Unione Zoologica Italiana
in collaborazione con
Comitato Scientifico per la Fauna d'Italia

Riassunti delle Comunicazioni e dei Poster

a cura di
Elvira De Matthaeis, Andrea Di Giulio, Marzio Zapparoli

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INDICE

Presentazione

Cari Colleghi,

con l'80° Congresso dell'Unione Zoologica Italiana, organizzato insieme al Comitato Scientifico per la Fauna d'Italia, l'UZI torna a riunirsi a Roma "la Città Eterna", dopo quattordici anni, presso l'Università Roma Tre.

Come da tradizione i Congressi UZI sono organizzati in Simposi che affrontano temi differenti, sempre interconnessi tra loro e legati da una lettura integrata delle numerose e diversificate attività di ricerca dei Soci UZI. I Simposi di questo 80° Congresso sono quattro, ognuno dei quali si aprirà con almeno una plenary lecture.

Il tema del I Simposio "Biologia dello Sviluppo e della Cellula" riguarda un ampio spettro di discipline che vanno dalla classica biologia dello sviluppo animale allo studio dei processi cellulari di base affrontati con un approccio integrato, evolutivo, comparato e molecolare.

Il tema del II Simposio è: "La Fauna in Italia", a dimostrazione dell'importanza che l'UZI ha da sempre attribuito ai problemi legati alla conservazione del patrimonio faunistico italiano, analizzati a diversi livelli. Al termine del Simposio ci sarà una rievocazione dei 150 anni della Società Entomologica Italiana. Non a caso l'intero Simposio è dedicato ad Augusto Vigna Taglianti, zoologo di fama internazionale recentemente scomparso.

Il Simposio III, "Zoologia Applicata", raccoglie le esperienze applicative di gruppi di ricerca diversi.

Il tema del Simposio IV è rivolto all'analisi delle possibili relazioni tra "Filogenesi, Biogeografia e Sistematica", ricordando che siamo a 160 anni dalla scomparsa di von Humboldt, insigne naturalista, esploratore e geografo.

Con la Tavola Rotonda "La fauna delle acque interne tra crisi della biodiversità, nuovi strumenti conoscitivi e primi consuntivi delle misure di gestione" si affronta un problema di grande attualità, legato all'uso della risorsa acqua.

In conclusione, nell'ambito del Congresso, che prevede oltre 150 partecipanti, ascolteremo complessivamente 43 comunicazioni e verranno esposti 65 poster, di cui 25 concorrono all'assegnazione dei diversi premi offerti dall' Unione Zoologica Italiana, dal Comitato Scientifico per la Fauna d'Italia, dalla UTET e dall'**Istituto Euro-mediterraneo di Scienza e Tecnologia.**

Come sempre, il Congresso annuale UZI costituisce un'occasione importante di incontro tra diverse generazioni di studiosi della Biologia Animale, con esperienze scientifiche differenti.

Desideriamo ringraziare l'**Accademia Nazionale Italiana di Entomologia**, la **Società Entomologica Italiana**, la **Federazione Italiana di Scienze della Natura e dell'Ambiente**, l'**Istituto Euro-mediterraneo di Scienza e Tecnologia**, l'**Università Roma Tre**, la **Sapienza Università di Roma** e l'**Università di Roma Tor Vergata** per il loro patrocinio e i due sponsor, Reborn e Zeiss.

Auguriamo a tutti un soggiorno piacevole e una partecipazione fruttuosa all'80° Congresso dell'Unione Zoologica Italiana.

Pietro Brandmayr
Presidente dell'Unione Zoologica Italiana

Marco Alberto Bologna
Presidente del Comitato Scientifico per la Fauna d'Italia

Elvira De Matthaeis
Presidente del Comitato Organizzatore dell'80° Congresso UZI

Roma, 23 settembre 2019

Opening lecture

ETTORE OLMO

Università Politecnica delle Marche

THE C VALUE ENIGMA A “COLD CASE OF THE EVOLUTIONARY BIOLOGY: DOES THE ENVIRONMENT SEE THE GENOME?”

The C value, known also as genome size is the amount of DNA contained within the haploid nucleus of a eukaryotic organism which is proportional to the number and length of chromosomes. Although the DNA has an informational role the C value has not ever had a clear relationship to the complexity of the organism or the number of coding genes. This apparent anomaly was called “C value paradox” or more recently “C value enigma. The more important cause of genome expansion is the repetitive DNA, especially the transposons. A very interesting correlation was found between the environmental stress and the activity of transposons. During vertebrate evolution several periods were characterized by environmental stress accompanied by strong reorganization of karyotype and by genome expansion as in the transition from the aquatic to terrestrial environment. During Carboniferous the genome of the Dipnoans showed a very great expansion from 2.5 to more than 100 picograms. This increase due to a rapid amplification of LTR transposons was accompanied by a progressive evolutionary decline and was possibly caused by drastic environmental changes. Great increases of the genome were also found in amphibians, especially in the three lissamphibian orders that differ in the genome sizes. It has been hypothesized that during Permian the amphibian genome had a first gradual expansion leading to the actual maximum levels found in frogs, afterwards during the Triassic a second great increase happened leading to the very large genome size found in Urodeles. An analysis of genome composition suggests that this second increase could be due to a rapid amplification of only one or few transposons families according to a mechanism remembering the saltatory evolution. Also in this class the transposons amplification and the resulting genome expansion would be due to environmental stress. The transposons activity has often a deleterious effects to the host genome. In the dipnoans a large increase in genome size had a retarding effect on the evolution. In amphibians and especially in the urodeles inverse relationships can be observed between genome size and evolutionary rate and species richness. These observations pose the important question to understand the meaning of the increase in genome size and which advantage, if any, the enormous genomes could give to lungfish and salamanders. Some theories suggest that most part of the genome is “junk” or “selfish” and is made by non-coding sequences that expand for its own benefit. The C value of a species would be a balancing between mutation pressure acting to increase the genome and physiological tolerance factors within the host cells. Other theories argue that besides the strict genetic role the nuclear DNA has a structural and quantitative role. This phenomenon was called nucleotypic effect. One of the possible nucleotypic effects of the genome size is the influence on the organization and mechanical function of the chromosomes based on special structural repetitive DNAs. Another important evolutionary parameter determined by genome size is the cell cycle length and through this the developmental and larval duration which in some animals are fundamental for the evolutionary adaptation to the environment. A relevant parameter for the evolution which is influenced by the genome and cell size is the metabolism. Genome and cell size may influence the metabolic level controlling the surface/volume relationship a fundamental parameter for the respiratory and food exchange between cell and the surrounding environment. The C value enigma is not yet fully clarified, but some interesting information are achieved: The expansion of the genome size depends mainly on the activity of transposons. The environmental variations influence the activity of the transposons which in turn influence the structure, size and composition of the genome. The current level of genome size found in eukaryotes is the result of a balance between the activity of transposons and selective pressure acting at various levels.

Simposio I

Biologia dello Sviluppo e della Cellula

Coordinatori:
Marco Colasanti, Mario Pestarino

Presentazioni orali

ROBERTO CHIARELLI, CHIARA MARTINO, LIANA BOSCO, MARIA ROCCHERI

Università degli Studi di Palermo

FROM STRESS PROTEINS TO APOPTOSIS AND AUTOPHAGY IN SEA URCHIN EMBRYOS

Marine invertebrates inhabit a key position as intermediate consumers in the pelagic as well as in the benthonic food chains, making them suitable model systems for ecotoxicological studies. Among benthonic organisms, echinoderms represent a simple, though significant, model system to test how specific stress can simultaneously provoke dangerous effects on growth and vitality of organisms. Sea urchins provide an attractive and exceptional model for investigating environmental pollution. Most studies investigating the effects of Cd stress were conducted on *Paracentrotus lividus*, one of the most important marine invertebrates used as bioindicator of metal/heavy metal pollution and an important model organism in developmental biology. It was shown that exposure to different toxics causes the synthesis of heat-shock proteins (HSPs), providing a protective role during stress conditions. The synthesis of a specific set of HSPs was demonstrated in *P. lividus* embryos continually exposed to 1mM of CdCl2 at the blastula (15 h) and gastrula (24 h) stages. Cd insult induces an alteration of normal development, probably inducing alternative pathways of growth, as demonstrated from the presence of several typologies of embryo morphology. Contextually, embryos activate other molecular defense mechanisms such as apoptosis. Studies on the apoptotic processes activated in *P. lividus* were conducted after a long-lasting exposure to low Cd concentrations, similar to those found in moderately or highly polluted seawaters. These exposures caused severe developmental delays and abnormalities in the larvae, suggesting that even very small amounts of Cd, if accumulated in cells, can produce significant cytotoxic effects and apoptosis. Finally, we can assume that in sea urchin embryos/larvae, apoptosis can be considered part of a defense strategy that, by sacrificing a few cells, can safeguard the whole organism and the developmental program, provided that the exposure to Cd is not excessively prolonged or too intense. Another molecular process studied in sea urchin embryos is autophagy, a mechanism of self-eating described as an important intracellular pathway responsible for degradation and recycling of long-term proteins and cytoplasmic organelles. Autophagy has been recently observed in eggs and embryos, in response to stress induced by Cd and other stressors. Results of these studies revealed a higher level of autophagosomes in embryos exposed to Cd for 18 h. Several experiments have been conducted on sea urchin embryos exposed to Cd to study the relationship between autophagy and apoptosis. Results suggest that autophagy may have a key role providing the energy supply necessary for apoptosis, delivering ATP molecules by recycling damaged cellular components. Recent studies suggest that autophagy is important for the clearance of protein aggregates that are formed in cells following stress, and in this process, the p62/SQSTM1 protein (sequestosome 1) appears to play a key role. p62/SQSTM1 is a multifunctional, multidomain adaptator protein which resides at the autophagosome membranes. It is an autophagosome cargo protein that targets other proteins that bind to it for selective autophagy. To highlight any protein aggregates concomitantly with the peak of autophagic vacuolation, control *P. lividus* embryos and embryos exposed to 1 mM CdCl2 for 18 and 24 h were submitted to the immunofluorescence/confocal laser scan microscopy protocol, using the anti-p62/SQSTM1 heterologous antibody. Qualitative analysis showed a diffuse globular signal that is often referred to as Ibs (inclusion bodies), suggesting that the presence of polyubiquitinated protein aggregates was intended for autophagic degradation. Taken together, these data indicate that in conjunction with the peak of autophagic vacuolation, there is an intense formation of protein aggregates that would be sent to degradation mediated by the autophagic process. Reported data about the defense mechanisms induced by Cd in *P. lividus* suggest a hierarchical choice of defense strategies; strikingly, the temporal choice of activation of different mechanisms depends on the fact that the embryo tries to face the stress conditions using, initially, defense strategies that are less deleterious to preserve the developmental program. If these processes are not sufficient to offset the damage, the autophagic and apoptotic mechanisms are activated.

BEATRICE CAMBIAGHI[1], CINZIA FERRARIO[1,2], SILVIA MERCURIO[1], FRANCESCO BONASORO[1], M. DANIELA CANDIA CARNEVALI[1], PAOLA OLIVERI[3], MICHELA SUGNI[1,2]

[1]Dipartimento di Scienze e politiche ambientali, Università di Milano
[2]Centro della Complessità e dei Biosistemi, Università di Milano
[3]Department of Genetics, Evolution and Environment, University College London, London, UK

LARVAL SKELETOGENESIS IN THE CRINOID *ANTEDON MEDITERRANEA* AND THE OPHIUROID *AMPHIURA FILIFORMIS*: A MORPHOLOGICAL, MOLECULAR AND EVOLUTIONARY APPROACH

The endoskeleton is a distinctive feature of echinoderms and consists of a true skeletal tissue with a complex microstructure (stereome). Skeletal elements can be present also in larval stages, although they differ markedly among classes. Therefore, the comparative study of larval skeletogenesis in different echinoderm classes can provide insights into the origin and evolution of this character. In this study we analysed the skeletogenic process in the crinoid *Antedon mediterranea,* which represents the basal echinoderm class, and the ophiuroid *Amphiura filiformis*. In *A. mediterranea* the doliolaria and the post-metamorphic pentacrinoid stages were mainly investigated, whereas in *A. filiformis* the blastula, gastrula, prism and pluteus stages were analysed. Detailed morphological investigations included both light and electron (SEM, TEM) microscopy techniques. Furthermore, a preliminary molecular analysis was carried out in *A. mediterranea* by cloning for the first time twelve skeletogenic genes already studied in *A. filiformis*, and studying their expression and presence by whole mount *in situ* hybridization (WMISH) and Quantitative-PCR (Q-PCR). Our results confirm that in *A. mediterranea*, primordial pentacrinoid skeletal elements are already present in the doliolaria stage. Spicule and plate formation starts in a planar multi-branched arrangement and continues throughout the metamorphosis process, until their complete three-dimensional stereome-like architecture in the pentacrinoid stage. Sclerocytes differentiate, produce short cellular expansions and develop calcitic elements in a highly cellularized and collagen rich mesenchyme. It is still unclear, however, if these elements originate within a syncytium. Differently, in *A. filiformis* thin tri-radiate and three-dimensional spicules are formed by primary mesenchyme cells (PMCs), following a process very similar to that described in sea urchins. PMCs differentiate in an extremely loose mesenchyme, produce long and branched fillopodia and eventually form a syncytium where calcium carbonate is deposited without furtherly developing in a stereom microstructure. Preliminary molecular analysis indicates that skeletogenesis gene orthologues of *A. filiformis* are present and expressed in areas of active skeleton formation also in the crinoid, suggesting that the overall genetic program for skeleton development was already present in the ancestor groups of echinoderms and has been conserved in all the five extant classes for several millions of years. Interestingly, in terms of skeletogenesis processes, the data obtained so far in different classes, comparing larval stages characterized by different life-style and cycle, indicate indeed that the cellular mechanisms can significantly diverge among groups adapting to specific functional requirements. In this view the skeleton of the lecitotrophic doliolaria, which represents the primordium of a persisting adult stereome structure, could be the primitive adaptive solution for these short-range dispersion larvae; in contrast, the specialized temporary skeleton of planktotrophic ophiuroid larvae could be an advanced strategy designed to maximize long-range dispersion capabilities.Further comparative studies on skeleton formation in different echinoderm classes, and particularly in crinoids, are necessary to complete the knowledge about its evolution.

ANNALISA CANDELORI[1], TAKAHARU G. YAMAMOTO[2], MASAAI IWAMOTO[2], PIERANGELO LUPORINI[1], ADRIANA VALLESI[1]

[1]Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino
[2]Advanced ICT Research Institute Kobe, National Institute of Information & Communications Technology, Kobe, Japan

NUCLEAR AND CILIARY LOCALIZATION OF THE *EUPLOTES RAIKOVI* KINASE *ER*-MAPK1, AS REVEALED BY EXPRESSION IN DIFFERENT CELL SYSTEMS

In cells of the ciliate *Euplotes raikovi* switching from the vegetative to the mating (sexual) stage of the life cycle, a 631-amino acid protein kinase, *Er*-MAPK1, was identified localized in the nucleoli of the transcriptionally active nucleus (macronucleus). While the *Er*-MAPK1 283-amino acid N-terminal domain revealed all the structural specificities of the mitogen-activated protein kinases required for a catalytic function, the 348-amino acid C-terminal domain appeared to be structurally unique. We analyzed its function by tagging the C-terminal domain coding sequence with the Green-Fluorescent-Protein (GFP) gene and expressing the construct in mammalian fibroblasts, the yeast *Schizosaccharomyces pombe* and the ciliate *Tetrahymena thermophila*. This function was identified with the *Er*-MAPK1 subcellular localization, most likely determined through information provided by two sequence segments of basic amino acids. This information, however, was differently interpreted by the three expression systems. Mammalian fibroblasts and *S. pombe* localized the GFP-construct to nucleoli; *T. thermophila*, to the basal bodies of both the somatic and oral ciliature. In the light of this evidence, the original observation of the *Er*-MAPK1 nucleolar localization in *E. raikovi* was revisited via new immunoreactions and shown to be ciliary besides that nucleolar. This double *Er*-MAPK1 subcellular localization adds functional significance to the close structural similarity that links *Er*-MAPK1 to animal intestinal cell kinases and male germ-cell associated kinases involved in multiple, nuclear and ciliary, activities.

ALESSIA GIANNETTO[1], SABRINA OLIVA[1], CARLOS FREDERICO CECCON LANES[2], FABIO DE ARAÚJO PEDRON[2], DOMENICO SAVASTANO, COSIMO BAVIERA[1], VINCENZO PARRINO[1], GIUSEPPE LO PARO[1], NUNZIA CARLA SPANÒ[3], TIZIANA CAPPELLO[1], MARIA MAISANO[1], ANGELA MAUCERI[1], SALVATORE FASULO[1]

[1]Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina
[2]Curso Superior de Tecnologia em Aquicultura, Campus Uruguaiana,
Universidade Federal do Pampa, Rio Grande do Sul, Brazil
[3]Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali,
Università di Messina

HERMETIA ILLUCENS **(DIPTERA: STRATIOMYDAE): MOLECULAR BASIS OF DIFFERENT BIOCHEMICAL PHENOTYPES IN DIFFERENT DEVELOPMENTAL LARVAL STAGES**

In recent years, a great interest in insects as alternative source of nutrients has been rising. Several insects species, indeed, especially during larval development, have been characterized by a high content in fats, proteins, fibres and other nutritive components. The Black Soldier Fly (BSF) *Hermetia illucens* has been recognized as a sustainable bio-converter of organic wastes and a valuable source of lipids at the larval stages with fat content accounting for up to 45%, making it a natural resource of biologically active hydrophobic substances and one of the most promising species for artificial breeding. In the present study, the differences among growth performances and nutritional values of *Hermetia illucens* V instar larvae and prepupae reared on vegetable waste were analysed and compared focusing on fat content. The capacity to bioconvert the substrate into biomass and the nutritional composition together with the fatty acid profiles were strongly dependent on the developmental stage. Moreover, the expression levels of key genes involved in the lipid metabolism pathway and herein identified, *namely acetyl-CoA carboxylase (acc), fatty acid synthase (fas), lipase (lip) and acyl-CoA dehydrogenase (acd),* were evaluated in order to understand the molecular basis underlying the observed differences in fatty acid profiles of BSF V instar larvae and prepupae. Our findings suggest that the different fatty acid profiles of the two developmental stages may be related to the modulation of the genes associated with the lipid metabolism during larval development. The obtained results highlight substantial differences between *H. illucens* V instar larvae and prepupae giving important features regarding the opportunity to select the preferable fatty acid profile to meet the industrial requirements.

ANNA PERONATO, NICOLA FRANCHI, LAURA DRAGO, LORIANO BALLARIN

Dipartimento di Biologia, Università di Padova

CHARACTERISATION OF COMPLEMENT SYSTEM OF A COLONIAL PROTOCHORDATE: STUDY OF THE EXPRESSION OF C3, CR1, C3AR AND THEIR ROLE OF C3 IN NONSELF RECOGNITION.

The complement system is one of the most ancient immune modulation mechanism of bilaterian metazoans. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways; all of them converge on the cleavage of C3. The compound ascidian *Botryllus schlosseri* is a reliable model organism for the study of immunobiology. It relies only on innate immunity for its defense and immunocytes. Recently, in the same species, we demonstrated of the lectin and alternative pathways. All the complement components identified so far, are expressed by morula cells, the most abundant circulating hemocytes. In mammals, once the complement system is activated, C3 is cleaved to C3a and C3b, the former exerting a chemokine–like activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. The best-known receptor for C3a in mammals is C3aR, whereas CR1 is the receptor able to recognize and bind C3b on the phagocyte surfaces. In the present work, we describe, in *B. schlosseri*, one genes showing similarity with vertebrate C3aR and three genes with similarity to CR1 (two soluble forms and one transmembrane), and studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that their mRNAs are located in different immunocytes suggesting the presence of a cross-talk between phagocytes and morula cells. In addition, we continued our analysis of the role of C3 in *Botryllus* immunity by studying the modulation of BsC3 transcription during the colonial blastogenetic cycle and the effect of *bsc3* knockdown on immune responses. Only morula cells, and no other immunocytes type, were labelled by the antisense probe for BsC3aR and the soluble CR1s, whereas phagocytes and young, undifferentiated cells known as hemoblasts were the cells stained by the probe for the membrane-linked BsCR1. Both the *bsc3ar* and *bscr1* genes are constitutively transcribed; however, a modulation of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at take-over, whereas no differences were observed when early-cycle and mid-cycle were compared. This is probably related to the renewing of circulating cells at TO, when 20-30% of hemocytes undergo cell death by apoptosis and are replaced by new, differentiating cells entering the circulation in the same period.

ROBERTA PIOVESANA[1], ALESSANDRO MATERA[1], CAROLINA UGGENTI[1], MARIO FIORE[2], JURGEN WESS[3], CLELIA DALLANOCE[4], ADA MARIA TATA[1]

[1]Dipartimento di Biologia e Biotecnologie "C. Darwin", Centro di Ricerca di Neurobiologia, Sapienza Università di Roma
[2]Istituto di Biologia e Patologia Molecolare, CNR, Roma
[3]NIDDK, National Institutes of Health, Bethesda, USA
[4] Dipartimento di Scienze Farmaceutiche, Sez. Chimica Farmaceutica P. Pratesi, Università di Milano

ACETYLCHOLINE MEDIATES CROSS-TALK BETWEEN NEURON AND GLIAL CELLS: IMPLICATION IN SCHWANN CELL DIFFERENTIATION AND PLASTICITY

ACh receptors both muscarinic and nicotinic types, were found in several glial cells during central and peripheral nervous system development as well as in the adult, suggesting a new role for ACh in glial cell proliferation, differentiation and physiology. Rat Schwann cells express different muscarinic receptor subtypes, such as M2 receptor which is the most abundant subtype. M2 activation causes in cultured Schwann cells, a reversible arrest of cell cycle in G1 phase. The negative effect of M2 agonist on Schwann cell proliferation appears dependent on its ability to decrease either the expression of NRG-1/1 or its receptors erbB2/erbB3. M2 agonist arecaidine caused also an increased expression of myelin proteins such as P0, PMP22 and MBP, suggesting that M2 receptor activation may induce a possible progression of Schwann cells towards myelinating phenotype. Support this hypothesis the observation that M2 receptor activation induces an up-regulation of transcription factors involved in the myelinating phase such as *krox 20* and *sox 10* and a down-regulation of the proteins involved in the maintenance of undifferentiated state such as *c-jun*, *Notch-1 and jagged-1*. By electron microscopy and morphometric analysis of the sciatic nerve of $M2^{-/-}$ mice we found an altered fiber morphology, with loose myelin, several myelin inclusions and degenerating axons. Recently we have also characterized the expression of α7 nicotinic receptor. This receptor is fainlty expressed in sciatic nerve fibers and in SCs *in vitro*, but its expression significantly increases both *in vivo* and *in vitro*, after nerve injury or in presence of Bradykinin (Bk), a neuropeptide known for its pro-inflammatory effects. The activation of α7 nAChR by selective agonist (R)-ICH3 after BK treatment, appears to promote the repair Schwann cells and to modulate pro-regenerative environment in terms of cytokines, growth factors and proteases production. These results suggest that α7 nicotinic receptor may be a cholinergic receptor expressed only by *repair Schwann cells* after peripheral axon injury. All together, these data suggest that ACh, probably released from cholinergic axons or by the Schwann cells themselves, may contribute to address Schwann cell differentiation and stabilization of compact myelin organization by M2 mAChR and to generate a microenvironment improving peripheral nerve regeneration by α7 nAChR.

Simposio I

Biologia dello Sviluppo e della Cellula

Coordinatori:
Marco Colasanti, Mario Pestarino

Poster

LAURA DRAGO, LORIANO BALLARIN, GIANFRANCO SANTOVITO

Dipartimento di Biologia, Università di Padova

STRESS GRANULES IN *CIONA ROBUSTA*: MOLECULAR EVOLUTION OF TIAR AND TTP AND EARLY EVIDENCE OF THEIR GENE EXPRESSION UNDER STRESS CONDITIONS INDUCED BY METALS

Stress granules are non-membranous cytoplasmic foci composed of messengers (not translated), ribonucleoproteins, translation initiation components and other additional proteins, that represent a primary mechanism by which gene expression is rapidly modulated when cells are subjected to adverse environmental conditions. Very few works have been devoted to study the presence of molecular components of stress granules in invertebrate animals. In this work, we characterized, for the first time in the solitary ascidian *Ciona robusta*, the genetic sequences of two important protein components of stress granules, TIAR (TIA-1 related to proteins) and TTP (tristetraprolin), and carried out the first studies on their gene expression. The sequences characterized for *tiar* and *ttp* genes have allowed to start a study on the molecular evolution of these proteins in animals: for TIAR the obtained results are consistent with recent phylogenetic analysis that place tunicates as sister group of vertebrates, whereas the phylogenetic position of TTP remains still uncertain. The data on mRNA expression, provided by qRT-PCR analysis, are absolutely the first obtained in non-mammalian animals. As expected, the exposure to each metal (Cu, Zn and Cd) led to a generalized decrease in mRNA expression levels for both TIAR and TTP, suggesting that the metal accumulation induce acute stress and the inhibition of the transcription of *tiar* and *ttp* genes. The data presented here improved our knowledge about the molecular evolution anti-stress proteins in metazoans and emphasize the importance of the transcription of *tiar* and *ttp* genes, which represents an efficient physiological response allowing *C. robusta* to survive in the presence of metals in the marine environment (Supported by M.I.U.R. grant).

PATRIZIA PAGLIARA[1], BERNARDETTA ANNA TENUZZO[1], JACOPO VIZIOLI[2]

[1]DiSTeBA, Università del Salento, Lecce
[2]Université Lille, Inserm, U-1192 - Laboratoire Protéomique, Réponse Inflammatoire et Spectrométrie de Masse – PRISM, F-59000 Lille, France

PRELIMINARY EVIDENCE FOR THE PRESENCE OF ALLOGRAFT INFLAMMATORY FACTOR-1 (AIF-1) IN *PARACENTROTUS LIVIDUS* GONADS

The allograft inflammatory factor-1 (AIF-1) is a protein involved in various aspects of inflammation and it was widely investigated in mammals. In these organisms AIF-1 has been described as mainly present in cells of monocyte/macrophage lineage, however it is also expressed in muscle, liver, spleen and thymus in rat and human. Interestingly, immunohistochemistry data showed that in normal mouse this protein is also present in spermatids, which are so far the only cells expressing AIF-1 not belonging to the monocyte/macrophage lineage mouse. In invertebrates, AIF-1 was reported in mollusks, annelids and echinoderms and is mainly involved in inflammatory response. The high and regulated expression level of AIF1 recently observed in green sea urchin *Paracentrotus lividus* hemocytes indicates that in this species it mainly plays a key role in immunity. However, the constitutive presence of AIF-1 transcripts in a variety of tissues including gills, digestive glands, adductor muscle and gonads, suggests that AIF1 might participate in other biological processes. Here we report on the presence of AIF1 protein in male and female gonads of the sea urchin *P. lividus*. In male gonads, the protein is localized in the cortical region, indicating the specific expression in spermatids, according to observations by Köhler (2007) in mouse gonads. In female gonads the protein is absent in oogonia and oocytes, but it is visible in the accessory cells. We have so far no evidence of AIF1 protein in mature gametes.

Simposio II

La fauna in Italia

Simposio dedicato ad Augusto Vigna Taglianti

Prima parte

Gli ambienti marini

Coordinatori:
Ferdinando Boero, Marco Curini Galletti, Marco Oliverio

Lettura plenaria

FERDINANDO BOERO[1,2,3,4]

[1]Dipartimento di Biologia, Università di Napoli Federico II, Napoli; [2]Stazione Zoologica Anton Dohrn, Napoli; [3]CNR – IAS, Genova; [4]CoNISMa, Roma

HOW THE SEA HAS CHANGED!

"Biodiversity is maintained" is the first descriptor of Good Environmental Status (GES) of the Marine Strategy Framework Directive (MSFD) of the European Union, issued in 2008 and compelling all states of the EU to reach GES in all their waters by 2020. It is unclear, however, what is the state that should be maintained. The present one? If, today, marine biodiversity were degraded, would it be wise to maintain it in this state? We need baselines, dating back to when human impacts were not as pervasive as they are today. Such baselines are not available for most marine systems, with some remarkable exceptions. In 1872, Anton Dohrn founded in Naples (Italy) the first modern infrastructure devoted to marine research: The Zoological Station Anton Dohrn. The scientists that worked there immediately started to explore the biodiversity of the Gulf of Naples and produced a series of Monographs on the fauna and flora that inhabited it. Chun's monograph on the Ctenophora opened the series in 1880. Brinckmann-Voss' monograph on the Anthomedusae closed the series in 1970, with a further off-series monograph on the Opistobranchs of the Mediterranean Sea authored by Schmekel and Portmann in 1982. The monographs udated and widened a wealth of knowledge built by the naturalists that worked at Naples in the past, such as Imperato, Poli, Delle Chjaie, Cavolini, Macrì and many others. Since its foundation, scientists from all over the world moved to Naples to perform experimental studies on the animals at the Zoological Station. Salvatore Lo Bianco, in 1909, published the catalogue of the animals of the Gulf of Naples, reporting on their period of sexual maturity and the environments where they were commonly collected. The catalogue was instrumental in experimental biology, informing scientists on the availability of organisms so as to programme their permanence at the Station. The Monographs on the Fauna and Flora and the studies of Lo Bianco are the benchmark against which the present state of biodiversity can be compared. The remake of the work of Lo Bianco and of the specialists that realized the 39 Monographs, updating their work, will allow to assess the present state of biodiversity of the Gulf and to compare it with the state of a century ago, before the age of global warming. Such studies are possible only at sites where the fauna has been studied long time ago, whereas new studies of little-known areas, as the Monographs of the Fauna d'Italia, will establish the baselines for future assessments. Long-term series of biological observations are crucial to evaluate the changes in biodiversity composition and will allow to better calibrate our efforts aimed at preserving the natural capital so as to reach GES. The publication of Faunas, also with the aid of modern molecular approaches, is indispensable for the evaluation of the state of biodiversity, as required by the Marine Strategy Framework Directive, along with the set-up of biodiversity observing systems based on both competent human capital and new technologies aimed at facilitating species identification.

Simposio II

La fauna in Italia

Simposio dedicato ad Augusto Vigna Taglianti

Prima parte

Gli ambienti marini

Coordinatori:
Ferdinando Boero, Marco Curini Galletti, Marco Oliverio

Presentazioni orali

WALTER RENDA[1], PAOLO RUSSO[1], MARCO OLIVERIO[2]

[1] Società Italiana di Malacologia, Napoli
[2]Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma

THE CHECKLIST OF THE ITALIAN MARINE MOLLUSCA: A SYNERGISTIC ACTION OF AMATEURS AND PROFESSIONAL MALACOLOGISTS

The marine mollusc fauna of the Mediterranean Sea is still considered as the best known in the world. The long-lasting European tradition in taxonomic research since the very beginning of modern systematics, yielded very solid bases for a framework of inventory of biodiversity. The Italian component is perfectly encased in this framework. The Golden Age of European Malacology in XIX century had the participation of a good number of specialists, at that time already including professional and amateur malacologists. The previous modern edition of the checklists of the marine Mollusca were produced by mixed teams of amateurs and professionals. During the last years the Italian Society of Malacology has maintained an updated version of the Mediterranean checklist, that served as the backbone for the development of the present project of Italian checklist. According to the current version of the checklist there are c. 1900 recognised species of marine molluscs in the Italian waters which represents an increase of 25% from the last checklist. This is largely due the new wave of studies based on Integrative Taxonomy approaches. The number of alien species is also very remarkable: c. 30 species, 1.6% of the fauna. All classes are represented, although the single monoplacophoran is still known only by empty shells. The families represented in the Italian fauna are c. 290, an increase of 12% from the last checklist, partly due to new records and partly to new phylogenetic systematics. Compared with the Mediterranean malacofauna, the Italian component represents 81% in species and 92% in families, which makes it a very remarkable part of the Mediterranean fauna. Only less than 2% of the species are strictly endemic to the Italian waters. The next step will be the building of a database of geo/chrono-referenced records based on a critical screening of the immense literature, but also on the digitalization of several, 'pedigreed' private and public collections, which may in principle yield millions of records, spanning the whole extension of the Italian coasts and a long temporal series.

