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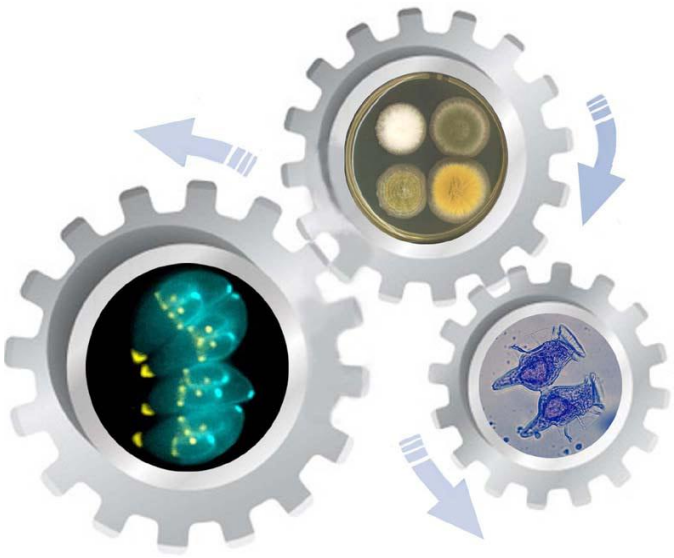

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CONFERENCE

PROCEEDINGS

LECTURE

How many Kingdoms?

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In this historical review the relationships between the taxa of animals, plants, fungi and protozoa/protoctista are briefly presented. The tradition of the division the living matter into animal and vegetables goes back for centuries. Nevertheless, it rapidly became evident that there are complex organisms, mainly microscopic and aquatic, with characteristic of plants, and the movements of animals that stretched the boundaries between the two main divisions. The greater morphological grouping into kingdoms is a reflection of our understanding of the living word, and it is part of the formal classification of living things since Linnaeus. In the last two centuries, the flourishing of researches induced the naturalists to classify these organisms outside the constraints of the plant and animal kingdoms and led to concept new kingdoms (Protozoa, Protista, Protoctista, etc.) in which these living things were included. R. Owen first proposed the new kingdom of Protozoa in 1860. In the same year J. Hogg proposed the Primigenial kingdom. E. Haeckel first proposed the Protist Kingdom in his three kingdoms scheme (1866), but the organisms included were different. The three kingdoms scheme has been modified in 20th century and four and five schemes were proposed. Whittaker (1969) proposed the widely used five kingdom hierarchy by introducing the kingdom Fungi. The refinement of endosymbiotic theory and improved researches led to a redefinition of Protozoa/Protoctist Kingdom and a variable number of kingdoms were proposed. In 1980s and 1990s the five kingdom Whittaker classification was re-interpreted by authoritative authors (Corliss 1986, Margulis 1990, Margulis and Schwartz 2001). In a series of works (1981-1986) T. Cavalier–Smith proposed a new kingdom and protists, which are to be viewed as heavily polyphyletic, were dispersed in five eukaryotic kingdoms. As often it happens, more taxonomic problems arose as knowledge of these organisms increased, and biological classification is never set in stone. However defined, the diversity of life is evidenced by our changing interpretation of the system of living organism, ripe for revision in response to the changing light of biological investigation.

DNA splicing contributes to the evolution of the *Paramecium* genome.

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Paramecium contains two genomes: a somatic genome housed in the macronucleus and a germline genome in the micronucleus. The germline genome contains thousands of short noncoding DNA sequences - called Internal Eliminated Sequences (or IESs) - which reside within and outside genes. After sexual reproduction, the germline DNA regenerates the somatic DNA, in a process whereby functional somatic genes are formed through the excision of IESs. In *Paramecium tetraurelia*, IES excision has been shown to be imperfect, as the removal of IESs from the regenerated somatic genome is often incomplete.
1I demonstrate that events of imperfect IES excision result largely from suboptimal excision signals combined with the preferential positioning of weakly excised IESs in intergenic regions. This latter observation suggests that natural selection shapes the distribution of imperfect DNA splicing in the *Paramecium* genome, i.e., selection acts against the accumulation of weakly excised IESs in the macronucleus. The extensive retention or incorporation of IESs in the somatic DNA of lines that were reared in selection-free conditions for several sexual generations supports this hypothesized selective signature.

Studying the marine environment: lessons from *Oxyrrhis*.

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Microzooplankton (20-200µm) are recognised as key players in most marine pelagic food webs, and protozoa such as ciliates and heterotrophic dinoflagellates are dominant members of the microzooplankton. Often, to assess the role of assemblages such as microzooplankton, researchers rely on "model organisms", i.e., species that reflect the key attributes of the dominant taxa. This presentation will focus on one such model: the heterotrophic dinoflagellate *Oxyrrhis marina*. First, I will briefly examine the use of protozoa as general models, supporting their versatility and encouraging us, as protozoologists, to consider protozoa as ecological models (see Montagnes *et al*. 2012, *The rise of model protozoa*, 10.1016/j.tim.2012.01.007). I will then specifically introduce the utility of embracing *Oxyrrhis* as an emerging model, by briefly reviewing the literature on this genus (see Montagnes *et al*. 2011, *An introduction to the special issue: Oxyrrhis marina, a model organisms?* 10.1093/plankt/fbq121). Finally, through a detailed example (that includes laboratory studies and mathematical modelling), I will illustrate two lessons that *Oxyrrhis* has taught us about methods associated with structuring marine pelagic ecosystem models. Lesson-one is that we should consider including independently determined functional and numerical responses (i.e., respectively, the responses of ingestion and growth to prey abundance) in ecosystem models, rather than determining the growth rate from ingestion by assuming constant assimilation and mortality, as is typically done. Lesson-two is that protozoa exhibit considerable strain-based variation; specifically, I will illustrate how temperature affects *Oxyrrhis* growth and ingestion and how this might impact on food-web models. In summary, I will reflect on the extent to which we should (or should not) use models like *Oxyrrhis* and invite discussion on it and other protozoa in this role.

Benthic microalgae in the marine environment: diversity, ecological role and relations with the substrate.

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Microalgae inhabiting sea bottom represent a highly diversified community, both from a taxonomical point of view, and for the variety of *modus vivendi*.
Benthic microalgae develop on all benthic substrata, especially at shallow depths where enough light penetrates, although they can also occur in subeuphotic conditions, due to adaptation to heterotrophic life style.
In the microphytobenthic communities, diatoms and cyanobacteria are the most important components, although almost all algal groups (dinoflagellates, euglenophyceans, cryptophyceans etc.) may occur. Benthic diatoms occur either as free living (motile forms) on sediments and hard surfaces, or as attached growth forms, comprising adnate forms (cells adhering to substratum through their valve face and having a limited movement) and erect forms (cells attaching to the substratum by one valve pole either through mucus pads or stalks).
Microphytobenthos play a key role in the primary production, especially in shallow waters, where their contribution may exceed that of phytoplankton in the overlying water column. Benthic microalgae mediate the oxygen and nutrient exchange between sediments and water column and are an important food resource for a number of meiofauna and microfauna grazers.
Microphytobenthos communities occur on a large variety of benthic substrata, such as soft bottom (epipelic, epipsammic, endopelic), rocks (epilithic, endolithic), marine plants (epiphytic), and animals (epizoic), often establishing specific relationships with their hosts.
Several animal phyla are known to host diatoms as epibionts: ciliates, sponges, hydrozoans, molluscs, crustaceans, and vertebrates. Living substrata offer a variety of advantages for epibiontic microalgae, among which the protection against grazing, a nutritional advantage, and the gain of an elevated position avoiding the resuspension of sediments. Hard-shelled metazoan groups generally support higher densities of epiphytes and normally the level of epibiosis is inversely related to the host motility.
Animal hosts offer a complex of microhabitats differently exploited by a variety of microalgae, indicating that a high spatial variations occurs even at small scales, both in terms of abundances and of species composition.

Towards a quality assessment of freshwater ecosystems through the morphological analysis of phytoplankton.

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Among the organisms living at low Reynolds numbers, those belonging to the ecological group of phytoplankton offer an amazing morphological diversity and all the scientists involved in phytoplankton research have commonly observed that they may express a quite high variability, both intra- and inter-specific, in their size and morphology. These features have been traditionally used just for taxonomic classification. However, the ecological value of morphological descriptors in phytoplankton is increasingly used to investigate their abilities in resource (light and nutrients) uptake as a result of natural selection and competition. At the same time, the analysis of suitable morphology and size descriptors can offer a useful tool to assess the ecological status of inland waters. In this presentation the relevance of phytoplankton size and morphology in depicting the ecological status of water bodies is shown by considering i) the role of their size in nutrient uptake and ii) the role of their morphology in light harvesting. The way in which the morphology of phytoplankton organisms cope with the hydrodynamic condition of a given water body is also shown. In addition, an account on the main research trends about the role of morphology in the definition of morpho-functional traits of phytoplankton is offered. Morpho-functional classification of freshwater phytoplankton can be viewed as complementary of taxonomy and is a promising tool to better understand the functioning of aquatic ecosystems. Investigating the variability of phytoplankton size and morphology and integrating these features in a coherent frame may offer a vast array of new perspectives in the field of phytoplankton ecology and aquatic research as well as a simplified, low-cost tool to perform water quality monitoring.

Testate amoebae communities in soils: from point to global scale.

