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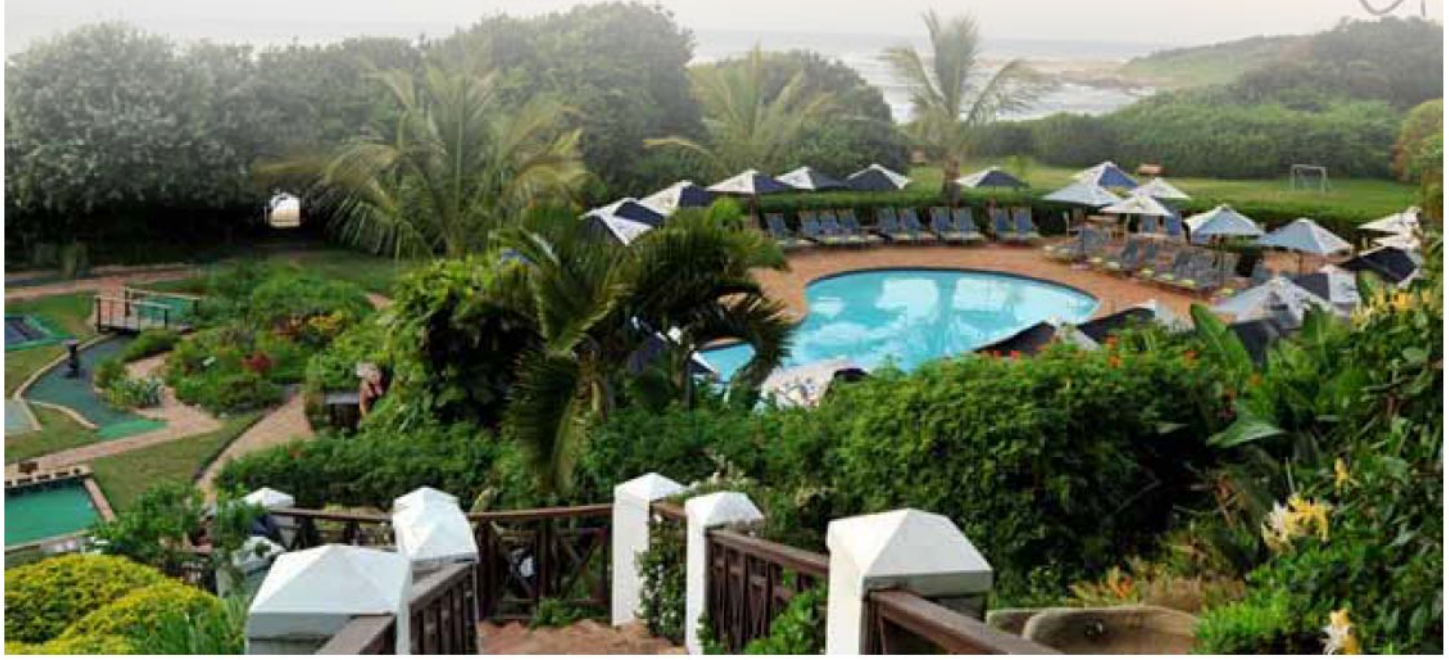

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Keynote

Marine fish parasitology in South Africa: history of discovery and future direction

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Almost 200 years have passed since the first description of a marine fish parasite from South Africa. It is therefore an opportune time to look back, take stock of and reflect on the history of discovery within this field, and, based on what we know, propose the future direction for research. The aim of this paper is hence to provide some background information on the growth in our knowledge and understanding of the major groups of marine fish parasites and to give an account of how pioneers such as Barnard, Stebbing, Fantham and Kensley, led the age of discovery and exploration in marine fish parasitology in South Africa. The paper also presents a brief overview of the contributions made by internationally acclaimed parasitologists, such as Rodney Bray and Angela Davies, to our knowledge of marine fish parasites from this region and also to acknowledge the role played by the South African parasitologists, especially over the past 30 years. A rich base of fundamental knowledge is available in South Africa and this research field continues to grow. The prognosis for the future of marine parasitology in South Africa is good; however, as we continue to acquire and record new information about species, it is proposed that future research should be more focused on the lesser studied groups such as monogeneans, protists and Myxozoans as these have received uneven attention to date. In addition, it is proposed that the scope of research on marine fish parasitology be broadened to include ecological and applied aspects, using modern techniques.

Review of *Mothocya* Costa, in Hope, 1851 (Crustacea: Isopoda: Cymothoidae) from southern Africa using morphological and molecular techniques

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Cymothoid isopods from the genus *Mothocya* Costa, in Hope, 1851 are known to occur inside the gill chambers of their host fish. Many of these gill-inhabiting species are characterised by the laterally twisted body shape of the female, resulting from the shape of the gill cavity in which they reside. Following collection of infected fishes from the Maputo fish market, Mozambique, as well as Sodwana Bay, St Lucia and Mhlathuze estuaries in South Africa, isopods were removed and preserved. Three species were identified using standard morphological and molecular technique; two known and one species new to science. *Mothocya plagulophora* (Haller, 1880) was obtained from Maputo, Mozambique, from the gills of *Hemiramphus far* (Forsskål, 1775) and can be identified by the characteristically large and extremely broad pleon and pleotelson. *Mothocya renardi* (Bleeker, 1857) was found at various localities in South Africa and Mozambique, parasitising *Strongylura leiura* (Bleeker, 1850) and *Tylosurus choram* (Rüppell, 1837), and can be distinguished by their large size (24–36 mm in length) as well as the narrow pleon and extended uropods. Lastly, a new species recently described as *Mothocya affinis* Hadfield, Bruce & Smit, 2015, was discovered from Sodwana Bay, South Africa, from the gills of *Hyporamphus affinis* (Günther, 1866), possessing distinctively large coxae which extend over the pleon. This study presents the first comprehensive morphological revision of all the *Mothocya* species from southern Africa, supported by the phylogenetic analysis of the mitochondrial cytochrome oxidase subunit I gene (COI) to compare the morphological and molecular results for these often misidentified species.

Symbionts on symbionts

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Copepods are the most abundant components of the marine zooplankton community. Their chitinous skeleton can serve as a suitable living environment for the settlement and growth of a diversity of symbionts. Reported symbionts on marine copepods include representatives of the classes Monogenea, Malacostraca, Dinophycaea, Oligohymenophorea, Diatomaceae and Protorophidaceae. They include *Udonella* species from sealice species such as *Lepeophtheirus* and *Caligus* (Caligidae: Siphonostomatoida), *Calinella* sp. on *Trebius latifurcatus* (Trebiidae: Siphonostomatoida); *Vampyrophrya* sp. on *Acartia* species (Acartiidae: Calanoida) and *Paracalanus* sp. (Paracalanidae: Calanoida). The current study aims to identify symbionts found in association with marine symbiotic copepods. Copepods were collected from elasmobranch host species caught in the nets of the KwaZulu-Natal Sharks Board and from hosts caught by staff of the Cabrillo Marine Aquarium (LA, USA). They were preserved in 70% ethanol and studied under the stereo microscope. Symbionts were collected from the surface of 10 copepod individuals belonging to the family Pandaridae i.e. *Achtheinus* sp., *Nesippus* sp. and *Pandarus* sp., stained in lactophenol blue, mounted on slides and examined through the light microscope. Their morphological characters were compared with those described in the available literature. Some copepod hosts harboured one symbiont species while other hosts haboured more than one species. The symbionts were found attached to both the dorsal and ventral sides of the copepod host. Although the identification of the symbionts on the copepod hosts proved to be difficult, most of the colonies resembled those of *Obelia* spp. and one of colony was identified as *Obelia* cf. *striata*. This species has a hydrocaulus arising from the stolon which has eight annulations at the base with 11 rings immediately above the origin of each hydrotheca and five to eight annulations at the base of each polyp. This is the first report of ciliates occurring in symbiosis with marine copepods.