MARCO CURINI-GALLETTI[1], TOM ARTOIS[2], MAIKON DI DOMENICO[3], DIEGO FONTANETO[4], ULF JONDELIUS[5], KATHARINA M. JÖRGER[6], FRANCESCA LEASI[7], ALEJANDRO MARTINEZ GARCIA[4], JON L. NORENBURG[8], WOLFGANG STERRER[9], M.ANTONIO TODARO[10]

[1]Dipartimento di Medicina Veterinaria, Università di Sassari
[2]Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium
[3]Center for Marine Studies, Universidade Federal do Paraná, Curitiba, Brazil
[4]CNR, IRSA: Istituto di Ricerca sulle Acque, Verbania
[5]Department of Invertebrate Zoology, Swedish Museum of Natural History, Stockholm, Sweden
[6]Ludwig-Maximilians-University, Munich, Germany
[7]University of Tennessee, Chattanooga, USA
[8]Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, USA
[9]Bermuda Natural History Museum, Bermuda
[10]Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia

CONTRIBUTION OF SOFT-BODIED MEIOFAUNAL TAXA TO ITALIAN MARINE BIODIVERSITY

Out of the approximately 34 metazoan phyla, 23 have at least some representatives in the meiofauna size-class – which includes organisms passing through sieves ranging from 22–44 µm to 500 µm - with a number of phyla (among which Gnathostomulida, Kinorhyncha, Loricifera, Rotifera and Gastrotricha) which are exclusively meiofaunal. The study of meiofaunal organisms is comparatively recent: the first naturalist who began to explore this field was REMANE (1933). Later researchers on meiofaunal taxa uncovered an astonishing diversity, whereby a mere tea-spoon of marine sediment or of sand in a beach could yield a bewildering biological richness. Most classic ecological studies have focussed on the so-called 'hard-bodied meiofauna' (notably Copepoda and Nematoda), whose cuticle allows retrieval from fixed samples in conditions suitable for identification at the species level. This is not the case for the 'soft-bodied' meiofauna, which most often loose any diagnostic character after fixation, and whose study must be performed exclusively on living material. This led to a vicious cycle, resulting in a general awareness of the prevalence of Nematoda and Copepoda in terms of biomass and diversity. Yet, recent metabarcoding studies of environmental DNA revealed a comparable diversity of soft-bodied taxa – although in the vast majority of instances no names can be attached to the sequences retrieved, due to lack of correspondence in GenBank. This problem is exacerbated by the few taxonomic authorities, in cases on entire Phyla, available worldwide. Here we present a specialist-based survey on the knowledge of the diversity of soft-bodied taxa along the Italian coasts. The level of knowledge differs widely, from taxa with species with good dispersal powers and wide ranges (such as Rotifera and Gastrotricha), whose level of knowledge may be considered comparatively satisfactory (although new taxa, even at the genus level, are continuously being added to the Italian census), to groups (notably Xenacoelomorpha and Platyhelminthes), where species with restricted ranges are on the contrary prevalent, and any sample from previously unessayed station along the Italian coast reveals many, if not most, undescribed species, making the catalogue of the biodiversity of the Italian fauna an apparently endless task.

MARZIA BO, GIORGIO BAVESTRELLO

Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università di Genova

DEEP SEA MEDITERRANEAN EXPLORATION: CONTRIBUTION TO SPECIES (RE-)DISCOVERY AND BIOGEOGRAPHY

The Mediterranean Sea represents the type locality for a wide number of marine species, among which there are taxa described in ancient times as well as during the golden age of the first scientific explorations, in the late 1800s. Deep investigations carried out with remote destructive sampling methods including experimental fishing were firstly employed to describe the deep sea fauna of the basin. This material was rarely examined living, in good conditions or in high quantities and only sporadically was put into a proper ecological context with the result of poor descriptions, which easily lead towards a rapid increase of the number of supposedly rare or endemic deep species. In this context, an emblematic group is represented by anthozoans, solitary or colonial benthic species accounting for over 200 taxa in the basin, many of which have been firstly described back in the 19th century, but have never been collected again since very recent times. Modern deep-sea explorations, carried out below the scuba diving bathymetric limits, have greatly boosted, in the last two decades, the collection of deep circalittoral, bathyal and abyssal specimens from remote, off-shore areas (such as canyons ad seamounts) coupling it with *in vivo* observations. As a result, a large number of new discoveries, as well as re-descriptions have been carried out in many cases redefining the concept of deep rarity. Multiple samplings have added significant biological and ecological addenda to the current knowledge of these species, highlighting their functional role in the deep ecosystems. In addition, the updated, basin-scale distribution of many deep taxa allowed for new considerations on the origin and spreading of the deep fauna of the basin giving a more consistent insight in the biogeography of the Mediterranean Sea.

FRANCESCO COLLOCA[1,2], FABIO FIORENTINO[1], FABRIZIO SERENA[1]

[1]Istituto per le Risorse Biologiche e le Biotecnologie Marine, Consiglio Nazionale delle Ricerche
[2]Dipartimento Biologia e Biotecnologie “C. Darwin”, Sapienza Università di Roma

OSTEICHTHYES AND CHONDRICHTHYES OF THE ITALIAN SEA: TOWARD A NEW CHECKLIST OF A COMMUNITY OF SPECIES UNDER FAST CHANGE

The Mediterranean Sea is regarded as a biodiversity hot-spot for many taxa of marine organisms, including bony and cartilaginous fish, which are represented by over 600 species and a conspicuous number of endemites. Despite the wealth of studies during the last 30 years, the actual number of species living in the basin is still debated, with estimates ranging from 650 to 681 (of which at least 80 Chondrichthyes). A steadily increasing number of non-indigenous species (NIS) is reported: recent estimates recorded 165 NIS, of which 71 Osteichthyes and 3 Chondrichthyes are non-occasional presences. Along the Italian coasts, ~75% of the Mediterranean species have been recorded (442 Osteichthyes and 70 Chondrichthyes) with an increasing trend for the bony fish and a decreasing one for the cartilaginous fishs, as highlighted by the 2009 checklist of the Società Italiana di Biologia Marina. The Osteichthyes are present with 26 orders and 109 families, whereas the Chondrichthyes are represented by 9 orders and 29 families. It is an extraordinary faunistic richness, the causes of which are bound in the paleoclimatic events of the Quaternary, which worked as “biological pump” of diversity, with multiple waves of colonisations and extinctions of species with distinct biogeographic affinities. The result is the current ichthyological fauna comprising species of a variety of chorological categories, and a heterogeneous distribution of the specie along the Italian coasts, with sharp differences among the biogeographic sectors. During the last 30 years we have witnessed remarkable changes in the faunistic composition of the fish communities in the Italian seas, due to the combined effect of the arrival of new species, of the climate change and the process of meridionalization and tropicalization of the biota, and finally of the anthropic impacts (fishery at first). From the conservation point of view, the data on the impact of fishery highlight a pervasive overfishing, and the decline (sometimes until disappearance) of many species of Chondrichthyes in some areas. Recent evaluations by IUCN at the regional scale have pointed out on the one side a low proportion (12%) of bony fish species threatened, but on the other side very alarming conditions of the cartilaginous fishes, with 39 species (53.4%) at risk of regional extinction, and among them, 20 (27%) Critically Endangered. One of the most important sources of data on the presence and the status of fish species in the Italian seas are scientific fishery campaigns carried out in the last decades, and integrated with faunistic surveys at local scale. We are witnessing a situation of rapid change in the composition of the fish community in the Italian seas, with the continuous arrival of new species and the rarefaction of several autochthonous ones. A continuous monitoring of their diversity requires the development of structured information systems, able to integrate traditional scientific data with those available from less consolidated sources (e.g. “citizen science” or “local ecological knowledge”) and from new tools of presence/absence data acquisition (es. DNA-barcoding).

SABRINA LO BRUTTO, DAVIDE IACIOFANO

Dipartimento STEBICEF, Università degli Studi di Palermo

SICILIAN AMPHIPODA FAUNA: GAPS AND CHALLENGES

The species richness, abundance and assemblage structure of the marine crustacean amphipods from Sicilian coast (southern Italy) were studied at different stations and from literature, comprising different habitats, such as *Posidonia oceanica* or *Sabellaria alveolata* reef. This dataset led to the identification of 127 species and 31 families, 16 species of which were new records for Sicily. Some species were present on the four sides of the island, North – East – South and Western coast, being present in the different biogeographical regions of the central Mediterranean (i.e. *Caprella acanthifera, Dexamine spiniventris, Hyale camptonyx* and *Ericthonius brasiliensis*). Other species, *Biancolina algicola, Microdeutopus anomalus, Tethylembos viguieri, Pseudolirius kroyerii, Cressa dubia, Guernea coalita, Gammarus insensibilis, Hyale pontica, Microjassa cumbrensis, Melita hergensis, Perioculodes aequimanus, Podocerus schieckei, Lepidepecreum longicornis* and *Urothoe elegans*, were present in single localities. The mostly diversified families are the Aoridae (with fiteen species), the Caprellidae (with fourteen species) and Maeridae (with twelve species). As a result of this study, new species can be added to the inventory of the Italian marine amphipod fauna. Some of the actual non indigenous species distribution hotspots are located in Sicily, which accounts for high number of alien taxa. Regarding the Sicily Island it has been outlined its role as crossroad for invasions routes from the Atlantic Ocean and the Indo-Pacific area. The role of Sicily Island as sentinel for Mediterranean bioinvasions is discussed. Unfortunately, literature on marine Sicilian Amphipoda fauna is limited. This southern Italian region has been scarcely investigated for the last fifty years, and scientific world should be aware of this gap.

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

[1]Dipartimento di Biologia e CoNISMa LRU, Università degli Studi di Bari "Aldo Moro"
[2]OCEANA, Madrid, Spain

DEEP-SEA FAUNA BEHAVIOUR: WHAT WE COULD NOT KNOW UNITL FEW YEARS AGO

The development of exploration technologies, from Remotely Operated Vehicles (ROVs) to landers and dropcams, is unveiling many aspects about the life of animals living in the mesophotic (ca. 50–200 m depth) and aphotic zones (>200 m depth) of the Mediterranean Sea. These places are not accessible to scuba diving, remaining mostly unseen until the last two decades. Together with the discovery of peculiar communities inhabiting and structuring the deep seabed, detailed studies are now allowing to deepen in the observation of the specimens, to see where do they live and how do they behave, *de facto* doing what has been done since many centuries on land and on coastal areas. This contribution reports some examples of unexpected behaviours in mesophotic and deep-sea species from four different phyla, along the Italian coasts. The red sea pen *Pennatula rubra* (Ellis, 1761) (Cnidaria: Anthozoa: Pennatulacea) has been observed performing a slow withdrawal behaviour after a mechanic disturbance. The study of a population off the coasts of Calabria (Ionian Sea) showed that this process requires from 3 to 6 minutes for the complete expulsion of the water from the colony, in order to significantly reduce its size and withdraw into its hole. Despite this species was first described at the end of the 17th century, such behaviour was never mentioned, differently from the rapid (i.e. a few seconds) withdrawal ability of certain pennatulacean species. Moreover, some colonies of *P. rubra* were observed out of the sediment, inflating themselves with seawater and getting carried by currents as a sort of dispersal behaviour. The elpidiid *Penilpidia ludwigi* (von Marenzeller, 1893) (Echinodermata: Holothuroidea: Elasipodida) was occasionally captured above the seabed in sediment traps and, based on these records, its swimming ability was assumed, even though it was under discussion. ROV surveys allowed to observe this species in the Aeolian Archipelago (Tyrrhenian Sea), documenting for the first time its behaviour and describing its mode of swimming. Active swimming using strokes of the tentacle crown is combined with drifting benefiting of the current, the former used for fast escape the latter mainly for energy-saving displacement. Swimming behaviour allows *P. ludwigi* to exploit various deep-sea habitats including seamounts, canyons and ridges inaccessible to non-swimming deposit feeders. The spider crab *Anamathia rissoana* (Roux, 1828) (Arthropoda: Malacostraca: Decapoda) is characterized by long, thin and pointed legs, not particularly efficient to walk on the muddy bottoms where the species live. This crab was observed climbing on the colonies of the bamboo coral *Isidella elongata* (Esper, 1788) and on antipatharians to catch its small preys. Such peculiar behaviour as coral-climber could justify the particular shape of its legs. The robust cusk-eel *Benthocometes robustus* (Goode & Bean, 1886) (Chordata: Actinopterygii: Ophidiiformes) is characterized by a particular shape with a slender tail and a robust head that induced the development of several theories to justify such unusual morphology. This species was observed swimming vertically behind coral branches as a camouflaging strategy, in association with alcyonaceans and antipatharians.

ELISA COLASANTO, LORIS GALLI

Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università di Genova

SOME NEW DATA ON THE ITALIAN PYCNOGONIDA

The Pycnogonida is a poorly known class of marine arthropods. Lehmann et al. (2014) report that 56 species are known for the Mediterranean Sea. In the Italian seas 45 species are recorded. Few cases of in-depth research on the Pantopoda of a specific area are known in the Italian literature and they are all focused on South-Central and Southern Italy. Other sporadic collections are reported in Faraggiana (1940), Krapp (1975), Chimenz & Cottarelli (1986), Arnaud (1987), and Chimenz Gusso (2000). Some new data on specimens collected in the Ligurian Sea (mainly in the Portofino shallow waters) and in Tyrrhenian Sea (especially near Torrevaldaliga, Civitavecchia, RM) allowed us to deepen the knowledge about the distribution of the already known species and to report the presence of a species not still recorded in the Italian fauna: *Endeis biseriata* Stock, 1968.

FRANCESCO TIRALONGO, GIUSEPPINA MESSINA, BIANCA MARIA LOMBARDO

Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università degli Studi di Catania

ABUNDANCE TRENDS AND NEW RECORDS OF COASTAL FISH IN ITALIAN SEAS: CONTRIBUTIONS OF UNDERWATER PHOTOGRAPHY AND LOCAL ECOLOGICAL KNOWLEDGE

In the Mediterranean Sea, the coastal fish community is threatened by several factors, such as overfishing, habitat destruction, pollutants, global warming and alien species. Although the study of littoral fish assemblages is complex and require a considerable effort, accurate measurements of fish diversity are of great importance in order to obtain useful data for conservation and management programs. Underwater photography can be a very useful tool to document the fish diversity in a certain area. Furthermore, the underwater photography can be particularly important for the detection of cryptobenthic species, such as some species of blennies, gobies and clingfishes. This technique, combined with the local ecological knowledge (LEK) from experienced divers, is an effective and low-cost tool to examine the variation of fish biodiversity over the time, thus obtaining precious information on spatio-temporal variation of fish communities abundance and diversity. In our study, we interviewed a total of 21 experienced divers (with at least 15 years of experience) in order to collect data about abundance's trends of seven selected coastal fish species in Italian waters, and to collect photographically documented records of rare and/or little known fish species. Among our main results, we find a positive trend over time of the Blue Runner (*Caranx crysos*) abundance. This thermophilic species appears to be more common than in the past and seawater warming could be the main responsible for the abundance increase observed. Contrariwise, the Dusky Grouper (*Epinephelus marginatus*) showed a negative trend in abundance over time. In this case, overfishing could be the main responsible of the collapse of the population. A total of 51 specimens and 30 rare and/or little-known species were recorded and photographically documented. In conclusion, even though visual census techniques are valuable tools for a quali-quantitative assessment of cryptobenthic ichthyofauna, if properly used, the underwater photography, combined with LEK, is an effective tool for monitoring spatio-temporal changes of coastal fish communities.

Simposio II

La fauna in Italia

Simposio dedicato ad Augusto Vigna Taglianti

Seconda parte

Gli ambienti terrestri e d'acqua dolce

Coordinatori:
Marco A. Bologna, Alessandro Minelli, Mazio Zapparoli

Lettura plenaria

ALESSANDRO MINELLI[1], MARCO A. BOLOGNA[2], MARZIO ZAPPAROLI[3]

[1]Dipartimento di Biologia, Università di Padova
[2]Dipartimento di Scienze, Università "Roma Tre"
[3]Dipartimento per la Innovazione nei sistemi Biologici, Agroalimentari e Forestali, Università della Tuscia

ITALY'S LAND AND FRESHWATER FAUNA: DIVERSITY AND PERSPECTIVES ON ITS STUDY AND CONSERVATION

A landmark in the history of studies on the country's fauna was the publication of the *Checklist della fauna italiana* (1992-1995). Of the 55.656 metazoan species in this list, to which contributed 272 specialists from 15 countries, 47.225 are nonmarine. The publication of the *Checklist* has stimulated additional work, often focused on hitherto less investigated taxa, and the birth of large-scale projects – the most important of which is *Fauna Europaea* – which have in turn produced a positive feedback on the knowledge of the Italian fauna. Impressive number of additions to the *Checklist* or to faunistic databases have been obtained in the framework of national or European projects. Sizeable numbers of species new to science have been described non only in groups rich in micro-endemics (e.g., peritelini weevils) but in virtually all major taxa, including vertebrates. In parallel, dramatic number of nominal taxa have been synonymized under valid names, e.g. in Chilopoda. As a result, the *Checklist* needs a radical refurbishment, a target currently being addressed by a dedicated project started in 2018. Dark holes still need be addressed, e.g. phorid and other flies, many groups of mites, and nematodes. Cryptic diversity is emerging in groups recently investigated with molecular tools, but the agreement between molecular operational taxonomic units and taxonomic species remains sometimes problematic, as exemplarily shown by a recent study in the scarab genus *Pachypus*. Progress depends on the availability of resources, the most important of which are large and well-curated collections and a substantial number of active and experienced taxonomists. As for collections, a nation-wide inventory of natural history collections is progressing under the care of the Associazione Nazionale Musei Scientifici, and the idea of a national museum of natural history has been refreshed of recent. As for human resources, worth mentioning is the strong contribution of nonprofessional taxonomists, among which are the authors of ca. 45% of the 53 volumes published to date in the *Fauna d'Italia* series by the Comitato Scientifico per la Fauna d'Italia, also responsible for the *Checklist*. Precious resources, not only for the specialists, are the identification keys, including the web-based interactive keys of which examples for the tachinid flies and the geophilomorph centipedes have been developed in Italy. While research progresses, the distribution ranges of many species is steadily changing, sometimes for natural reasons (especially climatic trends, e.g. *Dytiscus latissimus* and *Araschnia levana*, now extinct in Italy; *Leistus punctatissimus*, progressively restricted to higher altitude sites in the Eastern Alps), sometimes because of human agency, causing massive introduction of often successful aliens. Against this picture, the zoological community is called to establish and implement priorities in the study and in conservation activities, such as some recent LIFE projects aimed at the implementation of monitoring systems for species considered in the Habitat Directive, or the prioritization activities managed by ISPRA to control the spread of alien species.

Simposio II

La fauna in Italia

Simposio dedicato ad Augusto Vigna Taglianti

Seconda parte

Gli ambienti terrestri e d'acqua dolce

Coordinatori:
Marco A. Bologna, Alessandro Minelli, Mazio Zapparoli

Presentazioni orali

PAOLO PANTINI[1], MARCO ISAIA[2]

[1]Museo Civico di Scienze Naturali "E. Caffi", Bergamo
[2]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino

ARANEAE.IT: THE ONLINE CATALOG OF ITALIAN SPIDERS (ARACHNIDA: ARANEAE)

We here present the Catalog of Italian spiders, realized on the base of the information available in scientific literature (1125 references) regarding spider species distribution in Italy. At present, 1670 species and subspecies are known for Italy, of which 342 are endemics, grouped in 434 genera and 53 families. For mainland Italy we recorded 1538 species, 423 species for Sicilia and 520 for Sardegna. Thanks to recent efforts, knowledge in spider biodiversity in Italy has rapidly increased, going from 404 species known at the end of XIX century, to 1400 in the 1990s, and to the current 1670. However, knowledge on the distribution and the ecology of the Italian species is far from being complete and it seems likely that there are still new species to be found or described. The Italian spider fauna is highly characterized by the presence of endemic species (342), with a high percentage of species whose range falls entirely within the Italian territory. Concerning families, Linyphiidae present the highest number of species (477) and the highest number of endemics (114). Gnaphosidae (166) and Salticidae (144) follows in terms of species number, while Dysderidae (72) and Agelenidae (38) follows as number of endemics. Information regarding the regional distribution reveals great unbalance between northern and southern Italy, with very scarce records for some regions in the South. This work is accompanied by an online version where all the information is exhaustively listed and regularly updated by the authors. Moreover besides spiders, the online version reports the species lists of other Arachnid orders occurring in Italy, namely Opiliones, Palpigradi, Pseudoscorpionida, Scorpiones, Solifugae.

GIANANDREA LA PORTA[1], MATTEO PALLOTTINI[1], VALERIO ORIOLI[2],
ELDA GAINO[1], ENZO GORETTI[1]

[1]Dipartimento di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia
[2]Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano-Bicocca

USING N-MIXTURE MODELS TO EVALUATE POPULATION SIZE IN ODONATA: A CASE STUDY ON *COENAGRION MERCURIALE*

Accurate measures of species abundance are essential for studies of ecology and biodiversity management, but conducting a census (i.e. counting all individuals) of wild animal species is usually not possible. Hence, modeling of survey data can be a powerful method for estimating total abundances and population trends. Traditional approaches usually exploit sampling designs aimed to evaluate the factors influencing the detection of individuals. Over the past decade, imperfect detection and zero inflation in count data have been extensively discussed in the ecological literature and N-mixture models have been increasingly used to estimate abundance on the basis of repeated species counts. Case studies, which have tested these models, have demonstrated success for some taxa, but some doubts remain over the appropriateness of standard N-mixture models for many species. In the present study, we compared population estimates obtained using the capture-mark-recapture (CMR) approach and the N-mixture models. We focused on *Coenagrion mercuriale*, a damselfly classified as ‘Near Threatened’ by the Italian IUCN Red List and listed in Annex II of the EU Habitats Directive, for which accurate abundance data are needed for conservation assessments. Field sessions were carried out along five transects parallel to ditch banks from June to early July 2018 in Central Italy. A total of more than 800 adult specimens was counted and marked on eight different occasions. Our results revealed that estimates of abundance gathered by N-mixture models showed limited differences with the ones gathered through the mark-recapture approach, even though the estimates were sensitive to the maximal theoretical abundance cutoff. Detection probability for the species was almost constant across the transects and over time, with N-mixture estimates markedly higher than those of CMR models. It is known that N-mixture models perform well when basic assumptions are met and detection probabilities are moderate. The case study revealed that even in damselflies N-mixture models represent a promising alternative to the capture-recapture protocol when the main goal of the investigation is the determination of population size. Indeed, these models have the advantage to be cost-effective and less invasive than other methods, requiring only spatially and temporally replicated count data of unmarked individuals.

MATTEO BRUNETTI[1], MATTEO MONTAGNA[1], GIULIA MAGOGA[1], MAURIZIO BIONDI[2],
MATTIA IANNELLA[2]

[1]Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano
[2]Dipartimento di Medicina clinica, Sanità pubblica, Scienze della Vita e dell'Ambiente, Università degli Studi dell'Aquila

PHYLOGEOGRAPHY OF *CRYPTOCEPHALUS BARII* (COLEOPTERA: CHRYSOMELIDAE) AND INSIGHTS INTO THE RISK OF EXTINCTION FOR HIGH ALTITUDE ENDEMIC SPECIES

In the context of the actual global warming understanding how species, especially those adapted to cold or high-altitude environments, reacted to the climate changes in the past and trying to predict their future distribution can greatly improve the effectiveness of conservation actions. The series of glacial events separated by interglacial events that took place during the Quaternary period have significantly contributed in shaping the distribution and genetic diversity of animal and plant species that we can observe today. During this period, cold-adapted organisms experienced range expansion and contraction in response to the temperature decrease and increase, respectively. The aim of this study was to investigate the phylogeography of *Cryptocephalus barii*, a cold-adapted species endemic of Orobie Alps. We deeply explored the actual distribution of the species that results fragmented, with populations isolated on different mountain peaks. A portion of the mitochondrial marker COI was used to estimate the relationships among populations, their divergence time and the most probable migration model that led to the present distribution. Additionally, the current habitat suitability of *C. barii* was assessed in order to predict the future distribution in a global warming scenario. The major divergence events, occurred from ~750,000 to ~150,000 years ago, that characterized the evolutionary history of the species almost followed the pattern of temperature oscillations, with increased connections among populations during glacial periods and isolation on mountain massifs during interglacials. Moreover, since *C. barii* resulted to be highly sensitive to air temperature oscillations, the expected increase of temperature due to global warming will reduce the presence of suitable areas within the species range, leading to the possible extinction of the species in the next 50 years. This study shows how species adapted to high-altitude environment can react to changes in the climate and highlight the risk of extinction for such kind of species in a future global warming scenario.

EMILIO BALLETTO[1], FRANCESCA BARBERO[1], SIMONA BONELLI[1], LUCA PIETRO CASACCI[1.2], LEONARDO DAPPORTO[3]

[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino
[2]Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland
[3]Dipartimento di Biologia, Università degli Studi di Firenze

ITALIAN BUTTERFLIES (PAPILIONOIDEA) – AN OVERVIEW

Italy is home to 290 nominal species of Papilionoidea (or 280, according to Wiemers et al. 2019), but since as many as eight of these were first observed on Italian soil during the last 10 years, 'new' discoveries remain possible, in the future. Butterfly distribution on the Italian territory is not uniform and species richness follows a roughly North to South trend along the Apennines, with 227 species occurring in Piemonte and 115 (now probably 109) in Puglia. The least species-rich Italian administrative Region is Sardinia (58 sps), notwithstanding its high proportion of endemic and sub-endemic species. Italian butterflies generally have only one yearly generation (70%), but 16% have two generations and 14% are multivoltine or may reproduce and fly year-round. Some of the species occurring in the particularly harsh climates experienced above the treeline may have biannual or even tri-annual development, depending on conditions. The overwintering stage is normally that of caterpillar (70% of cases). Larvae of Italian butterflies feed on 53 plant families, in total, but 13 of the latter may be consumed, more or less occasionally, by only one species. Poaceae are food for as many as 89 butterfly species, followed by Fabaceae (47), Rosaceae (32) etc. From the dietary point of view, larvae are mostly oligophagous (feed on one hostplant family: 58%). Monophagous species, feeding on a single plant species, are 25 and occur only among the strictly sedentary (56%) or scarcely vagile ones (44%). Truly polyphagous species, consuming plants of more than two families, are only 11 and show no ecological regularity. Most Italian lycaenids are myrmecophilous, generally of the regularly myrmecophilous type (Fiedler's type 3: 29 sps), whose mature larvae are (almost) invariably associated to ants of one or more genera. Obligate myrmecophiles are only four and are all part of the genus *Maculinea*. In sharp contrast to tropical species, adults of Italian butterflies generally occur in transitional environments (e.g. grasslands below the treeline: 51%), while those characteristic of various types of woodlands are only 9% (90% in Madagascar). The most species-rich vegetational level is the lower montane (within or nearby deciduous oak woodlands: 30%); 17% of Italian butterflies occur within the Mediterranean vegetation (within or around ilex woodlands) as well as at or above the treeline, in the Alps. The most habitat selective species (e.g. microthermic, hygrophilous, etc.) and those occurring in the most extreme environments (e.g. screes) are also the most stenophagous and the least vagile. As expected, migratory species (7) do not show any altitudinal preference and most of them may occur indifferently in all vegetational belts, only excluding the few rather thermophilous ones. Italian species are generally mesophilous, both as concerns temperature and humidity levels. Thermophilous butterflies dominate the Mediterranean belt. Xerophilous butterflies are principally concentrated in the Mediterranean and Alpine vegetational levels. Few species are hygrophilous and occur mainly in the Padano-Venetian plains or in the subalpine vegetational belt. Only one Italian butterfly, *Lycaena helle*, is presumably extinct (RE) and only one*, Euphydryas maturna* (also known as *E. italica*) is critically endangered (CR).

PIERFILIPPO CERRETTI[1], YDE DE JONG[2], DAVIDE BADANO[3]

[1]Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma
[2]Department of Botany, Natuurhistorisch Museum, Rotterdam, Netherlands
[3]DISTAV, Università di Genova

TWO-WINGED INSECTS OF THE ITALIAN FAUNA IN NUMBERS

With ca. 160000 known species worldwide, Diptera are one of the hyperdiverse groups of hexapods and arguably the most important in terms of impacts regarding both human and animal health given the large number of hematophagous species transmitting devastating diseases (e.g., mosquitoes, sandflies, midges, tse-tse flies), or parasites causing myasis (e.g. blow flies, bot flies). Moreover, dipteran damages to managed and natural ecosystems such as crops and forests (mostly caused by nematocerans and acalyptrates) is nearly equally important. However, there are also miriads of beneficial species in this amazing group of insects, contributing to several fundamental ecosystem services such as plant pollination (e.g., flower flies, bee flies, as well as many calyptrates), population control of pests acting as predators and parasitoids (e.g., robber flies, bee flies, flower flies, tachinid flies), besides being an important component of freshwater and terrestrial trophic webs. The Italian fauna, with approximately 7000 dipteran species, is the most diverse among European countries. In this talk we will give an overview of the Diptera diversity in Italy, analyzing by family and genera for the first time the data from the Fauna Europaea project data base.

SANDRO TRIPEPI

Societas Herpetologica Italica

AMPHIBIANS AND REPTILES OF ITALY: KNOWLEDGE AND CONSERVATION

The SHI published in 2006 and subsequently updated in 2009 the “Atlas of Amphibians and Reptiles of Italy”, which marked the first milestone on the knowledge of the presence and distribution of herpetofauna in Italy. According to the atlas and other subsequent publications, the following species can be counted: 44 species of Amphibians (of which 19 are Caudata and 25 Anura) and 56 species of Reptiles (of which 11 Testudines and 45 Squamata). In recent years some new species have been established on the basis of genetic studies (i.e. the splitting of *Salamandrina*, *Hierophis viridiflavus*, *Hyla, Emys*). To these native species we must add 20 allochthonous specie (7 Amphibians and 13 Reptiles), introduced and naturalized in various parts of Italy. The isolation of Sardinia and the presence of climatic refugia, that occurred during the cold geological periods, were the main factors that allowed the survival of a consistent number of endemic species in Italy: 14 Amphibians (of which 11 are Caudata and 3 Anura) and 5 Reptiles (of which 1 Testudines and 4 Squamata). The threats to the survival of the reptilian populations are mainly due to the disappearance and alteration of habitats, including pollution, but the arrival of predators and alien competitors also begins to play an important role. In large areas of Po plain the native green frogs (*Pelophylax lessonae-esculentus* complex) are replaced by allochthonous Balcan green frogs (*Pelophylax “ridibundus” s.l.*). The main causes of decline of the amphibians, in addition to the disappearance and alteration of wetlands, which are also the main cause of the decline of *Emys* species too, are represented by the introduction of fish (in particular alien species, but not only) and shrimps, but, above all, by the fearsome pathology of chytridiomycosis, due to the fungus *Batrachochytrium dendrobatidis*, now widespread throughout the Apennines from Liguria to Calabria. Of the 14 species of amphibians analyzed with quantitative PCR, 8 are infected, including some Italian endemites such as *Salamandrina terdigitata* and *Lissotriton italicus*. However, the intensity of infestation relative to each individual seems to be low.

BRUNO MASSA

Dipartimento di Scienze Agrarie, Alimentari e Forestali, Università di Palermo

ITALIAN AVIFAUNA: CHANGES OF STATUS OF BREEDING SPECIES THROUGH THE LAST 100 YEARS

About 550 bird species are known from Italy; ca. 280 (51%) are breeding. Following the Italian Red List (Peronace et al. 2012) 240 species are considered at some threat degree: 3 became extinct, 6 are critically endangered (CR), 22 endangered (EN), 46 vulnerable (VU), 26 near threatened (NT) and 138 are classified as Least Concern (LC). BirdLife International (2017) updated the threat level of European species listing some of them within the "Spec1-3" (according to different threat level); BirdLife International (2017) included only 2 CR Italian species within Spec1 and another 1 within Spec3, 5 Italian EN species within Spec1, 1 within Spec2 and 7 within Spec3, while they listed 4 Italian VU species within Spec1, 5 within Spec2 and 13 within Spec3. Overall, only 38 out of 73 threatened species included in the Italian Red List are recorded within the Spec1-3 at European level. However, in some cases the knowledge of the historical status of species is basic to understand if the species are actually decreasing (= true diminution) or if through the decades they alternated periods of numerically low populations with other periods of increasing numbers (= fluctuation, outward diminution). Some examples of different threat level are reported by the author, some cases of true diminution and other cases of population fluctuations that suggest an intrinsic resilience, due to different factors, mostly unknown to us. Generally, sedentary species are the most threatened and at diminution risk [e g., *Alectoris graeca* (VU, Spec1), *Perdix perdix* (LC, Spec2)], while migrant species breeding in Europe may fluctuate over long lapse of time [e.g., *Coturnix coturnix* (LC, Spec3), *Streptopelia turtur* (LC, Spec1)]. There is some interesting case of species critically endangered (if not extinct) in some Italian regions, but increased in others [e.g., *Aquila fasciata* (CR, Spec3)] or that during the last century had periods with high populations and others declining [e.g., *Falco biarmicus* (VU, Spec3)]; in addition a group of breeding species, following a general expansion, colonized only recently Italy [e.g., *Ciconia ciconia* (LC, NonSpec), *Platalea leucorodia* (VU, NonSpec), *Hieraaetus pennatus* (NonSpec)] or increased unusually in the Italian territory [e.g., *Larus michahellis* (LC, NonSpec), *Columba palumbus* (LC, NonSpec), *Apus pallidus* (LC, NonSpec), *Merops apiaster* (LC, NonSpec)]. The decreasing of some species gives cause for concern and should deserve much attention both from the auto- and from the syn-ecological point of view [e.g., Laniidae (Spec2), *Alauda arvensis* (VU, Spec3), *Melanocorypha calandra* (VU, Spec3), *Luscinia megarhynchos* (LC, NonSpec)]. Finally, there are sedentary species of Passeriformes whose Italian populations cover an isolated southern geographical area in the Apennines and in the islands of Sicily and Sardinia; their taxonomic status should be studied in great detail; among them *Coccothraustes coccothraustes* (LC, NonSpec), *Pyrrhula pyrrhula* (VU, NonSpec), *Loxia curvirostra* (LC, NonSpec).

