# BOOK OF ABSTRACTS

## THE 45TH ANNUAL CONFERENCE OF THE PARASITOLOGICAL SOCIETY OF SOUTHERN AFRICA

### Lagoon Beach Hotel, Cape Town

### 28 – 31 August 2016

PARASITOLOGICAL SOCIETY OF SOUTHERN AFRICA · PARASITOLOGIESE VERENIGING VAN SUIDELIKE AFRIKA

PARSA

agriculture, forestry & fisheries
Department: Agriculture, forestry & fisheries
REPUBLIC OF SOUTH AFRICA

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

UNIVERSITEIT STELLENBOSCH UNIVERSITY

# PROGRAMME

| Sunday 28 August 2016 | | |
|---|---|---|
| 16:00-19:00 | Registration | |
| **Day 1 – Monday 29 August 2016** | | |
| **Time** | **Topic** | **Speaker** |
| 7:30 | Registration open | |
| 8:00 | **Keynote: An overview of recent applied parasitological studies on commercially exploited fish off southern Africa** | **Dr Carl van der Lingen** |
| | **Applied Marine Parasitology** | |
| 8:45 | Parasites of Cape (*Trachurus capensis*) and Cunene (*T. trecae*) horse mackerel in the Benguela ecosystem | **Cecile Reed** |
| 9:00 | Using parasites as biological tags for examining population structure of Cape hakes *Merluccius capensis* and *M. paradoxus* off South Africa | **Larvika Singh** |
| 9:15 | Parasites of *Genypterus capensis* (Kingklip) and assessment of their potential as biological tags | **Sizo Sibanda** |
| 9:30 | Comparison of two different parasite processing methods for stock assessment of South African kingklip, *Genypterus capensis* (smith 1874) | **Ayesha Mobara** |
| 9:45 | Investigating trophic interactions between parasites and their hosts using stable isotope analysis | **Mark Weston** |
| 10:00 | Investigating long-term host-parasite dynamics in odontocetes in southern Africa | **Inge Adams** |
| 10:15 | The development of a non-lethal diagnostic tool for the diagnosis of *Ichthyophonus hoferi* | **Nicholas Nicolle** |
| **10:30** | **Tea/posters (30 Min)** | |
| | **Marine Parasite Biodiversity & Taxonomy** | |
| 11:00 | A possible new species of *Trebius* (siphonostomatoida: trebiidae) infecting *Squalus acutipinnis* Regan, 1908 off South Africa | **Susan Dippenaar** |
| 11:15 | Review of the fish parasitic *Anilocra haemuli* (Crustacea: isopoda: Cymothoidae) species complex from the Caribbean, using morphological and molecular techniques | **Rachel Welicky** |
| 11:30 | Morphology of a Hemiurid found in the stomach of monkfish, *Lophius vormerinus* in South Africa | **Pieter King** |
| 11:45 | A parasitic nematode (Dracunculoidea) affecting captive bowmouth guitarfish (*Rhina ancylostoma*) | **Kevin Christison** |
| 12:00 | Eusocial behaviour of snapping shrimps parasitising sponges (Porifera) | **Jo van As** |
| 12:15 | *Fusarium solani* isolated from loggerhead sea turtles (*Caretta caretta*) in south africa | **Mariska Laubsher** |
| 12:30 | The value of museum collections: Fish parasitic isopods of *Ceratothoa* Dana, 1852 (Crustacea, Isopoda, Cymothoidae) as a case in point | **Kerry Hadfield** |
| **12:45** | **Lunch (60 Min)** | |
| **13:45** | **Guest: overview of marine mammal health studies in southern Africa** | **Dr Stephanie Plön** |
| | **Freshwater parasite Biodiversity & Taxonomy** | |
| 14:15 | Blood parasites of freshwater fish in South Africa: The lack of information | **Luthando Bopheka** |
| 14:30 | Haemogregarine parasites parasitizing frogs of the Hyperoliidae (sedge and bush frogs) from south africa | **Edward Netherlands** |
| 14:45 | Neotropical Polystomatidae: An introduction to the polystome diversity of South America with information on newly discovered species | **Louis du Preez** |

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| 15:00 | Parasite introduction to the endangered western leopard toad: Spill over or spill back? | **Natasha Kruger** |
| 15:15 | Morphological characters may provide new insights into chelonian polystome classification | **Carina Coetzer** |
| 15:30 | A comprehensive study of the parasitic mite *Xenopacarus africanus* (acari: Ereynetidae) parasitic in the African clawed frog *Xenopus laevis* | **Jani Reeder** |
| **15:45** | **Tea/posterS (30 Min)** | |
| **Macroparasite Diversity & Ecology** | | |
| 16:15 | Comparative helminth composition and prevalence of indigenous and invasive synanthropic murid rodents in urban Gauteng Province, South Africa | **Rolanda Julius** |
| 16:30 | Diversity and seasonal abundance of nematodes from *Rhabdomys dilectus* in South Africa | **Andrea Spickett** |
| 16:45 | Gastrointestinal parasites infecting ungulates, felids and avian species at National Zoological Gardens of South Africa | **Paballo Mosala** |
| 17:00 | Species interactions within the parasite community of eastern rock sengis (*Elephantulus myurus*) | **Heike Lutermann** |
| **17:15** | **Poster Session (60 Min)** | |
| **18:15** | **FREE TIME** | |

## Day 2 – Tuesday 30 August 2016

| Time | Topic | Speaker |
|---|---|---|
| 8:00 | **Keynote: What are the factors driving parasite diversity?** | **Prof Conrad Matthee** |

| **Parallel Session 1: Freshwater parasite Biodiversity & Taxonomy** | | | **Parallel Session 2: Medical & Veterinary Parasitology** | | |
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| **Time** | **Topic** | **Speaker** | **Time** | **Topic** | **Speaker** |
| 8:45 | Introduced vs indigenous fish trichodinids (Ciliophora: peritrichia) in southern Africa and Tasmania | **Linda Basson** | 8:45 | *Bartonella elizabethae* and *B. tribocorum* in *Rattus*-associated ectoparasites in gauteng province, south africa | **Asiashu Lithole** |
| 9:00 | Assessment of *Trichodina heterodentata* Duncan, 1977 (Ciliophora: peritrichia) using molecular and morphological taxonomy | **Gerhard de Jager** | 9:00 | The distribution of African horse sickness vectors in the protection and surveillance zones of the Western Cape province, south africa | **Karien Labuschagne** |
| 9:15 | A morphometric analysis of gill monogeneans infecting the redbelly tilapia, *Tilapia zillii* (gervais, 1848) from Lake naivasha, Kenya: new biogeographical records | **Nehemia Rindoria** | 9:15 | Redescription, molecular characterisation and taxonomic re-evaluation of a unique african monitor lizard haemogregarine *Karyolysus paradoxa* (Dias, 1954) n. comb. (Karyolysidae) | **Courtney Cook** |

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| 9:30 | The Diplozoinae of Africa – Solving the puzzle | **Quinton Dos Santos** | 9:30 | Molecular and morphological description of a likely new *Trypanosoma* sp. in *Cordylus tropidosternum* (Sauria:Cordylidae) | **Nokofa Makhahlela** |
| 9:45 | Effect of metal exposure on hatchability and survival of oncomiracidia of *Paradiplozoon ichthyoxanthon* (Monogenea; Diplozoidae) from the Vaal Dam: Field meets laboratory | **Beric Gilbert** | 9:45 | *Hepatozoon* species (Apicomplexa: Adeleorina: Hepatozoidae) infecting wild and captive leopards *Panthera pardus pardus* (Linnaeus, 1758) from the Free State, Limpopo and Mpumalanga, South Africa | **Michelle van As** |
| 10:00 | Effect of constant temperatures on *Bulinus globosus – Schistosoma haematobium* system: Implications for parasite transmission | **Chester Kalinda** | 10:00 | Plasmodiid infections of afromontane raptors in the eastern Free State, South Africa | **Teboho Lovemore** |
| 10:15 | *Tarebia granifera* – a successful invader free of parasite burdens and its implications for trematode transmission in the Lower Phongolo River and floodplain | **Christian Selbach** | 10:15 | Membrane-active chelators tighten brain microvascular endothelial barriers to African trypanosome infection | **Dennis Grab** |
| **10:30** | **Tea (30 Min)** | | | | |
| **Parallel Session 3: Freshwater parasite Biodiversity & Taxonomy** | | | **Parallel Session 4: Molecular Parasitology** | | |
| 11:00 | Brain infections with *Tylodelphys* spp. in *Clarias gariepinus* and *Nothobranchius orthonotus* from the Lower Phongolo River | **Olena Kudlai** | 11:00 | Molecular detection of *Anaplasma*, *Babesia*, *Neorickettsia* and *Theileria* infections in horses and donkeys in South Africa | **Malitaba Mlangeni** |
| 11:15 | Clinostomid metacercariae and larval *Contracaecum* sp. infecting the Cape kurper *Sandelia capensis* Cuvier, 1831 | **Candice Jansen van Rensburg** | 11:15 | Molecular characterisation, morphological description and life cycle elucidation of *Hepatozoon* (apicomplexa: Adeleorina) infections in three African snakes | **Johann van As** |
| 11:30 | Nematoda in fish from the okavango River system, Botswana | **Liesl van As** | 11:30 | Molecular analysis of cercarial stages of digenean trematodes from snails collected around the Tshwane area | **Baratwa Moema** |
| 11:45 | *Chonopeltis* larval development: spot the differences | **Lourelle Neethling** | 11:45 | Msp1as genotyping of *Anaplasma centrale* indicates a wildlife reservoir | **Khumalo Zamantungwa** |

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| 12:00 | *Neoergasilus japonicus* feeding structures and the associated pathology | **Annemarie Avenant-Oldewage** | 12:00 | Detection and molecular characterization of *Anaplasma phagocytophilum* in domestic dogs from the Mnisi community area, Mpumalanga province, South Africa | **Mamohale Chaisi** |
| 12:15 | Former Katanga Province in the Democratic Republic of Congo – rich in diversity and life! preliminary results on freshwater fish parasite diversity | **Wilmien Powell** | 12:15 | Molecular epidemiology of tick-borne pathogens infecting predators at the farmland interface | **Storme Viljoen** |
| 12:30 | Seasonal variation in metazoan parasite occurrence of tigerfish in Lake Kariba, Zimbabwe | **Nyasha Mabika** | 12:30 | Metagenomic diagnosis of microbiota of horse flies (Diptera: Tabanidae) collected in north-eastern KwaZulu-Natal, South Africa | **Moeti Taioe** |
| **12:45** | **Lunch (60 Min)** | | | | |
| **13:45** | **Guest: Consistently high prevalence of parasitic agents of infectious diarrhoea: The case of the Vhembe District in Limpopo** | | | | **Prof Amidou Samie** |
| | **Medical & Veterinary Parasitology** | | | | |
| 14:15 | *Leishmania* vaccine development using machine learning algorithms | | | | **Webster Nyakudya** |
| 14:30 | Validation of a urine circulating cathodic antigen cassette test for detection of *Schistosoma haematobium* in uMkhanyakude district of South Africa | | | | **Owen Rubaba** |
| 14:45 | The influence of life history characteristics on flea (Siphonaptera) species distributions | | | | **Luther van der Mescht** |
| 15:00 | Prevalence of gastro-intestinal parasites of livestock and dogs and risk factors for transmission with emphasis on *Giardia* and *Cryptosporidium* in Magude District, Maputo Province, Mozambique | | | | **Regina Miambo** |
| **15:15** | **Tea (30 Min)** | | | | |
| 15:45 | Host immune responses induced in mice mono- and co-infected with *Trichinella zimbabwensis* and *Plasmodium berghei* ANKA | | | | **Samson Mukaratirwa** |
| 16:00 | Prevalence and phylogenetic background of *Besnoitia besnoiti* isolates from different geographical regions of South Africa | | | | **Mokgadi Malatji** |
| 16:15 | Early differential changes in microvascular barrier function in response to Dengue virus | | | | **Dennis Grab** |
| **16:30** | **AGM (60 Min)** | | | | |
| **17:30** | **FREE TIME** | | | | |
| **18:30** | **GALA DINNER** | | | | |

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# PARSA 2016

# BOOK OF ABSTRACTS

## CONTENTS

# KEYNOTE AND GUEST PRESENTATIONS

## An overview of recent applied parasitological studies on commercially exploited fish off southern Africa

Carl D. van der Lingen[1,2]
*[1]Branch: Fisheries Management, Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa, [2]Marine Research Institute and Department of Biological Sciences, University of Cape Town, Cape Town South Africa; CarlVDL@DAFF.gov.za*

Whereas most historical research on parasitic species of marine fish off southern Africa has been taxonomic in nature and focussed on describing new species and recording new host records, several applied parasitological studies on commercially important fish in the region have been conducted in recent years. These have included (i) applications of the parasite biotag method to investigate population structure of several commercially harvested species including sardine, anchovy, horse mackerel, Cape hakes, kingklip, monkfish, snoek and St Joseph sharks; (ii) investigations into parasitic species that negatively impact fish physiology or product quality, or have human health implications; (iii) the identification of disease-causing parasites in marine aquaculture systems and the assessment of disease risks and potential impacts posed by the movement of aquatic animals; and (iv) inclusion of parasites in ecosystem studies, including early detection of pollutants such as heavy metals and investigations into topographical food web analyses. Particular focus is given to the parasite biotag method in this presentation, with sardine used as case study. A brief mention of similar studies on other fish species as well as other ongoing applied parasitological research is also presented. These studies demonstrate that applied parasitological research is useful for the sustainable management of fisheries, continued growth and development of marine aquaculture, and in ecosystem observations, in addition to making a significant contribution to documenting marine biodiversity.

**Biography:** Carl van der Lingen was awarded his PhD in Zoology by the University of Cape Town in 2000. He is presently a Specialist Scientist in the Directorate: Resources Research of the South African Department of Agriculture, Forestry and Fisheries, and is also an Honorary Research Associate at the Marine Research Institute of the University of Cape Town. Carl has been interested in fish from an early age, and his research has focused on the trophic and reproductive ecology of small pelagic fish species in the Benguela Current

ecosystem, their role in ecosystem functioning, and the impacts of climate variability and change on their population sizes and distributions. He has authored or co-authored almost 100 scientific publications, has co-supervised over 30 postgraduate students, is a member of the Editorial Board of Fisheries Oceanography and an Editor of the African Journal of Marine Science, and was awarded a B2 rating by the NRF in 2013. Carl has recently led research to examine the population structure of South African sardine and other commercially exploited species, and whilst he is not a parasitologist he is an enthusiastic exponent of the parasite biotag method for elucidating population structure.

**What are the factors driving parasite diversity?**

Conrad A. Matthee[1]

[1]*Department of Botany and Zoology, Evolutionary Genomics Group, Stellenbosch University, Private Bag X1, Stellenbosch, 7602, South Africa; cam@sun.ac.za*

The evolution of parasites is often closely linked to the evolution of their hosts. Parasites with a close host association and a narrow host range are predicted to show coevolution with their hosts while those who spend a considerable amount of time off the host, and have a broad host ranges, will show less co-evolutionary congruence. By making use of predominantly small mammal species and a mixture of ectoparasites occurring on them, the effects of species-specific life histories and environmental conditions on the diversification of parasites in southern Africa are investigated. Comparative population genetic studies between rodents, shrews, ungulates, mites, lice, fleas and ticks reveal that the evolution of ectoparasites is the result of a complex interaction between parasite and host life histories, ecological interactions among species concerned, and environmental processes that may affect the parasite and the host in the same way. This overview provides new insights into the ability of ectoparasites to disperse throughout the landscape and to maintain genetic connectivity among isolated populations. It is shown that host association alone cannot be used as proxy to accurately predict the dispersal potential of parasites. In some instances, other factors such as social structure of the host species, parasite prevalence on the host, sex biased dispersal, and sensitivity of different life stages to environmental factors off the host, plays an equally important role in driving parasite diversity.

**Biography:** Conrad Matthee graduated with a PhD in Zoology from the University of Pretoria in 1996. After spending 2 years as a postdoctoral fellow at the University of Pretoria he was awarded a Skye Scholarship to pursue further research at Texas A&M University. He returned to South Africa to take up a lecturing position

at Stellenbosch University in 2000 where he is currently a Professor in Zoology and also the Executive Head of the Department of Botany and Zoology. He is an evolutionary systematist who has published mainly in the fields of parasite-host codivergence at the phylogeographic and species level, stock assessment and conservation of marine resources, comparative phylogeography of terrestrial vertebrates and the evolution and systematics of free-living and parasitic arthropods. He served previously on the editorial board of Integrative and Comparative Biology and is currently on the editorial boards of Koedoe, African Journal of Marine Science and Molecular Phylogenetics and Evolution. He holds a B2 rating from the NRF and was recently elected as a Fellow of the Royal Society of South Africa.

**Overview of marine mammal health studies in southern Africa**

Stephanie Plön[1]

[1]*Research Fellow, School of Environmental Sciences, Nelson Mandela Metropolitan University, University Way, P O Box 77000, Port Elizabeth, 6031; Stephanie.Plon@nmmu.ac.za*

Globally the health of our oceans is of increasing concern, yet locally few studies have examined health parameters in top marine predators as indicators of ocean health. Often these animals are difficult to monitor, and their relative inaccessibility poses a hurdle to detailed scientific investigations of tissues and body fluids, which are required for many analyses. In this context, stranded animals are not representative, because most stranded animals show signs of illness, which probably led to the stranding. Under a long-standing agreement between the KwaZulu-Natal Sharks Board and the Port Elizabeth Museum, necropsies of incidentally-caught dolphins in bather protection nets off KwaZulu-Natal have been conducted since the 1980s. The most commonly caught species are the Indo-Pacific bottlenose dolphin *Tursiops truncatus*, the Indian Ocean humpback dolphin *Sousa plumbea* and the long-beaked common dolphin *Delphinus capensis*. During routine investigations of these animals in 2009, a series of unexplained lesions were observed in all species of dolphin commonly caught and in in all age groups. This prompted the first systematic health investigations of these carcasses, which have been conducted for the past six years. This research has shown that most lesions are parasite related, but more detailed investigations concerning the species involved are still being conducted. In addition to the histopathological analyses, investigations of diet, reproductive organs, and pollutant load are being carried out in an effort to gain a detailed picture of the biology and health of these animals, which are considered representative of the wild populations.