Fish Parasites

Metazoan parasites infecting the Southern mouth-brooder, *Pseudocrenilabrus philander* (Weber, 1897) from Nwanedi and Bubi rivers

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The importance of *Pseudocrenilabrus philander* (Weber, 1897) as an ornamental fish, has received attention due to its opalescence blue and pale yellow colour. This fish species is already utilised by subsistence fishermen in the Limpopo Province as an additional source of protein. Parasites cause huge economic losses in the agricultural industry with aquaculture being no exception. Determining the biodiversity of the parasite fauna of this fish enable better understanding of the interactions between parasite species and its host, ensuring better decisions about their management and where necessary, their control. Understanding the naturally occurring parasites of this fish species is therefore important. Eighty-six specimens of *P. philander* (mean total length = 5.46 ± 0.99 cm) were collected during summer period in November 2013 and February 2014 and during winter in July and August 2014 from Nwanedi River (South Africa) and Bubi River (Zimbabwe). Fish were sacrificed by severing the spinal cord. Recovered monogenea, digenea and cestoda parasites were fixed and mounted in glycerine ammonium picrate (GAP) solution and nematoda were cleared in lactophenol for examination. Parasite identification studying important structures were done using an Olympus BX50 compound microscope. Morphometric evaluations revealed the presence of six species of monogeneans, *Enterogyrus coronatus*, *Cichlidogyrus philander, Cichlidogyrus tilapiae,* Cichlidogyrus sp. 1, *Cichlidogyrus* sp. 2 and *Gyrodactylus* sp.; three species of digenean metacercariae, *Petasiger* sp., *Clinostomum* sp. and *Neodiplostomum* sp.; eight species of cestode larvae *Neogryporhynchus* sp. 1, *Neogryporhynchus* sp. 2, *Paradilepis scolecina*, *Paradilepis* sp., *Parvitaenia macropeos*, *Parvitaenia* sp. 1, *Parvitaenia* sp. 2 and *Valipora minuta*; L3 stage larvae of five nematode species, *Procamallanus* sp., *Camallanus* sp. 1, *Camallanus* sp. 2, *Camallanus* sp. 3 and *Contracaecum* sp.; and a single species of copepod, *Neoergarsilus japonicas*. Many of the observations are first geographical and host records for both localities.

Aspects of the ecology of monogenean parasites of *Labeo* species in the middle Limpopo River basin

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The study investigated ecological aspects of monogenean parasites of *Labeo* species from the middle Limpopo River Basin. Fishes were collected by gill netting and electrofishing from Bubiana Dam and Bubi River (Zimbabwe), and Nwanedi River and Nwanedi-Luphepe Dam (South Africa) in summer and winter surveys of 2014. A total of 149 *Labeo* spp. specimens were collected and of these, *Labeo rosae* (n = 46) and *Labeo ruddi* (n = 34) were only collected from Zimbabwe. *Labeo molybdinus* (n = 15) occurred only in South Africa, while *Labeo cylindricus* was collected from both Zimbabwe (n = 6) and South Africa (n = 48). A diverse monogenean fauna was found, comprising 11 species from the genera *Dactylogyrus*, *Dogielius* and *Diplozoon*. There was a marked reduction in the prevalence of monogeneans from all the localities during winter, attributable to low temperatures that inhibit egg-hatching. There was also a significant difference in the mean intensity of monogeneans between seasons. Significant positive correlations were observed between parasite mean intensity and standard length of most host species. Monogeneans did not affect the condition of their fish hosts. In all cases, the intensity of infection was not significantly correlated to the sex of hosts. The findings of this study contribute to the knowledge of the monogenean fauna in southern African fishes by reporting new host species and new geographical records. Future studies are essential in order to expand and intensify knowledge on taxonomy, faunistics and ecology of metazoan parasites infesting many fish species in this poorly studied area.

Redescription of *Parabrachiella supplicans* (Barnard, 1955) (Lernaeopodidae: Siphonostomatoida) parasitic on *Genypterus capensis* (Smith, 1847)

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Parabrachiella Wilson C.B., 1915 is the largest genus of the Lernaeopodidae Milne Edwards, 1840 and currently consists of 67 valid species which are generally host and site specific. Representatives of Lernaeopodidae have a long history of misidentifications and synonymies and in order to ascertain the number of valid species in *Parabrachiella*, a thorough re-examination of all the species is needed. *Parabrachiella* species were transferred to *Neobrachiella*, a new genus created to accommodate some species previously in *Probrachiella*; *Epibrachiella*; *Brachiellina*; *Brachiella*; *Lernaeopoda* and *Parabrachiella*. The genus *Parabrachiella* was however re-established in 2004. *Parabrachiella supplicans* Barnard, 1955 was described from *Genypterus capensis* (Smith, 1847) off Table Bay, South Africa with no subsequent reports. It is re-described based on the original material deposited in the Iziko South African museum, Cape Town. Nine females and two males collected from *G. capensis*, preserved in 70% ethanol, were examined. Dissected specimens were studied under the stereo- and light microscopes using the wooden slide technique. The armature of the antennule, antenna, maxillule and maxilla confirm the genus of these specimens as *Parabrachiella*. Even though examined males and females display morphological variations, *P. supplicans* can be distinguished from its congeners by possessing elongate, well developed, bilobed posterolateral processes which are the same size as the unilobed posteroventral processes. These characteristics are shared by *P. genypteri* (Capart, 1959) described from the same host off Fort Rock Point, Namibia and is therefore probably a synonym of *P. supplicans*.

A redescription of *Alella gibbosa* (Lernaeopodidae: Siphonostomatoida) and a comparison of the known species

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Alella (Lernaeopodidae) consists of seven species namely *A. pagelli* (Krøyer, 1863); *A. canthari* (Heller, 1865); *A. macrotrachelus* (Brian, 1906); *A. ditrematis* (Yamaguti, 1939); *A. pterobrachiata* (Kabata, 1968); *A. tarakihi* Hewitt & Blackwell, 1987 and *A. gibbosa* Van Niekerk & Olivier, 1995. However there has been uncertainty about the validity of some especially *A. canthari* and *A. macrotrachelus*. *Alella* specimens collected from *Rhabdosargus sarba*, *R. holubi*, *R. globiceps* and *Acanthopagrus berda* from South African waters, were examined using stereo- and light microscopes, dissected and drawn with the aid of drawing tubes. These drawings and illustrations and descriptions of the other species were compared. *Alella tarakihi* are very different from the other species and shows more similarities with *Clavellotis* species than with *Alella* species. Furthermore, as previously suggested, *A. pagelli* and *A. canthari* are most likely synonyms. Thus the remaining species are *A. pagelli*, *A. macrotrachelus*, *A. ditrematis*, *A. pterobrachiata* and *A. gibbosa*. The specimens from *R. sarba* are from the same host individuals that *A. gibbosa* was described but light microscopy did not reveal the details described for the armature of the appendages using scanning electron microscopy. Thus, comparing the armature of the appendages, no consistent and conclusive differences were found between *A. gibbosa* and the other species. Additionally, a comparison of the morphology of the appendages and their armature of the examined specimens and those described and illustrated for the known species also revealed limited differences which may be due to regional or intraspecific variation and inconsistent observations rather than distinguishing characteristics of different species. Hence, it seems like all the species of *Alella* should be synonymized with *A. pagelli*.