Simposio II

La fauna in Italia

Simposio dedicato ad Augusto Vigna Taglianti

Seconda parte
Gli ambienti terrestri e d'acqua dolce

Coordinatori:
Marco A. Bologna, Alessandro Minelli, Mazio Zapparoli

Poster

LUCA BORIO, MARCO ISAIA

Dipartimento della Vita e di Biologia dei Sistemi, Università degli Studi di Torino

A SURVEY OF THE SPIDER FAMILY DYSDERIDAE IN BASILICATA (ARANEAE, DYSDERIDAE)

Spiders of the family Dysderidae, also known as woodlouse spiders, primarily occur in Eurasia and Northern Africa. The core of their diversity is the Mediterranean basin, where they exhibit a very high rate of endemism, especially in Southern Europe. In Italy, Dysderidae are represented by 10 genus and 81 species, of which 72 are considered endemic (Pantini & Isaia, 2018). At the regional level, data regarding the region of Basilicata are very scarce, with only 6 species and 3 genera recorded. The high potential of the region in disclosing the diversity of Italian Dysderids stimulated the setting up of a faunistic survey in the region. The survey took place in spring of 2017 over an area of about 240 km^2 including the Regional Park of Gallipoli Cognato and Piccole Dolomiti Lucane, close to municipalities of Gorgoglione and Stigliano (Province of Matera), ranging from an altitude of 555 to 1088 m. We used pitfall traps, placed in pastures and broadleaved woods habitat. An amount of 242 specimen of Dysderidae were collected, belonging to 5 genera and 10 species. Several species found during the survey represents new regional records for Basilicata. In several cases we collected species that were previously known for one or a few localities: among these, it is worth to mention *Dysdera aberrans* Gasparo, 2010 that was uniquely known from one locality in Molise, *Dasumia diomedea* di Caporiacco, 1947, previously known for Isole Tremiti and one location in Calabria, and *Harpactea sardoa* Alicata, 1966 so far known for Sardinia and Circeo.

VALERIO CARUSO, PIERFILIPPO CERRETTI

Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma

FAUNA D'ITALIA: TACHINIDAE

In this work we provide an overview of the diversity of the parasitoid fly family Tachinidae in Italy, aimed at preparing an outstanding volume of the "Fauna d'Italia" series. Tachinids are a "megadiverse" family of flies with over 10.000 described species in the world, and one of the most diverse in morphology and natural history among Diptera. All tachinids develop as internal parasitoid of other arthropods, mainly phytophagous insects like Lepidoptera and Hemiptera, eventually killing their host. The adults, on the other hand, are mostly flower visiting, and feed on nectar and floral exudates. Given their astounding diversity and parasitoid habit, tachinids have attracted the interest of pure and applied biologists, especially for their potential applications in biological control programs of various agricultural and forestry insect pests. The Italian fauna counts about 650 species (in 236 genera) and is characterized by several endemic taxa; a key to the 236 genera and the Mediterranean species will be prepared and illustrated to make the identification of a notoriously difficult group easier, even for beginners.

PIERO COSSU[1], DARIA SANNA[2], ILENIA AZZENA[1], FABIO SCARPA[1], TIZIANA LAI[1], ALESSIO NIFFOI[1], ANTONIO VARCASIA[1], MARCO CASU[1]

[1]Dipartimento di Medicina Veterinaria, Università di Sassari
[2]Dipartimento di Scienze Biomediche, Università di Sassari

SEARCHING FOR MOLECULAR EVIDENCE OF A PURE POPULATION OF *ESOX CISALPINUS* INTRODUCED IN SARDINIA

The Cisalpine or Southern pike (*Esox cisalpinus*) (syn. *E. flaviae*) (Teleostei: Esocidae) is a species autochthonous to North-Central Italy, whose natural populations are increasingly declining. Our recent genetic study based on mtDNA markers recorded *E. cisalpinus* in the Sardinia island (Italy), where the species has been introduced from about a decade, starting from a conspicuous number of individuals. Here we report a preliminary survey carried out by means of 15 microsatellite markers aimed to ascertain whether the Sardinian population comprises only pure pikes belonging to *E. cisalpinus* or introgressive hybridization with the Northern pike (*E. lucius*), allochtonous to Italy, occurs among Sardinian individuals. Furthermore, we aimed to shed further light into the demography of this population. After having screened each individual for Cytochrome c Oxidase subunit I gene to assess the membership to *E. cisalpinus* or *E. lucius*, we compared individuals from the artificial lake of Alto Flumendosa (Sardinia) with individuals from the Trasimeno lake (Centro Ittiogenico, Umbria, Italy), which are known to be pure *E. cisalpinus*, and pure individuals of *E. lucius* from the Drava river (Northern Croatia). Results based on Factorial Correspondence Analysis (FCA) and Bayesian clustering showed a sharp separation between *E. cisalpinus* and *E. lucius*. In this context, the Sardinian population comprises only pure individuals of *E. cisalpinus*. Not surprisingly, genetic divergence between *E. lucius* and *E. cisalpinus* populations is more than twice that between conspecific populations of *E. cisalpinus*, as evidenced by Weir & Cockerham's and Jost's indices of genetic differentiation (FST = 0.129, DEST = 0.424, $P < 0.001$ for Alto Flumendosa; FST = 0.160, DEST = 0.489, $P < 0.001$ for Trasimeno). Furthermore, Sardinian individuals of *E. cisalpinus* appear to be genetically distinct related to their conspecifics from the Trasimeno lake (FST = 0.078, DEST = 0.209, $P < 0.001$), suggesting a different geographic origin of Cisalpine pikes introduced in Sardinia. The small population effective size recorded for the Sardinian population (Ne = 33, based on the Linkage Disequilibrium method), leads to assume that either overall abundance of Cisalpine pikes in the Sardinian lake is small, or few individuals might have contributed to the gene pool of this population. Such a finding likely reflects both a low recruitment rate in the Alto Flumendosa lake, as consequence of the absence of suitable spawning areas for pikes, and the disproportion in contribution to the reproductive output of large females in Esocidae. Results here obtained highlight some controversial issues. Indeed, in the first instance, the presence of a further non-native species in Sardinia should impel the regional government to implement freshwater controls. Yet, the fact that this population seems to be formed only by pure *E. cisalpinus* (i.e. not introgressively hybridized by Northern pike's gene pool as detected in other peninsular localities) outlines its potential importance for the conservation of Cisalpine pike, as in the near future it may be considered as a further natural reservoir for preserving the gene pool of *E. cisalpinus*.

GABRIELE COSTA, BIAGIO VIOLI, GIORGIO BAVESTRELLO, MAURIZIO PANSINI, MARCO BERTOLINO

DiSTAV, Università degli Studi di Genova

A NEW GROWTH FORM OF *APLYSINA AEROPHOBA* (NARDO, 1833) (PORIFERA, DEMOSPONGIAE) IN SEMI-SUBMERGED CAVES OF THE ITALIAN COASTS

For the lack of mineral skeleton (siliceous or calcareous) and presence of very similar spongine fibres, identification of the Verongida Bergquist, 1978 order sponges is always highly difficult; in the Mediterranean Sea, in fact, the two species *Aplysina aerophoba* (Nardo, 1833) and *Aplysina cavernicola* (Vacelet, 1959) (Aplysinidae) are distinguishable from their external morphologies and from the habitat in which they live, but often due to the high degree of phenotypic variability only differentiation based on morphological attributes is debatable. For these reasons, an integrated approach with morphological and genetic studies is needed. Recent studies have shown that the COI (cytochrome c oxidase subunit I) is an excellent marker for differentiating the species of various *taxa*. In this study, conducted in four semi-submerged caves in Ligurian Sea and Ionian Sea, we examined several specimens of sponge that we attributed to the genus *Aplysina*, but with a very particular growth form and different from that known for the species of this genus. Thanks to the DNA study (COI) it was possible to assign the studied specimens the name of *A. aerophoba*, one of the two species present in the Mediterranean Sea, thus documenting a new growth form and a habitat in which it had never been found before.

VIVIANA DI PIETRO[1], SILVANA PIERSANTI[2], MANUELA REBORA[2], GIANANDREA SALERNO[3], LEONARDO GIONTELLA[2], OLA M. FINCKE[4]

[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino
[2]Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia
[3]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università di Perugia
[4]Department of Biology, University of Oklahoma, Norman, U.S.A.

ANOTHER CHALLENGE IN THE SEXUAL LIFE OF A MALE DAMSELFLY: A FIELD STUDY ON FEMALE COLOUR POLYMORPHISM

Female-limited colour polymorphism is a character of many Odonata species, in particular damselflies. Typically, polymorphic species present one female morph that resembles the male (andromorph) and one or more females that have a different colour (gynomorph). Currently, there are two hypotheses on how polymorphism is maintained within populations which are based on frequency-dependent harassment on female morphs. (i) The male mimicry hypothesis, states that andromorph females mimic males and therefore they benefit from it when they are in small number in the population as, resembling males, they are less harassed and consequently less damaged by continuous copulas; (ii) the learned mate recognition (LMR) hypothesis (, claims that the most abundant morph in the population is harassed, as males are capable to form a search image of the most frequent morph. In view of the results obtained by Rebora and colleagues (2018) in a natural population of *Ischnura elegans*, in which they had questioned the validity of the two hypotheses, the aim of this study is to assess the presence of the search image formation in the same population, to verify the assumption of the LMR hypothesis. To form the search image, it is necessary for a male to encounter more than one female consecutively. From this assumption we tested if males encountered sufficient females to form the search image, following them visually and recording all their reactions to encountered individuals. Males encounter much more males than females and, among females, less than 50% are mature. The male performs significantly more non-sexual than sexual behaviours towards both sexes, so our data suggest that there is not a male preference towards females. The sex ratio at the study pond showed a presence of males much higher than that of females. This disequilibrium near the pond suggests that, when most of the mature females are mating, for many males the probability of finding a partner is reduced. So, from our results, it seems that males do not encounter a sufficient number of females to validate the assumptions of the LMR hypothesis and the rate of copula of the different female morphs suggest that this was not the mechanism that maintained the female polymorphism in our study population.

DOMENICO FULGIONE, MARIA BUGLIONE, VALERIA MASELLI, CLAUDIA TROIANO, ELEONORA RIVIECCIO, SIMONA PETRELLI

Dipartimento di Biologia, Università di Napoli Federico II

WILD BOAR, HOW MANY EVOLUTIONARY TRAJECTORIES MAY INVOLVE THIS SPECIES?

Wild boar (*Sus scrofa*) populations from southern Italy represent a hotspot of genetic variability as a result of several factors: phylogeography, hybridization with pigs and the management of populations according to hunting interests. The phylogeography of the wild boar is in agreement with post-glacial re-colonization theories, as well as with the long history of human-mediated translocations of *Sus scrofa* in the Mediterranean basin. Genetic variability has been already altered by interactions with the domestic forms during the Neolithic revolution, when specimens were first brought from the fertile crescent and then replaced by local forms throughout the Mediterranean area. Our results surprisingly indicate the presence of the Near Eastern haplotype Y1 on both Italy’s major islands (Sardinia and Sicily) during the Bronze Age, suggesting the seaborne transportation of domestic pigs by humans at least during 1600–1300 BC. The South Italian “centers of domestication originated” were characterized by an active selection by early breeders for fast growth rate in wild boars. Pigs belonging to the endemic E2 (Italian) clade were traded between the Peninsula and Sardinia by the end of the second millennium BC, and this genetic signature is still detected in Sardinian feral pigs.

Feralization, as well as continuous hybridization with the wild boars, in specific localities, have restored in domesticated animals their wilderness, as feral forms. This process was possible thanks to the adaptive plasticity that involved numerous phenotypic traits. Recent evidence allowed us to discriminate the form of hybridization (pig x wild boar) from feralization ones (pigs return to the wild), both characterized by adaptive plasticity, indeed they manage to reactivate the expressions of characters to contrast the expected reduction in fitness in the wild. For example, our studies concerning the sense of smell show that this trait is particularly important for a macrosmatic species both during development and during adulthood. Our results on adult individuals show a significant difference in the sense of smell between the domestic and the wild form [2 – 3 fold of mRNA expression of Olfactory Marker Protein, Odorant Binding Protein, Neuropeptide Y, in wild boar compared to pig] and reveal that in the feral/hybrid populations there is a recovery of sense of smell due to a morphological rather than functional recovery [number of cells in 5 mm^2 of the olfactory mucosa 9.5 ± 0.5; 9.3 ± 0.7; 6.0 ± 0.2 for wild boar, feral pig and domestic pig, respectively]. This return to the wild could represent a new frontier in the evolution of this suide, so worrying in the difficult management of its populations.

DOMENICO FULGIONE, SIMONA PETRELLI, VALERIA MASELLI, TOMMASO NOTOMISTA, MARIA BUGLIONE

Dipartimento di Biologia, Università di Napoli Federico II

THE WALL GECKO: CRYPTISM AND SYMPATRIC SPECIATION

The Wall Gecko (*Tarentola mauritanica*) is a common medium size lizard showing heterogeneous colour pattern. The species shows a gradient of skin colour ranging from dark geckos, living by day, to pale geckos that meet at night. Our studies have demonstrated for the first time how this species is able to adjust its dorsal skin colour in response to light and background conditions to match cryptically on substrate [interaction effect background × light, $F_{2,225} = 21.37$; $P < 0.001$], making it less conspicuous to predators. These modifications occur within 1 h, independently from visual inputs and they are driven by the levels of SWS1 opsin in the flanks [amount of mRNA in the flank 3-fold compared to belly and back]. Diurnal/dark and nocturnal/pale geckos have a different ability to change their skin colour, indeed the skin reflectance of diurnal/dark geckos shows a wider variability than nocturnal/pale ones [reflectance variation at 600 nm: nocturnal/pale geckos = 0.7 %; diurnal/dark geckos = 7.0 %], although, the diurnal/dark geckos cannot become as pale as the nocturnal ones and *vice versa*. Furthermore, these two morphotypes follow not only a temporal but also a precise spatial segregation. Field observations show that dark geckos are preferably found on the tree (86.2%), whereas pale ones are preferentially associated to the walls (73.4%). Capture-recapture experiments highlight that about 40% of pale marked geckos were found exclusively on walls of houses, at the site of release or close to it, during the first and second night after their release. Instead, dark geckos were found only on olive tree trunks (51% on the first day and 59% on the second day). We hypothesize that the diurnal/dark/trunk and nocturnal/pale/wall geckos represent the first described case of sympatric speciation among terrestrial vertebrates. This phenomenon seems to be in its incipient stage and this is confirmed by the moderate gene flow observed between these two morphs [$F_{st} = 0.08$, $p < 0.05$]. However, the evolutionary trajectories tend to diverge under the effect of a differential natural selection [predation events: pale models on wall house/night = about 1 %; pale models on tree/day = about 50 %; dark models on wall house/night = about 100 %; dark models on tree/day = about 50 %; chi-square test $p < 0.05$ within each morph]. Selective pressure is mainly due to predation and significantly affects pattern variability and mitochondrial DNA genome [Tajima D test on the whole mitogenome: diurnal population = -0.48280; nocturnal population = 2.0449]. Here, we presented experimental and field data on diurnal/dark and nocturnal/pale gecko's populations sampled in Punta Licosa, within Cilento, Vallo di Diano e Alburni National Park (Salerno, Southern Italy), belong to the same nominal species and biogeographical population.

DOMENICO FULGIONE, SIMONA PETRELLI, VALERIA MASELLI, MARION VAN VUGT, MARIA BUGLIONE

Dipartimento di Biologia, Università di Napoli Federico II

THE EVOLUTION OF *PODARCIS SICULUS* IN SMALL ISLANDS

The Italian wall lizard (*Podarcis siculus*) shows extreme polymorphism within its range distribution, such that 58 subspecies have been described, 47 of which live on islands. Thus, isolation is an important factor driving variation. When isolation is combined with high environmental unpredictability, the result is populations that fluctuate strongly from generation to generation [*P. s. klemmeri* population size varies yearly by up to 40%], inducing the so-called Reversed Island Syndrome (RIS). According to the RIS hypothesis, for lizard populations on small islands, like as *P. s. klemmeri* on Licosa, *P. s. coeruleus* on Scopolo, *P. s. salfii* on Vetara (and maybe other more) facing unpredictable resource supply and mortality schedules should evolve aggressive behavior (island lizards either threatening or attacking other individuals, on average, 1.95 times per hour, compared to 1.05 times on the mainland), high food intake rate (mainland lizards ate wax worms, on average, at 7'45" intervals, insular lizards at 3'2" intervals), and increased energy allocation to reproduction [in male from mainland 5a-DHT was 2.89 ± 0.63 ng mL-1, whereas in insular males was found to be 5 ± 0.5 ng mL-1; in females significant variation in clutch mass was observed between the two populations (analysis of covariance, $R^2 = 0.22$, N = 40, F = 7.09, $p < 0.05$)], that promote an increasing fitness. Here, we studied that the time needed for these adaptations was partially linked to the age of the islands that host these lizard populations, most of them in the order of few thousand years (Licosa split about 4,000 years ago from mainland; Scopolo split about 8,000 years ago from Capri). Gaining of features making lizards subspecies occurs thanks to the regulation of gene expression rather than mutational events on the genome, as resulting from our analyses both on single nuclear and mitochondrial DNA genes and on whole transcriptome. Indeed, sometimes, genomic variations seemed to be lower than expected, meanwhile differential gene expression lead main adaptation in response to natural selection on small island (divergence time between Scopolo and Capri lizards, using mtGenome alignment under HKY+G model, show approximately 4,000 years BP, less than the divergence time calculated by geological observation estimated as between approximately 10,000 and 8,500 years BP).

MARCO ISAIA[1,2], STEFANO MAMMOLA[1,2], FILIPPO MILANO[1,2]

[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino
[2]IUCN Species Survival Commission - Spider and Scorpion Specialist Group

CLIMATE CHANGE AFFECTS THE SURVIVAL OF *VESUBIA JUGORUM* (SIMON, 1881) (ARANEAE, LYCOSIDAE), ASSESSED FOR THE IUCN RED LIST

Vesubia jugorum (Araneae: Lycosidae) is a large-sized wolf spider that occurs in alpine rocky areas above 2,000 m altitude. The species is stenoendemic, with a limited number of populations documented for Maritime, Cottian and Ligurian Alps (Italy and France). We have studied the response of this species to climate change, in the perspective of its assessment for the IUCN Red Lists. We used a combination of climatic, topographical and geomorphological variables to model habitat suitability, using multiple SDM algorithms. Due to the climate change, the current observed Extent of Occurrence (EOO) and Area of Occupancy (AOO) are expected to face a significant decline in the next 30 years. According to the IUCN criteria, the small current observed range (<5,000 kmq), the expected future range reduction and the limited number of locations meet the standard for placing *Vesubia jugorum* in the IUCN category of "Endangered" species. In parallel, we carried out field samplings aimed at obtaining fresh specimens for measuring functional traits related to size and reproductive success. We tested the relationship between functional traits and habitat suitability predicted by the models using Linear Mixed Models (LMM). We demonstrated that optimal habitat conditions maximized both the individual performance— body size—and fitness—egg clutch size. In light of the IUCN conservation status of *V. jugorum* and the consequent need of monitoring future population trends in a climate change perspective, the relation between functional traits and climate herein demonstrated represent a promising approach for setting up monitoring programs. Indeed, measuring the variation of morphological traits offers a practical, non-invasive mean for assessing population health through time.

GIULIA LUZI[1], GIUSEPPE PUDDU[2], MARZIO ZAPPAROLI[1]

[1]DIBAF Dipartimento per la Innovazione nei sistemi Biologici, Agroalimentari e Forestali,
Università della Tuscia
[2]Ente Monti Cimini - Riserva Naturale Regionale Lago di Vico, Regione Lazio

SPECIES-HABITAT RELATIONSHIPS IN MESOMAMMALS OF FOREST ECOSYSTEMS IN VICANO DISTRICT (LAZIO, VT)

Aim of this work is the study of the relationship between four mesomammal with forest areas using camera-trapping techniques around “Lago di Vico” Natural Reserve (Lazio, VT). The targeted species are Crested porcupine (*Hystrix cristata* Linnaeus, 1758); European badger (*Meles meles* Linnaeus, 1758); European pine marten (*Martes martes* Linnaeus, 1758); Red fox (*Vulpes vulpes* Linnaeus, 1758). Through a systematic and stratified sampling design constituted by 1 km^2 square grid, 57 monitoring points were identified, located in the respective centroids. All the points fall in the wooded areas of the municipalities of Canepina, Caprarola, Ronciglione, Vallerano, Vetralla and Viterbo. The grid covers the whole forest compositions of the Vico district, with greater attention to forest entities of European beech (*Fagus sylvatica*), Chestnut (*Castanea sativa*) and Turkey oak (*Quercus cerris*). The monitoring was conducted starting on 07/31/2018 for a total period of 249 sampling days, divided into three different surveys with around 20 sites each, and an average of 66 sampling days (min = 57; max = 81). The data collected from the camera trapping monitoring, were elaborated using an occupancy model for each species investigated. Vico’s woods were analysed to collect descriptive dendroauxometric measurments, used as covariates in the statistical models. Were also investigated the possible correlation between presence/absence of the species and the ecological and management features of forest ecosystems.

FRANCESCO MASTROTOTARO[1], FEDERICA MONTESANTO[1], MARIKA SALONNA[2], GIOVANNI CHIMIENTI[1], CARMELA GISSI[2,3]

[1]Dipartimento di Biologia CoNISMa LRU, Università di Bari "Aldo Moro"
[2]Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università di Bari "Aldo Moro"
[3]IBIOM, Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari, CNR, Bari

INTEGRATED TAXONOMY AS USEFUL TOOL FOR ASCIDIAN MODEL ORGANISMS STUDIES

In recent years, solitary and compound ascidian species have been selected as chordate model organisms in a wide range of fields such as physiology, developmental biology, and chordate evolution. Indeed, the solitary species *Ciona intestinalis* (Linnaeus, 1767) and the colonial *Botryllus schlosseri* (Pallas, 1766), are two of the most powerful ascidian model organisms. Despite the large number of studies carried out on both species, in the last decades genetic studies revealed the presence of cryptic species under these two specific binomials.

In fact, several species belonging to the genus *Ciona* Fleming, 1822 were synonymized with the type species *C. intestinalis* for a long time, but starting from 2005 molecular studies showed that *C. intestinalis sensu lato* constitutes a complex of 4 cryptic species (named type A to D). Finally, in 2015 Brunetti *et al.* published the results of combined molecular and morphological analyses highlighting the presence of diagnostic characters able to discriminate type A from type B. Therefore, type A was identified as *Ciona robusta* Hoshino & Tokioka, 1967, and type B as *C. intestinalis*. Concerning the other two types (also named spC and spD), the rarity of these specimens has so far prevented morphological investigations. In this context, an integrated approach was used to study some peculiar specimens belonging to the genus *Ciona* collected from Sardinian coasts (Olbia, Italy). These specimens showed intermediate morphological characters compared to those of other moderate and shallow-water species, and several features in common with the species *Ciona edwardsi* Roule, 1884. Molecular characterization based on three mitochondrial regions confirmed that these *Ciona* specimens do not belong to any molecularly-characterized species within the genus, and that *C. edwardsi* is its sister-group. Even for *B. schlosseri,* molecular studies first highlighted the presence of five genetically divergent clades (named from A to E), that should correspond to five distinct cryptic species. On this regard, *B. schlosseri* neotype has been recently morphologically described and molecularly associated to clade A, although further studies are needing to check whether this association is univocal. Even for this species, further morphological analyses are ongoing to identify diagnostic morphological character(s) able to identify each molecularly-described clade. We emphasize that, as in in the case of *C. intestinalis* studies, it is fundamental to preserve samples in such a way that both molecular and morphological analyses can be performed. These results underline the importance of a synergistic approach between morphological and molecular studies especially for the study of species complex. Considering the relevance of these two genera in a wide range of fields, it seems crucial to gather additional knowledge regarding their taxonomy and evolutionary history, joining the competences of both taxonomists and experts in molecular evolution.

FILIPPO MILANO[1,2], MARCO ISAIA[1,2]

[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino
[2]IUCN Species Survival Commission - Spider and Scorpion Specialist Group

THE CONSERVATION OF SPIDERS IN EUROPE: AN OVERVIEW

Despite their ecological importance and their diversity, spiders are still underrepresented in conservation policies and conservation biology, especially compared to other invertebrate groups. The existing international legislation on spider conservation is rather poor, with only one species listed in the Bern Convention and in the EU Habitat Directive, and 22 species listed in CITES, none of which is naturally occurring in Europe. In addition, the risk of extinction of 274 species has been assessed by the International Union of Conservation of Nature, 65 of which occur in Europe. Global and regional Red Lists are valuable tools in nature conservation at different scales, aiming to inform and catalyse actions for prioritization, planning and implementation of conservation measures. The availability of both global and regional Red Lists is, therefore, of paramount importance for spider conservation. Here, we reviewed all existing regional, national and supranational Red Lists and Red Data Books focusing on spiders in Europe, with the goal of drawing a general picture on the status of conservation of this group in the Continent. Eigtheen European countries developed a national list of threatened species which includes spiders, and thirteen of them applied the IUCN Red List Categories and Criteria, whereas five other countries used specific national criteria or local expert judgements to produce Red Lists. Apart from national Red Lists, eleven countries consider spiders in regional or supranational lists of threatened species. Despite the general low level of spider diversity, Central and Northern European countries show the highest percentage of species mentioned in Red Lists. On the other hand, spider conservation is poorly developed in Mediterranean countries such as France, Italy and Spain, despite the generally high levels of spider diversity recorded therein. Based on our review, we highlight general patterns, limitations, gaps, and future directions about the conservation of spiders in Europe.

FILIPPO MILANO[1,2], STEFANO MAMMOLA[1,2], MARCO ISAIA[1,2]

[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino
[2]IUCN Species Survival Commission - Spider and Scorpion Specialist Group

DOLOMEDES PLANTARIUS (ARANEAE: PISAURIDAE) IN ITALY: TRENDS OF BIOCLIMATIC SUITABILITY IN A CONSERVATION PERSPECTIVE

The fen raft spider, *Dolomedes plantarius*, is a semi-aquatic species closely associated with standing oligo-mesotrophic water, from which many aspects of its life-history depend. In recent years, this Eurosibirian species has experienced population declines over much of its range, which is mostly related to the loss and the degradation of wetland habitats, to the fragmentation of the natural populations, and to water eutrophication. In Italy, this species is limited to the Northern regions, that also represent the southernmost limits of the species range. As the result of its extreme vulnerability, *Dolomedes plantarius* has been the subject of conservation programmes in many countries, and it is mentioned in several Regional Red Lists. In 1996, *D. plantarius* was listed as vulnerable species on the IUCN Red List, but despite the increasing pressure on European wetlands, the assessment has never been updated. Concerning Italy, *D. plantarius* is listed in the provisional Red List of Invertebrates and are under the protection of regional legislation of Regione Lombardia. Here we provide an updated frame of the existing knowledge on the distribution of this species in Italy and we point out the potential present and future distribution of the Italian populations obtained via Ecological Niche Modeling. We predict a declining trend in the current suitable range of this species in Italy, with a remarkable loss of suitability throughout the investigated area. Besides, the Italian situation is particularly critical due to the geographical isolation of the populations at the periphery of the species range, and to the presence of the Alpine barriers that prevent the species dispersion northward. On these results, we provide an estimation of the current and predicted Extent of Occurrence (EOO) and Area of Occupancy (AOO) by means of the newly developed R package *red - IUCN redlisting tools*, paving the way towards the assessment of the regional IUCN status of this species of elevated conservation concern.

Simposio III

Zoologia applicata

Coordinatori:
Paolo Bonivento, Maurizio Casiraghi

Lettura plenaria

PAOLO BONIVENTO

IEMEST - Istituto Euro Mediterraneo di Scienza e Tecnologia, Palermo

CREATIVE ZOOLOGY AND LATERAL THINKING

By tradition and method, in university courses there has always been an emphasis on vertical (logical) thinking, which is effective, certain and its learning can be verified in an objective manner. Thanks to this kind of thinking, university students are prepared to continue on the academic path or to school education, which always includes the same logic. The number of graduates in science subjects, and in particular in zoology, means that the selection does not allow everyone the opportunity to transform the effort of years of study into the basis of a career, for which many young people are forced to change course, putting aside all the cultural heritage absorbed in years of study. So, the vertical thought, even though sectorially perfect, is an extremely selective type of thought. Given the needs of the era in which we are living, the best solution would be to integrate vertical thinking with the productive qualities of creative thinking. This integration begins to occur due to the good will of some teachers but, even in these rare cases, creativity is usually treated only as something desirable, if possible achievable through vague exhortations. There is no prearranged practical procedure to achieve it. The report is entitled "creativity in Zoology" but, as an object, lateral thinking, which is the process of using information to achieve creativity and intuitive restructuring. Lateral thinking can be learned, practiced and used. It is therefore possible to acquire skills in this field as it is possible to acquire them in Zoology by identifying the "applied Zoology" with the same dignity as the other disciplines, the search for a practical method to use this type of thought whose importance continues to grow. It should be emphasized that there is no antagonism between the two types of thought: both are necessary. Vertical thinking is immensely fruitful, but it is necessary to increase its usefulness by adding creativity and attenuating its rigidity. In the last 10 years, all the activities financed by public bodies foresee, in fact, a synergy between the Public Research Body (University, CNR, etc.), innovative start-ups or SMEs and a manufacturing company; in this way it is assumed that the research organization carries out, in general, applied and research, the innovative start-up or SMEs transforming, integrating it, what has been done by the body into a prototype aimed at the production and marketing by the production company. As you can see, it is a path that does not define who should have the innovative idea: in fact, if there is no real innovation that has a final benefit on the economic and social growth of a population, nothing is paid. On this point all the auditors are extremely strict and operate in bureaucratically pre-established evaluation schemes. Therefore, an evaluation of vertical thinking is requested for a project that must absolutely be the result of creative thinking. Without creativity, no innovation, as J.S. Bach said, only infinite improvements in a given group of notes. Everyone (academies, start-ups / smes and manufacturing companies) is waiting for the innovative spark. This is rarely random. It is always the fruit of the fusion of vertical thought and lateral thinking. Therefore, we organically begin to apply ourselves in "creative Zoology and lateral thinking".

Simposio III

Zoologia applicata

Coordinatori;
Paolo Bonivento, Maurizio Casiraghi

Presentazioni orali

CINZIA FERRARIO[1,2], ALBANA PULAJ[1], RAFFAELLA MACCHI[3], PAOLO LANDINI[3], MOIRA PARONI[3], LUCA MELOTTI[4], M. DANIELA CANDIA CARNEVALI[1], FRANCESCO BONASORO[1], ILARIA IACOPETTI[5], MARCO PATRUNO[4], MICHELA SUGNI[1,2]

[1]Dipartimento di Scienze e Politiche Ambientali, Università di Milano
[2]Centro per la Complessità e dei Biosistemi, Dipartimento di Fisica, Università di Milano
[3]Dipartimento di Bioscienze, Università di Milano
[4]Dipartimento di Biomedicina Comparata e Alimentazione, Università di Padova
[5]Dipartimento di Medicina Animale, Produzioni e Salute, Università di Padova

SEA URCHIN-DERIVED COLLAGEN AND ITS POTENTIAL IN SKIN REGENERATION APPLICATIONS

Humans have always exploited marine ecosystems as source of inspiration, food, bioactive compounds and biomaterials. Marine organisms are interesting for their huge potential in both basic research and applied biotechnology/biomedicine. Sea urchins are currently actively investigated as eco-friendly source of valuable biomaterials, mainly collagen. This is one of the most used material/molecule to produce devices for different biomedical applications. For instance, collagen-based skin substitutes (SS) are currently considered promising in the skin injury care field to solve ulcers, burns and chronic wounds with tools alternative to skin grafts. However, the existing SS are mainly produced with mammalian collagen and lack important features that impair their overall performances. Hence, new sources of collagen as well as new SS design are largely explored. In this study, fibrillar collagen was extracted from sea urchin food industry wastes and employed to produce bi-layered SS (2D layer + 3D scaffold) that were characterised to evaluate their suitability for regenerative medicine in terms of aminoacid composition, ultrastructure, mechanical stability, permeability to water and bacteria, and cell viability, proliferation and infiltration. Our results indicate that the aminoacid composition and relative abundance of sea urchin collagen is rather similar to the human one. Furthermore, the thin and dense 2D layer, developed to resemble the skin epidermal layer, shows a high mechanical resistance, blocks water evaporation and bacteria infiltration. The sponge-like 3D scaffold, developed to resemble the skin dermis layer, shows good mechanical stability in wet conditions and it is biocompatible since mammalian cells are viable, proliferate and infiltrate within the scaffold. Preliminary *in vivo* tests with sheep skin models suggest a good biocompatibility and efficacy of the produced devices, although further studies must be addressed to carefully verify these results. Overall, marine skin substitute might be innovative and effective tools for future tissue regenerative medicine applications. Moreover, sea urchins might be considered an eco-friendly source of a high-value by-product and a pilot recycling food waste chain at a national level should be started to test the actual feasibility of this circular economy approach.