**Biography:** Stephanie Plön (PhD) was born and raised in Göttingen, Germany, and started her career in marine mammal science during her BSc in Marine Biology at the University of Wales, UK (1991-1994). During that time she conducted her Honours project at the Port Elizabeth Museum in South Africa during a three month internship under the guidance of Dr. Vic Cockcroft. This experience paved the way for further studies on marine mammals and for her love of the Eastern Cape Province of South Africa. She enrolled for an MSc degree, which was later upgraded to a PhD, on the natural history of pygmy and dwarf sperm whales off South Africa at Rhodes University, South Africa, also under the guidance of Dr. Vic Cockcroft and Prof. Ric Bernard (completed in 2004). During that time she received an Ernest Oppenheimer Student award to complete her PhD thesis at the University ofAuckland, New Zealand, with Dr. Scott Baker. Following an abbreviated stint as a Postdoctoral Research Associate at the University of Auckland, Stephanie took up the opportunity to return to South Africa as the marine mammal curator/ advisor at the Port Elizabeth museum. Here she advised on captive animals in the museum-associated oceanarium as well as the large Graham Ross marine mammal research collection. She re- established research on cetaceans in the Eastern Cape, which included field research in Algoa Bay as well as stranding response along the Eastern Cape coastline. Stephanie paid particular attention to the examination of humpback, bottlenose, and common dolphins incidentally caught in the shark nets off KwaZulu-Natal, South Africa, and started to work up material that had been collected since Dr. Cockcroft's departure in 1998. This resulted in a myriad of studies on the diet, life history parameters, anatomy, and health of the by-caught dolphins, laid the ground-work for ongoing research, and allowed the analysis of long-term datasets to examine possible trends in relation to changing environmental factors over time. In late 2008 Stephanie joined the South African Institute for Aquatic Biodiversity (SAIAB) (Grahamstown) to continue her research. This mainly involved the continuation of field studies in Algoa Bay, which she had initiated in early 2008, examining the temporal and spatial distribution of whales and dolphins in view of potential impacts resulting from the newly established deep-water Port of Ngqura. Since 2013 Stephanie is based at the Nelson Mandela Metropolitan University (NMMU) in Port Elizabeth as a Research Fellow in the School of Environmental Sciences, where she continues to conduct research on cetacean populations in Eastern Cape waters. She is currently the PI of four major research programmes along the KwaZulu-Natal and Eastern Cape coastline and supervises a number of postgraduate students. Her main interests are in cetacean ecology, population studies and health aspects.Stephanie has put much time and passion into teaching postgraduate students and has supervised numerous student projects to date. She has published a number of research papers, invited book chapters and presented at numerous international conferences.

**Consistently high prevalence of parasitic agents of infectious diarrhoea: The case of the Vhembe District in Limpopo**

Amidou Samie[1]

[1]*Molecular parasitology and opportunistic infections program, Department of Microbiology, University of Venda, Thohoyandou 0950, South Africa; samie.amidou@univen.ac.za*

Diarrheal diseases constitute an important cause of morbidity and mortality among children throughout the world and particularly in developing countries. Globally it is estimated that about 4 million children aged less than 5 years old die every year due to diarrhoea and its complications including dehydration and malnutrition. In the northern part of South Africa, diarrhoea still remains the main cause of death among children less than 5 years of age although in other parts of the country, HIV and lower respiratory infections come before diarrhoea. Among the infectious causes of diarrhoea, *Entamoenba histolytica*, *Giardia* and *Cryptosporidium* spp are the most common parasitic aetiology and have been found to be very common in our communities. The prevalence of *E. histolytica* varies between 8% and 38% in the stool while the seroprevalence varies between 30% and 86%. Although *Entamoeba* spp is very common in many rural areas, *E. histolytica* seems to be a common cause of diarrhoea in urban areas like those around Pretoria. *Giardia* is also common with a prevalence varying between 4% and 30% but seems to be a common cause of diarrhoea in the rural areas while it is less common in urban areas. Different genotyping systems have shown that *E. histolytica* has a complex genetic structure which might affect disease presentation. Similarly, host genetics might also have an impact on disease progression with different cytokines playing crucial roles according to the individual profile. In a recent study we showed for the first time that IL-10 promoter polymorphisms participate in the progression of amebiasis. The IL-10 -1082 A/G polymorphism was more associated with amebiasis susceptibility as compared to -592 A/C polymorphism. In conclusion, the parasitic aetiology of diarrhoea varies tremendously according to the population considered and probably with time and the complex interaction of host-parasite relationship. There is a need for collaborative research in order to increase our understanding of the role of intestinal and other parasites on the health of our communities.

**Biography:** Dr Amidou Samie is Associate Professor of Microbiology at the University of Venda, in South Africa. He gives lectures on topics covering Parasitology, Immunology and Industrial Microbiology. He is currently a rated researcher by the National Research Foundation of South Africa at the category C2, has published close to 100 research papers and book chapters in the field of microbiology and infectious diseases and has graduated several MScs and PhDs. His

research activities cover mostly topics in infectious diseases from epidemiology to control. His particular interest lies in the study of intestinal protozoan parasites and opportunistic infections among HIV patients as well as the potential impact of childhood diarrhoea on growth and child development.

# SESSION 1: APPLIED MARINE PARASITOLOGY

Oral presentations:

## (S1.1o): Parasites of Cape (*Trachurus capensis*) and Cunene (*T. trecae*) horse mackerel in the Benguela ecosystem

Jenna Bowker[1], Jessica le Roux[1], Cecile Reed[1,3], Carl van der Lingen[2,3], Willy Hemmingsen[5], Ken MacKenzie[4]
*[1]Department of Biological Sciences, University of Cape Town, Cape Town, South Africa; [2]Branch Fisheries, Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa; [3]Marine Research Institute, University of Cape Town, Cape Town, South Africa; [4]Department of Zoology, University of Aberdeen, Aberdeen, United Kingdom; [5]Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, University of Tromsø, Tromsø, Norway*

Two species of horse mackerel from the Benguela Upwelling Ecosystem off southern Africa were surveyed for parasitic infections. Cunene horse mackerel *Trachurus trecae* and Cape horse mackerel *T. capensis* were collected in the northern Benguela off the coasts of Namibia and Angola, whilst *T. capensis* only were collected in the southern Benguela off the South African west and south coast. Six parasite taxa were recorded for *T. trecae,* the most prevalent being the coccidian *Goussia cruciata* (68.1%) in the liver, the monogenean *Gastrocotyle trachuri* (45.8%) in the gills and an unidentified digenean(25.0%) found infecting the pyloric ceacae, intestine, gills and stomach. Twenty-four parasite taxa were found infecting northern Benguela *T. capensis*, the most prevalent being the nematode *Anisakis* sp. (83%), *G. cruciata* (72.3%) and the copepod *Lernanthropus trachuri* (34%). Twenty-one parasite taxa were recorded from southern Benguela *T. capensis*, the most prevalent being *Anisakis* sp. (85.9%), *G. trachuri* (56.4%) and *G. cruciata* (44.9%), and parasite assemblages were not different between fishfrom the west and south coast. Fish size had a significant effect on the parasite assemblages of both horse mackerel species. Parasite assemblages of *T. trecae* and *T. capensis* in the northern Benguela differed significantly, as did those of *T. capensis* from Namibia and South Africa. These results show that closely related fish species living in the same environment can have different parasites, and support the hypothesis of distinct stocks of Cape horse mackerel in the northern and southern Benguela subsystems but not within the southern Benguela itself.

## (S1.2o): Using parasites as biological tags for examining population structure of Cape Hakes *Merluccius capensis* and *M. paradoxus* off South Africa

Larvika Singh[1], Carl van der Lingen[1,2], Cecile Reed[2], Ken MacKenzie[3]
*[1]Branch: Fisheries, Department of Agriculture, Forestry and Fisheries, Cape Town, South*

*Africa;[2]Department of Biological Sciences and Marine Research Institute, University of Cape Town, Cape Town, South Africa;[3]University of Aberdeen, Aberdeen, UK*

Two species of hake, *Merluccius capensis* and *M. paradoxus*, occur off South Africa where they are targeted by the country's most valuable fishery. The two hakes are currently managed as a single stock that is not differentiated by species and does not take potential population structure of either species into account. These management practices are changing, however; in 2014 we conducted an initial study to assess the utility of applying the parasite biotag approach to elucidate population structure of the two hake species and identified seven parasite taxa as candidate biotags. Preliminary results show a spatial difference in the parasite assemblage of *M. capensis* off South Africa, with the monogenean *Anthocotyle merlucci* found attached to the gills of fish collected off the west coast but not the south coast. In contrast, the copepod *Neobranchiella insidiosa f. lageniformis* was found on the gills of fish off the south coast but not the west coast. These results suggest the presence of two stocks of *M. capensis* off South Africa, which is also reflected in current genetic results. Currently there seems to be almost no spatial difference in the parasite assemblage of *M. paradoxus*, which also agrees with genetic analysis that suggests that *M. paradoxus* is panmictic. Fish size and spatial effects on the occurrence of *Anisakis* spp, a potential human pathogen found in both hake species, will also be presented.

**(S1.3o): Parasites of *Genypterus capensis* (kingklip) and assessment of their potential as biological tags**

Sizakele Sibanda[1], Cecile Reed[1], Carl van der Lingen[1,2]
*[1]University of Cape Town, Cape Town, South Africa;[2]Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa*

Despite years of exploitation, stock discrimination of South African kingklip *Genypterus capensis* is yet to be resolved. The population of kingklip in South Africa is managed as a single stock even though there are morphological, meristic and behavioural indications of the existence of multiple stocks. This study explored the potential use of parasite biological tags for population structure studies of kingklip off South Africa by comparing the parasite assemblage of fish collected from five regions around the South African coast in April/May 2014. A total of 67 kingklip were examined for parasites, with 20 parasite taxa belonging to the Cestoda, Trematoda, Myxozoa, Secernentea, Copepoda, Acanthocephala, Rhabdotophora and Trypanoryncha recorded. Marked host-size effects were detected so spatial comparisons used adult fish only (n=57), and significant ($p < 0.05$) dissimilarities in the entire parasite assemblages of adult kingklip between regions were observed. Two parasites, namely *Lecithochirium genypteri* and *Anisakis* sp. were consistently the strongest discriminators in all paired regional comparisons, suggesting that these two taxa could be useful biotags and indicating possible population structure in South African kingklip. When

only the six parasites that had overall prevalence values of >10% were used (namely *L. genypteri*, *Anisakis* sp. *Cuccullanus genypteri*, *Myxidium* sp., *Diphyllobothrium* sp., and *Chondrocanthus* sp.), *Anisakis* sp. was the only significant discriminator between the five regions.

**(S1.4o): Comparison of two different parasite processing methods for stock assessment of South African kingklip, *Genypterus capensis* (Smith 1874)**

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The parasite biotag approach to examining population structure is presently being applied to South African kingklip, *Genypterus capensis* (Smith 1874), both as a single-method approach and as part of a multi-method approach. In Method 1 each fish was dissected and the entire carcass and internal organs immediately examined for parasites. In Method 2 the exterior and abdominal cavity of the fish were scanned for parasites following dissection, after which internal organs were removed and frozen for later parasitological analysis. Fish from three different areas off the South African coast were processed using one of the two methods. Their results are compared here. The two methods differed significantly in terms of species richness, with 20 parasite taxa found using Method 1 whereas only four of these parasites (*Anisakis sp., Cucullanus genypteri, Lecithochiriumgenypteri, Diphyllobothrium sp.*) and one acanthocephalanwere found using Method 2. Whereas mean infection intensities of these taxa did not differ significantly between methods, significant differences in the mean parasite abundance of three of them (*Anisakis sp*. $t = 4.46$, $df = 28$; $p < 0.005$; *Diphyllobothrium sp.* $t = -2.140$, $df = 73$, $p = 0.036$;and *L.genypteri* $t = 2.404$, $df = 29$, $p = 0.011$) were observed. Likely reasons for these differences are discussed. MDS reveal that there is no spatial effect on the parasite community structure of South African kingklip. Further parasitological data is required for stock structure analysis and will be done using Method 1 which proves to be the most comprehensive method.

**(S1.5o): Investigating trophic interactions between parasites and their hosts using stable isotope analysis**

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*[1]University of Cape Town, Cape Town, South Africa; [2]Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa*

Stable isotope analysis has been widely used to investigate the trophic interactions in ecological communities in a wide variety of ecosystems, both terrestrial and aquatic. There

has been a recent drive to include parasitic species into food-web studies as they have been largely ignored as essential components of ecosystems. A few studies on the isotopic signatures of parasite species have been published but these have provided contrasting results, with some parasite taxa showing enriched and others showing depleted isotopic signatures. The aim of this project is to investigate the trophic interactions between gill parasites and their marine fish hosts through the use of stable isotope analyses. In addition we observe the parasites' mouthpart morphology to gain insight into feeding mechanisms and how this might affect their isotope signatures. We hypothesize that the parasites will have enriched isotopic signatures of both $\delta^{15}N$ and $\delta^{13}C$ isotopes as a result of feeding on their fish hosts. Isotope signatures of copepod and monogeneaen parasites found on the gills and within the opercula cavity of several fish species will be presented and compared to gill and dorsal muscle tissue isotope signatures from their respective hosts. Initial results from two copepod and two monogenean parasites of three tuna species show significant $\delta^{15}N$ enrichment by the parasites of 0.4-1.4 ‰. Images of some parasite mouthparts/feeding apparatus are also shown. The utility of stable isotope data in understanding the role of and including parasites in pelagic food-webs will be further discussed.

**(S1.6o): Investigating long-term host-parasite dynamics in odontocetes in southern Africa**

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Globally, research into dolphin parasitology has received a significant amount of attention, however, investigations into the parasites infecting dolphins here in southern Africa have been lacking. Twenty-five dolphin species can be found in the South African subregion and most records of their parasites involve accounts of external parasites as they are the easiest to see and identify. Parasites have been collected both opportunistically and systematically over the past 40 years from odontocetes incidentally caught in shark nets off KwaZulu-Natal and are held in the Graham Ross Marine Mammal collection of the Port Elizabeth Museum. Parasites from the Risso's dolphin, Indian Ocean humpback dolphin, Indo-Pacific bottlenose dolphin, Pantropical spotted dolphin, striped dolphin, long-beaked common dolphin and pygmy and dwarf sperm whales were identified to species level where possible. To date, approximately 16 species of parasites have been identified across eight host species, with one, *Hymenolepis nana*, being recorded for the first time in *D. capensis* in southern Africa. Seven parasite species seem to be unique to a specific host, while the other nine are shared across hosts. Further data analysis is underway, including examination of spatial and temporal trends as well as between sexes and age groups of each host species. This study will provide us with valuable baseline data and allow us to shed some light on a largely unknown component of dolphin biology in this country. Knowledge about the parasites that infect cetaceans, specifically coastal species, is becoming increasingly important due to the continued degradation of our coastal environment.

**(S1.7o): The development of a non-lethal diagnostic tool for the diagnosis of *Ichthyophonus hoferi***

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*Ichthyophonus hoferi* has been diagnosed at the Two Oceans Aquarium. *Ichthyophonus* is a mesomycetozoan parasite that multiplies in blood-rich organs in the fish host causing a wide range of clinical signs relating to organ dysfunction. *Ichthyophonus* can be diagnosed from microscopic examination of tissue squash preparations, culture or PCR. In the literature only lethal methods of diagnosis are described. The development of a non-lethal diagnostic tool for disease monitoring is vital for collections where sacrifice of specimens is not possible. Liver biopsies were obtained from (n=30)white stumpnose (*Rhabdosargus globiceps)* comparing two surgical methods, coeliotomy (n=15) and coelioscopy (n=15), with ten fish used as a control group. Biopsy material for each fish was divided into three pieces for squash preparation examination, PCR and culture. All fish were monitored for43 days post-surgery and blood samples drawn at two week intervals. After 43 days fish were euthanized for full examination of the liver, kidney, spleen and heart allowing correct assignment to one of two groups; *Ichthyophonus*-infected fish and non-infected fish. PCR and culture of liver tissue was also performed. Preliminary results show a 64% sensitivity of the wet mount biopsy and a 38% sensitivity of biopsy in culture with 100% specificity for both. Wet mount and culture of the biopsy showed a sensitivity of 81%. Final post mortem on all organs showed 25 fish to be positive for *Ichthyophonus.* 5 fish were negative for *Ichthyophonus* in all diagnoses. Coelioscopy was less invasive and caused fewer organ adhesions than coeliotomy.

Poster presentations:

**(S1.8p): Establishing the effect of parasites on the health status, nesting behaviour and colony dynamics of African penguins (*Spheniscus demersus*)**

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The endangered African penguin (*Spheniscus demersus*) occurs naturally along the coast of southern Africa. Although the colony at Stony Point (SP) in Betty's Bay has experienced a gradual increment in penguin numbers (to 2,626 breeding pairs in 2015), conservationists

have observed an increase in nest and chick abandonment there. It is suspected that parasites, and in particular soft ticks, might be one of the factors behind this behaviour. However, the impact of parasites on African penguins is poorly investigated, and it is uncertain what the extent of the problem is within the SP colony. This study aims to: 1) collect comprehensive data on the ecto- and endoparasite diversity and abundance of penguins (adults and chicks) and soft tick abundances within different nest types (natural open, natural covered and artificial), 2) compare soft tick abundances within the nest to several parameters associated with the nest: microclimatic conditions, spatial position, density and occupancy of nests, 3) record the general health status and body condition of penguins, and 4) calculate the spatial covariance between regional colonies and regional population variability based on reported population counts. Parasite abundances and general health status of the SP colony will be compared to surrounding island and land-based colonies (Robben-, Dassen- and Dyer Islands and Boulders Beach). The study will be conducted during the main breeding season (May-October) in 2016 and 2017. Through this study we hope to better understand the importance of parasites in nest and chick abandonment, and the implications that this might have for African penguin conservation.