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Testate amoebae communities from different habitats (sphagnum bogs, soils, freshwaters) and biomes (tundra, forest-tundra, taiga, broad-leaved forests, forest-steppe, steppe) were studied in the Eastern-European plain, Western-Siberian plain, and Baikal region in Eastern Siberia. We aimed to clarify, what are the main environmental variables and spatial scales that affect community heterogeneity? We studied: i) patterns of testate amoebae communities along different biomes; ii) community changes along landscape catenas; iii) community modifications in accordance with successional stage of forest ecosystems; iv) seasonal patterns of community structure; v) spatial heterogeneity of communities at spatial scales ranged from 1 cm to 1000 km. Finally we compared patterns of testate amoebae heterogeneity with those of large-size organisms (springtails, mollusks, rotifers, crustaceans). We concluded that: i) several types of landscape heterogeneity affect testate amoebae community structure; ii) spatial distance itself is not major factor of testate amoebae community formation; iii) body size is a major factor that affects characteristics of community structure of soil-inhabited animals; iv) today, undersampling is the key factor affecting revealing patterns of diversity and distribution of protists.

Targeting the unique thiol metabolism in Leishmania, the Achilles' heel of the parasite. A structural biology study.

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The current repertoire of drugs *Leishmania* and *Trypanosoma* is limited and an increasing parasite resistance towards them has posed a major concern. *Leishmania* and *Trypanosoma* parasites possess a unique thiol metabolism based on trypanothione, replacing many of the antioxidant functions of glutathione in mammals. Trypanothione, synthesized by trypanothione synthetase and reduced by trypanothione reductase, is used as a source of electrons by the tryparedoxin/tryparedoxin peroxidase system (TXN/TXNPx) to reduce the hydroperoxides produced by macrophages during infection. This detoxification pathway is not only unique to the parasite but is also essential for its survival, therefore it constitutes a relevant drug target. Our group is focusing on the study of the trypanothione metabolism proteins with the aim of identifying lead compounds for developing new drugs against leishmaniasis.

We solved the crystal structure of *Leishmania infantum* TR in oxidized state and in reduced state in complex with Sb(III), with Ag(I) and with auranofin, an anti-rheumatic organo-gold compound and the structure of TXN and TXNPx from *Leishmania major*.

The resolution of the structure of reduced TR in complex with Sb(III), disclosed the molecular basis of the interaction of antimonials, the first choice drugs against leishmaniasis, with TR and of its inhibition. Sb(III) binds to Cys52, Cys 57, His461' of the two-fold symmetry related subunit and Thr 335, the residues of the active pocket involved in the catalysis, thereby inhibiting TR activity. The X-ray structures of TR in complex with Ag(I) and Auranofin have shown that also silver and gold inhibits TR by binding to the catalytic cysteines. Kinetic studies have shown that Silver and Gold are able to inhibit TR with higher affinity with respect to Sb(III) (Ki (Ag(I)= 50 nM, Ki (auranofin) =155 nM vs. 1.5 µM).

Further, the structure of TR in complex with auranofin, where Au (I) is complexed by the triethylphosphine and the 4,5-triacetyloxy-6-(acetyloxymethyl)oxane-2-thiolate, shows that TR inhibition takes place with a dual mechanism, i.e. Au (I), released by the complex, binds to the residues involved in T(SH)2 reduction, and the 4,5-triacetyloxy-6-(acetyloxymethyl)oxane-2-thiolate is trapped in the substrate binding pocket competing with the binding of T(SH)2.

TXNPx displays the classical fold of the 2Cys-peroxiredoxins family members and assemble to form the classical decamer, whereas TXN crystallizes as a domain swapped dimer, never observed among homologous proteins. Analysis of the electrostatic surface potentials of both TXN and TXNPx unveils the structural elements at the basis of functionally relevant interaction between the two proteins. Finally, the structural analysis of TXNPx allows us to identify the position of the epitopes that make the protein antigenic and therefore potentially suitable to be used in an anti-leishmanial polyprotein vaccine.

The knowledge of the structures of all the protein of the trypanothione metabolism offers the unique opportunity to study at molecular level the peroxide reduction in leishmania parasite and to find compounds able to inhibit more than one enzyme.

Our studies suggest that thiophilic metals that are Lewis soft acids, more generally, may serve as strong inhibitors of TR and may result in potential interest as antileishmanial agent since they are able to kill the parasite in both amastigote and promastigote stages. For these reasons, the use of gold- and silver-containing compounds represents a reasonable therapeutic approach against leishmaniasis.

Further, structure-based in silico docking experiments have been performed. A few compounds, selected for their ability to bind with high affinity to the active site of enzymes of the trypanothione metabolism, have been chosen to guide the synthesis of inhibitors, with increased solubility and higher specificity.

Dynamics of harmful algal blooms in the mediterranean sea.

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Harmful Algal Blooms (HABs) are recurring events in Mediterranean coastal waters and affect coastal areas. HABs pose a threat to human health, marine environment, and resources, such fisheries, mariculture and tourism. Due to their impacts, HABs are considered as important ecological events. When inserted into an ecosystem perspective, these bloom phenomena, can be seen as deviations from "normal" phytoplankton dynamics. Phytoplankton dynamics are regulated by seasonal conditions linked with environmental factors, by the recruitment of excisted resting stages, and grazing of zooplankton. HAB events, thus, can be considered as alternative pathways in ecosystem functioning, deriving from the alteration of the seasonal sequence of pulses that are considered as "normal". The exact causes of the HABs are still to be clarified. In the last decades, special attention was directed towards the human activities with habitat modifications and eutrophication input. Further, distinctive life-cycles and species strategies strongly modulate the bloom dynamics. Human mediated transports of resistant stages allow the dispersion of non indigenous species (NIS) that affect diversity and dynamic of phytoplankton function in different areas. The hypothesis that the induced modifications by Climate Change in the Mediterranean may provoke the intensive and recurrent blooming of new dominant toxic species is now under debate. New techniques and in particular, emerging molecular methodologies coupled with advanced modelling techniques (e.g. machine learning) are improving the detection of some HABs for the prediction of their occurrences. Besides, recent development in population genetic findings of HAB species can provide a basis for planning the control and management of HABs in the Mediterranean.
Therefore, with increasing pressures on the coastal landscape, it emerges that substantial measures to prevent and manage the recurrent HAB events have to be adopted through an integrated coastal zone management involving all players as scientists and managers to enact adequate policy decisions in the Mediterranean Sea.

Toxin production in dinoflagellates: focus on *Ostreopsis* cf. *ovata*.

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Marine protists produce a wide array of secondary metabolites, and many of these compounds possess high biological activity. The phycotoxins are among these bioactives although their toxicity do not necessarily reflect their ecological and evolutionary significance. Despite their currently unknown role, these bioactive secondary metabolites may play several roles in intracellular regulation of the cell growth and metabolism as well as in extracellular regulation of population growth via allelochemical interactions. Phycotoxins are mostly synthesized by marine phytoflagellates, and more than 80% of the eukaryotic algal taxa that are toxic belong to dinoflagellates. Most if not all polyether phycotoxins are produced via polyketide pathways, in which acetate units are added sequentially from acetyl-CoA within a pathway regulated by polyketide synthases (PKS). Algal toxins vary in chemical structure, atomic composition, functional activity, and show different toxic profiles depending upon the growth and environmental conditions of the producing species. *Ostreopsis* cf. *ovata* has recently become one of the most studied dinoflagellate species due to the recurrent blooms, which have been linked to human health problems, along the Mediterranean coasts, as well as other tropical and temperate areas worldwide. Their toxins belong to the palytoxins-like family and are among the most toxic natural compounds so far known. Recent studies have showed a marked variability in the toxin profile of several Italian strains, despite the similar behaviour during the algal growth which lead to an increase of the cell toxin content, accompanied by extracellular release, from the exponential to the stationary phase. Environmental factors (e.g. temperature, salinity, nutrients deficiency) have been reported to influence the toxin production. The results obtained by the associated presence of others algal species in *O.* cf. *ovata* cultures have so far excluded an allelopathic role of these toxins.

Mycotoxin producing fungi.

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Mycotoxins are secondary metabolites naturally produced by *Eumycota*, mainly belonging to 3 genera, *Aspergillus*, *Penicillium* and *Fusarium*. Humans and animals show toxic effects when exposed to mycotoxins, mainly after ingestion. Both chronic and acute effects are reported, due to the accumulation in time of these very stable compounds in the body, especially in target organs, or to the high concentration in ingested food or feed. The carry-over of mycotoxin in animals is also known for some compounds. *Aspergillus flavus* and *A. parasiticus* are probably the most studied fungi because of their involvement in aflatoxins production. B and G series of aflatoxins are described, with aflatoxin B_1 as the most hepatotoxic natural compound known. Cereals and maize in particular, peanuts, pistachio nuts, spices are the main products where they can be detected; they are the most xerophilic among the toxigenic fungi and their activity can continue post-harvest. *Aspergillus ochraceus* and *Aspergilli* section *Nigri*, in particular *A. carbonarius*, produce ochratoxins, with ochratoxin A as the most toxic and they colonise cereals and grapes, respectively. *Penicillium verrucosum* and *P. nordicum* are also able to produce ochratoxin A, they are morphologically undistinguishable, but they use different nutritional sources, cereals and meat respectively. A further *Penicillium*, *P. expansum*, produces patulin in fresh fruits, mainly in apples; it is considered less toxic compared to the previously cited compounds, but it is regulated by European Commission as all the others. Several toxigenic Fusaria are known and they all colonise cereals; their water request is high and consequently field conditions and early host crop stages are the most positive conditions for fungal growth and toxin production. *F. graminearum* is involved in Fusarium head blight of wheat and is the main producer of deoxynivalenol and zearalenone, while *F. verticillioides* and *F. proliferatum* are involved in maize ear rot and they produce fumonisins; all these Fusaria toxins are included in EC regulation. Ecological conditions and host-pathogen interaction strongly influence fungal growth and toxin production; therefore, meteorological condition determines the possibility of host crop contamination, but a proper cropping system can mitigate the mycotoxin content.