SILVANA PIERSANTI[1], MANUELA REBORA[1], GIANANDREA SALERNO[2]

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

A NEW METHOD FOR ELECTROANTENNOGRAPHIC RECORDINGS IN AQUATIC INSECTS

Insect extraordinary evolutionary success is related with their ability to receive and respond to a great variety of sensory cues, thanks to a high number of diversified sensilla mainly located on antennae. Insects belonging to twelve orders spend one, or more, life stages in freshwater and the successful invasion of lotic and lentic ecosystems required physiological constraints, also regarding antennal sensory systems. Chemoreception, in particular, allows organisms to respond to chemical cues and depends on the senses of taste and smell. In insects living in water all chemicals sensed are in aqueous solution, moreover, freshwater environmental conditions, such as turbidity of water, reduced light transmission, etc., prompts aquatic insects to use chemical cues for intra and interspecific relationships, such as foraging or predator avoidance. The ability to perceive chemical stimuli in water has been demonstrated in behavioural investigations in larvae of many aquatic insects; the morphology of potential chemoreceptors on the antennae has been described in Odonata, Ephemeroptera, Plecoptera and Trichoptera larvae. On the contrary, the classical electrophysiological techniques for insect chemoreception, such as extracellular recordings or intracellular patch-clamp method, have never been successfully performed in the aquatic stages of insects. In this context, the present research describes a new method, with a high rate of success, allowing the recording of global responses of the antennae of dragonfly larvae to chemical cues eluted in water. *L. depressa* was selected for the investigation because it is a common and widespread species in Italy and the composed coeloconic sensillum at the antennal apex of these larvae represents a good model to perform electrophysiological investigations on taste into the water. In our experiments, one larva belonging to the last stages of *L. depressa* was immobilized in a plastic tube, by dental wax and Patafix®, and its antenna was immersed in a continuous flow of a water solution 0.001M NaCl inside a plastic tip. The stimuli were waterborne; in detail, we inserted in the flow 10 µl of water solutions of chemicals potentially active, such as pentanoic acid, ammonia (28% in water) and L-glutamic acid. Dose-response experiments were performed, to analyse the effect of the dose on the response, stimulating the antenna by 8 doses of ammonia (from 10^{-5}M to 5M). Ammonia was selected because it is the compound that elicited the strongest responses between those that we tested. Additional recordings were done in order to analyse how the saline solution flow can affect the antennal response to chemicals (total depolarization, latency of the beginning and duration of the depolarization). To perform these essays, we used ammonia 10^{-3}M, and the flow rates tested were 20ml\h, 42ml\h, 83ml\h and 125ml\h. Our data show that *L. depressa* antennae clearly respond to ammonia, butyric acid, valeric acid and L-glutamic acid. Responses are stronger, shorter and faster when the flow rate increases. Responses to ammonia solutions at increasing concentrations start at 10^{-3}M and last at 5M. The present method allows to perform electroantennographyc recordings in water thus giving the opportunity to deep the knowledge on the chemical ecology of aquatic insects. In addition, it could be relevant for screening recordings of gustatory responses also in terrestrial insect, such as to investigate antennal gustatory sensilla not visible under stereomicroscope, and for this reason not suitable for taste-tip recordings (e.g. coeloconic sensilla).

STANISLAO DI AMATO, PAOLO BONIVENTO

Reborn srl – Start Up Innovativa, Trieste

THE USE OF DRONES FOR DETECTION AND IDENTIFICATION OF PESTS OF WOOD: EXPERIMENTS ON THE TERMITE *RETICULITERMES LUCIFUGUS*

Detecting termites is difficult. All termite species, be they dry-wood, damp-wood or subterranean, are cryptic, because this is their primary defensive strategy against predation. Detecting infestations in structures and locating the entrance routes used by the termites is often critical in effective management of the pest. Various detection methods are used, with some variation for different types of termites. Biological signs include: the termites themselves, including alataes or their discarded wings; their building activities such as ‘mudding’ that seals crevices and ‘mud tubes’ used to gain access by subterranean termites, or the expulsion holes of dry-wood termites; and their foraging activities – of course excavated wood will also indicate infestation. To detect termites using just these visual signs requires good eyesight and meticulous inspection methods. Such signs are more likely seen in a house when better access to wall cavities and other hidden areas is obtained, typically destructively with various tools such as knives and drills. Unfortunately, not all of these signs are present at all infestations; in fact no signs might be manifested at all. Often termites use construction beams as feeding areas. Many times the buildings, especially the Romanesque, Gothic and Baroque churches and the houses dating back to the Renaissance, have rooms with ceilings that are several meters high, so the main beams are difficult to reach. To carry out an entomological survey on these structures, aimed at understanding which is the infesting agent and at what point the infestation is, would be necessary support structures from the ground, very expensive and dangerous for the expert. In addition to this, the entomologist must collect material for identification, not knowing if the identified site is still populated or has been abandoned by the colony. Nowadays, the inspection methods are usually combined with the use of more sophisticated instruments, such as borescopes, moisture meters, electronic odour devices, acoustic emission detectors and infrared heat detection; in addition, dogs have been trained to smell termites. Borescopes enhance the visual inspection for biological signs whereas the other methods are electronic and measure secondary characteristics, viz water levels, snapping wood fibres, heat and so forth. These methods increase the reliability of detecting termites, but not all methods will work in all situations, and of course visual inspections can be somewhat destructive and may harm property unnecessarily. Each method of those highlighted involves tools and accessories that are difficult to transport and use at a height. Clearly, there is scope for improvement in termite detection. Our study focused on three themes that emerged from the expert experience: reaching the structures in height by drones, verifying the active presence and identifying the species of the pests with specific substances for qualitative and quantitative analysis.

NICOLA TOMMASI[1], PAOLO BIELLA[1], ANDREA TAPPARO[1], JULIUS VINCENT LASWAY[3,4] ,
LORENZO GUZZETTI[1], AIDANA NURTAZA[2], GIULIA AGOSTINETTO[1], HENRY NJOVU[3],
MAURIZIO CASIRAGHI[1], ANDREA GALIMBERTI[1],

[1]ZooplantLab, Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca
[2]National Center for Biotechnology, Laboratory of Plants Biotechnology and Breeding, Astana, Kazakhstan
[3]College of African Wildlife Management, Mweka, Tanzania
[4]Dpt of Animal Ecology and Tropical Biology, Biocenter, University of Wuerzburg, Am Hubland, Germany

A DNA-BASED APPROACH TO EXPLORE POLLINATORS DIVERSITY AND INTERACTIONS IN EUROPE AND SUB SAHARAN AFRICA

The increasing awareness about the importance of pollinator insects and on their global decline is leading researchers to a race against time, towards a deeper comprehension of the effect that human activities have on pollinator communities. The analysis of pollinator-plant interactions and their changes in relation to anthropogenic stressors is an issue of primary concern, as it allows to better plan and manage effective conservation efforts. Traditionally, the study of plant-pollinator networks requires long-term field observations, and challenging identification of entomological and palynological concern, with high costs and sometimes low data resolution. Nowadays, DNA-based tools have been successfully applied to characterize such interactions. Here we applied an ITS2 DNA metabarcoding approach to reveal the taxonomic composition of pollen carried by insects coupled with COI DNA barcoding to identify the pollinator species. This strategy allowed us to shade light on several features of pollinators interactions in multiple contexts. First, we analysed the response of Bumblebee (*Bombus terrestris*) colonies to a strong workforce reduction in a simulation of acute agrochemical intoxication. We identified 34 plant taxa (from 143 analyzed bumblebees) with most of pollen pellets of polyfloral origin. No significant changes in structure of insects-plant network were identified after colonies manipulation, suggesting that bumblebees do not adapt the foraging strategy after the colony halving, at least in terms of visited plant taxa. The second experiment was aimed at characterizing (for the very first time) plant-pollinators interactions from Sub Saharan Africa considering the effects urbanization and agricultural management. During the Tanzanian dry season and short rain season we sampled 285 wild pollinators, observed foraging on flowers, in three main landscapes: organic, intensive and urban. Despite the lack of knowledge in tropical taxonomy and the poor availability of DNA reference sequences from this region, we successfully characterized pollen composition and interactions of tropical bees with interesting conservation and sustainability outcomes. On the whole, our approach allows to increase the knowledge about declining pollinator insects and offer new insights for the formulation of landscape and biodiversity management policies.

MICHELE CESARI[1], LUCIA PIEMONTESE[1], LARA MAISTRELLO[1], IWONA GISKA[2], JOSÉ MELO-FERREIRA[2], EMILIANO TRUCCHI[3], LORENA REBECCHI[1], ROBERTO GUIDETTI[1]

[1]Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia
[2]CIBIO, Research Center in Biodiversity and Genetic Resources, InBIO Associate Laboratory, University of Porto, Vairão, Portugal
[3]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche

RAPID IDENTIFICATION, ROUTES OF INVASION AND DISPERSAL OF THE ALIEN STINK BUG *HALYOMORPHA HALYS* (HEMIPTERA, PENTATOMIDAE)

The number of exotic species which have invaded new territories has increased over recent decades, causing significant ecological and economic damages. Therefore, an effective control management of invasive species has to rely heavily on a rapid and accurate identification. Moreover, inferring source populations, routes of invasions and patterns of dispersal of founding populations are important issues for projecting their spread and potentially directing the focus on management strategies. *Halyomorpha halys* (Hemiptera, Pentatomidae) is a phytophagous insect native to East Asia which has rapidly spread around the world, being presently found in North and South America, and in Europe. *H. halys* has been detected in Italy for the first time in 2012, and it is now present in all Italian regions. The present study aimed at applying a high sensitivity molecular assay method (real-time PCR) for rapidly identifying the presence of *H. halys* in degraded samples, such as guano of Italian bats. Furthermore, the route(s) of invasion and dispersal of *H. halys* were investigated using highly polymorphic genetic markers, such as single nucleotide polymorphisms (SNPs). Previous molecular analyses on mitochondrial genes (*cox1*, *cox2*) led to the identification of 26 different haplotypes all over Europe, with haplotype TH1 being the most widespread in the world. Thus, the genetic diversity of invading specimens presenting the TH1 haplotype has been investigated by analysing 1422 single SNPs obtained by RADseq data from 28 specimens sampled in seven Italian regions and in Greece. The analysis pointed out the presence of four different clusters: while two clusters included individuals from different geographic regions, the third group included only individuals from Emilia-Romagna, and the fourth one included only specimens from Veneto region. The latter group was also characterized by the highest within-cluster differentiation. Present results show that *H. halys* invading populations have originated from multiple invasion events and highlight the high spreading ability of this species, likely enhanced by human activities. The application of the real-time PCR identified the presence of *H. halys* in guano samples of two genera of bats (*Myotis*, *Nyctalus*) in Piedmont and Tuscany, highlighting the high sensitivity of this technique and its potential for application in monitoring the presence of this important economic pest.

MANUELA REBORA[1], GIANANDREA SALERNO[2] SILVANA PIERSANTI[1], ELENA GORB[3], STANISLAV GORB[3]

[1]Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia
[2]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università di Perugia
[3]Dpt of Functional Morphology and Biomechanics, Zoological Institute, Kiel University, Kiel, Germany

INSECT ADHESION: FROM BASIC TO APPLIED RESEARCH

Among adaptations insects developed to survive in different environments, they evolved various types of leg attachment devices to achieve sufficient attachment for locomotion on widely different surfaces. Claws are adapted to interlock with rough surfaces, while on smooth surfaces, insects attach themselves by using their tarsal adhesive devices, such as hairy pads (for example, earwigs, beetles, flies), or smooth flexible pads called arolia, pulvilli and euplantulae (for example, cockroaches, ants, aphids, grasshoppers, bugs, butterflies, moths). In particular, insect adhesive pads are flexible areas of cuticle supplemented with cuticular secretions, whose presence has been demonstrated in both hairy and smooth pads. During the long period of coevolution between insects and plants, plants developed a wide diversity of features, not only to attract pollinators, but also to defense against herbivores. These features encompass chemical and physical barriers able to reduce insect performance on the plant surface. In this context, the coevolution of plant surfaces and insect attachment pads is an interesting example of competition between insect attachment systems and plant anti-attachment surfaces.

In the present research we tested the attachment ability of two economically important polyphagous insect pests belonging to two different orders, represented by the southern green stink bug *Nezara viridula* L. (Heteroptera: Pentatomidae) and the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). These two species are characterized by different tarsal attachment structures, whose morphology and attachment ability has been studied by us in detail in ultrastructural (SEM, TEM) and behavioural (traction force experiments) investigations, on natural (leaves and fruit surfaces) and artificial substrates (glass and epoxy resin) characterized by different roughness and wettability. The attachment structures of *N. viridula* comprise two sclerotised claws, a pair of smooth flexible pulvilli and a hairy adhesive pad located at the ventral side of the basitarsus, while *C. capitata* has claws and hairy pulvilli. Moreover, we investigated in detail under controlled conditions the effect of kaolin (nanoplate-like aluminosilicate mineral) particle film (particle film technology) on reduction of insect attachment ability. We performed traction force experiments on treated (covered with kaolin particle film) and untreated (control) natural (leaf surfaces with different morphological traits) and artificial (hydrophilic and hydrophobic glass) surfaces. In order to unravel the mechanism of action of the kaolin particle film in altering insect adhesion, we studied the initial wettability of the tested surfaces through measurements of water contact angle prior to the treatment, characterized the roughness of treated and untreated glass substrates using the white light interferometer and examined the natural surfaces and treated hydrophilic glass in cryo-SEM. To estimate the possible contamination effect of kaolin particles, we evaluated the attachment ability and observed the tarsal attachment devices of insects after walking on treated surfaces. Our data revealed that the kaolin particle film strongly reduces insect attachment ability on natural and artificial substrates with a degree of reduction depending on the initial wettability and morphology of treated surfaces.

Studies in the field of insect adhesion can contribute to develop in the future physical control barriers against pest insects, particularly important owing to the need to reduce the negative impacts of pesticides on environment and human health.

GIULIA AGOSTINETTO, ADAM CHAHED, DAVIDE MAGNANI, ANNA SANDIONIGI,
MAURIZIO CASIRAGHI

Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca

EXTAXSI: A TOOL FOR TAXONOMY DATA EXPLORATION IN APPLIED ZOOLOGY

DNA-based methods are nowadays a routine in taxa identification and biodiversity assessments, enabling to overcome issues lead to conventional approaches and extend surveys to complex multi-species matrices. Taking advantage of massive sequencing platforms, it is possible to move from a single species detection to the identification of a mixture of organisms, exploiting the informative content of DNA traces. In particular, the eDNA (environmental DNA) metabarcoding is a powerful DNA-based tool to explore biodiversity, allowing a rapid depth assessment with a reduction of costs and time. The applications are scattered among different fields like zoology, ecology, microbiome studies, food tracking and many others. The method depends on bioinformatic analysis of sequences, and its reliability is based on the quality of the information collected in reference databases. These allow the taxonomy assignments of the millions of generated sequences reads in a single run. A lack of reference sequences or an incorrect assembly of the reference database could lead to an incomplete or biased taxonomy identification. However, due to the advent of cheap sequencing techniques, huge amount of annotated sequences is produced every day. As a consequence, the public repositories are constantly enriched of data that could be exploited by both researchers and services. In order to perform a high-quality taxonomic assignment, here we present ExTaxsI (Exploration of Taxonomies Informations), a tool for taxonomy exploration of data that allows researchers (and other stakeholders) to explore NCBI Nucleotide references, visualizing both public and personal annotations. In fact, our tool is modular and could be used at any stage of taxonomy analysis of DNA sequences, during exploration of database composition, download of data, visualization of assignments considering all principals taxonomic ranks and, in addition, to survey the provenance of NCBI records, creating an easy-to-use application for data scouting. The tool allows to create a multi-species database and survey taxa distribution considering different marker genes, in order to better design experimental strategies applicable, for instance, to taxa identification, invasion biology, biomonitoring, conservation and biodiversity education.

PAOLO BONIVENTO, MONIA RUGGIRELLO

IEMEST - Istituto Euro Mediterraneo di Scienza e Tecnologia, Palermo

ARCHITECTURE, NANOTECHNOLOGIES AND BUILDING MATERIALS: HOW MUCH DO TERMITES TEACH US?

Termites give the impression of building nests with a marked design skill. Numerous regular shapes and geometries of a cell or compartment can be recognized, multiple spaces destined for housing and a complex system that provides ventilation. Among individuals there is a high range of coordination and apparent cognitions available to termites. In addition, it leaves the choice of the construction material and, above all, of the consolidation substances of the structure particularly admired and amazed. In the world, as far as we know, there are 2200 species of termites (a small thing, compared to 3100 varieties of amphibians or 4100 of mammals). It is one of the smallest orders of insects and, at the same time, one of the most varied and ingenious. These invertebrates draw their sustenance from cellulose, the most widespread carbohydrate on our planet. But, although cellulose is nothing but a very long sequence of glucose molecules, in the presence of oxygen it is indigestible. The bond that unites the sugars of cellulose cannot therefore be broken by conventional enzymes, therefore it is the building material par excellence. Termites do not directly digest cellulose (any more than cows, giraffes and antelopes do) but leave other organisms to perform chemical digestive reactions; for this purpose there are two strategies: the most primitive termites (for example the *Reticulitermes lucifugus*, present in Europe and, in particular, in the sub-Danubian area) ingest the cellulose and transfer it to a fermentation chamber in which anaerobic bacteria and protozoa demolish it; the most advanced termites (for example *Macrotermes bellicosus*, present in the whole sub-Saharan area of Africa) present a different feeding strategy digesting "outside". In fact these termites deposit food in termite mounds where they bring fragments of fungi which, precisely because of their essence, break down cellulose in the presence of air, heat and humidity. The structure of the nest, therefore, is modified not only to give maximum protection from the queen, to eggs and nymphs but also to create some spaces dedicated to the fermentation of food. If, on the one hand, *Reticulitermes lucifugus* uses its own digestive secretion as a building cement, on the other hand *Macrotermes bellicosus* uses the result of funginea fermentation not only as a food source but also as a substance for the construction of the termite mound. The molecular structure of this substance is being studied both to understand its "building" qualities (particularly surprising for the internal arches of the termite mound) and to verify its possible use as a nano-biomaterial in civil engineering. This communication will illustrate the recent discoveries whose results will be compared with the main building materials currently in use.

VALERIA MASELLI[1], EMILIA GALDIERO[1], ANNA MARIA SALZANO[2], STEFANIA GALDIERO[3], MARCO GUIDA[1], ANDREA SCALONI[2], DANIELE NAVIGLIO[4], AL-SAYED AL-SOUDY[1], DENISE SGUEGLIA[1], ANNA DI COSMO[1]

[1]Dipartimento di Biologia, Università di Napoli Federico II
[2]Laboratorio di Proteomica e Spettrometria di Massa, ISPAAM,
Consiglio Nazionale delle Ricerche, Napoli
[3]Dipartimento di Farmacia, Università di Napoli Federico II
[4]Dipartimento di Scienze Chimiche, Università di Napoli Federico II

OCTOPARTENOPINA: IDENTIFICATION AND CHARACTERIZATION OF ANTIMICROBIAL PEPTIDES FROM THE SUCKERS OF *OCTOPUS VULGARIS*

In the common octopus (*Octopus vulgaris* Cuvier, 1797) eight arms and soft body endow it with an amazingly extensive repertoire of behaviors. Each arm is equipped with a double-row of sophisticated active suckers, important manipulative and chemosensory structures that represent an evolutive innovation, which has no equivalent in all other animal taxa, that inspired bioengineers for soft robots, as powerful instrument for flexible movement and adhesion mechanism. Suckers collect and process many sensory information coming from millions of tactile and chemical sensory cells, and they are also involved in eggs taking care, touching continuously avoiding them to get any infections. Octopus, along with all other members of the phylum Mollusca, lacks a complete adaptive immune system, but they have an efficient innate immune system that allows them to act as the first line of defence against a wide spectrum of pathogens and interact with microorganisms, discern and remove pathogens, and repair wound and tissue damage. In cephalopods, the "white body" is considered the haemopoietic organ. Only granulocytes were observed in the haemolymph, and haemocytes were identified in *O. vulgaris* as the first agents responsible for innate immunity. However, studies investigating bioactive peptides with antimicrobial activity were those conducted on squids and cuttlefishes. The only data related to *O. vulgaris* concern haemagglutination activity in cell-free haemolymph, an antiprotease activity belonging to the α-macroglobulin family detected in the haemolymph and the bactericidal activity of haemocytes in the presence of Gram positive and negative bacteria strains. Therefore, the detailed identification of the cellular components and the characterization of defence mechanisms in octopus's suckers have received little attention. Probably octopus continuously fights against pathogens by secreting a wide range of antimicrobial peptides (AMPs) as an innate defence mechanism as observed in other aquatic animals as fish. AMPs have a key role in the innate immunity and can protect the hosts against a broad range of pathogenic infections, making them attractive as therapeutic agents. The present study describes the antimicrobial activity of proteic purified extracts from *O. vulgaris* suckers. We extracted small peptides with an innovative approach and tested different purified fractions against Gram-positive and negative species, their action on different membrane models and their potential intracellular targets. Our results highlight that these bioactive molecules can cover broad strategies to act against bacterial pathogens, combining membrane destabilization, and intracellular mechanism of action. Results support our hypothesis that sucker secretions are used by octopus to defence against pathogens in the environment and they may be a rich source of multifaceted peptides to develop alternative antimicrobial agents. In future, the identification of all proteins and the functional characterization of the broad bioactivity spectrum of peptides have high potential values, which may attract the attention of the pharmaceutical and nutraceutical industries, that can be used in treatment or prevention of various diseases.

FRANCESCO CERINI, MARCO A. BOLOGNA, LEONARDO VIGNOLI

Dipartimento di Scienze, Università Roma Tre

NESTEDNESS-PATTERN OF ODONATA COMMUNITIES IN ARTIFICIAL AND NATURAL AQUATIC HABITATS: THE POTENTIAL ROLE OF DRINKING TROUGHS FOR AQUATIC INSECT CONSERVATION

Pattern of nestedness between artificial and natural habitat may represent evidence of manmade habitats' importance in community assembly and conservation of animals inhabiting such sites. Odonata often colonize drinking troughs (artificial water reservoirs) and thus they are good study models as umbrella species. We investigated if a network of natural (pools and ponds) and artificial (troughs) aquatic habitats could create a nested subset pattern for Odonata communities. We surveyed all the troughs present in the Castelporziano Presidential Estate (Italy, Lazio, Roma): Odonata larvae have been collected and identified. Data of a previous paper on 18 natural pools and ponds, and our samplings of 16 troughs were transformed into a presence-absence matrix. The Odonata assemblage within natural and artificial habitats resulted nested with both NODF and T metrics. The 40% of the total species pool was found in the drinking troughs. Some of these artificial basins interspersed with the natural pools in the nested order: eight troughs were richer in species than some natural pools, despite the great difference in surface area. Thus, pristine water bodies and their area may not represent major constraints for Odonata species to lay eggs and for larvae to grow. Drinking troughs can be highly relevant in representing refuges in the absence or decline of natural ponds and pools: lacking in top-predators (fishes), they are small "island" habitats that support subsequent generations of Odonata (or other aquatic macroinvertebrates) during dry periods of natural water bodies. The use and focused management of such habitats can be a useful practice for freshwater ecosystems management and Odonata conservation.

ALESSANDRA TANCREDI[1], DAVIDE PAPANDREA[1], MICHELE MARCONCINI[1],
PAOLO L. CATAPANO[1], REBECA CARBALLAR-LEJARAZU[1], MAURICIO CASAS-MARTINEZ[2],
XIAOGUANG CHEN[3], ANNA R. MALACRIDA[1], MARIANGELA BONIZZONI[1]

[1]Department of Biology and Biotechnology, University of Pavia
[2]Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico
[3]Southern Medical University of Guangzhou, Guangzhou, China

TRACING TEMPORAL AND GEOGRAPHIC DISTRIBUTION OF RESISTANCE TO PYRETHROIDS IN THE ARBOVIRAL VECTOR *AEDES ALBOPICTUS*

Aedes albopictus (Skuse) is an important vector of many arbovirus such as chikungunya, dengue, yellow fever and Zika viruses. Since there are not specific antiviral treatments and vaccines are limited, vector control operations are critical to avoid disease transmission. The use of insecticides, primarily pyrethroids (PY), represents the mainstay for vector control (ECDC, 2017). As a consequence, the detection of phenotypic resistance to pyrethroids in *Aedes albopictus* populations since the early 2000 is a major concern and calls for continuous monitoring. Resistance to PY can be monitored in an unbiased manner by analyzing the frequency of *kdr* mutations in vector populations. *kdr* mutations are mutations in the PY target site (i.e. the *vgsc* sodium channel gene) that have been associated with phenotypic resistance. Considering that *Ae. albopictus* is an invasive species, with multiple introductions and genetic admixture among new and old populations, we used sequence data of the three domains of the *vgsc* gene to investigate the pattern of distribution of all PY-resistance predictive mutations in worldwide samples of *Ae. albopictus* and to analyze the temporal changes in the distribution of *kdr* haplotypes. Afterward, we derived haplotype networks to investigate whether *kdr* mutations arose once and then spread or they have evolved independently in different populations. For the first time, we detected mutations at all tested *vgsc* positions, with heterozygotes predominating and rare instance of double mutants. We observed an increase in the distribution and frequency of mutations at position 1534 and the appearance of the V1016G/I mutation as early as 2011 in Italy. Finally, haplotype analyses showed evidence for multiple origins of all *kdr* mutations. These results emphasize the importance of continuous monitoring because a global assessment of *kdr* mutations is fundamental to guide the set-up of national vector control programs.

Simposio III

Zoologia applicata

Coordinatori:
Paolo Bonivento, Maurizio Casiraghi

Poster

FRANCESCO CAVALIERE[1], PIETRO BRANDMAYR[1], MARIA LUIGIA VOMMARO[1],
PIERO GIULIO GIULIANINI[2], ANITA GIGLIO[1]

[1]Dipartimento di Biologia, Ecologia e Scienze della Terra, Università della Calabria
[2]Dipartimento di Scienze della Vita, Università di Trieste

PESTICIDE SUBLETHAL EFFECTS ON SOIL NON-TARGET INSECTS IN AGROECOSYSTEMS

The persistence and residual accumulation of pesticides in the soil are detrimental to the environment and pose a risk for human health and for species providing biocontrol ecosystem services. Carabid beetles are useful bioindicators to evaluate the ecological effects of agrochemicals due to their ecological role as predators in farmland. We performed an environmental monitoring over a period of 12 months in order to assess the sublethal effects on non-target species caused by regular pesticide application. To evaluate the impact of the agrochemicals at population level, activity density of *Harpalus (Pseudoophonus) rufipes* (De Geer, 1774) has been measured using *in vivo* pitfall traps in crops located in a conventional farm (treated; 39°16′58.05″N, 16°38′43.26″E, Società Cooperativa Orti dei Monti, Calabria,). Sampled fields were treated usually with herbicides (a.i. pendimethalin, metribuzin, cicloxidim), pesticides (a.i. imidacloprid, against aphids and potato beetle) and fungicides (a. i. cymoxanil, against potato blight). Specimens used as control were from an organic farm (control; 39°17′10.28″N, 16°42′28.33″E, Macchia di Tuono Farm). To quantify the physiological effect at organism level, we monitored spectrophotometrically the phenoloxidase (PO) activity involved in melanisation and in life trait such as egg production, moulting and cuticle sclerotization in haemolymph of adults from both treated and control sites. Our results showed that the activity density of *H. rufipes* over the experimental period is 4.5-fold higher in conventional field compared with organically managed fields. The value of PO activity in beetles from organic field was significantly higher than in samples from conventional field. However, the decrease of PO activity observed in specimens from treated field may be due to long-term residual effects of some active ingredients of commercial formulates. Even though decreasing immunocompetence will increase the risk of death, the population size of *H. rufipes* and its high seasonal activity density may be interpreted as an effect of residual pesticides in the soil. Low sublethal dose can induce a homeostatic modulation of physiological activities stimulating hormetically reproduction and population growth.

ALESSANDRO LUIGI HEINZL[1], ALESSANDRO VALIANI[1], MATTEO BRUNETTI[1], SUMER ALALI[2], GIULIA MAGOGA[1], MORENA CASARTELLI[3], STEFANO BOCCHI[2], ALBERTO SPADA[1], BIANCA ORTUANI[1], ROBERTO COMOLLI[4], PIETRO MARINO GALLINA[1], MATTEO MONTAGNA[1]

[1]Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano
[2]Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano
[3]Dipartimento di Bioscienze, Università degli Studi di Milano
[4]Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano

INFLUENCE OF GROUND COVER AND SOIL PROPERTIES ON BIOLOGICAL SOIL QUALITY IN ECOSYSTEMS OF THE PO VALLEY

The characterization of the soil physicochemical properties together with the knowledge of the biological diversity associated to this substrate are fundamental to assess the soil quality and to support political decision relative to agricultural and natural ecosystems. The soil is a complex environment in which different taxa (bacteria, fungi and metazoan) play a fundamental role in regulating the organic matter decomposition and nutrient recycling, influencing the ability to maintain the ecosystem services and agricultural production sustainability. In the early 2000s QBS-ar index (Qualità Biologica del Suolo – artropodi) was developed and arose as one of the most used indexes to assess soil biological quality. This index assigns a morphometric score, based on the level of adaptation to edaphic environment, to arthropods extracted through Berlese funnel from soil samples. In this project we collected soil samples from environments with different ground covers (grapevine, corn, rice, mixed forest, alfalfa, stable meadow), for each sample we measured physicochemical properties (i.e., texture, pH, organic carbon and nitrogen) and computed the QBS-ar index. The analysis of variance and a subsequent post-hoc comparison showed a subdivision of the crop into two different groups: the first containing corn, rice and alfalfa 1-3 years and the second with grapevine, mixed forest and alfalfa 4-5 years. The stable meadow is found in both groups. Among the different physicochemical properties analyzed, organic carbon and nitrogen positively correlate with the QBS-ar, while the percentage of sand in the soil correlates negatively with the index. These results support the conclusion that less disturbed soil can sustain higher biological diversity.

MARCO ISAIA, ELENA PIANO

Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino

FUNCTIONAL RESPONSE TO URBANIZATION IN SPIDERS: FROM COMMUNITY TO SPECIES LEVEL

The conversion of natural and semi-natural environments into urban areas results in profound changes in biotic and abiotic ecosystem components, with direct effects on local biodiversity and landscape structure. These alterations likely affect biological communities by filtering species based on their functional traits. This process is expected to deplete habitat specialists, which are often replaced by generalist and tolerant species. In particular, the latter take advantage of the reduced competition and enhance their fitness in urban environments. In order to verify these assumptions, we investigated the response of spiders to urbanization, here intended as a combination of patch isolation and increased urbanization density. We sampled ground spiders within the municipality of Turin (NW-Italy), wherein an isolated and a control subplot were repeatedly sampled across 15 sampling stations distributed along a gradient of increasing urbanization density. The analysis of the community patterns first revealed a decrease in species richness due to isolation, whereas urbanization density strongly reduced evenness. Second, we highlighted how increasing urbanization density essentially filters species based on their functional traits, causing functional homogenization and shifts in the functional composition of spider communities. According to our results, urbanization density alters the structure of spider communities in terms of composition of foraging strategies and increases the average dispersal capacity within the community. Lastly, we investigated whether a functional response to urbanization could also be highlighted at the species level. By considering femur length as a proxy, we showed how urbanization positively affects body size in a generalist species of wolf spider (*Pardosa proxima*), which we explained as a consequence of the reduced competition in urban patches. Being spiders top predators, the ecological consequences of these alterations are expected to be particularly dramatic because of cascading effects on trophic food webs and consequent alterations of ecosystem services.

EMILIANO MANCINI[1], MAURIZIO MUZZI[2], ANDREA DI GIULIO[2], EMILIANO FRATINI[2], MANUELA CERVELLI[2], PAOLO MARIOTTINI[2], FABRIZIO LOMBARDO[3], TIZIANA PERSICHINI[2], SILVIA GISONDI[1,4], TECLA GASPERI[2], MARCO A. BOLOGNA[2]

[1]Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma
[2]Dipartimento di Scienze, Università Roma Tre
[3]Dipartimento di Sanità Pubblica e Malattie Infettive, Roma
[4]Natural History Museum of Denmark, Copenhagen, Denmark

CANTHARIDIN IN BLISTER BEETLES: FROM BIODIVERSITY TO BIOTECHNOLOGY

Cantharidin (CA) is a toxic compound produced by blister beetles (Coleoptera: Meloidae) both as a defensive agent and nuptial gift and accumulated in large quantities in body compartments of these insects. This blistering substance, traditionally used as an aphrodisiac, is a promising biocompound to be used for several medical applications (e.g., dermatology and cancer treatment). However, many aspects regarding the biology of CA in blister beetle, like its endogenous metabolic pathway, site of production and storage, transport and detoxification mechanisms are still unclear. Moreover, the biodiversity of Cantharidin Related Compounds (CRCs) a group of molecules chemically similar to CA (e.g., palasonin, cantaridinimide, etc.) whose pharmaceutical properties are largely unexplored have been scarcely studied in blister beetles so far. Here we present some preliminary data obtained in the frame of the 'CANBBIO' project (Cantaridina: dalla Biodiversità alle Biotecnologie - Progetti Gruppi di ricerca, Conoscenza e Cooperazione per un Nuovo Modello di Sviluppo, L.R. Lazio 13/08, prot. n° 85-2017-14967), an interdisciplinary effort to outline: i) the chemical biodiversity of CA and CRCs in different Meloidae genera, ii) the morphological variability of CA-producing/storing organs and iii) the CA-related transcriptome. The acquired knowledge will aid, in collaboration with Takis Biotech s.r.l., to develop and test novel drug-delivery tools (to be transferred to I.D.I. Farmaceutici s.r.l.) aimed to optimize the therapeutic exploitation of CA and CRCs in oncology and dermatology.

