial membrane. Several studies carried out on seasonal-breeding vertebrates have confirmed the involvement of D-Asp in the endocrine control of reproductive activity. Because of their cyclic pattern of reproductive activity, the seasonal breeders are good models for studying the role of D-Asp in the testis. In both frog *R. esculenta* and lizard *P. sicula*, testis D-Asp concentration reached the highest levels in reproductive period when T gonadal and plasmatic levels were the highest. Intra-peritoneal injection of D-Asp (2.0 μmol/g b.w.) induced a rise of T and a fall in E2 synthesis in frog and lizard, both in pre- and post-reproductive phases. Exogenous D-Asp in reproductive frogs increased E2 levels. Increased spermatogonial mitotic activity has been reported in the testis of D-Asp-treated frog and lizard prevalently in post-reproductive phase. In duck testis D-Asp concentration was higher in the reproductive than non-reproductive phase, paralleling similar fluctuations in testicular T levels. Taking all together the data strongly suggest an endocrine role of D-Asp in vertebrate testis.

SARA FALVO, ALESSANDRA SANTILLO, GABRIELLA CHIEFFI BACCARI,
MARIA MADDALENA DI FIORE

Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Seconda Università di Napoli, Caserta, Italy

D-ASPARTATE IN MOUSE SPERMATOGENESIS: AN *IN VIVO* AND *IN VITRO* STUDY

D-aspartate (D-Asp) is an endogenous amino acid present in vertebrate tissues, with particularly high levels in the testis. Specifically, D-Asp was found in Leydig cells, Sertoli cells and germ cells. *In vitro* and *in vivo* studies have demonstrated that D-Asp up-regulates testosterone synthesis through hypothalamic-pituitary-gonadal axis and/or directly by stimulating in Leydig cells the protein expression of StAR, which is a key regulatory factor of cholesterol translocation to inner mitochondrial membrane. In order to elucidate the functional role of D-Asp in spermatogenesis, we used as model mutant mice (Ddo$^{-/-}$) with targeted deletion of D-aspartate oxidase, a peroxisomal flavoprotein which catalyzes the deaminative oxidation of D-Asp. Therefore, mice Ddo$^{-/-}$ show selective increase of D-Asp levels in numerous glandular tissues, including testis. Interestingly, we found that mice Ddo$^{-/-}$ of two months old showed higher levels of serum (5.7±1.4 ng/ml) and testis (193.3±37.2 ng/g) testosterone than wild type mice (0.2±0.1 ng/ml; 23.5±9.3 ng/g, respectively).

The morphological/morphometric analyses of germ epithelium revealed an increase of both spermatogonia mitotic index and seminiferous tubule diameters in mice Ddo$^{-/-}$. In contrast, mice Ddo$^{-/-}$ of six months old did not show significant differences in serum/testis testosterone levels as well as in spermatogenetic activity when compared to wild type mice. These results strongly suggest an age-dependent role of D-Asp in steroidogenesis and spermatogenesis. To investigate more direct involvement of D-Asp in spermatogenesis, a mouse cell line (GC-1) derived from immortalized type B spermatogonia that retains markers of mitotic germ were incubated in a medium containing the amino acid (200 μmol/l). Activity and protein expression of markers of cell proliferation were detected at various times of incubation. We found that D-Asp induced phosphorylation of ERK and Akt proteins, stimulated expression of PCNA and Aurora B, enhanced mRNA synthesis and protein expression of P450 aromatase and protein expression of Estrogen Receptor β. These results are the first demonstration of a direct effect of D-Asp on spermatogonial mitotic activity.

STEFANIA PUCCIARELLI, ANTONINO MIANO, FRANCESCO A. PALERMO,
PAOLO COCCI, DANIELA MICOZZI, GILBERTO MOSCONI

School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032 Camerino, MC, Italy

VITELLOGENIN IN GRAY MULLET (*MUGIL CEPHALUS*): ONE-STEP PURIFICATION, CHARACTERIZATION, AND ELISA DEVELOPMENT

Gray mullet, *Mugil cephalus* L., is a cosmopolitan fish species, widely distributed in coastal waters, lagoons, and estuaries between latitudes 42° N and 42° S. This mullet is an economically important species for both aquaculture and commercial fisheries around the world. A new single-step purification procedure was developed to purify gray mullet vitellogenin (VTG), from 17β-estradiol (E2)-treated gray mullet plasma. This method was performed by high performance liquid chromatography (HPLC) technique, using weak anion-exchange chromatography with a discontinuous gradient of NaCl (buffer B was 25 mM Tris-HCl, pH 8.5 + 0.416 M NaCl) with steps of 16.6 mM for 5.50 min from 0 to 0.416 M NaCl. SDS-PAGE electrophoresis revealed the presence of an intense protein band in E2-treated fish with a MW of 190 KDa. The 190 kDa is also the major protein band in the eluted fractions. Lower molecular weight proteins, corresponding to breakdown products of VTG, were also visible. However, by performing native-PAGE electrophoresis followed by Coomassie blue stain, two pure and non-degraded protein bands were detected. The two bands can be attributed to a monomeric and a dimeric form of VTG, as it has been already observed for VTG purified from other fish plasma samples. A polyclonal antibody against gray mullet VTG was raised in rabbits and found to be specific for gray mullet VTG through western blot analysis. Additionally, an enzyme-linked immunosorbent assay (ELISA) was developed for the quantification of VTG in plasma samples of gray mullet.

SUSANNA SALVADORI, ELISABETTA COLUCCIA, FEDERICA DEIDDA,
CINZIA LOBINA, ANNA MARIA DEIANA

Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, Via T. Fiorelli 1, 09126 Cagliari

SEX CHROMOSOME SYSTEMS IN OPHICHTHIDAE (ACTINOPTERYGII, ANGUILLIFORMES)

The Anguilliformes are the most speciose taxon (about 700 species) of the monophyletic group Elopomorpha and present a wide variety of sex-determining modes and reproductive behaviours. Considering chromosomal sex determination, differentiated sex chromosomes have been described in the 25% of the cytogenetically studied species. In this work we deepen the study of the karyotype and show the presence of XX/XY chromosomes in the snake eel *Ophisurus serpens* (Linnaeus, 1758) (Ophichthidae). The chromosome preparations were obtained from blood cell cultures and the karyotype was investigated by conventional (C and replication banding, CMA_3 fluorescence) and molecular (Fluorescence In Situ Hybridization with the two ribosomal gene families: 45S, 5S, and telomeric sequence probes) techniques. We demonstrated that the 5S ribosomal gene family is a useful sex chromosome marker being restricted to the X chromosome, and located in the heterochromatic region of the short arm. Beside terminal location, interstitial telomeric sequences (ITSs) were also found in the pericentromeric regions of five chromosome pairs, including the sex chromosomes. These ITSs might be the remnant of an intensively rearranged karyotype and related to the origin of the Y chromosome. The finding of a XX/XY sex determination system in *O. serpens*, shows that three different mechanisms of chromosomal sex determination are present among Ophichthidae; till now we showed ZZ/ZW sex chromosomes in *Dalophis imberbis* (SALVADORI *et al.*, 2009) and a multiple $X_1X_1X_2X_2/X_1X_2Y$ system in *Muraenichthys gymnotus* has been reported (MUROFUSHI and YOSIDA, 1984). These data highlight the lability of sex determination in this family and in general in fishes.

MARIA M. PALLOTTA, RAFFAELE RONCA, MIMMO TURANO,
ANIELLO MOCCIA, MARIA FURIA, TERESA CAPRIGLIONE

Dipartimento di Biologia, Università di Napoli “Federico II”, Complesso Universitario Monte S. Angelo, Via Cinthia,
80126 Napoli, Italy

AN UNBIASED APPROACH TO IDENTIFY GENES INVOLVED IN BRAIN SEXUAL DIFFERENTIATION OF *EUBLEPHARIS MACULARIUS*, A REPTILE WITH TEMPERATURE-DEPENDENT SEX DETERMINATION

Sexual differentiation (SD) during development results in structural, metabolic, and behavioural differences that involve not only the gonads, but also a variety of other biological structures, including the brain. In vertebrates, sex-related brain differences involve several spheres such as anatomy, behaviour, and physiological response in the breeding season. In many reptiles, whose gender is determined by egg incubation temperature, such as the leopard gecko, *Eublepharis macularius*, embryos incubated at different temperatures clearly differ in terms of behaviour and of the volume of the brain nuclei that modulate it. Based on the premise that “*the developmental decision of gender does not flow through a single gene*”, a large-scale analysis using several approaches was performed using *E. macularius* as a model to gain insights into the genes that may be involved in mediating brain SD during development. Our findings suggest that in reptiles, where offspring gender is determined by the sex-producing temperature, brain SD is influenced both by the genes that control gonad differentiation and by those modulating neurite outgrowth, synaptic plasticity, and metabolic activity. It is conceivable that the brains of reptiles incubated at different temperatures are sexually dimorphic at birth, and that postnatal behavioural experiences contribute to complete species-specific, gender-related, behavioural differentiation.

Symposium I

Reproductive strategies: behavioral, morpho-functional and evolutionary aspects

Coordinators:
Anna Maria Fausto, Gabriella Chieffi Baccari

Posters

CRISTINA GIOIA DI CAMILLO, SILVIA AROSSA, DANIELA PICA,
FABRIZIO TORSANI, AZZURRA BASTARI, CARLO CERRANO

Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, Ancona, Italy

REPRODUCTIVE CYCLE OF *ANEMONIA VIRIDIS* (FORSSKÅL, 1775) AND *AIPTASIA DIAPHANA* (RAPP, 1829) (CNIDARIA: ANTHOZOA) FROM THE NORTH ADRIATIC SEA

In lower metazoans the reproductive strategies are usually simple, without any peculiar ethological aspect. Reproductive patterns are strongly regulated by environmental factors and recent climate change is affecting the regularity of these events. The North Adriatic Sea is a eutrophic area subjected to cumulative impacts, and due to the reduced size of the basin, it is sensitive to fluctuations of physical parameters. Here, cnidarians are one of the most abundant taxa and sea anemones are the larger representatives. The aim of this study is to assess the abundance and the reproductive biology of the common sea anemones *Anemonia viridis* and *Aiptasia diaphana*. The field observations showed that *A. viridis* and *A. diaphana* were observed all year round in the study area but their abundances varied seasonally, with a peak in summer and a decrease in spring and autumn. Data showed any correlation with sea temperature and irradiance. The highest abundance values were respectively 150.45 and 534.84 individuals m^{-2}. The spring regression coincided with the maturation of gonads and hence with the highest reproductive effort. The autumnal decrease could be due to the waves action, that could dislodge and carry away the anemones, and to the low-salinity conditions due to intense rainfall and flood events. Both species are gonocoric. The gametogenesis of the two Adriatic species require different temperature threshold values. In *A. viridis* the oocyte production was continuous along the year except for July, and the spawning began from March (T = 12.5 °C). Male individuals were observed from January to May and the maturation of spermatozoans was favored by the temperature increase. Most of gametes (both male and female) were released in June. In *A. diaphana* the reproductive time is reduced respect to *A. viridis*, with female individuals found from January to May, and spawning in June. Male individuals were observed only in July, but the spermatogenesis was probably longer. Similarly to other invertebrates from the same area, the duration of the gametogenesis of the anemones from the North Adriatic Sea is longer in comparison to the same species studied in different localities, suggesting that the high food availability of the area optimize their fitness. The understanding and the monitoring of the dynamics of benthic species is the starting point to predict the effects of anthropogenic impacts and climate changes.

GIULIA FASSIO, VALERIA RUSSINI, MARIA VITTORIA MODICA,
MARCO OLIVERIO

Department of Biology and Biotechnologies "C. Darwin", Sapienza University, I-00185 Rome, Italy

LARVAL STRATEGIES AND CONNECTIVITY IN MARINE GASTROPODS

Connectivity is defined as the property and degree of interchange between populations. In the marine environment, this property is strongly influenced by the strategy of larval development. This is especially true for benthic organisms, which have a sessile adult lifestyle and can rely only on the larval phase for dispersal. Larval developments can be classified into two main types: planktotrophic and non-planktotrophic (mostly lecithothrophic) development. It is reasonable to hypothesize that different larval developments produce different patterns of connectivity. Several hypotheses can be tested on the relationship between genetic connectivity and duration of the larval phase: (i) isolation by distance occurs in species with non-planktotrophic development and not in species with planktotrophic development; (ii) genetic diversity and variance distribution are different in the two classes: low diversity and larger intrapopulation variance with planktotrophic development; high diversity and larger interpopulation variance with lecithothrophic development; (iii) different phylogeographic structure: phylogenetic trees not geographically structured with planktotrophic development and geographically structured with lecithothrophic development; (iv) different haplotypes networks: few haplotypes shared by most with planktotrophic development, and more haplotypes shared by geographically related groups with the lecithothrophic development. We first positively tested these hypotheses on literature datasets of three species of the genus *Crepidula* with different larval developments. Then we applied the same approaches on three original case studies: the sibling species *Columbella rustica* (Mediterranean, lecithotrophic) and *C. adansoni* (Atlantic, planktotrophic); and the Antarctic *Capulus subcompressus* and *Marseniopsis* spp. (both planktotrophic). All analyses positively tested the hypotheses of relationship between genetic connectivity and duration of the larval phase. Noteworthy, in Antarctica the planktotrophic development is usually severely counterselected, due the strictly seasonal presence of phytoplankton. *Capulus subcompressus* is the only Antarctic capulid with a planktotrophic development, and the genetics analyses confirmed the high connectivity patterns among populations as well as for species of the genus *Marseniopsis*.

FEDERICO ROMITI[1*], SARAH ROSSI DE GASPERIS[1*],
LARA REDOLFI DE ZAN[2], GIUSEPPE M. CARPANETO[1]

[1] Department of Science, Roma Tre University, Viale G. Marconi 446, 00146 Roma, Italy
[2] CRA - ABP Agricultural Research Council, Research Centre for Agrobiology and Pedology, Via di Lanciola 12/a, 50125 Firenze, Italy
[3] Corpo Forestale dello Stato, Centro Nazionale per lo Studio e la Conservazione della Biodiversita Forestale'Bosco Fontana', Strada Mantova 29, 46045 Marmirolo, Italy
* Equally credited authors

INTRASPECIFIC ALLOMETRY IN TWO SAPROXYLIC SPECIES *LUCANUS CERVUS* (LUCANIDAE) AND *MORIMUS ASPER* (CERAMBYCIDAE): EFFECTS ON DEMOGRAPHIC AND ECOLOGICAL PARAMETERS

Allometry studies the scaling relationship between organ size and body size. Positive allometry, related to male secondary sexual characters (SSC: ornament or weapon), evolves when a larger trait increases disproportionally with overall body size and confers a fitness advantage. Many studies on holometabolous insects reported examples of the advantage gained by larger males with exaggerated SSC, which scale non-linearly with body size. Non-linear allometries, with a threshold mechanism, lead to the expression of two or more morphs, identified by the estimation of one or more switchpoints and characterized by different ratio SSC/body size (allometric coefficient). We analyzed male SSC of two beetles species: mandible length (ML) for the stag beetle *Lucanus cervus*, and antenna length (AL) for the longhorn beetle *Morimus asper*, studying three populations for each species. Males of both species show high variation for these traits. Stag beetles use mandibles in combats to grab the opponent, whereas in longhorn beetles antennal lashing may be a form of mutual assessment of the resource-holding power. Morphological differences can affect behavioral and ecological parameters. We aimed to assess: i) if a threshold size mechanism exists and if is maintained between populations of the same species; ii) if the morphs have effects on ecological and demographical parameters. For this purpose, we combined a classic morphometric approach to population analysis. The allometric trajectories appeared opposite for the two species: positive curvature for ML in *L. cervus*, negative for AL in *M. asper*. The segmented regression strongly supported the presence of dimorphism in both species. For each species, the switchpoint value didn't vary significantly among populations. The phenology of the morphs didn't show significant difference for both species. Population parameters differ between morphs in *L. cervus*: higher survival probability and lower abundance for minor males. An higher copulation rate was recorded for major males of *M. asper*. Our results highlighted that the occurrence of two morphs is related to some ecological and demographical differences. The presence of polymorphism, balancing costs and benefits of developing exaggerated SSC, could be an evolutionary stable strategy for the species preservation.

VITO SCICCHITANO, ANDREA LUCHETTI, BARBARA MANTOVANI

Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Bologna, Italy

SEX INVESTMENT RATIO AND ASEXUAL QUEEN SUCCESSION IN ITALIAN COLONIES OF *RETICULITERMES LUCIFUGUS* (BLATTODEA, TERMITOIDAE, RHINOTERMITIDAE)

Termites are social insects living in colonies generally founded as simple families by a couple of primary reproducers (royals). Colonies, then, grow up in extended families because secondary kings and queens (neotenics) develop. In some species of the *Reticulitermes* genus, secondary queens are produced through parthenogenesis; they will mate with the primary king allowing the maintenance of a high level of genetic variability otherwise progressively reduced because of inbreeding (Asexual Queen Succession, AQS; MATSUURA *et al.*, 2009). These colonies show i) a sex ratio of swarming reproducers (future primary royals) significantly biased toward females and ii) fully homozygote secondary queens (because of automixis with terminal fusion of pronuclei). Here a detailed investigation with morphological/genetics approaches was carried out in Italian colonies of *R. lucifugus lucifugus* (Rossi, 1792) from the natural reserve of Migliarino-San Rossore (Tuscany), in the swarming seasons. Sex ratio calculated for the 26 collected colonies showed in 19 instances the diagnostic features of AQS strategy. Sex ratio was balanced in three colonies only and male-biased in four colonies. These results indicate the presence of AQS in the *R. lucifugus* population of San Rossore even if data suggest that *R. lucifugus* is in a particular evolutionary time of its reproductive strategies. Three colonies with a female-biased sex ratio and three male-biased colonies were genotyped at six MS loci using 10 winged reproducers of each sex and 10 neotenics, where present. Genetic analysis showed an overall excess of homozygote individuals. Furthermore, G-test evidenced a distortion in Mendelian genotype frequencies. This phenomenon may be a consequence of AQS, with some genotypes more advantageous, as a result of purging selection (MATSUURA, 2011). Morphometric/biomass analyses performed on winged reproducers of the genotyped colonies to verify the investment of each sex in the reproductive system pointed to significant differences between reproducers of both sexes belonging to colonies with different sex ratios, differences being not attributable to sexual dimorphism but to intrinsic characteristics of each colony. However, male biased colonies were collected in late swarming season and, thus, these differences could be correlated with environmental variables that might adversely affect the development of the size of individuals swarming in this time of year.

VALERIA TORTI[1], CRISTINA GIACOMA[1], GIOVANNA BONADONNA[1],
DARIA VALENTE[1], ROSE MARIE RANDRIANARISON[2], MARCO GAMBA[1]

[1] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Via Accademia Albertina 13, 10123 Torino, Italia

[2] Département de Paléontologie et d'Anthropologie Biologique, Faculté des Sciences, Université d'Antananarivo, Madagascar

NO FEMALES, NO CRIES: SEXUALLY DIMORPHIC SINGING IN THE INDRIS' SONG

Vocal signals mediate a broad range of behaviors, including mate attraction, reproductive behavior, spacing between competitors, and resources' defense. *Indri indri* is a socially monogamous lemur living in family groups that actively signal their presence with advertisement call. Loud singing in indris follows a sequential organization, which shows evident species- and sex- specific features. This outstanding vocal display is an excellent model to investigate the evolution of long-range signaling. To clarify the role of singing in regulating intra- and inter-group relationships, we investigated temporal and acoustical variability of modulated notes of the descending phrases (from now on DP-notes) of Indris' spontaneous calls. We studied songs of nine groups in the Andasibe-Mantadia National Park, two groups in the Mitsinjo 'Station Forestière' and seven groups in the Maromizaha Forest, all in Central Eastern Madagascar. This study shows that adult pair members mainly overlap each other while singing (N_{pair} = 8; 13±5%). Inspecting DP-notes, we found that indris' songs are sexually dimorphic (Discriminant analysis correctly classified calls ranging from 87% to 100%). The sex of the emitter could be discriminated by the acoustic modulation of the first two quartiles of the call while the mean fundamental frequency of the last two quartiles differentiates between note types across different descending phrases. The duration of each descending phrase allows discriminating between phrase types (88% - 97%), and between sexes (88% - 100%). The duration of DP-notes is significantly higher in males than in females. Finally, temporal and spectral features of the first note of each descending phrase allow discriminating between phrase types, for both sexes (females = 98%; males = 100%). We found evidence that the indris coordinate their singing during group chorusing and that adult female singing served as a template for the adult male contribution. As a measure of male/female cooperation, synchronous calling bouts may assure that a song is informative, influencing male or female dispersion and the formation of new groups.

DANILO G. M. VITALE, BIANCA BONACCORSI, RENATA VISCUSO

Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Sezione di Biologia Animale "Marcello La Greca", Università degli Studi di Catania, Via Androne 81, 95124 Catania

CYTOSKELETAL DISTRIBUTION AND MOVEMENT DURING SPERM DIFFERENTIATION AND MATURATION IN *TYLOPSIS LILIIFOLIA* (FABRICIUS) (ORTHOPTERA, PHANEROPTERINAE)

It is known that, within the cytoskeletal components, actin and tubulin play a major role in the organization and motility of the male gamete (AMOS and AMOS, 1991, THERIOT and MITCHINSON, 1991); therefore, their movements during spermatogenesis and their involvement in fertilization have been studied in many animals, including vertebrates and invertebrates (DVORÁKOVÁ *et al.*, 2005; DESAI *et al.*, 2009; TRIMMER *et al.*, 1986; TINLEY *et al.*, 1987; SAKAI *et al.*, 1993). Given the paucity of data on the issue, regarding the male gametes of insects, we deemed interesting to carry out an investigation in this regard, focusing our attention on the bush-cricket *Tylopsis liliifolia* (Tettigoniidae). Our recent surveys on this species, in fact, about genesis and organization of spermatozoa, their features in the male genital tract, in the spermatophore and in the female genital tract, have shown some peculiar characteristics with reference to Orthoptera (SOTTILE *et al.*, 2010; VISCUSO *et al.*, 2012; VISCUSO and VITALE, 2015). We deemed significant extending knowledge on this species and contextually using it as a model in this investigation, so comparing our results with what is known about other species of Orthoptera. Our investigation was carried out by means of immunofluorescence methods, aimed at detecting F-actin e α-tubulin; the examined germ cells were extracted from testes (during spermiohistogenesis), seminal vesicles, spermatophore and seminal receptacle. Our results have shown a peculiar distribution of F-actin e α-tubulin that seem to change during spermiohistogenesis of the gametes, from the different districts examined; this distribution also appears to be different, in part, respect to the Tettigoniidae *Platycleis albopunctata* (GUERRA and ESPONDA, 1999). Changes and distribution of the two investigated cytoskeletal molecules lead us to hypothesize their involvement in the control of the morphological changes occurring in the male gamete of *T. liliifolia* during its maturation and, in particular, in the structural rearrangement of the spermatozoa during the twisting of the acrosomal wings around the anterior region of the nucleus. Both molecules are also involved in spermatozoa locomotion through the male and female genital tracts and appear to be involved in the interaction with the female gamete during fertilization. Based on the latter consideration, further investigations are ongoing.

LILIANA MILANI, ANDREA PECCI, MARIA GABRIELLA MAURIZII

Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, via Selmi, 3 - 40126 - Bologna

EXPRESSION OF PL10 PROTEIN IN MALE GERM CELLS OF *PODARCIS SICULA* (REPTILIA, LACERTIDAE) DURING THE REPRODUCTIVE-CYCLE PHASES

PL10 is a DEAD-box protein that functions as ATP-dependent RNA helicase. DEAD-box proteins are involved in many processes related to RNA metabolism. In particular, some of them regulate the translation of multiple mRNAs allowing specific molecules to direct the process of differentiation in male and female germ cells. VASA, PL10, and P68 DEAD-box proteins are members of three closely related subfamilies: Vasa, in several animals, is exclusively expressed to the germ cell lineage, while PL10 and P68 expression are documented also in somatic tissues. In mouse, three PL10 related genes are identified: DDX3 and DDX3Y located on chromosomes X and Y and expressed in germ and somatic cells and the autosomal retrogene PL10 specifically expressed in testicular tissues at the pachytene stage of male meiosis. In non-mammal animals, *PL10* is the sole member of the subfamily and in *Xenopus* and in *Danio rerio PL10* expression is well documented in male and female germ cells but also in most embryonic and adult tissues. At the moment, there is no available data regarding PL10 in reptiles. We isolated *Podarcis sicula PL10* homologue gene (*Ps-PL10*), developed a specific antibody (anti-Ps-PL10) and analyzed, at confocal microscopy, the expression pattern of PL10 during spermatogenesis in all phases of adult reproductive cycle of *P. sicula* (1- full gonadal activity in the spring, 2- complete regression in the summer, and 3- slow autumnal recrudescence without spermiation) with the aim to identify when PL10 is expressed during the differentiation process of male germ cells. Moreover, to verify if this protein is also expressed in the somatic tissue of the testis, this analysis was extend to young testes when the walls of the seminiferous tubules were forming. The obtained results show that PL10 expression is present from spermatocytes I to spermatids. During full gonadal activity (spring), PL10 expression increases in spermatids in the final steps of spermiogenesis, the strong immunostaining accumulates in the cytoplasm of residual bodies. No stained is observed in spermatogonia and in spermatozoa as well as in the somatic cells of seminiferous epithelium in all phases of the reproductive cycle analyzed. The specific expression of PL10 in meiotic cells suggests that this protein is involved in the differentiation of germ cells, in particular, during the differentation process from spermatid to spermatozoa, given the massive expression observed in the cytoplasm of residual bodies.