SYMPOSIUM SEA WATER PROTISTS

SESSION 1

Evolution of mating systems in Euplotes.

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Ciliates control their sexual phenomenon of conjugation (or mating) through a genetic mechanism of mating types, which may either be only two within a species (recalling the duality of sexes in animals), or multiple (recalling self/non-self compatibility systems in plants and fungi). The nearly one hundred species of the most ubiquitously distributed ciliate, Euplotes, all evolved multiple mating types. Based on analyses of Mendelian genetics, these mating types have for long been assumed to be determined by multi-allelic series of genes inherited at a single genetic locus (i.e., the mating-type or mat locus) and responsible for the synthesis of mating type-specific signaling proteins. The chemical characterization of these signaling proteins (known as pheromones) from an array of Euplotes species has now permitted us to evolve in the study of Euplotes mating systems from an approach of Mendelian genetics to an approach of molecular genetics. In this new experimental context, we have cloned and structurally characterized the pheromone (mating-type) gene families of Euplotes species that take different positions in the Euplotes phylogenetic tree. In accord with predictions of Mendelian genetics, it appeared that early branching species (e.g., E. polaris, E. raikovi and E. nobilii) inherit their mating types at a single multi-allelic locus. However, in disagreement with predictions of Mendelian genetics, late branching species (e.g., E. crassus and E. focardii) showed to inherit their mating types at two distinct loci that are likely the result of an event of gene duplication in the germinal (micronuclear) genome. One locus appears to be structurally and functionally homologous with the multi-allelic locus of the early branching species, while the second locus appears to be structurally homologous but functionally divergent.

Phylogenetic systematics of the genus Condylostoma (Ciliophora, Heterotrichea) by means of a multidisciplinary analytical study of some marine morphospecies.

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The genus Condylostoma Bory de St. Vincent, 1824 is a well-known taxon of the class Heterotrichea consisting of medium- to large-sized organisms commonly found in various habitats. At present it comprises more than 20 species (Appeltans W, Bouchet P, Boxshall GA, Fauchald K, Gordon DP, Hoeksema BW, Poore GCB, van Soest RWM, Stöhr S, Walter TC, Costello MJ. (eds), 2010, World Register of Marine Species; accessed at http://www.marinespecies.org on 2012-07-31), but more than half of them are just superficially outlined, i.e. not described with modern morphological methods of investigation (protargol staining, electron microscopy). Species identification is sometimes very difficult because of: 1. unclearness/incompleteness of literature descriptions; 2. lack of easily recognizable diagnostic characters; 3. variability of cell shape (high to impressive tendency to contract); 4. frequent overlapping of diagnostic characters (cell shape and size, infraciliature, and cortical features). Recently, molecular analyses were performed on a few representatives of the genus, namely Condylostoma minutum, Condylostoma spatiosum, Condylostoma curva (by Guo et al. 2008), and Condylostoma sp. strain POE2.2 (by Modeo et al. 2006), which were all characterized based on 18S rRNA gene sequencing; this allowed a preliminary phylogenetic systematics of the genus. In the present research, two morphospecies of Condylostoma coming from the Mediterranean Sea and one morphospecies coming from the Atlantic Ocean were isolated, clonally cultivated, and characterized by means of a multidisciplinary analytical study (through optical investigation on in vivo and fixed/stained cells, SEM and TEM, and 18S rRNA gene sequencing). New interesting insights into the intrageneric phylogenetic relationships were gained, which also allowed some better understanding of intergeneric relationships within the class Heterotrichea. Protein cold adaptation of a novel eukaryotic

phospholipase from the psychrophilic Antarctic ciliate *Euplotes focardii* and catalytic activity optimization for industrial application.

Protein cold adaptation of a novel eukaryotic phospholipase from the psychrophilic Antarctic ciliate Euplotes focardii and catalytic activity optimization for industrial application

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The ciliated protozoon *Euplotes focardii*, originally isolated from the coastal seawaters of Terra Nova Bay in Antarctica, shows a strictly psychrophilic phenotype, including optimal survival and multiplication rates at 4-5 °C. This characteristic makes *E. focardii* an ideal model species for identifying the molecular bases of cold adaptation in psychrophilic organisms, as well as a suitable source of novel cold-active enzymes for industrial applications. This report is on the characterization of a cold-adapted patatin-like phospholipase (*Ef*pPLA) and it is a first attempt to optimize its catalytic properties for industrial applications. As far as we know, this is the first study on such a protein from eukaryotes. *Ef*pPLA shows a maximum activity at 25 °C and an extraordinary high stability even at higher temperatures up to 70 °C for 3 hours. This enzyme is stable between pH 9 and 11, and exhibits a maximal activity at pH 10.5. Comparatively to other psychrophilic enzymes, which usually have a limited application due to their thermolability, the *Ef*pPLA shows a remarkable activity also at higher temperature. We investigated the enzymatic activity of three engineered versions of the *Ef*pPLA through site-directed mutagenesis by performing substitutions with residues found in a homolog sequence from a closely related (mesophile) organism, *E. crassus*. It resulted that the introduction in the *Ef*pPLA of amino acids with rigid and bulky charged/hydrophobic side chains confers enzymatic properties similar to those of the mesophilic pPLA and still maintains the low temperature activity of the psychrophilic enzyme. The results reported in this work indicate that enzyme cold-adaptation is influenced by amino acid residues that typically confer structural polypeptide flexibility. To further optimize the catalytic activity for industrial application, we are now conducting experiments of directed evolution with a starting set of phospholipase genes from *E. focardii*, *E.crassus* and other organisms.

The analysis of the transcriptome from the Antarctic ciliate *Euplotes focardii*: hints of cold adaptation and regulation of the genetic information.

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As single cell directly exposed to environmental cues, protozoa represent excellent models to unravel the full suite of cold stable functions that allowed the ecological success of psychrofiles. To optimally address temperature adaptation, it is necessary to access to large sample sizes of sequences to enable evaluation via statistical and computational approaches. Only genome or transcriptome sequencing can provide such data sets. In this context, we analysed the transcriptome of *Euplotes focardii*, a hypotrichous ciliate isolated from the Antarctic coastal seawater. *E. focardii* is strictly psychrophilic: the optimal temperature for growth and reproduction is 4-5°C. From the sequencing of the transcriptome we obtained 7,158 assembled genes, 48% of which were identified as protein coding genes. To assist in functional interpretations and evaluate potential cold-adaptive response, we performed Gene Ontology annotation by means of the Blast2GO software. We found that the majority of the transcripts correspond to proteins involved in oxidoreductase activity. A similar finding emerged also from the analysis of transcriptome of the Antartcic fish *Dissostichus mawsoni* and from the krill. Among the sequences that respond to oxidative stress we include also the Heat-Shock Protein (HSP) 70 genes. Quantitative Real-time PCR shows that HSP 70 genes are expressed in high amounts when *E. focardii* cells are subjected to oxidative stress. In contrast, this ciliate lacks a visible classical heat shock response when subjected to heat. These results confirm that a major problem of Antarctic organisms is to cope with increased O_2 solubility at low temperatures, and suggest that an increased defence against oxidative stress likely constituted an important evolutionary aspect that allowed the adaptation of Antarctic organisms in their oxygen-rich environment. The analysis at nucleotide level showed that some of the *E. focardii* transcripts appear to derive from alterative intron splicing of the same

gene, or to require a +1 translational frameshift to produce a functional gene. Furthermore, many transcripts appear to rely on non-sense mediated mRNA decay (NMD) pathway to avoid premature termination of mRNA translation, as they retain introns in their sequence. All these results suggest that *E. focardii* represents a good model to unravel the evolutionary mechanisms that determine the adaptation of organisms to stable cold environments. In addition, the analysis of the *E. focardii* trasncriptome may help to better understand the regulation of intron splicing or, more in general, that of the translation of genetic information.

SYMPOSIUM SEA WATER PROTISTS

SESSION 2

Advances in the *Teredinibacter turnerae*/Shipwoms/*Boveria* interaction: An Inter-Kingdom Tripartite Interaction.

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Teredinidae mollusks have their wood-boring lifestyle supported by the symbiotic association with the cellulolytic/nitrogen fixing gamma-proteobacterium *Teredinibacter turnerae*. *T. turnerae* colonizes bacteriocytes within the invertebrate gills conferring nutritional support to the host. *T. turnerae* has been observed in all Teredinidae species analysed worldwide and a horizontal mode transfer of symbionts is speculated. Although this bacterium can be cultured *in vitro*, it has never been observed in a free living form or associated with other substrates in nature. In this work, we found three different species of ciliates in association with the mantle cavity of the mangrove shipworm *Neoteredo reynei* collected from different geographical localities in Brazil, including mostly *Boveria teredinidi* but also *Metanyctotherus rancureli* and *Trichodina* sp. PCR-based approach using specific primer set for *T. turnerae* identify the presence of this bacterium only within *Boveria* samples. Beside that FISH assays using specific probes for this bacterium identify its presence in the food vacuole of this ciliate. To evaluate the presence of a mechanism of phagocitose resistence in this bacterium an *in vitro* phagocytic assays were performed with *B. teredinidi* and *T. turnerae* Gfp+. Intracellular bacteria expressing Gfp were observed in approximately 70% of the Ciliates after 24h of incubation. Freshly collected *B. teredinidi* were observed under TEM and two different Gram-negative rod shaped bacterial morphotypes were detected in the ciliate cytoplasm. It is evident that both morphotypes are well established in the ciliate cytoplasm and we still do not know if they represent distinct bacterial species or distinct forms of the same bacterium. Taken together these data indicate that *B. teredini* may play a role in the Teredinidae/*T. turnerae* system and this is being deeply investigated.