PAOLO BONIVENTO[1], PAOLO GUGLIA[1], EDGARDO MAURI[1], SERGIO DAMBROSI[1], GIUSEPPE MASARIN[1], ISABELLA ABBONA[1], STANISLAO DI AMATO[2], MARCH DOUCHET[3], MICHEL PHILIPS[3], JÉRÉMIE PRIEUR DREVON[3] .

[1]Società Adriatica di Speleologia, Trieste
[2]Reborn srl, Trieste
[3]National Cave Diving Commette FFESSM, Marseille, France

MONITORING METHODS ON THE DISTRIBUTION OF *PROTEUS ANGUINUS* AND PRELIMINARY ESTIMATES IN THE TREBICIAN CAVE

Proteus anguinus has an evolutionary history of 11 million years, it is one of the largest stygobic amphibians, so far known and shares the dependence on underground fresh water with the human people of the karst areas. *Proteus* lives in the underground waters of the Dinaric Karst from the lower Isonzo (Soča), in Venezia Giulia, Italy, up to the Trebišnjica river in Bosnia Herzegovina. In Italy its presence is reported from 1826 in the Pozzo dei Frari in Gradisca; moreover a population, whose number is unknown, has naturalized in Veneto since 1850, when some specimens from Postojna, were introduced into the caves of the Oliero river. The observation and monitoring of *Proteus anguinus* occurs through the few windows (springs, caves and wells) that allow access (direct or indirect) to groundwater. Recent studies have been based on direct observation. However hypotheses exist on the presence of two distinct Olms' groups whose precise distribution and possible hybridization are not yet clear. The quantification of the population and its distribution remain objectives to be achieved. Modern sampling techniques with environmental DNA have given good results in Bosnia and Herzegovina and Slovenia, and studies are underway to extend the research also to the Italian Karst. Direct underwater observation can be considered optimal in accessible submerged stretches. For the first time in Italy in the summer 2018 March Douchet, Jérémie Prieur Drevon, experimented in the cave of Trebiciano, 17vg (Italy), the underwater counting of specimens of *Proteus anguinus* with the transept method. Recent observations lead us to believe that the Italian population of *Proteus anguinus* is in a satisfactory state of preservation. However the study is limited to specific situations and the values could be overestimated. This work aims to experiment methods of monitoring the hypogean fauna and the Proteus in particular, more and more precise to ensure their protection.

MARTINA PETRELLA[1], WALTER DI MARI[2], CLAUDIO GALLOTTINI[3], GRAZIA LUPOLI[1]

[1]Università degli Studi di "Tor Vergata", Roma
[2]Guardia di Finanza
[3]Eur servizi impresa

PATHOLOGIC MICROBIOMA IN DOGS. STEM CELL AND NANOBIOMATERIALS TO RECOVER BONE

The periodontal disease is characterized by progressive loss of bone and destruction of tooth-supporting tissues.When these chronic inflammations are left untreated, the consequences will be hard pain, difficulty to eat, and loss of teeth.Periodontal damage can affect the upper canine area where the sense of smell occurs. The result is a loss of the ability to perceive the palatability of food. This problem often appears in dogs in service with finance police's, anti-narcotics police's, or used to rescue missing people during natural disasters. In this case the disease would damage the upper olfactory canine, reducing the dog working capability.This leads to a reduction in working ability and an economic damage linked to the years spent on training.It is difficult to recover the correct structure and function of periodontal tissue using existing therapies, the cause is the complex structure of periodontal tissue. Through tissue engineering it is possible to repair the damaged tissues implanting stem cells, which are used in regenerative medicine. These could be applied alone or in combination with Nano-biomaterials, as scaffolds, creating a suitable 3-D environment in order to achieve complete regeneration.In this sense, every innovation is an exciting scientific challenge with the goal of producing bioinspired materials, early recognized and better integrated with living tissues: NHA can be one of these materials. Currently, clinical manipulation and in vivo NHA implant are still complex because Nano-structured materials tends to disperse. Multipotent mesenchymal stromal cells (MSC) from fat tissue have already been applied for periodontal regeneration in clinical trials. Based on these promising results, a clinical trial was carried on, and MSC were transplanted onto the cleaned dental root, like a plaster. This allogeneic transplantation of a MSC has become a good strategy. A clinical evaluation was carried out with imaging, histological and biochemical analysis in a follow-up. Thanks to the implant of NHA in the lesion site, through plaster mixed with a gel of autologous AMSC, was observed the function recovery of bone and olfactory. If this technique is practised on anti-narcotics dogs, it provides a considerable economic gain, allowing them to protract their activity for many years. Among the most compelling applications, we can find orthopaedic and maxillofacial reconstructions in veterinary dentistry and veterinary medicine-geriatrics. However, we can assist to a multitude of evaluations about applications in case of congenital malformations, genetic and degenerative solutions in order to improve the quantity and quality of bone tissue. Fusing the most advanced technologies, nowadays medical producers are manufacturing devices able to restore the functionalities of damaged body, towards self-regeneration. Tissue engineering with a combination of cells and Nha scaffolds is considered to be a viable alternative strategy and could promote and accelerate the healing process of bone tissue. To conclude, dental stem cells increase new bone formation in experimental design dysbiosis. We have evaluated the efficacy of AMSC autologous tissue stem cells for bone regeneration. This is an innovative application, different from traditional therapies to treat numerous injuries and it could become the "gold standard" therapy for many illnesses in veterinary. We propose to launch this pilot scheme in dogs treatment since it can be a protocol for other species too. The data obtained will be correlated with the standard intervention procedures and analysed in order to draw up a farsighted perception of new line of research.

LUCIA PIEMONTESE[1], SARA BORTOLINI[2], GIACOMO BULGARINI[1], LARA MAISTRELLO[1],
LORENA REBECCHI[1], ROBERTO GUIDETTI[1], MICHELE CESARI[1]

[1]Dipartimento di Scienze della Vita, Università degli Studi di Modena e Reggio Emilia
[2]Facoltà di Scienze e Tecnologie, Libera Università di Bolzano

IDENTIFYING NATIVE PREDATORS OF THE ALIEN PEST *HALYOMORPHA HALYS* (HEMIPTERA, PENTATOMIDAE) WITH REAL-TIME PCR ASSAY

In recent years, the establishment and diffusion in Italy of the invasive brown marmorated stink bug *Halyomorpha halys* is posing a serious threat to agricultural, urban and natural ecosystems. The difficulty to early detect the presence of new propagules, and the absence of native co-evolved antagonists (i.e. pathogens, parasitoids, predators) which can limit the populations growth, are among the main factors that drive an exotic species to become invasive. DNA-based methods can overcome the need of *a priori* information about the presence of a species by detecting its genetic signature in a large variety of biological and environmental samples. In this study, we tested the efficacy of a qualitative Real-Time PCR protocol to assess the predatory potential of several animals by detecting the presence of *H. halys* DNA in: i.) 27 guano samples from nine Italian chiropteran species collected in natural and agricultural areas (Piedmont, Aosta Valley, Tuscany), therefore assessing the potential chiropteran predation; ii.) gut-contents of 50 arthropod specimens collected in two urban parks in Emilia Romagna. For both assays, we used the protocol developed by Valentin *et al*. (2016) and based on a TaqMan minor groove binder (MGB) species-specific probe for identifying and amplifying a fragment of *H. halys* ITS1 rDNA. We identified two genera of bats (*Myotis*, *Nyctalus*) feeding on *H. halys*, with four positive hits found from agricultural sites in Piedmont and two positive hits in Tuscany parks. We also found positive results in 23 analysed gut contents, identifying eight *H. halys* predator species among insects (*Forficula auricularia,* Dermaptera; *Arachnocephalus vestitus*, *Yersinella raymondi*, Orthoptera; *Nagusta goedellii*, Hemiptera; *Harmonia axyridis,* Coleoptera) and arachnids (*Opilio canestrinii*, *Mitopus morio*, Opiliones; unidentified spider). Present results prove that the species-specific Real-Time PCR assay can address different biological questions (e.g. early detection of pests, predation rates) and operate on very different substrates, thus improving pest management efficiency.

DENISE SGUEGLIA, VALERIA MASELLI, ANNA DI COSMO

Dipartimento di Biologia, Università di Napoli Federico II

SPECIES IDENTIFICATION OF PRECIOUS BIOGENIC GEMS BY DNA FINGERPRINTING

Precious biogenic gems, like corals and pearls, are important for biodiversity and natural richness, but also for the jewelry industry, art and economy of many countries. As a collective term, “coral” is referred to most of the cnidarians of the Anthozoa class, and “precious coral” belongs to family Corallidae, that include many species such as *Corallium rubrum* or *C. japonicum*. Pearls, that are also considered very valuable gems, are produced by oyster species of Pteriidae family, like *Pinctada margaritifera, P. maxima* and *P. radiate*. Coral species included in CITES Annex III are *C. elatius, C. japonicum, C. konjoi*, and *C. secundum*, as recently requested by China and no precious oysters are included in CITES Annex. Due to the necessity to distinguish between protected and non-protected gems and between the fakes and the originals, the accurate identification of the species assumes a significant meaning, to protect the natural environment and to help both trade and law. Precious biogenic gems are studied to identify the species through various methods, analyzing the chemical-physical properties, the external morphology, and the internal structure. However, for species identification, the molecular approach such as DNA barcoding results to be the most assured. This work aims to describe a molecular approach for the species identification of precious biogenic gems (corals and pearls), using the DNA fingerprinting method. We extracted DNA from valuable gems, given by Bahrain Institute for Pearls and Gemstones (DANAT) and by Italian Gemological Institute (IGICOM). The coral samples provided were classified as *C. rubrum;* pearl samples were classified as unknown species or belonging to the following oyster species *P. margaritifera, P. maxima, P. radiata* or *P. fucata*. The extracted DNA were tested through a suitable protocol of PCR amplification, using as molecular markers mitochondrial COX1 and ND2 regions for corals, and mitochondrial 16S rRNA and COX1genes and the nuclear ITS1 and ITS2 regions for pearls. The purified PCR products were sequenced, and the assignment of species was obtained by comparison with our reference library and GenBank-BLAST database. The analysis of species identification for corals confirmed that all samples may be assigned to *C. rubrum* with 100% probability, meanwhile for pearls we confirmed, for most of the cases, the previous identification made by DANAT experts, in other cases our analysis has been able to assign the species more accurately. This is a significant achievement in order to demonstrate that the molecular approach is more reliable than others. In conclusion the use of molecular approach provides an effective method to identify precisely the species of precious biogenic gems, opening to numerous practical purposes. The DNA fingerprinting method provides an added value to precious gems and consequently for the jewelry industry. Moreover, it may also represent a strong tool to contrast the illegal market of protected species, contributing to the world jewelry sustainability.

MARIA LUIGIA VOMMARO[1], PIETRO BRANDMAYR[1], FRANCESCO CAVALIERE[1],
PIERO GIULIO GIULIANINI[2], ANITA GIGLIO[1]

[1]Dipartimento di Biologia, Ecologia e Scienze della Terra, Università della Calabria
[2]Dipartimento di Scienze della vita, Università di Trieste

HAEMOCYTE NUMBER AND PHAGOCYTING ABILITY AS MARKERS OF HERBICIDE EXPOSURE

Haemocytes are the first line of defence in invertebrate's immunity, and the changes in number, hemopoiesis and phagocytic ability are strictly connected with the developmental stage and physiological condition of the insects, for this reason, they may be good markers of exposure to xenobionts. Beneficial soil insects in the agroecosystems are inevitably exposed to herbicides, with a risk for the biodiversity. In this study, variations of haemocyte profile and phagocytosis activity in *Harpalus (Pseudoophonus) rufipes* (De Geer 1774) were assessed in order to estimate effects of pendimethalin exposure, one of the most commonly used herbicides in agriculture. Field collected specimens were housed in boxes containing soil treated once with Activus® (a.i. pendimethalin) at the recommended field rate (4L per ha of a.i., from 330gr/L of commercial formulation) and on clean soil as control (ctrl). Total (THC) and differential (DHC) haemocyte counts and phagocytosis activity have assessed 2, 7, 21 and 28 days after the initial exposure respectively and compared with non-treated control group. Morphotypes were identified evaluating differences in size, morphology and staining affinity. The percentage of phagocytizing cells was measured, as marker of immunocompetence, after *in vivo* injection of carboxylate-modified polystirene latex beads in beetles'abdomen. A significant reduction in THCs was recorded in treated group over exposure time. In DHCs, the relative percentage of immature cells increased 48h after initial treatment. These cells show high nucleus/cell surface ratio, bigger size than differentiated haemocytes and absence of granulations. DHCs in latex-injected animals have revealed a reduction in the relative percentage of granulocytes 2 days after exposure and a decrease in phagocytic ability 7 days after. Numerous cytological alterations, such as vacuolization of cytoplasm, clumping of chromatin and presence of cytoplasmatic inclusions, were observed in treated samples. This study confirms that the hemogram is a suitable marker to estimate herbicide harmful effects. Sublethal doses of pendimethalin affect haemocyte number and differentiation degree, with consequences on phagocytic ability. That compromises animal's immunocompetence and increases their vulnerability to pathogens, with potential effects on population dynamics and biodiversity in agroecosystems. Further investigation may elucidate cellular ultrastructure alterations following the exposure of tested herbicide.

PAOLA ZARATTINI[1], MATTEO CAMMARATA[2], GRAZIANO FIORITO[3], ANNARITA WIRZ[4],
MARIA AGNESE SABATINI[5]

[1]Dipartimento di Scienze della Vita, Università di Trieste
[2]Dipartimento della Terra e del Mare, Università di Palermo
[3]Stazione Zoologica Anton Dohrn, Napoli
[4]Fondazione Santa Lucia Irccs, Roma
[5]Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia

THE ANIMAL FACILITY: A PLACE WHERE ZOOLOGY IS (OR SHOULD BE) APPLIED

There are many different types and sizes of facilities that house animals utilized for research purposes. As a consequence, animals' care result to be more complex also considering the use of 'alternative' and genetically modified animal models. As animal husbandry and management have become more complex, staffing for animal facilities is required to be more specialized, thus to ensure the welfare of the species housed (e.g., rodents, non-rodent mammals, birds, reptiles, amphibians, fish and cephalopod molluscs). Animal care is a 365-day long process and the care-takers provide daily husbandry of laboratory animals, including observation of animals, handling and restrain, feeding and watering, changing cages/racks and sanitizing animal quarters and equipment. To do that a correct animal identification (genus and species recognition) based on zoological nomenclature, but also of each individual animal (same species) is needed. Furthermore, two different species might require different environments (e. g., appropriate micro- and macroenvironment) even though they belong to the same genus (e.g., *Xenopus laevis* Daudin, 1802 and *Xenopus tropicalis* Gray, 1864; *Octopus vulgaris* Cuvier, 1797 and *Octopus bimaculoides* Pickford & McConnaughey, 1949). Likewise, it is crucial to be able to carry out a correct genetic identification since the use of genetically modified animals and the amount of breeding have recently highly increased. An additional expertise that is starting to become more prevalent is the "animal behaviourist", that requires an educational background in ethology, the science of animal behavioural process and its causes. Thus ethology, closely related to other disciplines (e.g. neuroanatomy, ecology, evolutionary biology), is an essential part of the judgement of animal welfare in different husbandry animal systems. Based on all above considerations it is clear that zoological knowledge is increasingly required for care-takers and animal facility directors, as the consciousness and skill for animal well-being in laboratory animal facilities continue to improve and, it is at the foremost high attention for ensuring appropriate species maintenance and defining correct protocols and animal welfare overview including national committee participation and European rules and laws active actions.

Simposio IV

Filogenesi, biogeografia e sistematica

Coordinatori:
Giuliana Allegrucci, Vincenzo Caputo Barucchi, Ettore Olmo

Lettura plenaria

OMAR ROTA-STABELLI

Unità Entomologia Agraria, Centro Ricerche e Innovazione, Fondazione Edmund Mach,
San Michele all'Adige, Trento

THE MOLECULAR CLOCK: A POWERFUL TOOL TO STUDY THE EVOLUTION OF ANIMALS AND THEIR MICROBIOTA

The molecular clock is a powerful technique used in systematics and biogeography to estimate evolutionary timescales. Because the clock varies in different clades, it should be locally tuned using calibration points. In the case of animals characterised by a rich fossil records, the clock can be calibrated using paleontological data: this procedure was successful in the last two decades in estimating the timing of key macro-evolutionary events such as the origin of major Metazoa body plans or their colonisation of lands. For recent radiations, the clock can be calibrated using mutation rates and ancient DNA: this approach generally requires whole genome data and has the potential of revealing not only the timing of speciation, but also hybridisation and adaptive events as exemplified by various examples in Insecta and Mammalia. The clock can also be used to study the microbiota of animals: using complete genomes and ancient DNA it is now possible to reconstruct for example the origin of the *Wolbachia* infection in Ecdysozoa or the evolution of gut microbiota in primates. Overall, the molecular clock is a powerful and versatile technique for exploring the diversity of animals and tracing the timing of their evolution.

Simposio IV

Filogenesi, biogeografia e sistematica

Coordinatori:
Giuliana Allegrucci, Vincenzo Caputo Barucchi, Ettore Olmo

Presentazioni orali

RAZIANO DI GIUSEPPE[1], CLAUDIO ALIMENTI[2], ADRIANA VALLESI[2], ALESSANDRO VALBONESI[2], PIERANGELO LUPORINI[2]

[1]Dipartimento di Biologia, Università di Pisa
[2]Laboratorio di Microbiologia eucariotica e Biologia animale, Università di Camerino

MULTIPLE COLONIZATION EVENTS OF ANTARCTIC WATERS FROM THE CILIATE *EUPLOTES*: EVIDENCE FROM PHYLOGENETIC ANALYSIS OF ANTARCTIC AND NON-ANTARCTIC POPULATIONS

The Antarctic convergence together with the west-to-east circumpolar current provide a powerful barrier to the movement of marine life into, or out of the region. Despite this physical isolation, Antarctic coastal sea waters are extremely rich in biodiversity. Among unicellular eukaryotes, diatoms and dinoflagellates are the dominant autotrophic forms that bloom into huge biomasses in the water column, while filter-feeding ciliates are a dominant heterotrophic group that links the benthic and pelagic food webs by eating bacteria decomposers on the seabed and being, in turn, a relevant food source for sub-adult stages of benthonic and planktonic animals.-Through many years of sampling coastal sites of Terra Nova Bay (Ross Sea) for species of the most speciose ciliate, *Euplotes*, we established a numerous laboratory collection of strains representing five well-distinct morphospecies, *E. euryhalinus*, *E. focardii*, *E. nobilii*, *E. petzi* and *E. rariseta*, which manifest sex in the form of conjugation under the genetic control of high-multiple mating-type systems that, by greatly favoring outbreeding, generate an ample intraspecific genetic diversity instrumental to establish reliable inter-population phylogenetic links and phylogeographic patterns. By determining and comparing the small-subunit ribosomal RNA (SSU-rRNA) gene sequences of these strains with homologous gene sequences from strains of non-Antarctic congeneric populations, evidence was obtained that *E. euryhalinus*, *E. focardii*, *E. nobilii*, *E. petzi* and *E. rariseta* split into distinct clades of the *Euplotes* phylogenetic tree. This phylogenetic split implies that the evolutionary history of *Euplotes* entails multiple, independent events of colonization of the Antarctic waters and suggests, in a general perspective of microbial biogeography, that the ecological barriers to move into, or out of Antarctic waters are largely ineffective to disrupt a bipolar, cosmopolitan dispersal of eukaryotic microorganisms. Which reinforces the concept of "metapopulation" (i.e., a group of populations of the same species separated by space but linked by dispersal and migration) originally applied to protists' biogeography.

DAVIDE MAGGIONI[1,2], GIULIA LIGUORI[1], ROBERTO ARRIGONI[3,4], MICHAEL BERUMEN[4],
DAVIDE SEVESO[1,2], BERT HOEKSEMA[5], PAOLO GALLI[1,2], SIMONE MONTANO[1,2]

[1]Dipartimento di Scienze dell'Ambiente e della Terra (DISAT), Università degli Studi di Milano-Bicocca
[2]Marine Research and High Education (MaRHE) Center, Università degli Studi di Milano-Bicocca, Faafu Magoodhoo, Maldives
[3]European Commission, Joint Research Centre, Directorate D - Sustainable Resources, Bio-Economy Unit, ISPRA
[4]Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia
[5]Department of Marine Zoology, Naturalis Biodiversity Center, Leiden, The Netherlands

ONE PATHOGEN ONE DISEASE: SHEDDING LIGHT ON THE SKELETON ERODING BAND CORAL DISEASE

Coral reefs are declining worldwide, and coral diseases are emerging as one of the most affecting threats. Many coral diseases have been described so far, but their knowledge is often limited by the incomplete information reported in literature. The Skeleton Eroding Band (SEB) and the Caribbean Ciliate Infection (CCI) are two coral diseases caused by ciliates belonging the genus *Halofolliculina*. The SEB shows the widest host range and is distributed in the Indo-Pacific Ocean and Red Sea, while the CCI affects about half of the scleractinan species of the Caribbean Sea. Despite appearing very similar, the two diseases are considered different and their pathogens have been hypothetised to belong to different *Halofolliculina* species. In this work, we analysed the morphology and genetic diversity of *Halofolliculina* ciliates collected in the Caribbean Sea, Red Sea, and Indo-Pacific Ocean, to assess possible taxonomic affinities and to discuss a possible standardised disease name.

Fine-scale morphological and morphometric examinations revealed a conserved phenotype in all analysed samples, which were unequivocally identified as *Halofolliculina corallasia*. Phylogenetic analyses based on nuclear (ITS) and mitochondrial (COI) molecular markers consistently found all *H. corallasia* samples as monophyletic. The nuclear marker displayed an extremely low intra-specific diversity, supporting the belonging of all samples to the same species. The analyses based on COI showed a moderate divergence between samples from different localities, but this genetic diversity falls within the intra-specific range found for other heterotrich species. Overall, morphology and DNA support *H. corallasia* as the pathogen associated to both the Skeleton Eroding Band disease and the Caribbean Ciliate Infection. Therefore, we propose to synonymize the CCI with the SEB and to extend the distribution of the SEB to the Caribbean Sea, conferring to this disease the widest host range among all known coral diseases.

JOACHIM LANGENECK[1], FERRUCCIO MALTAGLIATI[1], FABIO SCARPA[2], DARIA SANNA[3],
MICHELE BARBIERI[1], PIERO COSSU[2], BARBARA MIKAC[4], MARCO CURINI-GALLETTI[2],
MARCO CASU[2], ALBERTO CASTELLI[1]

[1]Università di Pisa, Dipartimento di Biologia
[2]Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria
[3]Università degli Studi di Sassari, Dipartimento di Scienze Biomediche
[4]Università di Bologna, Centro Interdipartimentale di Ricerca per le Scienze Ambientali (CIRSA), Ravenna

EVOLUTIONARY COMPLEXITY IN MARINE INVERTEBRATES: THE CASE OF THE *SYLLIS GRACILIS* (ANNELIDA, SYLLIDAE) SPECIES COMPLEX

A striking abundance of cryptic and pseudocryptic species was highlighted in marine invertebrates by a number of studies, the majority of which focused however on cryptic diversity as such, without inferring on evolutionary drivers involved in organism diversification. The widespread polychaete *Syllis gracilis* Grube, 1840 was identified as a possible species complex by MALTAGLIATI *et al.* (2000), who identified Mediterranean marine and brackish populations as putative cryptic species on the basis of allozyme data. More recently, sequence data allowed to consider the allegedly cosmopolitan *S. gracilis* as a paraphyletic species complex, including at least three distinct Mediterranean lineages. To understand the role of biogeographical and ecological factors in the differentiation of the complex, populations of *S. gracilis* were sampled from eleven Mediterranean localities, and a combined morphological and molecular characterisation was carried out. Multivariate and univariate analyses on the morphological dataset highlighted that the majority of traits are fixed at population level, but it is possible to distinguish two morphotypes, one with short cirri, associated with intertidal coralline algae, and one with long cirri occurring on *Sabellaria* reefs and in brackish environments. On the other hand, 16S rDNA sequences highlighted the occurrence of three separated lineages, of which only two correspond to those identified by Álvarez-Campos *et al.* (2017). Therefore, *S. gracilis* includes at least four cryptic lineages. Molecular lineages did not show any correspondence with morphological diversity, and natural populations of *S. gracilis* usually included at least two cryptic lineages, without a clear distinction between marine and brackish-water populations. This outcome suggests that the relationship between the molecular pattern detected and ecological and biogeographical features is not straightforward. The observed morphological pattern is most likely due to local adaptations and phenotypic plasticity, while the separation between molecular lineages is possibly due to ancient geological events. The diversity and evolution of marine invertebrates is shaped by a number of different processes working and interacting at different spatial and temporal scales. The reconstruction of evolutionary processes should therefore take into consideration as many different evidence lines as possible. Although the inconsistent pattern detected make the integration of morphological and molecular patterns difficult, these patterns are the consequence of unpredictable interactions between different evolutionary drivers, thus representing an interesting case of irreducible evolutionary complexity in marine systems.

PIETRO PAOLO FANCIULLI, CHIARA LEO, FRANCESCO FRATI, ANTONIO CARAPELLI

Dipartimento di Scienze della Vita, Università di Siena

MORPHOLOGY AND GENETICS OF ANTARCTIC SPRINGTAILS

Among the life forms that adapted to the harsh Antarctic environment, springtails and mites are the most abundant arthropod groups of the terrestrial ecosystem, both in Continental and Maritime Antarctica. The study of the systematics of these organisms started at the beginning of the last century and, in more recent years, received new inputs. The taxonomy of the springtail species from the Continental Antarctica has undergone more systematic revisions using new and modern morphological characters and applying molecular techniques. The latter allowed us to study the evolution of populations of different species and have sometimes reported high values of intraspecific genetic differentiation, that would suggest that a larger number of "cryptic" species is not identified yet. In this study, a careful morphological analysis was therefore carried out on several populations of two Antarctic species, *Friesea grisea* and *Cryptopygus terranovus*, in order to further verify what emerged through the molecular approach and, possibly, to characterize from a morphological point of view these new taxonomic entities.

ANDREA GALIMBERTI[1], GIACOMO ASSANDRI[2,3], FAUSTO RAMAZZOTTI[1], ANNA SANDIONIGI[1], ANTONIA BRUNO[1], NICOLA TOMMASI[1], MAURIZIO CASIRAGHI[1]

[1]ZooPlantLab, Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano Bicocca
[2]MUSE – Museo delle Scienze di Trento
[3]Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia

FROM DNA BARCODING TO E-DNA MONITORING: NEW INSIGHTS ON ITALIAN ODONATE DIVERSITY

With about 90 resident species, Italy is among the richest European countries in terms of odonate diversity. However, many aspects regarding the occurrence and genetic structure of many species are still incomplete or unexplored. Given the peculiar distribution pattern of many dragonflies and damselflies species (some of which showing fragmented or relict populations in Italy, suggesting complex biogeographic histories), it is of fundamental importance to better characterize the genetic, morphological and ecological traits of Italian populations, in order to uncover possible taxonomic novelties and support conservation actions. Moreover, odonates are reliable bioindicators of environmental quality but their monitoring is often hampered by several detectability constraints and could be expensive in terms of time and resources. A DNA-based reference archive for Italian odonates is therefore required to shed light on this insect group; however, to date, only a few sequences referring to less than 10 species are currently available in international databases. Here we present the first results of the "DNA barcoding Italian Odonates" project which aims at i) creating a genetic reference dataset (DNA barcoding) for these insects to serve as starting point for further integrative phylogeographic studies and ii) using this dataset to taxonomically assign HTS reads from eDNA water samples collected at different study sites (DNA metabarcoding). eDNA offers many possibilities in terms of management and conservation applications in wetlands and, notably, for endangered and/or charismatic species. To date, more than 250 samples belonging to 80 Italian species have been sequenced at the mitochondrial COI barcode locus (658 bp) and, in case of high intraspecific or low interspecific divergence values, additional mitochondrial or nuclear loci were investigated. Highly divergent lineages between Italian/European populations have been found for some species (e.g., *Erythromma lindenii*) while unexpected and almost total genetic similarity has been observed among *Coenagrion* and *Somatochlora* species. Concerning the eDNA metabarcoding analysis, three universal primer sets targeting COI (250 - 322 bp) were tested on 10 water samples collected at study sites where the odonate assemblages are well-known. The preliminary results showed a good coverage of the species known to occur in the sample sites and thus the high potential of this approach in supporting biodiversity monitoring.

FAUSTO MOSCHELLA[1], ENZO COLONNELLI[2], ALESSIO DE BIASE[1]

[1]Dipartimentio di Biologia e Biotecnologie “C. Darwin”, Sapienza Università di Roma
[2]Via delle Giunchiglie 56, Roma

PHYLOGEOGRAPHY OF THE THISTLE WEEVIL *TRICHOSIROCALUS HORRIDUS* (PANZER, 1801) (COLEOPTERA: CURCULIONIDAE, CEUTORHYNCHINAE) IN EUROPE

Phylogeographical studies aim to elucidate the historical mechanisms and the processes responsable for the geographic distribution of genetic lineages, especially within and among closely related species. Frequent gene flow generally reduces differentiation level among populations, whereas other factors, such as historical processes, geographical barriers, and local adaptation, can lead to population subdivisions. Palaeoclimatic events and climatic changes have long been studied by evolutionary biologists, and in particular the role that glacial and interglacial phases played in the diversification of many plants and animals. The genus *Trichosirocalus* includes 16 species with a Palaeartic distribution that mainly feed on Plantaginaceae and Asteraceae. In particular *Trichosirocalus horridus* shows a distribution from Iberian Peninsula to Caucasus and an association to vegetal species belonging to the genera *Carduus*, *Cirsium* and occasionally *Onopordum* (Asteraceae, Carduae). Because these thistles are invasive in North America, Australia and New Zealand, *T. horridus* has been used as a biological control agent. Samples of adult weevils were collected during field trips carried out in 10 different countries (Armenia, France, Georgia, Germany, Greece, Iberian Peninsula, Italy, Romania, Slovakia, Turkey). We detect for the first time the phylogeography of *Trichosirocalus horridus* examining 621 bp of the mitochondrial cytochrome c oxidase subunit I (*cox 1*) for 215 individuals and 722 bp of the nuclear Elongation factor alpha subunit (*Ef1α*) for 185 individuals. Genetic divergence analysis between geographical groups was performed through MEGAX v10.1. Bayesian analyses were performed using BEAST v1.8.4 version under best-fit models of nucleotide substitution selected by jModelTest. The evaluation of intra and inter-populational genetic structure was performed by the construction of a haplotype network using TCS v1.21 and by Analysis of Molecular Variance (AMOVA) through ARLEQUIN v3.5.2.2. The inference of demographic history was carried out analysing the shape of the distribution of the number of observed differences between pairs of DNA sequences (mismatch distribution) and using D test and Fu test, through ARLEQUIN v3.5.2.2. Spatial diffusion processes out of the ancestral areas were estimated by means of Bayesian phylogeographic (BP) reconstructions. Historical demographic changes were inferred for each group based on mtDNA data through Bayesian Skyline Plot (BSP) analysis. Our data show that structure of genetic variation is consistent with geographical distribution of populations also suggesting an Eastern origin of this species. The detected pattern is probably correlated to the demographic histories of the analysed populations and very likely influenced by Pleistocene climatic oscillations. Moreover, the investigated Italian populations clustered in two groups, one exclusive of Italy and another showing relationships with other European populations.

ALESSANDRA RICCIERI[1], EMILIANO MANCINI[2], DANIELE SALVI[3,4], MARCO A. BOLOGNA[1]

[1]Dipartimento di Scienze, Università degli Studi Roma Tre
[2]Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma
[3]Dipartimento di Medicina clinica, sanità pubblica, scienze della vita e dell'ambiente, Università degli studi de L'Aquila, L'Aquila-Coppito
[4]CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal

BIOGEOGRAPHY OF *HYCLEUS* (COLEOPTERA: MELOIDAE): SPATIAL AND TEMPORAL DIVERSIFICATION OF A HYPER-DIVERSE GENUS

The genus *Hycleus* is a hyper-diverse genus of blister beetles including ~500 species with a wide geographic distribution in different biogeographic regions in the Old World, and with the highest diversity occurring in the Afrotropical Region. The phylogenetic relationships among the species and the biogeographic processes related to their diversification have never been investigated. In this study, to provide a biogeographic hypothesis that allow explaining the current diversity and the observed distribution patterns of *Hycleus*, we built a robust time-calibrated phylogenetic tree using mitochondrial and nuclear DNA obtained from 125 species. Phylogenetic results were subsequently used for biogeographic inference carried out with the R package BioGeoBEARS. Four main lineages were detected in our tree: A. including only Afrotropical species; B. including only Saharo-Sindian species; C. comprising Afrotropical, Palaearctic and Saharo-Sindian species; and D. comprising Afrotropical and Oriental species. According to our results, the genus *Hycleus* likely originated in the Afrotropical Region during the Early Miocene (~20 Mya), and subsequently spread in the Saharo-Sindian Transitional Region and in the Palaearctic Region. In the Late Miocene (~7.5 Mya) a second Saharo-Sindian group branched off from the Palaearctic lineage, whereas the Oriental Region was colonized (~7.8 Mya) following a dispersal event through the Arabian Peninsula from the Afrotropical Region.

