

crustacean health and disease assessment but new molecular genetic techniques are beginning to be integrated into this assessment. Modern genomics and transcriptomics have revolutionized the discovery of diagnostic and prognostic markers in human and terrestrial medicine and promise to drive crustacean health and diagnostic molecule discovery. We have recently begun to apply high-throughput transcriptomic techniques, such as microarray and RNA-Seq, to investigate American lobster health, disease and response to physiological and anthropogenic stressors. Our studies highlight the incredible potential that modern molecular biological approaches have for advancing our understanding of crustacean immunology and disease biomarker discovery.

Symposium. Thursday, 9:30. **206**

**Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns**

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The increasing application of massively parallel sequencing technology to environmental DNA samples (e.g. from water, sediment, soils, whole animals) is providing unprecedented resolution of microbial community structure, diversity and functioning. Application of general and specific primer approaches, amplicon sequencing and metagenomics have enormous potential for the detection of known, novel and otherwise cryptic pathogen lineages. We use such techniques to detect invertebrate pathogens of potential significance to fisheries and aquaculture. Using specific-primer approaches, we have revealed unknown diversity of haplosporidian parasites from eDNA and show shifts in parasite communities along an offshore gradient. At the other end of the spectrum, we have used a metagenomic approach to identify a mikrocytid pathogen of juvenile edible crabs that had eluded molecular characterization using specific- and general primer approaches. We highlight the current methods for discovery and detection of potential pathogens in eDNA samples and show how such studies can inform on ecology, life-cycle and transmission dynamics of aquatic pathogens. Finally, we predict a re-emergence in the importance of classical approaches to disease investigation (e.g. histopathology, electron microscopy) to enable meaningful links to be drawn between presence within the matrix and outcomes in hosts. eDNA analyses should therefore be considered as a 'tool in the box', rather than the toolbox per se, for investigating pathogens of concern to aquatic hosts.

CONTRIBUTED PAPERS Thursday 8:00-10:00

**Nematodes 3**

Contributed paper. Thursday, 8:00. **207**

**The Role of biocontrol agents within IPM of *Tuta absoluta* on tomato in Egypt**

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Since its arrival in Spain, the tomato leafminer *Tuta absoluta* has rapidly spread around Europe and has become an extremely important pest of tomato crops in Mediterranean Basin countries. This pest arrived to Egypt early in 2010 and there soon followed an outbreak in many tomato-planted areas where it caused extensive damage by mining in tomato leaves, stems and fruit. Egyptian entomopathogenic nematode species (EPN) induced 89.3-96.4% mortality to *T. absoluta* larvae. Also, the other biocontrol agents *Trichogramma achaeae* and *Macrolophus pygmaeus* are suggested as effective components within a new control strategy against the insect on tomato in the present study. *M. pygmaeus* may prey on *T. absoluta* eggs and larval stages, but due to more suitable climate of Egypt to *T. achaeae*, earlier release of the latter bug is preferable in order to start the control on the first generations of the pest eggs. EPN have both foliar and soil applications in the strategy. On the foliage, EPN can control efficiently feeding larvae of *T. absoluta* in and outside the leaf galleries while the soil nematodes kill both last instar larvae, when they slide down from the leaves to pupate, and emerging adults from the buried pupae. In addition to such natural enemies, the strategy is supported by prophylactic measures, light and pheromone traps, and IPM compatible insecticides.

Contributed paper. Thursday, 8:15. **208**

**Insecticidal activity of *Heterorhabditis bacteriophora* Shandong toward *Brontispa longissima* and *Cryptothelea variegata***

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*Heterorhabditis bacteriophora* nematodes kill many insect species, but its potencies toward *Brontispa longissima* and *Cryptothelea variegata* pests are unknown. Initially, four isolates of *H. bacteriophora*, UV resistant *H. bacteriophora* Shandong (HbSD), Hb I, Hb II, and Hb III were bioassayed against standard insect *Galleria mellonella*. The UV resistant HbSD isolate was chosen for next bioassay against the last-instar of *B. longissima* and *C. variegata* compared with *G. mellonella* in the laboratory. After exposure of insects to infective juveniles of nematodes (IJs) for six days, mortality was correlated with dosage, and the LC<sub>50</sub> was ≈ 9.35 IJs for *B. longissima* and ≈ 11.76 IJs for *C. variegata*, as compared with that ≈ 8.56 IJs for *G. mellonella*. There are no statistically different in potency among these three hosts. Thus, the insecticidal potencies of the nematodes to these three pests

were: *C. variegata* = *B. longissima* = *G. mellonella*. However, there is a significant dose-response in each treatment of the insect species. Two field trials were conducted in local residence yards in the Wanning City suburb of Hainan province, P. R. China. The results showed that after spraying *H. bacteriophora* SD IJs in the period of March and April, *Cinnamomum camphora* trees is significant difference in the survival rate between the treatment and untreated control ( $p < 0.05$ ). The technology presented may be of substantial interest to biological pesticide producers.

