

PARSA 2014

The following are abstracts of papers and posters at the 43rd Annual Congress of the Parasitological Society of Southern Africa (PARSA) 14–18 September 2014, Skukuza Rest Camp, Kruger National Park, South Africa.

Session 1

Keynote paper [A]: History and development of research on wildlife parasitology in southern Africa, with emphasis on terrestrial mammals

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After an early start (1896), when Bruce demonstrated the presence of trypanosomes in various wildlife species, the study of the parasites of wildlife in South Africa really commenced when Sir Arnold Theiler became the first director of the Veterinary Research Institute, Onderstepoort. Realizing the necessity of not only studying the parasites of domestic animals but of the indigenous fauna as well, he initiated the collection of parasites from wild animals in the Pretoria vicinity. Renowned early taxonomists at the Institute were GAH Bedford and G Theiler, who not only described many new ectoparasitic arthropod species, but their distribution in southern Africa as well. The helminthologist HO Mönnig studied the nematodes of a variety of wild animals, and described several new species. Records of the parasites of wildlife collected over a 40-year period published by RJ Ortlepp, one of South Africa's most productive helminth taxonomists, in an overview in 1961, indicated that more than 150 new species had been described. More than 100 of these had been recovered from mammalian hosts that at the time were receiving the most attention from zoologists and hunters, thus increasing the availability of parasites. Ortlepp concluded with an appeal to zoologists to study the parasites of wild animals that had hitherto not been given any attention. A number of South African parasitologists took up this challenge, often combining the efforts of various disciplines of parasitology, and with a slight shift in emphasis. Based on the ground work laid by earlier parasitologists in recording and describing the parasites of South African wildlife, in many instances based on incidental findings, they now followed a more structured approach, at the same time both increasing the host spectrum and complementing previous records. Studies were geared towards full parasite collections from a particular host species, including metazoan and blood parasites where possible, and hosts were examined in sufficient numbers to ensure detection of rarer parasite species. Examinations would be done over a period of 12 consecutive months or more, to account for parasites that may be plentiful but strictly seasonal, thus being easily missed in casual surveys, and to determine the seasonal abundance of parasites. Surveys of the same host species at different localities within South Africa would also provide an indication of the geographic distribution of its parasites. As a result, more and more data were accumulated that now allow us to gain insight into the ecology of parasites in at least some of the many and diverse hosts in South Africa and into the patterns and processes that shape their communities.

[1] Trypanosomes and the population decline of the woylie (*Bettongia penicillata*) in Western Australia

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The introduction of *Trypanosoma lewisi* to Christmas Island with fleas on ship rats (*Rattus rattus*) has been demonstrated to be a likely cause of the extinction of the native rat, *R. macleari*, a naïve host of this parasite (Wyatt KB *et al.* 2008. *PLoS One* 3, e3602). However, the pathogenic potential of indigenous trypanosomes to Australian wildlife has not been considered until recently. An ongoing investigation of the sudden and dramatic decline of the woylie (*Bettongia penicillata*) in Western Australia has shown that disease associated with infection with trypanosomes may be a contributory factor (Wayne A *et al.* 2013. *Oryx* doi:10.1017/S0030605313000677). Several species of *Trypanosoma* infect woylies but the species *T. copemani* appears to have the greatest pathogenic

potential (Botero A *et al. Int. J. Parasitol: Parasites Wildl.* 2, 77-89; Thompson CK *et al.* 2013. *Parasit Vectors* 6, 121) However, mixed infections are common in the declining population and thus polyparasitism may be exacerbating the situation. This investigation has also raised biosecurity concerns that the vectors of trypanosomes in wildlife could transmit introduced trypanosomes such as *T. cruzi* thus further demonstrating how little is known about their life cycle, evolutionary biology, host-parasite interactions and pathogenic potential.

[2] Diversity of *Theileria* in cattle and African buffalo (*Syncerus caffer*) using next-generation sequencing approaches

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Movement of African buffalo (*Syncerus caffer*) is controlled by the Department of Agriculture, Forestry and Fisheries of South Africa and testing for Corridor disease is mandatory. The current test uses a hybridization PCR assay that amplifies the species *Theileria parva*, *T. sp.* (buffalo) and *T. sp.* (bougasvlei). Questions raised include whether any other *Theileria* genotypes are amplified by the assay, whether all *T. parva* parasites are detected and whether diversity for *T. parva* exists in the probe region. Previously a conventional sequencing approach of the 18S rRNA gene from 705 nm *Theileria* positive samples was conducted. This study revealed the presence of 17 different genotypes in cattle and buffalo by conventional sequencing of ~1000 clones from ~50 cattle and ~50 buffalo. The current study expanded on this by sequencing ~576 cattle, 672 buffalo and 192 antelope samples from southern Africa using a next-generation sequencing approach. Approximately 900 000 informative sequences were generated of which ~24 000 were typed to *T. parva*. This study confirmed previous observations, notably that 17 genotypes exist in cattle and buffalo, that host-specific genotypes exist, that buffalo possess a greater diversity of *Theileria* genotypes than cattle and that polymorphisms occur in the hyper-variable regions of *T. parva*, albeit at low prevalence. No other genotypes were found to be amplifiable by the assay, while all *T. parva* genotypes possessed the conserved primer sequences. The results confirmed current specificity of the assay and suggest that next-generation sequencing may be a convenient tool to investigate *Theileria* diversity.

[3] Investigating non-specific interaction on the Indirect Fluorescent Antibody Test for *Theileria parva* in the African buffalo

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The African buffalo (*Syncerus caffer*) is a carrier of pathogenic and non-pathogenic tick-borne protozoan parasites of the genus *Theileria*. The specific diagnosis of the pathogenic species *Theileria parva* which causes Corridor disease in cattle has been complicated by the presence of non-pathogenic species in mixed infections. The movement of Cape buffalo in South Africa is regulated by using results from the real-time PCR hybridisation assay and the Indirect Fluorescent Antibody Test (IFAT). However, serologic tests may not be sensitive enough to detect all infected buffalo and cattle and cross-reactions can occur with other *Theileria* species and, therefore, a deeper understanding of serological specificity is needed. This study aims to investigate the non-specific cross-reactivity of sera using known immuno-dominant antigens such as p67, p104 and PIM. The protocol involves expression of recombinant antigens and testing of the antibody affinities for a panel of validated *T. parva* and *T. sp.* buffalo samples as well as negative and problem samples identified during routine diagnostics at the ARC-OVI. Affinities will be measured using the Biacore T200 and samples will be validated using IFAT, RT-PCR and DNA sequencing. A better understanding of cross-reactivity on the IFAT should be obtained and as well as investigation into the possibility of developing a specific serological test using plasmon resonance technology that will complement IFAT.

[4] Buffalo from National Parks – a cause of veterinary worry at the game-livestock interface?

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The co-infection dynamics of *Babesia* and *Theileria* species of cattle, dogs and various wildlife species are widely documented and increasingly studied. In a conservation-conscious era the focus on the risk at the game-livestock interfaces and trans-boundary animal diseases also fuels the study of reservoir disease hosts and accurate diagnosis of pathogens. Buffalo are notoriously known to harbour and act as a source of infection to vectors and other animals of especially foot and mouth disease, bovine tuberculosis, brucellosis and of particular interest, *Theileria parva* causing Corridor disease in cattle. They also harbour a number of other *Theileria* parasites complicating the diagnosis of *T. parva*. To this extent we examined the *Theileria* piroplasm burden of ~656 blood samples of buffalo herds across three national parks in South Africa and national parks from Zimbabwe, Botswana, Mozambique and Namibia to determine the extent of infection and diversity in parasite populations. We identified two main genotypes that interfered with accurate diagnosis of *T. parva* and calculated their relative parasitaemias for each park. The data generated supported a hypothesis that the two genotypes are different *Theileria* species and was confirmed using 'the barcode of life': the standard barcode for almost all animal groups of a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene ("CO1"). Although the vectors for these *Theileria* piroplasms are still unknown, possible reasons for the geographic distribution for the differences observed are noted.

[5] Corridor disease (*Theileria parva* buffalo-derived infection in cattle): Investigations on an outbreak in cattle introduced onto a game park located outside the demarcated Corridor line, KwaZulu-Natal, South Africa

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Corridor disease, caused by infection with buffalo-derived *Theileria parva*, is fatal in cattle. Corridor disease outbreaks have increased in disease endemic areas. A severe outbreak occurred in 2013 affecting cattle introduced onto a game park located outside the Corridor disease designated line. Monitoring of the outbreak included mortality, clinical signs, post-mortem, parasitological examination employing IFA test and real-time PCR specific for *T. parva*. Cattle were sampled early in the outbreak and twice during the following 75 days. Questing adult *Rhipicephalus appendiculatus* collected from the park were fed onto two susceptible cattle under controlled conditions. The ticks transmitted fatal Corridor disease to the two bovines. All buffaloes were confirmed *T. parva* carriers. Our observations confirmed the cause of death was due to Corridor disease. The prevalence was 62.3% and 29.5% cattle died due to Corridor disease. Thirty-five percent of cattle became positive by the IFA test only by day 40, indicating that they had lost their parasitaemia. These investigations demonstrated a classic occurrence of Corridor disease outbreak; however, the source of infections to buffaloes remains speculative.

[6] Investigating the possible presence of *Theileria parva* carrier cattle in Mnisi area

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Corridor disease (caused by buffalo-derived *Theileria parva*) is the most important *Theileria* sp. posing a threat to the cattle-farming industry in South Africa. The African buffalo (*Syncerus caffer*) is the reservoir host for this protozoan parasite transmitted by the three-host ticks *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis*. It is considered a self-limiting disease because most cattle die before the parasites reach the tick-infective stage. Recent experimental studies have shown that a carrier state can be attained in infected cattle that survive the disease. A study to identify *T. parva* carrier cattle in Mnisi, a wildlife/livestock interface area, was done in 2012–2013. Records from Hluvukani Animal Health Centre and Bushbuckridge State Veterinary office were scrutinized. Blood samples (n=670) were collected from herds that recorded Corridor disease cases in the past three years, as well as from herds that grazed in areas where buffaloes grazed when they broke out of Kruger National Park. The indirect fluorescent antibody test was used to check for *T. parva* antibodies. Deoxyribonucleic acid (DNA) was extracted from ethylene-diamine-tetra-acetic-acid (EDTA) blood samples collected from sero-positive herds and screened for the presence of piroplasm parasite DNA using a *T. parva*-specific quantitative real-time polymerase chain reaction (qPCR). The p67, p104 and PIM genes were amplified, cloned and sequenced. The sequences are being compared with those found in clinical Corridor disease cases in Mnisi as well as with those previously sequenced from isolates from buffalo.

Session 2

[7] Genetic characterization of avian *Babesia* species from several South Africa seabird species

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To determine if *Babesia peircei*, a potential pathogen of African penguins, also infects sympatric marine birds, we genetically characterized *B. peircei* from penguins, *Babesia ugwidensis* from Cape Cormorants (*Phalacrocorax capensis*), and Bank Cormorants (*P. neglectus*) and a *Babesia* sp. from Cape Gannets (*Sula capensis*). The 18S rRNA gene sequences were highly similar (>98.6%) between all of the African bird samples and also with *B. uriae* from common murres (*Uria aalge*) from California (USA) and *B. poelea* from brown boobies (*Sula leucogaster*) from the Pacific Ocean. Sequences of *B. poelea* and *B. peircei* were most similar (99.8% similarity). ITS-1 sequences from *B. peircei* were most similar to *B. poelea* (90.5–93.6%) and were only 83.8–87.4% similar to other African seabird *Babesia*. The ITS-1 sequence from a single gannet was very similar to some sequences of *B. ugwidensis* (98.4–99.7%). ITS-2 sequences from *B. peircei* were most similar to *B. poelea* (97.2%) and two sequences from gannets were highly similar to *B. ugwidensis* (99.3–99.6%). These data suggest that *B. peircei* and *B. poelea*, which are morphologically similar, are closely related and possibly synonymous. Data from two gannets suggest they are susceptible to *B. ugwidensis*. Of note, parasites from gannets are pleomorphic with some specimens having distal chromatin in the schizonts whereas some have proximal chromatin, a distinction typically used to distinguish avian *Babesia* species. Previous work on five South Africa species of cormorants showed that the chromatin in *B. ugwidensis* was always proximal. Additional testing of gannets with morphologically-distinct *Babesia* is needed to determine if *B. ugwidensis* is pleomorphic in gannets or if gannets are infected with two *Babesia* species. Additional surveillance and genetic characterization of avian *Babesia* would significantly improve our understanding of this understudied group of avian parasites.

[8] Molecular occurrence of haemosporidians in wild Orinoco geese (*Neochen jubata*) in Brazil

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Haemosporidia parasites are incriminated as causative agents of negative effects on host fitness, including reproductive success and immune responses in avian species. The present work aimed to investigate the occurrence of avian haemosporidians in wild Orinoco geese (*Neochen jubata*) in Brazil, using molecular techniques. Between 2010 and 2013, blood samples were collected from 41 Orinoco geese in Luis Alves - Goiás state. DNA was extracted from goose blood samples using a commercial kit and submitted to nested PCR assays for *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp., targeting mitochondrial cytochrome b (cytb) gene. Purified amplicons were submitted to direct sequencing and phylogenetic analysis. *Plasmodium* spp. cytb DNA (98% of identity by BLAST analysis) was detected in two goose blood samples collected in 2010. *Haemoproteus* spp. cytb DNA (98% of identity by BLAST analysis) was detected in 10 goose blood samples (six collected in 2010; four in 2013). The *Plasmodium* spp. lineage found was closely positioned to *P. elongatum*, although supported by a low bootstrap in the phylogenetic analysis using Maximum Likelihood method. The *Haemoproteus* lineage found in this study was placed in the *Parahaemoproteus* clade and constituted a distinct sub-clade in the *Haemoproteus* phylogenetic tree. The diversity of haemosporidian lineages found in the present study is important to assess the genetic variability of these haemoparasites in the Neotropical region. Financial support: FAPESP (Processo nº 2011/11230-1)

[9] Molecular detection of vector-borne haemoparasites in wild rodent species in South AfricaMilana Troskie¹, Ilse Vorster¹, Ray Jansen², Banie Penzhorn¹, Marinda Oosthuizen¹, Sonja Matthee³¹University of Pretoria, Pretoria, South Africa, ²Tshwane University of Technology, Pretoria, South Africa, ³Stellenbosch University, Stellenbosch, South Africa

It has long been recognized that wildlife can act as reservoirs for many human and livestock diseases and that arthropods are often involved in the transmission of pathogens. In recent years, vector-borne viral, bacterial and macro-parasitic diseases have emerged or re-emerged in many geographic regions causing global health and economic problems. The ecology and epidemiology of these diseases are affected by the interaction between the pathogen, the host and the vector, with the addition of the environment in certain cases and is therefore of major concern. In particular, rodents often harbour pathogens and parasites that can affect humans. *Babesia microti* is known to cause human babesiosis: a zoonotic, malaria-like illness that can be particularly severe and sometimes fatal in elderly, asplenic, or immunocompromised persons. The rare occurrence of human babesiosis in Africa may be due to the widespread occurrence of malaria which causes an underestimation of babesiosis. To date little is known with regard to zoonotic pathogens that naturally occur in rodents in South Africa and thus no information is available of the risk to livestock and/or humans. Therefore the pathogen diversity within wild rodent species in South Africa was recorded by screening for the presence of *Theileria*, *Babesia*, *Ehrlichia* and *Anaplasma* spp. using the Reverse Line Blot (RLB) hybridization assay. Preliminary results obtained revealed the presence of *Babesia microti*, *Anaplasma bovis* and *Ehrlichia ruminantium*, either as single or as mixed infections.

[10] Biodiversity of haematozoans parasitising South African frog species, with preliminary morphological and molecular data on haemogregarines of the Bufonidae

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Very little work has been done documenting South African frog haematozoan biodiversity, the only described haematozoan being *Hepatozoon theileri* (Laveran, 1905) of *Amietia queckettii*. The aim of this project was to screen a variety of different frogs representing different families for haematozoan infection. Frogs of 35 species were collected from five localities in three provinces. Blood was taken via the femoral artery or vein, thin blood smears made, stained, and parasites measured. Haematozoans, including microfilaria, trypanosomes, and apicomplexans including babesiasomes and haemogregarines, were found infecting members of the following anuran families: Bufonidae, Hemisotidae, Hyperoliidae, Phrynobatrachidae, Pipidae, Ptychadenidae and Rhacophoridae. Haemogregarines were found to be the most commonly recorded haematozoans along with trypanosomes, the former well represented by several species infecting various frog hosts from Africa, particularly within the Bufonidae. Of the bufonids occurring in South Africa, *Amietophrynus* species were the most frequently collected, the current study having examined 6/8 of these species thus far. Three of these, all from KwaZulu-Natal, were found infected with haemogregarines, 4/33 *A. garmani*, 3/8 *A. gutturalis* and 7/12 *A. maculatus*. Morphologically, using light microscopy features and measurements, and molecularly, using partial 18S rDNA fragments amplified with specific primer sets, haemogregarine isolates from all three hosts were phylogenetically compared, and were found to be conspecific belonging to the genus *Hepatozoon*. When compared morphologically with descriptions of other African bufonid haemogregarine species, they were found to be different and further molecular analysis is necessary to confirm this.

[11] Haemogregarines of the Hepatozoidae parasitising African frogs, redescribing South Africa's first recorded frog *Hepatozoon* species, *Hepatozoon theileri* (Laveran, 1905)

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Recently there has been a strong focus on amphibians as the most threatened vertebrate class. It is well known that frogs harbour several types of parasites, much attention given to their nematode and helminth parasite fauna. In contrast very little is known about the parasitic protozoa, particularly the apicomplexan haematozoans that infect them. Among these, haemogregarines are the most frequently recorded apicomplexans to parasitise frogs. Genera recorded from anurans include *Haemogregarina* Danilewsky, 1885, *Hemolivia* Petit, Landau, Baccam and Lainson, 1990, *Hepatozoon* Miller, 1908, *Lankesterella* Labbé, 1894, and *Schellackia* Reichenow, 1919. Before 1996 most frog haemogregarines were assigned to *Haemogregarina*, but Smith (1996) transferred all 42 of these to *Hepatozoon*. Of these, 15 are described from Africa, 73% from the Bufonidae, 13% from the Ptychadenidae, 7%

from the Pyxicephalidae and Hyperolidae, respectively. Furthermore, of the above, only one species has been described from South Africa, *Hepatozoon theileri* (Laveran, 1905). The aim of this project was to begin to describe South Africa's frog haematozoan biodiversity. This including redescrptions of 'old' species, such as *H. theileri* parasitizing *Amietia quecketti*. Wild frogs (n=20) were bled, thin blood smears prepared and stained, and parasites measured. Analysis of partial 18S rDNA fragments, amplified with *Hepatozoon*-specific, HEPF300 and HEPR900, primer sets followed. *Hepatozoon theileri* was the first redescrbed frog haematozoan from Africa using both morphological and molecular techniques, the findings of which proved to be significant, not only for Africa, but also on a global scale as well.

[12] Saurian malaria (Haemospororida: Plasmodiidae) in Cordylidae from the Free State Province, South Africa

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Saurian malaria is caused by intra-cellular apicomplexan parasites that produce haemozoin pigment as a metabolic by-product, and include species of the genera *Billbraya*, *Plasmodium*, *Haemocystidium* and *Haemoproteus*. Until now, 196 *Plasmodium* species have been described from lizards worldwide. While 21 species have been reported in African lizards, only one species has been described in a South African Cordylid lizard, *Cordylus vittifer*. Morphologically, *Plasmodium* infections can be difficult to identify, particularly if they form co-infections with haemogregarines, piroplasms and filarial nematodes, which was always the case in host lizards sampled in this study. This study aims to add new distribution and infection records by focusing on the unique morphological and morphometrical aspects of new *Plasmodium* species found in Cordylid lizards from the Free State Province. Detailed morphological studies revealed three unique endoglobular pigmented malarial parasites in the peripheral blood of *Cordylus polysonus*, *Pseudocordylus melanotus* and *Pseudocordylus subviridis*, from the Free State. The developmental stages in the peripheral blood were broadly similar to the described species in *C. vittifer*, but differed in staining properties, amount and distribution of pigment and general morphometrical dimensions. Based on the parasite morphology alone, no firm conclusions regarding the phylogenetic position are reached at this stage, but a molecular study is initiated to obtain sequence data in order to establish the true identity of these infections.

Session 3

[13] Biology of the kangaroo leech *Marsupiobdella africana* (Hirudinea: Glossiphoniidae)

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The South African leech *Marsupiobdella africana* is specific to the anuran host *Xenopus laevis*. We studied the morphology and biology of this leech species using various microscopy and histological techniques. Insemination takes place in that a leech plants a spermatophore on the surface of another. Sperm then penetrate the body and find their way to the ova. *Marsupiobdella* exhibits an advanced case of parental care by incubating its offspring in a brood pouch. This leech is a temporary ectoparasite of the amphibian *Xenopus laevis*, and has a phoretic association with a freshwater crab, *Potamonautes perlatus*. Because phoretic associations are usually regarded to favour the phoront's dispersion, its occurrence within the biology of a parasitic species reflects an intimate context of interactions. In addition to phoresy, attachment to the crab may confer other advantages pertaining to offspring development and predator avoidance, dispersion and the parasitic life cycle. The leeches detach from the crab in search of a *Xenopus*. When a leech makes contact with a potential host the brood pouch will burst open and the young released on the surface of the *Xenopus* where they start to feed and develop.

[14] The invasive American red-eared slider *Trachemys scripta elegans* in Southern Europe: A vector for parasite transmission in natural environments?Olivier Verneau^{1,2}, Louis du Preez² and Leon Meyer^{1,2}¹University Perpignan Via Domitia, Centre de Formation et de Recherche sur les Environnements Méditerranéens, UMR 5110, F-66860, Perpignan, France, ²Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

One of the most popular reptilian pets is the North American red-eared slider *Trachemys scripta elegans*. This turtle has been exported from the USA to destinations around the globe and in particular the Asian and European markets. Although many countries banned turtle imports, they were released or escaped accidentally, establishing feral populations. As with most introductions, invasive freshwater turtles pose a threat to local biodiversity and ecosystem functioning. In Southern Europe, i.e. in France and Spain, this exotic slider is indeed considered as a risk for the Mediterranean pond turtle, *Mauremys leprosa*, and the European pond turtle, *Emys orbicularis*, as they may compete for resources and habitat. Because freshwater turtles are hosts to a variety of parasites including protozoans and helminths, we hypothesized that *Trachemys* parasites could also spread to native host species. From extensive fieldwork in Southern France and Northern Spain, we sampled *M. leprosa* and *T. s. elegans* turtles that were examined for their parasites, especially platyhelminth polystomes of the class Monogenea. From DNA barcoding analysis based on the sequencing of the COI gene, we revealed a greater than expected polystome richness in *M. leprosa*, suggesting frequent host-switching in natural environments. Because most of the *M. leprosa* parasites were identified as American turtle parasites and because those parasites were also found within feral populations of *T. s. elegans*, we assume that the red-eared slider could serve as a carrier for a variety of not strictly host-specific parasites that may be transmitted to indigenous freshwater turtles in Southern Europe.