STEFANIA CHIESA[1,2], GIACOMO BERNARDI[3], ERNESTO AZZURRO[1,4]

[1]ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale, Roma
[2]Departimento di Scienze, Università "Roma Tre"
[3]Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, USA
[4]Stazione Zoologica A. Dohrn, Napoli

THE STATE OF THE ART ON GENETICS AND GENOMICS OF MARINE FISH INVASIONS

Biological invasions are unanimously considered a major treat to biodiversity and a driver of global environmental change but also a fascinating subject for ecological and evolutionary research. This also apply to marine exotic fishes, which have been the subject of an increasing number of studies, including molecular ones, which may provide key information for understanding and managing marine bioinvasions. Here, we searched the global literature to provide "the state of the art" of the genetics and genomics of marine fish invasions. Our review included information on 66 fish species belonging to 39 families and 52 orders, extracted from 80 published studies on both WoS (Web of Science) and Scopus databases. The majority of studies were performed in the Mediterranean Sea, which is considered one of the most invaded geographic marine regions on Earth. Furthermore, most studies were limited to taxonomic identification and the description of cryptic diversity of the species; and/or to the development of novel molecular markers. In contrast, few data are available on the genetic structure of introduced populations and the genetic mechanisms involved in the invasion of novel environments. When available, genetic/genomic data showed limited/no reduction of genetic diversity comparing native and introduced populations, suggesting the absence of bottleneck effects. Finally, the most recent investigations focused on the evolutionary changes associated with invasive lineages, opening new perspectives to investigate their mechanisms of adaptation.

TATIANA FIORAVANTI[1], FILIPPO BARGNESI[1,2], ANDREA SPLENDIANI[1], MASSIMO GIOVANNOTTI[1], FRANCESCA RENZI[1], VINCENZO CAPUTO BARUCCHI[1]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, UNIVPM
[2] Acquario di Cattolica, Parco Le Navi

HISTORICAL DNA SHEDS LIGHT ON THE ORIGIN OF THE MEDITERRANEAN SAND TIGER SHARK (*CARCHARIAS TAURUS*, LAMNIFORMES: ODONTASPIDIDAE): A SPECIES PROBABLY DISAPPEARED FROM THIS BASIN

The sand tiger shark (*Carcharias taurus*) is a coastal species distributed in temperate and sub-tropical waters, classified as "Critically endangered" in Mediterranean Sea. Six populations (Northwestern Atlantic, Brazil, South Africa, Japan, Eastern Australia and Western Australia) with low genetic diversity and limited gene flow were identified worldwide, but genetic information for many other geographic areas are still missing. Specifically, this species is listed in several reports as part of the Mediterranean fauna, even if there is a lack of catches and sightings in recent years in this basin. In order to clarify the origin of *C. taurus* individuals caught in the past in the Mediterranean Sea, historical samples were genetically analysed. Nine samples with a certain Mediterranean origin were collected from different European museums. Genomic DNA was extracted using a specific protocol for ancient samples and ~ 600 bp of the mitochondrial DNA control region was amplified using eight overlapping species- specific primer pairs. Sequences obtained were aligned with all the haplotypes globally known so far. Genetic analysis revealed the misidentification of one museum specimen. Among the remaining Mediterranean historical samples, three different haplotypes were recovered. Two of them previously observed only in South Africa and one described in both South African and Brazilian populations. Results obtained suggest a genetic relationship between Mediterranean sand tiger sharks and those from the Western Indian Ocean. According to previous studies, we hypothesized that during the Pleistocene the cold Benguela upwelling barrier was temporarily reduced allowing the passage of *C. taurus* from Indian to Atlantic Ocean. After the restoration of this phylogeographic barrier some individuals were trapped in the Atlantic Ocean and probably migrated northward colonizing the Western African coasts and the Mediterranean Sea.

LEONARDO DAPPORTO[1], ALESSANDRO CINI[1], RALUCA VODĂ[2], VLAD DINCĂ[3], MARTIN WIEMERS[4], MATTIA MENCHETTI[1], GIULIA MAGINI[1], GERARD TALAVERA[5], TIM SHREEVE[6], SIMONA BONELLI[2], LUCA PIETRO CASACCI[7,2], EMILIO BALLETTO[2], STEFANO SCALERCIO[8], ROGER VILA[5]

[1]Dipartimento di Biologia dell'Università di Firenze
[2]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino
[3]Department of Ecology and Genetics, PO Box 3000, University of Oulu, Oulu, Finland
[4]UFZ – Helmholtz Centre for Environmental Research, Department of Community Ecology, Halle, Germany
[5]Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Spain
[6]Centre for Ecology, Environment and Conservation, Faculty of Health and Life Sciences, Oxford Brookes University, Headington, Oxford, UK
[7]Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland
[8]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca Foreste e Legno, Rende

INTEGRATING THREE COMPREHENSIVE DATASETS SHOWS THAT MITOCHONDRIAL DNA VARIATION IS LINKED TO SPECIES TRAITS AND PALAEOGEOGRAPHIC EVENTS IN EUROPEAN BUTTERFLIES

Genetic diversity within populations and its spatial differentiation among populations are central concepts in biology. Understanding the dynamics determining and maintaining genetic diversity is critical for predicting responses to environmental changes and for effective conservation measures. This task requires mapping diversity differentiation, tracking its variation at large spatial scales and correlating it with species functional traits. We integrated three comprehensive resources to understand the determinants for mitochondrial DNA differentiation in butterflies represented by i) 15,609 COI sequences and ii) 14 traits belonging to 307 butterfly species occurring in Western-Central Europe and iii) the first multi-locus phylogenetic tree of all European butterfly species. By applying phylogenetic regressions we show that mitochondrial DNA spatial differentiation (as measured with Gst, G'st, D and Dst) is correlated with species traits determining dispersal capability and colonization ability. Due to the high spatial resolution of the COI data, we also provide the first zoogeographic regionalization maps based on intraspecific genetic variation. The overall pattern obtained by averaging the spatial differentiation of Western-Central European butterflies confirmed the hypothesis predicting the existence of glacial refugia in southern European peninsulas and large islands, and in general confirming the theory that long-term glacial isolation followed by rapid pulses of post-glacial expansion have been a pervasive phenomenon in European butterflies. The results and the extensive datasets we provide here constitute the basis for genetically-informed conservation plans for a charismatic group in a continent where flying insects are under alarming decline.

Simposio IV

Filogenesi, biogeografia e sistematica

Coordinatori:
Giuliana Allegrucci, Vincenzo Caputo Barucchi, Ettore Olmo

Poster

DAVIDE BADANO

Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università di Genova

EVOLUTION AND BIOLOGY OF THE LARVAE OF NEUROPTERA MYRMELEONTIFORMIA

Neuroptera, commonly known as lacewings, with ca. 6,000 described species, are a relatively small group by insect standards, although their morphological and biological disparity rivals that of larger clades such as their sister group, the Coleoptera. For example, they include diverse life-strategies, from predators of aphids (Chrysopidae and Hemerobiidae), to mantis-like predators with specialized larvae that feed on spider eggs (Mantispidae), to aquatic larvae that develop on freshwater sponges (Sisyridae). Myrmeleontiformia is the most species-rich clade within Neuroptera, comprising the largest and most iconic lacewings such as Myrmeleontidae (antlions) and Ascalaphidae (owlflies), which according to phylogenomic evidence form a monophylum, and a few small groups, such as Psychopsidae, Nymphidae and Nemopteridae, plus several entirely fossil representatives. Their larvae represent an important source of phylogenetic data, shedding light on the intricate relationships within this clade. Highly adept ambush hunters, larval myrmeleontiforms are characterized by strongly sclerotized head capsule, trap-like jaws and specialized body setae and protuberances. Moreover, they evolved remarkable strategies to increase the effectiveness of their hunting technique, such as camouflaging, burrowing and, in some myrmeleontids, the unusual ability to dig pit-like traps in the sand. Fossil and phylogenetic clues suggest that Myrmeleontiformia evolved as arboreal predators and later independently invaded soil in different lineages. Despite a few species are still associated their ancestral arboreal niche, most members of this group thrive in soil, especially in arid environments, where they are an important component of insect communities. From the shadows of Mesozoic forests to the shining glare of modern deserts, myrmeleontiform lacewings colonized a diverse array of terrestrial microhabitats despite their relatively conservative morphology.

EMILIA CAPOGNA, ALESSANDRA RICCIERI, MARCO A. BOLOGNA

Dipartimento di Scienze, Università degli Studi Roma Tre

EVOLUTIONARY HISTORY OF NEMOGNATHINAE SUBFAMILY (COLEOPTERA, MELOIDAE): NEW INSIGHTS FROM MOLECULAR PHYLOGENY

Nemognathinae is one of the three recognized subfamilies of blister beetles (Coleoptera, Meloidae) with a cosmopolitan distribution and including the only Meloidae in Australia and Pacific islands. According to morphological characters it was divided into four tribes: Stenoderini (one genus), Horiini (three genera), Nemognathini (27 genera) and Palaestrini (one genus). In this study, we explored for the first time with a molecular approach the relationships among some genera to assess the phylogenetic position of the tribes and to investigate the evolutionary history of the subfamily. For the purpose, we used two mitochondrial markers, 16S and COI, and two nuclear markers, 28S and ITS2. Preliminary analysis were performed following Maximum Likelihood and Bayesian Inference, with the software RaxML and MrBayes. Our first results show that Stenoderini and Nemognathini tribes are highly supported. In agreement with morphological evidences, sitarine beetles resulted included in the tribe Nemognathini rather than a distinct lineage. As for the tribe Horiini, well distinct by adult and larval morphological features, *Synoria senegalensis*, the only analysed species, appears referable to Nemognathini, but with a very low support. This preliminary research will be improved by the addition of other genetic markers, Palaestrini and Horiini samples and new Nemognathini genera.

LUCA PIETRO CASACCI[1,2], MARCO CHIADÒ CAPONET[2], LEONARDO DAPPORTO[3], ROGER VILA[4], RALUCA VODĂ[2], VLAD DINCĂ[5], EMILIO BALLETTO[2], MASSIMO MEREGALLI[2], SIMONA BONELLI[2]

[1]Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland
[2]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino
[3]Dipartimento di Biologia, Università di Firenze
[4]Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Spain
[5]Department of Ecology and Genetics, PO Box 3000, University of Oulu, Oulu, Finland

THE ITALIAN *EUPHYDRYAS MATURNA*: A NEW CASE OF CRYPTIC SPECIATION?

Recognizing cryptic species diversity is critical to better understand evolutionary processes but can also have deep consequences on biodiversity conservation. *Euphydryas maturna*, a Melitaeini butterfly, occurs in Italy at a single location and has been evaluated as the only critically endangered (CR) species in the first Red List of Italian butterflies. The need to protect this population has become essential after a recent work has hypothesized that it may represent a new Italian endemism (*Euphydryas italica*). To verify whether *E. italica* represents a good species, genetic analysis using four genes (COI, Wg, 28S, ITS2) have been carried out on *E. maturna* specimens sampled from over 30 populations from Italy, Central-Northern Europe and Asia. Specimens of congeneric species (*E. iduna*, *E. intermedia*, *E. cynthia*, *E. desfontainii*, *E. aurinia*) were also included in the analysis to investigate the phylogenetic relationship among the members of the *Euphydryas* genus. Bayesian inference revealed a discrepancy between the genetic patterns of *E. maturna*, *E. intermedia* and *E. iduna* obtained with different markers. DNA barcoding clearly separated the Italian population from other populations of *E. maturna* which grouped together with *E. intermedia* specimens. However, nuclear markers and genital morphology did not fully support a separation of *E. italica* from the other *E. maturna* populations. Our results will be compared with other cases of cryptic speciation within European butterflies and discussed in a wider evolutionary context.

DOMENICO DAVOLOS[1], ELVIRA DE MATTHAEIS[1], LEONARDO LATELLA[2], RONALD VONK[3]

[1]Dipartimento di Biologia e Biotecnologie “C. Darwin”, Sapienza Università di Roma
[2]Museo Civico di Storia Naturale of Verona
[3]Naturalis Biodiversity Center, The Netherlands

ON THE EVOLUTION OF TERRESTRIAL TALITRIDS (CRUSTACEA, AMPHIPODA) FROM ATLANTIC VOLCANIC ARCHIPELAGOS, WITH A NEW AZOREAN SPECIES.

The terrestrial talitrid amphipods from the Atlantic volcanic islands live in humid, evergreen broadleaf laurel forest. Currently, six autochthonous talitrid species from the Canary Islands (4), Madeira (1), and Azores (1) are validly described. Here, we propose a new terrestrial species, collected from the islands of Graciosa and Flores, Azores, with a small lobe on the merus of male gnathopod 1, a variable character within talitrid genera. To determine the evolutionary relationships, Bayesian phylogeny was carried out on the new taxon in relation to other talitrid species belonging to different genera including *Canariorchestia, Cryptorchestia*, *Macarorchestia*, *Orchestia*, *Palmorchestia*, and *Speziorchestia*. Our phylogenetic tree, inferred from a mitochondrial and nuclear gene dataset, supported major clades. The *Macarorchestia* taxa formed an independent monophyletic lineage. Our molecular analysis revealed the neotenic *Palmorchestia*, endemic to La Palma, Canary Islands, as the closest genus of the new Azorean taxon. They formed a clade with *Canariorchestia canariensis* and *Cryptorchestia stocki*, both endemic to Gran Canaria, and *Canariorchestia monticola* endemic to Madeira. Notably, our data confirmed *C. canariensis* and *C. stocki* as closely related species, indicating that the latter belongs to *Canariorchestia*. The phylogeny indicated another clade formed by *Canariorchestia gomeri* and *Speziorchestia guancha* from Canary Islands, and *Canariorchestia chevreuxi* from Azores, which share the absence of the lobe on the merus of gnathopod 1, and they have *Orchestia gammarellus* as sister species. They share a common ancestor with the clade formed by *Orchestia mediterranea* and *Orchestia aestuarensis*. Therefore, their putative marine common ancestor probably had the sexually dimorphic pereopods 1 (without the small lobe on the merus), 2, and 7. Our sequence-based tree showed that *S. guancha* is distantly related to *S. stephenseni*, indicating that the former is not a member of *Speziorchestia*. Finally, *Orchestia montagui* and *Orchestia xylino* from Crete showed identical mitochondrial and nuclear sequences; they formed a Mediterranean clade with *S. stephenseni*. Overall, our study suggested that the Atlantic archipelagos, with a sequence of island emergence and ageing over millions of years, might have been colonized by terrestrial talitrid lineages independently and on different times.

GIOVANNI FORCINA[1], MONICA GUERRINI[1], PANICOS PANAYIDES[2], PANTELIS HADJIGEROU[2], ALEEM AHMED KHAN[3], FILIPPO BARBANERA[1]

[1]Università di Pisa
[2]Ministry of Interior, Game and Fauna Service, Cyprus
[3]Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakariya University, Pakistan

MOLECULAR BIOGEOGRAPHY AND TRANSITIONS WITHIN ZOOGEOGRAPHIC REALMS: THE BLACK FRANCOLIN (*FRANCOLINUS FRANCOLINUS*) AS TOOL TO ELUCIDATE THE INTRA-PALAEARCTIC BOUNDARY

The transition zone between western and eastern Palaearctic is a topical and long debated issue among zoologists, with Iran recently proposed as entirely pertaining to the former (and featuring the “Great Western Palaearctic”) rather than to the latter. Further molecular and phenotypic information from sedentary and low-vagile polytypic taxa may help untangle the complex bioclimatic and environmental dynamics that shaped the distribution of faunal assemblages in this region to either validate the currently proposed boundary or suggest its reappraisal. In this study, we collected and analyzed 221 samples of black francolin (*Francolinus francolinus* Linnaeus, 1766), a galliform whose distribution range in Asia entirely encompasses Iran and that, by virtue of limited dispersal capabilities and high habitat fidelity, represents an ideal model to address this issue. This secretive and still scarcely known species has been the object of recent investigations at the University of Pisa addressing phylogeographic and conservation as well as historic-anthropological issues. Here, we compared the spatial genetic structure of birds sampled across the entire range (from Cyprus eastward to Bangladesh) as inferred at nine microsatellite loci with publically available vocalizations (namely male advertising calls) analyzed in a recent study. We found a striking overlap between the two sources of information pointing to the occurrence of a western and an eastern cluster including two and four morphological subspecies, respectively. The divide between these groups runs north to south across central Iran, and was found to match with that separating genetically dissimilar conspecific populations of other vertebrates. When framed within the paleoclimatic and geomorphological information of this region, this scenario suggests a generally higher connectivity favoured by warmer and more humid conditions preceding the cold-arid climatic phase associated with the last glacial stage that restrained most taxa to moist refuges. The subsequent increase of temperatures likely led to the substantial isolation of eastern and western black francolin populations with a drastic decrease of the gene flow between them. While the taxonomic significance of this split will be hopefully investigated further in a more in-depth study relying on whole genome data, in light of these results we propose to consider a western-eastern Palaearctic boundary crossing Iran longitudinally, thus reaching a compromise between the two traditionally most debated views.

ANNA MARIA PAPPALARDO, VENERA FERRITO

Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania

EVIDENCE FOR POSITIVE SELECTION AT NADH DEHYDROGENASE 2 (ND2) GENE IN *XIPHIAS GLADIUS* L., 1758.

The assumption of neutrality for mitochondrial DNA has been widely questioned due to the functional importance of the encoded proteins. Several studies have shown signature of positive selection in mitochondrial genes in species with large latitudinal cline and locally adapted and in highly aerobic species. In this study we search for evidence of positive selection at Cytochrome Oxidase I (*COI*), Cytochrome b (C*ytb*) and *ND2* mitocondrial genes involved in the OXPHOS pathway in *Xiphias gladius*. This species is widely distributed in tropical and subtropical areas of all oceans, included the Mediterranean Sea. The sequences of the three mitochondrial genes were obtained from 100 swordfish specimens belonging to Atlantic, Indian and Mediterranean stocks. To test for recombination we used the Genetic Algorithms for Recombination Detection (GARD) and to determine the site-specific selection pressures acting on genes, the Single-Likelihood Ancestor Counting (SLAC), the Fast Unconstrained Bayesian Approximation (FUBAR) and the Mixed Effects Model of Evolution (MEME) methods were applied. The Neighbour-Joining trees build for each gene showed the separation of all haplotypes in two main clades: the first (clade I) included the haplotypes from all the studied geographic areas and the second one (clade II) included only haplotypes from the Mediterranean Sea. In particular, the *COI* and *Cytb* haplotypes from Greece were all within the clade II, while the *ND2* haplotypes from Greece formed a sub cluster of the clade I. However, the *ND2* sequence analysis identified a non-synonymous transversion (T to A) at the nucleotidic position 293, resulting in a codon change. All *ND2* sequences of Greece had an asparagine (polar aminoacid) in position 98 of the translated sequences, while an isoleucine (apolar aminoacid) was present in the same position in the rest of the samples. Both MEME and FUBAR tests showed diversifying positive selection only for *ND2* dataset at 324 codon position. Similar results were recently obtained for the European anchovy where the selection tests applied to the *COI* dataset revealed that a codon was under positive selection. According to Murrel *et al.* (2012) and Grueber *et al.* (2014), mutations at such sites may experience transient positive selection, followed by purifying selection to maintain the change, and could play a key role in adaptive evolution.

IRENE PELLEGRINO, MARCO CUCCO

Dipartimento di Scienze ed Innovazione Tecnologica, Università del Piemonte Orientale, Alessandria

GENETIC CONTRIBUTION TO ZOOLOGICAL STUDIES: SOME APPLIED AND CONSERVATION CASE STUDIES

Zoology and conservation biology need knowledge on species and their phylogeography, phylogeny, taxonomy and population diversity. Genetic data can be extremely useful for these knowledges because genome contains vast information. For the last two decades, traditional Sanger DNA sequencing has been used to very accurately sequences a single target marker up to 1000 bases. Next generation sequencing, now, allows to sequence hundreds or thousands of genetic loci and to process difficult biological samples. In this context, the zoological group of University of Piemonte Orientale make an effort to improve the knowledge on animal diversity, as shown the carried-out studies using classical genetic methods (mtDNA and nuclear markers) and next-generation sequencing. We studied, through mtDNA, microsatellites and SNPs markers, phylogeography and populations genetic of Little owl *Athene noctua*, a species in decline in several European countries. Population genetic was examined in wintering Greylag goose *Anser anser* of W-Europe, and in the Italian Red-legged Partridge *Alectoris rufa*, two species that have been managed for hunting for decades. Another study was conducted on biogeography of endemic and endangered *Austropotamobius italicus* (*A. pallipex* complex) in northern Italy, a crayfish threatened by invasive species. Recently, we assessed the genetic differences and estimated the separation time between the cryptic species reed warbles *Acrocephalus scirpaceus* complex in W Africa, and swifts *Apus pallidus* and *A. apus,* these last two important species for urban biodiversity that are declining for disappearance of nesting cavities, and the genetic difference of the Jewel beetle *Agrilus viridis* hosted on different nurse plants. Most of these studies integrates genetics with other data such as morphology, ecology or paleontology. The information obtained from these studies, in a context of biodiversity crisis, have systematic and applied values, and the use of classical and modern genetics technique aid the increase on essential knowledges for conservations. Currently, we are working on malaria parasites in breeding birds and we collected samples from a population of the endangered butterfly *Zerynthia polyxena*. Moreover, we are planning to work on diet of a species of Chiroptera and on integrative taxonomy of different species of birds in order to contribute to Italian and European biodiversity characterization. As shown, zoology still require genetic data.

FABIO SCARPA[1], PIERO COSSU[1], DARIA SANNA[2], TIZIANA LAI[1], ILENIA AZZENA[1],
MARCO CURINI-GALLETTI[1], MARCO CASU[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari
[2]Dipartimento di Scienze Biomediche, Università degli Studi di Sassari

THE CONTRIBUTION OF THE MOLECULAR DATA TO THE SYSTEMATICS AND PHYLOGENY OF THE PROSERIATA (PLATYHELMINTHES): A CASE FROM THE GENUS *ARCHILINA* AX, 1959

Molecular information has been crucial in assessing phylogenetical relationships at the family-level within Proseriata (Platyhelminthes), one of the most species-rich and ubiquitous taxon of meiobenthic 'microturbellaria'. However, given the limited molecular taxonomic coverage available, reconstructions of intra-family relationships are still mainly based on morphological characters and a critical revision of the fine-level systematics of the Proseriata is still lacking. In addition, monophyly of many species-rich genera does not appear supported by the nucleotide sequence data available. Here we present the case of the genus *Archilina* (Proseriata Monocelididae), whose representatives show a comparatively simple morphology, with the aims of: i) ascertain the monophyletism of the genus, which includes a large number of species widespread in tropical and warm-temperate areas; ii) assess, with an integrative taxonomy approach, whether the morphological clues so far utilized in taxonomy (*i.e.* the fine details of the cirrus) may indeed support species decision; iii) reconstruct the ancestral state morphology for the system bursa-vagina for the Monocelididae, as a clue to possible speciation patterns in the group. In fact, part of the species presents a bursa not connected to the outside: in particular, species from the Canary Islands present a unique vacuolar 'tissue plug' blocking the access to the bursa. These species have a cirrus provided with an acuminate stylet, which may thus play a role in preventing interspecific fertilization. Molecular phylogeny, performed by means of rDNA 18S and 28S genes, clearly show that the genus *Archilina* is not monophyletic, as its representative are intermingled between species of *Duplominona* and *Duploperaclistus*. Furthermnore, the Caribbean species *A. stylifera* does not pertain to the same clade as the eastern Atlantic (Canary Islands) and Mediterranean sequenced species. Species delimitation methods yielded slightly different results on the whole dataset. The ST-GMYC model identified 36 entities (CI = 29–44), the PTP/bPTP model 37 entities (CI = 35–47), and the ABGD method 34 entities. It is noteworthy that, despite some discrepancies, all of the methods detected each nominal species of *Archilina* as a well defined molecular taxonomic entity. Dating indicates that the three species of *Archilina* from Canary Islands diverged about 7.97 mya, ranging 4.94–12.38 mya. Since the emergence of both Gran Canaria and Lanzarote is dated about 15 myr ago, divergence times of the Canarian species of *Archilina* are not conflictual with the hypothesis that their lineage may have evolved within the Archipelago. The ancestral state reconstruction provided interesting outcome. Indeed, in the common ancestor of the three species of *Archilina* from Canary Island, the presence of vagina as ancestral state (91% and 9% for presence and absence, respectively) is strongly supported, and the "plug", which blocks a direct access to the bursa, appears then as a clearly derived character in two species. In any case, a role of the stylet (39% and 61% for presence and absence, respectively) in preventing interspecific fertilization cannot be discounted. In conclusion, this study confirms, besides the obvious need of a reassessment of the systematics of Proseriata, the crucial importance of an integration of molecular data with careful morphological reconstructions, in order to shed light on the evolutionary pathways in the group.

VALENTINA SERRA[1], LEANDRO GAMMUTO[1], VENKATAMAHESH NITLA[1], MICHELE CASTELLI[2], OLIVIA LANZONI[1], DAVIDE SASSERA[4], CLAUDIO BANDI[2], BHAGAVATULA V. SANDEEP[3], FRANCO VERNI[1], GIULIO PETRONI[1], LETIZIA MODEO[1]

[1]Dipartimento di Biologia, Università di Pisa
[2]Dipartimento di Bioscienze, Università degli Studi di Milano
[3]Department of Biotechnology, Andhra University, Visakhapatnam, India
[4]Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Università di Pavia

CAN TRADITIONAL DESCRIPTIONS INTEGRATE WITH THE HOLOBIONT CONCEPT AND GENOMICS ANALYSES? THE “NEXT GENERATION TAXONOMY” APPROACH: CHARACTERIZATION OF THE CILIATE *EUPLOTES VANLEEUWENHOEKI* SP. NOV. AND ITS BACTERIAL ENDOSYMBIONT WITH HIGHLY REDUCED GENOME

Margulis (1991) defined holobionts as the assemblage of “two or more organisms, members of different species” which remain associate “throughout a significant portion of life history”. In recent times, holobionts have been described among many and far-related groups of living beings, such as plants, algae, insects, corals, and even humans. These studies have given rise to an increasing interest in different contexts, but to our knowledge, the holobiont concept has not been applied in taxonomy. Here we propose a new approach to modern taxonomy aimed to integrate the holobiont concept with genomic and bioinformatic analyses together with classical/morphological tools traditionally used in taxonomy. The inclusion of symbiont morphology, and of mitochondrial and symbiont genomes will allow the discipline to move towards what could become the “next generation taxonomy”. As an example of this new paradigm in the characterization of holobionts, we herein propose the in-depth description of the ciliate *Euplotes vanleeuwenhoeki* sp. nov. (Euplotia, Ciliophora). This novel *Euplotes* species, retrieved in the freshwater lake of Kolleru (Andhra Pradesh, India), shows the plesiomorphic features of the genus: 10 fronto-ventral cirri, double-argyrome *eurystomus*-type. It presents 3 deep longitudinal furrows in the dorsal region, recalling the morphology of *E. trisulcatus*, nevertheless other morphological traits and molecular analyses confirm the attribution to a novel species. *Euplotes vanleeuwenhoeki* belongs to the *E. trisulcatus* - *Euplotes* cf. *antarcticus* - *E. charon* (AF492705), *E. magnicirratus* (AJ549210), and *E. euryhalinus* (EF094968, JF903799) clade, based on 18S rDNA phylogenetic analyses. The complete mitochondrial genome (mitogenome) of this ciliate results in a single linear contig 41,682 bp long with a GC content of ~0.25%. This mitogenome shows an overall synteny with the mitochondrion of *E. minuta*, *E. crassus* and *Oxytricha trifallax* of previous studies, with the exception of the two terminal regions. A novel bacterial endosymbiont belonging to *Verrucomicrobia,* is hosted in the cytoplasm of *E. vanleeuwenhoeki*, “*Candidatus Pinguicoccus supinus*” gen. nov., sp. nov. (*Opitutae, Verrucomicrobia*). Interestingly, this endosymbiont shows an extremely reduced genome (~163 Kbp), which suggests a high integration with the host. Thus, we are presenting the first case of such an extreme reduction in *Verrucomicrobia* and the first case in a protist host.

GIULIA SIMBULA[1], EMANUELE BERRILLI[2], LEONARDO VIGNOLI[1], MARCO A. BOLOGNA[1],
FEDERICA BERRILLI[3], ALESSANDRA RICCIERI[1]

[1]Dipartimento di Scienze, Università di Roma Tre
[2]Dipartimento di Medicina clinica, Sanità pubblica, Scienze della Vita e dell'Ambiente, Università degli Studi de L'Aquila
[3]Dipartimento di Scienze Cliniche e Medicina Traslazionale, Università di Roma "Tor Vergata"

MOLECULAR IDENTIFICATION OF *MESOCESTOIDES* SP.: FIRST CASE REPORT FROM AN ITALIAN WALL LIZARD (*PODARCIS SICULUS*)

Mesocestoides spp. are cyclophyllidean tapeworms (Cestoda, Mesocestoididae) with an indirect life cycle probably requiring two intermediate hosts and a definitive one. Although their complete life cycle is not still clear, the first intermediate host is generally thought to be an arthropod, the second ones are commonly reptiles, amphibians, birds and micro-mammals, whereas the definitive hosts are wild and domestic carnivores (rarely humans). The tetrathyridium (second larval stage) may reproduce asexually spreading within the body cavity and provoking severe systemic infections. In this study, we report the first evidence of parasitic infection by several tetrathyridia of the genus *Mesocestoides* in a wall lizard (*Podarcis siculus*) collected in central Italy. Morphological identification at species level of this parasite is extremely difficult, and the taxonomy of *Mesocestoides* is still largely debated. In this work, we analysed ten tetrathyridia and assessed their phylogenetic position using the barcode region COI and species delimitation methods, such as Automatic Barcode Gap Discovery (ABGD) and Bayesian implementation of the Poisson Tree Processes model (bPTP). A first identification using the Basic Local Alignment Search Tool (BLAST) assigned the isolates from the wall lizard to *Mesocestoides litteratus* (100% of identity), and this result was confirmed by species delimitation analysis. According to our knowledge *M. litteratus* has been observed in Europe mainly in wild and domestic carnivores as dogs, cats and foxes, which represent the definitive hosts, but never identified in Italy. Consistent with our findings, *P. siculus* seems to have a role as second intermediate host in the transmission route of this tapeworm species in our country.

LUCA VECCHIONI[1], MARCO ARCULEO[1], FABIO STOCH[2], VALERIO KETMAIER[3],
FEDERICO MARRONE[1].

[1]Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Università degli Studi di Palermo
[2]Université Libre de Bruxelles (ULB), Evolutionary Biology & Ecology, Brussels, Belgium
[3]Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma

DIVERSITY AND PHYLOGEOGRAPHY OF THE FAIRY SHRIMP *CHIROCEPHALUS KERKYRENSIS* (BRANCHIOPODA: ANOSTRACA) FROM THE CENTRAL MEDITERRANEAN AREA

The central Mediterranean area is inhabited by the fairy shrimp *Chirocephalus kerkyrensis* Pesta, 1936, which is the westernmost representative of the *bairdi*-group of the genus *Chirocephalus*. The species is typical of temporary water bodies of the Italian and Balkan peninsulas and experienced a severe reduction in its populations' number and, possibly, distribution range due to the habitat loss linked with anthropogenic activities. Currently, the molecular diversity of the Italian, Albanian and Corfu Is. (Greece) populations is known; conversely, no information is available on the populations recently discovered in Epirus (continental Greece). The aim of this study was to provide a comprehensive survey of the genetic diversity and phylogeography of *C. kerkyrensis* throughout its known distribution range by including all known extant populations. Based on the analysis of partial sequences of the mitochondrial (mtDNA) COI gene, we obtained evidences supporting the existence of two main haplogroups within *C. kerkyrensis*: a first one including all the surveyed central Italian populations (Latium), the second one including all the populations occurring in the Balkan Peninsula and the single population to date known for southern Italy (Basilicata). This pattern suggests that the current Italian *C. kerkyrensis* populations originated from at least two allochronic, independent events. The first took place in the Late Pleistocene and gave origin to the highly-divergent "Latium" clade, which presents a sister-clade relationship to all the other currently known populations of the species. Conversely, a later post-glacial dispersal event allowed the secondary colonization of southern Italy, east of the Apennines mountain chain, from a source population located in Albania or northern Epirus. As a result, the Italian peninsula currently hosts two strikingly divergent evolutionary lineages of this rare anostracan species. Within the *C. kerkyrensis* Balkan clade, a noteworthy latitudinal structuring of mtDNA diversity is observed.