Symposium II

Physiological aspects and internal defenses system: evolutionary adaptations and applications in biotechnology

Coordinators:
Giuseppe Scapigliati, Giovanni Bernardini

Invited lectures

FRANCESCO PENNACCHIO

Dipartimento di Agraria, Università degli Studi di Napoli "Federico II"

IMMUNITY, STRESS AND HONEYBEE HEALTH

Honeybee colony losses are often associated with high loads of parasites and pathogens, which are favored by reduced immune competence. The association between the parasitic mite *Varroa destructor* and the Deformed wing virus (DWV) appears to play a pivotal role in the spread of viral infection and in the induction of an immunodeficiency status. The honeybee immune barriers can be further compromised by the negative impact that additional stress agents can exert. Among these latter, unbalanced nutrition and exposure to pesticides have received particular attention, as they can interfere with honeybee immune competence and trigger pathogen proliferation. Here we describe a functional model, which offers a new molecular framework for interpreting the complex multifactorial syndrome of honeybee colony collapse, modulated by multiple stress agents. This information will likely contribute to the development of additional guidelines for testing chronic or sub-lethal effects of pesticides on non-target organisms, and may set the stage for future studies aiming to define new strategies for alleviating the current problems of the beekeeping industry.

Symposium II

Physiological aspects and internal defenses system: evolutionary adaptations and applications in biotechnology

Coordinators:
Giuseppe Scapigliati, Giovanni Bernardini

Workshop *SPERA*

Organizer:
Anna Di Cosmo

ANNA DI COSMO[1], ANNARITA WIRZ[2], MARTA PISCITELLI[3]

[1] Dipartimento di Biologia, Università degli Studi di Napoli “Federico II”, Napoli, Italy
[2] IRCCS Fondazione Santa Lucia, Roma, Italy
[3] ENEA Dipartimento BAS, sez. Tossicologia e Scienze Biologiche, Roma, Italy

UNIONE ZOOLOGICA ITALIANA AND SPERA (SPERIMENTARE PER CURARE) FIRST STEPS TOGETHER

The SPERA mission is to promote the good science and explaining to the public the benefits of animal testing as a key element of research to face health problems and to counter animalist extremisms in favour of research and cure. Zoologists are life scientists who study animals, observing them in the laboratory and in their natural habitat. They study the origin and development of species as well as their habits, behaviour and interactions. Given that the UZI could not remain indifferent to SPERA call. These two associations and the others that share the same purposes are moving forward to constitute a Federation. This new Federation will promote the dissemination of scientific knowledge to counter false information that plays on emotion and affects public opinion. This joint path brought to the Federation's statute. The next step will be to promote initiatives at different levels, consisting of public debates, meetings, projects with schools and universities. The role of the federation is to defend the right of researchers to work in a calm atmosphere and without threats through the promotion of research, knowledge, education, and cure. The goal will be to abandon any kind of preconception and fundamentalism, to provide correct and accurate information to those who, rightly, want to make sure that animal testing is done only when absolutely necessary and in total respect of the laws and ethical principles.

MARTA PISCITELLI[1], ANNARITA WIRZ[2]

[1] ENEA Dipartimento BAS, sez. Tossicologia e Scienze Biologiche, Roma, Italy
[2] IRCCS Fondazione Santa Lucia, Roma, Italy

LEGISLATION ON ANIMAL EXPERIMENTATION: MEDIATING BETWEEN ANIMAL RIGHTS ASSOCIATIONS AND THE SCIENTIFIC COMMUNITY

Over the last 30 years, cultural changes have influenced the way we see animals and the way we value and respect their life. As a result, laws regarding the delicate subject of animal experimentation - both at a national and a European level - have become stricter than before and more oriented towards ensuring the wellbeing of the animals. It has gotten to the point where some of the limits and prohibitions included in the laws are almost incomprehensible for the scientific community. Protecting the animals and ensuring their welfare remains crucial for scientists and researchers as well, but unreasonable bans and laws that are too strict can be damaging for research itself. The EU Directive 63/2010 highlights two fundamental aspects of the legislation, that is, "animals are sentient creatures and as such they need to be considered" and "animals have an intrinsic value and for this they must be respected and protected for the only purpose of themselves". Nevertheless, animal rights associations are still battling against laboratory animal use by stating that it is absolutely immoral and unethical and most of all useless; whereas the scientific community is trying to speak in favour of this crucial aspect of biomedical research by underlying its necessity and its concrete application to everyday life. At this point, the question is whether or not it is possible to find a middle ground between the two opposite parties by encouraging an open dialogue based on accurate data and conducted in an honest and reasonable way. This is the hope of the scientific community and the aim of SPERA.

ANNARITA WIRZ[1], MARTA PISCITELLI[2]

[1] IRCCS Fondazione Santa Lucia, Roma, Italy
[2] ENEA Dipartimento BAS, sez. Tossicologia e Scienze Biologiche, Roma, Italy

SPERA FOR A CRITICAL AND WELL-INFORMED DEBATE ABOUT THE NECESSITY OF ANIMAL EXPERIMENTATION

In Italy, scientific research is often perceived as a theoretical activity with no application to everyday life - a reason for this is the lack of a clear and correct scientific elucidation. The public opinion seems to be particularly hostile to animal experimentation, the aspect of biomedical research that is most frequently misjudged and misrepresented. This also results from the strong anti-animal experimentation campaigns conducted by animal rights associations which play on the public's feelings through sensational and often inaccurate news stories. This whole situation can seriously damage research itself and its applications. That is why in October 2013 AISAL, the Italian Association for Laboratory Animal Science, launched the project *"SPERA – Sperimentare per curare",* which aims at providing accurate and understandable information, addressing all citizens, both experts and non-experts, adults as well as students, politicians as well as voters. For the first time, a large group of researchers from multiple experiences in different fields has assembled to work together on this project. Believing in networking among scientific associations, patients associations and schools, SPERA intends to increase the number of its participants to eventually evolve into a Scientific Federation. SPERA represents those who are morally and scientifically committed to the proper use of laboratory animals in scientific research; it supports their role through a campaign to raise awareness about the aims and instruments of scientific research and the results it has accomplished so far. Therefore, SPERA intends to promote curiosity with a series of different projects that would involve scientists and students at all levels. SPERA's main goal is to give people, the ultimate beneficiaries of biomedical research, the tools necessary to develop a personal critical opinion on the subject of animal experimentation, by encouraging an open dialogue and debate that will not resort to sensationalism and prejudice.

Symposium II

Physiological aspects and internal defenses system: evolutionary adaptations and applications in biotechnology

Coordinators:
Giuseppe Scapigliati, Giovanni Bernardini

Oral presentations

MATTEO CAMMARATA

Department of Biological Chemical Pharmaceutical Science and Technology, Animal Biology and Anthropology Division, University of Palermo, Italy

CNIDARIAN INFLAMMATORY REACTION AND BIOACTIVE MOLECULES: FROM MULTIFUNCTIONAL ROLE TO CULTURAL HERITAGE APPLICATIONS

The renewed interest in the study of genes of immunity in Cnidaria has led to additional information to the scenario of the first stages of immunity evolution revealing the cellular processes involved in symbiosis, in the regulation of homeostasis and in the fight against infections. We investigated the inflammatory response in *Anemonia sulcata* (Cnidaria: Anthozoa), following injection of various substances different in type and dimension, and observed clear, strong and specific reactions especially after injection of bacteria. The enzymes evaluation (protease, phosphatase and esterase), showing how the injection of different bacterial strains alters the expression of these enzymes suggesting a correlation between the appearance of the inflammatory reaction and the modification of enzymatic activities. Utilizing specialized penetrating nematocysts, cnidarians inject the nematocyst content or "venom" that initiates toxic and immunological reactions in the envenomed organism. These venoms contain enzymes, potent pore forming toxins, and neurotoxins. They could also take advantage of the multi-functionality of some of their toxins. The bioactive molecules were characterized and purified by biological assays, acid extraction, HPLC purifications, mass spectroscopy and peptide synthesis. Here, we show the cnidarian type of toxins regarding also to their multifunctional role as, for example, antimicrobial peptide and the future possibility of drawing important applications in fields ranging from pharmacology to cultural heritage. In particular, concerning biocleaning and antimicrobial growth control, representing a valid alternative to the traditional methods according to green restoration procedures for restoration of cultural heritage, safe for both operators and environment. Biomolecules from cnidarians have been tested to control the fungal growth, mimicking the relining of ancient/degraded paintings. Promising results were obtained from these molecules in order to control microbial colonies, growth in liquid media and on glue paste-canvas specimens.

NICOLA FRANCHI, LORIANO BALLARIN

Department of Biology, University of Padova

COMPLEMENT SYSTEM RECEPTORS C3AR AND CR1 IN TUNICATES: ANCIENT ROOTS OF IMMUNOCYTE DIALOGUE

Complement system is one of the most important humoral defense mechanism of innate immunity. Its evolutionary history is rooted in cnidarians where C3 molecules have been described. C3 is considered, in all taxa, the main protein of the complement system and the peptides C3a and C3b, derived from its proteolysis, are the effectors of all the complement-related responses towards microorganisms. These peptides have been described in many taxa, but only in vertebrates the complement system behaviour was deeply investigated, where more than 30 proteins were described. Conversely, in invertebrates, no more than 10 proteins in few organisms have been identified, all belonging to only two pathways of complement activation: the alternative and the lectin pathway. Recently we focussed our attention on the complement system of the colonial ascidian *Botryllus schlosseri*. We identified C3 and the serine proteases associated with the alternative and lectin pathways. In the present work, we identified the main receptors of the activated C3: BsCR1 and BsC3aR. The former is the receptor of C3b; the latter of the anaphylotoxin C3a. The sequence and the structure of BsC3aR and BsCR1 are highly conserved. Both genes are constitutively transcribed; CR1 and C3aR are transcribed only in haemocytes, the former in circulating phagocytes and the latter in cytotoxic morula cells. The different expression sites of CR1 and C3aR indicates a similarity with what reported in vertebrates, where C3 can contribute to the activation of phagocytes, and what happen in ascidians, where C3 is produced by morula cells which, through the complement system, can orchestrate a complex dialogue with phagocytes during the immune response.

MAGDA DE EGUILEOR[1], ANNALISA GRIMALDI[1], ENZO OTTAVIANI[2],
LAURA PULZE[1], STEFANO SALVIOLI[3], GIANLUCA TETTAMANTI[1],
ROBERTO VALVASSORI[1]

[1] Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy
[2] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[3] Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater studiorum-Universty of Bologna, Italy

FOCUS ON AMYLOIDOGENESIS AS A HIGHLY EVOLUTIONARY CONSERVED INFLAMMATORY MECHANISM

Amyloidogenesis represents a primitive, simple response, widespread in animals (invertebrates and vertebrates) where innate immune signalling pathways can be linked with stress responses. On the other hand "inflamm-aging" is a status characterizing old age that potentially triggers or facilitates the onset of the most important age-related diseases, as previously described. Thinking that amyloidogenesis is part of "inflamm-aging" and as such it can be partly responsible for the process of aging and a number of age-related diseases. Moreover, assuming that amyloidogenesis has beneficial effects early in life and eventually becomes detrimental late in life and that when deranged gives rise to a number of age-related pathologies such as cardiovascular diseases and Alzheimer disease. In other words the amyloid fibril synthesis is a physiological protective event aimed to weak ROS if at heavy load in the cytoplasm. This mechanism, often associated with autophagy, is normally balanced by physiological mechanisms involved in the clearance (amyloid fibrils can be cleaved by enzymes such as insulin or neprylisin-NEP) but when there is a slow down or a total block in the complex process of assembling and dismantling, amyloidogenesis occurs in wrong district and/or in wrong moment. Importantly we suggest that the amyloid fibril accumulation is one of feature of cell senescence.

ADRIANA VALLESI, FRANCESCA RICCI, PIERANGELO LUPORINI

Laboratory of Eukaryotic Microbiology and Animal Biology, University of Camerino, 62032 Camerino (MC) Italy

ANTI-OXIDATIVE DEFENSE IN A MARINE POLAR CILIATE, *EUPLOTES NOBILII*

Life in polar waters is permanently exposed to harsh environmental conditions, in primis sub-zero temperature, high oxygen concentration and enhanced UV radiation. These conditions are a general cause of intracellular accumulation of reactive oxygen species that are particularly deleterious to biological macromolecules. They have thus forced polar organisms to evolve specific mechanisms to cope with oxidative damage. We studied antioxidant mechanisms in a marine protist ciliate, *Euplotes nobilii*, in comparison with an its closely related species, *E. raikovi*, living in temperate seawaters. Doses and times of exposure to UV and hydrogen peroxide that did not affect at all *E. nobilii* viability were, instead, lethal to *E. raikovi*. To shed light on which genetic mechanism *E. nobilii* relies to face so effectively oxidative stress, particular attention was focused on methionine sulfoxide reductase (Msr) genes which are deputed to the repairing of proteins carrying oxidized methionines and basic genome components of every aerobic organism (from bacteria to mammals). Five different genes encoding type-B Msrs (specific for the reduction of methionine-sulfoxide R-forms) and five genes encoding type-A Msrs (specific for the reduction of methionine-sulfoxide S-forms) were identified from total DNA preparation of *E. nobilii*. The former gene set revealed unmistakable endogenous origins, since each gene carried telomeric C_4A_4 nucleotide repeats distinctive of every *Euplotes* gene of the transcriptionally active macronuclear genome. The latter gene set, instead, lacked these repeats and their sequences showed unequivocal prokaryotic origins. This observation suggested that *E. nobilii* reduced methionine-sulfoxide S-forms with exogenous MsrA enzymes produced by the *Francisella*-like bacterial endosymbionts that appear to be habitual host of its cytoplasm. We are currently verifying this hypothesis by screening the genome of the endosymbionts that we have successfully isolated and cloned into stable laboratory colonies.

MARIA VITTORIA MODICA[1], FABRIZIO LOMBARDO[2], PAOLO FRANCHINI[3], MARCO OLIVERIO[1]

[1] Department of Biology and Biotechnologies "C. Darwin", Sapienza University, I-00185 Rome, Italy
[2] Department of Public Health and Infectious Diseases, Sapienza University, I-00185 Rome, Italy
[3] Department of Biology, University of Konstanz, D-78745 Konstanz, Germany

VAMPIRES AT SEA:
BIOCHEMISTRY OF HEMATOPHAGY IN MARINE SNAILS

Hematophagy arose independently multiple times during metazoan evolution, with several lineages of vampire animals particularly diversified in invertebrates. However, the biochemistry of hematophagy has been studied in a few species of direct medical interest and is still underdeveloped in most invertebrates, as in general is the study of venom toxins. Adaptations to blood-feeding life style include behavioural traits, fundamental for host location, anatomical features, often involving specialized mouthparts and biochemical specialization, with the production of complex secretions (usually molecules acting as antihemostatic, anaesthetic and anti-inflammatory) in specialized glands. Among the less studied hematophagous groups, vampire snails belong to three lineages of Neogastropoda, namely Cancellariidae, Colubrariidae, and Cystiscidae that according to the available phylogenetic framework for neogastropods evolved hematophagy independently. We used a NGS RNA-Seq approach, integrated with differential expression analyses and custom searches for putative secreted feeding-related proteins, to describe in detail the salivary and mid-oesophageal transcriptomes of the Mediterranean vampire snail, *Colubraria reticulata*, with functional and evolutionary insights on major families of bioactive molecules. A remarkably low level of overlap was observed between the gene expression in the two target tissues, which also contained a high percentage of putatively secreted proteins when compared to the whole body. At least 12 families of feeding-related proteins were identified, including: 1) anaesthetics, such as ShK Toxin-containing proteins and turripeptides (ion-channel blockers), Cysteine-rich secretory proteins (CRISPs), Adenosine Deaminase (ADA); 2) inhibitors of primary haemostasis, such as novel vWFA domain-containing proteins, the Ectonucleotide pyrophosphatase/phosphodiesterase family member 5 (ENPP5) and the wasp Antigen-5; 3) anticoagulants, such as TFPI-like multiple Kunitz-type protease inhibitors, Peptidases S1 (PS1), CAP/ShKT domain-containing proteins, Astacin metalloproteases and Astacin/ShKT domain-containing proteins; 4) additional proteins, such the Angiotensin-Converting Enzyme (ACE: vasopressive) and the cytolytic Porins. *Colubraria* feeding physiology seems to involve inhibitors of both primary and secondary haemostasis, anaesthetics, a vasoconstrictive enzyme to reduce feeding time and tissue-degrading proteins such as Porins and Astacins. The complexity of *Colubraria* venomous cocktail and the divergence from the arsenal of the few neogastropods studied to date (mostly conoideans) suggest that biochemical diversification of neogastropods might be largely underestimated and worth of extensive investigation.

MARIA GIOVANNA PARISI[1], AITI VIZZINI[1], GIANLUCA SARÀ[2],
MATTEO CAMMARATA[1]

[1] Laboratory of Marine Immunobiology, Department of Biological, Chemical and Pharmaceutical Science and Technology, University of Palermo
[2] Laboratory of Experimental Ecology and Behaviour Department of Earth and Marine Sciences University of Palermo

αβ DEFENSIN ANTIMICROBIAL PEPTIDE (BPDEF) FROM THE INVASIVE RED SEA MUSSEL *BRACHIDONTES PHARAONIS* (P. FISCHER, 1870)

The immune system plays a major role in determining host fitness in the wild, i.e. under the constraints imposed by ecology and life history. Permanent conflict interactions with the environment are the natural situation for a living creature but the immune system is characterized by an enormous variety of mechanisms and effectors, including the Antimicrobial peptides (AMPs). These compounds, in fact, are extremely successful in dealing with a huge range of pathogens, including bacteria, fungi, protozoa and viruses. In the present study, using primers designed from coding sequences from *Mytilus galloprovincialis*, the cDNA sequence of the Defensin was cloned and characterized from the Red Sea mussel *Brachidontes pharaonis* living along the rocky shores of the Mediterranean. Analysis of the sequence of 262 nucleotides revealed in the *B. pharaonis* defensin (BpDef) the presence of an ORF coding for 81 amino acids consisting of a signal peptide of 23 aa in the amino terminal portion followed by the mature peptide of 35 a.a. and a carboxy-terminal extension position. Alignment with the sequences of *M. galloprovincialis* indicate high similarity with the antimicrobial peptide MGD1 and MGD2 of Mediterranean mussel. *In situ* hybridization analysis revealed BpDef mRNA expressed in circulating hemocytes with small intra-cytoplasmic granules and with large granules. Sequence identity and the common conserved sequence characteristics show that BpDef belongs to the αβdefensin family AMPs with a typical domain structurally characterized by α helix and two sheets αβ. Two predicted bactericidal stretches were found in correspondence of the mature peptide and the Boman index value indicated the strong antibacterial feature of defensin of *B. pharaonis*. This is the first report on the AMP of defensin family in lessepsian species *B. pharaonis*. The results enrich the basic research on the AMPs gene family in mussels, providing improved understanding of the immune system in invasive mollusks. The study is also a first step for further practical application of bioactive peptide derived from lessepsian species as alternative and/or complementary bioactive compounds in the vast reservoir of marine invertebrates antimicrobial peptides (AMPs).

VINCENZO ARIZZA, CLAUDIA MAZZARELLA, LUIGI INGUGLIA,
ANGELO SPINELLO, GIAMPAOLO BARONE, MARIA GRAZIA CUSIMANO,
DOMENICO SCHILLACI

Dipartimento STEBICEF Università di Palermo Via Archirafi 18, 90123 Palermo, Italia

ANTIMICROBIAL PEPTIDE FROM *PARACENTROTUS LIVIDUS* (ECHINODERMATA) AND BIOTECHNOLOGY APPLICATION FOR NEW GENERATION OF MEDICAL DEVICES

Implant-associated infections are one of the most serious complications in orthopaedic surgery. The increased use of orthopaedic devices, whose surfaces are an ideal substrate for the bacterial growth, has substantially increased the cases of infected implants. Therefore, the cost for the treatment of such infection is tremendous and it is estimated to be about $250 million per year in the USA. Common pathogens such as the *Staphylococcus aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa* are the most common bacteria that cause infections and may adhere to the surfaces of devices creating a bacterial biofilm resistant up to 1000-fold to antimicrobial agents. An ideal strategy to combat implant-associated infections would be prevention of infection at the site of the implant, thought local delivery of antibiotics by implant surfaces, has received much attention recently particularly due to their potential and low systemic side effects of this approach. A preferred solution would be to employ non-conventional antimicrobial drugs that do not potentiate the development of resistant phenotypes. One example of such non-classical drugs is the cationic antimicrobial peptides (AMP). Recently SCHILLACI *et al.* (2010) discovered that *Paracentrotus lividus* contains Paracentrin 1, an AMP with antimicrobial and antibiofilm activity against *Staphylococcus* strains and *Pseudomonas aeruginosa*. This study represent the results of the ASTED project, funded by the PO-FESR 2006-20013 whose main objective was to develop orthopaedic devices capable of delivering Parcentrin 1 from their surface. For this purpose, the delivers were coated with a polymer of Poly (D,L-Lactide) (PDDLA) containing recombinant Paracentrin 1 (rP1) produced in Sf9 cells. Our results showed that rP1 were released from PDLLA and was able to kill planktonic bacteria with a MIC value of 12.5 mg/lm and inhibit the biofilm formation of *S. aureus* and *S. aeruginosa* at sub MIC concentration. Biotechnology skills of this study were transferred to the small enterprises, Ge. Me. S, to create a process of innovation and technological development.

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PAOLO COCCI, GILBERTO MOSCONI, FRANCESCO A. PALERMO

Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino,
Via Gentile III da Varano, 62032 Camerino (MC), Italia

TOXICOLOGICAL APPLICATION OF LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) CULTURED ERYTROCYTES

Juvenile loggerhead turtles (*Caretta caretta*) can be considered a good indicator species for monitoring environmental contaminants that can mimic steroid hormone signaling. However, due to the legal constraints of their endangered status, the impact of environmental pollutants on nuclear steroid hormone receptor-mediated signaling is still poorly investigated in sea turtles. Here we describe the use of an *in vitro* toxicity testing for evaluating the effects of different environmental exogenous estrogens on the expression of *C. caretta* estrogen receptor (ER). In this regard, primary cultures of erythrocytes were exposed to increasing concentrations of 4-nonylphenol (4-NP), Diisodecyl phthalate (DiDP) and Tri-m-cresyl phosphate (TMCP) for 48 h. A real time quantitative PCR assay, optimized in the loggerhead turtles red blood cells (RBCs), showed significantly increased levels of ER mRNA in a dose-dependent manner after 48 h exposure to both 4-NP and TMCP. Interestingly, the dosage-dependent effects of DiDP on ER expression were opposite in comparison to that obtained following exposure to the other tested compounds. Our work demonstrates the validity of cultured erythrocytes in sea turtles suggesting that this approach could provide an understanding of the impact of environmental contaminants on an endangered species in a minimally invasive manner.

PATRIZIA PAGLIARA[1], CARMELA GERARDI[2], ANNA LUCIA DELOS[1],
LOREDANA STABILI[1, 3]

[1] DiSTeBA, Università del Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italia
[2] CNR-ISPA, UOS Lecce, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italia
[3] IAMC, U.O.S. di Taranto – CNR, Via Roma 3, 74123 Taranto, Italia

FIRST INSIGHT INTO THE IMMUNOLOGICAL DEFENCE SYSTEM OF THE SEA URCHIN *SPHAERECHINUS GRANULARIS* (ECHINOIDEA, ECHINODERMATA)

Echinoderms are an excellent model for studying innate immunity as invertebrates lacking adaptive immunity and as deuterosomes being more closely related to humans than any other invertebrate group. Several echinoderm species are able to differentiate self from non-self through the production of soluble molecules and coelomocytes response, exhibiting a robust innate immune system and playing an important role in the resistance to disease. Among sea urchins, *Sphaerechinus granularis* is one of the least studied with respect to immunological defense capabilities, particularly in the adult. Aim of this study was to investigate some immunological parameters in this sea urchin. Coelomic fluid and cell lysate were employed to investigate the hemolytic and agglutinating capability, antibacterial activity (lysozyme-like activity) as well as antioxidant activity. Our results evidenced that coelomic fluid possess the ability to lyse and agglutinate human red blood cells. In both cases, the activities resulted dependent from calcium: the presence of Ca^{++} increases both the hemolysis percentage and the agglutination title. Lysozyme-like activity was negligible in the the coelomic fluid; by contrast this activity was strong in the cell lysate. The antioxidant activity of *S. granularis* measured by two *in vitro* assays (the TEAC and the ORAC) was higher in the cell lysate than in the coelomic fluid. The obtained results represent a preliminary step in the knowledge the immunological competences of the examined species and its potential biotechnological exploitation.