Contributed paper. Thursday, 8:30. **209**

**Prospects for using Entomopathogenic Nematodes to Control the Vine Mealybug, *Planococcus ficus*, in South African Vineyards**

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*Planococcus ficus* (Signoret), the vine mealybug, is regarded a major pest insect of the South African grape industry. Mealybugs are difficult to control with chemicals due to their cryptic lifestyle of hiding in crevices, under bark and below ground on roots where chemicals battle to reach. Another problem in the use of chemical pesticides is the water repellent waxy secretions and the ability of mealybugs to rapidly build up resistance. Entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae can potentially be used within an integrated pest management system to control the vine mealybug, which not only occur mostly on the aerial part of plants, but also on the roots. Both local *Heterorhabditid zealandica* and *Steinernema yirgalemense* were able to move 15 cm downward in sand columns to infect *P. ficus*, with respective mortalities of 82% and 95%. Laboratory persistence of *S. yirgalemense* in sterile, moist sand in the laboratory remained high (> 85%) after 6 months, while that of *H. zealandica* dropped to 5%. When *S. yirgalemense* was applied to the soil of two vineyards with adult female *P. ficus*, contained in pierced Eppendorf tubes, buried at a depth of 15 cm in the soil, mortalities of up to 50% were obtained after 48 h. Persistence of *S. yirgalemense*, measured using codling moth larval mortality, was found to be zero in one vineyard, while in the other 70%, 12 weeks after application. These studies showed that entomopathogenic nematodes, specifically *S. yirgalemense*, have promising potential as biological control agents for *P. ficus* soil populations.

Contributed paper. Thursday, 8:45. **210**

**New data on *Steinernema ichnusae* distribution in the Mediterranean Area**

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The species *Steinernema ichnusae* (Tarasco, Mráček, Nguyen & Triggiani, 2008) have been isolated till now only from Sardinia (Tarasco *et al.*, 2014; doi: 10.1017/S0022149X

14000194). Recent molecular studies carried out on some strains isolated in other Mediterranean areas revealed this species is also present out of Sardinia island. Five strains of *S. ichnusae* were identified coming from different coastal sites in Algeria (ALG2, ALG3, ALG15, ALG 16 and ALG18), one from continental Italy (Campania Region, MU1) and two from Sicily (EMA 2 and CT026). All these strains had previously been only partially identified as belonging to a species of the *S. feltiae* group. The molecular studies showed that all the strains examined shared with *S. ichnusae* some nucleotide changes in the ITS1 region, including a very conserved 10 bp composite deletion. This makes it easy to setup a molecular assay to discriminate *S. ichnusae* from the close species *S. feltiae*. These new results show that this species is not endemic of Sardinia, as previously believed, and it might be widespread in other Mediterranean Countries as well.

Contributed paper. Thursday, 9:00. **211-STU**

**Evaluation of entomopathogenic nematodes for control of the diapausing overwintering codling moth population**

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In South Africa codling moth (CM) (*Cydia pomonella*) is the most important insect pest of apples and pears. During the winter months, from April to August, no fruit is on the trees, and the total CM population overwinters as diapausing larvae. During this period, entomopathogenic nematodes (EPNs) can be applied to reduce the number of emerging moths in the following season. The impact of aerial EPN application, and environmental conditions, on CM larvae mortality was investigated in an apple orchard. CM larvae were used to culture infective juveniles, used in the different field trials. As containment method, wire-mesh cages filled with apple tree bark and 20 last-instar CM larvae were used, while different nematode species and concentrations were used as treatments. The cages were kept moist, while temperature and moisture levels were recorded during 24 h in the field, after which they were retrieved, and the CM larvae removed and washed. After four days, infection was confirmed by dissection. Five *S. yirgalemense* concentrations and three nematode species (*Steinernema yirgalemense*, *S. feltiae* and *Heterorhabditis bacteriophora*) were investigated. *Steinernema yirgalemense* caused the highest level of mortality of CM larvae, with no significant difference being found between *S. yirgalemense* concentrations investigated.

Contributed paper. Thursday, 9:15. **212-STU**

**A new entomopathogenic *Oscheius* (Nematoda: Rhabditidae) from Italian cave**

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Specimens of nematode belonging to *Oscheius* genus was isolated through the *Galleria* bait method from soil collected

in a karst cave of Tuscany (Central Italy). Molecular and morphological analyses were performed. Total DNA was extracted from individual nematodes and the mitochondrial COI, the ITS containing region and the 18S rRNA gene were amplified and sequenced. BLAST search at NCBI discriminate this new taxon, similar to other *Oscheius*. This species belongs to Dolichura group. Cuticle is finely annulated, stoma is short and cheilorhabdion is simple, not well cuticularized. Female body is almost straight upon fixation, the reproductive system is didelphic and tail is short, conoid with pointed tip. Males are rare and similar to female in general morphology except for smaller size. Male body is straight when heat-killed, testis is single, ventral reflexed. They show peloderan bursa, tail short rounded and spicules slender and small. Infective Juveniles are slender with elongate tail and have stoma morphology similar to adult. The nematodes were cultured in Petri dishes on several substrates: Nutrient Agar, *Escherichia coli*, *Botritis cinerea*, meat baby food, without satisfactory results. Only Petri dishes method with *G. mellonella* larvae produced IJs, suggesting the entomopathogenicity of this new taxon.