Ecological, morphological and molecular characterisation of *Lamproglena cleopatra* (Copepoda: Lernaeidae) from selected fish species in the Limpopo and Olifants river systems

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The genus *Lamproglena* is the second largest and oldest member of the family Lernaeidae which consists of parasitic freshwater copepods. All *Lamproglena* species, except *Lamproglena lichiae* Von Nordmann, 1832, are gill parasites of freshwater teleosts in Europe, Asia and Africa. Fourteen of the 40 nominal species (and subspecies) occur in Africa, with six species reported from southern Africa. These include *Lamproglena monodi* Capart, 1944; *Lamproglena clariae* Fryer, 1956; *Lamproglena barbicola* Fryer, 1961; *Lamproglena comuta* Fryer, 1964; *Lamproglena hoi* Dippenaar, Luus-Powell & Roux, 2001 and *Lamproglena hepseti* Van As & Van As, 2007. It has become imperative to include deoxyribonucleic acid (DNA) based taxonomy, in addition to morphological studies, to distinguish among species of *Lamproglena*. Adult female *Lamproglena* specimens (n=56) were collected from *Labeo rosae* Steindachner, 1894 (31% prevalence), from Flag Boshielo Dam and *Labeo molybdinus* Du Plessis, 1963 (48% prevalence) from Nwanedi-Luphephe Dam in South Africa; and *L. rosae* (24% prevalence) and *Labeo ruddii* Boulenger, 1907 (8% prevalence) from the Bubi River and Bubiana Dam in Zimbabwe. Specimens were fixed and preserved in 70% ethanol for wooden slide technique and scanning electron microscopy, and in absolute ethanol for molecular studies. The allele-specific PCR amplification technique was utilised, and the phylogenetic relationships were made based on molecular data obtained from the sequences of the nuclear genes, 18S rDNA and 28S rDNA. Specimens were identified as *Lamproglena cleopatra* Humes, 1957 based on the free thoracic segments which are separated by lateral indentations; the caudal rami which have seta on the outer margin and terminally with 2 digitiform processes and 2 setae; the structure and number of setae on the antenna and antennule; and the lobed process on the ventral surface of the cephalothorax. Although the specimens from all localities were morphologically similar, they exhibited some morphometric intraspecies differences. *Lamproglena cleopatra* was originally described from specimens retrieved from the gills of *Labeo forskalii* from the Nile River. This is the first geographical record of *L. cleopatra* in southern Africa, and represent new host records for all three *Labeo* spp.

Haemoparasites

South African tortoise haemogregarines (Apicomplexa: Adeleorina): reassigning *Haemogregarina parvula* Dias, 1953 to the genus *Hemolivia*

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So far, only a single species of *Hemolivia*, *Hemolivia mauritanica* (Sergent and Sergent, 1904), had been described from African terrestrial tortoises. Although various haemogregarines have been described from southern African terrapins and tortoises including species from the genera *Haemogregarina* and one from the genus *Hepatozoon*, no species of *Hemolivia* have been identified as of yet. Since its morphological redescription, the taxonomic placement of one of these species, *Haemogregarina parvula* Dias, 1953, was questionable. Hence, research was undertaken to resolve the true taxonomic position of this haemogregarine. Blood smears from nine wild tortoises of two species, *Stigmochelys pardalis* and *Kinixys zombensis*, from South Africa were screened, with the focus on *H. parvula*. Parasite DNA was extracted from ethanol-preserved blood samples, and PCR was undertaken using two primer sets HepF300/HepR900 and 4558/2733 amplifying fragments of the 18S rDNA gene. The 18S rDNA sequences of *Haemogregarina parvula* fell with species of *Hemolivia* and not with those of *Haemogregarina* or *Hepatozoon*. It is thus recommended that this haemogregarine be re-assigned to the genus *Hemolivia*, making *Hemolivia parvula* (Dias, 1953) the first species of this genus recorded from southern African tortoises.

Molecular investigation of tick-borne haemoparasite infections amongst transhumant zebu cattle in Karamoja Region, Uganda

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Tick-borne diseases (TBDs) are a major constraint to cattle production in pastoral areas in Africa. Although information on tick-borne infections is important to prioritise control approaches, it is limited for transhumant zebu cattle in Karamoja Region, Uganda. The aim of this study was to determine the occurrence and level of tick-borne infections amongst transhumant zebu cattle in Karamoja Region. A total of 240 cattle were systematically sampled for blood in 20 randomly selected herds in two districts. The hypervariable V4 and V1 regions of the 18S rRNA and 16S rRNA genes for *Theileria*/*Babesia* and *Ehrlichia*/*Anaplasma*, respectively, were amplified and hybridized to genus- and species-specific oligonucleotide probes on a reverse line blot (RLB) membrane. A duplex real-time PCR (qPCR) assay based on *msp1β* and *groEL* genes was used for detection of *Anaplasma marginale* and *A. centrale*, respectively, while monoplex qPCRs based on pCS20 and 18S rRNA genes were used for detection of *Ehrlichia ruminantium* and *Theileria parva*, respectively. The full-length 18S rRNA genes from 10 out of 47 samples that were positive for the *Babesia* genus-specific probe and not for any of the *Babesia* species-specific probes on RLB were amplified, cloned and sequenced. The sequences were used to construct phylogenetic trees. The RLB hybridization assay demonstrated the presence of tick-borne haemoparasites in all but one sample (99.6%), mostly as mixed infections (97.5%). The most frequently detected species were *Theileria mutans* (88.3%, 95% confidence interval: 82.5-94.2%), *A. marginale* (73.7%: 67.9-79.6%), *T. velifera* (71.3%: 64.6-77.0%) and *Anaplasma* sp. Omatjenne (63.3%: 55.8-71.3%). *Babesia bigemina* (5.0%) and *T. parva* (2.9%) were also detected, but not *E. ruminantium*. The proportions of qPCR positive samples were 82.9% (*A. marginale*), 12.1% (*A. centrale*), 3.3% (*T. parva*), and 1.7% (*E. ruminantium*). Variations in the 18S rRNA gene sequences were identified in *B. bigemina*, which may explain the failure of the RLB hybridization assay to detect *B. bigemina*. In conclusion, this study demonstrated high and widespread occurrence of tick-borne haemoparasites amongst cattle in the pastoral area of Karamoja, which is useful for diagnosis and control of TBDs.

