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ELENCO CONTRIBUTI

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***Coenagrion castellani* Roberts, 1948 (Odonata: Zygoptera): storia e tassonomia integrata di un “nuovo” endemismo italiano**

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In Europa, gli Odonati sono uno dei gruppi di invertebrati meglio conosciuti; malgrado ciò, il loro studio attraverso metodi di tassonomia integrata può ancora fornire risultati inaspettati. *Coenagrion castellani* fu descritto nel 1948 dai dintorni di Roma. L'anno successivo il suo status tassonomico fu ridotto a quello di sottospecie (*C. mercuriale castellani*) per poi scivolare in un lungo oblio. Un recente inventario DNA barcoding sulla diversità degli odonati italiani ha mostrato come le popolazioni italiane di *C. mercuriale* siano distinte da quella d'oltralpe e dell'Africa settentrionale. Tale evidenza ha motivato il presente studio, volto a definire lo status tassonomico di questa entità e di valutarne distribuzione ed ecologia a scala nazionale. I risultati hanno mostrato come, a livello morfologico, *castellani* sia sempre distinguibile da *mercuriale* sulla base dell'analisi delle appendici addominali del maschio e della colorazione di capo e addome, sia nel maschio, sia nella femmina. A livello genetico, l'analisi di tre marcatori nucleari ha confermato quanto evidenziato a livello mitocondriale, con le popolazioni italiane nettamente distinte da quelle europee e nordafricane. *C. castellani* è distribuito esclusivamente in Italia, con popolazioni frammentate dall'Astigiano alla Calabria. È assente da nord-est e Sardegna, da riconfermare in Sicilia. *C. castellani* merita senza dubbio lo status di specie, la seconda, fra gli odonati, endemica a livello nazionale. Inoltre, essendo *C. mercuriale* inserito nell'allegato II della “Direttiva Habitat” e considerando le esigenze ecologiche comparabili tra le due specie e le minacce in atto, riteniamo che *castellani* meriti lo stesso trattamento e misure urgenti di conservazione. I risultati hanno mostrato come, a livello morfologico, *castellani* sia sempre distinguibile da *mercuriale* sulla base dell'analisi delle appendici addominali del maschio e della colorazione di capo e addome, sia nel maschio, sia nella femmina. A livello genetico, l'analisi di tre marcatori nucleari ha confermato quanto evidenziato a livello mitocondriale, con le popolazioni italiane nettamente distinte da quelle europee e nordafricane. *C. castellani* è distribuito esclusivamente in Italia, con popolazioni frammentate dall'Astigiano alla Calabria. È assente da nord-est e Sardegna, da riconfermare in Sicilia. *C. castellani* merita senza dubbio lo status di specie, la seconda, fra gli odonati, endemica a livello nazionale. Inoltre, essendo *C. mercuriale* inserito nell'allegato II della “Direttiva Habitat” e considerando le esigenze ecologiche comparabili tra le due specie e le minacce in atto, riteniamo che *castellani* meriti lo stesso trattamento e misure urgenti di conservazione.

Caratterizzazione di metaboliti secondari in *Carpodesmia crinita* es aggi biologici su *Arbacia lixula*

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Le alghe marine producono numerosi metaboliti secondari non solo per adattarsi alle situazioni di stress ambientale ma anche per difendersi dalle pressioni biologiche, quali competitori, patogeni, grazers, epifiti. Questo studio ha come scopo la valutazione dell'attività biologica dell'estratto cloroformico di *Carpodesmia crinita* vs il riccio di mare *Arbacia lixula*, un pascolatore di queste macroalghe, responsabile della degradazione dei cistosiereti in aree a basso indice di biodiversità (Barren habitat). I dati finora raccolti dimostrano che l'estratto di *C. crinita* è in grado di abbassare il livello degli sferulociti rossi, celomociti coinvolti nei meccanismi di difesa cellulari dell'echinodermi. Un abbassamento di questa popolazione, porterebbe all'inibizione dei processi di clearance dei corpi estranei e di espressione delle attività antimicrobiche abbassando, infatti, le capacità di difesa di *A. lixula*. Pertanto, il riccio in seguito al contatto con l'alga, potrebbe andare incontro a un abbassamento delle capacità difensive e quindi morire. Questo fenomeno potrebbe essere definito come un sistema anti-predatorio dell'alga nei confronti dei suoi possibili predatori, confermando l'attività anti-grazers e anti-microbica riportata per i metaboliti secondari prodotti dalle alghe brune. Ulteriori studi sono comunque necessari per chiarire il ruolo immunomodulante delle singole molecole presenti nell'estratto. Questo è il primo studio in cui viene valutato l'effetto di estratti di alghe sull'attività immunomodulante del riccio di mare *A. lixula*. I risultati da noi ottenuti, sebbene preliminari, sono certamente incoraggianti e ci spingono ad indagare ulteriormente sul potenziale che questi metaboliti estratti dalle Cistoseire hanno sulla fitness dei ricci di mare, considerati pascolatori di queste alghe.