PAOLA ZARATTINI[1], FULVIO FERRARA[2]

[1] Dipartimento di Scienze della Vita, Università degli Studi di Trieste
[2] Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Roma

ARTEMIA SALINA (BRANCHIOPODA, ANOSTRACA) FROM SALINE DI TARQUINIA (VITERBO): A SUITABLE FEED ORGANISM FOR ZEBRAFISH SURVIVAL AND GROWTH

The brine shrimp *Artemia* Leach, 1819 is the most widely used aquaculture live food organism because it is very convenient to use and readily available. *Artemia* is a small aquatic crustacean native to inland saline environments and during a portion of its life cycle produces a resting cyst that is able to withstand extreme environmental conditions. The cysts are cleaned and desiccated before they are canned for shipment. Moreover the fatty acids composition of *Artemia* adults, larvae and cysts is regarded as important in optimizing larval nutrition for survival and growth of aquaculture species. The aim of this work is to assess the nutritional quality of an Italian source of *Artemia salina* Linnaeus, 1758 occurred in the Saline di Tarquinia (Viterbo, Italy) and characterized by a suitable cyst diameter and an optimal biomass production (DAVIDDI, 1997; VITA, 1997). Ten samplings were performed from January to June at regular time intervals during the water phase of two main ponds and the biochemical composition of three different developmental stages (larvae, juvenes and adults) of the bisexual population were analysed by gaschromatography. The impact of water salinity, temperature, pH and lipid content of microalgal species (*pabulum*) on *Artemia* fatty acid profile was determined. The results show that HUFA profile of the examined samples is significantly influenced by the salinity and significantly correlated to the *pabulum* lipid content. The substantial levels of 20:5n, n-3 (4%) and 20:4, n-6 (1%) and the high ratio n-3/n-6 indicate that *A. salina* from Tarquinia is a marine-type strain and could be a valuable food source for larvae in aquaculture. The vast majority of *Artemia* cysts available to zebrafish culturists are of *Artemia franciscana* Kellogg, 1906 harvested from the Great Salt Lake in Utah. Annual harvests of *Artemia* cysts are unpredictable and can fluctuate greatly due to environmental factors. Nowadays the use of autochthon species as live food in aquaculture is recommended. A part from their substantial economic impacts, exotic invasive species may alter the evolutionary trajectory of natives through competition, displacement, hybridization and even extinction (AMAT *et al.*, 2005; MURA *et al.*, 2006).

ANNA MARIA TOLOMEO[1], RIGERS BAKIU[2], SEAN PLACE[3],
ESTER PICCINNI[1], GIANFRANCO SANTOVITO[1]

[1] Department of Biology, University of Padua, Padua, Italy
[2] Department of Aquaculture and Fisheries, Agricultural University of Tirana, Tirana, Albania
[3] Department of Biology, Sonoma State University, Rohnert Park, CA, USA

PEROXIREDOXIN IN THE EMERALD ROCKCOD *TREMATOMUS BERNACCHII*

Antarctic species are characterized by a large number of special physiological features that allow the life in their extreme environment. In particular, the low temperature and salt concentration are chemiophysical conditions that increase oxygen solubility and, consequently, the rate of ROS formation. With the aim to study the components of the antioxidant defense system in the Antarctic teleosts, we have characterized the genes codifying for peroxiredoxins (Prdxs) in the emerald rockcod *Trematomus bernacchii*. Prdxs are a family of small (22 – 27 kDa) non-selenium peroxidases thus representing a class of important antioxidant enzymes that protect cells against oxidative stress, in fact they are able to reduce hydrogen peroxide, organic hydroperoxides and peroxynitrite. In the genome of *T. bernacchii* we have verified the presence of five of the six Prdx isoforms known in vertebrates: Prdx2, Prdx3, Prdx4, Prdx5, Prdx6. For isoforms 3 and 6, we also characterized two variants (A and B). Multi-alignment analyses, performed with fish orthologous sequences, demonstrated high conservation of the amino acids involved in catalytic activity of the Prdx isoforms of *T. bernacchii*. However, some substitutions with polar amino acids, are characteristics of some residues close to the motifs important for the functionality of these proteins. The gene transcriptions of all s from various tissues (gills, heart, liver, spleen, and skeletal muscle) of *T. bernacchii* has been measured by RT-sqPCR. The gills are the organ in which the highest levels of Prdx mRNA accumulation is present, with the exception of isoforms 3B and 6A which have a low tissue-specificity. Prdx 2-Cys activity have been measured in the same organs. The highest levels are present in spleen and liver. The tissue-specific differences in the mRNA and active protein accumulations are probably related to the physiological function characteristic of these organs. (Supported by P.N.R.A. and M.I.U.R. grants).

AITI VIZZINI, FELICIA DI FALCO, DANIELA PARRINELLO,
MARIA ANTONIETTA SANFRATELLO, MATTEO CAMMARATA

Department of Biological Chemical Pharmaceutical Science and Technology, Animal Biology and Anthropology Division,
University of Palermo, Italy

CYTOKINES IN THE INFLAMMATORY RESPONSE OF THE ASCIDIAN *CIONA INTESTINALIS*

The *Ciona intestinalis* inflammatory response to several harmful agents have been demonstrated to be composed of a complex set reaction. The cellular reactions involve hemocyte activity and infiltration, cell disruption, while cell products can contribute to form capsule components and/or cause a wound. In this response the involvement of the pharynx, as the main immune-competent organ, has been disclosed by lipopolysaccharide (LPS) challenge that upregulates innate immunity genes. In vertebrate cytokines modulate the balance between humoral and cell-based immune responses, and they regulate the maturation, growth, and responsiveness of cell populations that have pleiotropic functions in a broad range of cell types. Proinflammatory cytokines, that are master key molecules of immune response and disease with large biotechnology and biomedical applications, plays a key role in the clearance of extracellular bacteria promoting cell infiltration and production of several cytokines. In this studies we focusing on identification and the role of Transforming Grow Factor (TGFβ), three form of Interleukins 17 (IL-17) and Tumor Necrosis Factor (TNFα) cytokines in the inflammatory process induced by LPS in ascidian *C. intestinalis*. Real time PCR analysis showed that *Ci*TNFα and *Ci*IL-17s were up-regulate at 1-4 hours post injection, suggesting that are involved in the first phase of inflammatory response; *Ci*TGF-β results transcriptionally up-regulated also in the first phase but significant in the secondary phase of inflammatory response (48-72 hours) in which a cell differentiation occur. *In situ* hybridization assays disclosed that the genes transcription was upregulated in the pharynx and expressed by hemocytes located in tightly packed cell groups within the vessel lumen inside the pharynx. The gene organisation, phylogenetic tree and structural modelling supported the close relationship with the vertebrate cytokines homologues.

CHIARA MANFRIN[1], FEDERICA PIAZZA[1], MORENO COCCHIETTO[2],
NIKOLINKA ANTCHEVA[3], DOMENICO MASIELLO[2],
ANDREA FRANCESCHINI[1], LUCA PERUZZA[1], LUCREZIA BONZI[1],
ALESSANDRO MOSCO[1], CORRADO GUARNACCIA[3], GIANNI SAVA[2],
PIERO G. GIULIANINI[1]

[1] Department of Life Sciences, University of Trieste, Via L. Giorgieri 5, 33127 Trieste, Italy
[2] Fondazione Callerio, Via Fleming 22-31, 34127 Trieste, Italy
[3] International Centre for Genetic Engineering and Biotechnology, AREA Science Park, Trieste, Italy

ORAL DELIVERY OF THE CRUSTACEAN HYPERGLYCEMIC HORMONE - CHH - IN *PROCAMBARUS CLARKII*

The red swamp crayfish (*Procambarus clarkii* Girard, 1852) is one of the most aggressive invasive species worldwide. Growing efforts are directed toward a better understanding of its endocrinology and physiology, in order to create new eradicative autocidal methods. The aim of the present study is to shed the basis for setting up hormonal baits for alien crayfish management. The CHH contains six cysteine, nine aspartic acid residues and a very hydrophobic region in the C-terminus. We synthetised the CHH in two parts: CHH1-38 and CHH39-72 in order to use a native chemical ligation to obtain the mature peptide. The hyperglycaemic activity of the synthetic neuropeptide has been carried out by testing two different injected doses (0.25 and 0.5 μg D-Phe-CHH/100 μl PBS per animal) in both *P. clarkii* (n=9) and in the Italian native crayfish *Austropotamobius italicus* (Faxon, 1914) (n=10). In *P. clarkii* the glycaemic levels raise the maximum values after 2 hours (151.00±7.00 mg/dL for 0.5μg and 94.12±13.58 mg/dL for 0.25μg), and high glucose levels have been maintained until 4 hours post injection, to revert the basal level after 8 hours. In *A. italicus* the maximum glycaemic peak for both doses is delayed to 8 hours post injection (75.46±9.30 mg/dL for 0.5μg and 62.36±9.64 mg/dL for 0.25μg). The comparison of the peak, for the 0.5 μg dose, at 2 hours in *P. clarkii* and at 8 hours in *A. italicus,* shows a significantly higher hyperglicaemic activity in *P. clarkii* of the synthetic D-Phe-CHH (Welch Two Sample t-test, p-value = 1.07e-05; Wilcoxon rank sum test, p-value = 0.00040). In the optic of using CHH as autocidal method to affect *P. clarkii* populations, we assayed an innovative system based on a specific microemulsion able to orally convey the D-Phe-CHH along the digestive system. After the administration of beads containing the neurohormone (20 to 50μg per animal), the glycaemia significantly increased at 6th hour from the ingestion, in eyestalkless animals (57.74±14.92 mg/dL vs 4.81±1.64 mg/dL, Welch Two Sample t-test, p-value = 0.0062; Wilcoxon rank sum test, p-value = 0.00097) and at 5th hour in intact crayfish compared to controls (16.88±2.54 mg/dL vs 8.22±2.08 mg/dL, Welch Two Sample t-test, p-value = 0.017; Wilcoxon rank sum test, p-value = 0.031). These encouraging findings are promising for the use of synthetic neurohormones administered via baits to hinder the spreading of invasive crayfish, as for the case of the red swamp crayfish.

MICHELA SUGNI[1], CINZIA FERRARIO[1], LIVIO LEGGIO[1],
CRISTIANO DI BENEDETTO[1], ROBERTA LEONE[1], SILVIA COLOMBO[1],
VALENTINA COCCÈ,[2], MIRIAM ASCAGNI[1], FRANCESCO BONASORO[1],
CATERINA LA PORTA[1], MARIA DANIELA CANDIA CARNEVALI[1]

[1] Department of Biosciences, University of Milan, Milan, Italy
[2] Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

BIOTECHNOLOGICAL APPLICATIONS OF ECHINODERM MUTABLE COLLAGENOUS TISSUES

Echinoderms possess unique connective tissues (Mutable Collagenous Tissues, MCT) which display striking passive mechanical properties. These tissues are widespread in all the five extant echinoderm classes and are fundamental for many aspects of their biology. Besides their evident physiological relevance, MCT can also be a promising source of inspiration and material, namely collagen, for biotechnological applications, as an alternative to the widely used mammalian sources. Collagen-made materials are the most promising in regenerative medicine: in the field of Guided Tissue Regeneration collagen barrier-membranes of mammalian origin (BM) are typically used to facilitate proper tissue regrowth in anatomically separated compartments. Recently, we have proposed the sea urchin peristomial membrane as a target MCT for the extraction of native collagen fibrils and the development of innovative membranes. In the present work we used and compared MCT from different echinoderm models, representative of the diverse classes (sea urchin, starfish and sea cucumber) to prepare echinoderm-derived membranes (EDM). The EDM were characterized and compared in terms of structure, biomechanics and human cell behaviour (skin-derived fibroblasts). BM or substrates made of soluble or re-fibrillated bovine collagen were used as further comparison. All the EDM were similar in terms of structure (highly dense fibrillar network) and mechanical performances, but all of them were much thinner (~20-30 folds) and mechanically more resistant (~20 folds) than the commercially used BM. Fibroblasts seeded on the EDM were generally less numerous than those on mammalian collagen substrates (except in the case of the sea urchin-derived collagen membranes, which were similar) and displayed a more elongated shape, less numerous filopodial processes and a different cytoskeletal organization, all these features suggesting a weaker cell attachment. Overall, our data indicate that all the echinoderm models can be considered potential alternative source of collagen to produce membranes suitable for Guided Tissue Regeneration, where high mechanical resistance, low cell attachment and dense fibrillar network for a barrier-effect are required. Nevertheless, sea urchins might display some advantage, also in terms of eco-sustainability (by recycling tissues from food wastes). Further *in vivo* tests are necessary to confirm the validity of this innovative marine biomaterial.

Symposium II

Physiological aspects and internal defenses system: evolutionary adaptations and applications in biotechnology

Coordinators:
Giuseppe Scapigliati, Giovanni Bernardini

Posters

ELENA GENTILI[1], MARTA PISCITELLI[2], ANNARITA WIRZ[3]

[1] SPERA
[2] ENEA Dipartimento BAS, sez. Tossicologia e Scienze Biologiche, Roma, Italy
[3] Fondazione Santa Lucia, Roma

SPERA:
IT IS POSSIBLE TO EDUCATE CITZENS ON HOW TO APPROACH SCIENCE AND BIOMEDICAL RESEARCH?

The SPERA Project is an information campaign started in 2013 by the Italian Association of Laboratory Animals' Science (AISAL). Its objective is to inform and educate the greater public about scientific research. What scientific research is, what are its results and what are the means through which they can be achieved, but also what could be its limits. The wider goal is to fight prejudice and allow citizens to develop their own critical judgment without preconceptions. This can only be achieved by involving researchers from many different fields, with different backgrounds and experiences. Activities will be presented that have already been carried out within the SPERA Project, as well as the short and mid-term planned activities. These will mainly concern projects about the understanding of science for middle and high schools.

ALICE MADONIA[1], CRISTIANO MELCHIORRI[1], MARCO GALEOTTI[2],
PIETRO-GIORGIO TISCAR[3], DONATELLA VOLPATTI[2],
CHIARA BULFON[2], ELISABETTA CACCIA[1], FRANCESCO MOSCA[3],
FILIPPO CASTIGLIONE[4], MARCO MARCELLI[1], NICLA ROMANO[1]

[1] Department of Ecology and Biology, Tuscia University, Viterbo
[2] Department of Food Sciences, Section of Veterinary Pathology, University of Udine, Italy
[3] Faculty of Veterinary Medicine, University of Teramo, Italy
[4] Institute of computing applications "M. Picone" CNR, Rome, Italy

IN SILICO MODELING OF ANTI-VIBRIOSIS VACCINATION IN FISH

The validation of vaccine protocols and the determination of the optimal dose for the populations to be treated represent a long-trial investigation often unproductive in term of time consuming and costs. Simulating the immune system is now one of the most innovative approaches in bioinformatics sciences, providing a new tool for immunological investigations. Vaccination of sea bass, *Dicentrarchus labrax* (L.), is generally limited to a single administration (or priming) and not adapted to the treated species. Recently we have formulated an innovative anti-vibriosis vaccine treatment for the farmed sea bass. Juveniles were vaccinated against *Listonella anguillarum* using a commercial formulation (Sharing-Plug) administered per double immersion (priming: 95 dph; booster: 165 dph) or by double immersion and subsequent i. p. injection (booster: 233 dph) (GALEOTTI *et al*., 2013). The *in vivo* findings were compared with the *in silico* experimentation through the use of the immunological model C-ImmSim. C-ImmSimis an Agent Based Model (ABM) which describes both the humoral and cellular response of a vertebrate immune system to the presence of antigens at the cellular level. The grid is three-dimensional with periodic boundary conditions, representing a known volume of mm^3 of a lymphoid organ or ml of peripheral blood. The vaccination steps were reproduced by injecting a quantity of "un-harmful" antigen at those t-steps corresponding to the immersion/i. p. injection dph of the *in vivo* experiment. The challenge was simulated by injecting a known amount of "active" antigen in the grid. Preliminary calibration tests were carried out to select the appropriate doses of vaccine and infectious bacteria concentrations to be used in the simulation experiments. The set up of the model parameters allowed to simulate the immune response of the sea basses subjected to vaccine treatments and to calculate the survival rate of each experimental group. The outputs of the model agree with the immunological analysis of Ab production (anti–L/serum), BcR and TcR gene transcripts in the spleen, allowing to follow the temporal evolution of the immunological response all over the trials. The use of an immunological simulator in fish vaccination trials confirms the suitability of ABM models for the simulation of biological systems, providing an useful tool to be exploited as a virtual laboratory where experiments can be made prior to the design of a real *in vivo* or *in vitro* experimentation.

PATRIZIA PAGLIARA[1], FRANCESCO DRAGO[2], FRANCESCA VACCA[1],
JACOPO VIZIOLI[2]

[1] DiSTeBA, Università del Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italia
[2] Université Lille 1, Laboratoire PRISM – Inserm U1192, Villeneuve d'Ascq 59655, France

PRELIMINARY INVESTIGATIONS ON THE ALLOGRAFT INFLAMMATORY FACTOR-1 (AIF-1): CONSTITUTIVE EXPRESSION IN ECHINODERMS COELOMOCYTES

The phylum Echinodermata is basal to the phylum Chordata. This relationship makes the analysis of echinoderms immune system relevant to understanding the evolution of deuterostomes immunity leading to Vertebrata. Like in other invertebrate species, echinoderms' circulating cells (coelomocytes) play a central role in host defense via a variety of immune functions, including immune surveillance by self/non-self-recognition, phagocytosis, and secretion of inflammatory cytokines. The Allograft Inflammatory Factor-1 (AIF-1) is an evolutionally conserved calcium-binding cytokine produced by activated macrophages during chronic transplant rejection and inflammatory reactions in vertebrates. Recent literature data evidence that AIF-1 is involved in immune response in phylogenetically distant Metazoan species such as fishes, mollusks, annelids or sponges. This cytokine is generally expressed in circulating cells (hemocytes) in physiological conditions and is further stimulated by bacterial infection, suggesting a conserved role in regulating hemocyte activity. Among echinoderms, Allograft Inflammatory Factor 1 genes have been recently cloned and characterized from the Antarctic sea urchin *Sterechinus neumayeri* and from the sea cucumber *Apostichopus japonicas*. In both species, the gene expression was up-regulated in coelomocytes upon bacterial challenge. We herein explore, by immunocytochemistry and through confocal observations, the constitutive expression of AIF-1 in the coelomocytes of some echinoderms species. The data evidence a positive reaction of cells to anti-human AIF-1 polyclonal antibodies in the studied animals with different cellular localizations, according to the coelomocyte populations and the species. These findings indicate that AIF-1 is a cytokine widely distributed among echinoderms and suggest its involvement in the innate immune responses of these metazoans.

ANNA MARIA TOLOMEO[1], RIGERS BAKIU[2], ESTER PICCINNI[1],
DIANA FERRO[3], GIANFRANCO SANTOVITO[1]

[1] Department of Biology, University of Padova, Padova, Italy
[2] Department of Aquaculture and Fishing, Agricultural University of Tirana, Tirana, Albania
[3] Institute for Evolution and Biodiversity, Westfälische Wilhelms-Universität, Münster, Germany

CHARACTERIZATION OF PEROXIREDOXIN GENES IN THE CILIATED PROTOZOAN *TETRAHYMENA THERMOPHILA*

Peroxiredoxins (Prdxs) are a family of small (22 – 27 kDa) non-selenium peroxidases that are able to reduce hydrogen peroxide, organic hydroperoxides and peroxynitrite. They represent a class of important antioxidant enzymes, that protect cells against oxidative stress. In order to obtain information about the molecular evolution of these proteins we studied four Prdx-like sequences from *Tetrahymena thermophila* present in GenBank database, by a bioinformatic approach. The nucleotide and aminoacid sequences of all genes (named *ttprdx1*, *ttprdx2*, *ttprdx3* and *ttprdx4*) were compared with Prdxs of other organisms and used for phylogenetic analyses. *Tetrahymena* proteins are all typical 2-Cys and show high homology with their orthologs. In particular, the residues important for the peroxidase activity, including the peroxidatic cysteine within the catalytic center and the resolving cysteine that composes the motif GEVCPA, are conserved. Phylogenetic reconstruction indicate that TtPrx1, 2 and 4 emerge as sister group of vertebrate Prx3s, while TtPrx3 is not strictly correlated with any isoform. Preliminary analysis of homology modeling do not support for the belonging of *Tetrahymena* Prdxs to a specific isoform. This could indicate that the molecular structure of *Tetrahymena* Prdxs has ancestral features, which could represent the result of a limited differentiation in the evolutionary process subsequent to the occurrence of these proteins. With the aim to obtain new information about the role of Prdx in the antioxidant system of this organism, other experiments will be performed. Time-course of gene expression will be analyzed by qRT-PCR in *T. thermophila* cells grown in normal conditions and after exposure of Cu^{2+}, used as pro-oxidant (Grant by M.I.U.R.).

MARIA ROSA TRAPANI, MARIA GIOVANNA PARISI,
DANIELA PARRINELLO, MARIA ANTONIETTA SANFRATELLO,
GIGLIOLA BENENATI, MATTEO CAMMARATA

Laboratory of Marine Immunobiology, Department of Biological, Chemical and Pharmaceutical Science and Technology, University of Palermo

SPECIFIC INFLAMMATORY RESPONSE OF *ANEMONIA SULCATA* (CNIDARIA) AFTER BACTERIAL INJECTION CAUSE TISSUE REJECTION AND ENZYMATIC ACTIVITY ALTERATION

The evolution of multicellular organisms was marked by adaptation to protect against pathogens. The mechanisms for discriminating the "self" from "non-self" have evolved into a long history of cellular and molecular strategies from damage repair to the co-evolution of host-pathogen interaction. The phylum of Cnidaria is one of the first branches in the tree of animal life to provide crucial insights on the evolution of immunity. Sea anemones (Anthozoa, Cnidaria) are benthic sessile species able to maintain the integrity of the tissues and allorecognition in colonial forms and to differentiate between symbionts and pathogenic intruders. We investigated the inflammatory response in sea anemone *Anemonia sulcata* following injection of various substances different in type and dimension, and observed clear, strong and specific reactions especially after injection of *Escherichia coli* and *Vibrio alginolyticus*. In particular we studied the effect of injection of different sized suspensions (LPS, Phenyl Sepharose, cellulose, red blood cells, bacteria) into the pedal disk of specimens in order to observe differential physical, biochemical and enzymatic reactions. The first phenomenon we observed was a body and foot swelling, followed by the formation of a yellowish rejection zone. A further study was also carried out by a histological analysis for the characterization of the area of rejection. This specific response suggested an extrusion of the material damaged by the animal, probably from the pores cinclides from which protrude the *acontia* in the pedal disk, a defense mechanism that has not yet been found in other cnidarians. Moreover, we analyzed enzymatic activity of protease, phosphatase and esterase, showing how the injection of different bacterial strains alters the expression of these enzymes, that catabolize prey tissues, suggesting a correlation between the appearance of the inflammatory reaction and the modification of enzymatic activities. Our study shows for the first time, a specific inflammatory-like response rejection and enzymatic responses following injection of bacteria in cnidarian.

Symposium III

The modern systematics: between structural morphology and molecular approaches

Coordinators:
Romolo Fochetti, Marzio Zapparoli

Invited lectures

ALESSANDRO MINELLI

Dipartimento di Biologia, Università di Padova, Via U. Bassi 58 B, 35131 Padova

THE GENOTYPE → PHENOTYPE MAP AND THE RECONSTRUCTION OF PHYLOGENY

The so-called developmental genes, especially the Hox genes, have long attracted interest as potential sources of phylogenetic information and have been used for example to revisit the relationships between the phyla of the Bilateria or the main clades of arthropods. The path is long, however, from a gene sequence to a morphological phenotype that may be considered an expression of the former. The relationships between genotype and phenotype (the genotype → phenotype 'map') are complex and leave room for a discontinuous and sometimes saltational (e. g., *Scolopendropsis*) evolution of morphological characters. This is the place where evo-devo biology can offer a valuable service to systematics. Beside the comparison of gene sequences, we can compare gene expression patterns, in order to trace homologies, especially in cases where morphology does not offer decisive clues. The following classes of evolutionary change of gene expression have been recognized: (i) changes in the spatial domain of gene expression, (ii) changes in the timing of gene expression (transcription heterochrony), (iii) changes in the level of gene expression. In different groups, different are the stages of development that can be more variable and informative. The most conservative stage is never the egg, but, often, a phylotypic stage (e.g. the pharyngula in vertebrates, the germ band stage in arthropods) on which it seems reasonable to focus if we are looking for characters useful for diagnosing taxa of higher level, while for information to be used in comparisons between closely related species we should look elsewhere. It is also useful to highlight changes of evolvability of a particular body plan along a phylogenetic tree; if a lineage, for example, is affected since the basal nodes by changes of regressive type, it is to be expected that evolvability will be rapidly reduced; an example are flukes and tapeworms. Particularly relevant for systematics is heterochrony, which contains in itself phylogenetic signal, detectable through appropriate models. Heterochronic effects, however, can also be misleading, especially in the case of paedomorphosis, which can create similarities because of convergence but also because of cancellation of the phylogenetic signal previously present in characters that in these groups are no longer expressed.