[15] Reproduction in *Chonopeltis australis* Boxshall, 1976

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Chonopeltis australis Boxshall, 1976 is endemic to the river systems of Southern Africa. In order to understand the biology of this species, an investigation into reproduction is required. Live adult specimens of *C. australis* collected in the Vaal River were transported to the laboratory to start a breeding colony. Male and female reproductive systems, sperm transfer, egg deposition and larval development was studied using behavioural observation, photography, light microscopy and scanning electron microscopy. The reproductive systems of both males and females showed similarities to other genera in the class and a spermatophore is used to transfer sperm. The spermatophore produced by *C. australis* is more malleable than that of *Argulus japonicus* Thiele, 1900 and does not harden on the female as in *Dolops ranarum* (Stuhlmann, 1891). *Chonopeltis* sp. are feeble swimmers, but the females of *C. australis* use the host's behaviour to distribute eggs as they were seen leaning off the resting fish to deposit eggs instead of climbing off. In this way the eggs and thus the larvae were at the same level as the fish, increasing the chances of infection. In addition, it was found that larval development is similar to the development in other species of this genus. However, a previous suggestion that juvenile specimens require a different host to those of adults was proven to be incorrect as the life cycle was concluded on a single host species. *Chonopeltis australis* therefore follows similar biological patterns to other Branchiura with adaptations to living in river systems.

[16] The effect of metazoan parasites on oxidative stress biomarkers and health of two cyprinids of the middle region of the Olifants River System, Limpopo Province, South AfricaTshepiso Ramalepe¹, Wilmien Luus-Powell¹, Lourens Erasmus², Anna-Maria Botha-Oberholster⁴, Joseph Sara³, Eunice Mogashoa², Martin Chabalala¹, Modibe Raphahlelo¹, Willem Smit¹¹Department of Biodiversity, University of Limpopo, Sovenga, South Africa, ²Department of Physiology and Environmental Health, University of Limpopo, Sovenga, South Africa, ³Aquaculture Research Unit, University of Limpopo, Sovenga, South Africa, ⁴Department of Genetics, University of Stellenbosch, Stellenbosch, South Africa

In biology, parasitism is referred to as the ecological relationship between the host and the parasite. This interaction has received extensive attention since the 1800s. Metazoan parasites are multicellular animals that have evolved to use different strategies in order to penetrate and invade their host's defences for the absorption of nutrients. This directly or indirectly affects the health of the host parasitized. As such, the effects of parasites on the health of *Hypophthalmichthys molitrix* (Valenciennes, 1844) and *Labeo rosae* Steindachner, 1894 in the Olifants River System, Limpopo Province were evaluated for the first time using oxidative stress biomarkers.

Concentrations of various biomarkers were used to assess the oxidative response in the gill and liver of these two fish species in relation to parasitic infections. In this study, considerable variation in biomarker concentrations between the two fish species and the various organ tissues (gills and liver) were noted. Results indicate that the specific response of each body tissue is associated with their susceptibility to oxidative stress and a resistance towards oxidative damage. Results also indicated that *H. molitrix* was affected more in terms of health and parasitism than *L. rosae*, an indication that alien fish are more susceptible than indigenous species. These findings, therefore, provide an indication of how adaptive these metazoan parasites are to new host species and to local conditions.

[17] Aliens on Aliens! Different species of Temnocephalans on Redclaw crayfish from the Komati River System

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Alien animals can cause a variety of problems by out-competing, consuming or parasitizing indigenous animals. In South Africa, four crayfish species were deliberately introduced from Australia for aquaculture purpose since the 1980s with all subsequently having escaped into the natural water systems. Temnocephalans are small, active ectosymbiotic flatworms occurring on freshwater crustacean hosts, particularly crayfish. One species of temnocephalan (*Diceratocephala boschmai*) was reported previously from Redclaw crayfish, *Cherax quadracarinatus*, in Nyamiti Lake (Ndumu Game Reserve) in South Africa (Du Preez and Smit 2013). In the current study, three different genera, *Diceratocephala boschmai*, *Craspedella* sp. and *Didymorchis* sp., were discovered from the branchial chamber, between the pleopods and legs and other external surfaces (i.e. carapace) of the same host in Komati River System, Mpumalanga Province. Parasite samples were either fixed in hot formalin or in cold 100% ethanol. Morphological characters relevant to the genera of temnocephalans were used to differentiate between different species, including locomotory cilia, tentacles, dorsal scales, dorsal papillate ridges, conical ciliated papillae in rows on tentacles and testes. Temnocephalans are considered as non-host-specific parasites, they can transfer to local decapods, especially to crustaceans which lack well grooming habits.

[18] African trypanosome-induced blood-brain barrier dysfunction under shear stress activates fractalkine but may not require ERK activation

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Background: African trypanosomes cause human African trypanosomiasis, a disease characterized by a plethora of neurological symptoms and death. How trypanosomes cross the blood-brain barrier (BBB) under shear stress (SS) flow conditions in the brain is unclear. *In vitro* studies using static models comprised of human brain microvascular endothelial cells (hBMEC) show that BBB activation and crossing by trypanosomes requires the orchestration of parasite cysteine proteases, cytokines and host calcium-mediated cell signalling. Here we examine hBMEC barrier function, cytokine expression and mitogen-activated protein kinase (MAPK) activation under SS flow trypanosome (*Trypanosoma brucei rhodesiense*) infection. Methods: Confluent hBMEC were cultured in electric cell-substrate impedance sensing (EICS) and parallel-plate glass slide chambers. The hBMEC were exposed to low or high SS in the presence or absence of trypanosomes. Real-time changes in transendothelial electrical resistance (TEER) as monitored and several cytokines and phosphorylation of MAPKs ERK 1/2 and ERK 5 analyzed by Luminex or immunoblot assay. Results: Starting from static conditions, hBMEC TEER rapidly increased when exposed low SS conditions mimicking postcapillary venules. Addition of trypanosomes caused a rapid drop in TEER. Increasing SS to brain capillary conditions, led to a transient increase in TEER in both control and infected hBMEC. Cytokine assays revealed that only fractalkine was induced by trypanosomes and under SS conditions. No differences in ERK1/2 and ERK5 activation were found under any condition tested. Conclusion: Under SS, African trypanosomes enhance blood brain barrier fractalkine expression but alter permeability under low shear conditions through an ERK1/2 and ERK5 independent pathway.

Session 4

[19] A medieval marauder or an ice age relic: the origins of the zoonotic blood fluke *Schistosoma turkestanicum* in Hungary, Eastern Europe

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Schistosomiasis is a debilitating parasitic infection caused by *Schistosoma* blood flukes infecting over 200 million people worldwide and over 165 million head of cattle globally, with particular high prevalence in Africa and Asia. Recently, a natural focus of schistosome infection caused by *Schistosoma turkestanicum* was found infecting red deer populations in the Gemenc Forest, Hungary. Initial comparisons of Asian and Hungarian populations using cytochrome oxidase 1 (cox1) sequences showed the two populations to be exceptionally diverse, with the Hungarian populations being more closely related to populations in Iran rather than China. Molecular clock analysis suggests that the Hungarian populations diverged from those in Iran around 63,000 years ago during the last ice age, coinciding with the invasion of Hungary by red deer from the near East and North Africa and establishing populations in Eastern Europe. DNA sequencing analysis reveals the Hungarian population of *S. turkestanicum* to be extremely genetically diverse; however, several haplotypes have been identified that appear to be more closely related to populations from the Far East, which could indicate the presence of a sub-population of recently introduced parasites. This is one of the first and most detailed molecular studies of schistosomiasis in wildlife, and highlights the importance of understanding the biology of causative agents of such diseases in populations of wild animals which could potentially act as reservoirs of diseases of public health concern.

[20] Inventory of avian schistosomes in France, and the role of migratory birds on parasitic transfers between the New and the Old World (Palearctic and Nearctic regions)

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Due to parasitic exchanges occurring through their definitive hosts, the study of parasites, including trematodes, cannot be limited to search on a single site or a single country. Among trematodes, the avian schistosomes are parasites of aquatic birds (in particular ducks and geese), which play the part of final host, without any apparent signs of disease. These parasites can be accidentally transmitted to humans by means of a larval stage, furcocercariae, whose transcutaneous penetration is responsible for cutaneous lesions, called cercarial dermatitis (or swimmer's itch) and regarded now as a re-emergent disease. By combining morphological and molecular approaches (ribosomal and mitochondrial domains), we were able to identify different species of bird schistosomes described in the past, but also describe new species never observed before. By associating the parasite species identified in different countries and their hosts along migration corridors, we try to understand how the parasitic transfers happen. The recent discovery of common parasitic species of avian schistosomes in France, Iceland and North America, has led us to assess the migration routes for different species of waterfowl passing through or settling temporarily in France [Greylag Goose (*Anser anser*), Red-breasted Merganser (*Mergus serrator*), Common Merganser (*Mergus merganser*), Tufted Duck (*Aythya fuligula*), Whooper Swan (*Cygnus cygnus*), Mute Swan (*Cygnus olor*) and Mallard (*Anas platyrhynchos*)] in order to consider the trades between the New and the Old World (Palearctic and Nearctic regions).

[21] Impact of blood-sucking nematode *Ashworthius sidemi* invasion on wild-living European bison (*Bison bonasus*) in the Białowieża Primeval Forest, Poland

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European bison is the largest terrestrial mammal in Europe. After becoming extinct in the wild it has been reintroduced to 30 locations in Eastern Europe. The number of bison is gradually increasing, but due to low genetic variation and many other threats it is still considered a high-risk species. Diseases and parasites are

major threats. Over the last decades bison acquired several new species of parasites, including the blood-sucking nematode of abomasums, *Ashworthius sidemi* (Trichostrongylidae), specific to Asian deer species. This parasite was first found in a culled bison in the Białowieża Forest in 2000. Four years later the parasite was found in all individuals examined. By 2011 the average infection intensity had risen to 12 000 (max. 77 600) nematodes. A decline of prevalence and intensity of infection has been observed since 2012, which suggests the beginning of the host-parasite balance formation. The rate of infection is influenced by age but not by the sex of bison. The highest infection rate was recorded in subadult bison in comparison to calves and adults. Our results showed that intensity of the infection significantly influenced blood parameters such as red blood count and haemoglobin. The higher the intensity of *A. sidemi* infection, the lower were the values of blood parameters. We assumed that the sudden appearance of a new and strong invasion of the parasite in the bison population in the Białowieża Forest had an impact on the overall condition of the animals, which is expressed in changes in blood parameters.

[22] Influence of management practices on parasite load in European bison (*Bison bonasus*) in the Białowieża Primeval Forest, Poland

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European bison (*Bison bonasus* L., 1758) – the largest terrestrial mammal in Europe – is managed as a forest specialist, although evolutionary history and morphological features indicate adaptation to open or mixed habitats. Bison are regularly provided with supplementary winter feed aimed at reducing damage to forest stands and agricultural crops, while also limiting bison dispersal. This leads to unnatural bison aggregations and in effect may lead to increase of parasitic load. In the last decade, invasion and rapid spread of a new blood-sucking nematode *Ashworthius sidemi* was observed. We investigated the influence of management practices and intensity of supplementary feeding on the load of *A. sidemi* and other parasites. Our results confirm that *A. sidemi* infection intensity is significantly higher (up to 7 times) in intensively fed bison, which spend up to five months at feeding sites, in comparison to less intensively or non-fed bison, which utilize much larger ranges during winter. We also found a significant influence of winter aggregations of bison on shedding of coccidian oocysts and gastro-intestinal nematode eggs. The prevalence and intensity was higher in bison congregating in large numbers around feeding sites in comparison to other sites. The results suggest the necessity for changing the general approach to the management practices of the bison population living in the Białowieża Primeval Forest and other locations, where similar actions are practiced. Splitting large winter aggregations may help to manage parasite infections and increase of bison fitness.

[23] Helminth parasites of cervids (roe and red deer) in France: a review of 30 years of survey

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Helminthological studies of parasitic community have often been done in many countries to estimate the potential reservoir for domestic ruminants. In Europe most studies have been carried out in sheep grazing mountain areas where chamois (*Rupicapra rupicapra*) and mouflon (*Ovis musimon*) are frequently observed. Since the 1980s a regular increase of roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) populations has been observed in France. Hence, the presence of deer must be integrated in parasite host-specificity investigations in all landscapes, especially in lowlands. Breeders worry about this situation and consider cervids as a potential reservoir of parasites involved in maintaining helminth infection in domestic ruminants (e.g., liver flukes, *Paramphistomum* spp and gastrointestinal nematodes such as *Haemonchus contortus* and *Oesophagostomum* spp.). On the basis of morphological and molecular approaches we review worm parasites found in roe and red deer in France and discuss particular situations noted in different regions and with the helminthofauna of domestic ruminants. The acquisition of new species such as *Ashworthius sidemi* and *Oesophagostomum sika* introduced via sika deer (*Cervus nippon* sensu lato) can be considered as tracers and markers of host population movements caused by their natural expansion or human impact. Financial support was provided by ONCFS (French game and wildlife agency) and by FNC (National federation of hunting). This work would not have been possible without the help of experts in parasitology who taught me the diagnosis of helminths, my colleagues and students in our laboratory, and agency staff who participated in sample collection.

[24] Nematode parasites of deer used as biological markers: studying population genetic structure of *Spiculopteragia spiculoptera* to map the social network of his hosts

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Following movement of wild species and understanding their population structure is not easy. Methods previously used (radiotracking, capture and analysis of host DNA) require a lot of resources and are often difficult to implement. Here, we employed a different method, tracking parasites to study movements, contacts, and population structure of their host species. Wild animals carry various endoparasites with a simple cycle requiring contact between their hosts to be transferred. Hence, sampling is much easier since one host contains many parasites. Moreover, because lifespan of parasites is shorter and reproduction frequency higher than that of their host, the turnover of their generations is faster, allowing us to observe quickly population structure modifications resulting from environment modifications. In the context of IN SITU program, we studied the helminthofauna from abomasum and caecum of roe deer (*Capreolus capreolus*) from eight sites in Champagne-Ardenne region (France). We inventoried all nematodes present and compared the parasitic community at each site in order to observe potential connections. Then, we performed a population genetic analysis on *Spiculopteragia spiculoptera*. Twelve new polymorphic microsatellite markers specific for this nematode were first developed, using the Next Generation Sequencing method, which were pooled in two multiplexes to analyse all samples. Studying the population structure of *Spiculopteragia spiculoptera*, we were able to infer contacts between roe deer, and map their social network. Financial support for this study was provided by Region Champagne-Ardenne as part of IN SITU program, and also by ONCFS.

[25] Gastro-intestinal parasitism in a population of Mediterranean mouflon (*Ovis gmelini musimon* x *Ovis* sp.) in France

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Gastro-intestinal parasites are common in wild ruminants. Most of the time, no particular sign of infection can be noticed. However, when the equilibrium between host-parasite-environment is altered, parasites can lead to poor body condition and reproductive success, and sometimes to death. Monitoring of gastro-intestinal parasites could thus be used as a measure of the relationship between a population and its environment. During spring from 2010 to 2014, we trapped wild Mediterranean mouflon (*Ovis gmelini musimon* x *Ovis* sp.) inhabiting the Caroux-Espinouse area, in South France, and collected more than 400 faecal samples. Faecal flotation was performed to isolate parasite eggs and cysts. The most prevalent species of parasites were the gastro-intestinal strongyles and *Eimeria* sp. We also isolated eggs of *Trichuris* sp., *Moniezia* sp. and of Trematodes (mostly *Dicrocoelium dendriticum*, but some individuals were infested by *Fasciola hepatica*). Cysts of *Giardia duodenalis* were also isolated, mostly in young lambs. Parasitism was mostly influenced by age and body condition of their host, with young and poor body condition individuals being the most parasitized. A higher sensitivity of lactating vs. non-lactating females to parasites was also noticed. Lastly, we focused on the relationships between parasites and habitat closure, and on the consequences of these parasites on population dynamic.

[26] Genetic diversity of *Echinococcus multilocularis* – comparative results from mitochondrial and microsatellite markers

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Echinococcus multilocularis is the causative agent of alveolar echinococcosis. The lifecycle involves canids as definitive hosts and small mammals as intermediate hosts. By analysis of microsatellite markers, high genetic diversity was detected in Europe which allows proposition concerning spatial-temporal spread of the parasite. With mitochondrial marker genes major clades have been identified, but only low genetic diversity was found. No conclusion concerning epidemiology was possible. To evaluate the use of mt markers for the description of

genetic diversity within small geographical units, a systematical analysis of samples with both systems was done to compare their discriminative potential. Around 540 samples from 7 European countries were analysed by partial sequencing of the mitochondrial genes *cox1*, *nd1* and *atp6* (~1600 concatenated bp's) and by analysis of EmsB profiles. In total, our samples contained 30 different EmsB profiles, with the highest diversity in the core area, whereas the number of profiles decreased towards the periphery. Across the mt genes >50 haplotypes were identified. Here the diversity was dispersed unequal across Europe. No obvious correlation was found between EmsB profiles and mt haplotypes, although some „pairs“ seem to be associated. Distribution of both ms profiles and mt haplotypes are highly uneven across Europe, but few variants occur only in one country.

[27] Long-term use of copper oxide wire to control parasites in hoofstock at the Johannesburg Zoo

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Copper oxide particles (CON, Copinox, Bayer, UK) have been used to treat internal parasites in both domestic and wildlife. Studies have shown a copper bolus to be an alternative treatment for anthelmintic-resistant *Haemonchus* infections in goats. Anthelmintic resistance resulting in ill thrift and death has been occurring primarily in four hoofstock species at the Johannesburg Zoo: Arabian oryx (*Oryx leucoryx*), scimitar-horned oryx (*Oryx dammah*), bontebok (*Damaliscus dorcas dorcas*) and aoudad (*Ammotragus lervia*). Anthelmintic resistance is an immense problem for zoos where rotation of enclosures is often not possible. Treatment of antelope with anthelmintic is often challenging as the animals cannot be handled and treatment has to be administered either remotely or in feed. Copinox boluses have been used to reduce *Haemonchus* spp and other nematode infections. The use of Copinox has resulted in reduced mortality and less frequent use of anthelmintics.

Session 5

[28] Molecular detection, genetic and phylogenetic analysis of trypanosome species in uMkhanyakude district of KwaZulu-Natal Province, South Africa

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African animal trypanosomosis is a disease caused by protozoan haemoparasites of the genus *Trypanosoma*. In South Africa, the disease is restricted to the north-eastern parts of KwaZulu-Natal Province. The current study aimed to determine the prevalence, genetic diversity and to conduct phylogenetic analysis of trypanosome species in KwaZulu-Natal Province as well as to determine preferred feeding host from tsetse fly bloodmeal. A total of 296 blood samples were collected from the north eastern parts of KwaZulu-Natal Province: 137 from cattle, 101 from goats, 9 from sheep and 49 from dogs, respectively. In addition, 376 tsetse flies (375 *G. brevipalpis* and 1 *G. austeni*) were also collected. Of the 137 cattle samples, 32 (23.4%) were positive by PCR for the presence of trypanosome infections. None of the samples collected from sheep, goats and dog tested positive by PCR. A total of 54/375 (14.4%) *G. brevipalpis* tested positive by PCR for trypanosomes. Detected species in both blood and tsetse flies included *T. congolense*, *T. theileri* and *T. b. brucei*. The genetic analysis of 18S rRNA and gGAPDH genes revealed that there is a high degree of genetic diversity amongst South African trypanosome species. Phylogenetic analysis from these two genes revealed that South African trypanosomes are genetically related to East African trypanosomes. They form isolated clusters when compared to each other, however, indicating that they are different genotypes. Bloodmeal analysis showed that *G. brevipalpis* preferred to feed on small mammals, birds and humans in the absence of livestock or other large wild mammalian hosts.

[29] Application of serological assays, ELISA and ICT for diagnosis of animal trypanosomosis in uMkhanyakude district of KwaZulu-Natal Province, South Africa

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A total of 231 serum samples were collected from sheep (n = 9), goats (n = 99) and cattle (n = 123) in the north eastern part of KwaZulu-Natal Province, South Africa. Trypanosome infection detection was conducted with two crude antigen (TbbCA and TcoCA) ELISA assays. Furthermore, recombinant antigen (TeGM6-4r) ELISA and immunochromatographic test (TeGM6-4r ICT) assays were also used. The crude antigen-based ELISAs detected 63/231 (27.3%) trypanosome-positive samples, whilst 67/231 (29%); and 46/231 (19.9%) were positive by TeGM6-4r-ELISA, and ICT, respectively. Trypanosome infection prevalence was the highest in cattle at 24.4–46.3% followed by sheep at 0–44.4% and lowest in goats at 0–9.1%. Detection performance of both crude and recombinant antigen ELISAs was relatively similar; both tests are recommended for reference diagnosis and large-scale epidemiological surveys. The ICT has a potential for on-site diagnosis application because of its simplicity but its sensitivity still needs to be improved.

[30] Molecular characterization of vaccine candidates from *Anaplasma marginale* strains in South Africa

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Bovine anaplasmosis is a tick-borne disease caused by the intra-erythrocytic rickettsia *Anaplasma marginale*. A blood-based vaccine is used for control of anaplasmosis though it does not offer protection against heterologous challenge by all *A. marginale* field strains, and has potential to transmit emerging diseases. Outer membrane protein (OMP) preparations of *A. marginale* have been shown to induce immune protection in nearly all animals tested, thus demonstrating the potential efficacy of a subunit vaccine. Eight OMP vaccine candidates have been identified from North American *A. marginale* strains, but it is not known if they are sufficiently conserved to be broadly useful worldwide or if vaccine development based on regional pathogen strains is necessary. Primers were designed and used to amplify DNA sequences for two of these OMP genes, Am779 and Am854. Genomic DNA was extracted from *A. marginale*-positive samples obtained from Gauteng, Mpumalanga and KwaZulu-Natal provinces, which were genotyped using the msp1a gene, and the most diverse *A. marginale* strains were selected for further characterization. PCR products obtained for Am779 and Am854 were cloned and sequenced. The South African Am779 gene sequences were very similar to the Am779 sequence of the US *A. marginale* St. Maries strain, with the exception of five Single Nucleotide Polymorphisms (SNPs). Three SNPs were identified between the South African Am854 sequences and the Am854 sequence from the St. Maries *A. marginale* strain. Six other OMP genes are currently being amplified and sequenced from the South African samples to enable comparative sequence analysis with U.S. strains.