Effects of polystirene nanoplastics on the brittle star *Ophiactis Vires*

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Nanoplastics are particles of less than 1µm, produced by the degradation/manufacture of plastic objects and represent an environmental issue of growing concern. The aim of this study was to investigate the effects of fluorescent polystyrene nanoparticles (PSNPs) on the physiology of *Ophiactis virens*, a filter-feeding and fissiparous ophiuroid widely distributed in the Atlantic-Mediterranean region. The animals were exposed to increasing concentrations of PSNPs (0,05 ug/ml;0,5 ug/ml;5 ug/ml) over a period of 2 weeks. Several parameters, at different biological levels, were analyzed: distribution of PSNPs, behavioural aspects (tipping time and moved distance), effects on arm regenerative development and biomarkers of oxidative stress. PSNPs apparently did not penetrate the epidermal layer, rather they “stick” to the body surface, with a higher presence over the stump than on the regenerating tissues. At 5 ug/ml accumulation of PSNPs was observed in the pre-buccal canal, thus suggesting their ingestion. A significant delay in tipping time was observed in specimens exposed to 0.5 and 5 µg/mL whereas no significant effects were found on the moved distance, the regenerative growth or presence of regenerate anomalies (external and internal anatomy). Furthermore, the highest tested concentrations significantly affected several biomarkers of oxidative stress, including SOD, GPx and lipid peroxidation level. In conclusion, PSNPs apparently do not cross the epidermal barrier therefore they do not accumulate in the inner tissues. However at high concentration they can be ingested or stick to the epidermis, thus leading to “more systemic” negative effects and stress conditions on behavioral and molecular parameters.

News and views on taxonomy and ecology of Italian bumblebees (Hymenoptera: Apidae)

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Species of the genus *Bombus* (Hymenoptera: Apidae) are charismatic pollinators that radiated mostly in cold habitats. Environmental changes provoked by recent geological phenomena left a deep mark on the populations' structure and isolation of these organisms, in some cases causing incipient speciation. On the other hand, climatic changes of the current time are driving dramatic changes in terms of bumblebee distribution. In this contribution, I will present the taxonomical changes in the genus *Bombus* with particular reference to the Italian fauna. Specifically, I will briefly present the cases of *Bombus bisiculus* and *Bombus konradini*, endemic to Italy, recently described based on integrative taxonomy (morphology, DNA analyses, and chemical profile of the cephalic glands). Furthermore, I will show the case of a recent range expansion of *Bombus haematurus*, a remarkable sentinel species of the quick warming climate of our times. Overall, this contribution will help understanding how evolution shapes insect populations in relation to a changing world.

Combined COI barcode-based methods to avoid mislabelling of threatened species of deep-sea skates