MARCO OLIVERIO

Department of Biology and Biotechnologies “C. Darwin”, Sapienza University, I-00185 Rome, Italy

MODERN CLASSIFICATIONS OF GASTROPODS

Recent paleontological discoveries shed ligth (surprisingly agreeing with molecular dating) on the origin of molluscs addressing to aenigmatic Ediacaran fossils and reinterpreting mouthparts of Cambrian representatives. However, relationships among molluscan classes are far from being settled. The same holds for gastropods, the largest extant class of molluscs. Gastropoda have been recovered as monophyletic by most cladistic analyses based on morphological data, but only recently, analyses of large molecular datasets were also able to retrieve such monophyly, which is supported by a major anatomical revolution: torsion of visceral mass. Early Cambrian weakly torted (10° rotation) pelagiellids, were probably the earliest stem gastropods. Thiele’s (1929-31) classification of gastropods with three subclasses, Prosobranchia (split into primitive Archaeogastropoda, middle Mesogastropoda and advanced Stenoglossa), Opisthobranchia and Pulmonata was maintained in textbooks until the end of the century. The major challenges to Thiele’s partitioning were by the late Salvini-Plawen and by his student Haszprunar. After their and other’s contributions many changes have been implemented in gastropod systematics. Archaeogastropods were proven to represent a grade (rather than a clade) including extant representatives of several early gastropod off-shoots. Most meso- and all neogastropods were reunited into the clade Caenogastropoda. Opisthobranchs and pulmonates were rearranged into a mostly euthyneuran clade Heterobranchia. A very primitive Eogastropoda (patellogastropod limpets) offshoot was morphologically defined, followed by the most modern Apogastropoda (all the rest). Analyses of molecular data in the last 20 years have challenged monophyly of almost all morphology-based clades. Few major clades have received support by both anatomical and (part of) molecular analyses: Eogastropoda and Apogastropoda; Caenogastropoda and Heterobranchia; Sorbeoconcha and Hypsogastropoda; Neogastropoda; Pulmonata. Access to genomes is currently providing unprecedented datasets. However, results from such studies are still not stable. Evidences for extremely diverse evolutionary dynamics in genomes of various groups may give reasons for odd and unstable results from their analysis. Although morphological characters seem to have lost importance in gastropod phylogenetics, it is also evident that recent advances may rescue morphology to an important role in studying gastropod evolution.

Symposium III

The modern systematics: between structural morphology and molecular approaches

Coordinators:
Romolo Fochetti, Marzio Zapparoli

Oral presentations

SERGEI FOKIN[1, 3], SASCHA KRENEK[2], THOMAS BERENDONK[2],
FRANCO VERNI[1]

[1] Department of Biology, University of Pisa, Italy
[2] Institute of Hydrobiology, Technical University of Dresden, Germany
[3] Department of Invertebrate Zoology, St. Petersburg State University, Russia

CURRENT SITUATION AND FUTURE PERSPECTIVE OF *PARAMECIUM* BIODIVERSITY AND SYSTEMATICS

The number of valid morphospecies within the genus *Paramecium* (Ciliophora, Oligohymenophorea) currently comprises 19 items, including the *P. aurelia* complex that encompasses 15 sibling species. While some of these species seem to have a cosmopolitan distribution, other *Paramecium* spp. are less widely distributed or might even be considered endemic. Still, many regions are either insufficiently sampled or have never been investigated by professional ciliate taxonomists. This suggests that the species diversity of genus *Paramecium* may be larger than our current knowledge suggests. Generally, it is more likely for new species to be discovered in more remote and therefore rarely investigated areas (all African species and *P. shewiakoffi* – China). However, *P. chlorelligerum*, a largely forgotten European species (KAHL, 1935) was recently rediscovered and redescribed from moorland in southern Germany (KREUTZ *et al.*, 2012), and now has been found also in Russia (St. Petersburg district) as well as *P. buetschlii* revealed from area near Oslo, Norway (KRENEK *et al.*, 2015). Therefore, this indicates the significance of field investigations not only in remote but in less frequently sampled areas to estimate species diversity and to seek out highly specialized or endemic congeners. Moreover, our data indicate that some unknown *Paramecium* species also can populate pure marine water. Careful investigations that use a combination of classical taxonomic and modern molecular approaches allow to detect not only phylogenetic connections between the genus representatives, but some cryptic diversity in *Paramecium.* Three cryptic *Paramecium* species were discriminated: *P. hungarianum*, *P. germanicum* and *P. brazilianum*. These herein investigated paramecia are rather difficult to discriminate from similar members of the genus by morphology. For them was proposed a new systematic term *"Eucandidatus"* as a component of the taxonomic name for eukaryotes to make a distinction between valid biological species or taxonomic units and the provisional cryptic species status. Before "*Candidatus*" term was established for uncultivatable prokaryotes. Within our comprehensive analyses based on current *Paramecium* COI gene sequences available, we may have revealed some misidentifications for *P. calkinsi, P. woodruffi*, *P. multimicronucleatum* and *P. chlorelligerum*. Thus, we urgently call for the use of comprehensive data sets when analyzing and publishing new sequence data of single or new species of protists.

GIUSEPPE CORRIERO[1], MARCO ABBIATI[2], GIORGIO BAVESTRELLO[3]

[1] Dipartimento di Biologia, Università di Bari, Via Orabona 4, 70126 Bari, Italia
[2] Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Via S Alberto 163, 48123 Ravenna, Italia
[3] Dipartimento di Biologia, Università di Genova, Corso Europa 26, 16132 Genova, Italia

LOWER INVERTEBRATES AND TAXONOMIC KNOWLEDGE: A STUDY ON THE GENUS *TETHYA* (PORIFERA, DEMOSPONGIAE) IN THE MEDITERRANEAN

The phylum Porifera (sponges) consists of about 8,500 species, with numerous critical cases in the taxonomic attribution. This is mainly due to the morphological plasticity frequently occurring in sponges at intraspecific level. Indeed, macroscopic traits like shape, size and color can greatly vary within the same species under the influence of environmental factors. At the same time, intraspecific variations of skeletal characters (spicule shape and size, spicular arrangement) have been repeatedly reported in the literature. Moreover, in many cases, it has been highlighted the lack of consistency between the phylogenetic arrangements provided by traditional taxonomy and by the molecular approach. In this work, traditional and ultrastructural morphological approaches have been used in association with molecular studies (COI), to assess the validity of the taxonomic reconstruction relative to the genus *Tethya* (Lamarck, 1815), one of the most studied among Mediterranean demosponges. The genus *Tethya* has a cosmopolitan distribution, and consists of 91 valid species. In the Mediterranean, two species are currently recognized: *T. aurantium* (Pallas, 1766) and *T. citrina* (SARÀ and MELONE, 1965), both subjected in the past to extensive taxonomic revisions. We compared a large number of *Tethya* specimens collected from several Western Mediterranean sites characterised by different environmental conditions. The study allowed to recognise a new species of *Tethya* on the basis of some skeletal characters (development of the cortical region; shape of some spicular elements), also supported by differences in the habitat and in the reproductive biology. By contrast, spicule size, usually considered a remarkable taxonomic feature, appeared highly variable at intraspecific level. In addition, genetic studies did not support the validity of this third Mediterranean species. On the whole, this work highlighted the lack of exhaustive taxonomic markers inside critical groups of the Mediterranean sponge fauna, confirmed some constraints of the traditional taxonomic approach, mainly based on the comparison of spicule size, and put in evidence, at least in the genus *Tethya,* the lack of consistency between taxonomic and molecular approaches.

FABIO SCARPA, PIERO COSSU, TIZIANA LAI, DARIA SANNA,
MARCO CURINI-GALLETTI, MARCO CASU

Dipartimento di Scienze della Natura e del Territorio – Sezione di Zoologia, Archeozoologia e Genetica,
Università di Sassari, Via F. Muroni 25, 07100 Sassari, Italy

MEIOFAUNAL CRYPTIC SPECIES CHALLENGE SPECIES DELIMITATION: THE CASE OF THE *MONOCELIS LINEATA* (PLATYHELMINTHES: PROSERIATA) SPECIES COMPLEX

Species delimitation is a critically important task in conservation biology, but its efficacy based on single lines of evidence has been questioned as it may not accurately reflect species limits and relationships. Hence, the use of multiple lines of evidence has been portrayed as a means to overcome identification issues, arising from gene/species tree discordance, morphological convergence, or recent adaptive radiations. The integrative taxonomic approach has been used to address the study of the taxonomically challenging *Monocelis lineata* species complex. Eighteen populations, which encompass most of the geographic range of the complex, were studied using morphology, karyology, crossbreeding experiments and molecular analysis (SSU and LSU sequencing). These different markers provided evidence of four (karyology) to eight (morphology) discrete entities, whereas crossings showed various degrees of intersterility among the tested populations. Molecular species delimitation revealed a different number of candidate species, spanning from five (ABGD and K/Θ methods) to 11 (GMYC method). The Atlantic populations were particularly controversial taxonomically, spanning from one (ABGD) to six (GMYC) candidate species. The genetic structure of fourteen North-East Atlantic populations was thus investigated using COI sequencing. Populations from Ferrol (Spain), Doolin (Ireland), and Helsingør (Denmark) showed a higher genetic variability, whereas a reduction in the number of haplotypes was found at the northernmost edge of the distribution and in northern Ireland and Scotland. Results provided evidence of three refugia (Iberian Peninsula, south-western Ireland and North Sea), with two different recolonisation pathways of north-western Europe during the post-Würmian glaciations, and the occurrence of secondary contacts which shaped the genetic variability of some of the populations examined. The incongruence among the different molecular species delimitation methods may thus reflect the multifaceted evolutionary history of *M. lineata s.l.* and hamper the full taxonomic resolution of the complex. However, two candidate species were consistently validated by all of the markers and have been formally described: *Monocelis algicola* and *M. exquisita*. The latter species appear to have a particularly restricted distribution, and the possibility that meiofaunal taxa may be of conservation concern should be considered.

ADRIANA GIANGRANDE

Department of Biological. and Environmental Sciences and Technology, University of Salento, Lecce

BACK TO THE PAST: APPRECIATION OF CLASSIC TAXONOMY

We are living in the biodiversity age but today, more than ever, the taxonomy, the science that traditionally studies biodiversity, is going through a very strong global crisis. In the identification of taxa morphological, geographical, ecological, behavioral and molecular information should be used. The morphological approach is undoubtedly the most operationally simple and therefore the most widely used. In many cases, however, the morphology does not reflect the degree of isolation between taxa and in this case the molecular analysis can be a great help. However, in recent years the genetic-molecular approach has become predominant, with a schism within the taxonomic research between the classical "morphological" branch and the "molecular" one, considered more innovative, The two approaches should instead proceed in parallel and coordinated. Beyond science, taxonomy has societal functions. Taxonomists play a larger role in connecting science to people in a way that they can appreciate the value of biodiversity conservation and I doubt the average man on the street appreciates beauty in "metabarcoded samples". Molecular biology applied to the systematic has (and will have) a significant impact on the development of taxonomic knowledge; but without the basic research on the morphology it will be difficult to test phylogenetic hypotheses, for proper systematic / molecular phylogeny. This predominant orientation towards molecular approach, that certainly has more appeal than the morphological analysis, also with regard to funding opportunities, is leading to the abandonment of the "old" system of identification and classification. In many centers when specialists retire there is no one who can really replace them. The consequence of this is reflected in the current lack of taxonomists. As an example that can describe this trend I will report my experience in the field of taxonomy of marine invertebrates, and more specifically on the group of annelid worms of which I am a specialist.

SALVATORE CAUSIO, ELISA GIANGRANDE, GENUARIO BELMONTE

Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, campus Ecotekne, 73100 Lecce

MOLECULAR TOOLS AND MORPHOLOGY IN MODERN TAXONOMY: IDENTIFICATION OF POLYCHAETA RESPONSIBLE OF BIOSTALACTITES GROWTH IN ITALIAN SUB-MARINE CAVES

Stalactites of organic origin were found in submarine caves of the Salento (S-E Italy). Their cores are formed by strings of Serpulidae tubes, around which a concretion coat was added with time. Generally, these structures were found embedded in the bio-stalactite matrix, but in some cases they presented living worms inside. A first morphological analysis led to their identification as belonging to the genus *Protula* Risso, 1826. A multidisciplinary approach was carried out to identify the worms that build the biostalactites because in literature there is not information about *Protula* gregarious behavior. The research was mainly based on molecular analysis: it compared DNA sequences of the cave worm with those of *Protula* genus present in GenBank, but provided also a morphological description of the specimens, and a comparison with specimens outside the cave. Barcoding genes as second Internal Transcribed Spacer (*ITS2*) and Cytochrome b (*Cyt b*), and nuclear genes as the Ribosomal Large Sub Unit 28S, were analyzed as molecular markers. Of the two *Protula* species reported from the Mediterranean Sea, there are not available sequences in genetic bank of *P. intestinum,* to compare with, nor Cytochrome b and ITS2 sequences of *P. tubularia*. Molecular analysis showed a significant divergence with the sequences of the Mediterranean *Protula tubularia* available in GenBank (up to 11% in *LSU28S*), two orders of magnitude higher than the intrapopulation one (0.3%). The morphological analysis examined several characters used to distinguish between species of the genus *Protula*: size, collar structure, abdominal chaetae type, number and arrangement of radioles, number of thoracic uncini, presence of radiolar and prostomial eyespots. They evidenced characters in common with both Mediterranean *Protula,* but not a complete coincidence with any of them. Both molecular and morphological analyses clearly defined the differences between the cave species and all the known species of the genus *Protula,* but phylogenetic analysis makes us confident about the membership to this genus. The present research demonstrates that the cave species is not attributable to any of the two known species of *Protula* reported for the Mediterranean Sea, thus these data can be considered as proof about the existence of a new species attributable to this *taxon*. In addition, it could represent a biological exclusive because up to now biostalactites are not reported from any other parts of the world.

LUCIO BONATO[1], GIUSEPPE FUSCO[1], MARCO ORLANDO[1],
MARZIO ZAPPAROLI[2], FRANCESCA BORTOLIN[1]

[1] Dipartimento di Biologia, Università di Padova, Via Bassi 58B, 35131 Padova
[2] Dipartimento per la Innovazione nei sistemi Biologici, Agroalimentari e Forestali, Università della Tuscia,
Via S. C. de Lellis, 01100 Viterbo

NEW INSIGHTS AND OLD MISCONCEPTIONS ON *PLUTONIUM*, THE LARGEST AND THE RAREST OF THE ITALIAN SCOLOPENDERS (CHILOPODA)

Plutonium zwierleini is a very little known scolopender of the Italian fauna, with remarkable traits: more than twelve centimetres in length, blind, it may pierce enemies with strongly uncinate rear legs in addition to the anterior venomous fangs. Its unique anatomical features, including supernumerary tracheal spiracles, have motivated speculations and debates in the context of both centipede phylogeny and arthropod evolutionary trends. However, despite the conspicuous body size and the distinctive morphology, specimens of *Plutonium* have been encountered very rarely, only in restricted areas of Sicily, Sardinia, Campania, allegedly also in Spain, and less than two dozen specimens are known to exist in zoological collections. A newly found specimen recently collected in Sardinia gave us the unprecedented opportunity to obtain the first molecular data for *Plutonium*. We also studied the morphological variation among previously collected specimens from all disjoint areas inhabited by the species. Integrating the two approaches allowed us to go past some misconceptions on *Plutonium* and to get a better understanding of its morphology, its phylogenetic position and the evolutionary origin of its unique features. A phylogenetic analysis of mitochondrial and nuclear DNA sequences confirms that *Plutonium* is a derived clade within a diverse group of blind scolopenders. In particular, molecular data agree with morphological evidence in resolving *Plutonium* within *Theatops*, which includes a few species of smaller scolopenders, with uncinate ultimate legs, occurring in fragmented areas throughout the temperate Holarctic. Besides morphological traits shared with *Theatops*, some previously overlooked characters are apparently derived in *Plutonium*, including elongated appendages and cutting-edged claws on the rear legs.

LORIS GALLI[1], DANIELA BARTEL[2], MATTEO CAPURRO[1], GÜNTHER PASS[2],
ANTONIO SARÀ[1], NIKOLAUS SZUCSICH[3]

[1] Dipartimento di scienze della terra, dell'ambiente e della vita, University of Genoa, Corso Europa 26, 16132 Genoa, Italy
[2] Department of Integrative Zoology, University of Vienna, Vienna, Austria
[3] Natural History Museum, Burgring 7, 1010 Vienna, Austria

INTEGRATIVE SYSTEMATICS IN PROTURA: FROM CHAETOTAXY TO DNA

The growth in knowledge about Protura, after their relative recent discovery (SILVESTRI, 1907), remained rather limited. Due to their small body size, and to their cryptic lifestyle in the soil, only few specialists are able to determine these insects to species level. Traditional identification is based on a combination of chaetotaxy, porotaxy, and morphometrics. The need to clear specimens before identification by using lactic acid, imposed an obstacle for the inclusion of a molecular-genetic approachin studies of Protura: no one is able to identify species before clearing specimens thereby destroying the DNA, and vice versa nobody was able to analyze DNA without destroying the specimens. The recent implementation of a non-destructive method (BÖHM *et al*., 2011) which enables the extraction of whole genomic DNA without destroying the cuticular exoskeleton, thus provides an unexpected opportunity to modernize proturan systematics. A first study using the DNA barcoding region of the mitochondrial CO1 gene, and a fragment of the nuclear 28S rDNA gene revealed a perfect match of molecular results with morphological identification, however, with unexpected high variation among different populations of the same species (RESCH *et al*., 2014). This presentation gives an overview of the problems and limitations of the traditional morphological methods and the first results of our integrative approach.

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ANDREA DI GIULIO[1], MAURIZIO MUZZI[1], ROBERTO ROMANI[2]

[1] Department of Science, University “Roma Tre”, Viale G. Marconi 446, 00146 Rome, Italy
[2] Department of Agricultural and Environmental Sciences, Perugia University, Borgo XX Giugno 74, 06121 Perugia, Italy

HOW MANY BOMBARDIER BEETLE LINEAGES? NEW MORPHOLOGICAL EVIDENCES SUPPORT INDEPENDENT EVOLUTION OF EXPLOSIVE DEFENSIVE SYSTEMS IN COLEOPTERA

The ground beetle subfamilies Paussinae and Brachininae, have always been of interest to scientists because of their ability to explosively eject a hot (up to 100°C) and irritating quinonoid secretion from pygidial glands, in response to attack by predators. Relationships of Brachininae with the rest of Carabidae, and especially with Paussinae, are still matter of debate since molecular analyses performed so far failed in clarifying their actual position. Despite the majority of biological and morphological traits would indicate a clear distance between brachinine and paussine clades, the peculiarity of their defensive system led many scientists to consider "bombardier beetle" as a monophyletic lineage, emphasizing the general similarities in morphology, function and chemical reactions. However, although the pygidial defensive system was used as the main evidence of relationship, studies concerning fine morphology and ultrastructure of its components (reaction chambers, valves, ducts, reservoirs, and glands that secrete H_2O_2, hydroquinones, catalases and peroxidases) are still lacking (paussines) or incomplete (brachinines). In order to render these issues available to a comparative advanced discussion, we performed a morphological and ultrastructural study of representative species belonging to the genera: *Metrius* (Metriini), *Pachyteles*, *Goniotropis* (Ozaenini), and *Paussus* (Paussini) for Paussinae; and *Brachinus*, *Mastax*, *Pheropsophus* (Brachinini) and *Crepidonellus* (Crepidogastrini) for Brachininae. Samples were examined through optical microscopy (dissections, classical histology), scanning (SEM) and focused ion beam (FIB) microscopy, this last replacing the traditional TEM analysis. The relative parts of the defensive systems for each paussine and brachinine species were described, illustrated and comparatively analyzed. We found significant differences between the two systems at both cellular and microstructural levels other than the different ways of ejecting the spray (directing the abdomen in brachinines; using the elytral flange of Coanda in paussines). Our preliminary results raise several challenges to the acknowledged hypothesis of evolution of this peculiar defensive system that parallel other evidences on the relationship between paussines and brachinines.

CLAUDIA PALESTRINI, ENRICO BARBERO, ANGELA ROGGERO

Department of Life Sciences and Systems Biology, Via Accademia Albertina 13, 10123 Torino, Italy

MODULARIZED TRAITS AND PHYLOGENETIC COMBINED ANALYSIS IN TWO DUNG BEETLES SPECIES-GROUPS OF ONTHOPHAGINE (COLEOPTERA, SCARABAEIDAE)

Arthropod insects are ideal and complicated model systems for studying phenotypical patterns in external and internal body traits. The present research was focused on the analysis of the phylogenetic relationships between dung beetles species-groups of Afrotropical onthophagine. The main aim was to test the applicability of a dataset of morphological continuous and discrete characters, desumed by employing different methodological approaches, and treated as distinct modules. The quantitative morphological variations were captured by the geometric morphometric method that proved to be a very useful tool to gain even small shape differences of several anatomical traits. In this framework, we examined the shape patterns of head, pronotum, right elytron, mouthpars (i.e., mentum and epipharynx), and genitalia of both sexes. The modularized traits were analyzed together by an innovative method, i.e. the combined phylogenetic approach (GOLOBOFF *et al.*, 2006). The use of quantitative data in phylogenetic analysis is a novelty since formerly those data could not be employed "just as they were" but were discretized during the analysis (GOLD *et al.*, 2014). The formalization of the combined approach (CATALANO *et al.*, 2010; GOLOBOFF and CATALANO, 2010) has opened up huge opportunities for the use of extremely diverse characters that were hitherto inapplicable to the phylogenetic analyses. Subsequently, the evolutionary and biogeographic patterns of these species was examined by combining the phylogenetic results with the distribution data (i.e., the georeferenced collection localities) to define what, and how, speciation processes have led to the current biogeographical ranges.

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GIULIANA ALLEGRUCCI[1], GIAN CRISTOFORO BOZANO[2],
DONATELLA CESARONI[1], LEONARDO LATELLA[3], BRUNO MASSA[4],
VALERIO SBORDONI[1]

[1] Dipartimento di Biologia, Università di Roma Tor Vergata
[2] Viale Romagna 76, 20133 Milano
[3] Museo Civico di Storia Naturale di Verona
[4] Dipartimento di Scienze Agrarie e Forestali, Università di Palermo

SPECIES DELIMITATION BY DNA BARCODE AND MORPHOLOGY: COMPARING BUTTERFLIES, GRASSHOPPERS AND CAVE BEETLES

Understanding the delimitation of species is crucial for several fields of biology and ecology and is important for both biodiversity studies and for conservation issues. Genetic data are often used to infer different species on the basis of reciprocal monophyly. However, several studies show incongruence between species inferred by genetic and morphological characters. Species delimitation can be especially difficult at early stages of population divergence, when both morphological and molecular characters show low levels of differentiation, due to genetic drift and/or gene flow. Among insects we studied three examples somewhat different from one another: the butterflies of the genus *Gonepteryx* (Lepidoptera, Pieridae), the grasshoppers of the genus *Eupholidoptera* (Orthoptera, Tettigoniidae), the cave beetles of the genus *Bathysciola* (Coleoptera, Cholevidae). By using a DNA barcode approach (a 648 base-pair region in the mitochondrial *Cox1* gene), we compared results from molecular and morphological characters in order to verify their potential congruence. The genus *Gonepteryx* includes 9-11 species distributed variously across Europe, temperate Asia, and north Africa; some species are endemic of islands of the Atlantic Ocean, others occupy mainly Western regions, others live around the Himalaya, others are purely Oriental and only one species is spread throughout the genus distribution range. From a morphological point of view, they are fairly similar to each other, sharing distinctive wing shape and genitalia morphology, whereas DNA barcode sequence differences can be considerable. The genus *Eupholidoptera* includes 46 Mediterranean species distributed from Turkey to Greece, Italy and southern France. Morphological analysis was carried out by considering genitalia structures usually used to distinguish different taxa. DNA barcode analysis showed that genetic differences between species were rather shallow, albeit contributing with other markers to infer phylogenetic relationships. On the whole we were able to distinguish nine lineages of Italian *Eupholidoptera*, of which *E. tyrrhenica* from Corsica was described as a new species. The Italian genus *Bathysciola* includes 43 known taxa which occur in all the Alps and Apennines. From a morphological point of view, they are exceedingly similar to each other in body shape, while show deep differences in DNA barcode sequences, thus confirming the taxonomic value of apparently small differences showed by genitalia.

VINCENZO CAPUTO BARUCCHI[1], ANTONELLA CAROSI[2], MASSIMO GIOVANNOTTI[1], GIANANDREA LA PORTA[2], MASSIMO LORENZONI[2], PAOLA NISI CERIONI[1], PAOLO RUGGERI[1], ANDREA SPLENDIANI[1]

[1] DiSVA, Università Politecnica delle Marche, Ancona
[2] Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia

A GENETIC AND MORPHOLOGIC STUDY IN A CONTACT ZONE BETWEEN TWO PUTATIVE ITALIAN BROWN TROUT SPECIES, *SALMO CETTI* AND *S. CENERINUS* (*SALMO TRUTTA* COMPLEX)

The brown trout, *Salmo trutta* Linnaeus, is a wide-ranging species complex native to Eurasia and North Africa. From a systematic point of view, various taxa were recently recognized as separate species within the *Salmo trutta* complex in Italy, with two species inhabiting the Apennine streams, namely *S. cenerinus* Chiereghini on the Adriatic side and *S. cetti* Rafinesque in the Tyrrhenian drainages (see KOTTELAT and FREYHOF, 2007). Due to stocking, also Atlantic brown trout, *Salmo trutta*, is now widely represented in Apennine river basins (e.g., CAPUTO *et al.*, 2004). Recently, within the "Life+ TROTA" (LIFE12 NAT/IT/0000940) aiming to put in place a strategy for conservation of the last central Italy native trout populations, we studied a contact zone between the two putative Apennine species along the watershed in the Sibillini Mountains National Park. In this context, we studied genetic and morphological differentiation of 16 local samples belonging to 3 river basins (Nera on the Tyrrhenian side of the Apennine, Tenna and Chienti on the Adriatic one) and over 400 specimens using molecular (nuclear and mitochondrial DNA) and morphologic approaches. The results of this survey allowed us to observe that, despite a long history of restocking with the alien Atlantic species, the native brown trout is still well represented in the central Apennine streams. In particular, the two groups representing alien and native trout were well separated from our analyses, suggesting the presence of biological barriers favoring the maintenance of separation despite hybridization due to restocking with Atlantic trout. However, the results obtained did not evidence a clear separation between the two putative species described for the two Apennine sides. Indeed, this is not so surprising, considering that this area of central Apennine could have acted as a semipermeable barrier between Adriatic and Tyrrhenian drainages, thus favoring gene flow in the past (BIANCO, 1995). Nevertheless, our data highlighted a marked differentiation between the main basins of this Apennine area, as a probable consequence of isolation. These significant differences at basin level should be considered in future plans for the management of brown trout species in order to preserve a rich biodiversity that endured both climate changes and the incorrect management policies.