[31] Molecular detection of *Anaplasma marginale* and *A. marginale* subspecies *centrale* in cattle in South Africa

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Bovine anaplasmosis is a potentially fatal tick-borne disease of cattle caused by the intra-erythrocytic rickettsia, *Anaplasma marginale*. It is widely distributed around the world; in South Africa it is endemic in most of the cattle-farming areas. *A. marginale* subspecies *centrale* causes a milder form of anaplasmosis in cattle and has been used as a vaccine in many parts of the world. Quantitative polymerase chain reaction (qPCR) is more sensitive and specific than conventional and serological methods in the detection of these organisms in infected hosts and vectors. We assessed previously described qPCR assays for the detection of *A. marginale* and *A. marginale* subsp. *centrale* in cattle samples originating from different areas in South Africa. *A. marginale* was detected in 97% of 75 known *A. marginale*-positive samples using the monoplex qPCR assay, and in 88% of 259 field samples. *A. marginale* subsp. *centrale* was detected in 32% of 114 field samples. Of 25 samples analysed using the duplex assay, 96% and 28%, respectively, were positive for *A. marginale* and *A. marginale* subsp. *centrale* while 28% had mixed infections. Six (24%) samples that tested negative for *A. marginale* subsp. *centrale* using the duplex assay were positive using the monoplex assay, albeit with high (>35) Cp values. The qPCR assays detected all samples that were positive using the reverse line blot hybridization assay. Sequence analyses of the msp1b and groEL genes are in progress to assess variation in these genes in *A. marginale* samples from cattle in South Africa.

[32] Molecular detection and characterisation of *Anaplasma* species in African buffalo (*Syncerus caffer*) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa

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Bovine anaplasmosis, caused by *Anaplasma marginale*, is the most prevalent tick-borne disease of cattle worldwide and causes significant economic losses in the livestock industry. *Anaplasma marginale* subspecies *centrale* is a closely related taxon of low pathogenicity that is capable of inducing protection against *A. marginale*. The African buffalo is the natural reservoir host of various tick-borne haemoparasites of veterinary importance. In this study, the occurrence of *A. marginale* and *A. marginale* ss *centrale* in buffalo (n=91) from two geographically isolated national parks in South Africa (Kruger and Hluhluwe-iMfolozi) was determined using the reverse line blot (RLB) hybridization assay, quantitative real-time PCR (qPCR) and conventional PCR targeting four different genes (*mosp1a*, *mosp4*, *groEL* and *16SrRNA*). RLB results indicated that 8% of samples were positive for the presence of *A. marginale* DNA, while 6% were positive for *A. marginale* ss *centrale* DNA. These samples also tested positive for *Ehrlichia*, *Theileria* and *Babesia* species. The qPCR assays indicated that 66% of the samples were positive for *A. marginale*, while 18% showed co-infection with both species. No *A. marginale* ss *centrale* single infections were detected. The PCR was used to amplify the four genes to generate sequence data and sequence differences will be discussed. Our results indicate that *A. marginale* and *A. marginale* ss *centrale* are prevalent in African buffalo populations in South Africa which suggests that buffalo are natural reservoirs of *Anaplasma* species infection and could play an important role in the epidemiology and spread of anaplasmosis to the livestock industry.

[33] Molecular detection of an *Anaplasma* sp. strain in domestic dogs in Mnisi, South Africa

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Some *Anaplasma* spp. are Gram-negative tick-borne zoonotic obligate intracellular parasites of dogs, predominantly found in the Americas. The aim of this study was to screen for haemoparasites in blood samples collected from domestic dogs in the Mnisi area of Bushbuckridge, Mpumalanga Province, South Africa. A total of 141 blood samples were collected from October 2011 through May 2012 and DNA screened for the presence of *Ehrlichia*, *Anaplasma*, *Theileria* and *Babesia* species infections using the reverse line blot (RLB) hybridization assay. The majority of samples reacted with the genus-specific probes for *Ehrlichia/Anaplasma* 70/141 (49.6%). Haemoparasite DNA could not be detected in 51/141 (36.1%) of samples. Sequences of the V1 hypervariable region from the 16S rRNA gene of selected *Ehrlichia/Anaplasma* positive samples were analysed and revealed an *Anaplasma* sp. South African dog strain, which is most closely related to *Anaplasma phagocytophilum* (98%). This finding reaffirms previous reports that *Anaplasma* sp. closely related to *A. phagocytophilum*, the cause of canine granulocytic ehrlichiosis and human granulocytic anaplasmosis in the United States and Europe, does occur in dogs in South Africa and its detection at a wildlife/livestock interface heightens the potential of transmission to humans.

[34] Characterization of *Babesia rossi* genotypes in dogs diagnosed with canine babesiosis

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Babesia rossi is a virulent tick-transmitted parasite responsible for causing canine babesiosis in dogs. Canine babesiosis still remains the cause of mortality and morbidity in dogs in South Africa. Preliminary results have suggested that there might be a link between the parasite genotypes and the disease phenotypes. Therefore, the aim of this study was to determine *B. rossi* genotypes in dogs diagnosed with canine babesiosis. Ten blood samples were collected from sick domestic dogs presented at the Onderstepoort Veterinary Academic Hospital. Six dogs were clinically classified as uncomplicated cases and four as complicated cases. DNA was extracted from the blood samples and *B. rossi* infections were confirmed using the Reverse Line Blotting assay. Only 7 *B. rossi* genotypes were amplified from 7 blood samples using real-time PCR, followed by sequencing of these samples. Based on the sequence analysis, *B. rossi* genotype 28 was identified in 5 dogs and genotypes 19 and 29 were identified in two dogs, respectively. Our results are in agreement with previously published findings that *B. rossi* genotypes are associated with disease phenotypes in dogs diagnosed with canine babesiosis.

[35] Tick-borne pathogens of camels in Sokoto, Nigeria: Updating some host-pathogen associations

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Numerous herds of camels (*Camelus dromedarius*) are reared in northern Nigeria, mostly for meat production. Here, their wellbeing and productivity are constantly challenged by several tick species. At present, however, no information is available on the occurrence of tick-borne pathogens in this livestock species. This study aimed to fill this major gap of knowledge. In October 2008, whole blood samples were collected from 36 camels in Sokoto, North West Nigeria. Collected samples were spotted onto FTATM cards and, once in the laboratory, subjected to three simultaneous PCRs followed by RLBs targeting *Ehrlichia/Anaplasma* spp. and *Rickettsia* spp. 16S and *Theileria/Babesia* spp. 18S fragments. Following RLB, amplicons were sequenced to ascertain their molecular identity. Twenty-two samples (61%) were positive for *Ehrlichia/Anaplasma* catch-all probe, and three samples (8%) for *Theileria/Babesia* spp. catch-all probe. Three cases of co-infections were also found. All *Ehrlichia/Anaplasma* –positive samples were identified as *Anaplasma platys* and all the *Theileria/Babesia* ones as *Theileria ovis*. To the best of our knowledge, this is the first report of the detection of *A. platys* and *T. ovis* in camels worldwide and in sub-Saharan Africa, respectively. The relevance of this finding is enhanced by the close living of these animals with both dogs and small ruminants, which may also be targeted host species for these pathogens. Results will also be put in relation with the competent tick species (e.g. *Rhipicephalus* spp., *Hyalomma* spp.) potentially acting as vectors for these two microorganisms in the study area.

Session 6

Keynote paper [B]: The zoo within: Evolutionary ecology of parasite-host relationships

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Field parasitological studies consistently show the reality of polyparasitism in natural systems. Starting with an overview of the ecological and epidemiological determinants of parasite species richness in mammals, with particular attention on small mammals, we show how the level of polyparasitism affects not only host body condition, survival or reproduction but also host metabolism, genetics or immune investment. Indeed, studies investigating the determinants of parasite diversity (i.e. polyparasitism) have helped to build simple causal chains of explanatory factors linked to epidemiology, biogeography and ecology. Major determinants of parasite diversity are found to be host population size (or host density) and host species geographical range, and more rarely host longevity. When investigated in comparative analyses, host home range and shape of host geographical range have been also found likely linked with parasite species diversity. On the other hand immuno-ecological studies have emphasized the importance of trade-off among costly traits through the maintenance of the homeostasis, immunity being one of these costly traits and the way that organisms invest in resistance or tolerance against parasitism. Here, we present a simple framework linking factors of parasite diversity and life-history trade-offs, including immunity, supported by the results of comparative analyses. Based on this review, we emphasize that polyparasitism should be considered more systematically in wildlife to better appreciate the importance of parasite diversity in wildlife through the cumulative effects of polyparasitism on the ecology and evolution of their hosts. We emphasize the main issues. For a more generic approach, studies of host-parasite interactions may lead to insight into the framework of the metabolic theory of ecology. And a better knowledge of polyparasitism will find applications in conservation biology or for a better control of zoonotic infectious diseases.

[36] The importance of understanding marine parasite biodiversity in commercial fishes

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Recent surveys of parasites infecting commercially exploited marine fishes in southern Africa highlighted that the number parasitic species are grossly underestimated components of biodiversity and that the usefulness of these species in applied marine research is generally overlooked. Five species were examined from 2010 to 2013. Sardine (*Sardinops sagax*) harboured seven parasite species. Two of these, a digenean “tetracotyle”-type metacercaria infecting the eyes, and a coccidian, *Eimeria sardinae* infecting the testes, met criteria for biological tags. Subsequent studies on spatial and temporal variation in infection of the “tetracotyle”-type metacercariae supported a two-stock hypothesis for South African sardine. Cape horse mackerel (*Trachurus capensis*) harboured 29 parasite taxa, of which 10 were identifiable to species level. Fish from Northern and Southern Benguela populations hosted similar parasite assemblages, but significant spatial differences were observed in infection statistics of four species [coccidian *Goussia cruciata*, monogenean *Gastrocotyle trachuri*, nematode *Anisakis* sp., copepod *Lernanthropus trachuri*] supporting the known existence of two populations in the Benguela Ecosystem and the use of parasites in distinguishing fish stocks. Cunene horse mackerel (*T. trechae*) from the Northern Benguela harboured similar parasite taxa to *T. capensis*, but with notably lower infection intensity of especially *Anisakis* sp., suggesting the existence of subtle and interesting interspecies host-parasite relationships. Cape hakes (*Merluccius capensis* and *M. paradoxus*) harboured nine parasite taxa, of which two (a myxozoan, *Pseudoalatospora* sp., and a coccidian, *Eimeria* sp.) are new to science. These findings emphasise the importance of documenting marine parasite biodiversity in multidisciplinary research on fish population structure.

[37] The role of ectoparasites in Caribbean coral-reef food webs: insights from stable isotope analysis

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In coral reef systems, ectoparasites directly impact food webs through their role in cleaning symbioses and can contribute to trophic linkages between habitats. However, parasites are typically overlooked in food-web models. Gnathiid isopods are common ectoparasites of reef fishes. They have benthic life history stages, can be transferred between habitats via hosts, and can be eaten by cleaning organisms or other predators during free-swimming stages. By comparison, cymothoid isopods of the genus *Anilocra* attach to hosts as juveniles and remain attached to the host. By including these parasites in coral reef food-web models via stable isotope analysis, we gain a better understanding of their role in transfer of energy through coral reef ecosystems. Gnathiid isopods and *Anilocra* spp. were collected from caged or free-living (respectively) individuals of 5 reef fish species. To evaluate how fish-derived carbon (energy) is transferred from gnathiids to parasite consumers, we fed gnathiids from specific host fish to captive Pederson shrimp (*Ancylomenes pedersoni*) for 1 month. Fish tissue (heart, gill, muscle, scales, blood), gnathiids, *Anilocra* isopods, and shrimp were analyzed for stable carbon and nitrogen isotopes. Isopods had $d^{13}C$ and $d^{15}N$ values similar to their host, comparable to results from other host-parasite studies. Gnathiid-fed Pederson shrimp also had $d^{13}C$ values consistent with their food source and enriched in ^{15}N as predicted due to trophic fractionation. These results indicate that stable isotopes can be an effective tool in deciphering cryptic feeding relationships and the role of parasites in carbon transfer among coral-reef ecosystems.

[38] General disease and parasite survey of commercially important fishes of the Free State, South Africa

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Due to the mismanagement of fisheries, there has been a decline in marine resources and the South African government has increased efforts to expand inland aquaculture activities. This resulted in the building of the Gariiep fish hatchery in collaboration with the People’s Republic of China. The hatchery is to become a fingerling supply station. In addition, several smaller hatcheries were opened and will be used as outgrowth stations. The forecasted aquaculture activities will take place around the Orange-Vaal River and it is therefore crucial to understand the effects it will have on the system as a whole. *Oncorhynchus mykiss* (rainbow trout), *Clarias gariepinus* (sharp-tooth catfish/baber), *Oreochromis mossambicus* (Mozambique tilapia) and *Cyprinus carpio* (common carp) were identified as commercially viable, though *C. carpio* and *O. mossambicus* have been cited in the Government Gazette as invasive species. The fact that South Africans have not developed a culture of consuming freshwater fish as protein, means that the industry is still relatively small. It can be said with certainty that catfish, trout and tilapia are delicious fish to eat, while carp is bony but still palatable. Parasites of fish have the potential of causing mass mortalities in artificial environments like aquaculture, leading to economic loss. This study focuses on the parasites found associated with commercially important fishes of the Free State. Preliminary results of the parasitological surveys conducted during November 2012 to March 2013 include: monogeneans, a

variety of peritrichs and, alarmingly, aliens such as *Bothriocephalus acheilognathi*, *Trichodinella epizootica*, *Argulus japonicus* and *Lernaea cyprinacea*.

[39] Parasitism and the conservation of native freshwater fishes in south-western Australia

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The south west of Western Australia contains a depauperate, but highly endemic freshwater fish fauna, as well as a large number of alien fishes. Until recently, almost nothing was known about the parasites of these fishes. We describe here the parasites of freshwater fishes in south-western Australia, compare the levels of parasitism between native and exotic fishes and examine the relationship between environmental degradation and parasite diversity. Almost 1500 fishes of 18 different species (12 native and 6 exotic) were sampled. Forty-four putative species of parasites were found. Forty-two of these species appear to be native parasites, most of which have been previously undescribed. Many of these native parasite species are host specialists, suggesting that threats to the survival of native fish species may also threaten a number of native parasite species. The two parasite species which were confirmed as introduced, *Lernaea cyprinacea* and *Ligula intestinalis*, are generalists which have transferred to a range of native fish species, where they have been associated with severe pathogenic effects. Levels of parasitism and parasite diversity were significantly greater in native fish species than in exotic fish species, and this may contribute to an enhanced demographic performance and competitive ability in invading exotics. Levels of parasitism and parasite diversity in native fish species were negatively related to habitat disturbance, in particular to a suite of factors that indicate increased human usage of the river and surrounding environment.

[40] *Lernaea cyprinacea*: An exotic parasite on our native freshwater fish

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Australia has a depauperate, although highly endemic, freshwater fish fauna. In the extreme south-west of Australia there are only 11 native freshwater fish species, with 9 endemic to the region, giving the south-west the highest level of endemism of any drainage division in Australia. One of the biggest threats brought by the introduction of exotic species is the introduction of exotic parasites. Recent studies identified the exotic parasite *Lernaea cyprinacea*, in the south west of Western Australia on four native freshwater fish species: Western Minnow (*Galaxias occidentalis*), Western Pygmy Perch (*Nannoperca vittata*), Nightfish (*Bostockia porosa*) and Freshwater Cobbler (*Tandanus bostocki*). This project focused on the infectivity, intensity and behavioural differences of *Lernaea cyprinacea* infections on its natural host goldfish compared with the native freshwater fish, pygmy perch. Differences in infection rate and intensity of infection were compared among species using a generalized linear model approach, with tank and fish species nested within tank as effects. Pygmy perch were significantly more likely to be parasitised ($c2 = 21.88$, $P = 0.04$), with an overall prevalence (i.e. across all tanks) of 55.3%, compared to 26.5% for goldfish. There were no significant differences in the intensity of infection ($F = 1.15$, $P = 0.37$), with a mean intensity of 1.92 (± 0.34) for pygmy perch and 2.20 (± 0.55) for goldfish. Behavioural differences were also seen when comparing the two species. Goldfish tended to group together and peck at one another, whereas pygmy perch were more likely to isolate themselves once infected.

[41] The potential threat of two alien fish parasites to cyprinids in the Eastern Cape of South Africa

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Deliberate introduction of alien fish species and their associated parasites to South Africa has been highlighted as a concern to local fish biodiversity and health. Aim of this study was therefore to determine the effect of the alien *Lernaea cyprinacea* on the health of *Labeobarbus aeneus* in Wriggleswade Dam, Eastern Cape, South Africa. *Labeobarbus aeneus* were sampled with the aid of gill nets between July 2011 and March 2012 ($n = 30$). Fish were examined using a macroscopic fish health assessment protocol (FHA). Necropsy results gave a FHA score of 59.0 ± 12.13 indicating poor health. This score is considered as high, the normal range is a FHA score of 17 or less. High FHA scores were largely due to the skin lesions caused by high infestations of *Lernaea cyprinacea* (100% of *L. aeneus* infected with >35 *L. cyprinacea*), greater than normal range hematocrit values, discoloured

livers and ruptured eyes. Parasite-infected Wriggleswade Dam *L. aeneus* (1.22 ± 0.11) also had a significantly ($p = 0.001$) lower condition factor when compared to uninfected *L. aeneus* (1.63 ± 0.46) from in natural distribution range in the Vaal River. The high FHI score indicates that the *L. aeneus* from the Eastern Cape are not in a healthy state and the results indicated that the primary threat to the health of *L. aeneus* in Wriggleswade Dam is the presence of the alien parasite *L. cyprinacea*. The presence of this alien parasite might have devastating effects on other endemic cyprinids of the Eastern Cape.

Session 7

[42] The zebra's amazing parasite fauna

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Equids, including zebras, are host to 83 helminth parasite species with 64 belonging to the family Strongylidae. This paper focuses on the zebra parasite biology. In addition to a wide biodiversity of parasite species, large burdens in zebras are not unusual. For example, it is not uncommon to find 100 million small pinworms, *Probstmayria vivipara*, and 4 million pinworms, *Crossocephalus viviparus*, in individual zebras. This paper highlights parasitic research on 3 zebra taxa that inhabit southern Africa: Burchell's zebra (*Equus quagga antiquorum*), Hartmann's mountain zebra (*Equus zebra hartmannae*) and Cape mountain zebra (*Equus zebra zebra*). The parasites of 78 zebras were studied in South Africa and Namibia using standard parasitological methods. These comprehensive studies led to discovery of 8 nematode species [including 4 cyathostomins (small strongyles), 2 large strongyles and 2 habronematids], all new to science. Significant differences in cyathostomin biology including the encystment, migration and site of location were identified when compared with domestic horses. Furthermore, unique microbial communities were identified attached to the reproductive organs of female cyathostomins in zebras; and may be a possible biological control. Understanding biological differences of nematodes between equine host species is critical today because both large and small strongyles cause serious clinical disease. The small strongyles are considered the most life-threatening parasite in horses in North America. This paper highlights biological differences of zebra parasites, which may provide needed insights to better understand biology of the cyathostomins in horses.

[43] Diversity and phylogenetic relationships within the parasitic nematode family Acuariidae Railliet, Henry & Sisoff, 1912 (Spirurida)

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The family Acuariidae is a morphologically and biologically homogeneous group containing 45 genera and more than 300 species occurring worldwide in the oesophagus and stomach of various birds and, rarely, mammals. The taxonomy of the group is mainly based on the morphology of anterior cuticular ornamentations (cordons, collarette or ptilina), used to assign the genera to the three recognized subfamilies, i.e. Acuariinae, Seuratiinae and Schistorophinae. This group has never been the subject of a cladistic analysis and questions about its origin, evolution of the main phyletic lineages and their associations with the host groups as a result of co-evolution or colonization remain unsolved. The aim of the present survey is to propose a phylogenetic hypothesis for the relationships within the Acuariidae on the basis of morphological and molecular approaches. To implement this task, representatives of the three subfamilies, including parasites of birds and mammals and such characterized by aquatic or terrestrial life cycles, were studied by light and scanning electron microscopy in combination with a molecular analysis of the 28S gene. We describe the first phylogenetic results revealing relationships among the acuariid genera and comment on the possible scenarios of the evolution of their morphological characters and life cycles.

[44] Life history strategy and landscape characteristics influence parasite responses to habitat fragmentation

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Anthropogenic habitat use is a major threat to biodiversity and is known to increase the abundance of generalist host species such as rodents, which are regarded as potential disease carriers. Parasites have an intimate relationship with their host and the surrounding environment and it is expected that habitat fragmentation and different landscape types will affect parasite infestation levels. We investigated the effect of habitat fragmentation on the ecto- and endoparasitic burdens of a broad-niche small mammal, *Rhabdomys pumilio*, in the Western Cape Province, South Africa. Our aim was to look at the effects of fragmentation on different parasite species with diverse life history characteristics and to determine whether general patterns can be found. Sampling took place within pristine lowland (Fynbos/Renosterveld) areas and at fragmented sites surrounded and isolated by agricultural activities. All arthropod ectoparasites and available gastrointestinal endoparasites were identified. Host density and body size were larger in the fragments. Combined ecto- as well as combined endoparasite taxa showed higher parasite species richness in fragmented sites. Parasite abundance was generally higher in the case of *R. pumilio* individuals in fragmented habitats but it appears that parasites that are more permanently associated with the host's body and those that are host-specific show the opposite trend. To examine the effect of anthropogenic habitat change on helminth species infestation pattern in more detail, additional investigations were conducted in three different human linked landscapes (crop, livestock and urban fragments) and an overview of results will be given.

[45] Helminth diversity of *Rhabdomys* spp. collected in Fynbos and Savanna Biomes in South Africa

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Rhabdomys spp. occur across South and southern Africa, occupying a wide range of habitats. These small mammals are highly adaptable to environmental challenges and contribute significantly to biodiversity, food webs and maintaining ecosystems. In South Africa, current information regarding helminths of small mammals is mainly taxonomic and little is known of the ecology that drives helminth communities. The aim of the study is to describe the helminth assemblages and infection levels within these murids, trapped in the fynbos and savanna biomes, where *Rhabdomys* spp. exhibit a more social structure in fynbos than in savanna where they are more solitary. One hundred and forty-eight *R. pumilio* (fynbos, 2003-2004) and 144 *R. pumilio* and *R. dilectus* (savanna, 2012) were dissected and helminths that were recovered from the gastro-intestinal tracts identified. In total 10 nematode species, representing four orders, were recorded, i.e. *Heligmonina boomkeri*, *Heligmonina spira*, *Neoheligionella capensis* and *Trichostrongylus probulurus* (Strongylida), *Syphacia obvelata* and *Syphacia* spp. (Oxyurida), *Streptopharagus* sp., *Abbreviata* sp. and *Protospirura numidica* (Spirurida) and *Trichuris muris* (Enoplida). Two of the 10 nematode species occurred in both biomes. The overall prevalence of nematodes in *Rhabdomys* spp. was markedly higher in the fynbos biome (90.5%) than in savanna (54.9%) as was the overall mean abundance in fynbos = 347, (\pm SD 403.9) compared to savanna = 16, (\pm SD 40.9). Nematode species richness and prevalence in populations of *Rhabdomys* spp. from the two biomes seem to be controlled by the diverse vegetation and climatic conditions as well as the habits of the rodents.