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Skates are characterized by conservative body morphology which hampers identification and leads to taxonomic confusion and market mislabelling. Accurate specimen classification is crucial for reliable stock assessments and effective conservation plans, otherwise the risk of extinction could be unnoticed. The misclassification issue is evident for the genus *Dipturus*, distributed worldwide, from the continental shelf and slope to the deep sea. In this study, barcode cytochrome oxidase I gene (COI) sequences were used along with species delimitation and specimen assignment methods to improve taxonomy and zoogeography of species of conservation interest inhabiting the Atlantic Ocean and Mediterranean Sea. We provided new evidence of the occurrence of *D. nidorosiensis* in the Central-Western Mediterranean Sea and the lack of Atlantic-Mediterranean genetic divergence. The Atlantic endangered species *D. laevis* and *D. batis* clustered together under the same molecular operational taxonomic unit (MOTU) with any delimitation methods used, while the assignment approach correctly discriminated specimens into the two species. These results provided evidence that the presence of the barcode gap is not an essential predictor of identification success, but the use of different approaches is crucially needed for specimen classification, especially when threshold- or tree-based methods result less powerful. The analyses showed how different putative, vulnerable, species dwelling across South-Western Atlantic and South-Eastern Pacific, are frequently misidentified in sequence repositories. Our study emphasized the limits associated to public databases, highlighting the urgency to verify and implement the information deposited therein in order to guarantee accurate species identification and thus effective conservation measures for deep-sea skates.

Un nuovo ordine di aracnidi per la fauna italiana: prima segnalazione di *Charinus ioanniticus* (Arachnida, Amblypygi)

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L'ordine di aracnidi Amblypygi è segnalato per la prima volta in Italia con il ritrovamento di una popolazione riproduttiva della specie *Charinus ioanniticus* (Kritscher, 1959) in bunker antiaereo sotterraneo risalente alla Seconda Guerra Mondiale sotto la città di Trieste. L'ordine Amblypygi conta circa 200 specie distribuite nella fascia tropicale e sub-tropicale di cui solamente tre sono presenti nel Paleartico Occidentale: *Musicodamon atlanteus* Fage, 1939, in Marocco ed Algeria, *Charinus ioanniticus*, in Egitto, Giordania, Israele, Turchia e Grecia e *C. israeliensis* Miranda et al. 2016, endemico di Israele. *C. ioanniticus* è l'unica specie presente in Europa, nota per le isole greche di Rodi e Kos e recentemente segnalata per la Grecia continentale. Questa specie ha abitudini sinantropiche e si riproduce prevalentemente per partenogenesi; tali caratteristiche la rendono una candidata ideale alla fondazione di popolazioni disgiunte in seguito a trasporto accidentale, tanto da rendere ancora poco chiaro per quali paesi la specie possa effettivamente essere considerata come nativa. Diversi esemplari a vari stadi di sviluppo, tra cui femmine adulte con uova, sono stati osservati, marcati e monitorati nel complesso di gallerie Klein Berlin a Trieste, alcuni esemplari sono stati prelevati e fotografati a fine identificativo e dati sono stati raccolti sui parametri ambientali e sulla presenza di potenziali prede nelle gallerie. Alcune ipotesi sono state avanzate sulle possibili modalità di arrivo dei primi esemplari nel complesso del Klein Berlin.

Validazione della metodica del metabarcoding di eDNA per il censimento della comunità di pesci e metazoi invertebrati di acqua dolce in un canale antropico dell'Emilia-Romagna.

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La validazione di protocolli di metabarcoding di DNA ambientale per l'analisi della biodiversità in habitat di acqua dolce dell'Emilia-Romagna è partita dall'individuazione dello Scolo Dosolo (Sala Bolognese, BO), canale di circa 205 metri compreso nella ZPS Cassa di espansione Dosolo, sito Natura 2000 (Direttiva Habitat; IT4050030, come sito-studio proprio perché parte di un'Area di Riequilibrio Ecologico dalla spiccata biodiversità in un contesto isolato dalle aree circostanti. Tre punti di campionamento sono stati scelti lungo il corso del canale sia per la raccolta di campioni d'acqua, sia per il censimento della fauna ittica tramite uso dello storditore elettrico. Gruppi target dell'analisi sono pesci d'acqua dolce e metazoi invertebrati; nonostante le indagini siano state complicate dall'emergenza Covid-19, tutto il 2020 è stato dedicato alla campagna di campionamento con una spedizione invernale, primaverile, estiva e autunnale.

Diverse metodiche per il filtraggio, preservazione dei campioni, estrazione del DNA, amplificazione, quantificazione, pooling e pulizia dei campioni sono state condotte per individuare quelle che più si adattavano a criteri di praticità, efficienza metodica, dispendio di tempo e forza lavoro. Preparazione delle library e sequenziamento Illumina Miseq sono stati svolti presso la Fasteris SA.