Contributed paper. Thursday, 9:30. **213**

**Genetic improvement of the entomopathogenic nematode *Heterorhabditis bacteriophora***

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**Abstract**-The entomopathogenic nematode *Heterorhabditis bacteriophora* has been genetically improved in beneficial traits, like heat and desiccation tolerance, by cross breeding and genetic selection. For instance, a final overall increase in mean heat tolerance of 5.5°C was achieved with *Heterorhabditis bacteriophora* by cross breeding the most tolerant five strains and then selecting for heat resistance. Success of breeding programmes largely depends on the heritability of the investigated traits. Advances in enhancement of desiccation and heat tolerance often have been lost again during mass production. For heterorhabditid nematodes methods have now been developed to stabilize the traits by selection of tolerant inbred lines. This technique provides a pathway to genetic improvement of commercial strains which will maintain the improved characters also during in vitro mass production. The methodology to produce stable inbred lines for steinernematids needs further investigation, as these nematodes are amphimictic and production of inbred lines is much more laborious. The reproduction potential in liquid culture was also successfully increased. Future targets for genetic improvement are prolongation of shelf life and field persistence and enhancement of virulence.

Contributed paper. Thursday, 9:45. **214-STU**

**Perspectives of new nematode formulation technology for biological control to pest insects in Georgia**

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In the result of route investigations the soil samples for searching of entomopathogenic nematodes (EPNs) have been collected in several agroecozones of different regions of Georgia. Samplings of testing material were done by using of

recent methods in insect nematology (Stock & Goodrich-Blair, 2012). According to preliminary data some active strains of *Steinernema* sp. have been obtained. EPNs extract efficiency was established on laboratory culture of *Galleria mellonella*. Further research directions for the identification of local strains (under the Project CRDF/DTRA/GRDF #GMG-01/13) have been conducted at the University of Arizona, laboratory of Entomology by two different ways: morphological and molecular diagnostic methods. It was established that four local EPNs isolates belong to the genus *Steinernema*. Furthermore partial sequencing of the ITS rDNA gene revealed they are closely related to the species *Steinernema feltiae*. This conventionally called - "Georgian strain", considered as a raw material will be base for local production of bioformulation - "*Geo-nema*". Provided technological product - environmentally safe nematode insecticide will be used for biological control to the pest insects of agricultural crops and ornamental plants. The researches will be continued under the projects CRDF/STEP and SRNSF/STCU financial support. The usage of nematode insecticide will take an important place in IPM (integrated pest management) system for agricultural crop protection in Georgia.

CONTRIBUTED PAPERS Thursday, 8:00-10:00

**Viruses 6**

Contributed paper. Thursday, 8:00. **215**

**Interactions between salivary gland hypertrophy virus and tsetse microbiota**

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Many species of tsetse flies are infected by a herpesvirus that causes Salivary Gland Hypertrophy (SGH) syndrome. Flies with SGH have a reduced fecundity and fertility. Due to the deleterious impact of the salivary gland hypertrophy virus (SGHV) on *Glossina pallidipes* colonies, several approaches have been investigated to develop a virus management strategy including the exploitation of endogenous microbiota. Tsetse flies harbor three symbiotic bacteria (*Wigglesworthia glossinidia*, *Sodalis glossinidius* and *Wolbachia*) in addition to trypanosome, the causative agent of sleeping sickness disease in human and nagana in livestock. The interaction of the tsetse microbiota (gut bacteria and symbionts) with the SGHV and / or trypanosome is largely unexplored. In the present study, we show that ampicillin treatment of *G. pallidipes* impedes the transgenerational transmission of the SGHV suggesting the involvement of tsetse microbiota in the virus transmission. Quantitative-PCR analysis of the levels of SGHV and *Wolbachia* in wild tsetse flies (mainly *G. morsitans morsitans* and *G. austeni*) clearly indicated a negative interaction between SGHV and *Wolbachia*: flies heavily infected with *Wolbachia* presented significantly low viral titers. In addition, injection of GpSGHV into different *Wolbachia*-infected *Glossina* species did not result to the transgenerational transmission of SGHV as normally occurs in *G. pallidipes* colony, which is free of *Wolbachia*. Taken together, these data