**(S1.9p): Seasonality of *Kudoathyrsites* and/or *Kudoa paniformis* infection in South African sardine *Sardinops sagax***

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Myoliquefaction of fish musculature results in product deterioration, customer complaints and significant economic losses, especially in the canning industry for South African sardine *Sardinops sagax*. *Post-mortem* myoliquefaction of fish muscle is associated with parasitic infection by *Kudoa thyrsites* and/or *K. paniformis*, but little is known about seasonal variation in the level of *Kudoa* infection in South African sardines. Given the present low population size of the sardine resource and food security as an important world-wide topic, it is crucial to study and implement food technology strategies which may reduce wastage of good-quality marine fish protein for human consumption due to *Kudoa* infection. The aim of this study was to investigate seasonal variation in infection of sardine by *Kudoa paniformis*and/or *Kudoa thyrsites*, and initial results are reported here. The genomic DNA of 74 sardine collected from the west and south coast during the first part of 2015 (11-15 samples per month from January, February, March, April, June and July) was investigated using custom-designed TaqMan® SNP Genotyping assays. Both assay mixes (designated AHLJ2B7_KP for *Kudoa paniformis* andAHKA35Z_KT for *Kudoathyrsites)* were designed from the nucleotide sequence of the small subunit ribosomal RNA genes (ssRNA) of the *Kudoa* parasites. *Kudoa paniformis* (AHLJ2B7_KP allele 1) could not be detected in any of the 74 samples, whereas *Kudoa thyrsites* (AHKA35Z_KT allele 1) was present in the majority (76%)

of sardine samples. Samples collected during the summer months (January-March) showed higher levels of infection compared to autumn/winter months (April-July), suggesting some seasonal variation.

**(S1.10p): Parasites of South African monkfish, *Lophius vomerinus* and their potential as biotags for stock discrimination**

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Monkfish*Lophius vomerinus* is a demersal fish distributed around South Africa and is the most economically important by-catch species taken in the demersal trawl fishery. Questions of whether there are one or more monkfish stocks off South Africa have prompted a multi-method approach to investigating the population structure of this species. This study aimed at documenting monkfish parasite assemblage and identifying which parasites might be useful as biotags for stock discrimination. Ninety five monkfish collected from research surveys conducted off the west and south coast in 2015 were examined for parasites, and prevalence, mean infection intensity and mean abundance of each parasite was compared between monkfish from the west and south coast. Five parasitic taxa were documented, including microsporideans, myxosporeans, nematodes, cestodes and digeneans. Whereas multidimensional scaling (MDS) and cluster analysis revealed a mixing of parasitic species between monkfish off the south and west coast, an analysis of similarity (ANOSIM) showeda significant ($p=0.04$) but weak ($R=0.044$) separation. Additionally, the prevalence of digenean parasites was higher in monkfish from the south than the west coast, and the mean intensity and mean abundance of Digenea sp1 (considered to be *Lecithochirium* sp.) from the stomach were significantly higher in south coast fish. Similarly, a similarity percentage (SIMPER) analysis indicated dissimilarity (67.44%) between monkfish from the two coasts and a discriminant function analysis identified Digenea sp1as a significant discriminating species ($F=4.44$; $p=0.03$).These results provide baseline data for monkfish stock identification and suggest that Digenea sp1 may have the highest biotag potential.

**(S1.11p): Applying the parasite biotag approach to investigate population structure in St Joseph shark (*Callorhinchus capensis*) in shelf waters off South Africa**

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The macroscopic parasite fauna of St Joseph shark (*Callorhinchus capensis*) has previously been examined for sharks collected from inshore waters in St Helena Bay, Saldanha Bay and False Bay off the South African west coast. One of the five parasitic taxa recorded the large (15-20cm) cestode *Gyrocotyle plana* was identified by previous researchers as having the highest potential as a biotag for population structure analysis of *C. capensis*. The present study applied the biotag approach to St Joseph sharks and documented the macroscopic parasites of 40 specimens sampled from shelf waters during demersal research surveys at 10 localities along the west coast, and 37 specimens from 13 localities off the south coast of South Africa in 2015. Observed macroparasites included *G. plana*, found in the spiral valve with an overall prevalence of 83%, and two monogeneans, *Callorhynchicotyle callorhynchi* and *Callorhinchicola multitesticulatus* infecting the gills, with overall prevalence values of 57% and 20%, respectively. Mean abundance and mean infection intensity observed were independent of sex and fish size for all three parasites. Whereas small-scale variation in the abundance of these parasites was observed for sharks off each coast, there were no significant differences in prevalence, mean parasite abundance and mean infection intensity between St Joseph sharks from the west and south coasts. These results suggest that the parasite biotag approach may not be a suitable method for application in future stock assessment studies for St. Joseph sharks in South Africa, or that this species comprises a panmictic population.

# SESSION 2: MARINE PARASITE BIODIVERSITY &TAXONOMY

Oral presentations:

### (S2.1o): A possible new species of *Trebius* (Siphonostomatoida: Trebiidae) infecting *Squalus acutipinnis* Regan, 1908 off South Africa

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Members of Trebiidae C.B. Wilson, 1905 and Caligidae Burmeister, 1835 are morphologically very similar by having free third and fourth thoracic somites and a biramous fourth leg. However, they have very different life cycles with that of Trebiidae without a chalimus phase while Caligidae have four chalimus stages, and copulation in Trebiidae takes place in the copepodid III and/or IV females while copulation in Caligidae only takes place in adult females. *Trebius* currently consists of 15 accepted species all infecting elasmobranchs. Most species are relatively host-specific and have been reported from only one or two host species. However, *T. caudatus* Krøyer, 1838 and *T. latifurcatus* Wilson, 1921 have been reported from 10 and eight host species, respectively. Specimens of *Trebius*, collected from *Squalus acutipinnis*, were examined through stereo- and compound microscopes, dissected, drawn and described. These specimens can be distinguished from the other known species by a combination of characteristics including an abdomen that is shorter than the genital complex, a maxillule with an endite that consists of a single-tined dentiform process, sternal furca tines that are blunt and as long as the base and with the innermost spine of the last exopodal segment of leg 1 the shortest, and hence may constitute a new species.

### (S2.2o): Review of the fish parasitic *Anilocra haemuli* (Crustacea: Isopoda: Cymothoidae) species complex from the Caribbean, using morphological and molecular techniques

Rachel Welicky[1], Kerry Hadfield[1], Nico Smit[1]
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The conspicuousness and global distribution of *Anilocra* isopods (fish ectoparasites) make them an excellent model for understanding the role of fish parasites in food webs. One of the greatest challenges with using *Anilocra* spp.as a model for trophic studies is that their high level of diversity and overall abundance has led to inconsistent taxonomic classification. Accordingly, *Anilocra* have been named after their host species, genus, and family. Without proper taxonomic and molecular classification, describing patterns of host-*Anilocra* interactions and the ecological role of *Anilocra* is haphazard at best. Given that our

previous ecological research demonstrates that *Anilocra* spp. have differential effects on fishes, we hypothesised that *Anilocra* are host species specific, which contradicts findings of previous taxonomic work on *Anilocra* spp. Specifically, we predicted that *Anilocra* spp.infecting *Haemulon flavolineatum* and *Epinephelus guttatus* are not both *Anilocra haemuli* as previously described, but two different species. To test our prediction, we used standard taxonomic and molecular approaches to describe and compare *A. haemuli* specimens obtained from both fishes, and we compared these findings to the original description of *A*. *haemuli*. Preliminary morphological analyses revealed differences between *A. haemuli* from *H. flavolineatum* and *A. haemuli* from *E. guttatus*. Preliminary molecular analyses support the morphological findings and suggest that the *Anilocra* spp. infecting these fishes may be different. Our study highlights the importance of using both taxonomic and molecular approaches to identify parasite species, as general patterns in host-parasite interactions cannot be determined without proper organismal identification.

**(S2.3o): Morphology of a Hemiurid found in the stomach of monkfish, *Lophius vormerinus* in South Africa**

Pieter King[1], Yusuf Agabi[2], Cecile Reed[2], Carl van der Lingen[3,4], Chantele Baker[1]
*[1]Sefako Makgatho Health Sciences University, Pretoria, South Africa; [2]University of Cape Town, Cape Town, South Africa; [3]Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa; [4]University of Cape Town, Cape Town, South Africa*

Hemiuroids are usually parasitic in the gut and stomach of marine fishes. The most characteristic feature of this parasite is the presence of an ecsoma. The parasite in the present study was found in the stomach of monkfish (*Lophius vomerinus*). Monkfish are an economically valuable species caught as by-catch in the offshore demersal trawl industry. Samples were collected from the west and south coasts of South Africa during demersal research cruises of the Department of Agriculture, Forestry and Fisheries. Fish were frozen on board, bagged and labelled. In the laboratory fish were thawed, dissected and hemiuroid parasites collected from the stomach and fixed in 70% alcohol and stained with haematoxylin for light microscopy. Specimens were also fixed in gluteraldehyde for scanning electron microscopy and were viewed using a VP FE-SEM. Excluding the distinct ecsoma, the present specimens resemble most characteristics shown by parasites of the genus *Lecithochirium*. Adult specimens from the stomach measure 3765-5075 x 721-1185 µm. Acetabulum is larger than the oral sucker and situated in the anterior third of the body. Testes are symmetrically posterior of the acetabulum with the ovary positioned distal and posteriorly. The bipartite seminal vesicle is large and leads to a pars prostatica and ejaculatory duct. The posterior half of the body is occupied with a large uterus filled with eggs. The vitellaria consist of seven oval to digitiform lobes. This study represents the first description of a *Lecithochirium* sp. found in the stomach of monkfish sampled near Cape Town.

**(S2.4o): A parasitic nematode (Dracunculoidea) affecting captive bowmouth guitarfish (*Rhina ancylostoma*)**

Kevin Christison[1], Francois Lampen[2], David Pearton[2], Rick Last[3]
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A heavy nematode infection on the gill arches of was observed on post-mortem examination of twobowmouth guitarfish (*Rhina ancylostoma* Bloch & Schneider, 1801) housed at uShaka Sea World, Durban. Histopathology of the gills confirmed a severe branchitis with moderate numbers of melanomacrophages and numerous cross sectional segments of nematode parasites. The gills further revealed thrombosis of many branchial vessels in the inflammatory zone. Initial diagnosis identified these parasites as dracunculoid nematodes. Several unidentified dracunculoid larvae have been reported from different organs of chondrichthyan fishes and many of these have been considered as belonging to the Philometridae. Both morphological characterization based on light microscopy and molecular characterization based on the phylogenetic comparison of a ~1400 bp fragment of the SSU-rDNA gene suggest that this parasite is more closely related to the Philonemidae and Daniconematidae than the Philometridae. Most dracunculoids are reported as being viviparous, however no first-stage larvae were observed *in utero* in any of the worms examined. This may be due to seasonal maturation as has been described for a few dracunculoid taxa, with gravid females only being observed within relatively short periods in spring or summer. Generally, information regarding the diversity, taxonomy and systematics of fish dracunculoids is sparse and although some Philometra species have been recorded from teleost hosts locally, this study represents the first record, to our knowledge, of a dracunculoid nematode from an elasmobranch host from South Africa.

**(S2.5o): Eusocial behaviour of snapping shrimps parasitising sponges (Porifera)**

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Social behaviour in terrestrial ecosystems iswell known amongst arthropod groups. This ranges from the simplest form of gregarious behaviour and extended brood care by individuals of the same generation, common amongst scorpions, spiders, mites, and different families of beetles, to very complex eusocial behaviour in bees, ants, termites and two species of mole rats. Until the early 1990's it was believed that there are no eusocial behaviours amongst aquatic organisms. This view was changed by the discovery of eusociality in the coral reef snapping shrimp *Synalpheus regalis.* These sponge-dwelling parasitic shrimps live in colonies of more than 300 individuals of mixed generations, with

only a single or a few females ("queens") reproducing. So far five species of the genus *Synalpheus* (Decapoda: Alpheidae) have been found to be completely eusocial, spending their entire life in the canals of sponges, feeding on particles of organic matter and mucoid secretions of the sponge. Fully grown large individuals do not reproduce, but defend the sponge on which they parasitise. Other non-reproductive adults remove debris from the sponge canals. This paper presents a review of this interesting parasitic association between crustaceans and sponges.

**(S2.6o): *Fusarium solani* isolated from loggerhead sea turtles (*Caretta caretta*) in South Africa**

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*Fusarium* species are fast growing, environmental saprophytic fungi. Along with many *Fusarium* species, members of the *Fusarium solani* species complex (FSSC) are filamentous fungi with a wide distribution, and are virulent in causing animal infections, especially in immunocompromised individuals. *Fusarium keratoplasticum,* a member of the FSSC, has previously been isolated from sea turtle nests along the Pacific and Atlantic oceans and has been associated with high egg mortality rates in sea turtles. In this study we characterise *Fusarium* species that were isolated from post-hatchling loggerhead sea turtles (*Caretta caretta*) that washed up on beaches along the Indian Ocean, South Africa. Macro- and micromorphological characterisation over a 21 day period on potato dextrose agar (PDA) and carnation leaf agar (CLA) revealed that isolates from this study are members of the FSSC. Two gene regions were amplified for more accurate characterisation, namely internal transcribed spacer (ITS) and a part of the nuclear large subunit (LSU). A third, less conserved gene region, namely translation elongation factor 1 α (EF), was amplified to identify possible differences between isolates from this study. Amplified products were submitted for sequencing. Resulting sequences were checked against the NCBI database using the BLAST option and aligned with closely related sequences. Results showed high similarity between isolates from this study and *F. keratoplasticum* isolates previously associated with high egg mortality rates in loggerhead sea turtles. To our knowledge this is the first record of *F. keratoplasticum* isolated from the Indian Ocean and from post-hatching loggerhead sea turtles.

**(S2.7o): The value of museum collections: Fish parasitic isopods of *Ceratothoa* Dana, 1852 (Crustacea: Isopoda: Cymothoidae) as a case in point**

Kerry Hadfield[1], Niel Bruce[1,2], Nico Smit[1]
*[1]North-West University, Potchefstroom, South Africa; [2]Museum of Tropical Queensland, Queensland Museum, Townsville, Australia*

The conservation of scientific collections, especially type material, is almost as important as the discovery of a new species itself. Without a type specimen to compare to, a species can be misidentified and all of the subsequent information would be applied to the incorrect species. This is most pertinent with parasites where a misidentification could lead to false information on the host, species distribution records, and the pathogenicity of the parasite. Within the parasitic isopod family Cymothoidae, there are a large number of species that have not been studied since their original description dating back more than 200 years. This could be due to a lack of cymothoid isopod specialists, or insufficient material, but it is most likely due to the fact that many of these species are difficult or near impossible to identify based on their original descriptions. A number of recent papers have aimed at alleviating this problem through re-descriptions of some of the more common species which have been incorrectly named or confused with other species. The present study focussed on the poorly known *Ceratothoa* species and aimed at clarifying some of the taxonomic uncertainties within this genus using type material. Results from this study emphasised the value of well-maintained museum collections, especially in regards to type material, when identifying and revising species.

Poster presentations:

**(S2.8p): A re-description of *Kudoa thyrsites* (Gilchrist, 1924) (Myxozoa: Kudoidae) from its type host, *Thyrsites atun***

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*Kudoa thyrsites* is a marine myxozoan that infects the muscles of several, commercially important fish species worldwide. While not generally pathogenic to the fish hosts, *K. thyrsites* induces post-mortem myoliquefaction, the enzymatic breakdown of infected tissue, resulting in severe economic losses in the salmon aquaculture industry and several wild fisheries. Despite its global economic importance, little is known about the biology of *K. thyrsites* and the description upon which its identification is based (Gilchrist 1923) is inadequate by modern standards. *Kudoa thyrsites* was initially described as *Chloromyxum thyrsites* from formalin-fixed *Thyrsites atun* tissue in South Africa. In 1949, Willis provided a

better description, including vegetative developmental stages, but both works lack detail, pre-dating the widely accepted guidelines provided by Lom & Arthur (1989) and updated by Burger & Adlard (2010) for descriptions in the Kudoidae. The identification of kudoid spores primarily relies on spore morphology along with tissue tropism and ribosomal DNA sequencing. As the genus *Kudoa* Meglitsch, 1947 grows, there is a need for more defining taxonomic characteristics to be recorded during species descriptions to enable an unambiguous classification of myxospores, especially amongst morphologically similar species. Consequently, the number of recommended measurements used to characterize kudoid myxospores has increasedfrom three to eight, which warrants a re-description of *K. thyrsites*. Here we seek to provide an updated description of *K. thyrsites* myxospores recovered from its type host, *Thyrsites atun* and type locality in South Africa.

**(S2.9p): Morphology of a Strigeid metacercaria found in the eyes of sardine, *Sardinops sagax* in South Africa**

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Strigeid metacercariae are parasites of many marine teleost fish, inhabiting the brain and eyes of their hosts. The present study provides a morphological study of a Strigeid 'tetracotyle-type' metacercaria found in the eyes of South African sardine, *Sardinops sagax*. Metacercariae were collected from fresh sardine sampled from a commercial landing in Gans Bay, South Africa, and were manually excysted, relaxed in warm water, fixed in 70% ethanol and stained with haematoxylin for light microscopy. The most characteristic feature of this metacercaria is its unique, large excretory bladder lobes situated on the lateral sides of the body. The metacercarial body is oval-shaped, measuring 762 - 967 x 512 – 677µm. It is divided by transverse folds into a forebody, midbody and hindbody. Other diagnostic features include: two large pseudosuckers in the anterior part of the midbody, an acetabulum which is larger than the oral sucker, and a large lobulated holdfast organ in the posterior half of the midbody. These characteristics are those of the metacercariae of the genus *Cardiocephaloides* sp. This study is the first description of a *Cardiocephaloides* metacercaria found in eye of *Sardinops sagax* in South Africa.

**(S2.10p): Hexabothriid parasites from Rajidae species of South Africa**

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Hexabothriid parasites have been described as some of the most host-specific parasites and can be found on the gills of chondrichthyan fish. This study aims to add information to the current scarcity of literature and to provide resolution to the systematics, particularly for South African species. Hexabothriid species already identified from South African Chondrichthyes include the genera *Callorhynchocotyle* and *Branchotenthes*. Both *Callorhinchus capensis* (Cape elephant fish)and *Rhina ancylostoma* (bowmouth guitarfish) play host to these two genera. Hexabothriid parasites were collected from *Rostroraja alba* (white skate)*, Leucoraja wallacei* (yellowspotted skate)*,* and *Raja straelini* (biscuit skate). The monogeneans were stained with alum carmine to aid in the identification process by a closer examination of their reproductive organs. Furthermore, morphometric analysis of the sclerotised haptoral armature including the hamuli was tested to assess the sensitivity of these characters to discriminate parasites from closely related host species. Measurements of the hamulus included, among others, circumferential length, shaft width, and aperture angle. The characters of the hamulus have the potential to provide species-level information previously not considered. The value in resolving an issue of systematics and identification cannot be underestimated due to the high monetary and educational value of aquarium species affected by these parasites. Ethical and responsible captive husbandry of these species may be well served by a targeted treatment for any infestation when correctly identified, thus reducing mortalities.