NICOLA ZADRA[1,2], ANNAPAOLA RIZZOLLI[1], OMAR ROTA-STABELLI[1]

[1]Dipartimento di Agroecosistemi sostenibili e Biorisorse, Centro di Ricerca e Innovazione,
San Michele all'Adige, Trento
[2]Centro di Biologia Integrativa, Università di Trento

PHYLOGENY AND DIVERGENCE OF AEDINI MOSQUITOES: EVOLUTIONARY SCENARIOS AND GENOMIC PERSPECTIVES

Mosquitoes are the indirect cause of more morbidity and mortality among humans than any other group of organisms. One-third of all mosquitoes species belong to the Aedini, a tribe comprising common vectors of viral zoonoses[1]. In order to improve our understanding of their evolution, we present a detailed multigene estimate of their phylogeny and divergence based on relaxed clocks and fossil calibrations. Our phylogenies using different replacement models and statistical frameworks recover some recognized clades such as *Stegomyia*, *Ochlerotatus*, clades A and B but also indicate unstable supports at many nodes: this is likely due to fast radiation events which should be investigated in future using whole genome data. Our divergence estimates point toward mosquito radiation in the mid-Jurassic and Aedini radiation from the mid-Cretaceous on: this is compatible with a paleo-ecological scenario in which first lineages of mosquitoes co-radiated with reptiles, followed by diversification of Aedini in a more modern ecosystem characterized by mammals, birds, and angiosperms. We observe both topological and chronological incongruence when comparing nuclear and mitochondrial data: this may be explained by either peculiar evolutionary events such as hybridization and/or by wrong taxa assignment in samples collections. Our divergence estimates indicate that emerging model Aedes albopictus can be currently compared from a genomic point of view only against distantly related A. aegypti: genome data from its sister species *A. flavopictus* would more than half the time from common ancestor increasing resolution of comparative genome studies. Overall, our results provide an updated view of Aedini molecular phylogeny and suggest paleo-ecological scenarios for their evolution; it also reveals some methodological issues which may be of help in setting the research agenda for future studies.

Poster a tema libero

ALESSANDRA ALEOTTI[1], IAIN C. WILKIE[2], LUIS A. YANEZ GUERRA[3], MICHELA SUGNI[1], FRANCESCO BONASORO[1], M. DANIELA CANDIA CARNEVALI[1], MAURICE R. ELPHICK[3]

[1]Dipartimento di Scienze e politiche ambientali, Università di Milano
[2]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, Scotland, UK
[3]School of Biological and Chemical Sciences, Queen Mary University, London, UK

FUNCTIONAL CHARACTERISATION OF A VASOPRESSIN-OXYTOCIN-TYPE NEUROPEPTIDE IN THE CRINOID *ANTEDON MEDITERRANEA*

A wide range of physiological and behavioural processes in animals are regulated by neuropeptides. The ever-growing availability of genomic and transcriptomic data has led to the identification of neuropeptide signalling systems in a vast number of animal species. This has unlocked the opportunity to reconstruct the evolutionary history of these molecules and understand their broad array of functions in different animals. Echinoderms are interesting invertebrate models due to their phylogenetic position close to vertebrates. Furthermore, echinoderms exhibit a variety of peculiar features, including remarkable regenerative abilities, which are of interest for physiological studies. While several studies in recent years have characterized numerous neuropeptide signalling systems in starfish, sea urchins, sea cucumbers and brittle stars, research on crinoids, the most basal class of the phylum, has been lagging behind. Here we have exploited recently obtained transcriptomic sequence data from *Antedon mediterranea* to investigate neuropeptide systems in this crinoid species endemic to the Mediterranean Sea. Transcripts encoding the precursor of a vasopressin/oxytocin (VP/OT)-type peptide (crinotocin) and its corresponding receptor have been identified. The expression of crinotocin in *A. mediterranea* was investigated using antibodies to the starfish VP/OT-type neuropeptide asterotocin, with immunostaining observed in the epithelium of the ambulacral groove, a structure that extends along the whole length of the oral surface of the arms and that is involved in capturing and directing food particles towards the mouth. Immunostaining was also observed in the epithelium of the mouth and of the oesophagus. *In vitro* pharmacological experiments aiming to explore the physiological roles of crinotocin, revealed that crinotocin induces dose-dependent destiffening of dissected arm preparations. Due to the complex anatomy of the arm, the exact mechanism of action of crinotocin is still not clear. However, our hypothesis is that this destiffening is due at least in part to effects on collagenous ligaments. Our work represents the first effort to characterize a neuropeptide signalling system in a crinoid, advancing our understanding of this signalling system within echinoderms and providing a basis for further investigation of neuropeptide function in crinoids.

ALESSANDRO ALLIEVI[1], MATTEO CANAVESI[1], CINZIA FERRARIO[1,2], MICHELA SUGNI[1,2], FRANCESCO BONASORO[1]

[1]Dipartimento di Scienze e Politiche Ambientali, Università di Milano
[2]Centro per la Complessità e dei Biosistemi, Dipartimento di Fisica, Università di Milano

COMPARED PATTERNS OF ARM REGENERATION IN DIFFERENT TAXA OF ARMED ECHINODERMS

Regeneration is a post-embryonic developmental process common in Metazoa, although it tends to be less widespread in the more complex-bodied phyla. An exception to this rule are echinoderms, which are known for phylum-wide and extremely advanced regenerative abilities, being able to regrow all appendages, and often large parts of the central body and viscera. Armed echinoderms (Crinoidea, Asteroidea, and Ophiuroidea) are especially practical models as their arms are easy to amputate, and their proximo-distal extension provides a useful reference point to describe the regenerative processes. Samples of four species from these taxa – the crinoid *Antedon mediterranea*, the asteroids *Echinaster sepositus* and *Coscinasterias tenuispina*, and the ophiuroid *Amphipholis squamata* – were subjected to arm amputation to study the progression of arm regeneration from a morphological point of view by means of different microscopy analyses. Particular attention was given to the "axial structures", defined as the continuous elements running along the proximo-distal axis of each arm, namely the radial water canal, the radial nerve cord, and the arm coelom, as they are believed to be fundamental for the re-organization of the regenerating arm. The comparison highlighted commonalities and differences of arm regeneration in the different taxa. Distal structures, represented in crinoids by the apical blastema and in asteroids and ophiuroids by the terminal ossicle and tube foot, form very quickly, whereas the proximal region develops later, in proximal-to-distal direction. This is in accordance with previously published models of echinoderm regeneration. These similarities suggest that the mechanism of regeneration has ancient origins and is extremely conserved through echinoderm evolution. Within the proximal region, the axial structures themselves develop earlier than the nearby discrete structures (e.g. ossicles and tube feet), and seem to have a crucial role in their organization, providing material and possible signaling molecules for the growing tissue. The cellular component of the nerve grows before any other structure, including its own fibers, thus confirming a primary role of the nervous system in the whole process. Molecular analyses must be combined to morphology data to improve our understanding of similarities and differences of the regenerative process as it occurs in the different echinoderm taxa, as well as in different animal phyla, and to identify related processes in both regeneration-competent and non-competent species.

AL-SAYED AL-SOUDY, VALERIA MASELLI, GIANLUCA POLESE, DENISE GUEGLIA,
ANNA DI COSMO

Department of Biology, Università di Napoli Federico II

SMELL BY TOUCH IN *OCTOPUS VULGARIS*: COMBINATION OF MOLECULAR AND BEHAVIORAL APPROACHES

The sense of smell enables all animals to recognize and discriminate a broad range of chemical stimuli in their environment, which provides essential information for survival profoundly influencing their behavior. There are a variety of odor molecules in the environment identified by olfactory receptors (ORs) and trace amine-associated receptors (TAARs), which are a member of the G protein-coupled receptor superfamily and known to be expressed in olfactory sensory neurons. Octopuses and other cephalopods possess a well-developed olfactory organ, that plays a crucial role in their life detecting widespread waterborne molecules from a distance source. However, many insoluble molecules (smaller than 300 Da) known as “odors” on the land must be perceived by touch in aquatic systems. Octopuses can “smell” them by touch using suckers, bearing diffuse chemical receptors, exhibiting a peculiar behavior that has been described recently as “smell by touch” based on the kind of molecules that they can perceive. Here we demonstrate the presence of olfactory receptors on the suckers and how they are employed in olfactory discrimination, combining genetics and behavioral approaches. Based on *O. bimaculoides* genome and transcriptome we analyzed expressions of the three TAARs genes by qPCR, and whole-mount *in situ* hybridization (WISH) technique. Our results revealed that all three TAARs genes preferentially localize on sucker’s rim and the infundibulum, suggesting that the epithelium rim of each sucker carries a considerable number of primary sensory cells devoted to chemical perception. Additionally, we designed a behavioral experiment to verify if octopuses are able to distinguish by touch between objects, differing only in the presence of insoluble odorant molecules that can be detected only smelling with suckers. The behavioral results show that octopuses learn to discriminate by touch between objects which differ only in their chemical characteristics ($P=75\%$, $p<0.05$) smelling by olfactory receptors born on their suckers. This study represents the first step for understanding the diffuse olfaction throw non-conventional olfactory organs in aquatic animals.

MARIA ASSUNTA BISCOTTI[1], ADRIANA CANAPA[1], FEDERICA CARDUCCI[1], MARCO GERDOL[2], MARCO BARUCCA[1]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche
[2]Dipartimento di Scienze della Vita, Università di Trieste

TRANSCRIPTOMIC ANALYSIS OF *CYNOPS ORIENTALIS* GONADS: SEXUAL DEVELOPMENT GENES

Among terrestrial vertebrates, amphibians have the largest genome sizes and thus whole genome sequencing is still excessively challenging. RNA sequencing technologies represent a cost-effective tool for large-scale transcriptome profiling that allows the rapid and complete elucidation of transcript sequence information. In this context, the high-quality transcriptome from the fire belly newt *Cynops orientalis* was generated to get precious information about genes and molecular mechanisms underlying gametogenesis and sex development in this species. Indeed, the data on the expression and function of genes participating in these processes in urodeles and in general in amphibians are very limited. The comparative analysis of testes and ovaries transcriptomes of *C. orientalis* evidenced that some patterns of expression are conserved in teleosts and sarcopterygians. Intriguingly firstly the *GSDF* gene is reported in a tetrapod species. Overall, our data significantly enhance the molecular resources for fire belly newt, a species lacking of genome information, representing a useful tool for future studies involving gene expression experiments, comparative transcriptomics, genomics, and metabolomics in amphibians.

BARBARA AUDAZIO[1], SIMONA SANVITO[2], FILIPPO GALIMBERTI[2]

[1]Dipartimento di scienze della terra, dell'ambiente e della vita, Università di Genova
[2]Elephant Seal Research Group, Sea Lion Island, Falkland Islands FIQQ 1ZZ

RELIABILITY OF KILLER WHALE (*ORCINUS ORCA*) PHOTO IDENTIFICATION

Artificial marking of cetaceans is logistically difficult and ethically questionable. Therefore, identification of individuals is normally based on natural marks observed in photos. Killer whales (KW hereafter) are a charismatic top marine predator with a complex life history and social behaviour, and are, therefore, an ideal subject for the study of the evolution of sociality. The study of behaviour and ecology of KW requires individual recognition and, therefore, photo-identification is frequently used. Notwithstanding this, the reliability of photo-identification of KW has been rarely tested. We studied KW at Sea Lion Island, in the Falkland Islands, that shelters a small KW population comprising both resident and transient individuals. We took pictures of the KW saddle patch and dorsal fin in 2013-2016. We extracted four batches of photos, each one comprising 300 randomly selected ones. An operator without previous experience of KW identification was given a brief training, the photo catalogue of the Sea Lion Island KW, and sample photos of each individual. He was asked to identify KW of the four photo batches, repeating the identification trial three times for each batch, leaving at least three days between each trial. We compared KW identifications of this operator to "true" identifications, obtained by consensus of three experienced operators. We calculated the intra-rater reliability, the agreement with true identifications, and the effect of various factors (e.g., trial order, photo quality, number of KW). The inexperienced operator identifications showed good reliability and agreement with true identifications. The only factor that affected the reliability of the identification was the uncertainty of the "true" identification, that was related to photo quality. The error rate was not affected by the number of KW or the increase in the operator experience of consecutive trials. Overall, our results confirm that photo-identification is a reliable technique to study KW, and that even inexperienced operators can achieve good identification success with a modest training, although there is heterogeneity between individuals due to the different level of scarring.

FEDERICA SEMPRUCCI[1], LUCIA CESARONI[1], ELEONORA GRASSI[1], VALENTINA LOSI[2], LORETTA GUIDI[1], MARIA BALSAMO[1]

[1]Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino
[2]Dipartimento di Scienze della Terra dell'Ambiente e della Vita, Università di Genova

TAXONOMIC COMPOSITION AND MORPHO-FUNCTIONAL TRAITS OF NEMATODES IN MALDIVIAN SUBTIDAL HABITATS

Nematodes are one of the most abundant animal phyla in marine bottoms, and show a remarkable diversity in carbonate sediments. The Maldivian Archipelago is experiencing major environmental changes and habitat loss, which requires great attention. Previous studies on meiofauna and nematodes from Maldives have revealed a considerable biodiversity in these sediments that are exclusively carbonate with a highly porous structure, which offers many microhabitats to meiobenthic organisms. Recently, an important relation between the morpho-functional traits of nematodes and the loss of ecosystem functioning has been proven. Thus, the taxonomical structure of the nematode communities of subtidal habitats of Maldives and five morpho-functional traits (cuticle morphology, tail shape, amphid type, structure of buccal cavity, lifestyle) were studied. The Maldivian nematode community included 174 genera in 37 families, mainly represented by Desmodoridae, Chromadoridae and Xyalidae, a faunistic composition in line with that usually found in this type of sediments. As for morpho-functional traits, the most common cuticle morphology was dotted/annulated with or without lateral differentiation (80% of the community).The most common type of amphid was loop-like in 42% of the community, followed by spiral (21%), circular (16%) and slit (11%). The analysis of trophic groups showed the prevalence of epistrate feeders (2A, 54%), followed by selective deposit feeders (1A, 22%), omnivorous (2B, 14%) and non-selective depositories (1B, 11%). As for life strategy, the colonizer-persisters class 3 (c-p 3) made up the 57% of the community, the c-p 2 22%, and c-p 4 20%. Statistical analysis allowed to detect interesting relations between nematode morpho-functional traits, especially amphid type and cuticle morphology, and some environmental features. For instance, a significant presence of nematodes with the peculiar cuticle provided with desmi was found in the outer reefs, likely related to the high abundance of Desmoscolecidae, a family typical of very heterogeneous sediments and highly hydrodynamic habitats just like the outer reefs. Instead, nematodes with a bacteria-coated cuticle were more numerous in the inner reefs: this cuticle occurs in the subfamily Stilbonematinae (fam. Desmodoridae), that is often associated to sediments with low hydrodynamism and hypoxic conditions as are those found in inner reefs. The bladder-like amphid typical of Desmoscolecidae appeared relevant to discriminate the habitat of the outer reef, whereas the pocket-like amphid was definitely more represented in nematodes of the inner reefs. No literature data about the relation amphid -habitat are available, but a correlation of the amphid type with the hydrodynamic regime is likely.

FEDERICA CARDUCCI, MARIA ASSUNTA BISCOTTI, MARIKO' FORCONI, MARCO BARUCCA, ADRIANA CANAPA

Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche

THE RELATIONSHIP BETWEEN TELEOST *REX3* RETROELEMENT AND ENVIRONMENTAL TEMPERATURE

Among vertebrates, teleosts represent a successful group of animals adapted to a wide range of different environments and their genome is constituted by a rich repertoire of transposable elements (TEs) which are known to be responsive and susceptible to environmental changes. However, the correlation between environmental conditions and TE sequence evolution remains still an unexplored field of research. The *Rex3* retroelement is a lineage specific non-LTR retrotransposon and thus represents a valid candidate for performing comparative sequence analyses between species adapted to diverse temperature conditions. In this study partial reverse transcriptase sequences of the *Rex3* retroelement belonging to 39 species of teleosts were investigated through phylogenetic analysis. Our findings highlighted an intriguing behavior of the analyzed sequences showing a clusterization of *Rex3* sequences isolated in species living in cold waters (Arctic and Antarctic regions and cold waters of temperate regions) compared to those isolated in species living in warm waters. This is the first evidence of a correlation between environmental temperature and *Rex3* retroelement evolution.

ELISABETTA COLUCCIA, FEDERICA DEIDDA, SUSANNA SALVADORI

Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari

COMPARATIVE MAPPING OF 5S RDNA IN MURAENIDAE BY SINGLE, AND DUAL COLOR FISH

The minor ribosomal gene family (5S rDNA) codes for the smallest ribosomal RNA component and comprises tandem repetitions of one transcriptional unit of about 120 base pairs separated by a non-transcribed spacer (NTS). The coding sequence is highly conserved, even among unrelated species, whereas the NTS is highly variable both in sequence and length, even in closely related species. These features make it a useful marker in species identification as well as phylogenetic and evolutionary studies. Furthermore, it is one of the few DNA sequences that can be localized by FISH in species with no genomic data. Within the order Anguilliformes, Muraenidae is one of the largest and ancient family, with about 200 extant species and the genus *Gymnothorax* is the most species-rich. The phylogenetic relationships within the family are still under debate with *Gymnothorax* genus considered to be not-monophyletic; in this respect, cytogenetic studies can help in speciation analysis and assist in taxonomic problems, such as complex or cryptic species. Cytogenetically, only the 10% of morays have been karyotyped, and in far less species FISH analyses have been employed mainly for the localization of major rDNA and telomeric sequences. In this study, 5S ribosomal genes have been hybridized on metaphase chromosomes, obtained from lymphocyte cell cultures, of Muraenidae species representative of *Muraena* and *Gymnothorax* genera. Furthermore, experiments of dual color FISH were conducted for the simultaneous chromosomal localization of the major (28S) and minor (5S) ribosomal genes. Different patterns of 5S rDNA location, in some cases associated to heterochromatic regions and/or to major ribosomal genes, were detected among morays species. In this family, despite a general conservation of the diploid number (2n =42 in most species) and the number of major ribosomal genes (one cluster in all species), the karyotype structure as well as the heterochromatin and 5S rDNA patterns are highly diversified among species. These data could be very useful in understanding the karyotype evolution of Muraenidae family.

GIANMARCO DEL SETTE, LISA POLA, CARLO CERRANO, BARBARA CALCINAI

Dipartimento di Scienze della Vita e dell'Ambiente (DiSVA), Università Politecnica delle Marche

MORPHOMETRIC ANALYSIS OF SOME INQUILINES INHABITING *SARCOTRAGUS SPINOSULUS* (DEMOSPONGIAE; IRCINIIDAE)

During a characterization, lasting one year, of the biodiversity of the macrofaunal organisms associated with *Sarcotragus spinosulus* (Schmidt, 1862) (Demospongiae; Irciniidae), some dominant species have been detected. On these species a morphometric analysis has been carried out with the purpose of defining the role of *S. spinosulus* in the life-cycle of these organisms. Sponges were sampled along the coast of the Ancona Promontory (Adriatic Sea) at around 6 m depth from July 2016 to July 2017. The associated species we investigated were the nereidid *Ceratonereis (Composetia) costae* (Grube, 1840), the amphipods *Gammaropsis maculata* (Johnston, 1828), *Leucothoe spinicarpa* (Abildgaard, 1789) and *Ericthonius brasiliensis* (Dana, 1853) and the isopod *Janira maculosa* Leach, 1814. For the measurements and the analyses of the results the digital image processing program, ImageJ, and Fisat II program with the Bhattacharya method were used.The dimensions of three anatomical structures of the species *C. (C.) costae* have been considered: total body length, width of the 3rd chaetiger (head) and of the 10th chaetiger; while for the crustaceans only the total length of the body was considered, defined as the distance, along the dorsal side, from the base of the antenna I to the base of the telson. *S. spinosulus* hosts the whole life cycle of *C. (C.) costae* and acts as a nursery ground for the polychaete; in fact, specimens were located on the canals of the sponges and juveniles were more abundant than the adults. The 3° chaetiger of *C. (C.) costae* resulted a statistical valid descriptor for morphometric analyses, permitting to estimate the length of broken specimens (regression analysis R^2=0.849), and to identify several frequency classes and three different cohorts (juvenile, mixed and adults). Furthermore, through the use of the K-means cluster test, and the subsequent discriminant analysis, it was possible to identify two groups, highlighted by a discontinuity in growth at a total length of approximately 30 mm, that is the size at which 50% of individuals reach sexual maturity, as reported in literature. Results about the amphipods *Gammaropsis maculata* and *Leucothoe spinicarpa* showed a single cohort of individuals made up of male and female adults, while *Ericthonius brasiliensis* was characterized by two cohorts, the first consisting of juveniles and the second of adults both male and female. For the isopod *Janira maculosa* only one cohort made up of adult males and females was detected; nevertheless, the non-parametric Kruskal-Wallis test highlights a significant difference between the median lengths of the sample, assuming that there are two size classes of individuals not clearly separated. In conclusion, it is clear the key role of the sponge *S. spinosulus* as optimal refuge habitat for its inquilines, highlighting once again the importance of sponges in the structuring and functioning of benthic assemblages.

LUIGIA DONNARUMMA[1], LUCA APPOLLONI[1,] ELISA BALDRIGHI[2], DANIELA ZEPPILLI[2],
GIOVANNI FULVIO RUSSO[1], ROBERTO SANDULLI[1]

[1]Dipartimento di Scienze e Tecnologie, Università di Napoli "Parthenope", Napoli
[2]IFREMER, Centre Brest, REM/EEP/LEP, Plouzané, France

MOLLUSC DIVERSITY CHARACTERIZING THE SHALLOW HYDROTHERMAL AREA OF SECCA DELLE FUMOSE (GULF OF NAPLES, ITALY)

In the Mediterranean Sea, very shallow hydrothermal vents are reported for the Tyrrhenian and Aegean Sea. They are related to tectonically active coastal zones, with volcanic fluids composed mainly by sulphide and/or CO_2. In the present study, the distribution and diversity of mollusc assemblage inhabiting the Secca delle Fumose (SdF), a shallow hydrothermal area (9 – 14 m water depth) within Baia Underwater Archaeological Park, were investigated for the first time. The SdF is a submarine relief consisting of a dense aggregation of Roman pillars, among which thermal vents discharging hot gas-rich hydrothermal fluids occur. We selected 4 sites, two characterized by the presence of vent emissions (H, with white bacterial mat scattered on the bottom and CO_2 gas bubbling, and G, geyser with a yellow substrate around the opening) and two controls without vent emission, at comparable water depth. The highest mollusc abundance (79.51%) and species richness (90.79%) were reported at control sites. Decreased abundances (18.85%) and species richness (30.26%) were recorded at site G, while the lowest mollusc values were detected at site H (1.64% and 3.95% respectively). Furthermore, the only species occurring exclusively at sites H and G was the nassariid gastropod *Tritia cuvierii* (Payraudeau, 1826), probably due to the presence of microbial mats as additional food source, as it was previously reported for the congeneric species *T. neritea* (Linneus, 1758) in other vents systems. Our results show that extreme environmental conditions of hydrothermal vents strongly affect mollusc biodiversity, originating a very peculiar assemblage of species at site G and H.

LEANDRO GAMMUTO[1], VALENTINA SERRA[1], VENKATAMAHESH NITLA[1], MICHELE CASTELLI[2], OLIVIA LANZONI[1], DAVIDE SASSERA[3], CLAUDIO BANDI[2], LETIZIA MODEO[1], GIULIO PETRONI[1]

[1]Dipartmento di Biologia, Università di Pavia
[2]Centro di Ricerca Pediatrica Romeo and Enrica Invernizzi, Dipartimento of Bioscienze, Università di Milano
[3]Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Università di Pavia

MULTIDISCIPLINARY CHARACTERIZATION OF A NOVEL CILIATE ENDOSYMBIONT: "*CANDIDATUS* PINGUICOCCUS SUPINUS" A VERRUCOMICROBIAL SPECIES WITH HIGHLY REDUCED GENOME

Members of genus *Euplotes* (Euplotia, Ciliophora) are known to frequently host endosymbionts, including eukaryotes (such as green algae or trypanomes), or, more commonly, one or even multiple bacterial symbionts belonging to diverse lineages. Here we characterized the morphology, phylogeny and genome features of "*Candidatus* Pinguicoccus supinus" gen. nov., sp. nov. (*Opitutae, Verrucomicrobia*), a bacterial endosymbiont of the novel species *Euplotes vanleeuwenhoeki*. This bacterium shows several intriguing features. The 16S rRNA gene based phylogeny, and the phylogenomic analysis, collocate this endosymbiont as a member of the family *Puniceicoccaceae* (*Verrucomicrobia*, *Opitutae*) clustering with sequences from uncultured organisms, and forming a clade related to the genera *Coraliomargarita*, "Fucophilus", *Cerasicoccus*, and *Ruficoccus*. We suggest that the long branch of "Ca. Pinguicoccus supinus" may implies a higher evolutionary rate with respect to related Verrucomicrobia. "Ca. Pinguicoccus supinus is located in the host cytoplasm, usually in clusters beneath the cortex, and sometimes in close contact with mitochondria or lipid droplets. It presents irregular roundish-ovoid shape and is not enclosed in a host symbiosome. The genome of "*Candidatus* Pinguicoccus supinus" was found to be extremely small (163,218 Kbp), being, to our best knowledge, the fourth smallest bacterial genome sequenced up to now. It represents also the endosymbiont with the smallest genome found in a protist host, and the first case of such an extreme reduction in *Verrucomicrobia*. Respect with other bacterial genomes of comparable size, the genome of this endosymbiont shares the same metabolic core of basic gene expression and chaperons. However, it possesses some peculiarities: it lacks biosynthetic pathways of nutrients or metabolites potentially useful for the host, and lacks several genes considered essential for bacteria, in particular any catalytic subunit involved in DNA replication. On the other side, it retains some genes involved in lipid metabolism, which might possibly correlate with the occasionally recorded "Ca. Pinguicoccus supinus" subcellular localization in close contact with lipid droplets. All these aspects taken together, in particular the extreme genome reduction, suggest a high integration with the *Euplotes* host, although the nature of this relationship has not yet been disclosed.

ILARIA GIOVANNINI[1], MICHELE CESARI[1], ELENA CENNI[1], TAKAHIRO KIKAWADA[2], RICHARD CORNETTE[2]; KOYUKY KONDO[3]; DAVIDE MAMELI[1], TIZIANA ALTIERO[4], ROBERTO GUIDETTI[1], LORENA REBECCHI[1]

[1]Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia
[2]Institute of Agrobiological Sciences, National Institute of Agriculture and Food Research Organization (NARO), Tsukuba, Japan
[3]Institute of Advanced Biosciences, Keyo University, Japan
[4]Dipartimento di Educazione e Scienze Umane, Università di Modena e Reggio Emilia

A NEW *MACROBIOTUS* SPECIES (EUTARDIGRADA, PARACHELA) FROM JAPAN REVEALS NEW INSIGHTS ON TARDIGRADE STRESS RESISTANCE

Tardigrades are small micrometazoans able to resist various environmental stresses in any stage of their life cycle. More than 1200 tardigrades species are known, with the family Macrobiotidae being one of the most speciose. However, the globally distributed *Macrobiotus hufelandi* species complex is represented in Japan only by the nominal species (*M. hufelandi*) and a recently discovered species, *Macrobiotus shonaicus*. An integrated analysis of tardigrade specimens collected in Tsukuba (Japan) revealed a peculiar morphology of the IV pair of legs and of the egg shell. The population was gonochoric and amphimictic. Moreover, molecular taxonomy and phylogenetic analysis on different genes (COI, ITS2, 18S and 28S) revealed that the Japanese population is made up by only one species, very well differentiated from all other known species, belonging to a well defined group within the *M. hufelandi* complex. The stress resistance capability of this new Japanese species has been tested by submitting animals to desiccation and ultraviolet radiation (UV-C and UV-B) stresses. Animals were able to survive desiccation (survivorship range: 87.3-95.5%), regardless of the desiccation method tested. Both hydrated and desiccated animals showed a good tolerance to increasing UV radiation, even though a decrease in survival was detected as UV radiation doses increased. Hydrated animals survived up to a UV dose of 1500 J cm^{-2}, while desiccated specimens survived up to a dose of 3500 J cm^{-2}. Nonetheless, the animal survival decreased with the increasing of the time after the end of irradiation, since many animals which were alive immediately after the irradiation, died 1 hour or 24 hours later. This indicates that tardigrades accumulate damages during UV exposition, and they are unable to repair them. This result is at variance with previous studies on the tardigrades *Paramacrobiotus spatialis* and *Acutuncus antarcticus*, where the survival increased with time, indicating the capability of damage repairs. Present data show the presence of a new Japanese species of *M. hufelandi* species complex, therefore contributing to the discovery of a larger tardigrade biodiversity in Japan. Desiccation and UV tolerance capabilities of this new species point out that it could become a new emerging model for studies on stress resistance. This project has received funding from the European Union's Horizon 2020 under the Marie Skłodowska-Curie "Research and Innovation Staff Exchange (RISE)" Grant agreement No 73443 – DRYNET.

CINZIA GRAVILI[1,2] MARIA FLAVIA GRAVINA[2,3], CRISTINA BARLETTA[1], ADRIANA GIANGRANDE[1,2]

[1]Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DiSTeBA), Università del Salento
[2]CoNISMa, Consorzio Nazionale Interuniversitario per le Scienze del Mare, Roma
[3]Dipartimento di Biologia, Università di Roma "Tor Vergata", Roma

MACROFOULING COMPARISON: THE CASE OF THE TAXON HYDROZOA IN TWO MEDITERRANEAN FARMING PLANTS

Aquaculture is increasing rapidly to meet global seafood demand and requires an increase in studies concerning the colonization by the macrofouling of artificial substrates. In fact, these organisms often represent negativity because they weigh down the floating structures and hinder the activities of the plant workers. Among macrofouling invertebrates some hydroids have been linked to mortality and health issues in finfish and shellfish, but their dynamics in and around aquaculture farms remain understudied. In the present work, two colonization experiments were conducted, respectively the first one in the Gulf of Taranto and the second one in the Gulf of Gaeta, by means of artificial panels hanging from a floating cage and placed at different depths and season of immersion, examined at different submersion duration over one year. The assemblage of hydrozoans was species-poor, although species richness, frequency and abundance increased with time, paralleling the overall increase in structural complexity of fouling assemblages. Submersion duration and season of immersion were particularly important in determining the species composition of the assemblages in the succession and colonization experiments, respectively. Altogether, 14 species of Hydrozoa have been recorded, of which 3 species are present in both aquaculture facilities, 7 species only in the Gulf of Taranto aquaculture plant and 4 species only in the Gulf of Gaeta one. On the artificial substrates of the two plants, the role of "facilitators" in the development of the macrofouling community can be recognized for hydroids: their colonies, in fact, modified the substrate during the initial phases of colonization, favouring the subsequent settlement of the macrobenthos, which turned out to be characterized by the abundant settlement of amphipods and the dominance of mussels, especially in the final stages of colonization. In the Gaeta plant many colonies of two hydrozoan species (*Kirchenpaueria halecioides* and *Aglaophenia octodonta*) have adhered to the *Anomia ephippium* shells that have extensively colonized the surfaces of the summer series panels. Moreover, production of water-borne propagules, including medusae, from the hydroids was observed from locally abundant colonies in both sites, among them the well-known fouling species *Obelia dichotoma*, potentially representing a nuisance for cultured fish through contact-driven envenomations and gill disorders. The results illustrate the potential importance of fouling hydroids and their medusae to the health of organisms in the aquaculture industry.

LORETTA GUIDI, MARIA BALSAMO

Dipartimento di Scienze Biomolecolari, Università di Urbino

PHENOTYPIC PLASTICITY AND MORPHOLOGICAL EVIDENCES OF SPERMATOGENESIS IN *TRICHOPLAX ADHAERENS* SCHULZE, 1883 (PLACOZOA) AFTER TREATMENT WITH ETHANOL

Trichoplax adhaerens (Placozoa), a non-bilaterian marine species, has the simplest body plan among metazoans. Only seven somatic cell types are organized into two pseudo-epithelial layers, a marginal cell cord, and an inner tridimensional network of star-shaped fiber cells. Placozoa reproduce by binary fission; budding occurs only in unfavourable conditions. Genetic data show that sexual reproduction may take place in nature and oocytes production has often been observed, especially under stressful conditions. A recent study on the possible effects of the treatment of *T. adhaerens* with ethanol extract of *P. spinosa* or with only ethanol was carried out. Results highlighted clear differences in cell morphology between the untreated and the treated animals. The phenotypic plasticity of the untreated animals *vs.* those treated with only ethanol was investigated through microscopical and ultrastructural TEM observations. Untreated animals maintained a large and flattened body with irregular shape, whereas specimens treated with ethanol showed a much smaller body with rounded shape. Under TEM, the treated animals showed a marginal cell cord clearly thicker than untreated ones, because composed of a higher number of cells. Moreover, several cells underlying the marginal cord appeared to undergo a spermatogenetic process. In fact, they showed a nucleus with condensing chromatin, a well-developed Golgi apparatus with numerous vesicles, progressively increasing in number, few mitochondria near a basal body and a developing flagellum. Near these cells, in one specimen a putative spermatozoon was observed, with a round central nucleus, a single, large electron-dense vesicle, and few mitochondria close to the flagellum. A peculiar feature, never observed before, was a single membrane surrounding both the nucleus and the large vesicle. These observations support a great phenotypic plasticity of *T. adhaerens* under different laboratory conditions and suggest to verify the spread of these cells in the ethanol-treated specimens and to focus their ultrastructural details in order to confirm these preliminary observations. That would be of great interest taking into account that signals of sexuality of *T. adhaerens* have been reported mainly in animals stressed by altered environmental conditions. In fact, the appearance of sexual reproduction in unfavourable life conditions is already known for a number of animals that usually reproduce by asexual modalities.