[46] Multiparasitism in Saffron Finch (*Sicalis flaveola*) from Brazil

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The Saffron Finch *Sicalis flaveola* (Linnaeus, 1766) (Passeriformes: Thraupidae) is a common passerine species widely distributed in the Neotropical region, bred in captivity as a songbird. This study reports three different parasite species in one adult female Saffron Finch: two nematodes, one parasitizing the gizzard and the other in the blood vessels, and one trematode in the kidneys. The bird showed nonspecific neurological signs. Undigested seeds were observed in the faecal portion of the droppings and the direct faecal smear revealed the presence of embryonated, elliptical eggs with thin and translucent shells, compatible with Spirurida nematodes. Microfilariae were observed in stained thin blood smears. The bird died 47 days after the clinical examination and was necropsied. Viscera samples were used for imprint smears and fixed in 10% buffered formalin for histopathology. The imprint smears revealed several nematode larvae with low tissue differentiation and high cellularity, typical of

the Onchocercidae. In the histopathology, Onchocercidae microfilariae were observed in blood vessels of the brain, liver, lung, heart, pelvic limb extremities, and also in the perivascular space. The nematodes found in the gizzard, under the koilin membrane, were identified as *Acuaria mayori* (Spirurida: Acuariidae). The renal trematodes found parasitizing the collecting ducts are probably *Tanaisia* or *Paratanaisia* species belonging to the family Eucotylidae. References on parasites of these birds are uncommon. Once the clinical signs of parasitism are nonspecific, a full investigation is necessary in order to establish an appropriate treatment.

[47] Gapeworm infection in mammals of Central African forest

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Gapeworms of the genus *Mammomonogamus* (Syngamidae) are parasitic nematodes found in upper respiratory tract of ruminants, felid carnivores, elephants and primates including humans in tropical areas worldwide. Although several species were named based on morphology of adults, taxonomy of the genus is rather obscure and deserves a deep revision. The host specificity of *Mammomonogamus* is not clear, partially due to the fact that lifecycle of this parasite is not known, yet even existence of intermediate host has not been proven. In Dzanga Sangha Protected Areas, Central African Republic, we detected eggs of *Mammomonogamus* using basic coproscopic methods such as flotation and sedimentation in faecal samples of African forest elephants (*Loxodonta cyclotis*) and western lowland gorillas (*Gorilla gorilla gorilla*). Prevalence in elephants was quite low, varying between 12.5% and 27%, depending on the season, whereas in gorillas, prevalence varied between 60% and 90%, depending on the particular group of gorillas. *Mammomonogamus* has previously been recorded also in forest buffaloes (*Syncerus caffer nanus*) in the Central African forest. These three animal species share the forest environment very closely, which raises a question about possible interspecific transmission. Since it is so far not possible to distinguish particular species of *Mammomonogamus* based just on morphology of eggs, we applied molecular methods in order to obtain information on both ecology and taxonomy of this parasite. Preliminary DNA sequence data indicate that elephants and gorillas share the same species of *Mammomonogamus*.

Session 8

[48] Transmission of *Trichodina heterodontata* between fish and tadpoles

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Ciliophoran peritrichs comprise two sub-orders, the Mobilida and Sesselida, found associated as epibionts on a wide range of aquatic hosts. The mobiline peritrichs are active movers, gliding over the surface of their hosts, whilst the sessiline peritrichs are attached to the host by means of a scopula, some stalked and in colonies and others solitary. It is not uncommon to find a host harbouring representatives of more than one species of both peritrich groups. Species of both groups display varied degrees of host specificity. Some are specific to a few related host species whilst others appear to be non-specific with a wide host range and geographical distribution. One such species is the mobiline peritrich *Trichodina heterodontata* Duncan, 1977 originally described from cichlid fishes from the Philippines and in the mid 1980s also found on a broad spectrum of host fishes in South Africa, Israel and Taiwan. Later we also found it associated with tadpoles of the platanna, as well as other frogs and toads. Recently a comprehensive study was undertaken on material collected at 35 localities from fish and tadpoles. The results confirmed that all the trichodinids from all the localities are the same species *T. heterodontata*. This paper reports on the results of transmission experiments to determine if naïve tadpoles could be infested with *T. heterodontata* from fish hosts and *vice versa*. All the tadpoles also harboured an infestation of a *Vorticella* sp. Linnaeus, 1767 which did not transmit to the fish.

[49] Peritrich (Ciliophora) diversity on cichlid fingerlings in the Okavango River, Botswana

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The subclass Peritrichia contains two orders, i.e. Sessilida and Mobilida. The highly mobile mobilines have a prominent aboral adhesive disc, while sessilines are permanently attached to the host with a scopula. While peritrichs occur on fish of all ages, infestation levels are very low and sporadic in older fish. In fingerlings and small fish that tend to school peritrichs are far more common symbionts. During fish parasitological surveys in the Okavango River in Botswana, a large number of trichodinid (Mobilina) and sessiline species have been found on especially young cichlid fingerlings. Surveys spanned a period of more than 15 years. Trichodinids from these cichlid fingerlings are represented by three genera. Some of these species show very little host specificity, such as *Trichodina heterodontata*, while other species, such as *T. minuta* and *Hemitrichodina robusta* are more host specific. A spectrum of sessilines representing two families and three genera have also been collected from cichlid fingerlings, with most of these representing new species. Peritrichs show very little harm to their hosts as all species are filter feeders. Only a small number of trichodinid species have been implicated in mortalities under aquaculture conditions where the damage was mostly due to adhesion to the host's epithelium. Sessilines are recognised as ectocommensals rather than ectoparasites as their attachment never harms the host's epithelium.

[50] Population genetics of *Achtheinus pinguis* Wilson, 1912 from different elasmobranch host species

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Even though numerous population genetics studies have been done on members of the class Copepoda, few of these studies focused on the population genetics of symbiotic copepods. One of the genera of the family Pandaridae is the genus *Achtheinus* consisting of three species (*A. oblongus* Wilson, 1908; *A. dentatus* Wilson, 1911 and *A. pinguis* Wilson, 1912) with *A. pinguis* being the only species previously recorded from the Indian Ocean. Population genetics focuses on evolution and the role played by the evolutionary forces in gene flow and shaping of the genetic structure of populations. This study attempts to investigate if there is any population structure between *A. pinguis* individuals collected from different elasmobranch host species. Collected copepods preserved in 70 % ethanol, from different elasmobranch host species, were used to extract DNA where after a 670 bp fragment of the mitochondrial gene, cytochrome oxidase I (COI), was amplified through PCR using primers LCO 1490, HCO 2198, LCO 1496 t1 and HCO 2198 t1. The haplotype and nucleotide diversities indicated little diversity among the in-group individuals. Similarly, the estimated pairwise sequence divergence values among the in-group taxa falls within the range of values estimated for intraspecific levels while those between the out-group and in-group taxa falls within levels expected for interspecific relationships. The estimated topologies showed no specific population structure among the in-group taxa in relation to the host and/or location from which the hosts were caught thus indicating a panmictic population of *A. pinguis*.

[51] How *Achtheinus* spp. Wilson, 1908 females attach to their elasmobranch hosts and the effects on the hostSusan Dippenaar¹, Anine Jordaan²¹*University of Limpopo, Sovenga, South Africa,* ²*North-west University, Potchefstroom, South Africa*

Achtheinus sp. (Pandaridae) is a copepod parasite found attached to the fins, gill slits and around the nostrils of their elasmobranch hosts. They are cosmopolitan in their distribution in terms of hosts and localities. Currently there are three accepted species with only *A. pinguis* being reported from the Indian Ocean. When they are removed the damage that they caused to the host's skin is clearly visible. Several specimens were collected with some still attached to the skin and others with placoid scales still attached to the maxillipeds. These were examined using histological sections and scanning electron microscopy to determine their way of attachment to the host and the effect on the host. They insert their antennae deep into the dermis of the hosts' skin which cause the most damage due to cell compression, proliferation and erosion of the dermal cells. Additionally, the presence of the copepod on the skin causes cell erosion of the dead epidermal cells and thus reduces the number of epidermal layers. The maxillipeds are used to attach to the placoid scales that cover the host's skin and probably serve to keep the copepod and inserted antennae into position. This is accomplished by the insertion of the placoid scales into the flaccid corpus of the maxillipeds.

[52] Symbiotic copepods associated with South African marine bony fish (Osteichthyes: Actinopterygii)

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The majority of South Africa's marine symbiotic copepods remain unidentified due to the scarcity of qualified taxonomists actively working on them. Symbiotic copepods are mostly found in the orders Siphonostomatoida and Poecilostomatoida with about 2200 known species in each. Previous reports included 89 species of copepods from 70 of the known 2500 marine bony fish species from South Africa. This study therefore aims to contribute to the limited taxonomic knowledge of the symbiotic copepods associated with marine fish in South African waters. A total of 46 infected host individuals representing 16 genera were collected from demersal and commercial prawn trawls off the coasts of South Africa and examined. Collected copepod specimens were fixed and preserved in 70% ethanol and studied with the stereo- and light microscopes. They represent both the Poecilostomatoida (families Bomolochidae, Chondracanthidae and Philichthyidae) and the Siphonostomatoida (families Hatschekiidae, Lernaepodidae, Pennellidae, Pseudocycnidae and Sphyrriidae). The collected copepods include species from nine different genera (*Nothobomolochus*; *Chondracanthus*; *Sarcotaces*; *Hatschekia*; *Parabrachiella*; *Cardiodectes*; *Cybicola*; *Lophoura* and *Sphyrion*). Seven out of the nine genera represent new geographical records and eight represent new host records.

[53] Siphonostomatoid copepods infecting *Squalus megalops* off South Africa

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The body of an elasmobranch offers a diversity of sites that can act as habitat to symbiotic copepods. Certain organs and organ systems of many elasmobranchs, such as the skin, digestive system and gills tend to host particularly diverse faunas of parasites. *Squalus megalops* is an indigenous, widely distributed species along the west, east and south coasts of South Africa. Copepods previously reported to infect this shark species include *Eudactylina vilelai*, *Pseudocharopinus bicaudatus*, *Trebius caudatus* as well as *Achtheinus pinguis*, *Perissopus dentatus* and *Lepeophtheirus longispinosus* from South African waters. In this study, hosts that were caught from several localities along the coasts of South Africa were examined for infection by symbiotic copepods. The collected copepod specimens were examined and identified using the wooden slide technique under both the stereo- and light microscopes and compared with previously described species. The prevalence, mean intensity and mean abundance were determined for collected copepods. *Squalus megalops* was infected by five genera of copepods, representing three different families (Eudactylinidae, Lernaepodidae and Pandaridae) of which three have not been reported before, namely *Eudactylina acanthii*, *Nemesis* sp. and *Lernaepoda* sp. *Achtheinus pinguis* exhibited the highest prevalence of all collected copepods with the highest prevalence of total copepods occurring along the west coast.

Session 9

[54] Scale-dependence of assembly rules in flea communities on two spatial scales: combining information on abiotic factors, host composition, geographic space, phylogeny and traits

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We investigated the role of environmental filtering as an underlying mechanism of assembly of compound communities of fleas parasitic on Palearctic small mammals at two spatial scales: a continental scale (regions across the entire Palearctic) and a regional scale (sampling localities within Slovakia). We used an expanded version of three table ordination that links species occurrences with geographic space, environmental variables, and species traits and phylogeny. We asked whether environmental filtering acts as an assembly rule of compound communities of fleas and, if yes, (a) whether the effect of environment on species composition of compound communities of fleas differs between spatial scales and (b) what are the relative importance of the abiotic and host environments. We found that compound communities of fleas are, to a great extent, assembled via environmental filters that represent interplay between filtering via abiotic environment and filtering via host composition. These

filters allows a community to contain only species that share certain phylogenetic affinities and/or certain adaptive traits that are necessary for persistence in that environment. The relative importance of these two components of environmental filtering differed between spatial scales. Host composition had a stronger effect on flea assembly than abiotic environment on the continental scale, while the opposite was true for the regional scale. The likely reason behind this scale-dependence is that communities on the regional scale are mainly governed by ecological and epidemiological processes, while communities on the continental scale are mainly affected by evolutionary, biogeographic and historical forces.

[55] Phylogeographic distribution of fleas associated with small mammals in South Africa

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Fleas are obligate arthropod ectoparasites of mammals and birds. They can act as vector for various pathogens which include the plague-causing bacterium, *Yersinia pestis*. Within Siphonaptera, different species show differences in life history and particularly also the level of host association which may affect their distribution at spatial and temporal scales. The aim of the study was to investigate the phylogeography of different flea species to gain more insights into the evolutionary and ecological processes governing the dispersal potential of fleas. Rodents and insectivores were trapped at 32 localities in South Africa. *Listropsylla agrippinae*, *Chiastopsylla rossi* and *Dinopsyllus ellobius* were found to be regionally widespread and locally most abundant. Mitochondrial (CO-II) and nuclear (EF-1 alpha) DNA sequences were generated and used for phylogenetic reconstructions. The mtDNA haplotype network of *L. agrippinae* indicated a strong geographic pattern with higher levels of divergence in the south-western part of the country. This pattern is congruent with previous observations for multiple host species, supporting a close correlation between parasite and host evolution. It further emphasizes the significance of the 'bedford-gap region' as a genetic hotspot in terms of conserving biodiversity. The other two flea species, *C. rossi* and *D. ellobius*, showed more complex patterns without clear geographic structure. Broad correlations were seen between locality specific genetic patterns and paleoclimatic oscillations. It is proposed that these changes in climate resulted in the expansion and contraction of suitable host habitat which influenced the ability of the latter two species to disperse across the landscape.

[56] Diversity of fleas on Southern African birds, new records for birds and the Limpopo Province

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In a three-year study of birds in South Africa, 989 birds, including 833 passerines and 156 non-passerines, were captured using mist-nets at 16 localities in four provinces, namely the Limpopo, North West, Northern Cape and Western Cape. Each bird was inspected visually and placed in a fumigation chamber; collected fleas were preserved in ethanol. Following DNA extraction, fleas were mounted as conventional mounts to be the voucher specimens to back the DNA. After PCR and sequencing, sequences were confirmed in GenBank. Three birds (all Passeriformes) harbored fleas; all records were collected during winter. Two flea species were found on three bird species: *Chiastopsylla rossi*, (Waterston, 1909): one male on a Rufous-vented Warbler (*Parisoma subcaeruleum*), one female on a Rattling Cisticola (*Cisticola chiniana*) both in Polokwane Game Reserve; and *Xenopsylla mulleri* DeMellion, 1947: one female on a Terrestrial Brownbul (*Phyllastrephus terrestris*) in Golwe. Both localities were in Limpopo province. All three records are new for these host species. *C. rossi* is the most common species of the genus and occurs throughout South Africa. *X. mulleri* has been reported from Botswana, Northern Cape (South Africa) and south-east Angola, but this is the first report in Limpopo. This flea is rarely found and is a parasite of the Highveld Gerbil (*Tatera brantsii*). These fleas do not normally occur on birds and their presence on them is likely an accidental association and birds could potentially disperse any fleas that stay on them long enough to be transported.

[57] Competitive interactions between ectoparasites of the eastern rock sengi (*Elephantulus myurus*)

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Infestations with more than one parasite species are the rule in wild animal populations. Since parasites rely on host resources and host size is finite, competition for both space and resources among parasites infesting the same host individual are likely. Although such competition has been shown under laboratory conditions studies data from wild hosts remain scarce and are often correlational. In the current study we monitored the ectoparasite community in a population of eastern rock sengis (*Elephantulus myurus*) from the Limpopo Province, South Africa, over 14 months. Half of the animals (125 individuals) were treated against ectoparasites with Frontline® while the remainder was left untreated. Changes in ectoparasite community as well as host body condition were recorded for short-term (i.e. days) and long-term (several months) intervals. Sengis harboured 10 tick, one mite (chiggers) and one louse species (*Neolinognathus elephantuli*) with two tick species (*Rhipicephalus warburtoni* and *Rhipicentor nuttalli*), chiggers and lice being the most prevalent and abundant. As short-term effect of our treatment the abundance of *R. warburtoni* was significantly reduced, chigger abundance increased while abundance remained unchanged for *Rc. nuttalli* and *N. elephantuli*. Conversely, long-term effects were only apparent for lice with louse abundance being significantly reduced by treatment. At the same time significant short-term improvements of body condition in treated animals were apparent that persisted over several months. These findings suggest direct competition between *R. warburtoni* and chiggers. Only short-term disturbances of the ectoparasite community were apparent while treatment benefits to the host were persistent.

[58] Increased infestation of Mediterranean mouflon (*Ovis gmelini musimon* x *Ovis* sp.) by ticks in FranceGilles Bourgoin^{1,2}, Mathieu Garel³, Antoine Mercier², Raphaël Massot^{2,5}, Marie-Thérèse Poirel^{1,2}, Christian Itty³, Jeanne Duhayer^{3,4}

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Ticks are major vectors of pathogens for wild and domestic animals, but also for humans. The impact of this parasite is rising due to higher local densities and larger spatial distributions of the different tick species. In this study, we focused on tick infestation in a population of wild Mediterranean mouflon (*Ovis gmelini musimon* x *Ovis* sp.) inhabiting the Caroux-Espinouse area, in South France. We trapped mouflon each year during spring since 2003, and counted on each mouflon the number of ticks attached on the head, armpit and groin. In addition, in 2010, 2012 and 2014, we also collected for identification all ticks attached to all mouflon trapped during 1–2 weeks. During this study period, we observed a strong increase of tick infestation in this population of mouflon. The number of ticks on mouflon was proportional to their body mass. Collected ticks were morphologically identified as nymphs and adults of *Haemaphysalis punctata*, *Ixodes ricinus* and *Rhipicephalus* sp. We also focused on the spatial distribution of the tick species according to the environmental conditions (vegetation, topography and local climate) in our study area. The high increase of the presence of ticks can be explained by higher densities of potential hosts (e.g., roe deer) and global warming.

[59] Oocyte maturation of the sloth's giant tick *Amblyomma varium* (Acari: Ixodidae) in an ecological context

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The sloth's giant tick *Amblyomma varium* Koch, 1844, a Neotropical species that inhabits tropical rainforests, is considered to be the largest tick reported so far. Its adult stage exclusively parasitizes mammals from the families Bradypodidae and Magalonychidae. These hosts are found in the treetops, descending only every three to eight days to defecate. This study aimed to analyse, in an ecological context, the oocyte maturation in this tick species. The ovaries dissected from three fully engorged females collected from *Bradypus variegatus* captured at Serra do Japi Biological Reserve, municipality of Jundiá, São Paulo, Brazil (23°13'S; 46°53'W) were processed for histological and transmission electron microscopy analysis, according to routine techniques. The ovary is a long single tubular organ with a large number of oocytes attached to the ovary wall by the pedicel. This tick species probably increases the reproductive capacity producing lots of eggs by increasing of the engorged female body size to compensate the difficult to meet its host. *Amblyomma varium* oocytes were classified into five development stages, and specific characteristics were observed: processes of yolk and chorion deposition starts early from the oocytes stage II; oocytes V with a very thick chorion (responsible for preservation of the egg); and eggs of moderated to large size. These are adaptations that enhance the survival and the reproductive success of this very host-specific tick, which is limited in a particular environment. Supported by FAPESP and CNPq.

[60] Impact of *Culicoides* species (Diptera: Ceratopogonidae) on wildlife

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It is well known that *Culicoides* biting midges can act as transmitters of disease-causing organisms that include viruses, protozoa and filarial nematodes. The main research focus in South Africa is primarily on the diseases caused by the viruses that can be transmitted by these midges. African horse sickness virus (AHSV), bluetongue virus (BTV) and equine encephalosis virus (EEV) can cause major outbreaks of disease mainly involving livestock such as equines and sheep. The role of zebra in the epidemiology of African horse sickness (AHS) has been documented and it was shown that zebra can act as cycling hosts of AHSV. But antibodies of AHS have also been found in the blood of camelids, bovids, elephants and various carnivore species. In the USA and Europe wild ruminants have been shown to be infected with BTV and outbreaks occur regularly. With the movement of wildlife across South Africa to areas where these animals normally do not occur, it is possible that the distribution of *Culicoides* species may change and new species be introduced to an area. In this presentation we look at the distribution of the known vector species and the species that utilise dung (wildlife and livestock) as a breeding medium to establish if any species are restricted to game areas. Long-term data are required to establish whether distribution patterns are changing.

[61] Historical overview of parasite research in Kruger National Park

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Parasite research dominated the ecological research taking place in Kruger in the late 1970s and early 1980s. At the time, more than 70% of the research registered in the KNP was either macro or micro-parasite research. These were largely descriptive studies and taxonomic inventories when very little was known about parasites of wildlife. Since then parasite research has been on a declining trend relative to other areas of research. In this paper, we will unpack the reasons for the decline in parasite research over the last two decades and highlight why this area of research needs re-invigoration within Kruger and more broadly throughout protected areas. We hypothesize that the conservation paradigm shift in the early 21st century from a "species" focus to a "process" focus and the

adoption of strategic adaptive management in SANParks, highlighted gaps in our understanding of savannah ecology, stimulating other areas of research. Past parasite research was aggregated to a few highly-productive individuals. These individuals were also KNP research department heads at the time and were able to drive the parasite research agenda internally. But we also know that modern parasite research is constrained by the need for large sample sizes, large research costs associated, shortage of trained taxonomists and the general lack of societal acceptance of lethal survey techniques. But we beg the question, can we afford to not be engaging in parasite research inside protected areas, when increased globalization, habitat transformation and climate variability are changing disease and parasites patterns throughout the world.