**(S2.11p): Parasites of South African angelfish *Brama brama***

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Angelfish (*Brama brama*; also known as Atlantic pomfret) is a pelagic species that is distributed from depths of 0 to 1000 m in temperate waters across the globe. Off southern Africa they primarily occur in continental shelf edge and upper slope waters of the Benguela Current ecosystem off Namibia and the South African west coast. Whilst not targeted, angelfish are caught as by-catch in the demersal trawl industry in both countries. Little is known about the biology and ecology of angelfish in the Benguela, and we are not aware of the parasite assemblage of this species having previously been documented from this region. This presentation describes the parasite assemblage of angelfish collected during research surveys conducted on the western Agulhas Bank in August 2015 and off the west coast in

March 2016. A total of six parasitic taxa were documented, the most prevalent (>95%) being *Hatschekia conifera*, a copepod species observed in angelfish gills with a mean infection intensity of 166 per fish. A presently unidentified monogenaen was also found in the gills, although at much lower prevalence. Other parasites include *Anisakis* sp. and *Hepatoxylin* sp., both found in the viscera. These results are compared to documented parasites of *Brama* sp. elsewhere, and will contribute towards understanding the population structure of this species within the entire Benguela upwelling ecosystem.

**(S2.12p): A new species and first record of the fish parasitic isopod *Pleopodias* Richardson, 1910, from the southern hemisphere**

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Over the last few years, several of the cymothoid isopods from southern Africa have been revised. A number of new species have been identified and new host and locality records have been noted; however, all of these new records were from genera already known to occur in this region. For the first time in almost a century, a new genus record of cymothoid isopod has been identified from South Africa. The cymothoid genus, *Pleopodias* Richardson, 1910, is a small group of parasitic isopods known to occur on the external surfaces of their fish hosts. Only three species are currently recognised, namely, *P.diaphus* Avdeev, 1975; *P. elongatus* Richardson, 1910; and *P. vigilans* Richardson, 1911. These species are known only from Japan, the Philippines and off the coast of Sudan, respectively. Recently a specimen from the South African Museum was positively identified as a *Pleopodias* sp., collected off the south coast of South Africa. Morphological variations as well as the geographical distribution confirm this species as not only new to South Africa but also new to science.

**(S2.13p): Investigating the parasite assemblages of two round herring species (*Etrumeus whiteheadi* and *E. wongratanai*) off South Africa**

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There are approximately 20 species of the Order Clupeiformes (anchovies, herrings and sardines) that occur in South African marine waters. Some of the Clupeiformes are of great ecological and economic importance. While studies have been done to determine the parasite assemblage of one of the most important clupeid species, sardine *Sardinops sagax*, knowledge of the parasite assemblages of other clupeiform species is lacking or is minimal.

This study reports on the parasite assemblage of two round herring species, west coast round herring, *Etrumeuswhiteheadi*, and east coast round herring, *Etrumeuswongratanai*. The assemblage of the two *Etrumeus* species will be compared to other clupeids in the waters around southern Africa, as well as *Etrumeus* species found elsewhere in the world. Initial investigations of the 11 west coast and 24 eEast coast round herring suggest that these two species have relatively low parasite loads, both in terms of taxa diversity and parasite numbers.

# SESSION 3: FRESHWATER PARASITE BIODIVERSITY &TAXONOMY

Oral presentations:

**(S3.1o): Blood parasites of freshwater fish in South Africa: The lack of information**

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Since the description of trypanosomes and haemogregarines from marine fish of South Africa by Fantham in 1918 and 1919, only a handful of research regarding fish blood parasites has been conducted in South Africa. A large majority of the research that has been done has concentrated on marine fish species around the coast of South Africa. This includes the works of JR Baker, NJ Smit, A Davies, PM Hayes, A Avenant-Oldewage and M Ferreira. Baker's research in the 1960s in Natal, Mozambique and throughout Africa focused on trypanosomes. In the late 1990s and early 2000s Smit and Davies described new haemogregarine and trypanosome species from intertidal pool fishes and established that a Gnathiid isopod is the vector. Compared to the rest of the world, the information on fish blood parasites in southern Africa is quite poor, within the major blood parasite groups. To date, three species of trypanosomes, four haemogregarines species and a single dactylosomatid have been identified in South Africa. Since 1919 less than 50 publications have been written on fish blood parasites in southern Africa, and of those less than 10 were from freshwater fishes in southern Africa, let alone South Africa. In comparison to other fish endo- and ecto-parasites the world of blood parasites is still to be explored. With over 200 fish species in southern Africa river systems, there is still a lot of work to be done to documentblood parasites of freshwater fishes in South Africa.

**(S3.2o): Haemogregarine parasites parasitizing frogs of the Hyperoliidae (sedge and bush frogs) from South Africa**

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Haemogregarines comprise a large group of apicomplexan blood parasites, with *Hepatozoon* being the most commonly reported genus to parasitize anurans. Sixteen *Hepatozoon* species have been described from anurans in Africa, with only a single species, *Hepatozoon hyperolli*, infecting a member of the Hyperoliidae. Furthermore, only two *Hepatozoon* species are known from South African anurans, namely *H. theileri* and *H. ixoxo*, from *Amietia quecketti* and three *Sclerophrys* species, respectively. A total of 225 individuals representing nine hyperoliids were collected from seven localities throughout northern KwaZulu-Natal, South Africa. Blood was drawn from each frog, thin blood smears prepared and the remaining blood fixed in 70% molecular grade ethanol. Giemsa-stained blood smears were screened for blood parasites using a compound microscope and micrographs of the parasites were taken and subsequently measured (*n*=50). Samples found positive were molecularly characterised using PCR amplification of the 18S rRNA gene (1,640 nt). Twenty frogs from three species were found positive for haemogregarines, namely *Afrixalus fornasinii* (6/11), *Hyperolius argus* (2/39), and *H. marmoratus* (12/74). Morphological characteristics, morphometrics, and molecular findings support that the haemogregarines from the three host species represent three different, but closely related *Hepatozoon* species. Furthermore, these species do not conform morphologically to *H. hyperolii*. The phylogenetic analysis showed that worldwide, all anuran *Hepatozoon* species sequenced so far form a monophyletic clade, within a larger clade comprising of *Hepatozoon* species from mammals, reptiles, birds and amphibians. These findings illustrate the close-knit evolutionary history shared by anuran *Hepatozoon* species, not only from Africa but also globally.

**(S3.3o): Neotropical Polystomatidae: An introduction to the polystome diversity of South America with information on newly discovered species**

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Polystomatids (Monogenea, Polystomatidae) are represented in the Neotropical Realm by the genera *Mesopolystoma, Parapseudopolystoma, Polystoma, Riojatrema* and *Wetapolystoma* from anuran hosts; *Nanopolystoma* from caecilians; and *Neopolystoma* and *Polystomoides* from chelonian hosts. The genus *Polystoma* has a widespread occurrence in all zoogeographical realms except the Australian realm, and 11 of the 65 currently known species are from South America but none are known from Brazil. A collecting trip to the Amazon led to the discovery of a new species of *Polystoma* from the pepper frog *Trachycephalus typhonius.* Although this new parasite is significantly smaller than other members of the genus known from the continent, it shares the basic body features. The far majority of neotropical *Polystoma* spp. have a highly diverticulated intestine that forms a

network of anastomoses between the gut caeca, a characteristic that is quite rare in other parts of the world.

**(S3.4o): Parasite introduction to the endangered Western Leopard Toad: Spill over or spill back?**

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*Sclerophrys gutturalis* (guttural toad, GT) was introduced into Constantia, Western Cape, South Africa, from Durban, KZN, South Africa. The toad is spreading at an alarming rate and is invading the natural distribution area of the endangered western leopard toad (WLT; *Sclerophrys pantherinus*). The WLT is endemic to the Fynbos Biome and in early spring they breed for a period of one week. Researchers have become increasingly concerned with directs effects of GT on WLT, such as predation and competition. However, indirect effects such as interaction with infectious agents and parasites carried by GT may also have impacts on the WLT. Frogs harbour variety of parasites such as protozoans, nematodes, acanthocephalans, flukes, cestodes, mites and leeches, and they may either be the intermediate or the definite host. Introduced hosts may act as vectors and/or reservoirs for introduced parasites, and when these introduced parasites are transmitted to the native host this is known as 'spill-over'. However, native hosts can transmit native parasites to the introduced host and this can then be a threat to uninfected native individuals; this is known as 'spill-back'. As we are dealing with different host species, results can differentiate between spill over and spill back, as parasite occurrence and infection intensity are determined by numerous factors such as host-infectious agent specificity, host density and invasion pathway. An introduced parasite might be life threatening for the endangered WLT.

**(S3.5o): Morphological characters may provide new insights into chelonian polystome classification**

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Chelonian polystomes (Monogenea: Polystomatidae) inhabit three different locations on their respective hosts, namely the conjunctival sacs under the eyes, oral region and pharyngeal pouch, and the urinary bladder. Although chelonian polystomes form a monophyletic group, the genera *Polystomoides* and *Neopolystoma* are, according to published molecular studies, not monophyletic. With the exception of one species, those from the conjunctival sacs form a distinct clade belonging to *Neopolystoma*. Other clades

contain combination of both *Polystomoides* and *Neopolystoma* and from both the oral region and the urinary bladder. The objective of the current study was to study the morphology of chelonian polystomes and determine whether species cluster according to shared morphological characters or combinations thereof. Then to compare these morphological clusters according to sites where species are found on the host and published molecular evidence. Preliminary morphological evidence supported by statistical analyses indicates that several characters may be of taxonomic value and may aid to resolve the taxonomic uncertainties surrounding chelonian polystomes. Characters that show potential to be of taxonomic importance include size of the marginal hooklets, number of genital spines, intestinal caecum length, hamulus shape, sucker diameter as a percentage of the body length, and testis shape.

**(S3.6o): A comprehensive study of the parasitic mite *Xenopacarus africanus* (Acari: Ereynetidae) parasitic in the African clawed frog *Xenopus laevis***

Jani Reeder[1], Edward Netherlands[1], Louis du Preez[1]
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*Xenopacarus africanus*, a parasitic mite occurring in the nasal and eustachian passages of the African clawed frog *Xenopus laevis*, was examined from six localities in and around Potchefstroom, North West Province, South Africa. The pH, conductivity and temperature were measured at each locality. Prevalence of infected hosts and parasite burden was recorded. An experimental study was also conducted to determine the effect of temperature on parasite numbers. No statistical correlation was found between the water parameters from the sites and infection levels. However, in the experimental study temperature was shown to have an effect on level of infection. Different life stages of mites were collected and preserved for both morphological and molecular characterisation. Scanning electron micrographs were taken of eggs, larvae and male and female adult mites. Phylogenetic analysis was conducted using fragments of the mitochondrial cytochrome b (cytb) gene.

**(S3.7o): Introduced vs indigenous fish trichodinids (Ciliophora: Peritrichia) in southern Africa and Tasmania**

Linda Basson[1]
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Trichodinids are mobiline unicellular organisms found worldwide on a wide range of hosts, but the majority are described from marine and freshwater fishes. Mobiline ciliophorans belonging to the family Trichodinidae are amongst the most common and widely distributed fish symbionts. These trichodinid species from fish number more than 260, represented by seven genera. Some fish trichodinids are more host specific than others. We have described

50 species from marine and freshwater fishes from 10 countries worldwide over the last three decades. Thirty-eight of these fish trichodinids were encountered in southern Africa and Tasmania collectively. When trying to determine which fish trichodinids might have been introduced and which are indigenous, these two regions both present challenges for two main reasons; i) most water masses contain introduced fish, and ii) no trichodinids were identified before the settlement of introduced fish. However, deductions can be made based on the worldwide occurrence of certain species as well as the specificity shown by some trichodinid species. Furthermore, there are a small number of water bodies in southern Africa where no alien fish have been introduced to date. Two case studies will be presented to speculate on the possible introduced *vs* indigenous trichodinid species, one from southern Africa with a proven record of new introductions, and a second where trichodinids from two introduced fish were identified and described which also represents the first records of freshwater trichodinids in Tasmania, Australia.

**(S3.8o): Assessment of *Trichodina heterodentata* Duncan, 1977 (Ciliophora: Peritrichia) using molecular and morphological taxonomy**

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The family Trichodinidae (Ciliophora, Peritrichia) consists of ten genera, of which the cosmopolitan genus *Trichodina* Ehrenberg, 1830 has the largest number of species. A substantial majority of trichodinid species are associated with marine and freshwater fish hosts, while some are also found associated with amphibians, as well as a variety of invertebrate hosts. Some trichodinid species show high levels of host specificity, whilst others occur on a range of hosts. *T. heterodentata* Duncan, 1977 is a globally distributed species associated with numerous freshwater fishes represented by various families, and it is also found on several tadpoles in southern Africa. This species shows a wide range of morphological variation, which appears to be valid. Recently, some workers have started to use molecular studies to analyse the taxonomic status of some trichodinid species, which leads to doubt concerning the validity of some species complexes, including *T. heterodentata*. Most fish species harbouring *T. heterodentata* display multi-trich infestations, however on its amphibian host, *T. heterodentat*a is a single infestation, which makes it perfect for using it as a model species. The present study therefore aims to use *T. heterodentata* as a specific model to investigate this question by incorporating ultrastructure (Nano Sam) and molecular DNA analyses (standard 18S SSU rDNA as well as complete genome) together with established morphological methodology.

**(S3.9o): A morphometric analysis of gill monogeneans infecting the redbelly tilapia, *Tilapia zillii* (Gervais, 1848) from Lake Naivasha, Kenya: New biogeographical records**

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A total of fifty specimens of the introduced redbelly tilapia, *Tilapia zillii* (Gervais, 1848) were collected from Lake Naivasha, Kenya from January to May 2015, and studied with the aim to collect and identify the first data on their monogenean parasites. Standard methods of parasitological examination were used in the identification of gill monogenean species. The collected monogeneans were preserved in 4% formalin prepared from formaldehyde solution for morphometric analysis. Seven *Cichlidogyrus* species were identified from the gills based on morphometric features of the opisthaptor and copulatory organs using identification keys. These include: *C. sclerosus* Paperna & Thurston, 1969; *C. tilapiae* Paperna, 1960; *C. digitatus* Dossou, 1982; *C. aegypticus* Ergens, 1981; *C. vexus* Pariselle & Euzet, 1995, *C. arthracanthus* Paperna, 1960 and *C. yanni* Pariselle & Euzet, 1996. These monogeneans form the first biogeographical record on the host (*T. zillii*) in Lake Naivasha and from the Republic of Kenya. *Cichlidogyrusdigitatus* was the most dominant *Cichlidogyrus* species in *T. zillii* in Lake Naivasha.

**(S3.10o): The Diplozoinae of Africa: Solving the puzzle**

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Currently, only fourspecies of the genus *Paradiplozoon* are known to occur in Africa, two of which were described early in the second half of the 20th century, while two more descriptions were published only recently. The latest species, *Paradiplozoon ichthyoxanthon* and *Paradiplozoon vaalense*, were described sufficiently from a taxonomic perspective, including morphology, morphometry and molecular aspects. The taxonomic information available for *Paradiplozoon aegyptense* and *Paradiplozoon ghanense*, the two historic species, are lacking in many crucial regards. By obtaining specimens from the Royal Museum of Central Africa, an attempt was made to add some of the missing taxonomic information for these species. Using these specimens, a re-description of the sclerite detail of *P. aegyptense* was possible. In addition, one specimen incorrectly identified was found to represent *P. ghanense*, allowing for a similar re-description of this species. Additionally, specimens collected from *Labeo rosae* and *L. congoro* in the Olifants River within the Kruger National Park in the early 1990's were also studied and described as *Paradiplozoon krugerense*. As such, the is represented by five species in Africa, for which the morphology and morphometry are now more holistically known. Unfortunately, molecular information is only available for the three recent species, with representative sequences of the internal transcribed spacer 2 (ITS2) published inGenBank. Using the ITS2 sequences for these three

species, as well as additional sequences for the 28S rDNA and cytochrome oxidase 1 (CO1) markers, comparative phylogenies were produced and also compared to the phylogeny of their fish hosts.

**(S3.11o): Effect of metal exposure on hatchability and survival of oncomiracidia of *Paradiplozoon ichthyoxanthon* (Monogenea; Diplozoidae) from the Vaal Dam: Field meets laboratory**

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Monogenea show potential as sentinel organisms for monitoring environmental health, especially concerning heavy metals. During field excursions in the Vaal Dam, 10 *Labeobarbus aeneus* were euthanised, and their gills excised and assessed for *Paradiplozoon ichthyoxanthon*. Parasites were removed from the gills and flash frozen. Another 10 *L. aeneus* were maintained in 160lplastic containers filled with dam water and were transported back to the laboratory where they were used to establish a laboratory culture of *P. ichthyoxanthon*. Parasite eggs were collected and exposed to varying concentrations of aluminium in static systems and checked every 24h. Frozen parasites were embedded in optimal-cutting temperature compound, sectioned on a cryomicrotome (5µm) and exposed to a Phenanthroline based Phen-Green fluorochrome for detecting metals. Absence of fluorescence reactions for metals associated with eggs were observed. Following laboratory exposure of eggs to aluminium, decreased survival of larvae and reduced hatching of eggs occurred, but it did not affect larval development. Normal embryonic development of *P. ichthyoxanthon* within eggs exposed to aluminium indicates moderate impermeablity of the egg shells to metals, and metal interaction with embryos. Even though parasites are accumulating metals, as indicated by vitellaria fluorescence, the eggs are apparently not involved in metal homeostasis. The egg shell is therefore an effective barrier, protecting developing embryos from the external environment. Higher larval mortality at120µg/lexposure related to aluminium crossing the egg shell and probably causing death of unhatched yet fully developed embryos, indicating a change in permeability of the egg shell towards the end of larval development.