The Dactylosomatidae (Apicomplexa: Adeleorina) of African Amphibians

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The Dactylosomatidae is a small family of blood infecting protozoans comprising the genera *Babesiosoma* and *Dactylosoma*. Species of this family have to date only been recorded from amphibians and fishes, with the vectors suggested to be haematophagous leeches. Although globally, several different frog species have been recorded to be parasitised with *Babesiosoma* species, in Africa, only fish have been recorded infected by representatives of this genus. In contrast, members of *Dactylosoma* have been reported from a variety of frog and fish species throughout Africa. For the current study thin blood smears and whole blood were collected from 476 frogs from 30 species in the Ndumo Game Reserve (KwaZulu-Natal Province), and North-West University Botanical Gardens (North-West Province), South Africa. Light microscopy and molecular analysis (using fragment 18S rDNA) were used to characterize haemogregarine isolates from the blood of collected frog species. Resulting morphometrics and sequences were compared to each other as well as to comparative haemogregarine sequences from GenBank. Both morphological characteristics and molecular findings supported that only *Ptychadena anchietae* and *Xenopus laevis* were parasitised with *Dactylosoma*and *Babesiosoma* respectively. This work thus represents the first report of a *Babesiosoma* species parasitising an African frog, as well as being the first molecular and phylogenetic analysis of species of the Dactylosomatidae from African frogs.

Detection of single and multiple drug resistance of *Trypanosoma congolense* populations in Zambezia Province, Mozambique

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Trypanosomosis is a tsetse-transmitted debilitating parasitic disorder affecting domestic and wild vertebrates. In Africa, it affects the efficient food production with three million cattle deaths per year attributed to it. Treatment and prevention of trypanosomosis in domestic animals, in Africa, is essentially done with isometamidium chloride and diminazene aceturate which are drugs introduced more than 40 years ago and with resistance cases reported in several countries, including in Mozambique. Drug resistance has shown to be an important drawback to the agriculture development in Zambezia province where it has been causing a drastic reduction in the cattle population. So, to achieve success in any intervention towards control of trypanosomosis in Zambezia province, it is essential to characterize the distribution and nature of drug resistance. A total of 798 animals were screened for trypanosomosis, using microscopical examination, in eight villages of three districts of Zambezia Province, namely Nicoadala, Maganja da Costa and Mopeia. The trypanosome-positive animals were randomly allocated into two groups, one group treated with 0.5 mg/kg of body weight (b.w.) isometamidium chloride and a second group treated with 3.5 mg/Kg b.w. diminazene aceturate. The cattle were monitored for the presence of trypanosomes at days 14 and 28 post-treatment, and on day 28 a drug swap was performed in order to verify multi-drug resistance through microscopical examination on day 42 post-treatment. From the 798 animals, 107 (13%) revealed to be positive for the presence of *Trypanosoma* spp. in three of the villages, namely Botao (n = 66), Mungama (n = 21) and Namitangurine (n = 20). Only *T. congolense* infections were found during the screening with prevalence ranging from 0% to 44%. On day 14, nine animals in Botao and seven in Mungama were positive. On day 28, in Botao the number decreased to eight and in Mungama to four. Microscopic analysis on day 42 revealed that six animals in Botao and two in Mungama remained positive after drug swap. No relapses occurred in Namitangurine. The results above confirm the existence of single and multi-drug resistance in Zambezia province and this is a fundamental information to take into account when considering the control of trypanosomosis in the area.

Economic Burden of Malaria to Households in Gwanda District, Zimbabwe

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Malaria still remains a challenge to public health in sub-Saharan Africa and is one of the leading causes of morbidity and mortality. About 50 % of the population in Zimbabwe lives in malaria prone areas and are therefore at high risk of infection. In addition to being a major health problem, malaria imposes an economic burden in populations vulnerable to the disease. We estimate the household economic burden due to malaria in a rural community set-up. A cross-sectional household survey was conducted to provide data on household economic costs due to malaria. Households (n=78) were purposively selected based on health facility data. Interviewer-administered closed-ended questionnaires were used to ascertain expenditure on malaria treatment, transport costs related to malaria, loss of productive time as well as failure to perform normal daily activities due to malaria. The data was analysed using IBM SPSS version 22. Malaria is still a health problem in Gwanda despite the ongoing interventions to eliminate the disease. Households spend an average of US$4 as direct cost for managing a malaria episode per family member. A household loses an average of 7 productive working days per episode of malaria per person resulting in loss of an average of 24% of the monthly household income through this indirect cost. Malaria imposes a significant economic burden on households in poor and vulnerable communities of Gwanda. Policy makers have to take this information into consideration when considering the impact of the burden of malaria.

Ticks and Mites

Tick-borne pathogens of zoonotic importance in eastern and southern Africa: A review

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The aim of this review is to provide information on the tick-borne pathogens of zoonotic importance present in eastern and southern Africa mainly focusing on their geographical distribution, host range and identification of research gaps. The following tick-borne zoonoses have been reported to occur in eastern and southern Africa based mainly on case reports: Crimean-Congo haemorrhagic fever (CCHF) caused by Crimean-Congo haemorrhagic fever virus; Q fever caused by *Coxiella burnetii*; erhlichiosis caused by *Erhlichia ruminantium, E. canis and Anaplasma phagocytophilum*; babesiosis caused by *Babesia microti;* relapsing fever caused by *Borrelia duttonii;* and rickettsioses caused by *Rickettsia africae*, *R. aeschlimannii* and *R. conorii.* The epidemiological factors influencing their occurrence are reviewed and discussed. Ecological and anthropogenic factors that increase the chances of human-tick contact are discussed, including bush encroachment, urbanization, agricultural intensification, increased trade and travel, proximity to livestock and ecotourism. The distribution of tick-borne zoonotic pathogens is also reviewed in relation to the climatic conditions of the region. Whilst there are a number of case reports of tick-borne zoonoses in humans in the region, very few surveys have been carried out to determine the distribution and threat of tick-borne zoonoses within the at-risk populations in eastern and southern Africa.

Genotyping of *Ehrlichia ruminantium* strains in the ticks *Amblyomma hebraeum* and *A. variegatum* from Mozambique

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Amblyomma hebraeum and *A. variegatum* ticks are the main vectors of Heartwater in Mozambique, an important ruminant disease caused by the bacterium *E. ruminantium*. In the country, the two tick species are present in the South (*A. hebraeum*) and in the North (*A. variegatum*) of the Save river with a thin overlap. The main objective of this study was to genotype Mozambican strains from different localities using multi locus typing and compare with *E. ruminantium* from other geographical origins (Africa, Indian Ocean and Caribbean region). *E. ruminantium* tick infection rate in several localities and the genetic diversity according to the tick vector species was evaluated. One thousand three hundred and eighty six (1386) ticks from cattle and wild ruminants collected in 41 localities throughout Center, Southern Mozambique and the Kruger National Park were screened for their positivity to *E. ruminantium* using PCR targeting the pCS20 region. Strong positive samples were typed by MLST and MLVA. For the MLST, 5 housekeeping genes, lipA, lipB, secY, sodB and sucA were sequenced. Seven VNTRs, RU6, RU11, RU12, RU13, RU14, RU15 and RU19, were systematically amplified and compared with reference strains. Globally, the *E. ruminantium* tick infection rate was 19% (140/732) and it varied from 4 to 30% between the localities. From the MLST analysis of Mozambican and other worldwide strains, we demonstrate that there is a correlation between genetic strain clusters and geographic origin for Mozambique, Southern Africa and Indian Ocean strains. Specifically, most of the Mozambican strains are unique to the country. The MLVA allow a discrimination of strains from the same MLST clusters showing a more recent evolutionary event. For instance, the results support the hypothesis of cattle introduction from Eastern Africa to Indian Ocean islands. In conclusion, *E. ruminantium* tick infection rate in Mozambique is similar to other countries such as Gambia, Nigeria and Burkina Faso. There is a correlation between geographical and genetic markers diversity for Mozambique, Southern Africa and Indian Ocean strains. More strains are currently being typed to evaluate the influence of tick vector species and the role of wildlife on the genetic structuration of *E. ruminantium* strains.