Session 10

[62] Ichthyophonosis in a multi-species exhibit in a public aquarium

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Ichthyophonus is a parasite of fish that belongs to the mesomycetozoa group of organisms. It has a worldwide distribution in marine fishes and low host specificity. It has caused disease in a variety of marine and estuarine fish and has been the cause of mass mortality in wild Pacific herring and been found to adversely affect spawning in Chinook salmon in the Yukon River. It is not frequently reported in the literature as affecting fish in public aquaria. *Ichthyophonus* spherical bodies were seen on wet mount of brain tissue from a Sand steenbras *Lithognathus mormyrus* that died after exhibiting neurological signs at the Two Oceans Aquarium. The presence of *Ichthyophonus* was confirmed by histopathology. A second Sand steenbras from the same exhibit displaying neurological signs and skin ulcers was euthanased and *Ichthyophonus* seen on wet mount examination of tissues post mortem. The prevalence of *Ichthyophonus* in this 211-fish, multi-species display was investigated and found to be 90.9%. Twenty-two fish of 6 species were sampled and the organism was found in the tissues of 20 fish. A very high density of spherical bodies was seen in some of the sampled tissues. None of the fish culled for sampling exhibited any discernable behavioural changes but some dark spots were observed on the skin of a number of Zebra fish, *Diplodus cervinus hottentotus*.

[63] *Kroyeria* sp. collected from *Galeorhinus galeus* with additional notes on all collected *Kroyeria* species and their parasite-host relationships

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Currently *Kroyeria* (Siphonostomatoida: Kroyeriidae) consists of 21 nominal species. These species infect the gill filaments of a wide variety of hosts belonging to the Carcharhinidae, Triakidae and Sphyrnidae. However, most species are relatively host-specific, infecting a single host or related group of host species. Previously *K. brasiliense*, *K. lineata* and *K. rrophemophaga* have been reported from *G. galeus*. Seventy *G. galeus* hosts caught by commercial fishermen along the west coast were examined for copepod infection. Collected copepods were fixed and preserved in 70% ethanol and examined using both stereo- and light microscopes. These specimens were dissected and drawn to be compared to the descriptions of the previously reported *Kroyeria* species. They share morphological features with *K. lineata*, *K. triakos* reported from *Triakis scyllium* (banded houndshark), *K. branchiocetes* from *Carcharhinus amblyrhynchos* (blacktail reef shark) and *K. cresseyi* from *Triakis semifasciata* (leopard shark). *Kroyeria* sp. can easily be distinguished from *K. lineata*, *K. branchiocetes* and *K. cresseyi*. However, they are quite similar to *K. triakos* and *K. rrophemophaga*. Comparing the illustrations made with the aid of drawing tubes revealed that *Kroyeria* sp. differs from *K. triakos* in the armature of the legs but are similar to *K. rrophemophaga*. From all collected species there are two new host records and two new geographical records. Prevalence values vary between 5.9 and 95.7% while mean intensity and mean abundance varies between 4 and 75 individuals and 0 and 71 individuals, respectively.

[64] Histopathological changes in the stomach of the southern mouthbrooder, *Pseudocrenilabrus philander philander*, naturally infected with the monogenean, *Enterogyrus coronatus*

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The southern mouthbrooder, *Pseudocrenilabrus philander philander* (Weber, 1897), is valued in fisheries. A total of 40 *P. p. philander* specimens were collected in April 2013 from Padda Dam, Johannesburg, transported to the laboratory and anaesthetized. Following examination, a stomach-inhabiting monogenean, *Enterogyrus coronatus*, originally described from *Tilapia guineensis* in Ivory Coast, was encountered. The monogenean occurred at a prevalence of 52.5% and mean intensity of 4.2. The histopathology of the stomach of this fish, naturally infected with *Enterogyrus coronatus*, was studied. Pieces of stomach tissue with parasites *in situ* were preserved in 70% ethanol. These specimens were prepared for scanning electron microscopy by dehydrating in a graded series of hexamethyldisilazane. Thereafter, the samples were coated with gold and studied with the aid of a JEOL 5600 microscope. Sections of tissue with parasites *in situ* were fixed and processed for routine histological investigation. The worms penetrated deep into the mucous membrane with their ornamented haptors, resulting in deep pits. This attachment resulted in acute mechanical effacement of the epithelium in the region of attachment. Desquamated cells mixed with mucus were encountered in close proximity to the attachment site. The changes observed were consistent with forceful penetration observed by Molnár *et al.* (2003), as evidenced by compression of the epithelial layer. This attachment resulted in injury as demonstrated by the presence of desquamated cells mixed with mucus and the disruption of the lamina propria. In areas more distant from the point of entry the stomach wall was covered by an intact epithelium.

[65] Parasites of the snoek (*Thysites atun*) in the southern Benguela

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Historically, the role of parasites in shaping ecosystems and determining the population structure of host species has been dismissed as unimportant. Following decades of research, however, parasites are now accepted as an important component of biodiversity. Worldwide, parasites are increasingly used as an early warning system for environmental pollution and as biological tags in fishery science. A survey of the parasite fauna of snoek (*Thysites atun*), an important species in the inshore linefishery, from the southern Benguela revealed 16 parasite taxa (Copepoda, Cestoda, Nematoda, Myxozoa). Amongst these, 10 were identified to species level and 11 taxa constitute new host records. Descriptive infection statistics for each parasite taxon are presented. Multivariate analysis (MDS, ANOSIM) of the parasite community was also conducted. Analyses revealed a seasonal increase in parasite diversity although the MDS and ANOSIM (Global R = 0.119, p = 0.035) suggested no significant difference in the community structure of snoek parasites in the southern Benguela. The seasonal pattern observed in parasite diversity could be explained by the acquisition and loss of parasite taxa as a result of the southward movement of snoek. The results of multivariate analyses suggest that snoek available to off South Africa constitute a single, nomadic stock. Future research should aim to compare the parasite assemblages of snoek from the southern and northern Benguela in an attempt to elucidate its stock structure in the South East Atlantic.

[66] Parasites of *Barbus* spp. (Cyprinidae) of Southern Africa

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Cyprinidae is the largest freshwater fish family worldwide with a cosmopolitan distribution, also occurring abundantly in most of southern Africa's water bodies. This fish family is represented by seven genera and 80 species in South African rivers, consisting of a few large fish species (more than 150 mm) of the genus *Labeo* and the yellow fish species *Labeobarbus*. The majority of the cyprinids are small (20–50 mm), however, and belong to the genus *Barbus*. Barbs have little commercial value and are thus overlooked by researchers. They are of great importance in the ecosystems, because they occupy a variety of niches within the aquatic habitats. Many are endemic, often restricted to a single river. Some are rare or endangered and at least two species are critically endangered. This study focuses on the parasites of *Barbus* in southern Africa. The aim was to expand our knowledge on the parasite biodiversity and to provide valuable information about potential threats to the barbs, especially alien species. Data on the fish parasites of *Barbus* spp. was collected within the Orange/ Vaal, Pongola and Okavango River Systems, and is being processed for better understanding of their taxonomy, distribution and

abundance. *Bothriocephalus acheilognathi* associated with cyprinid hosts, were introduced to Africa and are now found widespread in southern Africa's river systems, including in members of the genus *Barbus*. Other internal parasites including cestodes, acanthocephalans, trematodes and nematodes will be described and discussed, as well external parasites including peritrichs, monogeneans, myxozoans and copepods.

[67] Molecular detection of haemoparasites from South African squamates

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Haemoparasites are found in all vertebrate groups, worldwide. A large number of species have been recorded in squamates on the African continent. Relatively little work has been done on haemoparasites in South African squamates. This study will focus on blood infections of squamates by Apicomplexa (Levine, 1970) and Onchocercidae Leiper, 1911. Traditionally, malarial and haemogregarine species descriptions are based solely on morphologic characteristics of life stages in the peripheral blood, as well as novel records in new host species. Molecular detection is an essential method of differentiating between morphologically similar species. The aim of this study was to determine the molecular identity of members of the genus *Hepatozoon* (Miller, 1908), *Plasmodium* (Marchiafava and Celli, 1885), and Onchocercid infections occurring in squamates from South Africa. Bloodsmears were obtained from reptiles and DNA extractions were done by scraping fixed blood from Giemsa-stained bloodsmears. To date 39 samples have been collected and preliminary results indicated that (56.4%) of the samples were positive by microscopy for the presence of *Hepatozoon*, (38.5%) *Plasmodium* and (7.7%) filarial worm species. Thus far, 2.5% of samples were PCR positive for *Hepatozoon* species. A tentative phylogenetic tree constructed with the 18S rRNA gene sequence confirmed that *Hepatozoon* spp. belongs to the squamata clade as expected. Ongoing work includes PCR screening of *Plasmodium* and filarial nematode infections.

[68] Haemoparasites from captive and free-ranging leopards *Panthera pardus pardus* (Linnaeus, 1758) of the Free State, Mpumalanga and the Limpopo Provinces, South Africa

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The African leopard *Panthera pardus pardus* (Linnaeus, 1758) remained relatively unstudied until the early 1970s, and the majority of research up to date has been conducted on behavioural traits and feeding ecology. This paper aims to contribute to existing knowledge of leopard parasite diversity. During the initial phases of this ongoing study, blood was collected from nine leopards under sedation: three free-ranging and six captive individuals. Sampled leopards included melanistic, regular as well as one erythristic individual. Blood samples were obtained from adult males, females and cubs. A number of tick species, including members of the genera *Rhipicephalus*, *Haemaphysalis*, *Rhipicentor* and *Ixodes* were collected by hand. Giemsa-stained bloodsmears were inspected for haemoparasites. Preliminary molecular screening of blood samples indicated the presence of haemoparasites and the same samples were later molecularly screened for *Hepatozoon* and *Plasmodium* infections. *Hepatozoon felis* sequence data was obtained from all three free-ranging leopards and no *Plasmodium* DNA was detected by PCR techniques. Future work will include further molecular screening for related haemoparasites as well as relating parasitic infections with metabolomic analysis. Determining how the health of leopards, whether captive or free-ranging, is affected by parasites and different conservation management practices, may contribute to a holistic approach to leopard conservation.

[69] Helminth prevalence in captive primates of southwest Nigeria

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Primates are held in captivity around the globe in zoos for entertainment, conservation, education and research due to their psychological and physiological similarity to humans. Helminths are among the most pressing challenges affecting the health of primates. The study was conducted to assess the helminth infestation in captive primate of different species in 3 selected zoological gardens in southwest Nigeria. Forty-three freshly voided faecal samples were collected from captive primates and examined using direct wet mount, formal ether sedimentation and flotation technique. Out of 43 only 23 animals were found to be positive for helminth infestation. The study recorded 5 nematodes (*Trichuris trichuria*, hookworm, *Ascaris* sp., *Heterodera* sp., *Strongyloides* sp.) and 1 cestode

(*Taenia* sp.). Among the captive primate observed, *Papio anubis*, *Pan troglodytes*, *Erythrocebus patas*, *Cercopithecus aethiops*, and *Cercopithecus mona* monkeys were infected at different level with all the helminths recorded. This study shows that *Trichuris trichiura* had the highest prevalence (23.26%) followed by *Strongyloides* sp. (16.28%), hookworm (14.29%), *Ascaris* sp. (4.65%), *Heterodera* sp. (4.65%) and *Taenia* sp. (2.33%). In order to know the pathogenic role of identified helminths on captive primates and the zoonotic risk linked to their presence regular parasitological examination are necessary in order to prevent infection in the zoological garden because the identified helminthes are public health concern.

Session 11

Keynote paper [C]: The role of wildlife in the transmission of parasitic zoonoses in peri-urban areas

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The last decades have seen an unprecedented alteration of anthropogenic rural landscapes which has endangered many species of wild animals. In contrast, peri-urban areas in the industrialized countries have become increasingly attractive habitats for a variety of reasons, resulting in the paradox situation that heavily populated areas can be strongholds even for endangered species of vertebrates. Peri-urban areas are attractive because of the general "greening" of our immediate surroundings, the diversity of plant life, the presence of sheltering structures, the almost unlimited food supply for carnivores and omnivores and the increasing acceptance of wild animals. Species with adaptable behaviour benefit from this development, the main requirements being tolerance against disturbances by people, their pets, and traffic. Red foxes, raccoons and coyotes are now a common sight in many urban areas where they can reach far higher population densities than in rural landscapes. However, wild animals harbour parasites and the species composition and frequency of parasites in a given host species may differ drastically between urban and rural areas. Usually parasites with direct lifecycles are favored in periurban areas compared to those which need intermediate host species which have specific habitat requirements. Therefore the impact of a particular urban host animal on zoonotic disease transmission cannot be extrapolated from data obtained in rural areas. The situation gets more complex with regard to the role of pet animals, which may acquire parasites from peridomestic wildlife cycle and expose humans to disease agents which were not previously present in urban areas.

[70] Spatial heterogeneity and temporal variations in *Echinococcus multilocularis* infections in wild hosts in a North American urban setting

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Echinococcus multilocularis, the causative agent of human alveolar echinococcosis, has the potential to circulate in urban areas where wild host populations and humans coexist. The spatial and temporal distribution of infection in wild hosts locally affects the risk of transmission to humans. We investigated spatial and temporal patterns of *E. multilocularis* infection in coyotes and rodent intermediate hosts within the city of Calgary, Canada, and the association between spatial variations in coyote infection and the relative composition of small mammal assemblages. We examined *E. multilocularis* infection in small mammals and coyote faeces collected monthly in five city parks from June 2012 to June 2013. We analyzed coyote faeces (N=385) using a ZnCl₂ centrifugation and sedimentation protocol. We assessed infection in intermediate hosts (N=982) through lethal trapping and post-mortem analysis. Parasite eggs and metacestodes were morphologically identified and molecularly confirmed through species-specific PCR essays. We detected infection in 2/305 (0.66%) deer mice (*Peromyscus maniculatus*), 2/267 (0.75%) meadow voles (*Microtus pennsylvanicus*), and 1/71 (1.41%) southern red-backed voles (*Myodes gapperi*). Infections peaked in intermediate hosts in winter (3.33%), and in coyotes during spring (43.47%). Overall prevalence in coyotes was 21.42%, with clear variations across sites (5.34% to 61.48%). Relative composition of small mammal assemblages, rather than abundance of intermediate hosts *per se*, seems to shape parasite transmission and result in spatial heterogeneity of definitive host infection. We obtained the first evidence of a sylvatic life-cycle of *E. multilocularis* in a North American urban setting, and provided new insights into the parasite transmission ecology.

[71] *Rickettsia* diversity in small mammals from South AfricaSandra Essbauer¹, Mirja Hofmann¹, Sonja Matthee²¹Bundeswehr Institute of Microbiology, Department Virology and Rickettsiology, Munich, Germany, ²Stellenbosch University, Department of Conservation Ecology and Entomology, Stellenbosch, South Africa

Rickettsioses are recognized as emerging infections in several parts of the world. *Rickettsia conorii* and *R. africae* are known disease-causing pathogens of humans in several African countries, including South Africa. To date, little is known about the diversity of *Rickettsia* species that are naturally associated with small mammals in southern Africa. The aim of the study was to screen rodent and insectivore species for the presence of rickettsial DNA in South Africa. We sampled various small mammals in six different vegetation types (35 localities) in South Africa. In total 1616 small mammals belonging to 13 genera were tested using various molecular techniques. From the 1616 samples 251 (15.5%) were positive in the initial PCR. Prevalence values varied across localities with a few localities recording between 38.1–50.1%. Rickettsial DNA was detected in all the small mammal species that were tested. Several known *Rickettsia* species (*R. conorii*, *R. massiliae*, *R. felis* and *R. helvetica*) were obtained. In addition, six undescribed *Rickettsia* species were also recorded. In conclusion, a surprisingly high diversity of rickettsia in small mammals in South Africa was found that confirms the role of these animals as reservoir for rickettsia.

[72] Molecular detection and characterization of tick-borne Rickettsiales agents in wild Orinoco geese (*Neochen jubata*) in BrazilKarin Werther¹, Marcos André¹, Mayara Luzzi¹, José Roberto Alves Júnior², Eliane Sousa¹, Juliana Oliveira¹, Keyla Sousa¹, Rosangela Machado¹¹Faculdade de Ciências Agrárias e Veterinárias/ Universidade Estadual Paulista (FCAV/UNESP), Jaboticabal, SP, Brazil, ²Instituto Federal Goiano, Urutai, GO, Brazil

Recently, the role of migratory birds in the dispersion of tick-borne agents has been investigated. The present work aimed to investigate the occurrence of Rickettsiales agents in wild Orinoco geese (*Neochen jubata*) in Brazil, using molecular techniques. Between 2010 and 2013, blood samples were collected from 41 wild Orinoco geese in Luis Alves, state of Goiás, Brazil. DNA was extracted from goose blood samples and submitted to nested PCR assays for *Ehrlichia* spp. and *Anaplasma* spp., targeting rrs, omp-1 and groesL genes. Purified amplicons were submitted to sequencing and phylogenetic analysis. A multiplex 5' nuclease real-time PCR was performed for spotted fever (ompA) and typhus group (17-kDa lipoprotein gene) *Rickettsia*, and for *Orientia tsutsugamushi* (56-kDa outer membrane protein gene). *Ehrlichia* spp. rrs DNA phylogenetically closed to *E. chaffeensis* (97–100% of identity by BLAST) were detected in blood samples from 12 geese (five collected in 2010; seven in 2013). *Ehrlichia* spp. rs DNA phylogenetically closed to *E. canis* (97–100% of identity by BLAST) were detected in eight goose blood samples (two collected in 2010; six in 2013). *Ehrlichia* spp. omp-1 DNA phylogenetically closed to *E. canis* (97–98% of identity BLAST) was detected in blood samples from 4 geese collected in 2013. *Anaplasma* spp. rrs DNA phylogenetically closed to *A. phagocytophilum* (98–100% of identity by BLAST) were detected in blood samples from 3 geese collected in 2010. All samples were negative for *Rickettsia* spp. and *O. tsutsugamushi*. Orinoco geese may play a role in the dispersion of Anaplasmataceae agents in their migratory routes. Financial support: FAPESP

[73] Distribution patterns and predilection muscles of *Trichinella zimbabwensis* larvae in experimentally infected Nile crocodiles (*Crocodylus niloticus* Laurenti)Louis La Grange^{1,2}, Samson Mukaratirwa¹¹University of KwaZulu-Natal, Durban, South Africa, ²Department of Agriculture, Rural Development and Land Administration, Nelspruit, Mpumalanga, South Africa

No controlled studies have been conducted to determine the predilection muscles of *Trichinella zimbabwensis* larvae in Nile crocodiles (*Crocodylus niloticus*) or the influence of infection intensity on the distribution of the larvae in crocodiles. The distribution of larvae in muscles of naturally infected Nile crocodiles and experimentally infected caimans (*Caiman crocodilus*) and varans (*Varanus exanthematicus*) have been reported in literature. To determine the distribution patterns of *T. zimbabwensis* larvae and predilection muscles, 15 crocodiles were randomly divided into three cohorts of five animals each, representing high infection (642 larvae/kg of bodyweight average), medium infection (414 larvae/kg of bodyweight average) and low infection (134 larvae/kg of bodyweight average) cohorts. Predilection muscles in the high and medium infection cohorts were similar to those reported in naturally infected crocodiles despite changes in infection intensity. Results from this study show that, in Nile crocodiles, larvae of *T. zimbabwensis* appear first to invade predilection muscles closest to their release site in the small intestine before occupying those muscles situated further away. The recommendation for the use of

masseter, pterygoid and intercostal muscles as sampling sites for the detection of *T. zimbabwensis* in crocodiles is in contrast to the results from this study, where the fore- and hind limb muscles had the highest number of larvae. This study also supports the use of biopsy sampling from the dorso-lateral regions of the tail for surveillance purposes in both wild and commercial crocodile populations.

[74] First report of a mixed infection of *Trichinella nelsoni* and Genotype T8 in a leopard (*Panthera pardus*) from South Africa

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At least three *Trichinella* species (*T. nelsoni*, *T. britovi* and *T. zimbabwensis*) and one genotype (*Trichinella* T8) have been isolated from sylvatic carnivores on the African continent. With the exception of *T. britovi*, the other species are known to circulate in wildlife of the Kruger National Park (KNP), South Africa, and neighbouring game reserves (collectively known as the Greater KNP Area). Lions (*Panthera leo*) and spotted hyaenas (*Crocuta crocuta*) appear to be the most important reservoirs of *T. nelsoni* and *Trichinella* T8 in the KNP and surrounding areas. Interspecies predation between lions and hyaenas has been implicated as a primary mode of maintaining the life cycles of these two *Trichinella* species. We describe the first report of a mixed natural infection of *T. nelsoni* and *Trichinella* T8 in a leopard (*Panthera pardus*) from South Africa. The larvae were identified to species level by multiplex PCR. We also discuss probable sources of infection based on the known dietary preference and prey species' range of leopards. The described occurrence of *Trichinella* species in a leopard from the Greater KNP area raises the question of possible sources of infection to this predator species.

Session 12

[75] Review of parasitic crustaceans as definitive, intermediate and paratenic hosts, and as vectors

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Parasitic crustaceans are found among the Class Maxillopoda, Subclass Copepoda, and its constituent families. Further parasitic examples occur among crustaceans in the Class Ostracoda and particularly in the large Class Malacostraca, the latter containing, for example, the orders Amphipoda, Isopoda and Decapoda. In assessing the role of parasitic crustaceans themselves as definitive, intermediate and paratenic hosts, and as vectors, it is interesting to note that the relevant literature appears limited, but focussed on the following: Copepods of the genus *Caligus* and *Lepeophtheirus* (Copepoda, Caligidae) may be mechanical vectors or alternative hosts of a number of viral diseases between fishes and *Lepeophtheirus* may also transfer some bacteria between fishes. Ergasilids (Copepoda, Ergasilidae) parasitic on the gill filaments of fishes can support the replication of shrimp viruses, and likely act as viral vectors; they may also transmit lymphocystis virus of fishes. Branchiurans of the genus *Argulus* are thought to serve as mechanical vectors of several viruses to fishes, especially carp, as well as acting as intermediate hosts for dracunculoid nematodes of fishes. Barnacles (Thecostraca) on the carapace and gill filaments of crabs can also support shrimp viruses. Isopods of the genus *Gnathia* (Family Gnathiidae) likely act as definitive hosts and vectors of fish blood parasites of the genus *Haemogregarina*; they may also transmit some fish viruses, and may be paratenic or intermediate hosts for nematode larvae. Cymothoids (Isopoda, Family Cymothoidae) may transmit lymphocystis virus to fishes. This review will detail the examples cited above and identify areas of future research.