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SANTO CARACAPPA[1], ANNALISA PISCIOTTA[2],
MARIA FLAMINIA PERSICHETTI[1], GIULIA CARACAPPA[1],
ROSA ALDUINA[2], MARCO ARCULEO[2]

[1] Istituto Zooprofilattico Sperimentale della Sicilia, Via G. Marinuzzi 3, 90129 Palermo, Italia
[2] Dipartimento STEBICEF, Università di Palermo, Via Archirafi 18, 90123 Palermo, Italia

CAN DNA METHYLATION CONTRIBUTE TO EXPLAIN MORPHOLOGICAL VARIABILITY? THE CASE OF LOGGERHEAD TURTLE *CARETTA CARETTA*

The loggerhead turtle *Caretta caretta* is a common and threatened sea turtle in the Mediterranean Sea. It is listed in the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN 2012) and, although it is widely studied, little is currently known about the relationships between its phenotype and genetic variability. In the last few years some authors observed the presence of individuals, collected in different parts of the Mediterranean Sea, with a variable number of carapacial and plastron scutes. In some cases, this variability makes the sound identification of the species difficult (e.g. MARGARITOULIS and CHIRAS, 2011; TURKOZAN *et al*., 2001; OLIVER, 2014). To date, no one tried to correlate these morphological anomalies with the genetic diversity of the species, even if several studies indicate that, beyond genetic variation, natural phenotypic variations can be generated through a suite of epigenetic mechanisms. Here we present some results about the possible correlation among the variability of the number of carapacial and plastron scutes and cytosine DNA methylation. Twelve specimens of *Caretta caretta*, collected from different parts of Sicily, were analysed. Morphological characters were used for the identification of the collected specimens according to the guidelines of MARQUEZ (1990). Four out of the twelve individuals showed an unexpected number of carapacial and plastron scutes making difficult the immediate and sound identification of the specimens as *C. caretta*. Afterwards, all the individuals were subjected to genetic analysis by the sequencing of a 243 bp fragment derived from the mitochondrial cytochrome b gene. Sequences analysis confirmed that all the individuals belong to the species *Caretta caretta*. Genomic DNA methylation level of cytosines was evaluated by dot blot analysis using antibodies against methylated cytosines. This analysis showed a reduced global level of methylated cytosines in the four "abnormal" individuals in respect to the other ones. We hypothesize that the variability of the number of scutes could be ascribed as an epigenetic effect probably due to the environmental parameters experienced by the embryos during incubation. This finding could have implications in our understanding of the pathways of morphological evolution and diversification in the chelonians.

ANNA LOY[1], TASSOS KOTSAKIS[2], PAOLO PIRAS[2, 3],
GABRIELE SANSALONE[2], PAOLO COLANGELO[4]

[1] Dipartimento Bioscienze e Territorio, Università del Molise, Pesche, Italy
[2] Dipartimento di Scienze, Università di Roma Tre, Roma, Italy
[3] Dipartimento di Scienze Cardiovascolari, Respiratorie, Nefrologiche, Anestesiologiche e Geriatriche, Università Sapienza di Roma, Roma, Italy
[4] National Research Council, Institute of Ecosystem Study, Verbania-Pallanza, Italy

THE PRODIGIOUS ALLIANCE: MOLECULAR SYSTEMATICS AND MORPHOMETRICS DIG OUT THE PUZZLING ORIGIN AND DIVERSIFICATION OF EUROPEAN MOLES (SORICOMORPHA: TALPIDAE)

The Eurasian genus *Talpa* is characterized by high levels of endemism and morphological convergence related to the constraints of the subterranean environment. Molecular systematics suggests a monophyletic Asian origin of the genus in the early-middle Miocene, with *T. altaica* and *T. caucasica* basal to an eastern (*T. stankovici* and *T. davidiana*), and a western European clade (*T. europaea*, *T. occidentalis*, *T. caeca* and *T. romana*). *T. europaea* is widely distributed throughout Europe and is parapatric to three endemic moles: *T. romana* in South-Central Italy, *T. occidentalis* in the Iberian peninsula, and *T. stankovici* in the Balkans. The fifth and smallest European species *T. caeca* is scattered distributed in South eastern Europe and its range overlaps with the three large sized endemisms. Although this distribution pattern suggests a diversification during the Pleistocene climate oscillations, molecular clock dated it back to the early Pliocene, advocating for a more complex framework on events that driven mole's evolution. Also, in contrast with previous morphometric evidences, molecular data documented *T. occidentalis* the sister species of *T. europaea*, and *T. romana* the sister species of *T. caeca*, suggesting that convergence played a major role in shaping the morphology of this taxon. The relative contribution of phylogeny and convergent adaptation in extant and extinct moles was further explored through the morphometric and mechanical performance analyses of the humerus, a highly derived bone well preserved in the fossil record. Results provided strong evidence that the ability to dig complex tunnels reached a functional optimum in distantly related taxa, and that evolutionary allometry played a major role in shaping the moles' humerus. In contrast, size differences among species appear to be more related to selection pressures related to competitive interactions rather than to phylogenetic relationships.

FRANCESCO FRATI

Dipartimento di Scienze della Vita, Università di Siena

30 YEARS OF MOLECULAR SYSTEMATICS AND PHYLOGENY

It was exactly thirty years ago, with the pioneer work by Kary Mullis and his collaborators (SAIKI *et al.*, 1985; MULLIS *et al.*, 1986), that molecules burst into animal systematics and phylogeny. The advent of a new technique, the Polymerase Chain Reaction (PCR), suddenly allowed a new generation of zoologists, even without a specific molecular background, to have access to a relatively simple method for obtaining DNA sequences of homologous genes in different species. This provided the basic material (characters) for inferring phylogenetic relationships at all taxonomic levels, from population genetics to the molecular Tree of Life. While small molecular biology laboratories were being established in zoology departments, phylogenetic theory produced more efficient methods of phylogenetic inference, suited for the analysis of DNA and amino acid sequence alignments, and bioinformaticians developed softwares capable of implementing these analyses on exponentially growing data sets. For quite some time, the limiting factor to molecular phylogeny was the availability of molecular data, i.e. the length of the sequences and the number of taxa. In the last decade, more efficient methods of DNA sequencing have been developed (Next-Generation Sequencing), paving the way for more comprehensive phylogenetic analyses. Now, the limiting factor is time and quality of tree reconstruction. All through these 30 years of molecular phylogeny, the debate over the superiority of one character set over the other(s) (typically molecules vs. morphology) hasn't cooled down, and many examples are known of cases in which molecular phylogeny has promoted new phylogenetic views, as well as cases in which the interpretation of molecular data sets is misled by peculiar processes of molecular evolution. At the same time, molecules have become useful for systematics, as sources of unambiguous characters for species diagnosis. The so-called "barcode" approach has provided a useful tool in the hands of zoologists and ecologists for a relatively quick diagnosis of species. Thirty years of molecular phylogeny and systematics have been lived with great excitement by a couple of generations of zoologists; these contribution now represents the basis for an improved understanding of phylogeny and evolution.

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Symposium III

The modern systematics: between structural morphology and molecular approaches

Coordinators:
Romolo Fochetti, Marzio Zapparoli

Posters

VALENTINA SERRA[1], VENKATA MAHESH NITLA[2], SERGEI I. FOKIN[1, 3],
CHARAN KUMAR BASURI[2], CHAGANTI KALAVATI[2], B. V. SANDEEP[2, 4],
MICHELE CASTELLI[1], FRANCO VERNI[1], GIULIO PETRONI[1]

[1] Department of Biology, University of Pisa, Pisa, Italy
[2] Department of Zoology, Andhra University, Visakhapatnam, India
[3] Department of Invertebrate Zoology, St. Petersburg State University, St. Petersburg, Russia
[4] Department of Biotechnology, Andhra University, Visakhapatnam, India

MORPHOLOGICAL AND MOLECULAR DESCRIPTION OF A NEW SPECIES OF ANOXIC CILIATE FROM INDIA, BELONGING TO *METOPUS* GENUS (CILIOPHORA, ARMOPHOREA), WITH HINTS ON ITS ARCHAEAL ENDOSYMBIONTS

Species of protists belonging to *Metopus* genus are known to be anaerobic organisms, with a low degree of tolerance of dissolved oxygen. They occupy the anoxic part of the water column, often nearby sapropel-like sediment (dark-coloured sediments, rich in organic matter). Anaerobic ciliates are interesting organisms to study, although they are not so easy to detect and manipulate: they are never very abundant and most species are relatively rare, if compared to aerobic ciliates. Furthermore, anaerobic ciliates requires special cares to be cultured in laboratory condition, especially for their sensitivity to oxygen exposure. In this work we present a new method for keeping and growing *Metopus* spp. populations in laboratory as a part of the original sample. This innovation helped us to characterize a new species collected from Kolleru lake (India) by means of morphological and molecular tools. This is clearly a new morphospecies of the genus as its combination of morphological characteristics does not fit to any of described *Metopus* spp. It belongs to morphogroup III or IV according to the genus redescription (JANKOWSKY, 1964; ESTEBAN *et al.*, 1995). General morphometrical data collected on this *Metopus* strain do not deviate very much from *M. contortus* or *M. fuscus*. However, it shows particular ventral right side kinetom composition (several kineties terminate before posterior end of the cell), as well as majority of ventral kineties, formed by sets of 3-4 closely located cilia. As all representatives of the class Armophorea, this new ciliate bears in its cytoplasm methanogenic microrganisms, for which we provided molecular data, identifying them as Archaea members of *Methanoregula* genus.

VENKATA MAHESH NITLA[1], VALENTINA SERRA[2], SERGEI I. FOKIN[2,3], CHARAN KUMAR BASURI[1], CHAGANTI KALAVATI[1], B. V. SANDEEP[1,4], FRANCO VERNI[2], GIULIO PETRONI[2]

[1] Department of Zoology, Andhra University, Visakhapatnam, India
[2] Department of Biology, University of Pisa, Pisa, Italy
[3] Department of Invertebrate Zoology, St. Petersburg State University, St. Petersburg, Russia
[4] Department of Biotechnology, Andhra University, Visakhapatnam, India

REDESCRIPTION OF *PLAGIOPYLA NASUTA* (STEIN, 1860) (CILIOPHORA, PLAGIOPYLEA) USING A COMBINED APPROACH OF MORPHOLOGICAL AND MOLECULAR TECHINIQUES AND DETECTION OF A POTENTIAL NEW SPECIES OF PLAGIOPYLID FROM INDIA, WITH ARCHAEA ENDOSYMBIONTS

Members of order Plagiopylida (Class Plagiopylea) are known to be characterized by an ovoidal, often elongated, body shape, with varius degree of dorsoventral flattening and by their peculiar oral opening, an oblique slit in the apical part of the cell. Most part of them can be found in anoxic environments, thanks to the presence in their cytoplasm of hydrogenosomes (specialized organelles) and methanogens. We carried out samplings in India's largest freshwater lake (Kolleru Lake, Andhra Pradesh) and we developed an effective method by which we were able to grow plagiopylids ciliates and to carry out complete morphological and molecular studies. We found and redescribed *Plagiopyla nasuta,* studied for the first time by Stein (STEIN, 1860) and later by other authors (KAHL, 1931; JANKOWSKI, 1964; BORROR, 1972; DRAGESCO, 1972; AGAMALIEV, 1978; DRAGESCO and DRAGESCO-KERNEIS, 1986; SOLA *et al.*, 1988), but never fully redescribed. We realized a complete morphometry of this strain of *Plagiopyla nasuta* using traditional methods such as live observations under differential contrast microscope, silver impregnation and Feulgen staining to reveal, respectively, infraciliature and composition of nuclear apparatus. In our study, we were able to focus on characteristics never analyzed before such as the cortical region close to the cytoproct, which was precisely investigated with the aim to find apomorphic features. Moreover, we employed, for the first time on this species, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM was employed to reveal the structure of somatic and buccal ciliature, TEM was informative about the presence of possible Archeal endosymbionts in contact with hydrogenosome-like structure. This morphological data set was also supported by molecular data, obtained by 18S rRNA gene sequencing. Furthermore, we detected a potential new species of plagiopylid, with peculiar morphological traits, such as 2-3 small micronuclei and curved extrusomes. We also performed molecular analysis on 18S rRNA gene to improve species description. Moreover, we detected in the cytoplasm of this new plagiopylid hundreds of Archaea endosymbionts, ascribable to *Methanocorpusculum* genus, by mean of 16S rRNA gene sequencing and fluorescence in situ hybridization (FISH) analysis.

ANDRÉ R. S. GARRAFFONI[1], THIAGO Q. ARAUJO[2], ANETE P. LOURENÇO[3],
LORETTA GUIDI[4], MARIA BALSAMO[4]

[1] Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Caixa Postal 6109, 13083-970, Campinas, São Paulo, Brazil

[2] Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil

[3] Departamento de Ciências Biológicas, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Campus JK, Rodovia BR-367, CEP: 39.100-000, Diamantina, MG, Brazil

[4] Dipartimento di Scienze della Terra, della Vita e dell'Ambiente, Università di Urbino, Campus Scientifico, loc. Crocicchia, Urbino, Italy

AN ENIGMATIC FRESHWATER CHAETONOTIDA (GASTROTRICHA) FROM BRAZIL: A NEW SPECIES IN A NEW GENUS

The *phylum* Gastrotricha includes over 800 free-living species of worm-like, actively moving microinvertebrates that represent an important component of the meiobenthic fauna of marine, brackish-water and fresh-water habitats. Global diversity of Gastrotricha from inland waters is less known than that of marine forms, and available information is extremely heterogeneous: in particular South America has been poorly studied. A new freshwater psammic species of Gastrotricha Chaetonotida was collected from sediments of high altitude streams in the Minas Gerais State, Brazil. A set of peculiar morphological features clearly distinguishes this taxon from all the other Chaetonotida: very large and closely juxtaposed cephalic plates wholly cover the head region both dorsally and ventrally; cuticular large, spineless, hexagonal scales partially overlapping and similar to each other coat the anterior and central trunk region both dorsally and ventrally; posterior trunk region coated by extremely large dorsal and ventral scales; two pairs of terminal ventral keeled scales, the external ones exceptionally large and double in size than the medial ones; long, thin caudal adhesive tubes composed of three sections. The taxonomic position of the new species within Chaetonotida and in particular into the family Chaetonotidae is discussed on morphological bases, and the reasons for its possible assignment to a new genus are advanced. Preliminary molecular data obtained from 18S rDNA analysis of the new taxon are presented within a phylogenetic tree of Chaetonotida, and its phylogenetic relationships are discussed.

MATTEO VECCHI[1], MICHELE CESARI[1], APARNA PALMER[2],
ROBERTO BERTOLANI[3], GIOVANNI PILATO[4], LORENA REBECCHI[1],
ROBERTO GUIDETTI[1]

[1] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2] Department of Biological Sciences, Colorado Mesa University, Grand Junction, U.S.A.
[3] Department of Education and Humanities, University of Modena and Reggio Emilia, Italy
[4] Department of Biological, Geological and Environmental Sciences – Section of Animal Biology "Marcello La Greca",
University of Catania, Italy

THE PHYLOGENETIC POSITION AND IN DEPTH MORPHOLOGICAL ANALYSIS OF THE RARE GROUND DWELLING TARDIGRADE *HAPLOMACROBIOTUS UTAHENSIS* (EUTARDIGRADA, PARACHELA)

Eutardigrades systematics is based mainly on the morphology of the cuticular sclerified structures, especially of the buccal-pharyngeal apparatus and claws (formed by the fusion of a primary and a secondary branch). In particular, the main division of the class Parachela into four superfamilies relies heavily up on claw morphology (i.e. claws symmetry respect to the median plane of the leg and insertion angle of the secondary branch of the claws on the primary branch). However, these characters, alone, are either inadequate or useless for tardigrades with reduced claws, as in the case of the genus *Haplomacrobiotus*, or lacking claws. *Haplomacrobiotus* is a very uncommon soil-dwelling genus, reported so far only in arid areas of Mexico (Hermosillos) and U.S.A. (Moab, Utah). *Haplomacrobiotus* specimens have the secondary branch of the claws very reduced or absent, making the determination of the claw symmetry impossible. The systematic position of this genus has been debated since its description, having been firstly placed in the family Macrobiotidae (Macrobiotoidea with symmetrical claws) and then in the family Calohypsibiidae (Hypsibioidea with asymmetrical claws). Currently the position of the supposedly related genus *Hexapodibius* is still debated, being it attributed to Isohypsibiidae (Isohypsibioidea), or to Calohypsibiidae, i.e. to two different superfamilies. The recent discovery of an abundant population of *Haplomacrobiotus utahensis* in Snow Canyon State Park (Utah, U.S.A.), with also a great number of eggs-bearing exuvie, allowed us to perform an in-depth morphological analysis with light microscopy and scanning electron microscopy on the claws and buccal-pharyngeal apparatus of the animals, and to perform maximum likelihood and bayesian inference phylogenetic analyses based on fragments of the 18S and 28S nuclear genes, in order to validate the position of this genus within the proper superfamily. Our integrated findings place the genus *Haplomacrobiotus* in a close relationship with the genus *Hexapodibius* within the superfamily Isohypsibioidea. This new record for *H. utahensis* in the opposite side of Utah, U.S.A. respect to the previous finding, also suggests that this species may be distributed in all the arid area of the state.

DAVIDE IACIOFANO, EUGENIA SCHIMMENTI, SABRINA LO BRUTTO

Dipartimento STeBiCeF, Sezione di Biologia Animale, Via Archirafi 18, University of Palermo

INTEGRATIVE TAXONOMY: A MULTIDISCIPLINARY APPROACH USED TO DETECT INTRA-SPECIES MORPHOLOGICAL VARIATION ON *ORCHESTIA MONTAGUI* AUDOUIN, 1826 (CRUSTACEA: AMPHIPODA)

Marine talitrid amphipods have been demonstrated to be a key-information for ecological studies and for monitoring biodiversity at different levels, thus a correct identification at species level is a pre-requisite in conservation planes. Mediterranean species of the genus *Orchestia* Leach, 1815 are often syntopic; and their dichotomous keys are built only on adult males using the shape of merus/carpus of the seventh peraeopod and of propodus/dactylus of the second gnathopod. Along the coast of Trapani and the Stagnone of Marsala (Sicily, southern Italy), from 2011 to 2014, some specimens of *Orchestia* have been collected. Following traditional dichotomous keys it has been possible to identify *O. stephenseni* Cecchini, 1928, *O. mediterranea* A. Costa 1853 and *O. montagui* Audouin, 1826. However, many specimens presented characters not described yet. Specifically, they presented different shapes of merus/carpus of the seventh peraeopod and of propodus/dactylus of the second gnathopod. Consequently, an approach of Integrative Taxonomy, combining molecular, morphological and biometric analyses, was followed to identify these specimens. The specimens were stored in absolute ethanol for genetic analyses, and successfully amplified for a 650bp fragment of the mitochondrial gene cytochrome c oxidase subunit 1 (COI) using the pair of primers LCO-1490 and HCO-2198. The specimens were measured from tip of rostrum to apex of telson, and flagellum articles of second antennae were counted. Finally, through the camera lucida-microscope, iconography was produced. DNA-based approach authenticated that the specimens with characters not described in dichotomous keys were *O. montagui*. Biometric analyses confirmed that the variation of the shapes in Gnathopod 2 and Peraeopod 7 developed during growth. *Orchestia montagui* has been re-described with remarks on morphological variation. The smallest specimens showed macroscopic characters ascribable to *O. stephenseni* and other specimens showed macroscopic characters ascribable to *O. xylino* Lowry and Fanini, 2013, that should be considered *species inquirenda*.

ALESSANDRA RICCIERI[1], MICHELA MAURA[1], DANIELE SALVI[2],
MARCO A. BOLOGNA[1], EMILIANO MANCINI[1]

[1] Dipartimento di Scienze, Università Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy
[2] CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal

INVESTIGATING THE SICILIAN-MAGHREBIAN BIOGEOGRAPHIC PATTERN IN BLISTER BEETLES (COLEOPTERA, MELOIDAE)

Several animal and plant species show a West Mediterranean distribution restricted to Maghreb and Sicily. According to geological evidences, these lands became separated after the opening of the straits of Gibraltar at the beginning of Pliocene (5.33 million years ago). During Pleistocene glaciations, apparently, no land connections occurred between Sicily and Tunisian coasts, even if these lands were greatly approached. Therefore, the presence of species with a fragmented distribution at both shores of the Sicilian channel stimulated two zoogeographic hypotheses: (a) a pre-Pliocene origin of these species, whose range was subsequently fragmented by a vicariance event at the beginning of Pliocene (which implies a quite old species age); (b) Pleistocene dispersal events, during glacial stages, across the Sicilian channel. In order to test these hypotheses, we performed molecular analysis in two blister beetle species (subfamily Meloinae) having a Sicilian-Maghrebian distribution, but belonging to distinct phylogenetic lineages (BOLOGNA, 1991; BOLOGNA and PINTO, 2002): *Mylabris schreibersi* Reiche, 1866 (tribe Mylabrini), referred to a Palaearctic, widely distributed and speciose genus, and *Cabalia segetum* (Fabricius, 1792) (tribe Lyttini) belonging to a small Afrotropical genus distributed in eastern Africa, SW Arabian peninsula and Sahara, with the single *C. segetum* exclusive of Maghreb and Sicily. We sequenced one mitochondrial (*COI*) and three nuclear (*CAD*, *Wingless*, *RpS9b*) gene fragments in 9 Sicilian and 6-8 Maghrebian (from Morocco and Tunisia) populations of both species. Preliminary results (mainly based on *CAD*, the most informative gene) show that both species represent cohesive genetic units with a shallow divergence between Maghrebian and Sicilian populations supporting a Pleistocene dispersal scenario. North-African populations show higher genetic variability than Sicilian populations, suggesting an older history of these species in the Maghreb. Additional data and statistical analyses will allow to better understand the phylogeography of *M. schreibersi* and *C. segetum*, to estimate timing of population divergence, as well as to infer colonization routes between Maghreb and Sicily.

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MARIA ASSUNTA BISCOTTI[1], MARCO GERDOL[2], ADRIANA CANAPA[1],
MARIKO FORCONI[1], ETTORE OLMO[1], ALBERTO PALLAVICINI[2],
MARCO BARUCCA[1], MANFRED SCHARTL[3]

[1] Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy
[2] Dipartimento di Scienze della Vita, Università di Trieste, Trieste, Italy
[3] Department Physiological Chemistry, Biocenter, University of Würzburg, and Comprehensive Cancer Center Mainfranken, University Clinic Würzburg, Germany

THE REFERENCE TRANSCRIPTOME OF THE WEST AFRICAN LUNGFISH *PROTOPTERUS ANNECTENS*: A SOURCE TO INVESTIGATE MOLECULAR EVOLUTION EVENTS IN THE TRANSITION FROM WATER TO LAND

Besides the coelacanth, lungfish are the only other living sarcopterygian fish species. Due to possession of lobed fins and functional lungs they are able to move and live for extended periods outside of the water. The phylogenetic relationship to the last common ancestor of all tetrapods and the morphological similarity to their fossilized ancestors make lungfish of outstanding scientific interest. Unfortunately, because of their genome size, the largest among vertebrates, the generation of a whole genome sequence is precluded at this time. To compensate for the lack of a full genomic resource we have generated a highly complete reference transcriptome from the West African lungfish, *Protopterus annectens*. Using this dataset we were able to independently confirm that lungfish and not coelacanths are the closest relatives of the land-adapted vertebrates. Analysis on transposons showed that these elements are active and high diverse pointing to a role in the enormous expansion of lungfish genome. Moreover the investigation of specific gene families highlights changes connected to the water to land transition and demonstrates the importance of the lungfish reference transcriptome for comparative studies on vertebrate evolution.

Open session

Oral presentations

BELLA GALIL[1], ANNA OCCHIPINTI-AMBROGI[2], STEFANO PIRAINO[3]

[1] National Institute of Oceanography, Israel Oceanographic and Limnological Research, Haifa, Israel
[2] Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Italy
[3] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DISTEBA), Università del Salento, Lecce, Italy

THE EXPANSION OF THE SUEZ CANAL AND MARINE BIOINVASIONS IN THE MEDITERRANEAN SEA

A few weeks ago, media spread information that on August 6th 2015 Egypt will inaugurate a "new Suez canal" shipping route, which required a 37 kilometers of dry digging and 35 kilometers of expansion and deepening. Since last year, scientists have joined biodiversity, engineering, and hydrology experts from all over the world to caution that the enlargement of the Suez Canal will determine unintended adverse consequences to the Mediterranean marine biodiversity, by providing a wider 'corridor' to new cohorts of Erythraean biota.