Infracommunity dynamics of chiggers (Trombiculidae) parasitic on a rodent

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We studied the structure of chigger mite (Trombiculidae) communities parasitic on a South African rodent, *Rhabdomys pumilio*. The aims were to determine whether: (a) different chigger species differ in preferences for certain body areas of a host and (b) chigger assemblages among body areas of the same host individual, are structured and if so, whether the structure of these assemblages is aggregative or segregative. *Rhabdomys pumilio* is parasitized by seven chigger species belonging to six genera. The three most abundant species (*Leptotrombidium muridium*, *Schoutedenichia* spp. and *Neoschoengastia* sp. A) displayed a non-random distribution across the host body, with the two most abundant species (*L. muridium* and *Schoutedenichia* spp.) significantly associated with the tail area. In addition, whenever non-randomness of chigger co-occurrence in the same body area was recorded, it indicated positive but not negative co-occurrences of different species. This might be due to similarity of chigger species in resource needs and strategies to avoid host defence efforts.

Acaricide resistance in a resource-limited community and its implications for sustainable tick and tick-borne disease control in cattle

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Short-interval compulsory dipping is enforced in many rural cattle-farming communities as an important disease-surveillance tool. Intensive dipping, however, is contra-indicated in tick-borne disease endemic areas, as it limits exposure to tick-borne pathogens and thus predisposes to a state of endemic instability. In addition, frequent exposure of tick populations to acaricides inevitably selects for resistance, potentially resulting in severe economic losses amongst livestock. Due to financial and environmental constraints, however, strict adherence to the protocol of compulsory dipping is seldom maintained. This may have an unintended benefit of ensuring endemic stability to the important tick-borne diseases of cattle. However, failure to ensure availability of acaricides, or perceived lack of efficacy of the compounds, may lead to increased non-compliance of farmers in presenting their animals for inspection, as well as encourage uncontrolled use of acaricidal remedies, thus enhancing the development and spread of acaricide resistance. The Shaw Larval Immersion Test (SLIT), the standard test procedure used during the National Acaricide Resistance Survey, was used to screen for acaricide resistance at several sites in the Mnisi area of the Bushbuck Ridge district. Despite a fairly recent change to pyrethroid (after prolonged use of amidine) as the main active in state-funded dip tanks, resistance to both actives was detected in *Rhipicephalus microplus* collected from a number of locations. No resistance to organophospate was detected. Limited screening of *Amblyomma hebraeum* revealed no resistance to any of the actives. This suggests that both amidine and pyrethroid could be used more cost-effectively to control *A. hebraeum* (which is responsible for most mechanical damage and secondary infection) using spot-treatment on vulnerable areas such as the udders and genitalia of cattle. Strategic use of endectocides or chitin synthesis inhibitors, although costly, could possibly be used in conjunction with these to control excessive burdens of resistant populations of *R. microplus*, without reducing the current high level of endemic stability to tick-borne diseases. Such proposed changes, however, will require extensive consultation with both farmers and government departments to find a strategy that is both economical and sustainable.

Helminths

Helminthiasis and Schistosomiasis in pregnant women from informal settlements in South Africa: a cross sectional study

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Parasites in pregnancy are a devastating problem leading to severe adverse effects for both the mother and foetus. The lack of prevalence data on parasites in pregnancy prevents South Africa from introducing any interventions to prevent these adverse outcomes. A cross-sectional study was conducted to determine the prevalence, types and factors for soil-transmitted helminths and schistosomiasis in pregnant women of South Africa. 143 pregnant women attending antenatal care clinics near informal settlements around Durban, KZN Province, South Africa, were enrolled. After obtaining written consent, stool and urine samples were collected and analysed using the formal ether concentration method for intestinal parasites, and urine sedimentation method for *Schistosoma haematobium*. Additional data were obtained by interviewing participants and from their clinic records. Data were analysed using SPSS version 21. Descriptive statistics was used to show prevalence, co-infection and parasite species infecting pregnant women. Associations of exposures and parasites were analysed through Pearson's Chi square or Fisher's exact test. The statistical significance was considered to be $p<0.05$. More than forty percent parasitic infections were observed. The most common infections detected were due to *Ascaris lumbricoides* (36.4%) and *Trichuris trichiura* (18.9%). Very low (0.7%) infections of hookworms were observed and only 7.7% participants were infected with *Schistosoma mansoni*. More than 20% of women were infected with 2 or more parasite species. High infections were seen in women aged between 18-23 years, from informal settlement and in multi gravid women. However, associations were not statistical significant for all factors examined ($p >0.05$). The findings show moderate level of parasitic infections in pregnant women. The study provides baseline data which can be used to plan a comprehensive study to establish provincial and national prevalence. These data are critical for implementing the national strategy for which was finalised and approved by National Department of Health in 2014.

What is to be known and how do we know it? Understanding bilharzia using social sciences epistemology

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In the context of climate change it is beyond reasonable doubt that neglected parasitic diseases such as bilharzia will cause serious public health challenges. Bilharzia has dominantly been studied in the natural sciences domain. This brings a bias towards understanding the disease, as a result, the only viable solution to the bilharzia epidemic has dominantly been centred on the drug praziquantel. More recently, scholarship started engaging in promotion of behaviour modification targeted at reducing open water contact by human, mainly without the social science. Yet, this falls squarely in the domain of social science. Social sciences have been limited to a quick short questionnaire on risk factors. We ask are the 'risk factors' the only knowledge required to prevent, mitigate impacts, and promote coping and adaptation mechanisms that assist in reducing schistosomiasis. Thus, this paper seeks to answer the following questions, what socio-economic issues needs to be known? And how do we know the socio-economic issues that influence the schistosomiasis lifecycle? The study adopts a desk study methodology. Literature will be reviewed to draw what is known on bilharzia and what methodology was used. This data will be used to draw the research gaps and the role of social sciences in the understanding and analysis of bilharzia. It is known that the earliest social science research on bilharzia started in the late 1970s with a study on the social aspects of bilharzia. Most of these studies have adopted positivistic approaches. Yet constructive methodologies may offer an in-depth understanding into the lived realities. Thus, to a large extent social science did not contribute much to schistosomiasis studies. We contend that social sciences can contribute to the understanding and development of anthropocentric interventions to curb schistosomiasis.

Development of a real-time PCR for diagnosis of bovine cysticercosis

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Cysticercosis is emerging as a public health and agricultural problem of concern in lesser developed areas where animals are raised for consumption under traditional husbandry practices. Live cattle having cysticercosis show no symptoms, however, heavy infection by *Taenia saginata* larvae may cause myocarditis or heart failure. Currently the diagnosis of bovine cysticercosis is by meat inspection, a post-mortem examination carried out by meat inspectors to detect the presence of cysts at different predilection sites. During meat inspection there is a possibility of mis-taken identification of specific taeniid species involved due to cysts having died or degenerated or morphological similarities in lesions caused by taeniid larvae and other tissue larvae. Molecular tools such as PCR have already been developed for the specific diagnosis of taeniid metacestodes, hence, the current study aimed at developing a real-time PCR (qPCR) which is more sensitive and does not require post amplification processing, thus decreasing turnaround times and contamination risks. To this effect, DNA was extracted from both the metacestode and adult stages of *T. saginata*. The qPCR primers and probes were designed from the *COX*1 gene of *T. saginata* and amplified in LightCycler® 96 Real-Time System (Roche). The optimal annealing temperature of the qPCR assay is 56°C at 45 cycles, it produced a standard curve with an efficiency value of 1.98 and R^2 value of 1.00 and can detect the lowest DNA concentration of 0.013ng/µl. This assay specifically amplifies only DNA of *T. saginata* and does not cross react with DNA of *T. solium*, *T. hydatigena* and uninfected beef DNA. Futhermore, it can amplify DNA extracted from both *T. saginata* adult and cysticerci collected from cattle muscle.