Molecular monitoring for microbial community in the mucus of *Ostreopsis ovata* (Dinoflagellate) mediterranean isolate

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Ostreopsis sp. is a benthic Dinoflagellate forming mucous matrix where it grows and multiplies. This Genus caused several bloom in the Mediterranean sea since 1970 until 2005 year due to the *O. ovata* species that is considered an unicellular alga, mixotrophic and naturally fluorescent. The alga produces ovatoxin, a chemical variant of palytoxin (PLTx) and its presence in the Italian swimming sites has caused severe respiratory symptom in the population

The matrix where this protist grows is characterized by presence of bacteria and other dinoflagellates species and when the *O.ovata* culture is in vitro amplified it become disaggregated with free algae in the medium. Re-aggregation and characteristic mucosal structure is reached after few days.

We cultivated a D849 strain of *Ostreopsis ovata*, kindly provided by the SZN of Naples. In vitro culture was standardized in 750 ml flask provided of light\dark period is 14\10 h, with a luminous intensity about 3000 Lux, as usual. Count of the cultured cells and DNA extraction could be difficult in presence of the mucous. Therefore aliquots of *Ostreopsis* culture were treated with final 4mM Hall and protocol was standardized comparing PCR amplification or treated and not-treated samples. DNA was extracted according to Wizard® Genomic DNA Purification Kit after centrifugation and PBS washing to recover a hypotonic pellet. Following this method PCR was made by Its1/Its2 region amplification to check for Dinoflagellates DNA. In order to research for protocol and look for some associated microbial clades we choose primer specific for bacterial genes. Particularly we used primer for 16s RNA specific for coli-like bacteria and for PYR gene of Vibrio sp. bacteria. Vibrio is a Genus of bacteria living everywhere in the Ocean and some species have been found also in the Mediterranean Sea. These bacteria produce PLTx and is frequently associated to coelenterates of *Palythoa* genus.

With this molecular approach we obtained some preliminary results on the mucus nature were the *O.ovata* strain lives both as biological then biochemical components. Qualitative PCR discovers the presence not only of coli-like but also vibrio-like bacteria and a glycoprotein matrix in the mucus.

Meanings of these results obtained on a cultured alga strain are under study in our laboratory

Intracellular protein transport mechanisms in eukaryotes: ciliates and mammals use the same translocation and nuclear localization signals.

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In *E. raikovi*, the mechanism of signal transduction which promotes cell proliferation and is triggered by the autocrine interactions between signaling protein pheromones and their membrane receptors, involves the phosphorylation of a nuclear protein kinase (designated as Er-MAPK1). This kinase of 631 amino acids is composed by two distinct domains coincident with the N- and C-terminal halves of the molecule. The first (N-terminal) domain includes all the canonical sub-domains typical of every serine/threonine protein kinase, and shows a high sequence identity (more than 65%) with nuclear mammalian kinases such as the "Male germ cell-Associated Kinases" that are directly implicated in the control of spermatogenesis and the "Intestinal Cell Kinases" involved in the regulation of cell proliferation and differentiation of intestinal epithelial cells. The second (C-terminal) Er-MAPK1domain has no sequence similarity with other known kinases, is particularly rich in glycine and basic residues, and contains repeated sequences and putative phosphorylation sites.

We observed that *Euplotes* cells committed to grow by autocrine pheromone/pheromone-receptor interactions carry phosphorylated Er-MAPK1 in their transcriptionally active nucleus (macronucleus) and, in addition, that phosphorylated Er-MAPK1 localizes in particular at the level of nucleoli. Consistently with this observation, two putative motifs similar to the nuclear localization signals (NLS's) of many nuclear proteins were identified in the Er-MAPK1 C-terminal domain and expressed as GFP-tagged recombinant proteins in mammalian fibroblast cells. It was found that these cells recognize and translocate one of the two NLS motifs of Er-MAPK1 into the nucleus, thus implying that this motif is evolutionarily conserved from uni- to multi-cellular eukaryotes.

SYMPOSIUM FRESH WATER PROTISTS

SESSION 1

Distinct Functional Roles of Beta-Tubulin Isotypes in Microtubule Arrays of *Tetrahymena thermophila.*

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The multi-tubulin hypothesis proposes that each tubulin isotype performs a unique role, or subset of roles, in the universe of microtubule function(s). To test this hypothesis we are investigating the functions of the beta-tubulin isotypes of *Tetrahymena thermophila*. In *T. thermophila*, beta-tubulin is represented by two identical isotypes named BTU1 and BTU2, which are the most conserved, and six more divergent beta-tubulins, named beta-like (BLTs), from 1 to 6. Prior to sequencing of *T. thermophila* macronuclear genome, it was thought that the generation of a microtubule cytoskeleton with diverse functions was due to specific post-translational modifications of microtubules and/or to the binding of specific MAPs, but the presence of these BLTs, raises the possibility that diversity of tubulin isotypes in ciliates may contribute to the formation of functionally distinct subsets of microtubules. Our attention focused on the role of BLT1 and BLT4 in *T. thermophila*, in comparison with the canonical BTU2. To assess the roles of these tubulins *in vivo*, we transformed *T. thermophila* with expression vectors that direct the synthesis of GFP-tagged versions of the isotypes. We showed that GFP-BLT1 and GFP-BLT4 are not detectable in somatic cilia and basal bodies, whereas GFP-BTU2 strongly labels these structures. During cell division, GFP-BLT1 and GFP-BLT4, but not GFP-BTU2, are incorporated into the microtubule arrays of the microtubules of the mitotic apparatus of the micronucleus. GFP-BLT1 also participates in formation of the microtubules of the meiotic apparatus of the micronucleus during conjugation. Our results suggest that beta-tubulin isotypes possess intrinsic and different structural properties and/or sequence signals that specifically deliver them into axonemes or nuclei. We investigated the role of the axoneme motif EGEF, present at the carboxyl-terminal of BTU2, but not in the BLT1 and BLT4, in the specific localization of the ciliary beta-tubulin isotypes. By site-directed mutagenesis, we demonstrated that the EGEF motif is not sufficient to incorporate tubulin dimers into cilia. This result is also confirmed by the GFP-tagged chimera made between the N-terminal domain of BLT1 and the C-terminal of the BTU2, which localizes at the longitudinal bundles and into the mitotic spindle of the micronucleus during cell division, but not in cilia. This result suggests that not only the EGEF motif, but also the C-terminus itself, is not sufficient for the ciliary localization of BTU2. We conclude that *Tetrahymena* uses a family of distinct beta-tubulin isotypes to construct subsets of functionally different microtubules. We need further analysis to identify sequence signals for specific microtubule cell localization.

***Paramecium* genus. Main features to the morphospecies discrimination.**

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On the basis of morphological, morphometric, biological and molecular differences the genus *Paramecium* was subdivided into four subgenera (Fokin et al., 2004). So far we can record 19 morphospecies in the genus. For correct identification of even such ‘‘well-known’’ ciliates as members of the *Paramecium* genus, protozoologists must have an adequate general taxonomic knowledge and practice dealing with a particular group of ciliates. This study represents a simple way to discriminate *Paramecium* spp. For the matter the variation in morphology of the micronucleus (Mi) and contractile vacuole (CV) of the ciliate should be considered first of all. Both of the features are very easy to check using living and fixed (stained by Feulgen or protorgol impregnated) cells. In the *Paramecium* genus we can find all the possible variations of the Mi types and CV structure occurring within all the Ciliophora (Fokin, 2011). There are four main morphological types of the Mi organization: large “compact” type of the Mi can be found in *P. bursaria, P. chlorelligerum, P. putrinum, P. caudatum* and in “*P. butschlii”*- ciliate from Norway which still not fully described. The second type of the Mi is the large “chromosomal” nucleus corresponding to characteristic of *P. jenningsi* and *P. schewiakoffi.* Some of the common morphospecies, euryhaline, but mainly brackish water have a relatively small “endosomal” type of the Mi – *P. woodruffi, P. nephridiatum, P. calkinsi* as well as *P. duboscqui*. The small “vesicular” type of the Mi was recorded for *P. aurelia* complex spp., *P. polycaryum* and *P. multimicronucleatum.* Structure of CV in the genus members can also be of different types (Fokin, 1986). These organelles are commonly two in number and are located close to the dorsal surface in the

endoplasm, usually directly beneath the cortex. CV connects with the outside through pores permanently located in the cortex. The number of pores per one CV in a species is a distinctive feature. For example, *P. caudatum* and *P. aurelia* complex spp. always have one pore per CV, but *P. bursaria* and *P. nephridiatum*, from 2 to 5. Two types of CV within the *Paramecium* genus are known. The first one, called canal-fed type, a number of radial (collecting or nephridial) canals radiate from the vacuole and empty into it. The second type is a vesicle-fed one, in which a number of small vesicles (or vacuoles) lie close to the main vacuole and empty into it (*P. chlorelligerum, P. putrinum, P. duboscqui* and "*P. butschlii*"). Inside group which has canal-fed type of CV some variation in the number and size of canals could be recorded. According to the results of my investigation, it is possible to propose the new discrimination key to the main representatives of the *Paramecium* genus.