[76] Prey-to-predator isopod transfer: A previously unrecognized, but very important life cycle strategy in *Cymothoa* spp. and possibly other cymothoids and fish-associated isopods

Ernest H. Williams, Jr, Lucy Bunkley-Williams

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We believe we have solved 2 fish-parasitic isopod mysteries. Juvenile *Cymothoa* spp. in the mouths of small Caribbean and Pacific fishes are not a dead-end, as has been assumed, but a means of transferring the isopods to

appropriate predator hosts. This also explains how fast-swimming pelagic predators are infected by slow swimming *Cymothoa* juveniles. These transfers represent an unrecognized, but common life-cycle strategy infecting many commercially important fishes. We described what may have been the first published, natural prey-to-predator transfer of a cymothoid. We have transferred thousands of adult female and juvenile isopods. We have collected many specimens of small anchovies and herrings with *Cymothoa* spp. in their mouths. In Okinawa, we found hundreds of *Cymothoa* sp. in five small species of cardinalfishes. These juvenile isopods barely fit in the fishes' mouths and could never grow large enough for the isopods to mature. In aquaria juvenile *Cymothoa oestrum* infected the mouths of any small fishes. We demonstrated that fish-parasitic juvenile isopods react to an attack on their host with an escape mechanism. If juveniles react to an attack by escaping their brood pouch and isopods often abandon their hosts when captured, then juveniles in the mouths of small fishes may abandon their host and attach in the predator. Infecting slow-swimming anchovies, cardinalfishes, and herrings is relatively easy for free-swimming juvenile *Cymothoa*, as evidenced by an ~5% prevalence. Infecting large, fast swimming, often pelagic, final host directly seems almost impossible.

[77] Phylogeny of five species of *Anilocra* Leach 1818 (Isopoda: Cymothoidae) from Puerto Rico and the Virgin Islands

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Members of the genus *Anilocra* Leach 1818 are large external isopod parasites of a variety of coral reef and other habitat-associated fishes. In the Caribbean Sea, this genus is represented by nine species whose descriptions are based solely on their morphology. Their disjunct geographic distributions and host specificity suggest varying degrees of incipient speciation. This study used mitochondrial cytochrome c oxidase subunit 1 gene sequences to elucidate phylogenetic relationships of five species of *Anilocra* from Puerto Rico and the Virgin Islands through parsimony, maximum likelihood, and Bayesian inference. The results show that the Caribbean *Anilocra* species form a monophyletic group and are not closely related to *Anilocra physodes* from the Mediterranean Sea, the type species of the genus. Parsimony and Bayesian inference analyses recovered three clades: clade A (*A. chromis*), clade B (*A. holocentri*), and clade C (*A. acanthuri*, *A. chaetodonti*, *A. haemuli*), while maximum likelihood analyses only recovered clade A and C. These analyses depict *A. chromis* as the basal species and *A. chaetodontis* as the most recently evolved species of the Caribbean *Anilocra*. Phylogenetic reconstructions show population structure based on host for *A. haemuli* and based on geographical location for *A. chaetodontis*, which suggest that each of these species might represent cryptic species with morphological stasis.

[78] The potential role of parasitic crustaceans in feeding migrations and trophic connectivity in nocturnal reef fishes

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Many species of reef fishes in the families Haemulidae (Grunts) and Lutjanidae (Snappers) remain quiescent on reefs during the day and migrate to seagrass beds at night where they feed on benthic invertebrates and small fishes. It has been hypothesized that the advantage of such nocturnal migrations is an increase in food availability or a decrease in predation risk. We tested predictions of an alternative hypothesis that nocturnal migrations reduce exposure to blood-feeding gnathiid isopods. When departure and return times of grunts were compared with diel activity of gnathiid isopods, the departure of grunts from the reef coincided with increasing gnathiid activity, and fish returned to the reef just as gnathiid activity was declining. In field experiments, fish placed in cages and deployed on the reef at night harbored significantly more parasitic gnathiid isopods than those placed in the seagrass. However, this was not the case when the experiment was repeated during the day. These findings suggest that reduction in exposure to parasites constitutes at least one advantage of nocturnal feeding migrations in these fishes. However, the fact that they do not avoid infestation by gnathiids entirely, and that gnathiids are in turn eaten by other organisms, provides a means for ectoparasites to contribute to the transfer of energy between seagrass beds and reef. Future work will examine the extent of the role of gnathiids and other parasitic crustaceans in this trophic connectivity.

[79] Review of the fish parasitic genus *Ceratothoa* Dana, 1852 (Crustacea, Isopoda, Cymothoidae) from South AfricaKerry Hadfield¹, Nico Smit¹, Niel Bruce²¹North West University, Potchefstroom, South Africa, ²Museum of Tropical Queensland, Townsville, Australia

Cymothoid isopods are obligate parasites of both freshwater and marine fishes. They can be found on the external surfaces, gills or inside the buccal cavity of their fish host. Members of the genus *Ceratothoa* Dana, 1852, are located inside the buccal cavity of the fish hosts, attached to the tongue. *Ceratothoa* has long been considered to have three species in South Africa: *Ceratothoa imbricata* (Fabricius, 1775), *C. retusa* (Schioedte & Meinert, 1883) and *C. trigonocephala* (Leach, 1818). After comparisons of the type material of these species to material available in local museums, as well as freshly collected material from the south coast of South Africa, two main discoveries were made. Firstly, none of the material agreed with the descriptions of *C. imbricata* and *C. trigonocephala*, and no positive identification for these two species in South Africa could be made. These species are therefore excluded from the South African fauna. Secondly, fresh sampling revealed two species from the region new to science which were subsequently described as *C. africanae* sp. nov. and *C. famosa* sp. nov. With the removal of two species, plus the addition of two new species, the total number of *Ceratothoa* species in South Africa remains at three.

[80] *Chonopeltis* (Branchiura) species found on the genus *Labeo*

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The genus *Chonopeltis* is endemic to Africa, with 14 known species, all described from freshwater fish. Compared to the rest of the members of the class Branchiura, *Chonopeltis* species show a high degree of specificity to hosts, with some species restricted to a single host species and a specific river system. In contrast to some *Argulus* species, no record exists of any mortalities or pathology due to *Chonopeltis* infestations. Fishes that serve as hosts for *Chonopeltis* species include the endemic African families Mormyridae and Mochokidae, as well as the Clariidae and Cyprinidae. Six *Chonopeltis* species have been described from 11 cyprinid hosts. This paper deals with those species found associated with the genus *Labeo*. The high level of host specificity and selectivity of attachment sites suggest that these branchiurans have had a long evolutionary association with their hosts. Attention is given to the similarities and differences of *Chonopeltis* species found on these fish species as well as their distribution in the different river systems in Africa.

Session 13

[81] Parasites as sentinels of the environment – how far are we?

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Although chemical water analysis is extremely sensitive it is only accurate at the moment of sampling. It furthermore requires both highly trained technicians and costly apparatus. Changes in water quality may affect organisms and therefore a study of biological indicators may provide evidence of changes in water quality particularly if the incidents are sporadic. Fish are well-respected sentinels and the parasites of fish have also received attention in various laboratories. The effect on population structure, prevalence, infections rates and so on have received attention. In our studies we have recently concentrated on the use of parasites as accumulators of metals. We were able to show that the fish tapeworm *Bothriocephalus acheilognathi* accumulates metals at concentration many times more than the host, indicating that this organism has potential as an early- warning sentinel. Thereafter, the various sections of the worm were analysed to ascertain the effect of time on metal accumulation and also to determine the mechanism of excretion of metals. Recent studies concentrated on ectoparasites and in this regard *Argulus japonicus* and *Paradiplozoon vaalensis* were evaluated for metal accumulation. Both ectoparasites showed potential and this finding may eliminate the need to sacrifice hosts in future, particularly if the fish are red data species.

[82] Environmental Parasitology: understanding the interaction between parasites and pollutants

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In recent years there has been an increasing number of papers showing how parasitism and pollution can interact with each other in aquatic organisms. Apart from parasitological aspects these interactions are also important in terms of ecological and environmental research. The current presentation aims at identifying three promising directions for future research in the interdisciplinary field of environmental parasitology. 1. Parasites as sinks for pollutants within their hosts: Some parasites are able to reduce pollutant levels in the tissues of their host. The reduction of pollutants is an interesting implication since parasites might be beneficial to their hosts from this perspective. In other cases free-living accumulation indicators may erroneously indicate low levels of pollution if they are infected with parasites. 2. Parasites as a diagnostic tool to test bioavailability of substances. In order to take up and accumulate pollutants the substances have to be metabolized by the host in an initial step. Accordingly, the detection of substances within endoparasites is a sign for the biological availability of pollutants which justifies the establishment of parasites as new sentinel organisms for environmental monitoring. 3. Changes of biomarker responses of the host against pollutants. Parasites can alter physiological reactions of their hosts against pollutants in different ways. Therefore, studies examining the question whether exposure to certain chemicals affects the physiological homeostasis of a test organism have to consider parasites as important stressors.

[83] Trace elements in *Contraecum multipappilatum* (L3) in relation to its fish hosts from two Kenyan lakesElick O. Otachi^{1,2}, Christof Plessl¹, Wilfried Koerner¹, Annemariè Avenant-Oldewage³, Christine Fellner-Frank¹, Franz Jirsa^{1,3}¹University of Vienna, Vienna, Austria, ²Egerton University, Egerton, Kenya, ³University of Johannesburg, Johannesburg, South Africa

During a parasitological survey of fish from Lakes Naivasha and Turkana, Kenya, a high prevalence of the parasitic nematode *Contraecum multipappilatum* (L3) was recorded from the two fish species *Oreochromis leucostictus* (L. Naivasha, P = 51.8%, MI = 2.3 ± 1.6) and *Hydrocynus forskahlii* (L. Turkana, P = 83.7, MI = 46.0 ± 58.0). In *O. leucostictus* the parasite occurred exclusively in the pericardium, whereas in *H. forskahlii* it was aggregated in the body cavity and the intestine. The concentrations of 12 trace elements (Li, Al, Si, Mn, Fe, Cu, Zn, Rb, Sr, Mo, Cd and Pb) were determined in sediments, fish muscle, liver and the nematode. For both lakes sediments did not show any significant sign of pollution, except slightly elevated levels of Zn and Cd in Lake Naivasha. Compared to sediment concentrations, fish muscle showed a bioaccumulation factor (BAF) >1 for Li, Zn and Cd in Lake Naivasha and Li, Zn, Rb and Cd in Lake Turkana. The parasite showed BAF > 1 for Fe, Cu, Cd and Pb in relation to fish muscle from both lakes, with highest mean values for Pb: 7.0 (L. Naivasha) and 4.0 (L. Turkana). In relation to fish liver *C. multipappilatum* showed a poor accumulation ability, with no BAFs >1 from *O. leucostictus* and values >1 for Sr and Pb (1.6 and 3.2 respectively) from *H. forskahlii*. Compared to other intestinal helminths as acanthocephalans or cestodes, the bioaccumulation ability for the nematode of this survey must be called rather poor.

[84] Use of fish parasites as bio-indicators of heavy metals in South African marine ecosystemsThomas Morris¹, Stephen Lamberth³, Annemariè Oldewage²¹University of Cape Town, Cape Town, South Africa, ²University of Johannesburg, Johannesburg, South Africa, ³Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa

Changes in the diversity and structure of parasite communities of different fish hosts have received increasing attention due to the possible application of parasites as indicators of ecosystem integrity and health. Free-living invertebrates, notably bivalve molluscs, are commonly employed in this role as 'sentinel organisms' to monitor the concentrations of bio-available metals in aquatic ecosystems. Also certain parasites, particularly intestinal acanthocephalans of fish, can accumulate heavy metals to concentrations orders of magnitude higher than those in the host tissues or the environment. Concentrations of lead and cadmium in *Callorhincus capensis* (St Josephs shark) tissues (muscle, liver and intestine) were compared with those of selected parasites. Metal concentrations in the parasites were orders of magnitude higher than those in the host tissues or the aquatic environment. There does, however, need to be a greater accumulation of knowledge of the varying ecology and physiological effects of parasites to truly realise the effects of contaminants. Utilizing parasites as biological indicators to quantify the effect of anthropogenic impact on our aquatic resources is an important component in the measure of climate change. This can have direct implications for aquatic conservation as well as can be used in many commercial industries, for example marine fisheries. This study aims to identify parasitic biomonitors within the

marine environment of two anthropogenic impacted bays in South Africa and investigate if there is a parasite that can be used as a bioindicator for the measure of heavy metal toxicity.

[85] Eels (*Anguilla* spp.) in South Africa: distribution and parasite communities

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Globally eels of the genus *Anguilla* are of great economic importance. For stock enhancement and aquaculture live eels are traded transcontinentally which has led to the introduction of eel-specific parasites to novel areas and hosts. In South Africa we investigated populations of the two native eels *Anguilla mossambica* (n=375) and *A. marmorata* (n=73) from five rivers in the East Cape. In four habitats *A. mossambica* dominated over the latter species which mainly occurred in the coastal sections of the rivers and *A. marmorata* was more abundant than *A. mossambica* only in the Mngazi River in the Port St Johns area of the former Transkei. Each host species harboured a distinct parasite assemblage of eel specialists showing little overlap with that of the other. This seems to reflect a very long host-parasite co-evolution and little influx from abroad. Interestingly, the richness of the infra- and component communities was correlated with the dominance status of one or the other eel species. In *A. marmorata* the highest parasite richness was encountered in the rivers of the former Transkei. In addition we only detected one alien macroparasite, the monogenean *Pseudodactylogyrus anguillae*.

[86] Understanding parasite invasion success: Lessons learned from two *Anguillicola* species

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Anguillicola crassus is the most invasive species of its genus and it is a successful colonizer of different eel species worldwide. It has been so far the only species of the genus *Anguillicola* whose life cycle was studied completely under laboratory conditions. To analyse if life cycle characteristics may explain invasiveness success, we collected *Anguillicola novaezelandiae* from naturally infected short-finned eels (*Anguilla australis*) in New Zealand. Interestingly, infrapopulations in eels of the North and the South Island were dominated by larval parasite stages. This unique composition of infrapopulations has never been described for any *Anguillicola* species before and suggests a seasonal occurrence as a possible reason. Apart from studying life cycle characteristics of *A. novaezelandiae* under natural conditions European eels were infected under laboratory conditions with *A. novaezelandiae*. Again, *A. novaezelandiae* shows a more synchronised development in the European eel than *A. crassus*. No density-dependent effect in parasite development could be found for *A. novaezelandiae*. Finally, different stress markers such as serum cortisol and heat shock proteins were analysed in these eels following experimental *Anguillicola* spp. infections. Taken together, our results show that the life cycle of *A. crassus* differs on final host level compared to *A. novaezelandiae* in ways which result in a more successful invasion of new host species. However, if both *Anguillicola* species were experimentally introduced into a single eel, they can mature together in the same swim bladder. Furthermore, hybridization of *A. crassus* males with *A. novaezelandiae* females occurs as shown by microsatellite analysis.

[87] Parasites and fisheries

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Two aspects of the relationship between parasites and marine fisheries are presented: the effects of parasites on fisheries, and the effects of fisheries on parasites. Parasites can have negative effects on fisheries through loss of biomass due to pathogenic parasites, or through highly visible "spoilage" parasites reducing the market value of the product. While the effects of parasites on fisheries have been fairly well documented, little consideration has been given to date to the effects of fisheries on parasite biodiversity, largely because of the lack of historical data on infection levels. Until at least the late 1960s, the aspidogastrea parasite *Stichocotyle nephropis* was a common parasite of the decapod crustacean *Nephrops norvegicus* (intermediate host) and the elasmobranch fish *Raja clavata* (final host) in the Northeast Atlantic. In recent years, however, examinations of thousands of *N. norvegicus* and of 32 *R. clavata* have failed to find any *S. nephropis*. *Raja clavata* has been heavily overfished in this region since about the 1950s and is currently described in the IUCN Redlist as "Near threatened". The evidence suggests that *S. nephropis* may be extinct in the Northeast Atlantic, probably as a result of overexploitation of its final host.

The absence of any recent record of this parasite in the Northwest Atlantic, where its final host, the elasmobranch *Dipturus laevis*, is listed as “Endangered”, suggests that it may also be extinct there.

[88] Variation in population structure of *Paradiplozoon ichthyoxanthon* Avenant-Oldewage, 2013 infecting the gills of *Labeobarbus aeneus* (Burchell, 1822): The influence of an altered environment

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Alterations in water quality and increased metal concentrations influence population dynamics and infection biology of ectoparasites. Diplozoids are ectoparasitic Monogenea with direct lifecycles, infecting the gills of cyprinid fish hosts and are subsequently exposed to the macroenvironment. Study aims were thus to determine if there are noticeable alterations in infection biology of *Paradiplozoon ichthyoxanthon* infecting the gills of *Labeobarbus aeneus* and whether observed differences correlate to macroenvironment alterations. Sampling was undertaken during high and low-flow events at two sites along the Vaal River using gill nets. Gills were excised on dissection and *P. ichthyoxanthon* were removed while observing with a dissection microscope. At each site, water quality parameters were measured, and sediment and water samples were collected for metal analysis. Infections differed between locations, with a total absence of parasites at the Vaal River site. Water quality parameters were similar over both periods, except for oxygen content which was higher at the Vaal Dam site during the high-flow period. Metal analysis showed that most metals were present at higher concentrations at the Vaal River site, with aluminium being highest. Similarly increased metal and organic pollution have been associated with deformities in anterior and posterior body regions, and increased infection levels of other diplozoid parasites. Water quality between sites is comparable to previous work. The present study, however, reports on the absence of *P. ichthyoxanthon* from an area impacted by metal pollution. This finding possibly relates to elevated aluminium levels in the environment, to which other monogeneans are sensitive.

Posters: Aquatic Hosts

[201] Metazoan parasites of the Mozambique tilapia (*Oreochromis mossambicus*) from Massingir Dam, Mozambique

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Massingir Dam, situated in the great Olifants River, is located in the Gaza Province of Mozambique. The dam is the last impoundment along the Olifants River before it meets the Limpopo River and drains into the Indian Ocean. Mozambique tilapia (*Oreochromis mossambicus*) were collected from Massingir Dam and investigated for metazoan parasites. Monogeneans were mounted in either glycerine ammonium picrate or glycerine jelly. All other parasites removed were fixed and preserved according to standard methods for each parasite group. The recorded parasites included six different species. Ectoparasites comprised of *Cichlidogyrus* sp. (monogenean) from the gills and *Lernaea cyprinacea* (copepod) from the skin. Endoparasites comprised of *Contracaecum* larvae (nematode) and an unidentified digenean metacercaria from the body cavity as well as *Acanthogyrus* sp. (achanthocephalan) and gryporhynchid cestode larvae from the intestine. The current results represent new parasite distribution records for *O. mossambicus* in Mozambique.

[202] First record of metazoan parasites from *Clarias gariepinus* from the Bubi and Bubiana rivers in Zimbabwe

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The sharp-tooth catfish *Clarias gariepinus* (Burchell, 1822) is generally tolerant of extreme environmental conditions and therefore one of the preferred species for aquaculture. This fish species is widely distributed in the world after introduction from Africa and Asia. However, several countries reported adverse ecological impact

after introduction. Due to its importance a study on their parasites were conducted in Zimbabwe. *Clarias gariepinus* specimens were collected from the Bubi (n = 10) and Bubiana (n = 5) rivers in the southern part of Zimbabwe during February 2014. Standard methods were used for fixing and staining of parasites and they were identified based on available taxonomic keys. The following parasites were recorded: nematodes (L3 stage *Contracaecum* from the body cavity and *Procamallanus laevionchus* from the intestine), monogeneans (*Macrogryodactylus clarii* and *Quadriacanthus* sp. from the gills and *Gyrodactylus* sp. from the fins), echinostome digenean larvae from the gills, a branchiuran (*Dolops ranarum*) from the fins, copepods (*Lamproglana clariae* and *Ergasilus* sp.) from the gills and an unidentified leech from the body and fins. The echinostome digenean larvae, with a prevalence of 80% and mean intensity of 105, were the dominant species followed by *Contracaecum* larvae with a mean intensity of 24 and prevalence of 40%. The parasites recorded from these two rivers represent new distribution records for *C. gariepinus* in Zimbabwe. The echinostome and *Ergasilus* sp. are new host records for *C. gariepinus*.

[203] *Clarias gariepinus*: A single fish species but how many *Gyrodactylus* species?

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Sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), is widely distributed in Africa and is currently known to be a host to seven species of the genus *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae). During the period August 2011 – May 2013, several localities were sampled to establish gyrodactylid parasites diversity in the southern African region. In total 28 specimens of *C. gariepinus* were collected on several spots in South Africa (Flag-Bushiello Dam and Loskop Sand River, Limpopo Province) and Zimbabwe (Zambezi River and Lake Kariba). The *Gyrodactylus* prevalence was noted to be 43%. Species identification based on haptor sclerites morphometry and nuclear rDNA internal transcribed spacer (ITS) sequences identified the presence of 8 different *Gyrodactylus* species, of which three are currently known, i.e. *G. gelnari* Přikrylová, Blažek & Vanhove, 2012, *G. rysavyi* Ergens, 1973 and *G. transvaalensis* Prudhoe & Hussey, 1977. Present findings reveal unexpected *Gyrodactylus* species diversity and intensive parasite speciation among one host genus. Detailed morphological analyses revealed clear differences in the shape and size of taxonomically important structures between the species examined. Phylogenetic analyses, the maximum likelihood, Neighbour joining and Bayesian interference analyses, assisted in revealing interspecific relationships.

[204] Three worms from three localities: Nematodes of *Clarias gariepinus* (Burchell, 1822)

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The sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), is a widely distributed fish species within Africa and is an excellent aquaculture species. The study of parasites of this fish species is therefore of importance. Nematodes of the gut and body cavity of *C. gariepinus* were collected from three localities within the Gauteng Province, namely the Vaal River, Vaal Dam and Roodeplaat Dam. Catfish were examined for intestinal and coelomic nematodes. Fish collection was performed with the use of gill nets. On dissection the intestines were removed and placed into a saline solution, and subsequently opened to expose nematodes. Three nematodes were found at all three localities, but infection rates varied: *Procamallanus* in the stomach, *Paracamallanus* in the intestine, and *Contracaecum* in the caecum. The morphology of these species was studied with light and scanning electron microscopy. The infection rates were compared to those in previous reports for these localities and in the region. More nematodes occurred in fish in the river sites than in the dam and this correlates with the occurrence of piscivorous birds. The morphology was compared to previous descriptions to identify the current infections.

[205] Hidden diversity of gyrodactylid parasites on cichlids in Zambezi and Limpopo River basin

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In order to understand the diversity of viviparous monogeneans parasitizing cichlids in southern Africa, various

cichlids were collected during 2011–2012 in South Africa and Zimbabwe. Viviparous monogenean of the genus *Gyrodactylus* Nordmann, 1832 were recorded and found on *Oreochromis niloticus* (L.), *Pharyngochromis acuticeps* (Steindachner), *Pseudocrenilabrus philander* (Weber), *Tilapia rendalli* Smith, *Tilapia sparrmanii* Smith and *Tilapia* sp. Species identification based on haptor sclerites morphometry and nuclear rDNA internal transcribed spacer (ITS) sequences identified the presence of 11 *Gyrodactylus* species, of which three are currently known, i.e. *Gyrodactylus nyanzae* Paperna, 1973, *Gyrodactylus sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011, and *Gyrodactylus yacatli* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011. This study is the first record of *G. yacatli*, found on *O. niloticus* and *P. philander*, in Africa. Identification of eight new species of *Gyrodactylus* is indicative of high diversity of these parasites in the southern region of the continent. Based on the shape of the haptor sclerites complex, the species studied were divided into 5 morphological groups distinguishable according to the dimensions of haptor structures or the shape of marginal hook sickles. With the use of canonical discriminative analyses (CDA) *Gyrodactylus* species were separated into distinguishable morphological groups. Phylogenetic analyses, the maximum likelihood and Bayesian interference analyses, assisted in revealing interspecific relationships. The formation of clusters of particular species was in agreement with morphological divisions into groups.