Metabolic and adaptive immune responses of BALB/c mice infected with *Trichinella zimbabwensis*

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Tissue-dwelling helminths are known to induce intestinal and systemic inflammation accompanied with host compensatory mechanisms to counter balance nutritional and metabolic deficiencies. The metabolic and immune responses of the host depend on the parasite species and the organs or tissues affected by the parasite. The present study investigated metabolic and immuno-inflammatory responses of mice infected with tissue-dwelling larvae of *Trichinella zimbabwensis* and explored the relationship between infection, insulin signalling pathways and Th17 immune responses. A crocodile-derived *T. zimbabwensis* strain (Code ISS1209) was used in the study. Sixty 6 - 8 week old female BALB/c mice were randomly assigned into two equal groups; *T. zimbabwensis*-infected group (n = 30) and the non-infected control group (n = 30). Levels of Th1 (IFN-γ) and Th17 (IL-17) cytokines were measured in the two groups to determine and compare the immune-inflammatory response and the levels of insulin, blood glucose, food and water intake. Body weights were also measured to determine and compare the metabolic response in the two groups. Results showed that during the enteric phase of infection, insulin and IFN-γ levels were significantly higher ($P < 0.001$) in the *Trichinella*-infected group accompanied with hypophagia and weight loss compared with the non-infected control group. During systemic larval migration, food and water intake were significantly altered ($P < 0.001$) and this was attributed to compensatory feeding resulting in weight gain, reduced insulin levels and increased IL-17 levels. Larval migration also induced a Th1/Th17 derived inflammatory response. It was concluded that *T. zimbabwensis* alters insulin signalling pathways instigating host compensatory feeding. Furthermore, we showed for the first time that non-encapsulated *T. zimbabwensis* parasite immunomodulate host Th1/Th17 responses during chronic infection.

Spatial and seasonal variation of *Bulinus globosus* and *Biomphalaria pfeifferi* in Ndumo Area of Umkhanyakude District, Kwazulu-Natal, South Africa

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Density and abundance of intermediate host snails for schistosomiasis (*Bulinus globosus* and *Biomphalaria pfeifferi*) are influenced by climatic and environmental factors that include rainfall and temperature. However the extent of this variation may differ spatially and temporally due to changes in exploratory variables. The aim of this paper was to describe and analyse the spatial and seasonal variation of *Bulinus globosus* and *Biomphalaria pfeifferi* as well as their *schistosome* infection rates and related factors. The analysis was based on data collected between May 2014 and May 2015 in Ndumo area in uMkhanyakude district of KwaZulu-Natal province, South Africa. *Bulinus globosus* and *B. pfeifferi* snails were collected from 16 sentinel sites every month. The sampling was done by two people using scoops and handpicking for 30 minutes on each sampling day. The snails caught were expressed as number of snails per man hour search. The snails were screened for infection by the shedding process (exposure to light and darkness). *Bulinus globosus* was found at 75% of the sites (n=16) compared to *B. pfeifferi* (43.75%, n=16). Both species were sensitive to previous months rainfall and temperatures as indicated by changes in their numbers in the subsequent months. More snails were found during the dry season – (May to August 2014) with a sharp decrease in the period September 2014 to May 2015 mainly due to drying up of rivers. More *B. globosus* (8.9%, n=861) shed mammalian cercaria (especially during the dry period) compared to *B. pfeifferi* (0.1%, n=985), indicating that *schistosoma heamatobium* might be more prevalent compared to *schistosoma mansoni*. The transmission implications of the two forms of schistosomiasis in the study area are discussed.

DNA sequence analyses reveal co-occurrence of novel haplotypes of *Fasciola gigantica* with *F. hepatica* in South Africa and Zimbabwe

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The aim of this study was to identify and determine the genetic diversity of *Fasciola* species in cattle from Zimbabwe, the KwaZulu-Natal and Mpumalanga provinces of South Africa and selected wildlife hosts from Zimbabwe. This was based on analysis of DNA sequences of the nuclear ribosomal internal transcribed spacer (ITS1 and 2) and mitochondrial cytochrome oxidase 1 (CO1) regions. The sample included flukes collected from livers of 57 cattle at four abattoirs in Zimbabwe, 47 cattle at four abattoirs in South Africa and three alcohol-preserved duiker, antelope and eland samples from Zimbabwe. Aligned sequences (ITS 506 base pairs and CO1 381 base pairs) were analysed by neighbour-joining, maximum parsimony and Bayesian inference methods. Phylogenetic trees revealed the presence of *Fasciola gigantica* in cattle from Zimbabwe and *F. gigantica* and *Fasciola hepatica* in the samples from South Africa. *Fasciola hepatica* was more prevalent (64%) in South Africa than *F. gigantica*. In Zimbabwe, *Fasciola gigantica* was present in 99% of the samples; *F. hepatica* was found in only one cattle sample, an antelope *(Hippotragus niger*) and a duiker (*Sylvicapra grimmia*). This is the first molecular confirmation of the identity *Fasciola* species in Zimbabwe and South Africa. Knowledge on the identity and distribution of these liver flukes at molecular level will allow disease surveillance and control in the studied areas.

Haemoparasite Genetics

18S rDNA sequencing data reveal possible novel *Babesia* spp. in domestic cats

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Feline babesiosis is of major clinical significance in certain areas of South Africa. When the first clinical cases were reported (1937), the causative agent was presumed to be *Babesia felis*, described from an African wild cat. Before the advent of molecular characterisation, small intra-erythrocytic piroplasms seen on felid blood smears were usually named *B. felis* or (occasionally) *Cytauxzoon felis*. It is now known that not only *B. felis*, but also *B. leo, B. lengau* and *B. microti* can occur as single or mixed infections in domestic cats. These findings, together with that of *B. lengau* associated with clinical signs in domestic cats, challenge the long-held belief that *B. felis* is the only causative agent involved. This study was conducted to document the occurrence of *Babesia* species in domestic cats in South Africa. Blood specimens from 20 cats showing clinical signs compatible with feline babesiosis were confirmed to contain a *Babesia* spp. either by light microscopy and/or the reverse line blot hybridization assay. They were subjected to cloning of the full-length 18S rDNA, sequencing and phylogenetic analysis. Ten clones were selected from each sample. Sequence data from the near-full-length 18S rRNA gene was assembled and edited to a total length of 1,600 bp. Similarity matrices were constructed using the two-parameter model of Kimura and the Jukes and Cantor correction model for multiple-base changes. Phylogenetic relationships between known *Babesia* spp. and sequence data obtained were analysed using both neighbour-joining and maximum parsimony phylogenetic analyses. Although most of the sequences grouped together with *Babesia* species previously described from domestic cats (*B. felis*, *B. leo*, *B. lengau* and *B. microti*), a large number grouped together with sequences of *Babesia* species such as *B. divergens* and *B. odocoilei* that infect dogs, ungulates and humans. Results suggest the existence of novel *Babesia* spp. in domestic cats. The possibility of more than one parasite being the causative agent of babesiosis in domestic cats, and single infection or mixed infection with other *Babesia* parasites, cannot be out ruled.