Molecular monitoring for pathogenic protozoa in lake water

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Since several years it has been revealed the role that inland surface waters have in the transmission of several protozoan parasites infection. In the United States and in Europe (England) has been indicated water for different purposes (drinking, bathing, use for hygienic purposes or hospital) as causing of outbreaks with gastrointestinal or neurological symptoms. Outbreaks of giardiasis or cryptosporidiosis of high entities were easily traced as due to water contaminated by these pests through the fecal-oral route. This situation is not evident in Italy where does not exists a system to notify these infections. Nevertheless the environmental conditions which favor these infections are easily identifiable. This study is carried out to examine a volcanic lake near Rome which water is used in summer to supply two small town of drinking water. We performed a molecular analysis of the lake water sampling each month in a year in different sites including two bathing stations.
Ten different protozoa parasites species were monitored in the lake water by specific PCR amplification using also specific primer for genetic assemblages and strains. Some of that are recently considered as emergent parasites. Only Giardia duodenalis, Assemblage A, was amplified by the water samples showing the presence of a zoonotic risk factor. The lake is normally used to graze livestock of different species, particularly in winter, autumn and spring. The access of animals to the lake is interdicted in summer when the beaches are open and Giardia is not more recoverable from water.
This finding suggests that a risk for infection is present just when the zoonotic cycle of the parasite is maintained. The touristic population, in summer, is then not exposing to the giardiasis risk.
This is an example of quite good management of the environment and his usage.

Copper induces two copper-zinc superoxide dismutase genes in *Tetrahymena thermophila.*

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High rate of reactive oxygen species (ROS) formation may produce cell damages but the organisms evolved protective antioxidant systems. Among those, superoxide dismutase scavenge superoxide anions. With the aim to study this enzyme in the ciliated protozoan *Tetrahymena thermophila*, we characterized the genes codifying for two copper-zinc superoxide dismutase (Cu,Zn-SOD). Deduced amino acid sequences were compared to orthologous genes in other species to verify the presence of conserved residues, essential for enzyme activity. Copper-dependent regulation of Cu,ZnSOD expression was investigated by measuring mRNA accumulation and enzyme activity in response to chronic exposure to subtoxic doses of the metal (500 μM). Total RNA and cell-free extract have been purified from *T. thermophila* cells (SB210 strain) cultured in PPYG medium and harvested after different times from 30 min up to 48 h. Time-course of mRNA accumulations were analyzed by RT-qPCR. The presence of active antioxidant enzyme was verified by measuring Cu,ZnSOD activity spectrophotometrically. The data were correlated to Cu accumulation, measured by atomic absorption spectrophotometry, and *in vivo* ROS formation, determined after exposure of cells to dihydroethidium. The results emphasise the importance of an efficient detoxification pathway by complex Cu regulation of Cu,ZnSOD transcription, which enables *Tetrahymena* to survive in the continued elevated presence of Cu in the environment. (Grant by M.I.U.R.)

SYMPOSIUM
SOIL PROTISTS

SESSION 1

Ciliate community structures correlates with environmental variables: investigation on agricultural and natural soils.

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Ciliated protozoa are ubiquitous eukaryotic microorganisms, which constitutes an essential component of aquatic and soil ecosystems. Ciliates are very sensitive to any change in their habitat, fluctuations in their communities can affect the food web and energy transfer within the ecosystem. Thus, monitoring the structure of ciliate communities can represent a valuable tool to assess ecosystem quality and functioning. Currently, few studies have been addressed to the analysis of soil ciliate communities and in particular, from agricultural soils under organic and conventional management. In our study, the first one to be performed in Italy, the ciliate communities in three fields under organic and one field under conventional management located in a hill area (300-550 m asl) of the provinces of Macerata and Ancona (Marche), were investigated by means of qualitative method. Furthermore, six more sites representative of natural and semi-natural soils were sampled for comparison. Soil samples were taken twice in autumn and spring. Chemical-physical parameters were analysed for all sampled sites. Our surveys showed a total of 68 species belonging to 9 classes, 19 orders, 45 genera from all sites under study with the dominance of the ciliates genus: *Colpoda*, *Gonostomum*, *Oxytricha*, and *Halteria*. The species richness ranged from 37 to 14 and it is higher in agricultural sites as compared with the natural habitats. Principal Component Analysis (PCA) was able to discriminate between agricultural (organic and conventional) and natural sites. Furthermore, Canonical Correspondence Analysis (CCA) and Generalized Procrustes Analysis (GPA) showed strict correlations between environmental variables and distribution of ciliate species. Altogether, these results highlight the importance of environmental variables in shaping the ciliate communities in the investigated soil types.

Ciliate biodiversity and preliminary behavioral observations from the chemoautotrophic cave ecosystem of Frasassi, Italy.

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Chemoautotrophic cave organisms requires specific adaptations to tolerate the stress of living in darkness and extreme environmental conditions, such as nutrient and energy limitations, low temperatures (12-13°C), highly variable sulphide concentrations (from 0 up to 415 μM H2S), toxic levels of gases (H_2S, CO_2, CH_4), lethally low oxygen concentrations and variable hydrogen ion concentrations. Up to now, very few study attempted to describe ciliate communities from caves as well as, their fluctuation with respect to environmental factors. The aim of the study was to identify, spatio-temporal characterize and observe the behavioural differences of ciliate species from the Frasassi caves. Four sampling locations within Frasassi caves were selected these include Pozzo dei Cristalli, Lago Verde, Ramo Solfureo and Grotta Solfureo. Sampling site Pozzo dei Cristalli was studied in detail for spatio-temporal distribution, since it is highly diversified and include several microhabitats represented by small sulfidic (H2S-rich) ponds, streams and spring as well as, deep and shallow muddy, stagnant lakes. Periodic sampling was realised from 2009 to 2011 in the form of water-sediments, picked up by scraping the surface. Classical culturing, silver staining methods and 18S rRNA gene (for some selected species) for phylogenetic analysis were employed. A total of 31 species were identified belonging to 9 classes, 15 orders and 23 genera. The different ciliate communities were recorded from Pozzo dei Cristalli during various sampling occasions, this could be due to changing environmental conditions, which leads to shifting of ciliate species, those unable to encyst, to a favourable environment within these microhabitats. It was observed that some species e.g. *Urocentrum turbo*, *Coleps hirtus hirtus*, *Oxytricha* sp, *Euplotes* sp, showed adaptation for the cave environment (Photo-sensitivity, sulphur tolerance, feeding behaviour, morphological difference). The results provide a platform for various in-depth studies of ciliates to understand potential role in ecosystem functioning, nature of chemical compounds secreted, dispersal pattern, adaptations within cave.

Immunohistochemical detection of aquaporin-like proteins in *Dictyostelium discoideum* and *Paramecium primaurelia.*

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Since the first description of the aquaporin (AQP) by Agre and colleagues, which was awarded the Nobel Prize in Chemistry in 2003, much information on the physiological significance of these channel proteins in the transmembrane water transport has been amassed. The AQPs are a family of small pore-forming integral membrane proteins. They have representatives in all kingdoms of living things and although they all share structural similarities their expression is tissue specific. According to their structural and functional properties, AQPs are classified into three subgroups. The classical AQP, selective for water and in some cases also permeable to anions and urea. The aquaglyceroporins, permeable to water, urea, glycerol, monocarboxylates, purines and pyrimidines. The super-AQPs, a subgroup recently proposed whose permeability has not yet been fully determined. Nevertheless, this classification is only a simplification and different molecules, such as ammonia and hydrogen peroxide, arsenite and antimonite, carbon dioxide and nitric oxide gases are able to cross the AQPs. Concerning protozoa, AQP-like proteins have been described in parasitic protozoa such as some *Plasmodium* and *Toxoplasma* species, *Leishmania major*, *Trypanosome cruzi* and *Encephalitozoon cuniculi* and in the free living protozoa *Dictyostelium discoideum* an AQP protein has been sequenced. In this study we identified by confocal laser microscopy and immunoblot analysis the presence of AQP1- and AQP2-like proteins in the protozoa *Paramecium primaurelia* and a AQP1-like protein in *D. discoideum*. These data are a preliminary work aimed at identifying cellular models for studying the role of AQPs in cell growth and migration, to develop new therapeutic strategies against metastasis of cancer cells.

SYMPOSIUM PATHOGENIC PROTISTS

SESSION 1

Shift towards zymodeme homogeneity of *Leishmania infantum* from HIV-positive patients in the HAART era.