[206] The use of *Argulus japonicus* as a sentinel organism for metal pollution

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Previous studies indicated that various endoparasites (Acanthocephala and Cestodes) are good sentinels for heavy metal pollution, capable of accumulating metals to concentrations far above that of their respective hosts. There has yet to be any conclusive result regarding the use of branchiuran ectoparasites as sentinels. Thus the use of *Argulus japonicus*, collected off of the body surface of *Labeo capensis*, as a possible sentinel organism was evaluated. Both parasite and host were collected with the aid of gill nets, and water and sediment samples were taken from the Vaal Dam. This locality was selected as a study site due to its vast economic and social importance. *Argulus japonicus* specimens were stained with a fluorescent probe, Phen Green FL cell permant diacetate stain, to visually identify metal binding sites with the aid of fluorescent microscopy. The use of the fluorescent probe indicated that metals are bound to the carapace, the eggs, and that metals are present within the blood meals. This provides justification that metals are sequestered by *A. japonicus*, of which the sentinel status is still under investigation. Analysis of the host and parasite tissues confirms this observation.

[207] Monogeneans from *Labeo* spp. in the middle Limpopo River Basin (Bubiana Conservancy, Zimbabwe and Nwanedi Nature Reserve, South Africa)

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Monogeneans are host-specific and naturally occur in intensities that are not very harmful to their fish hosts, but they may cause epizootics under culture conditions. The aim of this study is to shed more light on the biology and ecology of monogeneans found on the gills of selected *Labeo* species in the middle Limpopo River System. Their identification is vital for any subsequent ecological studies and their prevention or treatment so as to avoid the fatal effects on hosts when cultured, as well as contribute to knowledge of the parasitic fauna of this neglected area. Fish (n=75) were collected from the Bubi River and a rocky Bubiana Dam in Zimbabwe and Nwanedi River and Nwanedi-Luphepe Dams in South Africa using gill nets and electro-fishing, sacrificed by severing the spinal cord behind the head. The gills were removed and examined for worms under a stereomicroscope with both incident and transmitted light sources. The collected specimens were individually mounted in GAP or glycerine jelly. Identifications were based on morphological analyses with the help of drawings, micrographs and dimensions. *Dactylogyrus* spp, *Diplozoon* spp. and *Dogielius* sp. were recovered from the various *Labeo* sampled. Future molecular studies are essential in order to verify the diverse spectrum of monogeneans infesting the gills of *Labeo* species in this ignored area.

[208] Soft tissue digestion for the study of haptor sclerites using SEM and simultaneous molecular analysis of larger monogeneans

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Classification of Monogenea is primarily based on the morphology, morphometry and arrangement of their sclerites. However, these structures can be obscured or misinterpreted when studied using light microscopy, particularly in larger species. In the last four decades, the use of scanning electron microscopy has been employed for the study of sclerites. In these studies the main focus has been on haptor sclerites, specifically the hooks and anchors of smaller monogeneans. The sclerites of the family Diplozoidae have not been studied using SEM. However, these parasites do not display anchors but instead large, complex clamps. The haptor sclerites of diplozoids collected from South Africa were released from the surrounding tissue using the digestion solution from a DNA extraction kit and thereafter prepared for SEM. Both poly-L-lysine coated and concavity slides were employed to limit drifting and loss of sclerites, with the latter being more effective. The sclerites were successfully studied, providing new knowledge about ultrastructure. In addition, viable genetic material was extracted, amplified and sequenced from the digested haptor tissue. The results indicate that this method holds promise for future research such as the study of other clamp-bearing genera and sclerites such as cirri present in other monogenean families. Funding from NRF and UJ is acknowledged.

[209] Species of *Cichlidogyrus Paperna, 1960* and *Enterogyrus Paperna, 1963* (Monogenea: Ancyrocephalidae), infecting *Pseudocrenilabrus philander* (Weber, 1897) in Nwanedi River

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The Southern mouthbrooder, *Pseudocrenilabrus philander* (Weber, 1897) with its opalescence blue and pale yellow colours could become an important ornamental fish. This fish species is already utilized by subsistence fishermen in the Limpopo Province as an additional source of protein and they are valuable in behavioural and evolutionary research. Parasites (including monogeneans) cause huge economic losses in the agricultural industry with aquaculture being no exception. The crowded circumstances in culture systems may lead to an increase in monogenean infestation, with consequent high mortalities and economic losses. Understanding the naturally occurring parasites of this fish species is therefore important. Forty specimens of *P. philander* (total length = 5.46 ± 0.77 cm) were collected in November 2013 (summer) and August 2014 (winter) from Nwanedi River, Limpopo Province. Fish were sacrificed by severing the spinal cord. Recovered parasites were fixed and mounted in glycerine ammonium picrate (GAP) solution and some were preserved in 96% ethanol. Examination of fish with the aid of a stereomicroscope revealed the presence of monogenean parasites of two genera, *Enterogyrus* Paperna, 1963 and *Cichlidogyrus* Paperna, 1960. The structures were studied and drawn with the aid of an Olympus microscope. Based on the morphological features of the dorsal and ventral bar, the copulatory complex and marginal hooks, four species of monogenean parasites were distinguished among collected specimens. In the stomach *E. coronatus* Pariselle, Lambert & Euzet, 1991 was found. From the gills three *Cichlidogyrus* species, *C. philander* Douëllou, 1993, *Cichlidogyrus* sp. 1 and sp. 2 were identified. *Enterogyrus coronatus* and *Cichlidogyrus* spp. had a prevalence of 85% and 38% in summer and 50% and 10% in winter, respectively. The finding of *C. philander* and *E. coronatus* represents a new distribution record for *P. philander* in Nwanedi River, South Africa.

[210] Morphological characterization of *Lamproglena* species (Copepoda: Lernaecidae) from selected fish species in the Limpopo and Olifants river systems

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There are currently 39 species of the genus *Lamproglena* Von Nordmann, 1832 described worldwide, six of which

are from southern Africa. These include *Lamproglena monodi* Capart, 1944; *Lamproglena clariae* Freyer, 1956; *Lamproglena barbicola* Freyer, 1961; *Lamproglena comuta* Freyer, 1964; *Lamproglena hoi* Dippenaar, Luus-Powell and Roux, 2001 and *Lamproglena hepsetii* Van As and Van As, 2007; recorded from different fish hosts. Minor morphological differences between most species of this genus have complicated species identification. Due to this, some species have been synonymised, whereas others have been transferred to other genera. Furthermore, the characterization of *Lamproglena* spp. is perplexing due to the partial and inadequate descriptions of many of the existing species. In the current study, specimens of *Lamproglena* spp. were collected from the gills of *Labeo rosae* from Flag Boshielo Dam and *Labeo molybdinus* from Nwanedi-Luphephe Dam in 2013–2014 and preserved in 70% ethanol. Light as well as scanning electron microscopy were used to compare the morphological features of these specimens to known species of *Lamproglena*. Morphological differences were found between the *Lamproglena* spp. collected during the current study and known species but molecular work is planned for future studies to see if these are new species. This study also served as a new host record for *Lamproglena* sp. from *Labeo molybdinus*.

[211] Two new records of gill-attaching isopods from the Caribbean

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Members of the genus *Mothocya* Hope, 1851 are usually found inside their host branchial cavities where they can cause detrimental effects to the gill tissue. While sampling on St John Island in the Caribbean, new records of these isopods were recorded. *Mothocya xenobranchia* is recorded for the first time from this region on the redfin needlefish, *Strongylura notata*. This host was previously unconfirmed for the species but is now validated and added to the known host records too. Another *Mothocya* species was also collected which does not conform to other known species and is new to the Caribbean and science. This species is small in size and was removed from the gills of the redlip blenny, *Ophioblennius atlanticus*. This record is also the first record of a *Mothocya* species on a blenny host.

[212] Blood parasites of reef associated fishes of the eastern Caribbean

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Although Apicomplexans are common parasitic protozoans of the blood of many vertebrates, no systematic surveys of their occurrence in Caribbean fishes have been undertaken since the 1960s. A total of 1513 fishes representing 83 species, 26 families, and 5 orders were collected by divers or obtained from local fishermen from the northeast Caribbean. Among the samples examined, 60 (6.3%) were infected with blood parasites. Species infected included damselfishes: *Stegastes diencaeus* (12/39), *S. fuscus* (16/32), *S. leucostictus* (8/18), and *S. planifrons* (4/4); parrotfishes: *Scarus taeniopterus* (1/4), and *Nicholsina usta* (3/4); the mugilid mullet, *Mugil curema* (1/2); the clupeid scad *Selar crumenophthalmus* (1/1); blennoid fishes of the families Blenneidae (*Ophioblennius macclurei* 3/5) and Labrisomidae (*Hypoleurochilus bermudensis* (1/6), *Malacoctenus triangulatus* (1/2), and *M. macropus* (1/4)); the lutjanid snapper *Ocyurus chrysurus* (6/7); and the pomacanthid angelfish *Pomacanthus arcuatus* (2/8). None of the blood parasites found conformed to the description of any of the previously reported parasites from the Caribbean. Parasites found resembled in morphology typical *Haemogregarina*-like intraerythrocytic parasites as well as *Haemohormidium*-like parasites collected from fishes on the Great Barrier Reef, Australia. In total, eight distinct types of blood parasites were found and future work will include classifying these to species level through molecular analysis. Current focus is on molecular characterization of the blood parasite in damselfishes, which exhibit higher parasitemias and are the most common reef inhabitants.

Posters: Terrestrial hosts – ectoparasites

[213] Ecology of chigger mites (Trombiculidae) on rodents and insectivores in the Cape Floristic Region, South Africa

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Trombiculid mites are a large group of arthropods consisting of more than 3000 described species. Only the larval stage (also known as chiggers) is parasitic and occurs on mammals, birds and reptiles. Research on chigger mites occurring within South Africa is currently restricted to taxonomic descriptions and biased towards reptilian hosts. The aims of the study were, firstly, to record the diversity, abundance and temporal variation of chigger mites on small mammals in the Cape Floristic Region (CFR) and, secondly, to determine the relationship between the length of the mite's cheliseral blade and exposure of the parasitope to grooming activities. Rodents were trapped at two localities in the fynbos biome of South Africa during February, May, August and November 2011 and again in February 2013. Sherman-type live-traps were used and each animal was euthanized and carefully examined under a stereomicroscope. Chigger mites were counted and removed. They were mounted onto microscope slides and identified to genus level. Seven morphospecies including two undescribed species, belonging to five genera were recorded. Host infestation was found to be significantly higher (363.17 ± 268.25) between scrotal males compared to non-perforated females (195.99 ± 175.01). Chiggers were only present on rodents during the warm dry season as opposed to the wet and cold season. Cheliseral blade length differed between parasitopes with significantly longer cheliseral blades recorded for taxa that occurred at exposed parasitopes compared to taxa in more protected parasitopes. The present study provides novel data on the ecology and diversity of trombiculid mites on rodents in the CFR.

[214] Digging for answers: what are the impacts of ectoparasite burdens on social, subterranean hosts?

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Common patterns in parasite ecology are difficult to find, as the distribution of parasites amongst hosts can be influenced by several abiotic (e.g., rainfall, temperature) and biotic factors (e.g., host sex, breeding condition and sociality) simultaneously. Several studies have indicated that variation in ectoparasite loads of small mammal populations are primarily due to environmental fluctuations. However, it is unknown whether these patterns are retained in subterranean habitats, where individuals are protected from extremes of ambient conditions. This study aimed to answer this question by analysing ectoparasites of common mole-rats (*Cryptomys hottentotus hottentotus*). Samples were collected seasonally from populations inhabiting both an arid and a mesic area along the western coast of South Africa. Effects of environmental (location, rainfall, temperature) and host-related factors (sex, breeding status, group size) on ectoparasite abundance were assessed. Only five parasite species from three taxa were found. All taxa were more abundant in the wet season possibly due to increases in host dispersal. In contrast, both species richness and abundance were greater at the arid site. The effects of biotic factors differed between ectoparasite taxa probably as a result of different life-history strategies of the different taxa. For the most common parasites (*Androlaelaps* spp.) the abundance decreased significantly with group size. These results defy the consensus that gregarious behaviour is costly in terms of parasite infestation and suggests that environmental factors may affect ectoparasites of subterranean host both directly and indirectly.

[215] Sarcoptic mange vulnerability in carnivores of the Białowieża Primeval Forest, Poland

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Sarcoptic mange, caused by *Sarcoptes scabiei*, is a highly contagious mite infection responsible for epizootic skin disease in populations of wild and domestic mammals worldwide. It causes skin disorders that may lead to severe

generalized skin disease (mange). We investigated the spread and dynamics of sarcoptic mange in one of the best preserved carnivore communities in the Białowieża Primeval Forest, Poland, over the last 20 years. Sarcoptic mange was found in 6 of 10 species investigated. The highest percentage of infected individuals was recorded in red foxes (19%), European badgers (9%), grey wolves (7%), raccoon dogs (6%), and stone martens (5%). Other species were not infected (least weasel, American mink, European polecat, pine marten) or infected sporadically (Eurasian lynx - 1 case). Rate of infection was correlated with the sum of ecological and behavioral features which may increase the spread of infection. Outbreaks of sarcoptic mange occurred simultaneously in several species of carnivores which indicates the occurrence of transmission between species. We found that the source of investigated individuals (trapped, culled and found dead) may influence results of analysis. Our study shows that in carnivore communities such as in Białowieża Forest, numerous species may serve as a reservoir of the pathogen and favour its persistence in the environment. This study indicates that sociality and use of den sites are important factors affecting sarcoptic mange spread and prevalence in wild populations of carnivores.

[216] Occurrence of *Haemaphysalis juxtakochi* in *Mazama gouazoubira* in midwest of Paraná State, Brazil

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Ticks are parasites considered important to public health and the economy. These parasites require blood of vertebrates, having significant degree of specificity and can also use alternate hosts, like humans. The genus *Haemaphysalis* has the second highest number of species in the family Ixodidae. Among them, the species *Haemaphysalis juxtakochi* prefers parasitizing members of the family Cervidae in Neotropical regions. It was found that *H. juxtakochi* may carry strains of *Rickettsia* and may become potential transmitters of diseases caused by these bacteria. This study aims to report the occurrence of the tick *H. juxtakochi* two grey brocket deer (*Mazama gouazoubira*) received by the Service of Wildlife Animals (SAAS), of UNICENTRO in Guarapuava, Paraná State, Brazil. After being collected, ticks were placed in bottles containing alcohol 70°GL and were identified by means of dichotomous keys as being the adult stage of *H. juxtakochi*. Four or 5 rows of teeth were observed on each side of the midline of the hypostome, based on the rectangular section, and spines were present on trochanters located in thighs number I to IV. This is the first report of adult *H. juxtakochi* in the Midwest of Paraná State. The presence of this parasite has been reported in Southeastern Brazil, parasitizing one red brocket (*Mazama americana*), and a grey brocket deer (*Mazama gouazoubira*). This shows the importance of *H. juxtakochi* as potential transmitters of diseases caused by these intracellular bacteria.

Posters: Terrestrial hosts-Helminths

[217] Woylie worms – Applying parasitology to the conservation of a critically endangered Australian marsupial

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The woylie (*Bettongia penicillata*) is a critically endangered Australian marsupial which has declined by over 90% in recent times. Evidence is growing that parasitic disease may be a significant threat to the species' survival. Many recommendations have been made to integrate parasitology in wildlife conservation. This study demonstrates wildlife conservation parasitology in action. Depending on a suite of host, parasite and environment interactions, normally innocuous parasites have the capacity to diminish individual and population health and this may be of critical importance in endangered species conservation. Extensive field and laboratory work will be undertaken to investigate parasites (including helminths, protozoa, haemoparasites) in woylies in the context of their decline. Characterising these parasites and their infection dynamics will help us apply parasitology to wildlife conservation and, ultimately, help secure the health and survival of endangered species now and into the future.

[218] North American bears and their hookworms (Nematoda: Ancylostomatidae): a phylogenetic hypothesis on parasite evolution and life-history traitsStefano Catalano¹, Pdraig Duignan^{1,2}, Manigandan Lejeune², Christopher Pagan³, Paolo Tizzani⁴, Steven Nadler³¹University of Calgary, Calgary, Alberta, Canada, ²Canadian Cooperative Wildlife Health Centre - Alberta Node, Calgary, Alberta, Canada, ³University of California Davis, Davis, California, USA, ⁴University of Turin, Turin, Italy

The hookworm species *Uncinaria rauschi* Olsen, 1968 and *Uncinaria yukonensis* (Wolfgang, 1956) have been formally described from grizzly (*Ursus arctos horribilis*) and black bears (*Ursus americanus*) in North America. Further reports of these ursine hookworms are available, but there is a little information on the life-history traits of *Uncinaria* spp. in bears, and on the evolution of this host-parasite system. Phylogenetic relationships among hookworm species have already been used to infer host affiliations and parasite life-history traits; our investigation explored whether a similar inference based on phylogenetics could support specific transmission patterns of *Uncinaria* spp. in grizzly and black bears. We used the internal transcribed spacers (ITS-1 and ITS-2) of the nuclear ribosomal DNA to explore the evolutionary relationships between the ursine *Uncinaria* spp. and other hookworms from carnivore hosts. The phylogenetic analysis of the concatenated ITS-1 and ITS-2 datasets supported a clade of *U. rauschi* and *U. yukonensis* with *Uncinaria stenocephala* (Railliet, 1884), a parasite primarily of canids. Maximum parsimony and maximum likelihood phylogenetic trees showed these three species nested within a clade distinct from the monophyletic group composed by *Uncinaria* spp. from otariid pinnipeds, hosts that shared common ancestry with bears until 25 million years ago. The close relationship between the ursine hookworms and *U. stenocephala* led to the following postulations: (I) incongruent co-phylogeny between carnivore hosts and their *Uncinaria* hookworms; (II) similar transmission patterns for the closely related *U. rauschi*, *U. yukonensis* and *U. stenocephala*.

[219] Occurrence of *Tripaxyuris* sp in brown howler monkey (*Alouatta guariba*) received by the Service of Wildlife Animals (SAAS), of UNICENTRO in Guarapuava, Paraná State, Brazil

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With a wide variety wild species, Brazil is home to the greatest biological diversity in the world. Encroachment of urban areas into wild areas could cause the emergence and re-emergence of various diseases, including parasites. Wild animals are hosts to a variety of parasites. Despite the increased number of publications on investigations of parasitic diseases, knowledge about them is still far from satisfactory. This paper reports a case of infection *Tripaxyuris* sp in two babies of brown howler monkey (*Alouatta guariba*) received by the Wildlife Animal Service (SAAS) of UNICENTRO, Guarapuava, Paraná State, Brazil. The animals had become lethargic and anorexic. Faecal samples for parasitological examination were collected for three consecutive days. Double-shell eggs, unembryonated, without operculum and larvae were detected, characterized as *Tripaxyuris* sp, a parasite of the family Oxyuridae. The animal was treated with Levamisole (10 mg/kg), with repeat dosing after 15 days. Twenty days after treatment, faeces and perianal material were collected; results were negative. The nematodes of the genus *Trypanoxyuris* are exclusive parasites of families Cebidae and Atelidae, described extensively in South America. The parasite colonizes the caecum. Female larvae migrate to the rectum, and lay eggs in the perianal region. Primates are generally very vulnerable to infection by parasites, since many of the species live in social groups, which facilitates orofaecal transmission, e.g. *Trypanoxyuris* sp. This is the first report of *Trypanoxyuris* in *Allouatta guariba* in the state of Paraná, Brazil.

[220] *Echinococcus equinus* and other taeniid cestodes in wildlife of the Etosha National Park, NamibiaMarion Wassermann¹, Ortwin Aschenborn², Julia Fellhauer³, Ute Mackenstedt¹, Thomas Romig¹¹University of Hohenheim, Dept. Parasitologie, Stuttgart, Germany, ²Bwabwata Ecological Institute, Susuwe, Caprivi, Namibia,³University of Veterinary Medicine, Hannover, Germany

Various species of *Echinococcus* and *Taenia* have been described from wild mammals of sub-Saharan Africa. However, only few isolates have become available for molecular identification in recent years, which means that the involvement of wildlife in the lifecycles of the various cryptic species within *E. granulosus* sensu lato is still little known. A preliminary survey was done in Etosha National Park, Namibia, from August to October 2012. Faecal samples were obtained from 34 individual wild carnivores. In addition, carcasses of 18 culled herbivores were examined for larval taeniids. Single eggs and metacestode tissue were lysed and identified by sequencing of the nad1 gene and comparison with GenBank entries. Several haplotypes of *Echinococcus equinus* were found in

lions, black-backed jackals and Burchell's zebras. The frequency of this parasite in the absence of domestic dogs strongly indicates its transmission in a wildlife cycle. Further, a variety of sequences were obtained from eggs and cysticerci from lions, cheetahs, caracals, spotted hyaenas and oryx, which most closely clustered with species of *Taenia* and *Hydatigera*. Only two of them, of lion origin, could be allocated to *H. taeniaeformis* and *T. regis*, respectively. *T. regis* cysticerci were also found in oryx. For the first time, lions are confirmed as suitable hosts for *Echinococcus* species other than *E. felidis*, and the first wildlife cycle of *E. equinus* is described. The diversity of other taeniids in wild mammals was found to be far higher than expected.

[221] Seroprevalence and distribution of bovine and porcine cysticercosis in the Free State Province

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Serological tests have been developed for detection of specific antibodies or antigens specific for *Taenia saginata* and *T. solium* and detection of the parasite products associated with current infection. Although these assays have been reported to be less sensitive in animals infected with fewer cysts, they have been shown to be three times more sensitive than meat inspection and have been successfully used in epidemiological surveys. It was therefore the aim of this study to use a monoclonal antibody based (HP10) antigen-detecting enzyme-linked immunosorbent assay (ELISA) to determine the prevalence of both bovine and porcine cysticercosis in Free State Province. Blood samples were collected for a period of 12 months from randomly selected cattle and pigs belonging to smallholder farmers in Xhariep, Fezile Dabi, Lejweleputswa and Motheo districts. Results showed respective prevalence of 30 and 36% of bovine and porcine cysticercosis in the province. Of the four districts, Xhariep had the highest prevalence (10%) of bovine cysticercosis, whilst the Fezile Dabi showed the highest porcine cysticercosis prevalence of 17%. The main risk factors associated with *Taenia* infections in cattle and pigs include the limited use of toilets by humans and access to human excreta by cattle and pigs. Furthermore, the free-roaming behaviour of these animals creates an infection opportunity through ingestion of *Taenia*-contaminated herbage or water, especially after floods. Public awareness programmes on life cycles of *T. saginata* and *T. solium* are therefore recommended as part of effective control strategies against *Taenia* infections in the province.