**(S3.12o): Effect of constant temperatures on *Bulinus globosus* - *Schistosoma haematobium* system: Implications for parasite transmission**

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The impact of climate change may alter schistosomiasis transmission patterns because of its influence on host-parasite interactions. Although empirical evidence suggests that the

response of hosts and parasites to climate change will be different owing to their size difference, predicting the overall effect on transmission dynamics still remains a challenge.Using a host-parasite system involving *Bulinus globosus*, a freshwater snail, and its trematode parasite *Schistosoma haematobium*, we experimentally evaluated the effect of different constant temperatures on snail fecundity, mortality and shedding of cercariae. Infected snails subjected to 30 and 25$^{o}$C released a higher number of cercariae and had high mortality compared to those subjected to 20$^{o}$C. We observed peak shedding of cercariae at 25$^{o}$C and a delayed commencement of egg laying was observed in snails at 20$^{o}$C. The interaction between infection and temperature influenced egg laying and mortality among infected snails.Our results show variable responses to temperature on host-parasite interactions. Although temperature enhanced egg production and shortened the onset period for egg laying, it had an overlapping effects with parasite production that led to reduced egg laying and increased and host mortality.

**(S3.13o): *Tarebia granifera*: A successful invader free of parasite burdens and its implications for trematode transmission in the Lower Phongolo River and floodplain**

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The south-east Asian gastropod *Tarebia granifera* is a highly successful invader in many parts of the world, including South Africa. In its native habitats *T. granifera* harbours a diverse and prevalent fauna of trematodes that, as parasitic castrators, control population size. Free of its predator and parasite burdens, invasive species often hold competitive advantages over their native counterparts. To date, no trematode infections have been recorded from *T. granifera* populations in South Africa. It is possible, however, that trematode species can make their way to South Africa via migratory birds, as has happened in other regions. Furthermore, as an aggressive invader, *T. granifera* competes with other snail species that harbour trematodes in South African waterbodies and might thus relieve other hosts' parasite burdens. It is not understood to what extend this complex interplay shapes parasite communities under natural conditions. The present study aims at addressing the following research questions to shed some light on the ecological role of *T. granifera* in the transmission of trematodes: Does *T. granifera* serve as an intermediate host for trematodes in South Africa? What are the densities of *T. granifera* in relation to snails that harbour trematodes? What is the potential effect of *T. granifera* on trematode transmission? During preliminary sampling in May 2016, snails were collected from the Phongolo River floodplain and analysed for trematode infections. Results showed that *T. granifera* harboured no trematodes and its densities far outnumbered other snail species, which implies a potential diminishing effect on trematode diversity and community structure.

**(S3.14o): Brain infections with *Tylodelphys* spp. in *Clarias gariepinus* and *Nothobranchius orthonotus* from the Lower Phongolo River**

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Diplostomid metacercariae are important fish pathogens that can, at high densities, cause significant morbidity and mortality in fish populations. This group of parasites has become in recent years a focus of intensive studies on most continents, especially in Europe and North America. However, Africa remains the continent where a paucity of information on these parasites exists. To rectify this a large-scale study using modern molecular tools in combination with quality morphological analysis, an approach that proved to be extremely successful, is required. The diversity of diplostomoids in South Africa is poorly understood, with just two forms from fish and amphibians identified to the species level, i.e. *Tylodelphys xenopi* and *T. mashonensis*. In order to elucidate the species diversity of diplostomids in fishes from South Africa, a pilot study was performed at the Lower Phongolo River applying morphological and molecular approaches. Two species of fish, the African sharptooth catfish *Clarias gariepinus* (7 specimens) and spotted killifish *Nothobranchius orthonotus* (6 specimens) were found to be infected in the cranial cavity with metacercariae of *Tylodelphys* spp. Overall parasite intensity was remarkably high (7–249 metacercariae per fish for *N. orthonotus*; 151–1,343 metacercariae per fish for *C. gariepinus*). In this study the metacercariae of *Tylodelphys* spp. are described and their association within diplostomatids tested with phylogenetic analyses of ribosomal and mitochondrial DNA. This study is the first to report metacercariae of *Tylodelphys* from *N. orthonotus*.

**(S3.15o): Clinostomid metacercariae and larval *Contracaecum* sp. infecting the Cape kurper *Sandelia capensis* Cuvier, 1831**

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*Sandelia capensis* is endemic to the southern tip of South Africa and occurs within a narrow range from the Coega River (Algoa Bay) to the Cape Flats and north to Verlorenvlei. During fish parasitological surveys in the De Hoop Nature Reserve, *S. capensis* was found to be harbouring infections of trematode metacercarial cysts and larval nematodes. Forty seven specimens of *S. capensis* with an average body length of 75mm were examined for the presence of parasites. Metacercarial cysts were removed subdermally from all regions of the body and excysted and fixed in 10% buffered neutral formalin. Nematode specimens were collected from the viscera of the abdominal cavity and fixed in 10 % formalin or 70%

ethanol. Both digeneans and nematodes were prepared for light and scanning electron microscopy using standard techniques. The metacercarial cysts collected were identified as belonging to the Family Clinostomidae (prevalence 45%, intensity 1-5 cysts per fish), while the only nematode species found was the third stage larvae of *Contracaecum* sp. (prevalence of 23%, intensity 1-4 nematodes per fish). The Cape kurper represents a new host record for both the *Contracaecum* sp. larvae as well as the clinostomid metacercariae.

**(S3.16o): Nematoda in fish from the Okavango River System, Botswana**

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The Okavango Basinis the world's largest inland delta. The river rises on the Angolan Highlands, from where small streams converge into two rivers, the Cubango (west) and the Cuito (east) that subsequently flow into the Kavango River (Namibia), before reaching Botswana where it is known as the Okavango. The river meanders within the papyrus swamp of the Panhandle, dotted with lagoons, oxbow lakes and channels. About 150 km downstream, the river spreads out into the Okavango Delta where the sediment is deposited as an alluvial fan across the Kalahari Desert. The fish fauna of the Okavango can be considered as being part of the Zambezi system, which has 134 fish species. Of these, 86 are found in the Okavango basin and 71 species within the Okavango Panhandle and Delta. This presentation deals with representatives of the phylum Nematoda (*Camallanus, Cithariniella, Falcaustra, Labeonema, Paracamallanus, Philometroides, Procamallanus, Spinitectus* and *Synodontisia*)that were found in17 fish species collected over the past two decadesfrom the Okavango system. The nematode fauna that parasitise African freshwater fish species still remains fairly unknown, however since 2000 new species have been described. The current presentation will not be presented as a checklist, but will rather attempt to use the data collected to refer to some of the interactions and ecological aspects of these roundworms and their fish hosts.

**(S3.17o): *Chonopeltis*larval development: Spot the differences**

Lourelle Neethling[1], Annemariè Avenant-Oldewage[1]
[1]*University of Johannesburg, Johannesburg, South Africa*

Development in *Chonopeltis* spp. has been described for four species namely *C. inermis* Thiele, 1900, *C. brevis* Fryer, 1961, *C. minutus* Fryer, 1977, and *C. lisikili* van As and van As, 1996. The development of the trophic appendages of *C. australis* Boxshall, 1976 has also been described. This study provides further information on the lifecycle of *C. australis*. Adult

specimens of *C. australis* were collected from *Labeo capensis* (Smith, 1841) and brought back to the lab to start a breeding colony. The development of eggs was monitored daily. Seventeen juvenile specimens were collected before they attached to the host and were studied using light and scanning electron microscopy techniques. During the course of the study four life stages were identified, namely larval stage 1, larval stage 5, larval stage 9, and the sub-adult stage. The first larval stage bears prominent maxillulae and maxillae. By larval stage 5, two cephalic bars appear between the antennae, the maxillulae develop sucker "anlage" and the maxillae begins to take on the adult form. By the ninth larval stage the specimen bears fully functional suckers and some gender characteristics start to develop; while the sub-adult stage shows the ornamentation of the head increase to four bars and the gender characteristics become prominent. The result of this study showed that development in *C. australis* follows a similar pattern to that in other species of the genus with the differences occurring in the species details of the male copulatory structures.

**(S3.18o): *Neoergasilus japonicus* feeding structures and the associated pathology**

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*Neoergasilus japonicus* is a recent introduction to South African freshwater and was first recorded in 2009 on *Labeo capensis, L. umbratus* and *Labeobarbus aeneus* in the Vaal Dam. It has since then been found in a variety of other fish host species and also in the Limpopo River system, where it also appears to be not host specific. Parasites and host tissue were collected and fixed in a formalin-based fixative, thereafter dehydrated in acetone and embedded in resin before sectioning. The sections were stained with either Heidenhein trichrome stain or haematoxylin and eosin. Additional specimens were prepared for scanning electron microscopy using HMDS as the dehydrating agent. The remainder were fixed in 70% ethanol, cleared in lactic acid with cotton blue, dissected and studied by light microscopy. Female parasites attach with their antennae to the host's fin attachment sites and sites show little evidence of pathology. However, the mouthparts comprise highly modified mandibles, maxillulae and maxillae that facilitate feeding with a brush like harvesting of host tissue. This is enhanced by the unique musculature of the oesophagus and midgut funnel. It is concluded that although the attachment of the parasite appears to have little impact on the host, the feeding structures are capable of rupturing blood vessels, allowing the female parasite to acquire the blood meal required for egg production.

**(S3.19o): Former Katanga Province in the Democratic Republic of Congo: Rich in diversity and life! Preliminary results on freshwater fish parasite diversity.**

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The copper-producing former province of Katanga is characterised by several lakes and rivers with abundant and diverse fish species with *Oreochromis macrochir* economically the most important. Fish is an essential source of protein and also the livelihood for many households from this province with a population amounting to 5 million inhabitants. Fish were collected with seine and gill nets from Lubumbashi Zoo, an aquaculture farm and two lakes, Tshangalele and Koni, some 100 km from Lubumbashi. Standard procedures were followed for parasite collection; prevalence of infection indicated. Eight fish species were collected from the Zoo with *Lamproglena* sp. (100%) and a trichodinid (33.3%) recorded from *Oreochromis niloticus*; a diplozoid (100%) from *Barbus paludinosus*; *Macrogyrodactylus* sp. (50%) from *Clarias gariepinus*. The latter species was also infected with *Paracamallanus* sp. (20%); *Macrogyrodactylus* sp. (40%) and *Quadriacanthus* sp. (40%) from the aquaculture farm. Except for *Lamproglena* sp. (100%), *Cichlidogyrus* sp., *Enterogyrus* sp., and *Gyrodactylus* sp. were recorded in low numbers from *O. niloticus* from the farm. Six fish species were collected from Lake Tshangalele with digenean larvae the most prevalent from several hosts, including*Lamproglena* sp. from *Sargochromis maylandi* (100%) and *Cichlidogyrus* spp. (66.6%) from *Coptodon rendalli*. From the eight fish species collected from Lake Koni, *Synodontis lufirae* was infected with *Camallanus* sp. (33.3%) and a cestode (33.3%) while *Clinostomum* sp. and *Enterogyrus* sp. were recorded from *O. macrochir*. A large parasite diversity was recorded, all comprising first records for Katanga. Diversity indices analysed from data from the lakes are linked to mining-related pollution.

**(S3.20o): Seasonal variation in metazoan parasites' occurrence of tigerfish Hydrocynus vittatus (Castlenau 1861) in Lake Kariba, Zimbabwe**

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*[1]University of Johannesburg, Johannesburg, South Africa; [2]University of Zimbabwe, Harare, Zimbabwe*

The tigerfish is one of the most important commercial and game fish species in Lake Kariba and one of the top predators in the ecosystem. However, information on its parasitofauna is scanty. This study investigated the seasonal occurrence of metazoan parasites of Hydrocynus vittatus in the Sanyati basin, Lake Kariba. Eighty fish specimens were collected by seine netting over four seasons between October 2014 and July 2015 and examined for metazoan parasites. Mean seasonal water temperatures ranged from 24.1°C in winter to 31.2°C in spring, with slight variations among the seasons. Identified parasites included Annulotrema sp.1 and Annulotrema sp.2 (Monogenea), Contracaecum larvae (Nematoda), Lamproglena hemprichii (Copepoda), larval cestodes and Ichthybothrium sp. (Cestoda) and pentastomid larvae(Pentastomida). Larval cestodes were recorded in autumn and spring, while pentastome larvae were recorded in summer and spring. The Ichthybothrium sp. was

recorded once in winter. Annulotrema sp.1 had a100% prevalence in all the seasons and showed significant differences in mean abundance among the seasons ($P<0.05$). Annulotrema sp.2, Contracaecum larvae and L.hempricii were recorded in all the seasons, with slight variations in seasonal prevalence, mean abundance and mean intensity. The slight variations in the occurrence of the parasites could probably be due to the thermal stability of the lake where the variation in temperature between seasons was small. Annulotrema sp.1, Annulotrema sp.2, and Contracaecum larvae were aggregated on the fish host whereas L. hemprichii exhibited a random distribution.

Poster presentations:

**(S3.21p): Ultrastructural comparison of *Hepatozoon ixoxo* and *Hepatozoon theileri* (Adeleorina: Hepatozoidae), parasitising South African amphibians**

Roxanne Conradie[1], Courtney Cook[1], Anine Jordaan[1], Louis Du Preez[1], Edward Netherlands[1]
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To date, only two haemogregarine species have been described from South African frogs: *Hepatozoon ixoxo*, infecting toads of the genus *Amietophrynus*; and *Hepatozoon theileri*, parasitising the common river frog, *Amietia quecketti*. Both species have been characterised using limited morphology, as well as genetically using fragments of the 18S gene. However, no ultrastructural work has been performed thus far. The aim of this study was to add descriptive information on the two species by studying their ultrastructural morphology. Mature gamont stages, common in the peripheral blood of infected frogs, were examined by transmission electron microscopy. Results indicate that *H. ixoxo* and *H. theileri* share typical apicomplexan characteristics, but differ markedly in their external cellular structure. *Hepatozoon ixoxo* is an encapsulated parasite presenting a prominent cap at the truncate pole, and shows no visible modifications to the host cell membrane. This is in contrast to *H. theileri*, which does not present a capsule or cap, but does produce marked morphological changes to its host cell. Scanning electron microscopy was performed to further examine the cytopathological effects of *H. theileri*, and results revealed small, knob-like protrusions on the erythrocyte surface, as well as notable distortion of the overall shape of the host cell.

**(S3.22p): The occurrence of *Hirschmanniella* and *Tylenchorhynchus* spp., plant parasitic nematodes from the Seekoeivlei Nature Reserve, Memel**

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The occurrence of phytoparasitic nematodes in freshwater habitats is a rather neglected area in nematology. They may be major pests of aquatic or wetland plants and can act as biological control agents of economically important aquatic weed pests such as the water

hyacinth. During three surveys to the Seekoeivlei Nature Reserve near Memel, soil samples were collected from three different localities within the wetland area. Soil samples were processed for nematodes using the sieving-centrifugal-flotation method and fixed using standard techniques. A total of 36 genera belonging to 21 families were identified, including the families Pratylenchidae and Telotylenchidae to which the genera *Hirschmanniella* and *Tylenchorhynchus* belong. *Hirschmanniella* specimens were collected from all three localities but only during the winter survey, while *Tylenchorhynchus* spp. were collected from all three localities in the spring, summer and winter surveys. To date 35 species belonging to the genus *Hirschmanniella* have mostly been described from subtropical and tropical regions of the world, while 111 species belonging to the genus *Tylenchorhynchus* have been described worldwide. Only two species of *Hirschmanniella* have been described from the Kwa-Zulu Natal midlands and 15 *Tylenchorhynchus* species have been described throughout South Africa. This is the first report of these two genera from a wetland within the Free State Province.

**(S3.23p): The effect of pollution on parasite diversity of four fish species in Barotta and Lutanandwa rivers, Venda, Limpopo Province, South Africa**

Magdeline Takalo[1], Sareh Tavako[1], Moses Matla[1], Wilmien Luus-Powell[1]
[1]*University of Limpopo, Sovenga, South Africa*

Aquatic ecosystems are affected by increasing levels of pollution with ecological consequences and should be monitored. Fish parasites are a useful biomonitoring tool and the diversity of parasites can be used as a potential pollution indicator. Global change is expected to disrupt the balance between hosts and parasites and the rate of some parasites' life-cycle may increase in warmer water with an increased probability of parasitic outbreaks. To gain more knowledge on the diversity of parasites and global change, four fish species were collected with the aid of electrofishing from two tributaries of the Luvuvhu River. Surface water samples were analysed for selected water constituents. Standard methods were followed for fixing and preservation of parasites. Nine different parasite species were recorded. Ectoparasites comprised of monogeneans on the gills, i.e. *Schilbetrema* sp. from *Chiloglanis pretoriae*;and*Dactylogyrus spinicirrus* and *Afrodiplozoon* sp. from *Labeobarbus marequensis*. Endoparasites included nematodes, i.e. *Contracaecum* larvae from the body cavity of *Amphilius uranoscopus* and *L. marequensis*; digeneans *Clinostomum* sp. and *Diplostomum* sp. from the body cavity and eyes, respectively, and an unidentified digenean larva from all the fish species except *Barbus lineomaculatus* and echinostome larvae embedded in the mesenteries of *B. lineomaculatus*. Higher diversity indices were recorded at the less impacted site. The current results represent new parasite distribution records for these two rivers and South Africa.

**(S3.24p): Metal contamination, in particular arsenic, in dams along the Vaal River.**

Mahlogonolo Molefe[1], Beric Gilber[1]t, Annemariè Avenant-Oldewage[1]
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Previous parasitological studies in the Vaal Dam have indicated that *Labeo capensis* is infected by: *Dactylogyrus iwani,D. larindae, D.nicolettae* and *Dogielius intorquens* (Crafford et al. 2014), *Chonopeltis australis*, *Argulusjaponicas* (Neethling & Avenant-Oldewage 2015) and *Paradiplozoonvaalense* (Dos Santos & Avenant-Oldewage 2015). A recent study showed that the arsenic concentration in the Vaal Dam has risen sharply; the reason for this increase is not known but may be due to additional mining activity within the catchment. Sampling took place at six impoundments (Grootdraai Dam, Vaal Dam, Vaal Barrage, Bloemhof Dam, Vaal-Harts Dam and Douglas Weir) along the Vaal River. Water, sediment, fish and parasite samples were collected. The aim of the study was to evaluate the effect of a change in water quality on the parasite assemblages. Twenty fish per site were collected with the aid of gill nets and electroshocking. Fish were euthanized and parasites were collected and identified in the field. Water quality measurements and collection of water and sediment samples for metal analysis were done at each site. The water quality at each site was related to the surrounding human activities. The result of this study showed that parasite numbers are related to water quality. Thus this corroborates with the use of parasites as bio-indicators.