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About 2,000 HIV-*Leishmania* coinfection cases were reported through 2001 in southern Europe. In Spain, Italy and France similar increasing trends were observed since mid-1980s which peaked in the period 1996–1998. Since the advent of HAART therapy in Europe in 1997-1998, the number of coinfections reported in the area has fallen dramatically (Alvar et al, Clin Microbiol Rev 2008; 21:334-59). In Italy, cases recorded at ISS Leishmania Reference Centre have been 329, with two peaks in 1994 (33 cases) and 1997 (34 cases) followed by a decrease in 1998–2001, and a low-incidence plateau of about 10 case/year till present. The characterization of *L. infantum* isolated from HIV-patients in Italy until 1998 has been extensively reviewed (Gramiccia M, Ann Trop Med Parasitol. 2003; 97:S65-73), showing the high genetic polymorphism of these strains. In subsequent years, 51 new strains were isolated and identified by MLEE and/or molecular methods. We aimed to compare zymodeme composition of *L .infantum* strains from coinfected patients before and after the HAART era. Eighty-four strains collected through 1998 (pre-HAART group) have been compared with 42 strains obtained in 1999-2007 (post-HAART group) through MLEE analysis. All strains have been isolated from primary infections (126 patients). The pre-HAART group consisted of 16 *L.infantum* zymodemes, of which MON-1 represented 60/84 strains (71%); the remaining ones (1-6 strains each) showed variations in 1-4 enzyme patterns and were identified mainly as already known dermotropic (4), viscerotropic (1) and viscero/dermotropic (2) zymodemes, and 8 new zymodemes. Conversely, the post-HAART group consisted in 3 zymodemes only, of which MON-1 represented most of them (40/42, 95%) and the only 2 variant zymodemes were known dermotropic ones. The difference in zymodeme composition ("MON-1" versus "non-MON-1") between the 2 groups was found highly significant at the Fisher's test ($P<0.001$). Our findings suggest that HAART therapy while conferring an immune restoration may also operate a negative selection towards less virulent agents of VL in treated HIV-patients.

This study was partially funded by EU grant FP7-261504 EDENext.

Molecular diagnosis of *plasmodium vivax* infection in the frame of european project pregvax (*Plasmodium vivax* infection in pregnancy).

M.MENEGON, Istituto Superiore di Sanità, Rome, *not received*

SYMPOSIUM PATHOGENIC PROTISTS

SESSION 2

Detection of parasitic protozoa DNA in sea water of the Taranto Bay

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Several not obligate protozoa parasite species have been found in surface freshwaters of both river and lakes but also in piping. These protozoa utilize water to disperse them self (*Giardia, Cryptosporidium)*

Sometimes it has also been found an obligate parasitic protozoon as *Toxoplasma gondii* it is. The presence is, in this case, occasional and casual but this highly pathogen agent has been recovered both from surface and deep waters and when used to infect mice it shows to be still infectious and pathogen.

For a long time it was believed that the species in question are not able to live in certain doses of salinity and therefore not to be found in seawater. Nevertheless this view has been challenged by the discovery *of Cryptosporidium, Giardia, Acanthamoeba* and *Toxoplasma* and in seawater.

In this study we analyzed the waters of the Mar Piccolo and Mar Grande of the Taranto Bay, in Apulia, to search for possible contamination by pathogen protozoa.

The waters were collected at different sites, different depths of the sea coast and we examined also mussels hepatopancreas to research for this parasite.

The mussels, in fact, are capable of concentrating the particles present in many liters of water and then also the suspended cells in water.

The analysis of monitoring was conducted using qualitative gene amplification by PCR using primers specific for the different species.

Giardia, Cryptosporidium, Toxoplasma and *Acanthamoeba* protozoa have been investigated and was only *Toxoplasma* found.

This parasite (oocystes) recovered from mussels hepatopancreas was also inoculated in vivo and mice observed for infection developing The bay of Taranto shows particularly favorable conditions to contamination from cat feces infected with *Toxoplasma* for geographical factors (semi-enclosed basin) , salinity degree and poor hygiene..

Studies of this type must be repeated in other marine areas to understand the health risk that derives by the sea use both for the aquacultures and bathing

Use of photosensitized processes in the prevention and control of water- and vector-borne diseases caused by protozoa.

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Despite major advances in medicine, infectious diseases continue to present enormous global health problems. Two groups, according to their main ways of transmission to humans, of water- and vector-borne diseases can be distinguished. Malaria, amoebiasis and leishmaniasis are among the most dangerous diseases and antibiotic therapy for these infections caused by protozoa is not always successful. The antimicrobial photodynamic therapy (PDT) as a novel mode of treatment has been demonstrated highly effective against some protozoa, offering alternative approaches in the medical and environmental control of pathogens. PDT with aminolevulinic acid (ALA), Al(III)-phthalocyanine or phenothiazinium analogues was proposed to treat cutaneous leishmaniasis with good cosmetic outcomes as well as retinochoroiditis of *Toxoplasma gondii* origin. The combined action of visible light and photosensitizers was also demonstrated to disinfect blood products through the photoinactivation of *Plasmodium falciparum*. The sensitivity exhibited by *Acanthamoeba palestinensis* in both vegetative and cystic stages to irradiation with a cationic Zn-phthalocyanine emphasizes the promising role of PDT based on phthalocyanines to disinfect wastewater. In order to reinforce the efficacy of major tools in the integrated vector control strategy, a novel porphyrin, namely meso-tri(N-methyl-pyridyl),mono(N-dodecyl-pyridyl)porphine, C12, associated with specifically selected carriers, was tested against *Anopheles gambiae* and *An. arabiensis* larvae, both laboratory reared and collected from malaria endemic sites in Burkina Faso. C12 porphyrin formulates, when administered at a 50 µM C12 dose, were accumulated in the gut. Subsequent exposure of the porphyrin-loaded larvae to sunlight for short times (0.5 – 3 h) led to a complete mortality. The high efficacy exhibited by porphyrin formulates

also in the presence of typical larval food particles opens promising perspectives for the development of an effective photolarvicide. Studies on C12 photosensitization of ciliates and crustaceans, as a model of organisms from larval habitats, showed that photosensitivity occurs for free porphyrin concentrations in the μmolar range, which is by far larger than the amount released from the C12 formulate.

Toxoplasma gondii DNA in goats: a preliminary report

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This report aims to evaluate the seroprevalence of Toxoplasma gondii infection in a herd of goats in Tuscany as well as to detect and genotype parasite DNA in their blood and milk samples.
Fifty-nine serum and milk samples from adult (1-3 year old) Chamoisee (shamwahzay) brown lactating goats, from a herd in Tuscany, with an anamnesis of abortions, were investigated by a modified agglutination test (MAT) for T. gondii antibodies, starting to a dilution 1/20. A PCR assay was performed on all blood samples as well as on milk samples from PCR blood positive goats. PCR was performed as described by Jones et al (2000 Invest Ophthalmol Vis Sci. 41:634-44), with slight modifications. Determination of genotypes was carried out according to Su et al. (2010 Parasitology 137:1-11).
Forty two sera scored positive for anti T.gondii antibodies, with a seroprevalence of 71.2%. Ten animals out of 42 (23.8%) yielded PCR positive blood samples, 10 milk specimens from them scored PCR positive too. Genotyping of DNA showed genotype III.
The seroprevalence was higher when compared with a recent survey performed by the same serological technique (Dubey et al, 2011 Int J Parasitol.,41:827-833). The occurrence of protozoan DNA in goat milk could be suggestive of viable parasites presence, but to evaluate the effective risk a mouse bio assay will be recommended. However, the finding of PCR positive milk samples out of PCR positive blood specimens appears as a very interesting feature, underlining the importance of milk pasteurization before any processing or ingestion.
Food-borne toxoplasmosis in humans may result from the ingestion of tissue cysts or tachyzoites contained in meat, primary offal, or meat-derived products of many different animals (Tenter, 2009 Mem Inst Oswaldo Cruz, 104: 364-369) and consumption of unpasteurized milk (Santos et al., 2009 Vet Parasitol, 161: 324-326). The risk of acquiring an infection with T. gondii by drinking cow's milk, if any, is minimal (Jackson & Hutchison 1989 Adv Parasitol, 28:55-105), however, it cannot be excluded that any type of milk is a potential source of infection, if consumed raw. A study assessing risk factors associated with primary T. gondii infections in women of childbearing age suggested that drinking milk may be a potential risk factor for horizontal transmission to humans (Paul,1998, Przegl Epidemiol 52:447-454). Thus far, clinical toxoplasmosis in humans has been associated with consumption of unpasteurized goat's milk (Riemann et al., 1975, Sacks et al. 1982, De Andrade et al., 1984, Skinner et al., 1990 cited by Tenter, 2009). Even if raw goats' milk is a proven vehicle for pathogen transmission raw dairy products are frequently considered healthier than pasteurized ones (Basnet et al., 2010 Pediatrics 125:973-977). Furthermore goat's milk is believed to be better to use in fresher cheese. There are only few data about the resistance of different Toxoplasma stages (Pettersen, 1984 Acta Pathol Microbiol Immunol Scand B 92:175-176), during the production process and storage of fresh cheese (Hiramoto et al., 2001 Rev Saude Publica 35:113-118) demonstrating that untreated milk and dairy products could be an important source of T. gondii in human infection.

Detection of* Toxoplasma *DNA in serum samples of sheep from South Africa

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During the 2006 a number of 600 sheeps coming from abbatoirs of 5 different provinces in South Africa were studied for acquired toxoplasmosis. Results of the serodiagnosis with IFA and ELISA showed a per province seroprevalence for *Toxoplasma* infection in sheep of 5.6 % and 4.3 % . This value is lower than in other countries, but titres were generally very high as it does during an active infection.

These results were published the year after and the serum samples have been kept for more then two year in a refrigerator and not frozen. The sera were then given to an Italian scientific expedition in South Africa to be processed for molecular research of *Toxoplasma* DNA. Then, these samples were frozen and stored for two more years frozen at -20°, until the current year they were processed for DNA research.

The samples has been treated with the kit for extraction of DNA for forensic samples which is of high sensitivity (DNA IQ System, PROMEGA) and amplified by PCR for *Toxoplasma* DNA research. The B1 highly repeated gene has been targeted and all the positive sera were positively amplified.

The B1 positive serum samples were then successfully amplified by SAG3 gene specific primer and the genotype characterization were performed by endonuclease digestions.