Le reads ottenute sono state analizzate usando una pipeline bioinformatica basata su OBITools arrivando ad individuare 7 specie di pesci e 54 specie di invertebrati.

I risultati ottenuti sono stati comparati con i dati derivanti dai campionamenti faunistici tradizionali e dai record bibliografici.

Complessivamente, la metodica validata fornisce una fotografia affidabile della biodiversità del sito-studio e un supporto efficiente alle metodiche tradizionali.

Motility and acoustic signals of the decapod *Cherax destructor*

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The present study examined for the first time the sound production and motility of the freshwater decapod crustacean *Cherax destructor*. An acoustic and video system was used to monitor the behavior of 10 individual (5 males and 5 females) in an experimental tank. Video data were used to analyze three motility variables (velocity, distance moved, and angular velocity). Acoustic data were analyzed to obtain abundance and these acoustic parameters: duration, 1st and 2nd peak frequency, sound pressure level (SPL) and the bandwidth. Both males and females produced short-duration pulses that could be grouped into three categories, high, medium, and low frequency. Males showed a higher rate of emission of mid-frequency sounds than females. The sounds emitted by females had significantly higher 1st and 2nd peak frequencies (Mean± DS: F1= 23±32 kHz; F2= 17±20 kHz) than males (F1= 10±15 kHz Mean± DS; F2= 11±16 kHz), sound duration was also higher in females (10±7 ms) than in males (8±4 ms), otherwise, SPL was higher in males (133±5 dB re 1µParms) than in females (132±7 dB re 1 µParms). Concerning the motility, there is no particular differences in velocity and distance moved, on the contrary, angular velocity was greater in males (21±39 deg/s) than in females (18±41 deg/s). This study is part of a wider one on the behaviour associated with the production of sounds by these animals observed both individually and in groups and on the behavioural and biochemical responses to potential sources of anthropogenic noise disturbance.

Sabellid polychaetes as new habitat-forming species from the mesophotic Ligurian Sea

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Marine animal forests have recently received significant attention, but scarce information is currently available on those dominated by sabellid polychaetes. A detailed ecological description of extensive sabellid fields from the mesophotic Ligurian Sea was made using ROV, autonomous lander, and scuba diving. The aggregations develop in areas with high trophic input, on horizontal muddy bottoms between 56 and 85 m depth, with extensions ranging between 0.5 and 23 m² and densities up to 943 individuals m⁻². The canopy formed by the sabellids ranges from 0.6 to 20.2 cm, with the intermediate size class (10-15 cm) resulting the most common. The continuous monitoring of this species suggests an intense feeding activity: the branchial crown remains open for most of the day, with feeding phases of approximately 3.4 hours, alternated by intervals of retraction of about 0.5 hours. The activity resulted positively affected by temperature and current, providing the first insights into the functioning of these mesophotic forests. The sustained filtering activity combined with the high density of individuals suggests a considerable impact on the pelagic-benthic coupling. Indeed, meiofaunal abundance and diversity within the aggregations resulted significantly higher than in outer stations, with differences also recorded in the community composition.

Finally, the taxonomic analysis of the structuring species supports the creation of a new taxon within the genus *Bispira* (Canalipalpata, Sabellidae). In conclusion, sabellid aggregations produce a strong effect on the surrounding biodiversity. These findings highlight the importance of these animal forests and claim adequate conservation measures against possible threats.

Macroevolutionary Analyses Provide New Evidences of Phasmids Wings Evolution as a Reversible Process.

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The concept that complex ancestral traits can never be re-acquired after their loss has grown popular since its initial formulation and it's often referred to as Dollo's law. Nonetheless, several macroevolutionary evidences - along with molecular ones - suggest instances where complex phenotypes could have been lost throughout a clade evolutionary history and subsequently reverted to their former state in derived lineages. One of the first and most notable rejection of Dollo's law is represented by wing evolution in phasmids: this polyneopteran order of insects – which comprises stick and leaf insects - has played a central role in initiating a long-standing debate on the topic. In this study, a new and comprehensive molecular phylogeny of over 300 Phasmatodea species is used as a framework for investigating wing's evolutionary patterns in the clade, taking into consideration several sources of uncertainty and all the methodological recommendations which have been proposed to test Dollo's law rejection. Macroevolutionary analyses support a dynamic and reversible evolution of wings, with multiple transitions to ancestral states taking place after their loss. Our findings suggest that neither wings or flight have acted as drivers of Phasmatodea species diversification and that brachyptery is an unstable state, unless when co-opted for non-aerodynamic adaptations. Our findings demonstrate that wings evolution can be a reversible and dynamic process in phasmids and contribute to shape our understanding of complex phenotypes evolution.