The evaluation of the effect of cattle and buffalo host cells on gene expression in buffalo-derived and cattle-derived *T. parva* isolates

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Theileria parva is a haemoprotozoan parasite that affects cattle in eastern, central and southern countries of Africa causing serious mortality. The African buffalo is the natural reservoir host of *T. parva* which is not affected by the parasite. The cattle-derived *T. parva* is responsible for East Coast fever (ECF) while the buffalo-derived parasites cause Corridor disease (CD). Although *T. parva* parasites cause different disease syndromes they are indistinguishable by morphological or serological criteria and molecular characterization has not been successful in distinguishing ECF and CD parasites. Consequently, transcriptome profiles of two *T. parva* isolates representing ECF- and CD-causing parasites were investigated to identify gene expression profiles that can help distinguish these parasites. Transcriptome analysis revealed differentially expressed genes; however, it is suspected that variation in gene expression could have been influenced by the hosts of origin, which were cattle and buffalo. Hence, the aim of this study was to evaluate the effect of cattle and buffalo host cells on gene expression in *T. parva* isolates. A microarray comprising of 4061 *T. parva* genes was developed to analyse gene expression profiles for comparison of cattle-derived (Muguga and Schoonspruit) and buffalo-derived (7014, SA8200, SA8301 and SA8610) *T. parva* isolates. All isolates were maintained in cattle lymphocytes except *T. parva* 7014, which was maintained in buffalo cells. Thus the analysis was based on 3 groups of parasites maintained in different hosts cells including cattle-derived maintain in cattle, buffalo-derived maintained in cattle and buffalo-derived maintained in buffalo. The analysis of cattle-derived isolates gene expression profiles revealed 744 differentially expressed genes (DEGs) compared to 763 detected from buffalo-derived isolates maintained in cattle. The expression between the two groups is similar however the buffalo-derived isolates have more DEGs than the cattle-derived isolates. The analysis of buffalo-derived maintained in cattle versus buffalo-derived maintained in buffalo show on average 802 differentially expressed genes. The comparison of cattle-derived and buffalo-derived expression profiles demonstrated more differentially expressed genes in latter compared of the differentially expressed genes identified from cattle-derived isolates. The preliminary data indicates that the host of origin could be influencing the gene expression in the parasite.

Molecular detection of trypanosomes in tabanid flies collected from Phinda and Charters Creek Game Reserves in Kwazulu-Natal Province, South Africa

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Tabanid flies are mechanical vectors of several disease causing pathogens that infect livestock worldwide. Due to their persistent biting behaviour affected livestock have poor body condition, reduced meat and milk yields therefore affecting the agriculture as well as the economy of the affected nations. The current study was aimed at characterizing tabanid flies collected from Phinda and Charters Creek game reserves in KwaZulu-Natal Province and to determine trypanosome parasites they are harbouring. A total of 256 tabanid samples were collected and the most abundant species were *Tabunus par* 37% (95/256) and *T. taeniola* 27% (68/256) whilst *Haematopota decora* 1.1% (3/256) and *Philoliche aethiopica* 1.9% (5/256) were the least. Each tabanid fly specimen was homogenised and genomic DNA was extracted using a commercial kit. PCR was used to screen for trypanosome infections from tabanid DNA. As a result trypanosome infection rate in tabanid flies was 10.9% (28/256), with 57.1% (16/28) and 42.9% (12/28) for *Trypanosoma theileri* and *T. congolense* (Savannah) 42.9% (12/28), respectively. Data obtained from this study highlights the possible role of tabanid flies in transmission of trypanosome parasites in livestock and wild life and they should be considered when formulating trypanosome and tsetse control strategies in South Africa.

Human blood-brain barrier brain gene expression in response to African trypanosome infection and shear stress flow

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Trypanosoma brucei rhodesiense and *T. b. gambiense* are vector-borne parasites that cause human African trypanosomiasis (HAT; sleeping sickness). Dissemination into the brain leads to neurologic signs characterized by concomitant psychiatric disorders, progressive coma and, if untreated, death. How African trypanosomes alter blood-brain barrier (BBB) function to disrupt brain homeostasis to cause central nervous system (CNS) disease remains unresolved. The effect of *T. b. rhodesiense* on human brain microvascular endothelial cell (HBMEC) gene expression under pulsatile flow was analysed under brain capillary (high), venule (low) and arteriole (low to high) shear stress (SS) conditions: high SS flow (14 dynes/cm^2), low flow (2 dynes/cm^2) and static (0 dynes/cm^2; a condition used for gold-standard Transwell-based in vitro BBB models). It was found that 333 to 491 genes that were differentially expressed at > 3 standard deviations (SD) for each condition and Ingenuity Pathway-enrichment analysis of these genes identified 405 canonical pathways covering the three conditions primarily represented by 6 broad functional classes; *Cellular Mechanics and Molecular Transport* (119 enriched pathways), *Cell Cycle* (107 pathways), *Immune System* (51 pathways),*Vascular Remodeling* (33 pathways), *Signal Transduction* (32 pathways) and *Nervous System* (29 pathways). Thirty-four pathways altered in HBMEC by trypanosomes were specific to high brain capillary high SS flow conditions in brain capillary, of which 26 were metabolic in nature suggesting that the parasites could alter overall brain homeostasis. The ratio of metabolic to signalling pathways was lower in trypanosome infected HBMEC exposed to low SS conditions as found in venules, a known trypanosome brain entry site: 25 of 42 pathways specific to this condition were metabolic. For comparison, only 12 out of 65 static condition specific pathways were metabolic in nature. Pathways that under low and high SS that might regulate smooth muscle tone in arterioles and thus regulate brain blood flow were also identified. Overall, these data begin to reveal how trypanosomes under SS conditions, as they occur in the brain, might remodel the vasculature and trigger molecular and cellular events that regulate brain blood flow, brain homeostasis and parasite brain entry that are the signature of CNS HAT and identify novel therapeutic targets.

A new 5.8S rRNA-internal transcribed spacer 2 gene lamp assay for *Trypanosoma brucei gambiense* detection

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Highly sensitive LAMP reactions specific for *T. b. rhodesiense* or that recognize but do not discriminate between *T. b. brucei, T. b. rhodesiense, T. b. gambiense* and *T. evansi* have been developed. While sensitive LAMP assays targeting the *T. b. gambiense* 5.8S rRNA-internal transcribed spacer 2 (5.8S-ITS2) gene are available these assays do not target binding sites that span the CCCA (556-560 bp) insertion site that further differentiates *T. b. gambiense* from other *T. b. brucei*. We have developed a 5.8S-ITS2-targeted LAMP assay that fit these criteria. The LAMP primer sets containing the *T. b. gambiense*-specific CCCA tetranucleotide at the start of the F3 primer sequences show high specificity and sensitivity for *T. b. gambiense* genomic DNA.