**(S3.25p): Introduction and invasion of the red-eared slider and its parasites in freshwater ecosystems of southern Europe: risk assessment for the European pond turtle in wild environments**

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The red-eared slider *Trachemys scripta elegans*, which is nowadays considered among the world's worst invasive alien species, could constitute a real threat to native freshwater turtles. Because the species was introduced in the same habitats of the European pond turtle *Emys orbicularis* in European wetlands, we have conducted a parasitological survey of several populations of the indigenous species in France and Spain in order to determine the parasite diversity of platyhelminths (Monogenea, Polystomatidae) and to carry out risk assessment of turtles in natural environments. From a DNA barcoding procedure based on the haplotypic diversity of the COI gene, ten polystome species were evidenced within wild *E. orbicularis* populations and/or captive animals among which nine species could be considered as introduced parasites from American freshwater turtles. Results indicated that host switching could have occurred either in natural environments, or following the release

of infected indigenous turtles kept in captivity with exotic species, demonstrating that turtle farms could act as reservoirs of parasites. The presence of non-native polystome species within indigenous wild populations of *E. orbicularis* of the European freshwater ecosystems also highlights the risks that these parasites may pose on the survival of natural populations but also on the biodiversity as a whole. *In fine*, polystomes could serve as markers of turtle's releases in freshwater environments.

# SESSION 4: MACRO-PARASITE DIVERSITY &ECOLOGY

Oral presentations:

### (S4.1o): Comparative helminth composition and prevalence of indigenous and invasive synanthropic murid rodents in urban Gauteng Province, South Africa

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Although, synanthropic rodents such as indigenous *Mastomys coucha* and invasive *Rattusnorvegicus*, *R.rattus* and *R. tanezumi* are well-known to host various micro- and macroparasites, their helminth parasite fauna is understudied in South Africa. As these synanthropic rodents are abundant in urban areas and frequently encountered in human settlements, the aim was to investigate the helminth fauna of invasive and sympatric indigenous synanthropic rodents sampled from informal settlements of Alexandra, Tembisa and Diepsloot, and residential suburbs of Pretoria and Hammanskraal in Gauteng Province. Helminths were recovered from the urinary bladder, liver and gastrointestinal tract and were morphologically and molecularly identified. All rodent species examined showed a high helminth infection prevalence (≥ 70%) with generally higher nematode than cestode prevalence ($\chi 2 = 29.90$; df = 3, $p < 0.05$). However, *M.coucha* showed significantly lower cestode prevalence than *Rattus* spp.($\chi 2 = 18.19$; df = 3, $P < 0.05$). Nematodes were all rodent-specific and a novel spiruroid species was recovered from *M. coucha*. *Nippostrongylusbrasiliensis* and *Syphacia muris*, both originally described from invasive rodents, were shared among indigenous and invasive rodents and as such may have been co-invaders. Strobilar stages of cestode species found included *Hymenolepis diminuta*, *H. nana* and *Inermicapsifer madagascariensis,* which have zoonotic implications. Metacestodes recovered included *Taenia taeniaeformis* and *T. parva*. The acanthocephalan, *Moniliformis moniliformis*, was found in *R. rattus* only. Interspecific transmission of helminths likely occurs with invasive rodents possibly transferring helminths to indigenous rodents. Several helminths found in synanthropic indigenous and invasive rodents have zoonotic implications.

### (S4.2o): Diversity and seasonal abundance of nematodes from *Rhabdomys dilectus* in South Africa

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*Rhabdomys dilectus* is a solitary grassland species that occurs in the northern and eastern mesic parts of South Africa. To date little is known with regard to the helminth diversity associated with this species. The present study discusses the nematode assemblages and diversity of *R. dilectus* trapped in the mesic eastern region of South Africa. Sherman-type live traps were used to trap the murids. One hundred and seventy-three *R. dilectus* were trapped at seven localities in the Savanna, Grassland and Albany thicket biomes. Hosts were dissected and the gastro-intestinal tracts removed and examined for helminth parasites. In total 16 nematode species, representing four orders, were recorded in *R. dilectus*. The most abundant species were *Neoheligmonella* sp. with a mean abundance of 41.43 (0-3856) and *Heligmonina spira* with a mean abundance of 32.74 (0-4501). Only *Syphacia* sp. occurred in all seven localities. Nine of the species occurred only in the northern localities whereas four species were exclusively found in the three southern localities. One locality was sampled during spring, summer, autumn and winter. Nematode infection varied within the seasons with the highest mean abundance recorded during autumn, followed by summer. It is evident that *R. dilectus* harbours a large nematode species richness. This might be due to increased contact between host and parasite governed by the solitary nature and large home range size of the rodent.

**(S4.3o): Gastrointestinal parasites infecting ungulates, felids and avian species at National Zoological Gardens of South Africa**

Paballo Mosala[1,4], Essa Suleman[1], Ana Tsotetsi-Khambule[2,3], Oriel Thekisoe[4]
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Gastrointestinal tract (GIT) parasites are one of the leading factors that threaten health of wildlife, especially in captivity, and some are zoonotic with potential to infect staff and visitors. This study investigated the prevalence and seasonal abundance of GIT parasites in selected captive animals at the National Zoological Gardens of South Africa (NZG). A total of 497 faecal samples were collected from felid (n=58), ungulate (n=265) and avian species (n=174) at the NZG. Egg-floatation techniques (Faecalyser and McMaster) were used to estimate the parasite load in sampled animals. Faecal analysis revealed an overall prevalence of 30.6%, 60.8% and 6.9% of GIT parasites in felid, ungulate and avian species, respectively. The GIT parasite prevalence (eggs per gram of the faeces, EPG) was slightly higher in warm summer months (63.7% in ungulates,47.2% in felids and 10.8% in avians) as compared with the cool winter months (60% in ungulates,27.3% in felids and 1.0% in avians). Warm and moist weather conditions facilitate the development of parasitic eggs; hence the GIT parasite prevalence is higher in summer months. The helminth eggs most commonly encountered via

microscopy were *Haemonchus contortus* in ungulates, *Toxascaris leonina* in felids and *Capillaria*sp. in avian species with prevalence levels of 38.5%, 37.9% and 4.1%, respectively. The data from this study combined with regular monitoring and treatment of captive wildlife for GIT parasites is very important for understanding and maintaining the welfare of the animals, staff and visitors of facilities such as the National Zoological Gardens.

**(S4.4o): Species interactions within the parasite community of eastern rock sengis (*Elephantulus myurus*)**

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*[1]University of Pretoria, Pretoria, South Africa; [2]National Zoological Gardens of South Africa, Pretoria, South Africa*

Most hosts are simultaneously infected with several parasite species. However, it is not always apparent whether coinfection patterns arise because host susceptibility to parasite infection is modulated by an already present infection or because of intrinsic differences between hosts. We collected data on tick and endoparasites burden from wild eastern rock sengis (*Elephantulus myurus*) over the course of one year to evaluate the contributions of abiotic (*i.e.* season) and host factors (*i.e.* sex) as well as the abundance of other parasite species on the parasite community. From 121 sengis we collected a total of 21,312 ticks (*Rhipicephalus warburtoni*), 15,559 nematodes from nine taxa (*Maupasina weissi*, *Syphacia minuta*, *Moaciria* sp., *Spinicauda* sp., *Abbreviata* sp., *Physolapetra* sp., *Trichuris* sp., Physalopterinae and Spiruriod larvae), cestodes (Hymenolepididae) and 21 pentastomids (*Armillifer armillatus*) with *R. warburtoni*, *M. weissi*, Physalopterinae and Spiruriod larvae being the most common. Seasonal effects were apparent for all of these parasite taxa while host sex affected all but the ticks. We found no evidence for interactions between ticks and any of the helminths. In contrast, there appeared to be a mutually beneficial relationship between *M. weissi* and Spiruriod larvae. Conversely, our data suggested a competitive relationship between Physalopterinae and Spiruriod larvae. The latter two taxa occur in the host stomach while *M. weissi* lives in the caecum. Thus, our results suggest that competition for space may explain the relationship between the former while immunomodulation may account for the facilitating relationship between *M. weissi* and Spiruriod larvae.

Poster presentations:

**(S4.5p): Annual cycles of three flea species parasitizing the Namaqua rock mouse (*Micaelamys namaquensis*)**

Stephanie Wilson[1], Heike Lutermann[1], Dina Fagir[1]
[1]*University of Pretoria, Pretoria, South Africa*

Abiotic and biotic factors have an effect on the survival and reproductive success of parasites often resulting in seasonal patterns of prevalence and/or abundance. *Micaelamys namaquensis* is a host of *Xenopsylla brasiliensis, Chiastopsylla godfreyi* and *Epirimia aganippes* flea species. Little is known about the biology of these species, which is a concern since *X. brasiliensis* is a vector of plague with a global distribution while the latter two are endemic to southern Africa. This study aims to document and compare the annual cycle of these species as well as evaluate the effect of abiotic and biotic factors on their abundance and prevalence. Rock mice were sampled four times a year between April 2010 and February 2012 and between April 2015 and August 2016 in Ezemvelo/Telperion Nature Reserve, Gauteng Province, and fleas stored in 70% ethanol for later identification. Morphometric measurements were carried out and the body condition and blood digestion scored. For females, we determined their reproductive condition. All three flea species exhibit seasonal variation in prevalence and abundance, with *X. brasiliensis* exhibiting peaks in spring while the other two species peaked in winter. The three flea species differed markedly in size with *C. godfreyi* being the smallest. All species exhibited a sexual dimorphism, however, this was much less pronounced in *C. godfreyi*. Preliminary data suggest that body condition can vary even within a season and may be linked to reproductive condition in females. The implications of these findings for the life cycle of the three flea species will be discussed.

**(S4.6p): The phylogeographic pattern of the gastro-intestinal nematode, *Neoheligmeonella capensis,* and possible co-phylogeny with its host genus *Rhabdomys.***

JC Bothma[1], Conrad Matthee[1], Sonja Matthee[1]
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Nematodes can be regarded as permanent parasites with only the egg and larval stages occurring in the external environment. Given the close association with their hosts it is predicted that the dispersal of nematodes may be determined by host vagility. Little information exists on the dispersal ability and subsequent gene flow patterns of rodent-associated nematodes in South Africa. This study will investigate geographic genetic connectivity of a gastro-intestinal nematode, *Neoheligmeonella capensis*, in South Africa. We will determine the phylogeographic structure of *N. capensis* recorded from the two most distinct *Rhabdomys* species (*R. pumilio* and *R.dilectus*). A total of 15 *N. capensis* specimens have been removed from the gastro-intestinal tract of 5-6 *Rhabdomys* from each of 10 localities. The mitochondrial DNA CO1 marker will be sequenced. Using the

sequences, a haplotype network will be constructed for *N. capensis* and compared to a haplotype network of the host. A Bayesian tree will be constructed to test for evolutionary relationships among clades. To test for mechanisms responsible for genetic structure we will perform an isolation by distance analysis and we will also calculate pairwise ST values to test for differentiation among sites. An AMOVA will be conducted to test for differentiation between nematodes sampled from the two *Rhabdomys* species. This study will allow us to make inferences on the life history characteristics effecting gene flow in the poorly studied *N. capensis* and give us a better understanding of the mechanisms that play a role in the dispersal of nematodes across the landscape.

**(S4.7p): An introductory study on the host factors that influence parasite body size: An example of a host-specific rodent mite, *Laelapsgiganteus***

Sonja Matthee[1], Evan Sorour[1], Luther van der Mescht[1]
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Mesostigmatid mites that occur on rodents are generally regarded as broad-niche temporary parasites. However, recent studies have indicated that *Laelaps giganteus* is in fact host-specific to the rodent genus *Rhabdomys* in southern Africa. Within-species difference in the body size of *Rhabdomys* have been recorded along a rainfall gradient in that there appears to be a significant negative correlation in weight along an environmental gradient from south (more mesic, cooler) to north (more xeric, hotter) on the subcontinent. Adult female mites are mainly recorded from the body of the rodent and it is surmised that they spend prolonged periods on the host. Given the close association with the host and lower surface area to volume ratio, in contrast to most other ectoparasites, it is predicted that the body size of the mite will track the body size of the host within localities and across the landscape. Parasites were removed from the body of adult *Rhabdomys* individuals trapped at 8 localities along the western part of southern Africa. The mites were mounted using standard techniques and identified. Seven independent morphological characters were measured from each of more than 10 adult female *L. giganteus* per locality, where possible. The weight and total- and tail lengths were measured for each rodent host. Statistical analyses followed standard procedures implemented in widely cited software packages in R. It is anticipated that the study will provide novel data on the importance of host body size for the size development and fecundity of a host-specific rodent mite.

# SESSION 5: MEDICAL &VETERINARY PARASITOLOGY

Oral presentations:

## (S5.1o): *Bartonella elizabethae* and *B. tribocorum* in *Rattus*-associated ectoparasites in Gauteng Province, South Africa

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Bartonellae is known to be transmitted through bites and contact with faeces of infected ticks, lice, mites and fleas. The present study investigated *Bartonella* genome presence in *Rattus*-associated ectoparasites from invasive *Rattus* species sampled in Gauteng Province, South Africa by targeting the *gltA* and *nuoG* gene regions. A total of 1400 ectoparasites were collected from 342 *Rattus* hosts (males = 186 and females = 156), these included fleas (*n* = 112), ticks (*n* = 25), mites (*n* = 392), and lice (*n* = 871). *Bartonella* genome presence in fleas (*Xenopsylla brasiliensis*, and *X. cheopis)* and in *Haemaphysalis elliptica* ticks was 84.3% and 0%, respectively. In contrast, *Bartonella* prevalence in the *Rattus* host was intermediate, at 54.1%. There was a ~50% match between the *Bartonella* strains detected in the vertebrate host and the corresponding flea vector. These results indicate that fleas play an important role in vectoring *Bartonella* in commensal invasive rats, and that they feed on multiple hosts infected with different *Bartonella* species. Phylogenetic analysis confirmed that the *Bartonella* species in fleas and *Rattus* hosts correspond to *B.elizabethae* and *B. tribocorum*. The high levels of infection with two zoonotic *Bartonella* species, *B. elizabethae* and *B.tribocorum* detected in rats and their ectoparasites, are of public health concern.

## (S5.2o): The distribution of African horse sickness vectors in the protection and surveillance zones of the Western Cape Province, South Africa

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African horse sickness (AHS) is a devastating viral disease occurring annually in South Africa. The disease is more prevalent in the northern areas, though outbreaks have been recorded throughout South Africa. To facilitate the export of horses, an AHS-controlled zone was established in 1997 in the Western Cape Province. Since its establishment nearly 20 years

ago, 8 outbreaks have been reported in the protection and surveillance zones. The results from surveys conducted across South Africa, since 1990, show that *Culicoides* species can be collected at nearly every site surveyed. Looking specifically at the protection and surveillance zones in the Western Cape Province, the results clearly indicated the presence of both the vectors of AHS virus. In these two zones 1 408 collections have been made at 101 sites. Some 6 305 738 specimens of 47 species were collected, with *C. imicola* the dominant species at 91.64% (5 778 665), present in 1 037 collections and were recorded at 92 sites. Only at Zoetendalvallei, Struisbaai was *C. imicola* absent in 133 collections made over a three-year period from 1996-1999. The largest collection of *Culicoides* was 211 280, made in the Robertson area with *C. imicola* the dominant species (209 8333). *Culicoides bolitinos* was the fourth (1%) most common species collected, being present in 749 collections made and recorded at 83 sites. The largest collection (14 460) of *C. bolitinos* was made in the Stellenbosch area. With both the vector species present, this area remains vulnerable to AHS outbreaks.

**(S5.3o): Re-description, molecular characterisation and taxonomic re-evaluation of a unique African monitor lizard haemogregarine *Karyolysus paradoxa* (Dias, 1954) n. comb. (Karyolysidae)**

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Within the African monitor lizard family Varanidae, two haemogregarine genera had been reported, comprising five species of *Hepatozoon* Miller, 1908 and a species of *Haemogregarina* Danilewsky, 1885. Until recently, three *Hepatozoon* species were known from southern African varanids. One of these, *H.paradoxa* (Dias, 1954), however, shared morphological characteristics with species of the family Karyolysidae, particularly to species of the genus *Karyolysus* Labbé, 1894. *Karyolysus* species have to date been formally described and named only from lizards of the family Lacertidae, including nine species of which only two have undergone molecular characterisation. The recent study thus aimed to morphologically re-describe and characterise *H. paradoxa* molecularly, so as to determine its taxonomic placement. Specimens of *Varanus albigularis albigularis* (rock monitor) and *Varanus niloticus* (Nile monitor) were collected from the Ndumo Game Reserve, South Africa. Blood was collected for both comparative morphometrics and molecular characterisation of parasites. *Hepatozoon paradoxa* was identified infecting two out of eight (25%) *V. a. albigularis* and a single (100%) *V. niloticus* examined. Phylogenetic analyses revealed that *H. paradoxa* clustered with the '*Karyolysus*'clade, and not with those of reptilian *Hepatozoon* spp., and as such was transferred to the genus *Karyolysus*. In addition

to this being the first morphological and molecular characterisation of a haemogregarine within the African Varanidae, it is the first report of a species of *Karyolysus* infecting this monitor lizard family. Furthermore, this constitutes now only the third described and named *Karyolysus* species for which there is a nucleotide sequence available.