Transcriptomic response of *Trematomus bernacchii* to a short to mid term mild heat stress and bias of experimental design

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Many stenotherm marine species live in the cold and stable environment of the Antarctic Ocean which could be impacted by climate change in the upcoming years. To investigate this issue, gene expression analysis was carried out on *Trematomus bernacchii* specimens caught near Mario Zucchelli Station. Brain, gill and muscle tissues were sampled from naïve animals and from those kept in control (-0.9°C) and experimental (+0.6°C) tanks for six hours, seven and twenty days, post acclimation. RNAseq data showed that the temperature increase mostly affected the brain, with a time dependent response. Immune response was up-regulated at mid-term; protein turnover and energy management were down-regulated and neuronal remodelling related genes were up-regulated at 20 days, where the highest number of differentially expressed genes was observed. Gill tissue showed a mild response after 20 days, mostly involving DNA replication. A great early-starting response to stablign was also observed across the entire experiment in brain and gills, with the latter being the most affected. The expression pattern clustering analysis showed that many synapse-related genes were down-regulated and energy related genes were up-regulated, while several binding processes were up-regulated at the latest time point in brain. The response in gills was milder and mostly involved cytoskeleton and glycolysis. No significant change was observed in muscle. These results identify the brain as the tissue most affected by both heat and stablign, evidencing its great sensitivity to the slightest environmental change, and highlight the importance of a careful experimental design when working with captive wild organisms.

Insight of the structural organization of the feeding apparatus of tardigrades.

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The feeding apparatus [FA] of tardigrades, the apomorphy of the phylum, is composed of chitinous structures (sometime embedded with calcium) and aragonite piercing stylets. It contains autofluorescent molecules historically considered to be α -chitin bound with proteins. Nevertheless, its exact molecular composition and evolution are still unclear. The morphological comparison of the FA in different tardigrade lineages is here explored to increase our knowledge on its molecular nature and evolution. Alive animals of both classes (Heterotardigrada, Eutardigrada) were analysed via confocal laser scanning microscope after staining with the chitin binding Calcofluor White (CFW). Imaging was performed exiting both autofluorescent molecules and chitin bound with CFW. CFW stained specimens fluoresced, confirming the presence of a chitinous lining in the FA of animals and revealing chitinous flexible structures that were rarely observed before and usually too thin to be appreciable with light microscopy. Autofluorescent areas never overlapped the areas stained with CFW, but perfectly fitted all the harder opaque structures visible in light microscopy. The different emission spectra of the autofluorescent and CFW stained molecules hence allowed to distinguish a multilayered structure in the FA, to determine that the autofluorescence is probably derived from a stiffening proteic matrix, and not from the chitin as thought, and to identify structures specific of different lineages suitable for systematic purposes. Future comparison with the claw's emission spectra could allow to explore the hypothesis of a cephalic appendages internalization that led to the unique FA in Tardigrada.

Morphometric analyses indicate a relationship between autumn-winter availability of trophic resources and bone size/shape in Roe deer (*Capreolus capreolus*) fawns

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The roe deer is a widely distributed ruminant described as “concentrated selector”. It chooses plant species or their parts with high protein and low fiber contents. The most critical period for feeding is autumn-winter, when feed is scarce or covered by snow. Management decisions in Italy are based on annual trophic availability, without considering alimentary selectivity and the critical period. To contribute to a more appropriate management, we analysed biometric data of roe deer fawns (age<11 months), in a Central Italy population, evaluating the relationship between size of body/cranial parameters and trophic resources available during the first critical autumn-winter period. Additionally, to verify whether shape data could be used as a supplementary tool, we applied a geometric morphometry approach to mandibles of fawns. Each sample was assigned to an environmental category based on the carrying capacity estimation of the hunting management unit in which the animal was shot down. Statistical analyses were performed on obtained data. Our findings indicated a possible relationship between feed availability during the autumn-winter period. The length of hock of roe deer fawns showed significant differences among environmental categories. Geometric morphometry data analysis showed that the mandible shape of samples under 8 months of age is likely influenced by the availability of trophic resources during the first critical period. Results suggested that morphometric analyses could help to identify suitable parameters for defining optimum management plans for roe deer, and that both feed selectivity and trophic resource availability in critical autumn-winter period should be taken into account.