Genotyping has revealed the presence of only genotype 1, the most virulent agent of toxoplasmosis. This result is in agreement with what we already knew by the geographical distribution of *Toxoplasma* genotypes and is according with the high serological titres previously found

This work shows also that by the use of very sensitive techniques for DNA extraction is possible to obtain results also by very old and not well preserved samples. The epidemiological result is the correspondence between high serological titres and the high degree of the isolated strain belonging to the genotype 1.

SYMPOSIUM
PATHOGENIC PROTISTS

SESSION 3

Antimycotic activity of some Mediterranean autochtonous plants essential oils against canine isolates of *Malassezia pachydermatis.*

L. MUGNAINI[1], S. NARDONI[1], L . PISTELLI[2], A. BERTOLI[2], M. LEONARDI[2], F. PISSERI[3], and F. MANCIANTI.[1] [1]Dipartimento di Patologia Animale, Profilassi ed Igiene degli Alimenti, Università di Pisa, Italy; [2]Dipartimento di Scienze Farmaceutiche, Università di Pisa, Pisa, Italy; [3]Dipartimento di Clinica Veterinaria, Università di Pisa, Pisa, Italy.

Malassezia pachydermatis is a fastidious opportunistic yeast involved in canine otitis externa, whose recurrence rate is very high in predisposed subjects. Considering that commonly used drugs show a number of drawbacks, the request for alternative antifungals is high. The antifungal activity and the chemical composition of essential oils (EOs) from some Mediterranean autochtonous plants were investigated against *Malassezia pachydermatis*, a fastidious opportunistic yeast usually involved in canine otitis externa.
Minimal inhibitory concentrations (MICs) of *Anthemis nobilis, Citrus limon, Citrus paradisi, Illicium verum, Lavandula hybrida, Mentha piperita, Ocimum basilicum, Origanum vulgare, Origanum majorana, Rosmarinus officinalis, Salvia sclarea, Thymus serpillum* were assessed by microdilution test; fungicidal activity (MFC) was also determined. *O. vulgare, T. serpillum* and *O. basilicum* showed the lowest MIC/MFC values (0.8%) followed by *C. limon* and *M. piperita* (1%).
EOs from the tested plants showed variable degrees of anti-malassezia activity, putatively related to their chemical composition.
The results obtained show that some essential oils appear advisable as new natural antifungal drugs in canine malassezia otitis
The effectiveness, manageability and pleasant organoleptic properties of *O. vulgare, T. serpillum, O. basilicum, C. limon* and *M. piperita* EOs make them advisable as promising new natural antifungal drugs in the management of *M. pachydermatis* otitis in dogs.

Identification and susceptibility to some antifungal drugs of yeasts isolated from healthy animals.S. NARDONI, L. MUGNAINI, R. PAPINI, F. MANCIANTI. Dipartimento di Scienze Veterinarie, Università di Pisa,Pisa, Italy.

Domestic and wild birds, as well as domestic mammals, are known to be possible carriers of fungi pathogenic to humans and others animals. Opportunistic fungal infections resistant to antifungal agents have been increasingly documented in recent years, and their frequency will likely continue to increase. The aim of the present survey was to investigate the antifungal drug susceptibility profile of some yeasts isolated from farm animals and pet birds.
Fifty-two samples were collected from healthy animals and cultured for yeasts, including conjunctival swabs from 17 cattle (Bos taurus) and 15 swine (Sus scrofa domesticus), and faecal matter from 16 canaries (Serinus canaria), 3 grey parrots (Psittacus erithacus) and 1 house sparrow (Passer domesticus). Macro and microscopic features of colonies grown on Malt Extract Agar, micromorphology on rice agar, presence of capsule, and assimilation of carbon sources were evaluated for identification. Susceptibility to four antifungal drugs was determined using Etest strips (AB Biodisk) containing amphotericin B, caspofungin, fluconazole and voriconazole. Tests were performed on RPMI 1640 agar plates as recommended by the manufacturer. Debaryomyces hansenii, Pichia etchellsii, Candida albicans, Candida catenulata, Candida krusei, Cryptococcus laurentii, Trichosporon cutaneum, Candida colliculosa, Candida parapsilosis, Pichia membranaefaciens, Candida tropicalis and Candida pelliculosa were recovered and identified. Twenty six isolates were resistant to at least one of the four tested antimycotic agents; one isolate of D. hansenii cultured from a canary sample was found to be resistant to all the drugs, while 22 isolates were fully susceptible. These latter included 8 isolates of D. hansenii from cattle and canaries, 4 of P. etchellsii, 2 of C. catenulata, 2 of C. krusei and 2 of C. colliculosa from swine, and finally 3 of C. albicans and 1 of T. cutaneum from birds. Three D. hansenii and one C. catenulata

isolates resulted dose dependent susceptible to fluconazole. The present results indicate the occurrence of a variety of yeasts in different animal anatomical sites. Among yeasts belonging to Candida genus, both albicans and non-albicans species were recovered. Cryptococcus neoformans was never isolated. During the past decade, there has been an increasing trend of human systemic and fatal diseases due both to non-albicans Candida species and to non-neoformans cryptococci. Most of the isolated organisms are known to be widespread environmental contaminants and saprophytic commensals of different animal species with worldwide distribution. As many of them are documented human pathogens, animals could play a role in the zoonotic transmission of fungal agents sharing the same environment with humans. Furthermore, healthy animals seem to harbour potentially zoonotic yeasts with variable antimycotic susceptibility pattern within the same species.

ROUND TABLE 1

Academic and educational activities to the Protistological Science

Protists in classroom: single-celled organisms for biology teaching.

F. BUONANNO, M. AQUILANI, M. G. TROTTA, C. ORTENZI**.** Laboratory of Protistology and Biology Education, University of Macerata, 62100 Macerata, Italy.

Microscopic life forms are usually represented by single-celled organisms: protista and bacteria, and they are present in all environments. In this study, we point our attention on eterotrophic protista, usually indicated as "protozoa", which are very common in soil, in fresh- and salt water, simple to collect, to grown and to manipulate. Though protozoa consist of only one cell, they are extraordinarily different in form and activity. In fact, these microscopic organisms carry out all the functions of life within the single cell. For example, we need to get food and to digest it to provide energy for our cells; we need to release indigestible matter from our bodies by excretion; we respond to environmental stimuli by nervous system and muscles; we grow, differentiate, and reproduce. Protozoa perform all of these activities within the small space of the single cell that forms their body. They have no nervous system or muscles, yet they respond and move in very complex ways. They also get food and energy, excrete wastes, differentiate, and reproduce to survive in their environment. Since all life is made up of cells, the study of protozoa may therefore help students to understand how larger forms of life survive and maintain their health.

The hall of Protistology at the Museo di Storia Naturale e del Territorio di Pisa: developments and correlated initiatives.

GRAZIANO DI GIUSEPPE. Dipartimento di Biologia; Università di Pisa, Pisa, Italy.

The educational exhibition hall of Protistology, which opened in 2011 at the Museo di Storia Naturale e del Territorio di Pisa and actually the first in Italy, is regularly included in the teaching offer, scheduled annually by the museum itself. During the year, about 15 classes of children school of the Pisa's district for a total of about 350 students have attended the educational courses related to the hall and entitled "*Microcosm of a water drop*" for the Primary School, and "*Discovering the eukaryotic microorganisms*" for the Secondary School. The educational visits, lasting about an hour and a half each, were coordinated by the Museum staff trained and generally composed of young graduates in Biological Sciences or Natural Sciences. Each educational trail was organized in such a way as to provide, in an appropriate language to the level of acquisition to the interested users, the necessary tools to develop the scientific and application knowledge on the importance of protists in various fields, from the environment to the health. In satisfaction questionnaires distributed to the completion of the courses, participating teachers have expressed very favorable opinions regarding to the discussion of the topics, the clarity of objectives consistent with the contents, the acquisition of new knowledge and the professionalism of the speakers. The answers of the students involved have confirmed that it is widespread in children's interest to work experimentally, suggesting the need to include new contents, such as the preparation of slides for observation under the optical microscope and the DNA extraction from protist cultures. The museum hall of Protistology was also involved in various initiatives of scientific-informative character, such as "II Congresso La ricerca scientifica in Museo, quale la realtà toscana?", "Amico museo 2012", "Calci …in festa 2012", "Campi solari CUS Pisa", "Pianeta Galileo 2012".

Protist tracks.

F. CANTARANO, Pisa

What arouses curiosity to anyone to explore the world of eukariotic micro-organisms?
The school makes only some accents, often considering only the pathogens , with the effect of alarm also versus the non-hazardous protists of which many teachers know little or nothing and they are not equipped or motivated to address their biology
People that know that the microscope is not only the toy given to children at Christmas, they believe that it is a tool of hard use and basically useless.
The Editors of the scientific divulgation of any channel always ignores the matter.

Therefore, in spite of their considerable importance for understanding the evolution and the origin of life, the protists are not interesting for people even when they indicate the meaning of the research in the life in the Universe.
It is essential, therefore, that this interest can be finally encouraged and the importance of research in this field be underlined. Therefore, I decided to collect my notes in the publication "The Invisible Planet - the pond's microorganisms narrated by an illuminist Amoeba". I showed also the lives of free-living microorganisms in the movie "The Invisible Planet - the secret life in the pond ", sponsored by the Regional Park of Migliarino, San Rossore and Massaciuccoli.

ROUND TABLE 2

Secondary metabolites of protists: new tool in pharmacology?

Ciliate lipidomics: the case of *Coleps hirtus*.