**(S5.4o): Molecular and morphological description of a likely new *Trypanosoma* sp.in *Cordylus tropidosternum* (Sauria:Cordylidae)**

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At least 80 species of *Trypanosoma* have been described in reptilian hosts, of which 48 infect lizards alone. A likely new species of *Trypanosoma* was studied from the peripheral blood of *Cordylus tropidostenum* Cope, 1869 collected from Ndumo game reserve in KwaZulu-Natal, South Africa. In Ndumo Game Reserve, these arboreal lizards were found to be infected with trypanosomes, which were detected by light microscopy. This study was aimed at describing new trypanosome species using morphological and molecular characterisation. A total of 8 lizards were collected at Ndumo Game Reserve where 4 lizards where found infected with *Trypanosoma* by microscopic examination. Morphological measurements were compared to already-described *Trypanosoma* spp.infecting lizards of the Ethiopian region. This trypanosome measured 15.5 µm in length with a maximum width 8.4 µm. The free flagellum averages 5.4 ± 0.2 µm (2.1 - 8.6); the kinetoplast is situated 3.8 ± 0.2 µm (1.1 - 6.9) from the posterior end. The nucleus is situated 11.3 ± 0.4 µm (6.2 - 15.3) from the anterior end, with nuclear index 1.2 ± 0.07 µm (0.5 - 2.9). Morphological measurements of the detected trypanosome found in *C. tropidosternum* do not match any known described trypanosomes. DNA was extracted from same lizard blood samples and were amplified by PCR targeting the 18S rDNA of trypanosomes. Phylogenetic analysis indicates that cordylid trypanosomes appeared to be in the same clade with *T. varani* Wenyon, 1908. The current data indicates that this trypanosome is possibly a new species.

**(S5.5o): *Hepatozoon* species (Apicomplexa: Adeleorina: Hepatozoidae) infecting wild and captive leopards *Panthera pardus pardus* (Linnaeus, 1758) from the Free State, Limpopo and Mpumalanga, South Africa.**

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Leopards, *Panthera pardus pardus* (Linnaeus, 1758), were once the world's most widespread solitary cats, but is today considered a near threatened species due to illegal hunting and habitat loss that lead to an ongoing decline in population numbers throughout large parts of their natural range. Members of the genus *Hepatozoon* Miller, 1908 are intracellular apicomplexan blood parasites, widely reported from wild carnivores. The aims of this study were to determine whether captive and wild leopards in South Africa are infected with *Hepatozoon* spp. and to identify any infections found based on morphological characteristics of the gamont stage in peripheral blood smears as well as phylogenetic analysis of partial 18S rRNA gene sequences. Blood samples of seven wild and nine captive individuals, including adult males, females and cubs representing regular, melanistic and erythristic leopards, were collected while under sedation. Leopards were also screened for haematophagous ectoparasites that might serve as vectors. Ectoparasites found were squashed on clean microscope slides and Giemsa-stained as for bloodsmears. Intraleucocytic *Hepatozoon* gamonts were detected by microscopy from bloodsmears, and measured 10,62 ± 2,11 (5,7-20,85) ųm long by 4,84 ± 0,58 (2,65-6,13) ųm wide and surface area of 41,55 ± 6,89 (14,72-57,55) ųm (n=124). The *Hepatozoon* 18S rRNA gene was amplified by PCR and sequences obtained matched with *Hepatozoon* species in GenBank. Sporogonic stages were documented in squashes of ticks that fed on infected leopards. This is the first report on molecular and morphological characteristics of *Hepatozoon* in captive and wild leopards in South Africa.

**(S5.6o): Plasmodiid infections of Afromontane raptors in the eastern Free State, South Africa.**

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Haemosporidians have the potential to cause severe pathology and epizooties resulting in death in wild and captive birds. Knowledge on the biodiversity of bird haemosporidians is therefore important. This study aimed to determine this biodiversity in raptors housed at a wildlife rehabilitation center in Kestell in the eastern Free State, South Africa.Blood samples collected from seven birds, a Cape eagle owl (*Bubo capensis*), five spotted eagle owls (*B. africanus*) (Strigiformes) and a red-chested goshawk (*Accipiter toussenelii*) (Accipitriformes), were prepared for the morphological identification of possible haemosporidians, and these findings tested with molecular techniques, amplifying fragments of the parasites' cytochrome b gene using a nested-PCR approach employing avian-haemosporidian specific primers.Screening showed that both owl spp. (1/1 Cape eagle owl and 4/5 spotted eagle owls) were infected with a *Parahaemoproteus* sp. (Parasitaemia: 8.76% Cape eagle owl, mean 6.9% spotted eagle owl), whilst the goshawk tested negative for haemosporidians.

Molecular characterisation supported the identification of the owl haemosporidians as species of *Parahaemoproteus*, and furthermore recognized the presence of two genotypes. Two *Parahaemoproteus* spp. have been described from Strigiformes, *P.* (syn. *Haemoproteus*) *noctuae* Celli & Sanfelice, 1891, and *P*. (syn. *Haemoproteus*) *syrnii* (Mayer, 1910), both recorded from all zoogeographical regions except the Antarctic for both species and Australia for the latter species. The genotype found infecting the Cape eagle owl was according to the phylogenetic findings closely related to *H. syrnii* and that of the spotted eagle owl to an unidentified *Haemoproteus* sp. isolated from *Athene noctuae*, the type species for *H. noctuae*.

**(S5.7o): Membrane-Active Chelators tighten brain microvascular endothelial barriers to African trypanosome infection.**

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Found safe in humans, BAPTA-based lipophilic Membrane Activated Chelators (MACs) modulate cell membrane $Ca^{2+}$, $Zn^{2+}$ and $Cu^{2+}$ ion homeostasis by adopting an active conformation only in the lipid environment of cell membranes, resulting in excellent tolerability. While MACs at high concentrations are cytotoxic for African trypanosomes *in vitro*, at 100-fold lower concentrations, MACs (DP-b99/DP-460) tighten brain microvascular endothelial cell (MEC) barriers that form the blood-brain barrier (BBB). Human brain MEC monolayers were incubated with 0 to 8 µg/mL DP460 and transendothelial electrical resistance (TEER) measurements taken by Electrical Cell-Substrate Impedance Sensing (ECIS). Compared to controls, TEERs for MECs exposed to 2 and 4 µg/mL DP-460 increased, reaching values 15 to 20% higher than the controls. Higher drug concentrations appeared to have a more biphasic effect, initially tightening the barrier over the first 3-4 hours before returning to baseline levels. The data suggested that MACs could protect BBB barrier function in response to BBB traversing/compromising neurotropic pathogens. As 'proof-of-concept' the MAC drug DP-b99 (5 µg/mL) inhibited/reversed the drop in human brain MEC TEER induced by the human neurotropic African trypanosome, *Trypanosoma brucei rhodesiense*, showing that MACs may have use as an adjunct therapy to tighten vascular barrier as a protective response to these pathogens. MACs should be studied to better delineate their mechanisms of action with regard to *in vitro* TEER changes and as potential adjunctive therapies to diminish severe consequences of African trypanosome infections and others where vascular permeability leads to disease.

**(S5.8o): *Leishmania* vaccine development using machine learning algorithms**

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Protozoa of the genus *Leishmania* are obligatory intracellular parasites transmitted by the bite of phlebotomine sand flies. Clinical manifestations of the disease include cutaneous lesions, of a muco-cutaneous form and acvisceral form. Worldwide, there are twomillion new cases each year and one tenth of the world's population is at risk of infection. Chemotherapeutics are available but show high toxicity, costs and are prone to resistance. Healing is associated with life-long resistance to re-infection and this argues for the feasibility of vaccination. Despite all the efforts and advances, there is no vaccine against Leishmaniasis for use in humans. Reverse vaccinology has attracted much attention since the term was introduced and the approach tested by Rappuoli and colleagues. This *in silico* selection of antigens from genomic and proteomic data sets was also adapted to aim at developing an anti-*Leishmania* vaccine. We will review the application of Vacceed, a recently developed vaccine prediction tool which provides a flexible and automated process to predict worthy vaccine candidates for eukaryotes from large volumes of superfluous and disseminated data. Given thousands of protein sequences from the target pathogen as input, Vacceed's main output is a ranked list of protein candidates determined by a set of machine learning algorithms. Vacceed has the potential to save time and money by reducing the number of false candidates allocated for laboratory validation.

**(S5.9o): Validation of a urine circulating cathodic antigen cassette test for detection of *Schistosomahaematobium* in the uMkhanyakude district of South Africa**

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Genus-specific circulating cathodic antigen (CCA) tests for schistosomiasis are fast and less complicated allowing them to be used by personnel with little technical training. They are therefore good candidates for routine qualitative screening for schistosomiasis at health centres. The urine-CCA has been evaluated for detection of *Schistosomamansoni* with promising results. Its specificity and consistency in detecting *S. haematobium* infection in different endemic regions has been variable. This study validated a rapid urine-CCA cassette test for qualitative detection of *S. haematobium* infection in an *S. haematobium* endemic area with low *S. mansoni* prevalence. The standard urine filtration technique was used to validate the commercially available urine-CCA cassette test (Rapid Medical Dianostics®). The validation was done in a sample of primary school pupils (n=420) aged 10-15 years from schools in the Jozini Municipality, KwaZulu-Natal. Using the urine filtration method as a gold

standard, the overall accuracy of the CCA kit was 54.8%, sensitivity was 68.1% and the specificity was 45.8%. The positive predictive value was 45.8 while the negative predictive value was 68.1%. The urine filtration and the urine-CCA methods detected heavy (≥ 50 eggs/10 mL urine) and light infections at statistically significant levels. The overall accuracy, sensitivity and specificity of the urine-CCA cassette test were low. The urine-CCA cassette test performed much better for heavy infection intensity than low infections intensity ($p < 0.05$) implying that the kit may not be suitable for with low schistosomiasis prevalence.

**(S5.10o): The influence of life history characteristics on flea (Siphonaptera) species distributions**

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Ectoparasites exhibit pronounced variation in life history characteristics, including traits such as time spent on the host and host range. Since contemporary species distribution (SD) modelling does not account for such differences in life history, the accuracy of predictions of species' current and future ranges could differ considerably between life history groups. Flea occurrence records for 83 small mammal species from 1109 localities across South Africa were digitized from published literature. Climate and landscape variables that are considered important for flea survival were selected for SD modelling. SD model performance was compared between 21 flea species that differ in microhabitat preferences and level of host specificity. Distribution models generally performed well, with no significant differences in model performance based on either microhabitat preferences or host specificity. However, the relative importance of predictor variables was significantly related to host specificity, with the distribution of host-opportunistic fleas strongly limited by thermal conditions and host-specific fleas associated with conditions that restrict their hosts' distribution. The importance of temperature was even more pronounced when considering microhabitat preference, with the distribution of fur fleas being strongly limited by thermal conditions and nest fleas more strongly associated with variables that affect microclimatic conditions in the host nest. Contemporary SD modelling, that includes climate and landscape variables, is a valuable tool to study biogeography and future distributions of fleas and other parasite taxa. However, whenever possible life history characteristics should be considered during modelling as groups of species may be differentially sensitive to environmental conditions.

**(S5.11o): Prevalence of gastro-intestinal parasites of livestock and dogs and risk factors for transmission with emphasis on *Giardia* and *Cryptosporidium* in Magude District, Maputo Province, Mozambique**

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The objective of this study was to determine the prevalence of gastrointestinal parasites and risk factors for transmission of *Giardia* and *Cryptosporidium* in livestock and dogs of Magude District, Maputo, Mozambique. A total of 696 fecal samples (480 from calves, 60 from goats and 156 from dogs, ≤7 months) were randomly collected from February to September, 2015. The flotation method using NaCl solution was applied to identify gastrointestinal helminthic and protozoal infections. The formol-ether method was applied and the sediment obtained was used for the modified Ziehl Neelsen (mZN) for detection of *Cryptosporidium*, and direct immunofluorescence (DIF) and indirect immunofluorescence (IIF) tests for both *Cryptosporidium* and *Giardia*. To determine the risk factors, a questionnaire was administered to dog owners and livestock farmers. Using flotation, IIF and DIF, the prevalence of *Giardia* in calves was 0%, 8.1%, and 6.04%; in dogs 0.64%, 8.3%, and 5.7%; and for goats it was 0% and 13.3% respectively and the IIF was not done. The prevalence of *Cryptosporidium* in calves using flotation, mZN, IIF and DIF was 0%, 3.75%, 4.7% and 0.41%; in dogs it was 0%, 0.64%, 6.4% and 0.64% respectively; and in goats it was 0% for all tests. All positive samples fromDIF, IIF and mZN were negative when usingPCR. The lack of regular treatment against parasitic infections in calves and the source of water was identified as risk factors. *Giardia* and *Cryptosporidium* are prevalent in Magude District, although assessing the risk of zoonotic transmission through molecular techniques was not done due to low numbers of oocysts/cysts.

**(S5.12o): Host immune responses induced in mice mono- and co-infected with *Trichinella zimbabwensis* and *Plasmodium berghei* ANKA**

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Parasite-host-parasite interactions during malaria co-infection with tissue-dwelling helminths still remain unclear. This study sought to determine the differential immune responses induced in mice mono- or co-infected with *Trichinella zimbabwensis* and *Plasmodium berghei* ANKA and further explored the effect of antihelminthic treatment on immunity and malaria disease outcomes. Mice aged between 6 to 8 weeks old were assigned into mono- or co-infected with/or without treatment groups. At day 0, mice were infected with *T. zimbabwensis or P. berghei* parasites. At 42 dpi, mice in non-infected, malaria mono-infected, *Trichinella*-mono-infected and co-infected groups were treated

with three doses of 7.5 mg/kg/bwt of fenbendazole (FBZ). Levels of IFN-γ, TNF-α, IL- 4 and 10, IgG total and IgG isotypes in sera were measured. Malaria disease outcomes were determined by monitoring parasitaemia and survival rate. Results showed that co-infecting *T. zimbabwensis* with *P. berghei* induced mixed Th1/Th2 immune responses that were responsible for the altered survival rates and parasitaemia profiles. Co-infected mice had prolonged survival and sustained parasitaemia compared with malaria mono-infected mice of which all mice died within 5 to 7 dpi. All mice in the co-infected group died by day 13 dpi compared with those of the co-infected-treated group which had a 42.85% survival rate. This showed that chronic *Trichinella* infection and antihelminthic treatment decreased *Plasmodium* parasite load and peak parasitaemia by delaying onset of patent parasitaemia and enhanced survival. The co-infected and treated group had significantly elevated levels of cytokines and parasite-specific antibodies compared with the *Trichinella* and malaria mono- and co-infected groups.

**(S5.13o): Prevalence and phylogenetic background of *Besnoitia besnoiti* isolates from different geographical regions of South Africa**

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This study was conducted to determine the prevalence of *Besnoitia besnoiti* infection in cattle, as well as to establish the phylogenetic background of *Besnoitia* spp. emanating from different geographical regions of South Africa. A total of 688 cattle were randomly sampled from Limpopo, Mpumalanga, North West, Gauteng, KwaZulu-Natal and Eastern Cape provinces of South Africa, from which 688 blood samples and 376 skin samples were collected. Based on the analyses of DNA sequences of the nuclear ribosomal internal transcriber spacer 1 (ITS-1), it was observed that 15.7% (108/688) of the sampled animals were positive, with 5.3% (20/376) and 14.4% (99/688) of the animals being positive on skin and blood samples, respectively. Of the animals that were sampled, 2.9% (11/376) were positive on both blood and skin samples. The disease was more prevalent in exotic breeds and communal farms than in indigenous breeds and commercial farms. Phylogenetic analysis of the isolates based on the ITS-1 region using maximum parsimony, neighbour joining and maximum likelihood methods, demonstrated that our isolates were closely related to the wildebeest strain used the vaccine, forming a clade which is separate from the European strains. One of our isolates from Limpopo province was closely related to the European strains, forming a sister clade for the European strains from sourced from sequences deposited in GenBank. Our study therefore showed the distribution of *B. besnoiti* infection in different regions of South Africa and the strains circulating in these regions.

**(S5.14o): Early differential changes in microvascular barrier function in response to Dengue virus**

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Dengue viruses (DENV) are mosquito-transmitted *Flaviviruses* that cause dengue fever (DF) and dengue hemorrhagic fever (DHF) for which organ-specific vascular leakage is a hallmark feature. In this study, we examined the hypothesis that DENV differentially modulates the permeability characteristics of microvascular endothelial cells(MECs) derived from different areas along the vascular tree. We studied the effects of DENV on the barrier function of MECs derived from human brain and lung using Electric Cell- Impedance Sensing (ECIS) to monitor transendothelial electrical resistance (TEER) in real-time. ECIS was also used to monitor changes in cell-cell adhesion ($R_b$) and cell-substratum (a) interactions, which are not well discerned via microscopy. It was found that human brain and pulmonary MEC displayed rapid, differential barrier responses to DENV. The TEERs of brain MEC immediately decreased upon DENV infection. After 5h post-infection, resistance spiked and tapered to a constant gradual increase.By 24h TEERs of infected brain MEC recovered and were well-over controls up to 72h. ECIS modeling revealed that these TEER changes were due to alterations in cell-cell and cell-substratum interactions. In contrast to the brain MEC response, a rapid, transient increase in pulmonary MEC monolayer resistance was noted upon DENV infection. After an initial peak between 1 to 1.5h, TEER rapidly decreased to below control levels, a phenotype that was maintained for up to 72h p.i. and these changes were attributed to variations in cell-cell (a) interactions only. The findings suggest that DENVs differentially disrupt the barrier functions of MECs from different organs immediately upon infection.

Poster presentations:

**(S5.15p): Ticks and tick-borne diseases: Knowledge and perceptions of communal cattle farmers in the Eastern Cape Province, South Africa**

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The objective of the study was to assess farmers' perceptions on ticks and tick-borne diseases (TBDs) prevalent in the Eastern Cape Province, South Africa. Face-to- face interviews were conducted with fivefarmers in 20 communities which were selected randomly within four vegetation zones in the Amathole District Municipality. The respondents were mostly males (85%) compared to females and there were no significant

differences between age groups of farmers in the four vegetation types (p = 0.195). The mean number of cattle per farmer ranged between 12.8 (± 1.17) and 15.6(± 1.35) for the different vegetation types. There was no significant differences in the mean number of cattle (p = 0.596) and goats (p = 0.524), while there was a significant difference in the mean number of sheep (p < 0.001) between the different vegetation types. Significantly more respondents ($\chi 2$ = 15.98, p < 0.001) confirmed that adult animals were more affected by ticks compared to calves. Redwater and Gallsickness were the most common TBDs within and between vegetation types, while Heartwater was less commonly reported and absent in one of the vegetation type, Amathole Montane Grassland. However, this is confirmed by the observation of most common tick species, *Amblyomma hebraeum* (44%) and *Rhipicephalus* (*Boophilus*)spp (36%), while *R. appendiculatus*, *Hyalomma* spp and *R. evertsi evertsi*were less prevalent (9%, 8% and 3%, respectively). From this study it is evident that cattle farmers do regard ticks as a problem and have noted an increase in severity with a change in climate.