[222] Prevalence of *Fasciola jacksoni* of wild elephants (*Elephas maximus maximus*) in Sri Lanka: is it an important disease for public health, wildlife conservation and the livestock industry?

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Throughout its large geographical distribution, fasciolosis is a well-known veterinary problem. Moreover, studies carried out in recent years have shown it to be an important public health problem as well. Fasciolosis is an important disease caused by two trematode species: *Fasciola hepatica* and *F. gigantica*. There is no record on any of these parasites present in Sri Lanka. However, our study found *F. jacksoni* in wild elephants (*Elephas maximus maximus*) in Sri Lanka. The shape of *F. jacksoni* differs significantly from the other two species: it is more circular while others are elongated, the average size being 12–14 mm x 9–12.5 mm. However, microscopic morphological features of *F. jacksoni* are more similar to those of the other two species. Similarly, genomic study revealed that *F. jacksoni* is very close to other two species. We have been conducting studies to determine prevalence, pathological importance and life cycle of *F. jacksoni* among wild elephants in Sri Lanka since 2000. The liver was examined for the presence of adult liver flukes in 104 wild elephants that died naturally or through anthropogenic interventions. Fifty-nine animals of all age categories were infected with the fluke. The severity of the infestation varied: most severe (>100 flukes) in 7 animals, moderate (50–100 flukes) in 11 animals, mild (10–50 flukes) in 12 animals and low (<10 flukes) in 29 animals. According to the history and post-mortem findings four elephant deaths were due to *F. jacksoni*.

[223] Presence of *Paragonimus westermani* in free-ranging leopards (*Panthera pardus kotiya*) in Sri Lanka

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Lung flukes of the genus *Paragonimus* are a pathogenic agent of human paragonimosis, one of the medically important food-borne trematode diseases in tropical, subtropical, and some temperate countries. This infection is often confused diagnostically with tuberculosis. The prevalence of human paragonimosis is currently estimated at 20 million. To date, four species of *Paragonimus* have been reported in Sri Lanka. They are identified based on the morphological features of adult flukes, such as the branching pattern of ovaries and the arrangement of cuticular spines. Humans and other mammals become infected by eating raw or undercooked crayfish or freshwater crabs that harbour the parasites. Paragonimosis most frequently involves the lungs, but can affect other organs, including the brain and skin. In this investigation, we examined the prevalence of *Paragonimus* worms in the lungs of free-ranging leopards (*Panthera pardus kotiya*) in Sri Lanka. The 11 animals examined originated from different areas of the country. The causes of death of these animals included health complications associated with noose trapping (n=4), gunshot (n=1), vehicle collision (n=1), poisoning (n=1) and intraspecies trauma (n=4). Worms were identified in the lungs and determined to be *Paragonimus westermani*. Infected animals originated from the Nawalapitiya and Yala National Park indicating the worm is widely distributed. This is the first report of *Paragonimus* from free-ranging leopards of Sri Lanka. Further studies are currently underway to determine the prevalence and mode of transmission in leopards, and its zoonotic importance in Sri Lanka.

Posters: Protozoa

[224] The diversity of *Hepatozoon* genotypes in *Caiman crocodilus yacare* (Crocodylia, Alligatoridae) from North Pantanal, Brazil

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Hepatozoon species are the most common intracellular haemoparasite found in reptiles. Blood smear examination of crocodile blood smears in Brazil revealed a high prevalence of *Hepatozoon caimani*, whose vectors are *Culex* mosquitoes. The present work aimed to detect and characterize the *Hepatozoon* sp. found in 33 crocodiles (*Caiman crocodilus yacare*) (24 free-ranging and 9 captive; 28 males and 5 females) sampled at Poconé, North Pantanal, state of Mato Grosso, Brazil, using blood smear examinations and molecular techniques. *Hepatozoon* sp. gametocytes were found in 70.8% (17/24) and 88.8% (8/9) of blood smears from free-ranging and captive crocodiles, respectively. *Hepatozoon* sp. 18S rRNA DNA was found in 79.2% (19/24) and 88.8% (8/9) of free-ranging and captive crocodiles, respectively. Thirteen (54.2%) free-ranging crocodiles showed *Hepatozoon* sp. gametocytes in blood smear examinations and were also positive to 18S rRNA PCR for *Hepatozoon* sp.; four (16.6%) showed positive results only in microscopic examination. Seven (77.7%) captive crocodiles showed *Hepatozoon* sp. gametocytes in blood smear examination and were also positive to 18S rRNA PCR for *Hepatozoon* sp.; one (11.1%) showed positive results only in microscopic examination. Comparative analysis of parasitized and non-parasitized erythrocytes showed that all analyzed features were significantly different ($p < 0.05$) for both linear and area dimensions. The Maximum Likelihood phylogenetic analysis based on 18S rRNA sequences showed a separation of *Hepatozoon* spp. sequences in two groups, also supported by the pairwise distances. Through morphological, morphometric and molecular approaches, the present work showed a high diversity of *Hepatozoon* genotypes that could parasitize crocodiles in Brazil. Financial support: FAPESP

[225] Cold-blooded parasitism! Molecular identification of *Hepatozoon pettiti* (Hoare, 1932) and its evolutionary interactions with the Nile crocodiles (*Crocodylus niloticus*) of the Okavango Delta

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Hepatozoon pettiti is an apicomplexan intraerythrocytic parasite of the Nile crocodile (*Crocodylus niloticus*) with high prevalence throughout Africa and is transmitted by tsetse flies, although leeches are suspected to also play a role in transmission. The aims of this study were firstly to identify *H. pettiti* in blood-fed leeches using 18S rDNA sequences and match them with parasites in blood films from the crocodiles, and secondly to understand the evolutionary interactions between *H. pettiti* and the crocodiles of the Okavango by measuring variation in the major histocompatibility complex (MHC) II b gene of both infected and uninfected individuals. DNA was extracted from archived material from a previous study in 2006 which included leeches preserved in ethanol and blood films

from the crocodile hosts. *Hepatozoon* 18S rDNA sequences from leech blood meals were obtained and were successfully matched with corresponding blood films from infected crocodiles. Also a small fragment of MHC was sequenced from the blood films and specific genotypes that appeared to be more susceptible to the parasite than others were observed. There was reduced variation in infected MHC genotypes potentially indicating that parasites are adapting to the most common genotypes within a genetically diverse crocodile population. This is of considerable concern as Nile crocodiles that are being commercially bred are shown to have a substantially genetic variation which could lead to parasites such as *H. pettiti* becoming highly pathogenic and causing considerable economic losses as an emerging disease.

[226] Wild ruminant species as reservoir hosts of tick-borne haemoparasites

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Tick-borne diseases (TBDs) of ruminants pose important constraints to livestock production. Although tick-borne haemoparasites have also been implicated in losses amongst wild animals, the epidemiology and phylogeny of piroplasms of wildlife are largely unknown. A total of 56 antelope (eland, waterbuck, impala and blue wildebeest) and buffalo blood samples from the National Zoological Gardens and SANParks Biobanks were screened using the reverse line blot (RLB) hybridization assay for the presence of known and novel haemoparasites. DNA was extracted; the V4 hypervariable region of the 18S rRNA gene of *Theileria* and *Babesia* species as well as the V1 hypervariable region of the 16S rRNA gene of *Anaplasma* and *Ehrlichia* species were amplified and subjected to the RLB assay. The RLB results revealed the presence of *Theileria*, *Babesia*, *Anaplasma* and *Ehrlichia* species, either as single or mixed infections. *Theileria* sp. (sable) (0.12%) and *Theileria ovis* (0.1%) were the most prevalent parasites found (waterbuck and blue wildebeest samples only). Other *Theileria* species present at very low parasitaemia included *Theileria seperata* (0.08%) in waterbuck and blue wildebeest, *Theileria* sp. (kudu) (0.07%) in eland and waterbuck, *Theileria mutans* (0.07%) in buffalo and *Theileria bicornis* (0.05%) in eland and impala. Furthermore *Theileria buffeli*, *Theileria taurotrogi* and *Babesia occultans* were detected. Importantly, 25% of PCR products failed to hybridize with any species-specific probes, and only hybridized with the *Babesia/Theileria* and/or *Anaplasma/Ehrlichia* group-specific probes suggesting the presence of a novel species or variant of a species within those particular samples. These are currently under investigation.

[227] Attempted infection of common waterbuck (*Kobus ellipsiprymnus*) with buffalo-derived *Theileria parva*

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Following reports that defassa waterbuck (*Kobus defassa*) may play a role in the epidemiology of *T. parva*-group infections in cattle in East Africa, common waterbuck (*Kobus ellipsiprymnus*) in Kruger National Park (KNP) were investigated as potential carriers of *T. parva*-group infections. Seven adult and sub-adult common waterbuck were captured and screened by conventional and molecular diagnostic techniques for *Theileria* spp. infections. Laboratory-reared *Rhipicephalus zambeziensis* nymphs were fed in ear bags on 4 captive buffalo (*Syncerus caffer*) confirmed to be naturally infected with *T. parva*. The ensuing adult ticks were fed on 4 captive sub-adult waterbuck and 2 cattle. All the waterbuck were found to carry microscopically detectable *Theileria* sp. piroplasm infections, found by PCR diagnosis to belong to a hitherto uncharacterised *Theileria* species. *R. zambeziensis* adults which fed as nymphs on the buffalo transmitted fatal *T. parva* infections to cattle. However, no transmission of *T. parva* to the waterbuck could be demonstrated clinically or by PCR diagnosis. Also, *R. zambeziensis* nymphs which were subsequently fed on the waterbuck failed to transmit *T. parva* to cattle in the ensuing adult stage, confirming the absence of *T. parva*-group infections in the waterbuck. The results suggest that buffalo in KNP probably do not carry *T. parva*-group parasites which are readily transmissible to common waterbuck which are therefore unlikely to play an important role in the epidemiology of *T. parva*-group infections in cattle in South Africa.

[228] Characterization of South African cattle and buffalo-derived *Theileria parva* using microsatellites

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Buffalo-associated Corridor disease in South Africa is a fatal disease of cattle caused by buffalo-derived *Theileria*

T. parva transmitted by *Rhipicephalus appendiculatus*. The objective of this study is to describe the population structure of *T. parva* in South Africa using mini- and microsatellites. Host specificity and geographic differentiation of cattle and buffalo-derived *T. parva* samples were determined using 23 mini- and microsatellite markers by fragment size analysis. Current hypotheses tested are that host specificity between cattle and buffalo exist due to selection of sub-populations in cattle, and that geographic differentiation in buffalo might exist due to the quarantine-like nature of the historical buffalo projects and whether Corridor disease has been endemic in South Africa or introduced in the last 100 years. This information could assist in determining population structure of *T. parva* and give possible solutions to clarify the epidemiology of the disease and the parasite carrier-state in infected cattle.

[229] Characterization and annotation of *Babesia orientalis* apicoplast genome

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Babesia orientalis is a new haemosporidian parasite infecting water buffalo, and is specifically epidemic to the southern part of China. As a member of the Apicomplexa, *B. orientalis* possesses a relatively independent and prokaryotic-derived organelle, called the apicoplast. The apicoplast, which was acquired by secondary endosymbiosis from a eukaryotic alga, participates in the metabolism of fatty acids, isoprenoids, iron-sulphur clusters and haem. These metabolic pathways are essential for parasite survival. To develop novel drug targets against the parasite, it is essential to gain more genetic information from the apicoplast. A 33 kb circular DNA, which showed high identity with the *B. bovis* apicoplast genome, was obtained by analysis of the full genome sequence of *B. orientalis*. Bioinformatic analysis indicated that the circular DNA contains 1 LSU rRNA, 1 SSU rRNA, 38 ORF and 24 tRNA genes. The 38 ORF genes include four DNA-dependent RNA polymerase beta subunits which were rpoB, rpoC1, rpoC2A and rpoC2B, respectively, 16 ribosomal proteins, and a translation elongation factor (TufA), 2 Clp protease genes, 15 hypothetical proteins. No SurfB fragment was found. This is the first report of the *B. orientalis* apicoplast genome.

[230] Using participatory epidemiology to investigate management options and relative importance of tick-borne diseases amongst transhumant zebu cattle in Karamoja region, Uganda

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A participatory epidemiological (PE) study was conducted with pastoralists in Moroto and Kotido districts, Karamoja region, Uganda, between October and December 2013 to determine the management options and relative importance of tick-borne diseases (TBDs) among transhumant zebu cattle. Data collection involved 24 focus group discussions (each comprising 8 to 12 people) in 24 settlement areas (*manyattas*), key informant interviews (30) and direct observation. Methods used in group discussions included semi-structured interviews, simple ranking, pair-wise ranking, matrix scoring, proportional piling and participatory mapping. The result of pairwise comparison showed the ngaKaramojong-named diseases, *Lokit* (East Coast fever, ECF), *Lopid* (anaplasmosis), *Loukoi* (contagious bovine pleuropneumonia, CBPP), *Lokou* (heartwater) and *Lokulam* (babesiosis), were considered the most important cattle diseases in Moroto in that order while ECF, anaplasmosis, trypanosomosis (*Ediit*), CBPP and nonspecific diarrhoea (*Loleo*) were most important in Kotido. Strong agreement among informant groups (Kendall's coefficient of concordance $W = 0.568$ and 0.682 ; $P < 0.001$) in pairwise ranking indicated that the diseases were a common problem in selected districts. East Coast fever had the highest estimated relative incidence score (18% [95% confidence intervals 11, 25]) in Moroto, followed by anaplasmosis (17.5% [12, 24]) and CBPP (9% [4, 16]). Most animals that suffered from ECF, anaplasmosis, heartwater and babesiosis died, as the respective median scores for case fatality rates were 89.5% (56.2, 100%), 82.8% (75,100), 66.7% (20, 90) and 85.7% (0,100%). In Kotido, diseases with high relative incidence scores were ECF (21% [16.5, 26]), anaplasmosis (17% [10, 20.5]) and trypanosomosis (8% [5, 13]). The case fatality median scores for ECF and anaplasmosis were 81.7% (57.7, 91.3%) and 70.7% (66.3, 90.9). Matrix scoring between informant groups revealed that disease indicators showed strong agreement ($W = 0.382-0.659$, $p < 0.05-0.001$). Inadequate knowledge, poor veterinary services and limited availability of drugs were key factors that hindered control of TBDs. Hand picking of ticks was done by all pastoralists while hand spraying with acaricides was irregular, often determined by availability of drug supplies and money. It was concluded that PE could efficiently be used to generate information on livestock diseases in transhumant systems thereby assisting the design of feasible control strategies.

[231] First report of *Trypanosoma congolense* in Temminck's ground pangolin (*Smutsia temminckii*) from the Kalahari DesertIlse Vorster¹, Marinda C Oosthuizen¹, Raymond Jansen²¹Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa,²Department of Environmental, Water and Earth Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001 South Africa.

Four of the eight extant pangolin species occur in Asia and four in Africa. The African species are Temminck's ground pangolin (*Smutsia temminckii*), giant ground pangolin (*Smutsia gigantea*), white-bellied tree pangolin (*Phataginus tricuspis*) and black-bellied tree pangolin (*Phataginus tetradactyla*). All four African pangolin species are listed on the 2008 International Union for the Conservation of Nature (IUCN) Red List of Threatened Species, with the giant ground pangolin and white-bellied tree pangolin listed as "Near Threatened". Not only are these animals' meat considered a delicacy in certain parts of the world and in central and West Africa in particular, but their scales and selected body parts are also used in traditional medicine and their skins for the production of shoes, belts and bags. Pangolins also appear in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora along with a zero annual export quota for wild captured individuals traded for commercial purposes. Although illegal intercontinental trade, subsistence hunting and habitat loss are major contributing factors to the decline of this species in Africa, very little is known about other threats such as the internal and external parasites they are associated with and the implications thereof. Tree pangolins from south Cameroon have previously been shown to be infected by *Trypanosoma brucei* and *T. vivax*. In this study, eight of 22 blood smear samples collected from Temminck's ground pangolins in the Kalahari Desert were screened for the presence of *Trypanosoma* spp. using PCR-RFLP analysis. Results indicate the presence of *T. congolense* DNA in one (12.5%) of the samples investigated. This serves as the first report of *T. congolense* in pangolins.

Posters: General

[232] Molecular detection of Anaplasmataceae agents in wild deer in Brazil

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In Brazil, the identification of wildlife reservoirs for Anaplasmataceae species is much needed. The present work aimed to detect *Ehrlichia* and *Anaplasma* in buffy-coat samples from 45 wild deer from central-western and south-eastern regions of Brazil, using molecular techniques. Between 1996 and 2011, blood samples were collected from two brocket deer (*Mazama gouazoubira*) and four small red brocket deer (*Mazama bororo*) from São Paulo (SP) state, four brocket deer from Goiás (GO) state, three red deer (*Mazama americana*) from Paraná (PR) state, and 23 brocket deer and 11 pampas deer (*Ozotocerus bezoarticus*) from Mato Grosso do Sul (MS) state. Thirty (66.6%) and 14 (31.1%) deer were positive to *Anaplasma* spp. and *E. chaffeensis* rrs nPCR, respectively. Phylogenetic analysis of 350bp of rrs confirmed the presence of *A. bovis*, *Anaplasma* sp. closed related to *A. platys* and *Ehrlichia* spp. phylogenetically related to *E. chaffeensis* circulating in sampled deer. *Anaplasma* sp. (~*A. platys*) was detected in five pampas deer and 16 brocket deer from MS. *A. bovis* was detected in one brocket deer and four small red brocket deer from SP, and three red deer from PR. *Ehrlichia* spp. was detected in 11 brocket deer from MS and in three small red brocket deer from SP. Sampled deer showed negative results in specific qPCR for *E. chaffeensis* (v1pt), *A. phagocytophilum* (msp-2) e *A. marginale* (msp1-b), suggesting that new genotypes of *Ehrlichia* spp. closely related to *E. chaffeensis* circulate in Brazil. The present work suggests Brazilian deer may play an important role in the maintenance of Anaplasmataceae agents in wildlife environment. FAPESP financial support.

[233] No kangaroos in Austria – examples of possible roles of wildlife for parasite transmission in Central Europe

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Austria is a comparatively small Central European country with high biodiversity and abundant wildlife. At the interphase of wildlife habitats and human activities a range of pathogens can be transmitted from wild to domestic animals and to humans. Wild animals serve as indicators for the presence of parasites; however, transmission risks

to domestic animals or humans are usually subject to speculation. This concerns food-borne parasites like *Trichinella britovi* (found in foxes) and *Alaria alata* (in wild boar), as well as *Echinococcus multilocularis* which is prevalent in Austrian foxes and may be spread by the newly introduced raccoon dog. Wildlife may also serve as reservoir for arthropod-borne infections than can be transmitted to domestic animals and/or humans. Tick-borne encephalitis (TBE) transmitted by *Ixodes ricinus* is highly endemic in Austria and roe deer is an important reservoir for this tick species. A lot of discussion surrounds the possible introduction of *Rhipicephalus sanguineus* into Central Europe. It represents the most important vector of canine tick-borne diseases worldwide and has been introduced several times into Austrian animal shelters in the past. The presence of foxes certainly supports the spread of the recently detected *Dirofilaria repens* in Austria east-to-west and there is growing concern that *Dirofilaria immitis*, the agent of canine heartworm disease, may establish in foxes and subsequently in dogs in temperate climate areas in eastern and southern Austria. A close monitoring is necessary to plan and carry out control measures to prevent excessive transmission from wildlife reservoirs to humans and their animals.

[234] Molecular detection of *Mycoplasma ovis* in wild deer in Brazil

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Haemotrophic mycoplasmas (haemoplasmas) are bacteria that attach to red blood cells of mammals, leading to acute and/or subclinical disease in infected animals. The present work aimed to detect *Mycoplasma ovis* in buffy-coat samples from 45 wild deer from central-western and south-eastern regions of Brazil, using molecular techniques. Between 1996 and 2011, blood samples were collected from two brocket deer (*Mazama gouazoubira*) and four small red brocket deer (*Mazama bororo*) from São Paulo (SP) state, four brocket deer from Goiás (GO) state, three red deer (*Mazama americana*) from Paraná (PR) state, and 23 brocket deer and 11 pampas deer (*Ozotocerus bezoarticus*) from Mato Grosso do Sul (MS) state. Eighteen (40%) deer were positive to *M. ovis*-PCR based on a 224 pb of 16S rRNA: 05 *Ozotocerus bezoarticus*, 11 *Mazama gouazoubira*, 01 *M. bororo* and 01 *M. americana*. In order to obtain a larger 16S rRNA fragment for phylogenetic analysis, positive samples were submitted to another PCR using primers which amplify a 1341 pb of 16S rRNA. Sequenced amplicons showed a 99% similarity to *M. ovis* by BLAST analysis. The phylogenetic analysis based on Maximum Likelihood method grouped the haemoplasma DNA sequences found in sampled deer in the clade (bootstrap 96/100) occupied by *M. ovis* previously detected in Brazilian marsh deer in Brazil. In conclusion, haemoplasma infections are common in free-ranging deer populations. The pathogenic potential of haemoplasma species in deer is still unknown. FAPESP financial support.

[235] The ecology of parasite transmission in fauna translocations: Investigating the impact of polyparasitism on host fitness and translocation success.

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The woylie or brush-tailed bettong (*Bettongia penicillata*) is currently listed as critically endangered by the IUCN, and parasitic disease is suspected of playing a role in the recent decline of this endemic Australian marsupial. Polyparasitism, in which a host is co-infected with various parasite species or intraspecific strains, is common in wild animal populations. Whilst parasites have been implicated in a number of species declines, the role of polyparasitism as a potential factor contributing towards translocation failures has never been investigated. This project will evaluate how fauna translocations impact the transmission of parasites in woylies and what consequences this has for translocated populations and other coexistent species. As we lack a rigorous understanding of whether current parasite management protocols enhance translocation success, we will also assess the impact of parasite removal on translocated hosts. We aim to test the hypothesis that fauna translocations lead to a higher diversity of parasites within the resultant host-parasite community, and therefore a higher incidence of polyparasitism; which in conjunction with the disruption of established host-parasite associations, and the exposure of hosts to novel parasite species and strains, may exacerbate the negative impacts of parasites on hosts to the detriment of translocation success.

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