Unfortunately, the Egyptian Canal authorities, scientists, media, or international enterprises involved in the project have made no reference to an Environmental Impact Assessment which would have been a crucial process towards risk identification, assessment, management, and development of mitigation countermeasures. In spite of the obligations of the Convention on Biological Diversity and the Barcelona Convention for the protection of the Mediterranean marine environment and coastal regions, the enlargement of the Suez Canal has been carried out and will be soon completed without any consideration for its trans-boundary impacts on the Mediterranean biodiversity. There is little doubt that the Suez expansion will determine strong consequences over the applicability of the European Marine Strategy Framework Directive, by negatively affecting several key indicators of the Good Environmental Status tool.

For these reasons, an urgent call to action is required by supporting shared initiatives of experts in the field of marine conservation biology and ecology to identifying critical issues (e.g. harmonized data collection and dissemination, parameters and protocols monitoring, management of vectors, NIS impact in marine conservation areas) and concerted strategies for a coordinated pan-Mediterranean management of marine bioinvasions.

FABIO STOCH

Unione Zoologica Italiana, Viale dell'Università 32, 00185 Roma, Italy

MONITORING UNDER THE HABITATS DIRECTIVE: THE ROLE OF THE ITALIAN ZOOLOGISTS IN COMPILING A NATIONAL MONITORING PLAN FOR THE ANIMAL SPECIES OF COMMUNITY INTEREST

European Union member states perform monitoring under the Habitats Directive both within and outside of the Natura 2000 protected areas network. In Italy, the responsibility for implementing the monitoring activity falls to the Regions and Autonomous Provinces. National government (Ministry of Environment, acting through the Institute for Environmental Protection and Research - ISPRA) is responsible for data aggregation and the final assessment of conservation status at national level. Unfortunately, a comprehensive, nationally uniform monitoring system meeting EU requirements has not yet been developed, as it is clear from the data assembled in the course of the last reporting period (2007-2012: GENOVESI *et al.*, 2014). Whereas Italy, with over 56,000 animal species, has the highest species richness in Europe (Fauna Europaea dataset), and about 200 terrestrial and inland water animal species are listed in the annexes of the Habitats Directive, the compilation of a monitoring plan is complex and requires the support of zoologists. The methodology adopted by Italian zoologists, in accordance with agreements signed by ISPRA and five representative organizations (Associazione Italiana Ittiologi Acque Dolci, Comitato Scientifico per la Fauna d'Italia, Societas Herpetologica Italica, Associazione Teriologica Italiana, and, as coordinator, Unione Zoologica Italiana), aimed to: 1) implement a final checklist of animal species of Community interest, including their synonyms and cryptic species emerged with the latest molecular research; 2) analyse inconsistencies between the latest report and the Nature 2000 network dataset; 3) develop a national monitoring system for each species, including field methods, survey intervals, and sampling design, tested in the field on a subset of 30 species; 4) support the participation of ISPRA to the activities of the European thematic workgroup for setting Favourable Reference Values (FRVs) for long-term conservation targets, testing the models on a subset of 20 species. As a consequence of the agreements, an important role in the implementation process of the Habitats Directive is assigned to science. This activity will help policy makers to take appropriate conservation measures in according to the results of the monitoring activity.

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Open session

Posters

CLAUDIO ALIMENTI[1], ADRIANA VALLESI[1], ROBERTA RUSSO[2],
CINZIA VERDE[2, 3], BILL PEDRINI[4], PIERANGELO LUPORINI[1]

[1] Laboratory of Eukaryotic Microbiology and Animal Biology, University of Camerino, 62032 Camerino (MC), Italy
[2] Institute of Biosciences and BioResources, CNR, 80131 Naples, Italy
[3] Department of Biology, Roma 3 University, 00146 Roma, Italy
[4] Paul Scherrer Institute, 5232 Villigen PSI, Switzerland

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF WATER-BORNE SIGNALING PROTEIN PHEROMONES FROM THE BIPOLAR CILIATE, *EUPLOTES PETZI*

Among numerous Antarctic and Arctic species of ciliates that we have stably adapted to grow in laboratory, *Euplotes petzi* has attracted particular interest because of its strict psychrophilic behavior (it does not tolerate temperatures above 8-9 °C) and capacity to constitutively secrete signaling protein pheromones. Using cultures of genetically different strains, we have isolated and structurally characterized four *E. petzi* pheromones as small proteins of 32 aminoacids including eight cysteines located in strictly conserved positions and predicted to form four intra-chain disulfide bridges. This high density of disulfide bridges would intuitively imply a quite compact globular molecular structure of these molecules. However, this hypothesis appears to be contradicted by nuclear magnetic resonance (NMR) analysis of purified *E. petzi* pheromone preparations. The NMR protein solution structures show that the regions devoid of regular secondary organization are spatially predominant over regions exhibiting regular α-helical organization. Considering that the pheromone structures of *Euplotes* species living in temperate waters are largely dominated by helical regions, our findings suggest that the *E. petzi* pheromone cold-adaptation is functionally correlated with an increased flexibility of the molecular backbone and, hence, with a decreased thermo-stability. This hypothesis is currently verified by analyzing the unfolding and refolding properties of *E. petzi* pheromones when exposed to increased temperatures and variations of other environmental parameters.

ELENA BARONI[1], SIMONA SANVITO[2], FILIPPO GALIMBERTI[2]

[1] Dipartimento di Biologia, Università degli Studi di Firenze, Firenze, Italy
[2] Elephant Seal Research Group, Sea Lion Island, Falkland Islands

PUP VOCALIZATIONS IN SOUTHERN ELEPHANT SEALS: COMMUNICATION IN A NOISY ENVIRONMENT

Vocal communication is a fundamental component of social behaviour, and pup vocalizations are particularly important because the mother-pup bond is essential for pup survival. In gregarious species communication happens in a noisy environment, with contemporary vocalizations by many individuals and, therefore, we expect signal structure to increase reliability of transmission. Southern elephant seals (*Mirounga leonina*, SES) breed in harems that include up to hundreds of individuals. Notwithstanding this, rates of pup separation and abandonment are low. Therefore, we expect SES to have an efficient mother-pup communication system, which permits safe recognition in a noisy environment. We studied SES at Sea Lion Island (Falkland Islands). We obtained 2,203 recordings of pup vocalizations. We identified three levels in the structure of vocalizations: *call*, i.e., a sound emission produced by a single air exhalation; *part*, i.e., a call component separated from other components by silence; *subpart*, i.e., a part component with a homogeneous acoustic structure. Based on recording quality, we selected 380 parts belongings to 40 pups. We measured time and frequency parameters using RAVEN software, and we classified parts based on their acoustic structure by inspection of waveforms and spectrograms. We observed three main part types: harsh (65.3%), tonal (31.6%), and pulsating (3.1%). Non-tonal parts (harsh + pulsating) often showed harmonics (13.5%) or inter-harmonics (58.9%), and tonal parts often had a harsh component (74.2%). A pulsating component was observed in most parts (69.0%). Formants were observed in most non-tonal parts (92.3%), and in many tonal parts (64.2%). Frequency modulation was observed in most tonal parts (76.7%), and in most cases (82.5%) the shape of the main spectrographic bands of tonal parts was non-linear. Parts were composed by 1-5 subparts, and we identified 39 subpart sequences. Tonal parts were longer, had a lower frequency in both the first and third quartile, and showed a narrower frequency range. All together, SES pup vocalizations showed a rich, hierarchical, highly variable, acoustic structure. Variability in the structure of signals is a pre-requisite of an effective recognition system. In the northern elephant seal (*M. angustirostris*) formants were identified as the most likely candidates for individual recognition, and the prevalence of parts with clear formant structure suggests that the same may hold for SES.

CARLA BERTAPELLE, GIANLUCA POLESE, ANNA DI COSMO

Dipartimento di Biologia, Università degli Studi di Napoli "Federico II", Napoli, Italy

"PROBLEM SOLVING" AND "PLAY-LIKE" IN *OCTOPUS VULGARIS*: FROM BEHAVIOR TO GENES

Adult neurogenesis occurs in organisms that have complex and centralized nervous system, as teleosts, amphibians, reptiles, birds and mammals, and among invertebrates, decapods and insects. Adult neurogenesis takes place in neural districts characterized by structural plasticity, involved in learning, memory and sensory stimuli integration. It suggests that the process plays a crucial role to support high cognitive capabilities. *Octopus vulgaris* has a complex and centralized nervous system, located around oesophagus, with a hierarchical organization. It is considered the most "intelligent" invertebrate due to its advanced cognitive capability, as learning and memory, and complex behaviour, as "problem solving" and "play-like". Neural plasticity and synaptic remodelling, the base of adult neurogenesis, underlie these sophisticated behaviour."Problem solving" is the capability to use cognitive processing to find a solution to a problematic situation and several behavioural experiments show that *Octopus* has this kind of skill: the animal is able to open jars to reach a prey. "Play-like" is a kind of playful behaviour that animals show toward non-food objects. It is spontaneous, voluntary, repeated, not stereotypic behaviour, observed in healthy subjects and in stress-free conditions. "Play-like" is observed among all taxa of vertebrates and invertebrates, including octopuses. We conducted experimental training to evaluate how enriched environment vs. standard environment influences adult neurogenesis. We also performed western blotting analysis to compare level of PCNA and PARP1, to evaluate both cell proliferation and synaptogenesis, and semiquantitative PCR and Real Time PCR, to compare expression of Oct- Elav1 in WILD, TRAINED and CONTROL groups. In conclusion we found cell proliferation in areas of brain involved in learning, memory and sensory stimuli integration. Performing behavioural experiments, we found that intellectual, physical and sensory stimuli increase neural plasticity and synaptic remodelling, affecting adult neurogenesis.

MARIA ASSUNTA BISCOTTI, MARIKO FORCONI, MARCO BARUCCA,
ETTORE OLMO, ADRIANA CANAPA

Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

CHARACTERIZATION OF REPETITIVE ELEMENTS IN POLAR SCALLOPS *ADAMUSSIUM COLBECKI* AND *CHLAMYS ISLANDICA*

Satellite DNAs (SatDNAs) and Transposable Elements (TEs) are the two major constituents of the heterochromatic fraction of the genome and are spread across all eukaryotes. SatDNAs and TEs have been investigated in a large number of organisms but data available are rather scarce for marine invertebrates. In particular, molluscs represent one of the largest groups of marine invertebrates and are the second most abundant phylum amongst metazoans. The genome of two polar scallops, the Antarctic *Adamussium colbecki* and the Arctic *Chlamys islandica*, was investigated through the construction of genomic libraries. In this frame we report the identification and characterization of two novel satDNAs, namely 4p1 and 4p3, in *A. colbecki*. These two satDNAs have a very low genomic abundance and a monomer length of 33 bp and 59 bp, respectively. Southern blotting analyses showed a characteristic ladder of tandemly arranged sequences for 4p3 and a more complex organization for 4p1 due to a variable number of a subrepeat within its monomers. CvA elements were identified in both libraries of the polar scallops. CvA represents an example of satDNA-TE link and was firstly discovered in the oyster *Crassostrea virginica* (GAFFNEY *et al.*, 2003). This element was classified as a new MITE-like family of non-autonomous transposable elements that harbours in its structure a tandemly repeated core. Southern blotting analysis confirmed the tandem arrangement of this element in *A. colbecki* while no bands were detected in *C. islandica*. The latter result might be explained by the very low presence of this element in the genome of the Arctic scallop evidenced through dot blot analysis. The sequence analysis revealed that the repeated sequences of CvA core are highly conserved not only in the two polar scallops but also in other species of the Pectinidae family. Finally in both species analysed the RT-PCR analyses evidenced a transcriptional activity of this ancient element arisen 540 years ago at the beginning of the bivalve radiation.

FERDINANDO BOERO[1, 2], PIETRO ALIFANO[1], GRAZIANO PIZZOLANTE[1],
MARCO LEZZI[1], MARIO DE STEFANO[3], CINZIA GRAVILI[1],
LOREDANA STABILI[1, 4]

[1] Dipartimento di Scienze e Tecnologie Biologiche e Ambientali, Di.S.Te.B.A., Università del Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy
[2] CNR-ISMAR, Genova, Italy
[3] Dipartimento di Scienze Ambientali, Seconda Università di Napoli, Caserta, Italy
[4] Istituto per l'Ambiente Marino Costiero, U.O.S. di Taranto – CNR, Via Roma 3, 70400 Taranto, Italy

MICROBIAL ASSOCIATIONS IN *AGLAOPHENIA OCTODONTA* (CNIDARIA, HYDROZOA)

Marine animals host a great diversity of microorganisms, with the establishment of specific animal–microbe interactions. Several papers describe the epibiotic community of a number of Mediterranean colonial hydroids, analysing mainly the spatial and temporal distribution of both diatoms and bacteria. In a previous study we observed a new association between the hydroid *Aglaophenia octodonta* (Cnidaria, Hydrozoa), the luminous bacterium *Vibrio* sp. AO1 and microalgae assigned to *Symbiodinum* sp. AO1 belonging to the Clade A. All specimens of *A. octodonta* observed under epifluorescence microscopy showed a clear green fluorescence on the external side of the perisarc (chitinous exoskeleton) around hydrocladia due to *Vibrio* sp. AO1 and a red fluorescence due to the presence of microalgae. In the present work we describe the diversity of other microorganisms associated to this hydroid. In particular, under optical and scanning electron microscopy the presence of about 4-5 peritrich ciliates attached to each hydrothecal rim was observed. Taxon specific primers for 18s rDNA were employed to identify the epibiotic peritrich ciliates, which were assigned to *Pseudovorticella* sp. AO1. Furthermore, it appears that the *Pseudovorticella* sp. AO1 contains endosymbiotic *Symbiodinium* sp. AO1. This is the first record of a similar potential association between a peritrich ciliate and microalgae belonging to the genus *Symbiodinium*. Treatment of *A. octodonta* with the antibiotic ampicillin results in a decrease of vibrios luminescence followed by a *Symbiodinium* expulsion and *Pseudovorticella* detachment suggesting an involvement of all these microorganisms in a consortium with beneficial metabolic interdependence. Presumably, in such association, peritrich ciliates play a role of reservoir of *Symbiodinium* avoiding their expulsion. These microalgae, performing photosynthesis, release oxygen but the activity of *Vibrio* presumably removes the excess of this compound. This bacterial species indeed may play the role of reactive oxygen scavenger species (ROS) thus avoiding oxidative stress and protecting the hydroid host. Studies are in progress to confirm this hypothesis.

MARIANNA CATASTI[1], MARTA D'ONOFRIO[1], JHEYSIS CARDENES[1],
ANGELISA FABRIZI[1], MATTEO PALLOTTINI[1], MARIO LUNA[2],
ENZO GORETTI[1]

[1] Dipartimento di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia
[2] Via Goffredo Mameli, Foligno (PG)

LEPIDOPTERA PAPILIONOIDEA OF THE UMBRIAN SIDE OF SIBILLINI MOUNTAINS

Investigations on the lepidopterans of Sibillini Mountains were often conducted on the side of Marche Region, much larger than the Umbrian side. The purpose of this study is to outline the checklist and distribution of Lepidoptera Papilionoidea in the Umbrian side of Sibillini Mountains and surrounding mountain areas. The investigated area shows a high natural value involving the whole Umbrian side of the Monti Sibillini National Park (17,790 ha, 24.90% of PNMS) and a Natura 2000 Site (Monti Pizzuto – Alvagnano, IT5210067), close to PNMS. The surveys were conducted during spring and summer between 2010 and 2011, mainly through butterfly-watching or capture/release techniques. Papilionoidea found, about 70% of what was known of the entire PNMS, amounts to 87 species: 4 Papilionidae, 9 Pieridae, 28 Lycaenidae and 46 Nymphalidae; only *Coenonympha rhodopensis* had not been reported to the PNMS (TEOBALDELLI, 2010). Many of these species are taxa of high natural value, relegated to high altitude environments in the Apennines, especially *Parnassius apollo, Lycaena hippothoe, Polyommatus eros, P. damon, Boloria pales, Brenthis hecate, Melitaea trivia, Coenonympha dorus, C. glycerion, C. rhodopensis, Hyponephele lycaon, Erebia alberganus, E. cassioides, E. epiphron, E. ligea, E. meolans, E. neoridas* and *E. pluto*. The georeferencing of the reports has made possible to delineate the relative distributions in the investigated area. Such detailed knowledge is a basic prerequisite for the preservation programs of these species, key elements of Apennines biodiversity. Indeed, a satisfactory condition of their conservation status is a useful reference system for an evaluation of the management policies of the natural heritage of the Apennines.

GIOVANNI CHIMIENTI[1], RICARDO AGUILAR[2],
FRANCESCO MASTROTOTARO[1]

[1] Department of Biology, University of Bari, Via Orabona 4, 70125 Bari, Italy
[2] OCEANA, Leganitos 47, 28013 Madrid, Spain

FACIES OF *ISIDELLA ELONGATA* (ESPER, 1788): WHAT ARE WE LOOSING?

Isidella elongata (Esper, 1788) is a Mediterranean near-endemic gorgonian (GRASSHOFF, 1989) that can characterize a *facies* on bathyal compact muds between 200 and 1,200 m in depth (PÉRÈS and PICARD, 1964; BO *et al*., 2015). About 45 years ago CARPINE (1970) reported that *I. elongata* was the most common gorgonian species of the Western Mediterranean bathyal zone. However, with the relentless development of trawl fishing technologies in the last century, the real exploitation of deep-sea species began and the strictly muddy *facies* such as *I. elongata* ones almost completely disappeared (MASTROTOTARO *et al*., 2014). In spite of this widespread decline, in some untrawlable areas such *facies* has been accidentally preserved. This is the case of the muddy bottoms located 20 nautical miles East off Ibiza (Balearic Sea, Western Mediterranean) where trawling activities are interdicted because of submarine cables occurrence. Several ROV dives from 2010 to 2014 revealed the presence of a wide *I. elongate facies* between 480 and 615 m in depth, with an extraordinary density of about 125,400-149,600 colonies/km^2. Thanks to visual surveys, it was documented how this *facies* provides several ecological niches for many species, playing important roles in the ecology of the bathyal zone. First of all, the branched colonies act as a secondary substrate for epibionthic species such the actinia *Amphianthus dohrnii* and provide support to lay eggs from oviparous sharks such as *Galeus melastomus*. Furthermore, *I. elongata* gardens act as trophic area where many species prey swimming among the colonies, such as *Merluccius merluccius* and *G. melastomus*, or climbing on them using *I. elongata* as a feeding podium, such as *Anamathia rissoana* and *Antedon mediterranea*. Moreover, the *facies* represents a recovery area for other species that camouflage or protect themselves, such as the ray-finned fish *Benthocometes robustus* observed swimming vertically behind *I. elongata* branches or several other species, such as *Plesionika* spp., *Nephrops norvegicus* and *Octopus salutii*, refuging beside the root-shaped bases of fallen colonies. The present ROV surveys confirm the fundamental ecological role of this habitat former species in the muddy bottoms of the Mediterranean Sea, underlining how few information we have about a peculiar habitat we are still losing.

MICHELE D'ERRICO, MARINA MAURI, LUIGI SALA,
MARIA AGNESE SABATINI

Dipartimento di Scienze della Vita, Università degli Studi di Modena e Reggio Emilia

EVALUATION OF TWO DIFFERENT STANDARD SOILS FOR THEIR USE IN ECOTOXICOLOGICAL TESTS

The quality of soil as a resource is evaluated with ecotoxicological tests, using soil organisms such as Anellida, Nematoda and Collembola. The International Organization for Standardization (ISO) has developed ecotoxicological tests to obtain information on biological effects of contaminants in soil and to improve the data obtained with the conventional chemical analysis. The ISO guideline 11267 (1999, 2014) describes a standardized method that is based on the determination of sublethal effects of contaminated soils on the collembolan *Folsomia candida*. However, the standard artificial soil OECD (1984), firstly proposed in ISO 11267 (1999), does not represent the heterogeneity of natural soils, possibly invalidating the transfer of laboratory evaluations to the natural environment. As a matter of fact, the second edition of the ISO 11267 (2014) involves the use of standard natural soils in addition to the artificial soil OECD. The aim of this work is to compare by soil ecotoxicological tests two standard soils included in ISO 2014: the artificial soil OECD and the natural soil LUFA 2.2. The comparison of the two soils was performed assessing the effect on survival and reproduction of *F. candida* of a secondary product, the digestate, which is used in agricultural practices and it is derived from the anaerobic digestion of agricultural crops and manure. The digestate (pH 7.3) was tested at two concentrations (2%, 4%) in the two soils; two control series without digestate were also prepared. The digestate added did not affect the survival of *F. candida* in both tested soils. However, the digestate added to the LUFA soil negatively affected the reproduction of *F. candida*: in the experimental series with 2% digestate, the number of juveniles dramatically decreased with respect to control group, and no juveniles were found in LUFA soil treated with 4% digestate. In the experimental series with OECD soil no effect on reproduction of *F. candida* was detected. Many Authors affirm that *F. candida* reproduction is negatively affected by soil pH values >6.6. In our experiment, the pH value of the OECD soil treated with digestate did not change compared to the initial pH value = 6, while the pH values of the treated LUFA soil reached 6.8 and 7.4 with 2% and 4% digestate, respectively. The negative effect on reproduction could therefore be attributed to the increase of pH values. Thus, our study deeply questions the interpretation of the results of standardized toxicity tests.

CHRISTIAN DE GUTTRY[1, 3], YAIR ACHITUV[2], FERRUCCIO MALTAGLIATI[1], FEDERICA G. PANNACCIULLI[3]

[1] Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy
[2] The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, 52900, Israel
[3] ENEA - Marine Environment Research Centre, PO Box 224, 19100 La Spezia, Italy

PHYLOGEOGRAPHY ON THE ROCKS: A LARGE SPATIAL-SCALE MOLECULAR SURVEY OF *CHTHAMALUS MONTAGUI* (CRUSTACEA, CIRRIPEDIA)

Chthamalus montagui Southward, 1976 is a barnacle living in the intertidal zone of rocky shores with a broad geographic distribution area. Its larvae, with a pelagic life of 2-4 weeks, give it high capability for dispersal. We analysed a total of 312 individuals of *C. montagui* from 13 locations from the NE Atlantic, Mediterranean and Black Sea by sequencing a 537bp region of the mitochondrial gene *COI*. Overall, we detected 130 haplotypes, 98 of which were location-private, while 32 were shared. High within-location genetic variability was found, with haplotype diversity $h = 0.750$ to 0.967. At the adopted spatial scale, the hypothesis of panmixia can be rejected, being the species genetically structured. In fact, considering three regions (NEA: NE Atlantic, WMIA: Western Mediterranean-Ionian-Adriatic and ABS: Aegean-Black Sea), AMOVA produced significant values of both Φ_{CT} and Φ_{SC}. Genetic divergence among regions was very high, with fixation indices $F_{ST} = 0.596$ to 0.886. The NEA showed slight genetic structuring, while the ABS region was homogeneous. The WMIA exhibited a much higher degree of genetic structuring, with the presence of three distinct subregions: Western Mediterranean, Adriatic-Ionian and central Mediterranean (Malta). Considering the entire study area, *C. montagui* fitted the isolation-by-distance model. The median-joining network of haplotypes and Bayesian assignment test detected three haplogroups corresponding to the three regions. Historical demography, obtained by mismatch distributions, neutrality tests and Bayesian skyline plots was consistent with past demographic expansions in the NEA and ABS areas. The WMIA showed a similar trend, despite the high degree of genetic substructuring observed in Malta. Phenomena of larval retention in the La Valletta Bay could account for the unique genetic cluster observed at this location. The results of this study highlighted *i*) that *C. montagui* is genetically structured in the three main biogeographic areas and *ii*) how the Mediterranean, given its particular oceanographic conditions, represents a heterogeneous basin, even for a broadcast spawning species. From a biogeographic perspective, we detected barriers to gene flow at the Strait of Gibraltar and in the system of the Turkish Straits; in addition, we found a genetic discontinuity in the Crete/Cyclades islands, which separates individuals of the Aegean-Black Sea region from the rest of the Mediterranean counterparts.