Recovering carbon and elemental sulfur from contaminated matrices by exploiting crude glycerol focusing on the Microbial Diversity Dynamics

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The combustion of sulfur-containing matter results in SOx formation which causes health impacts, acid deposition in the environment and visibility depletion if released in the atmosphere (EPA 2019). Pigments industry, coal-based power generation plants and waste incineration facilities are some examples of the industries that generate SOx containing emissions. The pigments industry, in particular, utilizes many different chemical compounds in innumerable formulations which are mainly synthesized from sulfur and other additives (Eastaugh et al., 2007). As such, sulfate is not a harmful compound, but if it is poured into rivers or sewage systems, it can generate an imbalance in the overall sulfur cycle. In this cycle, the last product after the reduction of sulfur compounds is hydrogen sulfide (H2S) which is corrosive, odorous and toxic at low concentrations. This research aims to study the SOx contained in flue gases focusing on the biological reduction sulfate to sulfide. This process was carried out in a 2,5L UASB reactor exploiting the recirculation approach for 258 days, using 1L of an anaerobic sludge and crude glycerol as carbon source. Both physical-chemical procedures and molecular biology techniques were used during the long-term performance of the UASB and short-term experiments to get a broad knowledge of the anaerobic process.

Moreover, Illumina analyses of the 16S and 18S rRNA gene were used to study the dynamics of the Bacteria and Eukarya community along with the whole operation.

This research could be not only technologically and economically feasible, but also beneficial from the environmental point of view.

Haemolymphatic parameters in two aquaculture crustacean species *Cherax destructor* (Clark, 1836) and *Cherax quadricarinatus* (von Martens, 1868)

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The growing demand for animal proteins of aquatic origin such as fish, molluscs and crustaceans have prompted some European and Italian companies to focus their attention on some species of freshwater crustaceans such as *Cherax destructor* and *Cherax quadricarinatus*. They are among the largest freshwater decapods, matures early, females can lay over a thousand eggs in a single brood and has a broad environmental tolerance. All these characteristics make it highly appreciated species for aquaculture and few years ago their breeding has spread to Europe and recently also in Italy (Sicily). Although some commercial yabby farms have been studied these species from different points of view (dietary supplementation, growth, environmental condition), to date no one has analysed the basic cellular and biochemical parameters of the haemolymph of these two species under standard farming conditions. Information about their hemolymphatics parameters is fragmentary and filling these gaps becomes important for breeding. Cellular and biochemical parameters were analysed in both species to create a reference baseline to identify the state of welfare or suffering of these animals. The results showed that total haemocytes count, haemocytes subpopulations, enzymatic activities and pH are similar between the two species while total protein and osmolality are higher in *C. destructor* respect to *C. quadricarinatus*. The knowledge of these parameters could be useful to evaluate the good health status of these species kept in aquaculture facilities.

Valorization of sea urchin wastes: characterization of marine collagen peptides

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We have recently started two research projects (CIRCULAR and BRITEs) addressed to the recycling of food wastes of the sea urchin industry and their valorization in different products, including collagen-based biomaterials for tissue regeneration applications. We have already developed and characterized these sea urchin-derived medical device prototypes in terms of structure, mechanical performances, efficacy and *in vitro* cytocompatibility. In the present work we further characterized them in terms of collagen aminoacidic composition, *in vitro* degradation rates in physiological (PBS) and enzymatic (collagenase) conditions and *in vitro* cytotoxicity (human fibroblasts) of the collagen peptides, which could in turn become "bioactive molecules" useful in the regenerating tissue microenvironment. Amino acid profiling confirmed that sea urchin collagen is mainly composed of glycine, hydroxyproline and proline and its overall aa composition is similar to that of human collagen, with a few small exceptions for alanine, arginine, methionine and glutamic acid. Degradation test showed that in collagenase the biomaterial is degraded by 66% after 48 hours, while in PBS by 54% after 10 days. A commercially available bovine collagen membrane (Integra) was used as control. In collagenase, Integra is degraded by less than 10% after 48 hours and in PBS it remains intact even after 10 days. Finally, the *in vitro* tests on human fibroblasts showed that at short time (24h) high concentrations(100 µg/mL) of collagen peptides increase vitality (and, indirectly, proliferation) of human dermal fibroblasts. Overall, this work has strengthened the potential usefulness of our innovative and environmentally friendly biomaterial in tissue regeneration.