**(S5.16p): *In silico* functional prediction and characterization of selected *Theileria parva* hypothetical proteins**

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Cattle theileriosis is infamous for hampering the economic development of south, central and east African countries due to exorbitant numbers of cattle mortalities. The disease is caused by *Theileria parva,* a tick-transmitted parasite. Infection of cattle with cattle-derived *T. parva* isolates causes East Coast fever while infections with buffalo-derived isolates results in Corridor disease. A transcriptome study comparing two *T. parva* isolates, representing cattle- and buffalo-derived parasites, identified 1089 differentially expressed transcripts (DETs). Analysis of DETs revealed 593 hypothetical proteins (HPs), believed to be crucial in understanding the different *T. parva* infections. Thus, this study proposed to characterize these proteins. The initial screening using sequence similarity led to designation of sequence descriptions for 284 HPs; this report focuses on the analysis of the remaining 309. Functions of these HPs were predicted using various bioinformatics approaches including domains discovery tools, protein family classification systems, approaches based on amino acid sequence characteristics and 3D structures predictions. Furthermore, information of functionally characterized homologs and subcellular localization was considered in the analysis. Overall, n=193HPs were successfully annotated for function and some of these are virulent proteins, significant in the survival of the pathogen within the host. Subcellular localization revealed three HPs that can be possible therapeutic targets. Secretome analysis revealed 57 HPs containing signal peptides, suggesting possible interactions with the host. Results of this study may facilitate a better understanding of the

mechanism of pathogenesis of cattle theileriosis and development of more effective disease control strategies.

**(S5.17p): Morphological identification of ectoparasites associated with three African pangolin species**

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Ticks are known to play a significant role in the transmission of pathogenic agents in animals, and may impact a population's survival rate. Endoparasites (primarily nematodes) have been identified and associated with pangolins in several studies; however ectoparasites have been poorly studied and only isolated and opportunistic sampling has been done in both Asia and Africa. This study aimed to morphologically and genetically identify a total of 140 ticks and mites collected from three African pangolin species, the white-bellied (*Phataginus tricuspis*), black-bellied (*P. tetradactyla*) and Temminck's ground (*Smutsia temminckii*) pangolins. Identification based on morpho-anatomical characteristics revealed the presence of three species of ticks, *Ornithodorus compactus,Rhipicephalus theileri* and *Amblyomma compressum.* In addition, one mite species, possibly *M. heterotarsus*, was found. *Ornithodorus compactus* and *R. theileri* were identified from 21 Temminck's ground pangolins. *Ornithodorus compactus* was the most abundant including both nymph and adult stages while only the adult stage of *R. theileri* was observed. This data suggests that the Temminck's ground pangolin may be a previously undescribed host for *O. compactus*, since this parasite has not previously been associated with pangolins. *Amblyomma compressum* (adult stage) was identified in both black and white-bellied pangolin samples. Further research is ongoing to verify these results by sequencing mitochondrial and nuclear gene regions from the ticks and mites collected in this study and to develop DNA barcoding tests for future research on pangolin ectoparasite associations.

# SESSION 6: MOLECULAR PARASITOLOGY

Oral presentations:

## (S6.1o): Molecular detection of *Anaplasma, Babesia, Neorickettsia* and *Theileria* infections in horses and donkeys in South Africa

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The main aim of this study was to determine the occurrence of *Anaplasma phagocytophilum, Babesia caballi, Theileria equi,* and *Neorickettsia risticii* infections in horses and donkeys in South Africa. A total of 256 blood samples were collected from horses (n = 222) and donkeys (n = 34) from the Free State (FS) [horse = 38 & donkey = 2], Mpumalanga (MP) [horse = 94 & donkey = 0], Northern Cape (NC) [horse = 42 & donkey = 12] and North West (NW) [horse = 48 & donkey = 20] provinces of South Africa. The *T. equi* infections detected by PCR were 35 (15.8%) in horses and 2 (5.9%) in donkeys. *Babesia caballi* infections detected by PCR were 19 (8.6%) in horses and none were detected from donkeys. ELISA with recombinant antigens detected 47/216 (21.8%) and 8/34 (23.5%) antibodies for *T. equi* in horses and donkeys, respectively, whilst 43/216 (19.9%) and 7/34 (20.6%) where positive for *B. caballi* antibodies in horses and donkeys, respectively. The *A. phagocytophilum* infections detected by PCR were 77 (34.7%) and 10 (29.4%) in horses and donkeys, respectively, whilst *Neorickettsia risticii* infections detected were 9 (4.1%) in horses and none were detected from donkeys. Data generated from this study indicates that *T. equi,* and *B. caballi* are still prevalent in South Africa despite extensive tick control measures practiced. This study reports for the first time the detection of *A. phagocytophilium* and *N. risticii* infections in equids by PCR in South Africa.

## (S6.2o): Molecular characterisation, morphological description and life cycle elucidationof *Hepatozoon* (Apicomplexa: Adeleorina) infections in three African snakes

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Over the last century, haemogregarines have been reported in the erythrocytes of African snakes across several families. Unlike *Hepatozoon* species of anurans, which form a monophyletic clade, those of snakes are distributed randomly throughout the larger

*Hepatozoon* clade. This raises the question whether this finding suggests a complex association between parasite, vector, intermediary host and/or predatory host, snakes predating on a wide variety of vertebrate prey, and does the phylogeny of these parasites mirror these associations? To address aspects of this question blood was collected from snakes from Ndumu game reserve, northern Kwa-Zulu Natal. Blood smears were prepared and a portion fixed in ethanol for molecular studies. Any ectoparasites present on the snakes at the time of capture were also collected and examined for additional parasite stages. This study reports three morphologically and molecularly distinct species of *Hepatozoon* in the African rock python (*Python sebae natalensis* Smith, 1833), Cape file snake (*Mehelya capensis capensis* (A. Smith, 1847) and Mozambique spitting cobra (*Naja mossambica* Peters, 1845). Morphological comparisons confirmed these to be *Hepatozoon sebai*(Laveran and Pettit, 1909), an unidentified species, and possibly *H. najae* (Laveran, 1902), respectively. All three snakes were infested with the snake tick *Amblyomma latum* Koch, 1844. This study further confirms the taxonomic placement of *H. sebai*(Laveran and Pettit, 1909) and *H. najae* (Laveran, 1902) via morphological and molecular methods, and highlight the ecological role of *A. latum* as possible vector of the different *Hepatozoon* species infections, regardless of the intermediary host transmission.

**(S6.3o): Molecular analysis of cercarial stages of digenean trematodes from snails collected around the Tshwane area**

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The classification and description of digenean trematodes is commonly accomplished by using morphological features, especially in adult stages. The aim of this study was to provide an analysis on the genetic composition of cercarial stages based on their sequences using PCR and sequence analysis. DNA was extracted from the cercariae found in *Lymnaea natalensis* snails collected from Boekenhoutskloof farm dam. PCR was done using primers targeted for regions of interest within the cercarial genomes. Agarose gel electrophoresis was used to determine the sizes of the sequences. Bands that appeared on the gel were excised and purified for sequencing. The Internal Transcribed Spacer (ITS-1), ITS-2, small subunit ribosomal DNA (ssrDNA) and the large subunit ribosomal DNA (lsrDNA) were amplified from the genomes of strigeidae, xiphidio and *Trichobhilharzia* cercariae. The sizes of the regions were variable; ITS-1 was ~1100 bp for most cercariae but was ~800 bp for some of the strigeidae, ITS-2 was ~600 bp, ssrDNA was ~1900 bp and lsrDNA was ~1300 bp. The sequence analysis showed multiple nucleotide variations. Molecular analysis using tools like PCR and sequence analysis provided key information on the genetics of these three types of cercariae which cannot be done using only morphological analysis.

**(S6.4o): Msp1aS genotyping of *Anaplasma centrale* indicates a wildlife reservoir**

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In seminal experiments in the 1900s Sir Arnold Theiler described the use of *Anaplasma centrale* as a vaccine against *A. marginale*. The vaccine strain that Theiler isolated has been disseminated throughout the world, and, over 100 years later, it is used in many countries to protect against severe anaplasmosis. There has been little interest in the epidemiology of *A. centrale*, and, as a result, there are few reports detecting natural infections. In this study we tested 380 animals using a duplex qPCR assay to screen for the presence of *A. centrale* and *A. marginale* in vaccinated and unvaccinated cattle and wildlife from South Africa. Positive samples were analyzed using a new test that we developed based on the homolog of *A. marginale* msp1α, msp1aS. Similar to *A. marginale*, the msp1aS gene of *A. centrale* contains repeats near the 5' end which vary in sequence and number that are useful for genotyping strains. We detected 47 msp1aS repeats corresponding to 32 different *A. centrale* genotypes that were found in cattle, buffalo and wildebeest in South Africa. We used a newly developed tool, RepeatAnalyzer, to catalog and manage the repeat data. Our results show that the vaccine strain is widely distributed across South Africa and is present in vaccinated cattle and in cattle and wildlife with no history of vaccination. A high degree of repeat diversity was detected, suggesting that *A. centrale* strains have been circulating in nature and evolving msp1aS repeat sequences that are distinct from the sequenced vaccine strain.

**(S6.5o): Detection and molecular characterization of *Anaplasma phagocytophilum* in domestic dogs from the Mnisi community area, Mpumalanga Province, South Africa.**

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*Anaplasma phagocytophilum* infection causes tick-borne fever in ruminants and granulocytic anaplasmosis in a wide variety of animals. Anaplasmosis is regarded as a re- emerging zoonosis. Blood samples from 56 domestic dogs from the Mnisi community area, a rural, high poverty area located at the livestock/wildlife/human interface of the Kruger National Park, were screened for the presence of tick-borne haemoparasites using the Reverse Line Blot (RLB) hybridization assay, and for specific detection of *A. phagocytophilum* by quantitative real-time PCR (qPCR) assay. The partial 16S rRNA and *msp2* genes of *A. phagocytophilum* from selected samples were amplified, cloned and the recombinants sequenced. A

phylogenetic tree was constructed from the 16S rDNA sequences and the *msp2* gene sequences were analysed for variations within the qPCR target region. The RLB assay detected *Anaplasma*, *Ehrlichia* and *Babesia* spp. in 48.2% of the samples, either as single or mixed infections; 26.8% samples hybridized with the *Ehrlichia*/*Anaplasma* genus specific probe only, and not with any of the species specific probes, and 25% of the samples were negative. The qPCR assay detected *A. phagocytophilum* DNA in 82.9% of the samples. Sequencing results indicated the presence of 16S rDNA sequences closely related to *A. phagocytophilum*, *A. platys*, and an undescribed *Anaplasma* sp. of dogs from South Africa. Sequence variations were observed within the qPCR target region; the effect of these on assay efficiency remains to be elucidated. This study further indicates that dogs may play an important role in the epidemiology of anaplasmosis in this community.

**(S6.6o): Molecular epidemiology of tick-borne pathogens infecting predators at the farmland interface**

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An increase in the emergence of infectious disease poses a threat to the persistence and functional ecology of both domestic and wildlife species. There is currently a shortage of baseline research on parasites and pathogens in wildlife species in many systems. This study examines the prevalence and distribution of pathogens at the critical interface between humans, wildlife and livestock in the Western and Northern Cape provinces of South Africa. Using molecular techniques, weexamined the prevalence and diversity of various tick-borne pathogens infecting black-backed jackals (*Canis mesomelas*) and caracals (*Caracal caracal*) living in and around farmland regions, protected areas and urban settlements. Among jackals, only 44% (n=46) of the population showed signs of infection with one or more of the ca. 40 parasites for which we tested, all of which fall into the *Babesia*, *Hepatozoon*, *Theileria*, *Anaplasma* or *Ehrlichia* species. In contrast, all three caracal populations (n=57), showed 100% prevalence of infection with at least one haemoparasite species. Phylogenetic sequencing of amplified pathogen DNA revealed an interesting diversity of Apicomplexan parasites. Tick diversity was also investigated. Preliminary data indicate at least four species found on farmland predators; including *Amlyomma marmoruem*, the South African tortoise tick, and *Ixodes rubicundus*, the Karoo paralysis tick. The implications of ticks as vectors of disease is discussed within the context of the "One Health" paradigm, with reference to imminent land-use and climate change impacts in the Karoo region of South Africa.

**(S6.7o): Metagenomic diagnosis of microbiota of horse flies (Diptera: Tabanidae) collected in north-eastern KwaZulu-Natal, South Africa**

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Tabanids, which are commonly called horse flies, belong to the family Tabanidae which is composed of more than 4 400 species belonging to 114 genera with a cosmopolitan distribution. They are nectar- and blood-sucking ectoparasites as well as mechanical vectors of several disease-causing pathogens that infect livestock worldwide. These pathogens range from viruses, bacteria, protozoa to nematodes. The current study used 16S rRNA based metabarcoding on an Illumina MiSeq platform to determine the microbial community found in seven different species of tabanid flies, namely, *Atylotus agrestis* (n=1)*, A. diurnus* (n=2)*, Philoliche aethiopica* (n=2)*, Tabanus taeniola* (n=3)*, T. par* (n=3)*, T. gratus* (n=1) and *T. conformis* (n=3) collected from north eastern KwaZulu-Natal Province. Dominant bacterial families detected across all tested tabanid flies included Spiroplasmataceae, Pseudomonadaceae and Enterobacteriaceae. Both gram negative (*Shigella flexneri*, *Klebsiella pneumoniae* and *Salmonella enterica*) and gram positive (*Streptococcus pneumoniae*, *Staphylococcus epidermidis* and *Bacillus subtilis*) bacterial species as well as *Spiroplasma* and *Wolbachia,*which have been previously reported to have symbiotic relations with tabanid flies, were detected. Despite their ability to transmit a variety of pathogens particularly in livestock, research on horse fly ecology, transmission of pathogens and possible control methods is still lacking in South Africa as compared to focus given to tsetse flies and ticks. Data generated from this study highlights the need to conduct vector (horse flies), host (livestock) and pathogen relationship studies in South Africa.

Poster presentations:

**(S6.8p): SEM study of haptoral sclerites and molecular characterization of *Cichlidogyrus philander* Douëllou, 1993**

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Hard sclerotized structures consisting of copulatory organs and haptoral parts (specialised attachment structures that anchor the parasite to its host) remain a key feature for identification. These parts are traditionally studied using different staining techniques and light microscopy which has led to the morphological description and the measurement of

their sizes, but these structures are sometimes misinterpreted using these techniques. Therefore, the aim of this study was to use the Scanning Electron Microscope (SEM) to further study the morphology of the sclerites and also to carry out DNA analysis from digested genetic material. *Cichlidogyrus philander* collected from Padda Dam, Gauteng, South Africa, were digested using a digestion buffer from a DNeasy® Blood and Tissue Kit to release the surrounding soft tissue from the sclerites. The digested tissue also provided sufficient genetic material for molecular characterization which was used to confirm the validity and provide the DNA details for the first time of the already-described species. Successful SEM study revealed better details of the surface morphology of the sclerites and the first description of the three-dimensional structure of the penis. The DNA analysis was used to compare the species with other species in the genus. This method, therefore, proved to be a useful tool for providing additional details of these internal structures. Previous scanty information on the morphology of the genus *Cichlidogyrus* using the SEM and molecular analysis was lacking, therefore, the SEM study and molecular characterization for *C. philander* add to the existing body of knowledge.

**(S6.9p): Determination of sequence descriptions and predicted functions of selected *Theileria parva* hypothetical proteins**

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The protozoan parasite *Theileria parva* is a causative agent of cattle theileriosis, a disease with destructive economic impact. In cattle, *T. parva* infection results in varied disease syndromes based on host of origin; cattle-derived *T. parva* causes East Coast fever while buffalo-derived parasites cause Corridor disease. Variation in the effect of *T. parva* infection raised an interest to understand proteins involved in disease manifestations. Consequently, a transcriptome study comparing the cattle and buffalo-derived *T. parva* isolates was undertaken which detected differentially expressed genes (1089). The DETs included 867 (74%) hypothetical proteins (HPs) and the aim of this study was to predict biological roles of these proteins. A combination of sequence analysis databases were employed to assign sequence descriptions (SDs) to HPs, confirm SDs by sequence homology and conserved domains; and identify associated gene ontologies (GOs). The initial sequence similarity search on BLAST2GO retrieved 397 HPs with SDs and comparison with KEGG and KOBAS output detected consensus SDs for 219 HPs. Of these, 105 SDs were confirmed by inferring homology to related species. Sequence homology analysis also revealed SDs for 54 HPs without consensus SDs from database analyses, while 91 HPs that failed to meet the sequence homology analysis criteria were assigned SDs based on conserved domains. Overall, 250 HPs were successfully allocated SDs and possible functions were predicted for

80% of these GOs. Hopefully, the functional annotation of HPs will unravel their role in the biology of *T. parva* and its effect in the infected host.

**(S6.10p): Laboratory colonization stabilizes the naturally dynamic microbiome composition of field-collected *Dermacentor andersoni* ticks**

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Ticks are obligate ecoparasitic arthropods that feed on terrestrial vertebrates and are of medical-veterinary importance due to their ability to inflict harm to humans and animals. The Rocky Mountain wood tick, *Dermacenter andersoni*, is a principal vector of bovine anaplasmosis, caused by *Anaplasma marginale*, the most widespread tick-borne pathogen of livestock worldwide. Although ticks are host to pathogenic bacteria, non-pathogenic microbes make up the majority of the microbiome. These endosymbionts have roles in fitness, fecundity, and pathogen acquisition. However, only a few studies have analyzed the ecology and ecological response of the bacterial microbiome of ticks. The goal of this study was to investigate the bacterial microbiome of *D. andersoni* ticks, focusing on different ecological factors, specifically on time and space. We compared the bacterial microbiome of two populations of *D. andersoni* ticks with differing *A. marginale* vector competence: Burns, Oregon USA had a high rate of acquisition (60%) and Lake Como, Montana USA a low rate (20%). We used Pacific Bioscience SMRT sequencing platform to sequence 16S rDNA to examine the microbiome. Our analysis has shown that the bacterial composition was tissue- and spatial-specific; however, generational variation was dependent on geographic location. Furthermore, the microbiomes of laboratory-reared populations were not necessarily representative of the respective field population. These results have demonstrated that the bacterial microbiome of *D. andersoni* was dynamic, with the ability to undergo significant change. In order to employ microbiome manipulation as a source of pathogen inference, ecological factors and variations need to be fully understood.