MARIA GABRIELLA DENARO, CLARA BERTUCCIO,
DEBORAH PALOMBIERI, IRENE PATERNITI, CONCETTA CALABRÒ,
PATRIZIA LO CASCIO

Department of Biological and Environmental Sciences, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy

THE EFFECT OF FASTING AND REFEEDING WITH *SPIRULINA* ON ENDROCRINE CELLS IN THE GASTROINTESTINAL TRACT OF ZEBRAFISH (*DANIO RERIO*)

Several studies on the gastrointestinal tract have documented that certain experimental procedures can act on morphological integrity (DENARO *et al*., 2006), metabolism, enzymatic patterns regulation, gastrointestinal hormones and peptides transport (CARUSO *et al*., 2014). *Danio rerio* (Hamilton, 1822) zebrafish, vertebrate model used in scientific research, has been also proposed for fish nutrition studies. PepT-1 is an oligopeptide transporter responsible for absorbing di- and tri-peptides through a brush border membrane of intestinal mucosa. Cholecystokinin (CCK-8), satiety hormone secreted from endocrine cells of the gastrointestinal tract, enhanced digestion by triggering the release of pancreatic enzymes, gallbladder contraction and inhibiting gastric emptying. The aim of this study was to determine the effect of short-term food deprivation and refeeding in adult zebrafish on the gastrointestinal expression of PepT-1 and CCK-8, in order to elucidate a potential mechanism driving compensatory growth. Samplings were repeated following 2 and 5 days of fasting and 2 and 5 days of refeeding with commercial Sera Spirulina tabs, in which the major constituent is *Spirulina* sp. which brings 50.2% in protein. The morphological analysis and immunohistochemical determination of PepT-1 and CCK-8 by Western blot were carried out. The intestinal tract from control specimens exhibites the simple architecture of digestive tract. Fasting 2 and 5 days caused morphostructural changes in folds and constriction of the intestinal lumen. Western blot analysis revealed a reduction in PepT-1 levels after fasting and an increase after refeeding, reaching very high levels after 5 days, compared to the control. The levels of CCK-8 decreased after food deprivation, while increased gradually after refeeding. The significant increase in expression of PepT1 in refeeding fish, following fasting, suggests a physiological mechanism contributing to compensatory growth for enhanced absorption efficiency and might influence CCK-8 secretion.

CINZIA FERRARIO[1], YOUSRA BEN KHADRA[2], FRANCESCO BONASORO[1],
MARIA DANIELA CANDIA CARNEVALI[1], MICHELA SUGNI[1]

[1] Department of Biosciences, University of Milan, Milan, Italy
[2] Laboratoire de Recherche, Génétique, Biodiversité et Valorisation des Bioressources, Institut Supérieur de Biotechnologie de Monastir, Université de Monastir, Monastir, Tunisia

MICROSCOPIC ANATOMY OF ARM REGENERATION IN THE RED STARFISH *ECHINASTER SEPOSITUS*

Starfish are armed echinoderms well-known for their striking regenerative ability. Despite this, the process of arm regeneration is still not well defined in terms of both morphological and molecular aspects. Indeed, also considering the limited number of species examined so far, a comprehensive account of the variety of possible regenerative models is still lacking. In this work, arm regeneration was studied in the red starfish *Echinaster sepositus* until 16 weeks post-amputation (p.a.) by means of a microscopic approach in order to deeply elucidate its cellular mechanisms and provide the necessary complement to the recently published molecular data related to this same species (BEN KHADRA *et al.*, 2014). As in other starfish, the regenerative process could be divided into three main phases. The repair phase (1 h-1 W p.a.) was characterized by a strong constriction of the stump sealing off the perivisceral coelom and a wound healing by stretched stump epithelial cells providing a thin epidermal monolayer covering the injury. Beneath the wound epithelium a network-like syncytium of phagocytes was detectable at 24 h p.a., whereas an oedematous area consisting of different cytotypes (including dedifferentiating myocytes) widespread in the newly-deposited extracellular matrix was evident between 72 h p.a. and 1 W p.a. The early regenerative phase (1 W-6 W p.a.) was characterized by further stump tissue rearrangement (mainly muscle components) and first signs of regenerative processes mainly involving the radial nerve cord, the ossicles and the terminal tube foot. This latter can be considered as the "driving distal structure" of the subsequent regenerative process: indeed, the new elements, such as ossicles, muscles and tube feet, were developed by gradual intercalation between the terminal tube foot and the stump with a proximo-distal gradient. The advanced regenerative phase (6 W-16 W p.a.) was characterized mainly by skeletogenesis, myogenesis and differentiation of neural structures (optic cushion) leading to the re-growth of a complete miniaturized arm-tip. Overall, *E. sepositus* arm regeneration p.a. can be considered mainly a morphallactic process during which the pre-existing tissues are rearranged and used as source of cells/materials to re-grow the new structures. Our findings indicate the distalization-intercalation model proposed by AGATA *et al.* (2007) as the most appropriate mechanism to describe the regenerative processes in this species.

PAOLA FERRI, PATRIZIA AMBROGINI, TIZIANA CECCHINI, SANDRA CIARONI, FLAVIA BUCCELLA, RICCARDO CUPPINI, PAOLO DEL GRANDE

Dipartimento di Scienze della Terra, della Vita e dell'Ambiente, Università degli Studi di Urbino "Carlo Bo"

NEWBORN CELL SURVIVAL REGULATION BY α-TOCOPHEROL IN THE DENTATE GYRUS OF OLD RATS

Hippocampus dentate gyrus is a brain region involved in learning and memory, where neurogenesis occurs during adulthood (AIMONE *et al.*, 2014). Aging severely affects proliferation of neural progenitor cells (NPCs) and survival of immature neurons, leading to a reduced number of neurons integrating into the neuronal network, which may contribute to age-related cognitive impairment (SEIB and MARTIN-VILLALBA, 2015). NPC proliferation and newborn cell survivalare regulated by a great number of different intrinsic and extrinsic factors (VARELA-NALLAR *et al.*, 2010). The identification of factors able to counteract the neurogenesis decline with age may have implications for restoring age-related cognitive impairment. Our previous investigations indicated that α-tocopherol is an exogenous factor affecting hippocampus neurogenesis in the adult rat, by inhibiting cell proliferation, but promoting newborn neuron survival (CECCHINI *et al.*, 2003). In order to examine α-tocopherol effects on NPC proliferation and newborn cell survival decline occurring during aging, in this study, an immunohistochemistry assay of PCNA-positive cells was performed in hippocampus dentate gyrus of old rats fed with α-tocopherol-supplemented diet for 3 months and of age-matched controls. In addition, to evaluate newborn cell survival, a quantitative analysis of BrdU-positive cells, detected by immunohistochemistry, was performed one month after BrdU administration. Although our results did not show any PCNA-positive cell both in α-tocopherol-supplemented old and in age-matched control rats, BrdU immunohistochemistry analysis demonstrated a significant increase in BrdU-positive cells in the dentate gyrus granule cell layer of α-tocopherol-supplemented rats with respect to controls. These results show that α-tocopherol is able to increase newborn cell survival during aging, suggesting a possible role of α-tocopherol in counteracting age-related cognitive impairment.

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ILARIA GIOVANNINI[1], THOMAS BOOTHBY[2], BOB GOLDSTEIN[2],
LORENA REBECCHI[1]

[1] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2] Department of Biology, University of North Carolina, Chapel Hill, North Carolina

SILENCING OF GENES POTENTIALLY INVOLVED IN ANHYDROBIOSIS IN THE TARDIGRADE *PARAMACROBIOTUS RICHTERSI*

Anhydrobiosis is a highly stable state of suspended metabolism in organisms involving a temporary suspension of active life and a developmental standstill. Oxidative damage is one of several severe stresses associated with water depletion. Although oxygen is a vital and fundamental molecule for aerobic organism, it can also cause several damages to biological molecules. Oxidative stress is a process resulting from an imbalance between the excessive production of reactive oxygen species and the limited action of antioxidant defenses. Anhydrobiotic organisms are able to recover successfully from extraordinary cellular stresses associated with desiccation, since cells are protected by a complex network of endogenous antioxidants that allow cellular, tissue and organ integrity and functionality to be maintained. In this study, RNA interference was utilized to silence the expression of genes potentially involved in desiccation tolerance in the anhydrobiotic eutardigrade *Paramacrobiotus richtersi* to understand if these genes play functional roles in mediating survival under desiccation. In order to obtain the sequences of these genes, RNA was extracted from groups of *P. richtersi*. Its transcriptome was then sequenced and genes encoding antioxidant enzymes, aquaporins, heat shock proteins and trehalose-6-phosphate synthase were identified and cloned. These clones were used to generate double stranded RNAs, and RNA interference was performed by injecting them into active adult animals. As controls, animals injected with RNAse free water and not-injected animals were used. All animals injected with RNAse free water recovered, suggesting that microinjections are not deleterious to tardigrades. Controls and experimental animals were desiccated using standard laboratory conditions and then rehydrated. The comparison of survival after rehydration among injected animals with double stranded RNA and controls will allow us to understand the involvement of these genes in anhydrobiosis. These data could give further evidences on the role of the molecular defense mechanisms in tolerant desiccation organisms, and the role of tardigrades as animal model to discover the secret of life without water.

CINZIA GRAVILI[1], LUIS MARTELL[1], FERDINANDO BOERO[1, 2]

[1] Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (Di.S.Te.B.A.), Università del Salento, Italy
[2] Istituto di Scienze Marine, Sezione di Genova, CNR, Genova, Italy

CHANGES IN THE TROPHIC STRUCTURE OF HYDROID (CNIDARIA: HYDROZOA) ASSEMBLAGES: LINKS WITH MEDITERRANEAN WARMING

Information on the feeding biology of hydroids in nature is scarce. Most studies on this subject focus on the feeding rates, diets, physiology, and energy budgets of individual species, with no integrative approach to identify feeding guilds. The hydrozoan species found along a vertical transect in 1980 and throughout 2004 in the Portofino Promontory (Ligurian Sea, Italy) were categorized based on trophic guilds and the changes among guild structure were analyzed to ascertain the trophic responses of hydrozoan assemblages to the parallel documented warming of the Ligurian Sea. Analysis tools included the graphic representation of the change in trophic and polyp-size structure, as well as chi square (X^2) tests that allowed to identify patterns of change in the community. The feeding habits and diet of the hydrozoan families found in Portofino can be ranked in the following categories: passive suspension feeders, active suspension feeders, benthic predators, symbionts, and multidirectional feeders. The general trophic and polyp-size structure of the hydrozoan assemblages in Portofino in 1980 and 2004 was very similar, but some species present in 1980 had disappeared in 2004, meanwhile other southern-affinity species, never recorded from the area before, became abundant in 2004. Not all trophic and size categories were affected in the same way during the study period, and the rate of disappearance and substitution of species was higher for benthic predators, active suspension feeders, and large-polyp species. This could lead to alterations on the abundance of the invertebrate prey with consequent perturbations of unknown magnitude in the benthic communities where hydroids are dominant, especially in the cold season. Therefore, further studies are needed to understand and forecast the effects of changing benthic-predator abundances, the relative strength of top-down control in hydrozoan-dominated habitats and, after all, the interactions of these changes with intensifying anthropogenic stressors such as climate change.

RACHELE MACIRELLA, ANTONELLO GUARDIA, ILARIA BERNABÒ, SANDRO TRIPEPI, ELVIRA BRUNELLI

Dipartimento di Biologia, Ecologia e Scienze della Terra, Università della Calabria, Via P. Bucci, 87036 Rende (CS), Italia

EFFECTS OF TWO SUBLETHAL CONCENTRATIONS OF MERCURY CHLORIDE ON THE MORPHOLOGY AND METALLOTHIONE IN ACTIVITY IN THE LIVER OF ZEBRAFISH (*DANIO RERIO*)

Heavy metals are ubiquitous pollutants that are introduced in to the aquatic environment by anthropogenic sources, and have become one of the most toxic substances found in our environment. Among heavy metals, mercury is one of the most detected pollutants and is mainly used in industrial, pharmacological and agricultural fields. Accidental and occupational exposures to mercury, both organic and inorganic (mercury chloride $HgCl_2$), cause acute damage in various human and animal organs (RAO and CHHUNCHHA, 2010). Mercury chloride is one of the most toxic forms, because it easily forms organomercury complexes with proteins. Zebrafish (*Danio rerio*) is a small teleost and is one of the most widely used vertebrate models for toxicological testing (HILL *et al.,* 2005). As the liver is the main organ that performs detoxification processes as well as regulation of metabolic pathways, it is important to understand toxicity effects and malfunctions on this organ. By analyzing zebrafish liver the main metabolic disorders caused by mercury exposition emerge. In this work adult zebrafish livers were examined, under basal conditions and after 96h of exposure to two mercury chloride concentrations (0.03-0.15 μM) from a morphological and ultrastructural point of view. With the aid of immunohistochemistry and in situ hybridization analysis, the induction of Metallothionein (MT), a key mediator for the detoxification of metals, was detected. Pathological alterations on liver histology and ultrastructure were observed at both tested concentrations. The main effects recorded were: increase in intercellular spaces, increase in nuclear size of the hepatocytes, accumulation of large lipid droplets, gradual accumulation of smooth ER, increase in melanin content and degenerative phenomena. Mercury chloride also induced a dose-dependent MT expression in all the liver regions, especially in the distal/external portion. This is the first report on the effects $HgCl_2$ has on zebrafish liver. It emerged that a short-term exposure to $HgCl_2$ induces severe histological alterations and also affects the expression pattern of MT in *Danio rerio* liver.

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MARIA MELE[1], GILDA FAZZARI[1], GIUSI IACHETTA[2], RAFFAELLA ALÒ[1], ENNIO AVOLIO[1], VINCENZA LAFORGIA[2], MARCELLO CANONACO[1]

[1] Comparative Neuroanatomy Laboratory, Biology, Ecology & Earth Science Department (DiBEST), University of Calabria, Ponte P. Bucci 4B, 87036 Arcavacata di Rende (CS), Italy
[2] Department of Biology, Section of Evolutionary & Comparative Biology, University "Federico II" of Naples, Via Mezzocannone 8, 80134 Napoli, Italy

CATESTATIN AND GABA$_A$R INHIBITORY SIGNALS ALTER FEEDING BEHAVIORS THROUGH THE MODULATION OF DOPAMINE, GHRELIN AND LEPTIN NEURORECEPTOR EXPRESSION CAPACITIES

Catestatin (CST), a small, hydrophobic and sympathoinhibitory peptide produced in neuroendocrine cells (MAHATA *et al.*, 2003) is capable of interfering with the major cerebral neuroreceptor-inhibiting site, i.e. γ-aminobutyric acid$_A$ receptor (GABA$_A$R) system (AVOLIO *et al.*, 2014). Such a feature is mostly evident in limbic brain areas such as the hypothlamus, amygdala, hippocampus and cortex that are largely involved with feeding behaviors (JONAIDI and NOORI, 2012). GABA$_A$Rergic-depending effects might very well be caused by an interaction with other neuroreceptor such as dopaminergic, ghrelin and leptinergic systems that have also shown to be tightly linked with eating and drinking behaviors (MUNZBERG *et al.*, 2007; JONAIDI *et al.*, 2012; CRUNELLI and DI GIOVANNI, 2014). On the basis of these considerations, this research study aimed to investigate the feeding responses induced by treating the valuable hibernating rodent model, i.e. the Syrian hamster (*Mesocricetus auratus*) with CST plus the principal GABA$_A$R antagonist bicucullin (BIC) alone or in combination (CST+BIC). Hamsters that received CST via intracerebroventricular infusions displayed a very great reduction of feeding and drinking bouts, with respect to its control (Ctr; NaCl treated hamsters), thus strengthening the anorexigenic role exerted by CST (MELE *et al.*, 2014). On the contrary, animals treated with BIC displayed a significant increase of feeding and drinking bouts. Interestingly, when both agents were given contextually (CST+BIC), it appeared that the sympathoinhibitory component (CST) predominated as shown by a notable reduction of feeding bouts flanked by a very great decrease of body weight. From a biomolecular point of view, these behavioral performances, especially those induced by the combined treatment seemed to be correlated with the significantly increased dopamine receptor 1 (D1) along with reduced ghrelin receptor (GhsR) and leptin receptor (LepR) transcript levels (HOLM *et al.*, 2004; AVENA and RADA, 2012). Overall, the predominant CST effects on D1 and GhsR expression levels tend to account for notable anorexigenic responses in our rodent model, very probably via its interaction with GABA$_A$Rs, thus proposing these neuroreceptor systems as key limbic molecular components implicated in the different feeding disorders.

GIUSEPPINA MESSINA[1], BIANCA MARIA LOMBARDO[1], ERMINIA CONTI[1],
MIRELLA CLAUSI[1], ANNA GUGLIELMO[1], GIORGIO TESTA[2],
PAOLO GUARNACCIA[2], SAVERIO SCIANDRELLO[1],
GIANCARLO ROCCUZZO[3], CRISTINA ABBATE[2], FILADELFO BROGNA[4],
GIUSEPPINA CAMIOLO[1], DIEGO LEONE[1], ANGELIKI DROUTSA[5],
VERONICA MAZZEI[1], MARIA VIOLETTA BRUNDO[1]

[1] Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università degli Studi di Catania
[2] Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania
[3] Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Acireale (CT)
[4] Azienda Foreste Demaniali per la gestione delle aree protette in provincia di Siracusa
[5] Agricultural University of Athens. Laboratory of Agricultural Zoology and Entomology

BIOLOGICAL INDICATORS AND BIOMARKERS FOR SOIL QUALITY EVALUATION IN NATURAL AND CULTIVATED AREAS OF THE ORIENTED NATURAL RESERVE "FAUNISTIC OASIS OF VENDICARI" (EASTERN SICILY)

The proposed research is related to the problematic of the soil quality degradation, in many systems, due to the increasing anthropic pressure. Such degradation is really alarming since soil is one of the environmental matrices of the terrestrial ecosystems richest in species, supports the biological productivity and preserves the environmental quality; its deterioration, therefore, has a negative, sometimes destructive, impact on the whole system functionality and on its biodiversity. Specifically, this research aims to investigate the effects that agricultural practices of different nature may have in sensitive ones such as the protected areas, where zones subject to integral protection are contiguous to crops and are consequently affected by the input of persistent organic compounds, pesticides and xenobiotic of various nature. The area selected for this study is the Oriented Natural Reserve "Faunistic Oasis of Vendicari", a humid area of eastern Sicily protected by the Ramsar Convention and declared ZPS according to the European Directive "Birds". It hosts in the B zone a wide agricultural area with different crops, subject to different cultural practices. This research intends to investigate the effects of the different cultural practices on the soil of the fully protected zone, adding to the traditional chemical-physical analyses and methods an analysis of the soil organismic component, based on different ecological and eco-physiological methods with an integrated approach. The analyses will concern: a) analysis of biodiversity of both soil microarthropods and flora by several ecological indexes; b) assessment of presence/absence of some species of Isopoda Oniscidea; c) identification of the principal classes of pesticide residues in animal and vegetal matrices by GC/MS; d) identification of effect and exposure biomarkers in the tissues of collected animals and plants; e) microbial biomass analysis; f) evaluation of the agronomic soil quality, even through the measurement of soil respiration by IRGA system and other numerous parameters. The outcomes of this research will provide: a) useful information on the main causes of the disturb induced by the agricultural practices in the fully protected area and b) indication on the most adequate cultural practices in problematic areas, so to combine the agricultural activity with the need of environmental protection, including the biodiversity maintenance.

VINCENZO MIGLIACCIO, RAFFAELLA SICA, LILLÀ LIONETTI,
ROSALBA PUTTI, ROSARIA SCUDIERO

Dipartimento di Biologia, Università "Federico II", Napoli, Italy

HIGH FAT DIET AND DDE COMBINED EFFECTS ON METALLOTHIONEIN EXPRESSION AND SYNTHESIS IN RAT TISSUES

Persistent organic pollutants (POPs) are a wide group of environmental pollutants that accumulate in the adipose tissue. POPs include the p,p'-dichlorodiphenyldichloroethylene (DDE), DDT's major metabolite with the highest persistence. DDT was extensively used for the control of malaria, typhus and other insect-transmitted diseases. Although it was banned in many countries since the 70s (in Italy in 1978), it is still used in developing countries because of its low cost and high efficiency. DDE has been listed as a pollutant for its persistence in the environment, potential to biaccumulate and toxicity for living organisms, including humans. Nowadays, the exposure is primarily through contaminated food, and several studies report DDE adverse effects on tissues and cells, especially in terms of inflammation and oxidative stress. In this study, we evaluated the effect of a possible synergic interaction between high fat diet and DDE treatment, two risk factors for cellular inflammation and oxidative damage, on expression and synthesis of metallothionein (MT). MT, in fact, is an inducible cysteine-rich protein, able to modulate the binding of both essential or toxic heavy metals and the removal of hydroxyl radicals. MT expression is also enhanced by inflammatory stimuli and is induced in adipose tissue during the development of obesity. Adult male Wistar rats were randomly divided in 4 experimental groups (n = 6) and fed for 4 weeks with: 1) control diet (N); 2) N plus DDE (10 mg/kg bw by gavage); 3) high fat diet (45% fat) (HF); 4) HF plus DDE (10 mg/kg bw by gavage). qPCR and western blotting analyses demonstrated a tissue-specific modulation of MT expression and synthesis. In fact, the changes in MT expression following high fat diet and DDE treatment depended on tissues. In liver, the major amount of MT transcripts and proteins was found in N rats: both HF and DDE treatment led to a decrease in MT expression; in kidney, where a low constitutive amount of MT was found, the DDE treatment greatly enhanced MT gene expression and synthesis, in both N and HF rats. Finally, immunohistochemical analysis showed a nuclear translocation of the MT protein, in particular in the hepatocytes of HF and DDE-treated rats. Nuclear translocation is also evident in kidney from DDE-treated animals. We demonstrated that, in these animals, the intake of DDE caused mitochondrial dysfunction and lipids peroxidation; possibly, the MT nuclear translocation protects DNA from oxidative damage.

MARIA M. PALLOTTA[1], RAFFAELE RONCA[1], ROSA CAROTENUTO[1],
CONCETTA AMBROSINO[2], IMMACOLATA PORRECA[2],
TERESA CAPRIGLIONE[1]

[1] Dipartimento di Biologia, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cinthia, 80126 Napoli, Italy
[2] IRGS, Biogem, Via Camporeale, 83031 Ariano Irpino, Avellino, Italy

EFFECTS OF EXPOSURE TO CHLORPYRIFOS ON *MUS MUSCULUS*

Cohort studies provided new evidence that prenatal exposure to OPs can cause developmental neurotoxicity. In fact, younger individuals may be more susceptible than adults due to biological factors and exposure settings. In particular, maternal exposure to CP was found to be dose-related to slower brain growth and associated to several structural abnormalities such as thinning of cerebral cortex. It has been demonstrated that even sub-lethal doses of neurotoxic pesticides affect behaviour by inhibiting acetylcholinesterase (AChE). While the cognitive short-term effects may be directly attributed to this inhibition, the mechanisms that underlie OP's long-term cognitive effects remain controversial and poorly understood. To elucidate through what mechanism CP induces alterations in neurogenesis, we used *Mus musculus* as animal model. F2 offspring of *Mus musculus* females, treated with low doses of CPF during pregnancy, were used to study the effects of chronic exposure to this compound. The analysis was performed on 84 genes associated to Parkinson disease. Our results highlighted a dose dependent down-regulation for many of the genes studied. It was interesting to note that, among the genes significantly down-regulated, there were some of those more directly related to Parkinson's disease, such as the Parkina 2, Pink1 (PTEN induced putative kinase 1) and the receptor Gabbr2 (Gamma-aminobutyric acid B receptor, 2). Only the expression of the Ubiquitin C gene, which encodes for a a polyubiquitin precursor with proteolitic activity, showed a significant increase. Ubc might have role in degradation of proteins, such as alpha-synuclein, which typically accumulates in many neurodegenerative diseases. The results of our study confirm that exposure to CPF can elicit alterations in the gene expression profile during neurogenesis and reinforce the view that the critical window of vulnerability of the developing brain to CPF extends from gestational exposure through postnatal period.

ANTONIO ROLANDO, MATTEO NEGRO, ELEONORA OPERTI,
KATIA LEO, ELENA CERRUTI, ANGELA ROGGERO, ROBERTA BERRETTI,
GIORGIO VACCHIANO, RENZO MOTTA, CLAUDIA PALESTRINI

Department of Life Sciences and Systems Biology, Via Accademia Albertina 13, 10123 Torino, Italy

BIODIVERSITY AND FOREST MANAGEMENT: THE CASE OF BRACHYPTEROUS GROUND BEETLES OF SESSERA VALLEY BEECH FORESTS (NW ITALIAN ALPS)

European beech forests are of particular importance for biodiversity, although relatively little is known about how beech forest management impacts on invertebrate communities. We focused on a group of localized, medium and large-bodied brachypterous ground beetles inhabiting beech forests in the north western Italian Alps. The study site, located in the Sessera Valley, is part of Natura 2000 ecological network. In particular, the site houses *Carabus olympiae*, classified as a priority species in Annexes II and IV of the “Habitats Directive” (92/43/EEC). We used baited pitfall traps to sample the carabid community and investigate the influence of beech forest management history [i.e. over-mature coppices (OC) and coppices in conversion to high forests (CCHF)], climatic, topographic and microhabitat characteristics on ground beetle diversity, measured as total relative abundance, species richness, Shannon diversity and abundance of the endangered endemic species *Carabus olympiae*. Moreover, we used radiotelemetry to evaluate *C. olympiae* movements in differently managed beech forest stands. The diversity of forest specialist carabids was higher in OC and in forest stands characterized by a higher mean temperature and lower relative humidity. Moreover, we detected a positive response of several diversity variables to coarse wood debris cover or volume, herb cover, and the standard deviation of tree diameter. Currently, OC seems to be a more favorable habitat for forest carabids, including *C. olympiae*, although succession over time can lead to a progressive homogenization of the vegetation structure, with negative consequences for the conservation of the forest carabid assemblage. Preliminary observations on *C. olympiae* movements seem to suggest they move more linearily in recently coppiced stands. Based on our results, we suggest that the traditional management of beech coppice and its conversion to high forest should be modified by including practices aimed at promoting structural and microhabitat diversity such as retention of large trees, creation of canopy gaps, retention of coarse wood debris and the preservation of ‘islands’ of older trees in the managed stands. The research was supported by a European grant (EU LIFE+ Project NAT/IT000213).