A. ANESI[1], F. BUONANNO[2], M. PLANCHESTAINER[1], C. ORTENZI[2] and G. GUELLA[1]. [1]Bioorganic chemistry Laboratory, Department of Physics, University of Trento, Povo, Trento, Italy; [2] Laboratory of Protistology and Biology Education, University of Macerata, Macerata, Italy.

Lipidomics aims to study the complete lipid profile of a cell, tissue or living organism, both on a qualitative and quantitative point of view. In this study we analyzed lipids extracted from toxicysts (extrusomes) of ciliate *Colep hirtus* using both liquid-chromatography-electrospray-mass spectrometry (LC-ESI-MS) and gas chromatography-mass spectrometry (GC-MS). Extrusomes are mainly located in the oral area of freshwater ciliate *C. hirtus*; these organelles help its carnivorous and scavenger feeding by releasing compounds with cytotoxic activity. Toxicysts lipids were extracted using a mixture of chloroform and methanol and analysed by LC-MS; fatty acid methyl esters were also obtained and analysed by GC-MS. Raw extracts resulted to be composed mainly by mixture of free fatty acid, mainly hexadecanoic acid (C16:0), octadecenoic acid (C18:1) and octadecatrienoic acid (C18:3). Very surprisingly, extrusomes also contained two diterpenes, 3,7,11,15-tetramethyl-2-hexadecen-1-ol and 3,7,11,15-tetramethyl-hexadecanoic acid, that counted up to 6% of total free fatty acids.

Mechanical and chemical interactions in offense-defense strategies of freshwater ciliates.

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In the past 30 years a number of studies have been devoted to analyzing the morphological and molecular basis of predator–prey interactions in ciliates, and particular attention was focused on the important role of specialized ejectable membrane-bound organelles, generally called extrusomes, in the immobilization and capture of prey, and in defense from predators. There are, essentially, two types of strategies adopted in predator–prey interactions among ciliates: 1) the first mediated by mechanical mechanisms involving trichocysts, the subpellicular non-toxic extrusive organelles, used for defense by some ciliates such as Paramecium, Frontonia or Pseudomicrotorax; 2) the second mediated by secondary metabolites (contained in different kinds of chemical extrusomes) used for offense or defense by other ciliates (such as Bepharisma, Climacostomum or Spirostomum). These interactions are, until now, mainly studied in unicellular predators-prey models. Here we report the defensive function of trichocysts in Paramecium tetraurelia against three metazoan predators and we compare the defensive efficiency of these mechanical extrusomes with that of chemical extrusomes in two toxic ciliates (Climacostomum virens and Spirostomum ambiguum). The results prove the defensive function of trichocysts against two of these metazoan predators (Cephalodella sp., Rotifera and Eucypris sp., Arthropoda) while they seems ineffective against S. sphagnetorum (Platyhelminthes).
In addition, with regards of chemical extrusomes in ciliates, we analyze the content of the cortical granules of the heterotrich ciliate S. ambiguum, represented by mono-prenyl hydroquinone, and for the first time, the content of the toxicysts (offensive extrusomes) of a predatory ciliate, the protostomatid Coleps hirtus. The data collected for this ciliate show the chemical composition of the toxicysts content as a free fatty acid mixture mainly represented by PUFAs, together with the presence of two diterpenes.

ROUND TABLE 3

Emergent Protists

Endemic and emerging Phlebotomine-borne diseases in Italy: a short review in the light of the EU projects EDEN and EDENext.

GRAMICCIA M. Dipartimento di Malattie infettive, Parassitarie ed Immunomediate; Istituto Superiore di Sanità, Rome, Italy.

In 2004 started the activity of the FP6 EU EDEN project with the goal to identify, evaluate and catalogue European ecosystems and environmental conditions linked to global change, which can influence the spatial, temporal distribution and dynamics of vector-borne diseases (VBD). It has continued with the FP7 EU EDENext project, based on the EDEN approach, but with additional goals to biological, ecological and epidemiological components of VBD introduction, emergence and spread, and to propose new tools for controlling them. The present review updates the knowledge on the endemic and emerging Phlebotomine-borne Diseases (PhBD) in Italy: leishmaniases and Phlebovirus infections. Principal objectives of the two projects have been to generate or update vector and PhBD risk maps, and to exploit genetic factors associated with spreading by various sand fly species. *Leishmania infantum* associated to *Phlebotomus* (*Larroussius*) vector species still represents in Italy the main risk for human infections. However due to increased migration and travelling, climate changes and globalization, there is an elevated risk for introduction and spread of infections by *Leishmania* species non-endemic in Italy. An example is given by *L. tropica* for which its natural vector *P. sergenti* is present in Italy. Zoonotic visceral leishmaniasis (ZVL) incidence has been increased in Italy, with new foci detected in northern regions previously recorded as non-endemic. The most competent *L. infantum* vector, *P. perniciosus*, was collected in both sub-Alpine and sub-Apennine areas, associated with *P. neglectus* in the former and *P. perfiliewi* in the latter. Canine leishmaniasis (CanL) surveys confirmed the northern spreading of ZVL, with seropositive CanL rates in pre-Apennine sites being significantly higher than in pre-Alpine sites. CanL distribution and seroprevalence risk maps were made. About Phlebovirus infections, previous studies have assessed the presence in Italy of five different *Phlebotomus*-transmitted viruses. During the first year of survey no Phlebovirus infections were detected in *P. perniciosus* or *P. perfiliewi* specimens collected in two biotopes of Rome province.

This study was funded by EU grant FP7-261504 EDENext.

Molecular tools for an environmental monitoring of emerging pathogens

M. C. ANGELICI, Istituto Superiore di Sanità, Rome

The appearance of new species of parasitic protozoa, often vehicle by water, or the changed frequency of species already known is introducing a new risk to the health in our country.
The problems of global health require an adjustment of the detection techniques of pathogens in an environmental matrix. For the latter, moreover, have already been developed guidelines and directives that do not take into account neither of parasitic protozoa nor of sensitive molecular techniques.
In this way we operate microbiological controls of a classic type directed only to coliforms and enterococci so everything else completely escapes to the control.
If you think that protozoa polluting water for human use, including drinking water, are resistant to normal chlorination and pass filter used for aqueducts, one perceives the risk to health.
Biotechnology, however, have gone a long way in optimizing the detection of microbial biodiversity in terms of both quality and quantity with the tools of last generation sequencing and metagenomics. We study their great potential.

AWARD

Multi-marker molecular characterization of ciliates and their endosymbionts.
VITTORIO BOSCARO. Dipartimento di Biologia; Università di Pisa, Pisa, Italy.

This presentation summarizes three projects performed during my Master Thesis in the laboratory of the Protistology/Zoology Unit of the University of Pisa. All of them concern the molecular characterization of ciliates and, where present, their bacterial endosymbionts. The first project was the multidisciplinary description of a nuclear *Holospora*-like symbiont detected in the macronucleus of Thai *Paramecium jenningsi* and *Paramecium aurelia* populations. The "Full-Cycle rRNA approach" (obtainment of the SSUrRNA gene sequences, and confirmation of the results through sequence-specific FISH experiments) was fulfilled, and a detailed phylogenetic analysis performed. The molecular data were congruent with the morphological and ultrastructural ones, and suggested the establishment of a new species and genus ("*Candidatus* Gortzia infectiva") for the novel symbiont, that is closely related to *Holospora*. The second project was the molecular characterization of the *Condylostoma* strain COL2. The SSUrRNA gene sequence of this strain, as well as those of other *Condylostoma* specimens, were compared in order to obtain the first sequence-rich molecular systematics of the genus. The results proved the existence of three separate clades; the genus is also paraphyletic, because the species *Chattonidium setense* reliably clusters inside one of these clades. Moreover, the SSUrRNA gene sequence of a putative bacterial symbiont was obtained, although FISH experiments didn't succeed to confirm this result. The third project focused on ciliates, and was the molecular survey of the morphospecies *Paramecium duboscqui*. Using three molecular markers (SSUrRNA gene, ITS1-5.8S-ITS2 and COI gene sequences) the intraspecific systematics was assessed and correlated to biogeography. The study strongly suggests that *P. duboscqui*, like other *Paramecium* species, is a complex of sibling species. The areals of these species are not overlapping, showing a possible pattern of geographic speciation that is uncommon in ciliates. A fourth marker (the mitochondrial LSUrRNA gene sequence) was obtained for two *P. duboscqui* strains as well as other *Paramecium* morphospecies, and a preliminary evaluation of its potentialities was performed.

Characterization of alpha-amylases from the psychrophilic Antarctic ciliate Euplotes focardii
V. TURTURO, Università Camerino, Camerino, *not received*

Discovering Protists: "model organisms" for an educational path in a second-year Scientific High School. Final-year student: CASTIONI MATTEO. Supervisor: Roberta Predonzan. Assistant supervisor: Olimpia Coppellotti. Faculty of Science MM.FF.NN.; University of Padua, Padua, Italy.

The main objective of this thesis is the planning of an educational path aiming at introducing the students of a second-year Scientific High School to the study of Biology.
The Protists are the focus of this educational path as they are the most apt microorganisms for introducing the students to a series of fundamental themes of Biology such as ecology, systematic and evolution. Moreover, the path emphasizes how these microorganisms can be considered suitable "model organisms" in lab activities as, through the investigation of their basic characteristics, the students will be able to approach various important biological phenomena in the school context. The study of Protists is often misled in the school contexts as it is considered of little significance in the process of learning. This can lead to the strengthening of unfortunately common misconceptions which often identify unicellular organisms as inferior and less evolved beings.