Absence of larval regeneration in the highly regenerative crinoid *Antedon mediterranea*

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Echinoderms are marine deuterostomes characterized by extraordinary regenerative abilities, both in adults and larval stages. Larval regeneration is well documented for all echinoderm classes, except Crinoidea, the most basal taxon. Therefore, the aim of this work was to assess if the larval stage (doliolaria) of the crinoid *Antedon mediterranea*, whose adults are perfectly able to regenerate, can regenerate as well. In normal conditions, doliolaria develops in a temporary post-metamorphic stalked stage (pentacrinoid) with an apical calix. Adults of *A. mediterranea* were collected at Le Grazie (SP, Liguria). After hatching, doliolariae were transversally bisected with surgical blades, the anterior and posterior fragments were monitored for 2-3 weeks and the survival rate was compared to non-bisected doliolariae. For both fragments we defined different post-amputation stages, which were characterized by stereomicroscopy and Scanning Electron Microscopy (SEM). Results indicate that less than 50% of the bisected larvae survived after 3 weeks and none of the halves was able to actually regenerate. Rather, after a wound-healing phase each half continued its pre-determined development and the obtained post-metamorphic stage lacked structures deriving from the missing half: anterior fragments originated a stalk without the calix whereas the posterior halves produced a calix without a stalk. These data suggest that: doliolaria cells are strictly committed to their original fate; cellular plasticity/dedifferentiation is temporary blocked and/or “stem cells” are missing or in a “stand-by” state. Considering the basal phylogenetic position of Crinoidea these results are particularly significant to better understand the evolution of (larval) regeneration in echinoderms and deuterostomes.

Approcci morfologici, cariologici e molecolari allo studio della biodiversità nei tardigradi del genere *Xerobiotus* (Eutardigrada, Macrobiotidae)

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Il phylum Tardigrada comprende circa 1300 specie adattatesi a diversi habitat, sia terrestri che acquatici. Il genere *Xerobiotus* (Eutardigrada, Macrobiotidae), il cui nome rimanda al fatto che gli organismi che vi appartengono sono frequentemente rinvenuti in muschi di ambienti aridi come le dune sabbiose o rocce esposte al sole, è relativamente raro e poco studiato e allo stato attuale include solamente quattro specie. Numerosi campioni provenienti da differenti località sia italiane che estere sono stati utilizzati per l'estrazione di tardigradi del genere *Xerobiotus*. Gli individui sono stati analizzati in modo integrato mediante osservazione con microscopia ottica ed elettronica a scansione e caratterizzazione molecolare e, dove possibile, è stato studiato il cariotipo e le modalità riproduttive. Le analisi di microscopia hanno evidenziato, oltre alle strutture morfologiche distintive del taxon, caratteri nuovi o ancora poco noti per il genere *Xerobiotus*. Le indagini molecolari, consistenti nel sequenziamento del gene mitocondriale *cox1* e dei geni nucleari rRNA 18S, 28S e ITS2, hanno permesso la caratterizzazione delle popolazioni e la costruzione di reti di aplotipi per gli individui analizzati. I dati mostrano come le specie gonocoriche anfimittiche presentino una distribuzione geografica limitata (locale), al contrario la specie partenogenetica è caratterizzata da un ampio areale (Italia, Svezia, Sudafrica e Australia). Questi studi permetteranno inoltre di chiarire la dibattuta validità filogenetica del genere *Xerobiotus* e di indagare le variazioni morfologiche, molecolari e cariologiche presenti tra gli individui campionati in diverse regioni del globo, che potrebbero portare all'identificazione di specie non ancora note.