FRANCESCO SACCO[1], EUGENIA SCHIMMENTI[1], FEDERICO MARRONE[1],
SABRINA LO BRUTTO[1], AMINA BESBES[2], AHMED NFATI[3], MARK GATT[4],
SAMAR SABER[5], FABIO FIORENTINO[6], MARCO ARCULEO[1]

[1] Dipartimento STEBICEF, Università di Palermo, Via Archirafi 18, 90123 Palermo, Italia
[2] INSTM, Centre de Monastir, Tunisie
[3] Marine Biology ResearchCenter, Tajura, Lybia
[4] Ministry for Sustainable Development, the Environment and Climate Change, Malta
[5] Instituto Espanol de Oceanografia, Malaga, Spain
[6] IAMC- CNR Mazara del Vallo (TP), Italia

GENETIC VARIATION OF THE DOLPHINFISH *CORYPHAENA HIPPURUS* (PISCES, CORYPHAENIDAE) IN THE MEDITERRANEAN SEA

Coryphaena hippurus is a cosmopolitan migratory epipelagic species and represents a target species for artisanal fisheries and recreational fishing. Moreover, in some parts of the Mediterranean Sea, it constitutes one of the most important species for commercial fishery. In the present study we analysed the mtDNA genetic diversity of dolphinfish throughout the Mediterranean Sea with the aim to support future management plans aimed at ensuring the sustainability of this important marine resource. Genetic variation was investigated by the sequencing of two mtDNA markers, i.e. the cytochrome c oxidase subunit 1 (COI) and the NADH dehydrogenase subunit 1 (ND1). Tissue samples were obtained from 10 different landing localities of the Mediterranean Sea: Palma de Majorca (Balearic islands, Spain), Ancona (Adriatic coast of peninsular Italy), Ischia (Naples, Tyrrhenian coast of peninsular Italy), Porticello (Palermo, Sicily), Capo Passero (Syracuse, Sicily), La Valletta (Malta), Teboulba (Monastir, Tunisia), Tripoli (Libya), Larnaca (Cyprus) and Izmir (Aegean coast of Turkey). A fragment of the mitochondrial ND1 was amplified using the primer described by DIAZ-JAIMES *et al.*, (2010), whereas a fragment of mitochondrial COI was amplified using the primer described by FOLMER *et al.*, (1994). Haplotype (*h*) and nucleotide (π) diversity, AMOVA, Mismatch analyses, and the neutrality tests were performed with ARLEQUIN 3.5 (EXCOFFIER *et al.*, 2010). Fragments of 751bp and 551 bp of the mitochondrial ND1 and COI were amplified and sequenced from 88 *C. hippurus* individuals. 26 polymorphic sites and 23 different haplotypes were scored for ND1, whereas for COI 6 polymorphic site sand 8 different haplotypes were identified. Populations structure and genetic differentiation was investigated using F-statistics with AMOVA. Comparison among the Mediterranean samples did not show any significant difference for both markers highlighting a general genetic homogeneity within the basin ($\Phi CT = 0.043$, $P = 0.179$ for ND1 and $\Phi CT = 0.072$, $P = 0.083$ for COI). In conclusion, based on our results, the Mediterranean dolphinfishes could be considered as a single-stock management unit, which needs a large regional scales management plan involving all countries interested in the exploitation of this important marine resource. Research realised with the financial support of RITMARE. We are grateful to CONISMA and FAO-MEDSUDMED project.

CLAUDIA SBROCCA, FEDERICA SEMPRUCCI, MARIA BALSAMO

Dipartimento di Scienze della Terra, della Vita e dell'Ambiente (DiSTeVA), Università Urbino 'Carlo Bo', Campus Scientifico, loc. Crocicchia, 61029 Urbino, Italy

HARPACTICOID COPEPOD ASSEMBLAGES ASSOCIATED TO HARD SUBSTRATES OF THE MARINE PROTECTED AREA OF PORTOFINO

Despite rocky shores are common littoral habitats, relatively little is known about the meiofauna living there. Thus, a study on the meiofaunal community of the marine protected area of Portofino (Genoa) was carried out. The taxonomic composition of the harpacticoid copepods fauna, a dominant meiofaunal component together with nematodes, was studied in detail in order to improve the knowledge of this group in the Ligurian coasts, and also to update the list of Italian species. Meiofauna samples were collected during summer 2009 and winter 2010 by SCUBA divers using an underwater suction sampler ("sorbona"). Sampling was carried out at 20 stations located at Paraggi and Aurora (Portofino promontory). The sampling stations were characterized by different depth, slope, latitude and algal covering. From each sample harpacticoids were counted, isolated and identified up to putative species level. Copepods (including Harpacticoida, Cyclopoida, Calanoida and Siphonostomatoida orders) represented the 31% of the whole meiofauna community exceeded only by nematodes (37%). Sixty putative species in 43 genera belonging to 22 families of harpacticoid copepods were identified. Seven species and 8 genera were reported for the first time in the Italian coasts, while 13 species were new reports for the Ligurian Sea. The most abundant families were Miraciidae, Laophontidae, Longipediidae and Thalestridae, while the most abundant genera were *Amphiascus*, *Laophonte*, *Longipedia, Thalestris* and *Ameira*. The Shannon-diversity was very high (H'> 3,04) in all samples along with the equitability (J> 0,73) and species richness (S> 14). The 2-way ANOSIM showed that the greatest differences in the copepods assemblage could be related to a different sampling season, while slight differences were revealed between sites or depths. In particular, the genus *Longipedia*, more abundant in summer, mainly contributed to the differences revealed in the seasonal comparison, followed by *Laophonte* that was more abundant during winter. This was probably related to the heterogeneity of the substrates in each season, that offer to copepods different food sources and habitats. In fact, different marine seaweeds harbour several species of copepods that differ in body shape and characteristics of the legs used for clinging to the substrate. This survey documents an overall good ecological quality of the whole study area and greatly increases our knowledge of marine biodiversity of harpacticoid copepods.

VALERIO SCALI[1], ELISABETTA COLUCCIA[2], FEDERICA DEIDDA[2],
CINZIA LOBINA[2], SUSANNA SALVADORI[2]

[1] Department of BiGeA, Università di Bologna
[2] Department of Scienze della Vita e dell'Ambiente, Università di Cagliari

CO-LOCALIZATION OF RIBOSOMAL AND TELOMERIC SEQUENCES IN *LEPTYNIA* (INSECTA PHASMATODEA)

Stick insects have been studied mainly for non conventional reproduction modes such as parthenogenesis, hybridogenesis and androgenesis (review in SCALI, 2009); parallel karyological investigations have also evidenced high numerical and structural chromosome re-patterning (WHITE, 1976; SCALI and MARESCALCHI, 1987; MILANI *et al*., 2009). Chromosome sets of strictly related *Leptynia* (Pantel) species, range from 40 to 36 chromosomes and are characterized by satellites of variable size and localization (SCALI *et al*., 2012). Chromosomal preparations of *Leptynia* were obtained by manual dissection of the gonads of anaesthetized specimens, gentle teasing of tissue fragments in drops of 60% acetic acid on a lab glass and subsequent cell drying on a hot plate. FISH of ribosomal 45S and telomeric (TTAGG)n sequences were carried out according to SALVADORI *et al*., 2014. In the three analyzed taxa [*L. attenuata attenuata* (Scali), *L. attenuata algarvica* (Scali) and *L. montana* (Scali)] FISH by 45S sequences constantly labeled large areas on a single chromosome pair, always corresponding to underlying satellites, which in different specimens - or even in plates of the same specimen - were often in a heterozygous condition. FISH of the telomeric repeats, beside ordinary telomeres, also labeled one/two large interstitial regions; both 45S and telomeric signals marked the 4th chromosome pair of the karyotypes. Double FISH tests fully confirmed the co-localization of ribosomal and telomeric highly repeated sequences on the satellites. In *L. montana* one additional chromosome pair, likely the 6th, sometimes showed a lesser expanded telomeric marking. In *Bacillus atticus* (Brunner) similar large satellites have been demonstrated to hybridize with $AgNO_3$, shift along chromosomes and also give origin to independent supernumerary chromosomes (TINTI and SCALI, 1991), thus suggesting transposon activity. Be this also the case of *Leptynia*, highly repeated NOR and telomeric sequences might represent "hot spots" for chromosome re-patterning with a bearing on karyotype evolution and speciation. Further targeted investigations are obviously needed.

ANNA SCANDURRA[1], MASSIMO ARIA[2], PAOLO MONGILLO[3],
LIETA MARINELLI[3], BIAGIO D'ANIELLO[1]

[1] Department of Biology, University of Naples "Federico II", Italy
[2] Department of Economics and Statistics, University of Naples "Federico II", Italy
[3] Department of Comparative Biomedicine and Food Science, University of Padua, Italy

INTRASPECIFIC OBSERVATIONAL LEARNING IMPROVES PERFORMANCE OF DOGS IN A TRAINING CONTEXT

The aim of the present study was to assess the potential benefit of conspecific observational learning in real dog training context where handlers are not professional trainers and dogs are requested to perform activities that may be worrying. Fifty Labrador retrievers were recruited, which were under training as water rescue dogs at the Italian Water Rescue School. Dogs were equally distributed between basic and advanced training level and assigned to demonstration and control groups. The experimental procedure consisted of two phases, which were intended to ascertain that dogs did not perform the selected exercise when requested by their handlers (Phase1) and then to assess whether they would perform the same exercise after the observation of a conspecific demonstrator (Phase2). Dogs of the control group (who have not witnessed the demonstration) were kept on a leash in the same place and for the same amount of time as dogs of the demonstration group, before moving to Phase 2. The tests were performed outdoors in an agility training fenced area and two dynamic exercises were selected. The outcome of Phase2 was coded into a binary variable as successful or unsuccessful and generalized linear mixed model was used to analyze outcomes. The model included the dog's age as a covariate and the dog's group, level of experience, sex and the interaction age*level of experience as fixed factors. Student t-test was used to assess age differences between groups. Dogs of demonstration group were more likely to perform the requested exercise (more than 62%) than those of control group (less than 23%) ($p < 0,01$). The dog's sex and level of experience and the interaction between age and training level had no effect on the likeliness of performing the exercise in the Phase2. The effect of the dog's age was close to statistical significance and a subsequent student's t test indicated that dogs who performed the exercise at Phase2 were older than those who could not perform the exercise ($t = 3.61$; $P = 0.001$). Dogs' performance improves after demonstration by a conspecific performing this action. Whereas the interspecific social observational learning was just demonstrated, this study supports also the ability of dogs in the intraspecific observational learning. If this effect is part of a mechanism of social learning, or simply reflected a stimulus or local enhancements should be determined.

MARCUS V. X. SENRA[1], ROBERTO D. P. JUNIO[1], MICHELE CASTELLI[2],
CARLOS A. G. SOARES[3], INÁCIO D. S. NETO[4], FRANCO VERNI[2],
GIULIO PETRONI[2]

[1] Departamento de Zoologia, Universidade Federal de Juiz de Fora, UFJF, Brazil
[2] Unit of Protistology-Zoology, Department of Biology, University of Pisa, Via A. Volta 4/6, 56126 Pisa, Italy
[3] Departamento de Genética - Universidade Federal do Rio de Janeiro - UFRJ, Brazil
[4] Departamento de Zoologia - Universidade Federal do Rio de Janeiro - UFRJ, Brazil

CHARACTERISATION AND PHYLOGENY OF BACTERIAL ENDOSYMBIONTS FROM A BRAZILIAN CILIATE

Eukaryotic organisms are involved in a high biodiversity of symbiotic associations with bacteria, with various effects on the host, ranging from beneficial to detrimental. Their degree of stability is also variable, alongside opportunistic interactions, some bacteria are well-adapted to a symbiotic lifestyle. An example is represented by *Rickettsiales* (*Alphaproteobacteria*), whose representatives are all obligate intracellular bacteria. In this work, we characterised the bacterial symbionts harboured by a strain of the ciliate *Euplotes woodruffi* Gaw, 1939 isolated from a Brazilian sample. The ciliate cells were screened for endosymbionts using fluorescent probes targeting the bacterial SSU rRNAs. The corresponding SSU rRNA genes were amplified by PCR and sequenced, and were employed to design species-specific probes, necessary to confirm symbionts' identity inside the host cells. Two different coexisting symbionts were retrieved in this *E. woodruffi* strain. The most abundant was *Polynucleobacter necessarius*, a betaproteobacterium present in certain *Euplotes* species, known to be necessary for host survival. The other symbiont was a new member of the recently described family "*Candidatus* Midichloriaceae" (*Rickettsiales*), which was named "*Candidatus* Bandiella woodruffii". Phylogenetic analysis revealed that "*Candidatus* Bandiella woodruffii" is a member of a well defined clade within "*Candidatus* Midichloriaceae", whose members were found in association with a wide range of aquatic organisms, such as sponges, cnidarians, echinoderms and euglenoids, while no direct relationship was found with other endosymbionts of ciliates belonging to the same family. These findings confirm the broad host range and natural abundance of "*Candidatus* Michloriaceae", suggesting that host shifts may have happened multiple times in nature. Considering also that there are hints for a potential pathogenic role by some members of this candidate family to humans and other vertebrates, we decided to test the ability of transfer of "*Candidatus* Bandiella woodruffii", employing as recipient a strain of *Euplotes encysticus* Yonezawa, 1985, sampled in the same location.

MARIA TERESA SPENA[1], ROSARIO GRASSO[1], ALESSIA BIONDI[1],
VANESSA CANNAVÒ[1], VEZIO COTTARELLI[2], MARIA CRISTINA BRUNO[3]

[1] Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Sez. Biologia Animale, Università degli Studi di Catania, Via Androne 81, 95124 Catania, Italy
[2] Dipartimento per l'Innovazione nei sistemi Biologici, Agroalimentari e Forestali, Università degli Studi della Tuscia, Largo dell'Università snc, 01100 Viterbo, Italy
[3] Fondazione Edmund Mach, Centro Ricerca e Innovazione, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy

THE EPIKARST OF SOME SICILIAN CAVES: AN "*ULTIMA* THULE" FOR CRUSTACEAN MEIOFAUNA?

Pollution and global change are strongly affecting groundwater habitats of several Italian regions, with impacts on their biotic communities and strong decrease of their biodiversity. Starting in 2009, our research group has been investigating the crustacean meiofauna collected from the epikarst of four caves in Sicily (Conza, Entella, Molara (PA) and Cavallo caves (AG)), representing the first research in this habitat for Sicily, and one in Calabria (Vucco Ucciardo Cave, CS). We focused our research on harpacticoid copepods, mainly of the family Parastenocarididae, a characteristic and highly specialized component of groundwater fauna worldwide. The results of our research, when compared with older records, highlight the conservation value of the epikarstic habitats and their taxocoenosis: a) *Stammericaris trinacriae* (Galassi, Pesce, Cottarelli, 1989) was collected only in 1987 from two wells in Trapani Province which have now been filled up. A new, very abundant population was collected from the epikarstic drip and rimstone pools of di Entella Cave throughout a 2-year sampling campaign conducted monthly in 2012-2014. This is the first record of a Parastenocarididae from an evaporitic cave; b) *Nitocrella stammeri* Chappuis, 1938 a representative of the family Ameiridae with perimediterranean geonemy, was extinct in one of the 19 recorded Sicilian populations (STOCH, 2003-2004): the well of Porto Palo (SR) (monitored in 2005), where it was originally collected in 1978. A new populations of this species was collected in 2014 from a typical carbonatic karstic fissured acquifer, in the rimstone pools of Cavallo Cave; c) a new species of *Cottarellicaris*, presently under study, was collected in 2013 and 2015 from rimstone pools in Vucco Ucciardo Cave; d) *Stammericaris diversitatis* (Cottarelli and Bruno, 2013) was collected in several occasions from 2009 to 2014 from the rimstone pools and epikarstic drip of Conza Cave, where it is endemic. All the caves where these species were collected are dry caves, exclusively recharged by percolating rainfall. Hence, the rainfall-fed epikarst underlying unfarmed soil could represent a refuge for sensitive taxa, when compared with other more easily polluted habitats (e.g., the hyporheic zone of streams and rivers), and should be carefully protected since it could harbor endangered, endemic or rare species.

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MARIA TERESA SPENA[1], ROSARIO GRASSO[1], VANESSA CANNAVÒ[1],
MANUEL ANDREA ZAFARANA[1], MARCELLO DIMARTINO[2],
CORRADO SANTORO[3]

[1] Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Sez. Biologia Animale, Università degli Studi di Catania, Via Androne 81, 95124 Catania, Italy

[2] Comune di Ragusa - Pianificazione Urbanistica e Centri Storici, Piazza San Giovanni snc, 97100 Ragusa, Italy

[3] Dipartimento di Matematica e Informatica, Università degli Studi di Catania, Via A. Doria 6, 95125 Catania, Italy

AUTONOMOUS SYSTEMS FOR NON-INVASIVE ZOOLOGICAL MONITORING

In the field of the zoological monitoring, a key aspect is the avoidance of any kind of interference with respect to the normal activity of the species to be monitored. The presence of the team of researchers would compromise the equilibrium of the dynamics of the population, annoying the individuals, which would change their behaviour during their biological functions. This aspect is more stressed in the case of species hard to monitor, for which the level of non-invasivity must be kept as minimal as possible. In such a context, the technology can provide useful support by considering the use of robotic autonomous systems, which can perform the monitoring activity in an autonomous way minimising the interference with individuals. To study certain avian species, particularly vulnerable, a joint research project is being conducted. We have prototyped some autonomous air vehicles (UAV) able to fly in formation, to monitor, by means of photo cameras, the habitat of wildlife of interest. Since a single UAV is able to monitor a limited space, when certain social dynamics have to be studied, a wide view is mandatory. To this aim, we use a set of UAVs to self-organise in order to make a collective monitoring of the wide area of interest. The avian species to be studied are those with terrestrial habits and characterised by a negative population trend: in particular the Stone Curlew *Burhinus oedicnemus* (L., 1758). The areas of interest are chosen among the most interesting, in Sicily, for the bird presence and preservation: peninsula Magnisi, plain of Gela, and extensive farming in Iblean region (Ragusa). The areas occupied are investigated geomorphologically and botanically, through the study of topographic-planimetric maps. A preliminary test has been performed by using a single UAV, which has been used for some missions to monitor the nesting sites with the objective of determining the number of individuals and the distribution of the nests (distance and allocation). We found 28 nests in peninsula Magnisi, 2 in plain of Gela and 4 in Iblean region. However, the most interesting aspect concerns the reaction of the individuals: according to the images acquired, the behaviour of the birds in hatching seems not affected by the presence of the UAV and the birds remain on the nest. This is an important result since it proves that such a technology can be used with success in monitoring these species without affecting their normal behaviour.

MARTA TOGNETTI[1], SIMONA SANVITO[2], FILIPPO GALIMBERTI[2]

[1] Dipartimento di Scienze Naturali, Università degli Studi di Milano
[2] Elephant Seal Research Group, Sea Lion Island, Falkland Islands

SOCIALITY IN KILLER WHALES: THE ROLE OF RECRUITERS

Killer whales are an apex predator species, with a worldwide distribution, a complex sociality and communication system, and advanced hunting techniques that let them prey on species of very different size and habits. Although killer whales were studied extensively in the northern hemisphere, knowledge about populations of the southern hemisphere is rather limited. We studied sociality of killer whales at Sea Lion Island (Falkland Islands; SLI hereafter) during two seasons (September 2013 to March 2015) for a total of more than 400 days spent in the field. We used a combination of photo-identification, surveys and observations from vantage points. We identified 31 killer whales, some of them observed the first time in 2004, using features of the saddle patch and the outline of the dorsal fin (DARWIN software). We assembled adjacency matrices of identified individuals, we calculated association indices, and we used social networks methodologies to visualize and quantify killer whales social structure. At SLI the basic social unit was the mother-calves group, that included a maximum of three calf generations. Mother-calves groups formed a hierarchical, matrilineal social structure. Males were quite irregular in their presence, association among themselves, and bond with other killer whales, although they were sometimes involved in social predation events. Killer whales were either regular visitors of SLI, spending an important share of their time close to the island, or occasional visitors appearing for just a very short time (few hours to few days). Different groups of occasional visitors were always observed together with a specific mother-calf pair (Lola and Ale), that had a high centrality in sociograms, and apparently acted as recruiter and social facilitator for the coming of new individuals. Group size and association patterns changed along each season, and between the two seasons, but the basic building blocks of the social structure remained the same The complex sociality observed at SLI was related to hunting tactics. At SLI, killer whales prey on two species of pinnipeds, the southern elephant seal and the southern sea lion. Predations were social events involving up to 13 individuals, lasted a long time in case of large seals (e.g., sub-adult males), and sometime involved the males, although actual killing was usually carried out by adult females. Social prey sharing was the norm.

MARIAILARIA VERDERAME, ERMELINDA LIMATOLA,
ROSARIA SCUDIERO

Dipartimento di Biologia, Università "Federico II", Napoli, Italy

MANURE FERTILIZING MAY CONTRIBUTE TO ESTROGENIC CONTAMINATION OF THE LIZARD *PODARCIS SICULA* INHABITING ORGANIC FARMING

Emerging data on the effects of chemical fertilizers and pesticides to human health and environmental impact led in recent years to the sharp increase in agricultural practices and animal husbandry of organic crops and livestock. Thus, in the last past years, the organic farming has exponentially grown and the manure as soil fertility source has become the principal agricultural choice. Unfortunately, overuse of animal manure as organic fertilizer may pose a serious ecological risk: the introduction of hormones (specifically, estrogens) into the environment. In addition, manures can contribute to heavy metals bioaccumulation in agricultural soils. Direct studies on wildlife exposed to runoff from manure-fertilized cropland are limited; so, in order to elucidate if the levels of estrogens being released into the environment from manure are capable of causing endocrine disruption, we decided to investigate about the presence of vitellogenin (VTG) and Estrogen Receptor alpha (ERα), good biomarkers of endocrine disruption, in the liver of the adult male lizard *Podarcis sicula*. Lacertids, in fact, are an important vertebrates group in agro-ecosystems and have been identified as potential model species for ecotoxicological studies. As key components of the food webs, they fill a critical role both as predator and prey species, being the main prey for birds, snakes and other animals and important predators of insects. Adult male lizards were captured in two organic farmlands (manure-treated sites) and in an uncultivated field (control site). Lizards from the two organic farms displayed the hepatic biosynthetic alterations typical of an estrogenic contamination: hepatocytes, in fact, contained both VTG and ERα transcripts and proteins, detected by ISH and ICC investigations. The same cells did not show cadmium, lead and metallothionein accumulation, as demonstrated by atomic absorption spectrophotometry and Real-Time PCR analysis, respectively; these findings are indicative of the lack of inorganic contamination. Taken together, these data suggest that manure-derived exogenous hormones could affect the welfare of wild animals and the animal breeding, leading to the bioaccumulation of estrogens in food chain, with possible risk for human consumers. For this very reason, it should be implemented in the organic farming the use of sustainable practices such as crop rotation to preserve the soil biological activity, rather than organic manure as fertilizer.

DAVID C. WILCOCKSON[1], LAURA S. HOELTERS[1], VITTORIO PASQUALI[2],
ALICE CIOFINI[3], ALBERTO UGOLINI[3]

[1] Aberystwyth University, Penglais Campus, Aberystwyth, Ceredigion SY23 3DA, UK
[2] Dipartimento di Psicologia, Università di Roma "La Sapienza", Via dei Marsi 78, 00185 Roma, Italia
[3] Dipartimento di Biologia; Università di Firenze; Via Romana 17, 50125 Firenze, Italy

ANTENNAL CLOCK IN *TALITRUS SALTATOR* (CRUSTACEA, AMPHIPODA)?

The supralittoral sandhopper *Talitrus saltator* (Montagu, 1808) uses the sun as an orientation cue during its excursions along the sea-land axis of sandy beaches. Obviously, its use requires the ability to compensate for its azimuthal variations. An endogenous biological clock is involved in the chronometric mechanism of the sun compass (UGOLINI *et al.*, 2007). The aim of this work is to evaluate the presence of an extra-cerebral clock, localised in the antennae, involved in the solar orientation, as has been demonstrated in the Monarch butterfly *Danaus plexippus* (Linnaeus, 1758) (MERLIN *et al.*, 2009). We tested the ability of solar orientation of intact and operated ("antennaeless" without: 1) second antennae and 2) first and second antennae) individuals, in phase with the natural L:D = 12:12 cycle or advanced by 6 hours. We also quantified mRNA levels of canonical genes (*Per*, *Tim*, *Clk* and *Cry2*) during the circadian time both in the brain and in the antennae. Results of behavioural tests show no relevant differences in directional choices between intact and operated individuals. Clock-shifted animals exhibit the expected 90° deflection in their orientation compared with directions assumed by individuals in phase with the natural photoperiod. Molecular analysis indicates that *Per* and *Tim* mRNA levels are subjected to time oscillations with similar trends in the brain and in the antennae. Therefore, while behavioural experiments do not give support to the hypothesis concerning the existence of an antennal clock involved in the solar orientation of *Talitrus saltator*, circadian oscillations of key clock genes' expression observed in the antennae can not be neglected.

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