DAVIDE MAGGIONI[1,2], SIMONE MONTANO[1,2], OLIVER VOIGT[3], DAVIDE SEVESO[1,2], PAOLO GALLI[1,2]

[1]Dipartimento di Scienze dell'Ambiente e della Terra (DISAT), Università degli Studi di Milano-Bicocca
[2]Marine Research and High Education (MaRHE) Center, Università degli Studi di Milano-Bicocca, Isola di Magoodhoo, Atollo di Faafu, Repubblica delle Maldive
[3]Department of Earth and Environmental Sciences, Palaeontology and Geobiology, Ludwig-Maximilians-Universität München, München, Germany

A MESOPHOTIC HOTEL: THE OCTOCORAL BEBRYCE GRANDICALYX AS A HOST

Mesophotic coral ecosystems are widespread and harbour rich coral reef communities below 30 m and down to 150 m depth. Although in recent years the knowledge of these charismatic ecosystems has increased, many aspects are still to be explored, including, for instance, symbiotic associations. During surveys of the upper mesophotic reef of Dharanboodhoo Island, Republic of the Maldives, a novel, previously unreported association between an octocoral, a sponge, and a hydrozoan was discovered. The octocoral *Bebryce* cf. *grandicalyx* was commonly found at depths ranging from 40 to 50 m. A closer inspection of the colonies revealed the presence of two frequent associates. The octocoral was always overgrown by a sponge, which fully covered the colony, leaving uncovered only the polyps. Additionally, in most of the observations, hydrozoans belonging to the species *Zanclea timida* were found as epibionts of the octocoral. Hydrozoan polyps reached high densities, covering almost all the available space, except for octocoral polyps, and were partially embedded in the sponge. The three organisms were well integrated with each other in a stable way, given also the high prevalence of the associations. Moreover, a parasitic interaction seems to be excluded and the possible advantages gained by each associate are discussed. In addition to the recurring three-way association, other invertebrates were found dwelling on the octocoral and sponge surface, including barnacles, entoprocts, amphipods, flatworms, foraminiferans, and ophiurans. It is difficult to define the ecological interactions among these organisms, but their presence is likely related to an increased habitat availability provided by the stable association between the octocoral and the sponge. This and other multi-taxa associations need further investigation to characterise the possible positive and detrimental effects on the whole association systems, especially in the light of the current decline of both shallow and mesophotic coral reefs.

CHIARA MANFRIN[1], PIERO GIULIO GIULIANINI[1], ILARIA GIOVANNINI[2],
THOMAS C. BOOTHBY[3], ROBERTO GUIDETTI[2], LORENA REBECCHI[2]

[1]Università di Trieste, Dipartimento di Scienza della vita
[2]Università di Modena e Reggio Emilia, Dipartimento di Scienza della vita
[3]University of North Carolina at Chapel Hill, Department of Chemistry, USA

NEW INSIGHTS ON THE MOLECULES ALLOWING DESICCATION TOLERANCE: THE CASE STUDY OF THE ANHYDROBIOTIC TARDIGRADE *PARAMACROBIOTUS SPATIALIS*

Anhydrobiosis is a highly stable state of suspended animation in an organism due to its desiccation, which is followed by recovery after rehydration. Among desiccation tolerant organisms, tardigrades are the most resilient known animals and are able to perform anhydrobiosis at any stage of their life cycle. However, physiological and biochemical/molecular mechanisms at the bases of extreme desiccation tolerance remain elusive. Using the anhydrobiotic tardigrade *Paramacrobiotus spatialis* as a model organism, we have re-investigated transcripts of desiccated specimens comparing the transcriptomes of hydrated active animals and desiccated ones. A *de novo* transcriptome from Illumina-sequenced reads (SRR5217911-12, SRR5367859-60-61) has been assembled through Trinity v2.4.0 and annotated with Trinotate v3.1.1. To identify high-quality transcripts, redundancy has been removed following Evidential Gene tr2aacds pipeline, and evaluated with BUSCO. In order to outline the highly perturbed transcripts in response to desiccation, data were analysed with RNA-seq tool from the CLC genomics workbench v12, selecting those transcripts with at least a 5-fold between hydrated and desiccated, and a False Discovery Rate p-value ≤ 0.01. About 50 transcripts resulted highly activated upon desiccation. As already reported by BOOTHBY et al. (2017), the most represented group belongs to the intrinsically disordered proteins as cytosolic-abundant heat soluble protein (CAHS), but a majority of transcripts is still reported as unknown. A transcript encoding for Reticulon, a group of evolutionary conserved proteins present in the endoplasmic reticulum which mainly plays a role in promoting membrane curvature, and two transcripts involved in collagen and connective tissue organization are also among the most up-regulated contigs. Transcripts related to the synthesis-pathways of the sugar trehalose (as bifunctional trehalose-6-phosphate synthase/phosphatase and alpha,alpha’-trehalose-phosphate synthase) have been also reported among differentially up-regulated, but with a lower fold change ≤ 3. These findings support and highlight previous hypotheses, pointing out that desiccation tolerance involves multiple molecules working together as bioprotectants.

EDOARDO MASSA[1,2], K. INGEMAR JÖNSSON[2], ROBERTO GUIDETTI[1]

[1] Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia
[2] Department of Environmental Science and Bioscience, Kristianstad University,
Kristianstad, Sweden

A FAUNISTIC SURVEY OF TARDIGRADES IN KRISTIANSTAD VATTENRIKE BIOSPHERE RESERVE (SWEDEN)

The Kristianstad Vattenrike Biosphere Reserve is an UNESCO designated area in the southernmost region of Sweden, known for its wetlands, rivers and lakes and for successful coadaptive management of its nature resources. Within the Biosphere reserve both residential areas and biotopes are included, the latter classified as possessing high biological values of international and national importance such as wetlands, forest, and sandy arable land. Tardigrades, also called water bears, are microscopic (50-1000 µm in length) aquatic animals, inhabiting a variety of freshwater, marine and terrestrial ecosystems and micro-environments. Species living in terrestrial habitats, such as mosses and lichens, are able to enter in a “suspended-life” stage, namely cryptobiosis. Around 1300 species of tardigrades have currently been described. The tardigrade fauna of Sweden has been investigated only scarcely in the last century and the Kristianstad common area (Skåne region) has been sampled just once. The review of tardigrades in Sweden by Guidetti *et al*. (2015) reported 101 species in the country. The aim of this work was to study the tardigrade fauna in the Kristianstad Vattenrike Biosphere Reserve. In 2014, 34 samples (mosses, lichens, leaf-litter, grass and freshwater sediments) were collected in four environments (*i.e.* not-disturbed sandy dune, human-disturbed sandy dune, not-disturbed forest, and human-disturbed forest) from five different locations. Thirty-two species of tardigrades were identified: all of them are new records for the Biosphere Reserve. In particular, 11 species are recorded in Sweden for the first time, 21 species are new records for the Skåne region, and 5 species are also new to science. Only two moss samples lacked tardigrade specimens. In addition, a quantitative analysis has been carried out on samples belonging to four locations, corresponding to the different environments mentioned above. The samples collected in human-disturbed areas showed a higher number of tardigrade specimens. However, the biodiversity found in human-disturbed areas was lower than that found in non-human-disturbed areas. The most common species in all samples was the eutardigrade *Ramazzottius oberhaeuseri* with 320 specimens found in 13 different samples. Even though present data are the first attempt to describe the tardigrade biodiversity of the Kristianstad Vattenrike Biosphere Reserve, they evidence that this area has to be considered a hotspot of biodiversity for Sweden. Our study has also resulted in a considerable increase (11%) in the number of tardigrade species recorded in Sweden and show how tardigrade biodiversity is still underestimated.

MANUELA MAURO[1], ISABEL PÉREZ-ARJONA[3], EDUARDO BELDA[3], MARIA CERAULO[2], MANUEL BOU-CABO[3], THOMAS BENSON[5], VÍCTOR ESPINOSA[3], GIOVANNI CUOMO[5], FRANCESCO BELTRAME[4], SALVATORE MAZZOLA[2], MIRELLA VAZZANA[1], GIUSEPPA BUSCAINO[2]

[1]Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università di Palermo
[2]BioacousticsLab, Consiglio Nazionale delle Ricerche, Torretta Granitola (TP)
[3]Polytechnic University of Valencia, Campus of Gandia, Spain
[4]ENR, Ente Nazionale di Ricerca e promozione per la standardizzazione, Palermo
[5]HR Wallingford, Howbery Park, United Kingdom

ANTHROPOGENIC NOISE: THE BEHAVIOURAL RESPONSES OF SPARUS AURATA JUVENILES AS THE BASIS FOR A NUMERICAL MODEL.

Underwater noise is mainly produced by anthropogenic activities and has increased in recent years. The Marine Strategy Framework Directive aims to monitor "continuous low frequency sound" trends in ambient noise in particular at 1/3rd octave bands centered at 63 and 125 Hz. The aim of this work is to evaluate the possibility of using the results of *Sparus aurata* juvenile's behavior for parameterizing a numerical model of the fish behavior. The fishes were stressed with white noise filtered with a band pass filter at 1/3 octave band centered respectively at 63 Hz, 125 Hz, 500 Hz, and 1 kHz (SPL: 140-150 dB re 1μPa). We performed three test replicas for the control (without any sound emission) and for each of the four frequencies. Each trial lasted 7:15 hours in total and included the following three periods in which two video cameras recorded: 30 min before the acoustic emission; 60 min in which the acoustic stress was dispensed; 6 hours after the acoustic emission divided into 6 periods of 1 hour. To obtain the behavioral data (cohesion, motility, swimming height) the fishes were video monitored 15 min before the sound exposure, one hour during the sound exposure and the final 15 minutes of each hour after the sound exposure. The bottom of the tank was divided into squares. Cohesion was evaluated by counting the number of squares occupied by the group; motility by counting the number of squares crossed by each fish; swimming height by counting the fish presence in three zones (deepest, intermediate, and highest). Using Kruskal-Wallis tests and multiple comparisons post-hoc tests we assessed different behaviors of the fishes reacting to different acoustic frequencies. Here, we propose the use of part of these data (the first hour of acoustic emission) for the parameterization of a numerical model using an adaptation of the Boids method. In the model the cohesion, motility and the swimming height are analyzed in the same way as they are analyzed for in vivo video recordings. Preliminary results showed that it is possible to reproduce in the new model the same qualitative behavior of the in vivo recordings with fish. In this way, it will be possible to understand the behavioral responses of fish in laboratory experiments and contribute to the prediction of the impacts of other possible acoustic sounds emitted by human activities through the application of the created numerical model.

FEDERICA MONTESANTO[1], GIOVANNI CHIMIENTI[1], MARIKA SALONNA[2], CARMELA GISSI[2,3], FRANCESCO MASTROTOTARO[1]

[1]Dipartimento di Biologia e CoNISMa LRU, Università di Bari "Aldo Moro"
[2]Dipartimento di Bioscienze, Biotecnologie and Biofarmaceutica, Università di Bari "Aldo Moro"
[3]IBIOM, Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari, CNR, Bari

THE PROPHECY CAME TRUE: *APLIDIUM ACCARENSE* (MILLLAR, 1953) SPREADS IN THE MEDITERRANEAN SEA

Ascidians are considered one of the major fouling pests. Several species can grow fast and spread rapidly, becoming invasive on both artificial and natural substrata thanks to their tolerance to a wide range of stresses. Furthermore, several ascidian species can be easily transported with the maritime traffic or with aquaculture products, making harbours and farms some of the main gateways for the spread of invasive taxa. The number of the so-called Non-Indigenous Species (NIS) recorded in Italian seas is rapidly increasing also thanks to an increased scientific effort which leaded to the recent reassessment of the alien or cryptogenic status of some species, as well as to the adoption of integrated approaches between morphological, ecological and molecular studies for taxonomic purposes. This is particularly true for ascidian identification, whose complex taxonomy is one of the major limiting factors in the early detection of non-indigenous species. *Aplidium accarense* (Millar, 1953) was firstly recorded along the locality of Accra (Gold Cost, western Africa), hence the name *accarense*. The geographical distribution of *A. accarense* includes also Brazilian and Venezuelan coasts, where it is suspected to be. This species can represent a real threat to the autochthonous diversity, since it forms large colonies that can cover and displace other species. Recently, *A. accarense* was reported for the first time in the western Mediterranean Sea, along the Catalan coasts, where it affected mussel farms within the Ebro Delta. Thus, it was hypothesized that *A. accarense* is currently undergoing an expansion in the Mediterranean Sea, and that it may constitute a pest in the near future. The present study confirms this hypothesis by reporting the easternmost record of the species *A. accarense* within the Taranto harbour, almost close to aquaculture facilities along the Mar Piccolo basin. Its presence was confirmed by morphological and molecular characterization, the last one based on sequencing of the mitochondrial COI gene (Cytocrome Oxidase subunit I), and comparisons with *A. accarense* sequences already available in the nucleotide databases. This study will allow future identification of *A. accarense* in other areas of the Mediterranean Sea. Indeed, monitoring the evolution of *A. accarense* populations within the Mediterranean basin will be a fundamental prerequisite to set appropriate management strategies to limit its spread to the neighbouring areas as well as its secondary spread by means of shipping or as "accidental stowaway" on commercial mussels during transfer of cultured bivalve and/or associated to aquaculture gears.

SIMONE MONTANO[1, 2], FEDERICA MANCA[3], ANNALISA CARAGNANO[3], DAVIDE MAGGIONI[1, 2], DAVIDE SEVESO[1, 2], DANIELA PICA[3], INGA DEHNERT[2], PAOLO GALLI[1, 2], STEFANIA PUCE[3]

[1]Dipartimento di Scienze Ambientali e della Terra (DISAT), Università di Milano – Bicocca
[2]MaRHE Center (Marine Research and High Education Center), Magoodhoo Island, Faafu Atoll, Republic of Maldives
[3]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche

FOOTPRINTS OF CORAL SYMBIOTIC HYDROZOANS HIGHLIGHT THE DIVERSITY OF THE *ZANCLEA*-SCLERACTINIANS ASSOCIATION

Hydrozoans of the genus *Zanclea* have been acknowledged only recently as a fundamental component of the highly diverse fauna associated with reef-building scleractinian corals. Although widely distributed in coral reefs, the biodiversity of these diminutive hydrozoans remains enigmatic due to the paucity of available morphological characters, incomplete morphological characterizations and the possible existence of cryptic species. Recently, molecular techniques have revealed the existence of multiple hidden genetic lineages but phylogenetic data are not yet corroborated by significant morphological results. In this work, we further explore the morpho-diversity of three lineages, namely *Zanclea* associated with the coral genera *Goniastrea*, *Porites* and *Pavona*. Aside from providing a complete classical characterization of either the polyp and medusa stage of each clade, we searched for new potential taxonomic indicators either on symbiotic hydroids and on host corals. On the hydroids, statistical analyses on almost 7,000 nematocyst capsules revealed a significant difference in terms of nematocyst size among the three *Zanclea* clades investigated. On each host coral genus, we identified peculiar skeletal modifications related to the presence of *Zanclea* symbionts and we assessed their footprint as diagnostic attribute in the characterization of the *Zanclea*-scleractinians symbioses.

CARLO POLIDORI[1], ALBERTO JORGE[2], ALEXANDER KELLER[3], JOSÉ LUIS NIEVES-ALDREY[5]

[1]Instituto de Ciencias Ambientales (ICAM), Universidad de Castilla-La Mancha, Toledo, Spain
[2]Laboratorio de Microscopia, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain
[3]Center for Computational and Theoretical Biology, University of Wuerzburg, Hubland Nord, Germany
[4]Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense de Madrid, Spain
[5]Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain

LARGE PHYLOGENETIC EFFECT ON THE DISTRIBUTION OF METAL-ENRICHED MANDIBLES IN SAWFLIES, WASPS, BEES AND ANTS (HYMENOPTERA)

In different insect groups, body structures such as mandibles and ovipositors are known to have a hardened cuticle by incorporation of transition metals, such as Zn and Mn. Since these structures are strictly associated with feeding (e.g. food chewing) and reproduction (e.g. ovipositing, emerging as adults from a substrate), it was suggested that metal incorporation represents an adaptive trait which improves resource use and larval breeding. In the Hymenoptera (sawflies, wasps, bees and ants), for instance, a functional link between metal enrichment and ecological pressures has been proved within some lineages, but only for ovipositors. It still unclear if also metal incorporation in the mandibles can be considered as adaptive. Here, we verified if Zn and Mn incorporation in the mandibles of Hymenoptera is associated with two physiological-ecological traits: larval diet (herbivorous vs. carnivorous) and larval development site (concealed vs. unconcealed). Across 87 species spanning most of the extant superfamilies, we found Zn enrichment to be widespread (57 species) and Mn enrichment to be rare (10 species). Metals lack in the mandibles of the most primitive “Symphyta”, suggesting (though not conclusively) that metal enrichment is not ancestral in the order. Furthermore, Zn enrichment was the ancestral state for the Apocrita, where it was lost and re-acquired few times. Interestingly, all bees lacked metals in their mandibles, while wasps vary in this trait. By reconstructing the phylogeny of the studied species, we found a strong phylogenetic effect on Zn % and Mn% variation in mandibles. On the other hand, the two considered physiological-ecological factors were unimportant: a causal effect of diet on metal occurrence was unlikely, and species emerging from concealed vs. unconcealed development sites had similar likelihood to have metal-enriched mandibles. Evolutionary history seems to constrain metal-enrichment in hymenopteran mandibles, yet the few observed losses and re-gains of this trait during evolution claim for deeper investigations. Financial support for this study comes from a Post-Doc contract to CP by Universidad de Castilla La-Mancha and ESF, and from the funded project CGL2017- 83046-P by Ministerio de Economía, Industria y Competitividad (España)

CARLO POLIDORI[1], KRZYSZTOF SZPILA[2], FEDERICO RONCHETTI[3]

[1]Instituto de Ciencias Ambientales (ICAM), Universidad de Castilla-La Mancha, Toledo, Spain.
[2]Department of Ecology and Biogeography, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Toruń, Poland
[3]Department of Animal Ecology and Tropical Biology, University of Wuerzburg, Hubland Nord, Germany

DO VISUAL AND OLFACTORY SYSTEMS VARY WITH HOST-FINDING BEHAVIOUR IN MILTOGRAMMINE FLIES (DIPTERA: SARCOPHAGIDAE)?

Many morphological features of parasitic insects are modified for host-finding and host-attacking activities, which are expected to change with the host type. The fly subfamily Miltogramminae (Diptera: Sarcophagidae) are an excellent group to study possible adaptations in relation to the resources used by females to feed their larvae. Indeed, in this subfamily there are three main groups of species that could be categorized in relation to the behaviour they use to find their resource. The necrophagous species use various types of animal carrion to feed their brood. The parasitic species, which attack wasps and bees, include the other two groups, which differ in their host-finding strategies: some species patrol the host nesting site and enter actively in the host nests (“hole searchers”), while others wait on perching sites, close to host nest entrances, for a nest-returning host female, then follow it in flight at a fixed distance behind (“satellite flies”), ultimately sneaking into the nest. We hypothesize that necrophagous and hole searchers rely more on olfactory cues to find their resource, while satellite flies would rely more on visual cues, given their particularly elaborated host-trailing behaviour in flight. To test for this hypothesis, we studied the visual (eye size, ommatidia size and number, ocellar size) and the olfactory (antennal size, sensilla size and number) systems in 18 species of Miltogramminae, through Electron Scanning Microscopy. We found differences in many of the analysed morphological variables. In particular, despite species from the two groups did not differ in body mass, satellite fly species had larger eye, larger ocelli and a greater number of ommatidia compared with necrophagous/hole searcher species. The latter, on the other hand, had some structures of the antennae which were larger than in satellite fly species. The available phylogenetic hypotheses for Miltogramminae evolution suggest that common ancestry may partially account for the observed morphological variation, so that a formal correction has to be applied to confirm our hypothesis. Financial support for this study comes from a Post-Doc contract to CP by Universidad de Castilla La-Mancha and ESF, and from the funded project CGL2017- 83046-P by Ministerio de Economía, Industria y Competitividad (España). KS was supported by National Science Centre, Poland (project 2015/17/B/NZ8/02453).

MATTEO PALLOTTINI[1], SARAH PAGLIARINI[1], MARIANNA CATASTI[1], GIANANDREA LA PORTA[1], ELDA GAINO[1], ALESSANDRO DI GIULIO[2], ARSHAD ALI[3], ENZO GORETTI[1]

[1]Dipartimento di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia
[2]USLUmbria1, Perugia
[3]University of Florida, USA

CHIRONOMIDS (DIPTERA) OF LAKE TRASIMENO: POPULATION STATUS OF THE PELAGIC ZONE

At Lake Trasimeno Chironomids (Diptera) are considered a cause of discomfort for residents and tourists because of their intense swarms attracted by light sources during summer, interfering with human activities. The shallow lake (maximum depth < 6 m), the formation of areas rich in organic material and the lack of an effective predation in the ecosystem determine an ideal environment for the proliferation of these insects. In particular, *Chironomus plumosus* is the species that causes the main nuisance. Since 2006, to reduce the density of the Chironomid larval populations, the biological larvicide product *Bacillus thuringiensis* var. *israelensis* has been used. This treatment is applied only to the littoral zone, where the water is not deeper than 2 m. Since the shallow waters provide a suitable habitat for Chironomid life cycle for all the lake extension, the Chironomid larval populations were investigated also in the pelagic zone. During 2018 and 2019 the macrobenthic community of the pelagic zone of Lake Trasimeno was investigated through 14 monthly samplings (May 2018 - June 2019) at 3 sites, with a depth of about 4-5 m. Samplings were carried out with a compressed air dredge (sampling surface 400 cm^2), at each site 5 samples were collected. The macrobenthic community was composed mainly of Chironomids and Oligochaeta. About 2,071 (30%) and 4,660 (70%) Chironomid and Oligochaeta specimens, respectively, were sampled. Negligible numbers of other macroinvertebrate taxa were found (mainly the alien invasive species *Dreissena polymorpha*). The average densities of the macrobenthic community were about 250 ind./m^2, 550 ind./m^2, and 0.4 ind./m^2, for Chironomids, Oligochaeta and other macroinvertebrate taxa, respectively. The Chironomid community was composed of only 3 taxa: *Chironomus plumosus* (95%), *Tanypus* sp. (4%), and *Cryptochironomus* sp. (1%). From the comparison of the larval densities in the months of July and August 2018 (when the nuisance problem caused by adult emergences is more consistent) between the littoral and pelagic zones, the total Chironomid density was almost equal, but regarding only *Chironomus plumosus*, the larval density of the pelagic zone was three times higher than the littoral one (about 600 vs 200 ind./m^2, respectively). Thus, the pelagic zone (90% of lake surface) represents the main area of *C. plumosus* adult emergences and therefore the source of the Chironomid-related nuisance problem.

FEDERICO RONCHETTI[1], CARLO POLIDORI[2]

[1]Department of Animal Ecology and Tropical Biology, University of Wuerzburg, Würzburg, Germany
[2]Instituto de Ciencias Ambientales (ICAM), Universidad de Castilla-La Mancha, Toledo, Spain

WEAK BUT SIGNIFICANT SPECIALIZATION IN HOST USE BY PARASITOID WASPS OF THE FAMILY MUTILLIDAE

The wasp family Mutillidae (Hymenoptera: Aculeata), known as velvet ants, includes approximately 4300 described, albeit their biology is still largely unknown. Almost all studied species are ectoparasitoids of mature larvae and prepupae of other aculeate Hymenoptera (bees, wasps and ants). The known host associations in general suggest that mutillids are quite polyphagous, but the lack of quantitative studies may be hiding tendencies to specialization among velvet ant lineages. Through an analysis of 408 host associations, we investigated, at the genus level, if a taxonomic bias in the host use exists, and how different ecological traits of the hosts (larval diet, type of nests and solitary/social condition) may have a role in the observed pattern. A network analysis showed an overall weak but significant host specialization by mutillids. Such global pattern arose from a combination of largely generalist taxa and highly specialized ones, both within velvet ant genera and within host subfamilies, each of the latter being attacked by one to many mutillid genera. The specialization index is not correlated to the number of observations, suggesting that our result is not a mere by-product of sample size variation. Among the main groups of Mutillidae, Kudrakumiini are clearly associated with herbivore and eusocial, ground-nesting hosts, while Myrmosini are less specialized. On the other hand, Spheropthalmini attack hosts building aerial nests, while Dasymutillini are associated with hosts that maintain ancestral parameters (carnivorous larvae, ground-nesting and solitary). Pseudomethocini shifted to solitary and eusocial hosts with herbivorous larvae. This carnivorous-herbivore diet shift seemed to have occurred in many mutillid tribes, even those phylogenetically closely related. Furthermore, there were shifts from solitary to social hosts (and *vice versa*) in many mutillid tribes. Overall, the data reveal a tendency to parasite ground-nesting hosts, while there is variability in larval diet and sociality, with social hosts that build aerial nests being very rarely parasitized. Financial support for this study comes from a Post-Doc contract to CP by Universidad de Castilla La-Mancha and ESF, and from the funded project CGL2017- 83046-P by Ministerio de Economía, Industria y Competitividad (España)

STEFANO RUBERTO, DANIELE BUONO, ALFREDO SANTOVITO

Dipartimento Scienze della Vita e Biologia dei Sistemi, Università di Torino

BISPHENOL A AFFECT THE REPRODUCTIVE FUNCION OF THE MARINE POLYCHAETE *OPHRYOTROCHA DIADEMA*

Polychaetes of the genus *Ophryotrocha* are small warms living among the interstitial fauna in nutrient-rich and polluted habitats, such as harbors. Owing to easy rearing in the laboratory and short generation times, several of the about 40 species currently described are model organisms for studying different biological aspects, including reproductive biology. The amount of plastic and microplastic dispersed in the sea is continuously increasing, strongly affecting the development and reproduction system of several marine organisms. Bisphenol A is one of the most used additives in the production of many plastic products and can be released into the environment by many products commonly used as a food containers and polycarbonate bottles. This compound was found to be an endocrine disruptor, able to produce adverse effects on the reproduction of benthonic and pelagic communities. In the present study, we describe, in *Ophryotrocha diadema,* the effects of different concentrations of BPA on eggs production. We tested six different concentrations of BPA in a 5-weeks experiment: 0.2 µM (45.7 µg/L), 0.4 µM (91.3 µg/L), 0.8 µM (182.6 µg/L), 1.6 µM (365.3 µg/L), 3.2 µM (730.5 µg/L) and 6.4 µM (1461.1 µg/L). We found a strong negative correlation between BPA concentrations and the number of eggs produced ($R^2 = 0.817$), with an average of 22.3 eggs/individual for controls and 3.3 for polychaetes exposed to the highest concentration. The Mann-Whitney test revealed a significant reduction in eggs production after exposure to BPA concentrations over 730.5 µg/L. This threshold concentration is higher than that observed in marine environment, although for many species of crustaceans, echinoderms and isopods, an impairment of reproductive function was observed even at very lower BPA concentrations. This apparent ability of *Ophryotrocha* to "withstand" at higher concentrations of BPA, may be related to the fact that these organisms are commonly found in highly degraded environments, and they probably evolved genetic mechanisms able to provide them a greater resistance to pollutants. For this reason, *Ophryotrocha* seems to be particularly useful as bio-indicators of the health status of the marine environment.

MANUEL SCARFO', MARTINA BUGLISI, ALFREDO SANTOVITO

Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino

CHRONIC STRESS INDUCE GENOMIC DAMAGE IN SHELTER DOGS

In mice and rats, exposure to chronic stress was found to induce alterations in gene expression, whereas, in humans, stress and anxiety conditions are associated to modifications in DNA methylation patterns and to increased levels of genomic damage and telomeres erosion. No data are present in literature about the possible correlation between stress and genomic damage in dog. For this reason, we decided to evaluate the level of genomic damage in shelter dogs (n = 30), by buccal micronucleus assay. As control group, we sampled family dogs (n = 30) matched for age and sex but without any experience of shelters. The tested hypothesis was that stress conditions, like those potentially present in shelter houses, could affect the levels of genomic damage. Moreover, for the first time in literature, the baseline frequencies of buccal micronuclei (MNi) and nuclear buds (NBUDs) were provided for non-human mammals. MNi represent chromosome fragments or whole chromosomes that fail to segregate properly during mitosis, appearing in interphase as small additional nuclei. NBUDs represent elimination processes from cells of amplified DNA and/or excess chromosomes. For both groups, at least 30.000 cells were analyzed. MNi and NBUDs frequencies (% ± SD) in shelter dogs were 0.300±0.233 and 0.280±0.220, respectively, whereas in family dogs were 0.083±0.095 and 0.130±0.154, respectively. Significant differences were found between shelter and family dogs in terms of MNi and NBUDs frequencies ($P<0.001$ and $P = 0.001$, respectively, ANOVA and Mann-Whitney tests), whereas sex and age did not influence the level of genomic damage in both groups. As general result, we provided evidences of a possible association between stress conditions and highest levels of genomic damage in shelter dogs. Results of this work, also considering the relatively low costs of laboratory procedures, could represent a stimulus for the introduction of genomic techniques in the evaluation processes of stress conditions of animals living in communities. These genomic techniques, associated with more traditional ones, such as cortisol level analysis and behavioral testing, could provide a more complete picture of the health status of animal communities of human interest.

GIULIA SCARPARO[1], PAUL RUGMAN-JONES[2], MARCO GEBIOLA[2], QUINN S MCFREDERICK[2], ANDREA DI GIULIO[1]

[1]Dipartimento di Scienze, Università degli Studi Roma Tre, Roma, Italia
[2]Department of Entomology, University of California, Riverside, USA

CHARACTERIZATION AND COMPARISON OF BACTERIAL COMMUNITIES OF *MICRODON MYRMICAE* (DIPTERA, SYRPHIDAE) AND ITS HOST, *MYRMICA SCABRINODIS* (HYMENOPTERA, FORMICIDAE)

Insects are the most abundant organisms of the animal kingdom, able to occupy and exploit a very wide variety of environments and resources. This enormous evolutive success is, almost partially, ascribable to their association with an exceptional diversity of symbiont microorganisms, with whom they coevolved since their appearance on Earth. Recently, many studies have highlighted how numerous bacteria supply their hosts with essential nutriments as well as protection against pathogens, parasites and predators. Nevertheless, the role of these symbiont microorganisms in the interactions between social insects and their parasites is still poorly known. *Microdon* Meigen, 1803 (Diptera, Syrphidae, Microdontinae) is a peculiar genus of hover flies counting about 300 species worldwide distributed, especially in South-America, while in Europe this genus is represented by only six species. The aberrant, slug-like larvae of these parasites, very different from standard larvae of other hover flies, are able to intercept and reproduce the communication of their ant hosts in order to successfully infiltrate the ant colony, feeding upon the ant brood and gaining other benefits, like shelter, favourable climatic conditions, and protection against predators. In order to find out if some bacteria could play a role in the host-parasite interaction*,* we performed the first microbiome survey of microbial communities of two populations of third instar larvae of *M. myrmicae* and larvae and workers of its host, *Myrmica scabrinodis,* using high throughput 16S amplicon sequencing. Furthermore, both for the host and the parasite we analysed separately the microbiome of the external surface of the cuticle and that of the internal part of the body. The results clearly show that the microbiome of the *M. myrmicae* is significantly different from that of its host, both ant larvae and workers. Moreover, while bacterial community of the ants doesn't change in the two populations, significant differences were observed in the external microbiome of the parasite, sign that the dominant groups of bacteria within *M. myrmicae* are affected by the surrounding environment rather than by a horizontal transmission between host and parasite. Families Streptococcaceae, Carnobacteriaceae and Rizhobiaceae are dominant in *Myrmica* microbiome, and *Spiroplasma* genus is preponderant in ant workers. Microbiome of *M. myrmicae* larvae is mainly characterized by high quantity of family Anaplasmataceae, of whom *Wolbachia* is the predominant genus. Further analyses are necessary to investigate if some bacteria harboured by *M. myrmicae* larvae could promote its survival inside the ant nests.

FRANCESCO TIRALONGO, GIUSEPPINA MESSINA, SARA IGNOTO, BIANCA MARIA LOMBARDO

Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università degli Studi di Catania

THE ROLE OF ROCKY INTERTIDAL POOLS AS NURSERY AREA FOR THE GIANT GOBY, *GOBIUS COBITIS* PALLAS, 1814, IN THE MEDITERRANEAN SEA

Tidal pools are very important marine habitats, in which rapid and dramatic changes in chemical and physical conditions deeply affect the biological communities. Notwithstanding these extreme oscillations, some marine species have the ability to adapt to these unstable conditions. In the Mediterranean Sea, the fish community that inhabits this particular environment is represented by several species. Some species of gobies (Gobiidae) and, in particular, of combtooth blennies (Blenniidae) are resident in intertidal pools. Here we present a preliminary study on the ichthyological community in two tidal pool systems of the central Mediterranean Sea (Sicily, Ionian Sea), with particular emphasis on very closed ones (connected with the sea only during high tide and rough sea conditions). Data were collected between April-July 2018. The abundance of specimens was estimated by visual observations using a quadrat (50x50 cm). A total of 68 juvenile specimens were randomly collected with a hand net (mesh size 1 mm), identified, measured (total length, TL) to the nearest mm using a meter scale and released (only five specimens of *G. cobitis* at different developmental stages were preserved in alcohol). The giant goby, *Gobius cobitis* Pallas, 1814, was the dominant species. The total length of juveniles of *G. cobitis* ranged from 14 to 43 mm and the mean abundance was 2.1 juveniles/m^2. In the Mediterranean Sea, there are very few studies on tidal pool systems and intertidal species. Our results show that, in high isolated tidal pools, there was the dominance of a single fish species; while, in less isolated pools, widely connected with the sea, the fish species diversity is significantly higher. In this preliminary study, we underline the role of rocky intertidal pools as nursery areas for this little studied species. Further investigations are needed in order to better understand the complexity and the ecological importance of this habitat.

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