Preliminary study of protozoan parasites in different wild and domestic animals in South Africa

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There is insufficient information available on protozoan parasites of African animals. Tissue samples collected from nine animals of eight different species were investigated for *Toxoplasma gondii* and *Neospora caninum* by polymerase chain reaction (PCR).*Toxoplasma gondii* was detected by PCR in tissue samples (brain, spleen, liver, lung) from three (33.3 %) of 9 tested animals: Greater Kudu (*Tragelaphus strepsiceros*), South African Hedgehog (*Atelerix frontalis*) and domestic cow. *Neospora caninum* was not detected in any of the tested tissues. Simultaneously stool samples collected from 23 carnivores and one tortoise were also checked for protozoan parasites with proof of *Isospora* sp. (16.7 %), *Toxocara* sp. (12.5 %) and *Cryptosporidium* sp. (12.5 %). *Isospora* sp. and *Toxocara* sp. oocysts were found in faecal samples of dogs *(Canis familiaris*), lion (*Panthera leo*) and Banded Mongoose *(Mungos mungo*) and *Cryptosporidium* sp. in Banded Mongoose, Spotted Hyena (*Crocuta crocuta*) and Striped Polecat *(Ictonyx striatus*). Eggs of an *Oxyurid* sp. were observed in Leopard Tortoise (*Stigmochelys pardalis*). This study increases our knowledge about the occurrence of protozoan parasites among population of different wild and domestic animals in South Africa.

Avian haemo- and ectoparasite prevalence in Mpumalanga, South Africa

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Despite its threat to avian diversity, little information is known about avian haemo- and ectoparasite prevalence especially in Africa. Avian malarial haemoparasites (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon spp*) cause anaemia in bird populations across six continents. Due to the massive scope of this disease, understanding factors that affect avian malaria prevalence and transmission is crucial. Though believed to be endemic in African passerines, true burden of malaria parasitemia in their native hosts is unknown. The factor of note is land-use types, the diversity of which is linked to varying abundance and distribution of parasite hosts and vectors, as well as to habitat quality. Our preliminary study investigated the influence of land-use type on avian parasite prevalence in Mpumalanga Province, South Africa. During the month of May 2015, we collected blood and feather samples, along with morphological measurements, from birds sampled from Skukuza, Kruger National Park (n = 160) vs those sampled from Mkhuhlu township waste disposal site (n = 109), an area with high levels of human disturbance. We compared the avian haemo- and ectoparasite prevalence between the two sites. We found that the overall parasite prevalence for both sites was 25.49% for haemosporidian parasites, and 20.59% for ectoparasites. Haemoparasites persisted in about the same number of individuals as inside (24.77% and 26.32%, respectively). Leucocytozoon prevalence, however, was about four times higher inside Kruger than outside (8.42% and 1.83% respectively). Haemoproteus (17.89% inside and 12.84% outside) and Plasmodium (11.58% inside and 11.93% outside) prevalence were similar between both sites. Ectoparasites infected more birds inside Kruger than outside (25.26% and 16.51%, respectively). However ectoparasite load did not vary between sites. Relative body condition was the same in infected birds as uninfected ones. Interestingly, parasite prevalence varied between avian families. Our initial results show that land use practice influences parasite prevalence in birds, with the pristine site having greater parasite prevalence overall. The mechanism behind this trend will need to be further elucidated.

Metazoan parasites of *Amphilius uranoscopus* and *Chiloglanis pretoriae* from four tributaries of the Luvuvhu River in the Vhembe biosphere reserve, Limpopo Province

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The parasite diversity of the small fish species in the Luvuvhu River tributaries (Barotta, Dzindi, Mvudi and Lutanandwa rivers) in the Vhembe Biosphere Reserve has never been studied. An assessment of the parasite composition of *Amphilius uranoscopus* (Stargazer mountain catfish) and *Chiloglanis pretoriae* (Shortspine suckermouth) was done in 2014 during April (high flow condition) and July (low flow condition). A total of 64 *A. uranoscopus* specimens were sampled (34 during high flow and 30 during low flow) and 56 *C. pretoriae* specimens (30 during high flow and 26 during low flow). The fish were sacrificed by severing the spine, weighed, measured and examined for parasites. All parasites were fixed and preserved according to standard methods for each parasite group. Parasites recorded from both fish species include *Contracaecum* sp., and *Clinostomum* sp. from the body cavity, an unidentified leech from the skin, *Diplostomum* sp. from the eye and unidentified digenean larvae from the gills as well as an unidentified monogenean from the gills. Higher parasite diversity was recorded for *A. uranoscopus* than for *C. pretoriae*. Monogeneans were the most prevalent parasites for *C. pretoriae* and were more prevalent during low flow conditions. Digenean larvae were the most prevalent parasite for *A. uranoscopus* and were collected in higher numbers during high flow than low flow conditions. No parasites were recorded from *A. uranoscopus* from Mvudi River (the more polluted site) during both high and low flow conditions. The current results represent new parasite distribution records for these fish species from these rivers and can consequently be implemented in the management and conservation of the Vhembe Biosphere Reserve.

Update of the parasitofauna of fishes in the middle Zambezi River and Lake Kariba

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In an on-going study to update the parasitofauna of Lake Kariba and the Zambezi River which started in February 2011, twenty two (22) fish species belonging to ten (10) families were sampled and examined for internal and external parasites. Thirty three parasite groups have so far been isolated from the fishes, of which 27 have been identified at least to genus level. Of these nine newmonogeneans belonging to the genus *Gyrodactylus* have been identified on six host species and we are currently working on their specific taxonomy. The African catfish *Clarias gariepinus* hosts the greatest diversity of parasites (17 species), followed by the squeaker *Synodontis zambezensis* (5) and the tigerfish *Hydrocynus vitattus* (4). Could this be an indicator that carnivorous fish are more susceptible to parasitism as a result of trophic transmission? This remains unanswered as more data are being gathered. The possible role of introduced species such as the Nile tilapia *Oreochromis niloticus* and the exotic snail *Melanoides tuberculata* in the transmission of novel parasites to the Zambezi fishes is also being investigated. Current data suggest that the Monogenea (14 species) is the most specious group, and these have a direct life cycle where parasites actually 'jump' from one fish to the next. Impacts of global environmental change are also hypothesized to affect the distribution of parasites and their host fishes in the middle Zambezi and this study will hopefully provide information that will be vital in unravelling this mystery.

Posters

Identification and molecular characterization of *Babesia* species in brown (*Parahyaena brunnea*), striped (*Hyaena hyaena*) and spotted hyaenas (*Crocuta crocuta*)

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Fifty-five blood and skin samples collected from three hyaena species were obtained from the biobanks of South African National Parks and the National Zoological Gardens of South Africa: 12 from brown hyaenas (*Parahyaena brunnea*), four from striped hyaenas (*Hyaena hyaena*) and 39 from spotted hyaenas (*Crocuta crocuta*). These were screened for the presence of *Babesia* and *Theileria* species using the Reverse Line Blot (RLB) hybridization technique. Samples were simultaneously screened for the presence of *Ehrlichia* and *Anaplasma* spp. The RLB results revealed that the PCR products of 74.5% of the samples hybridized only with the *Theileria/Babesia* genus-specific probe, and not with any of the species-specific probes, suggesting the presence of a novel species or variant of a species. No *Ehrlichia* and/or *Anaplasma* species DNA could be detected. The parasite 18S rRNA gene of five spotted and seven brown hyaena samples was subsequently amplified, cloned and the recombinants sequenced. Homologous sequence searches of databases that were performed indicated that the obtained sequences were most closely related to *B. lengau*, previously identified in cheetah in South Africa. The observed sequence similarities were subsequently confirmed by phylogenetic analyses which showed that the obtained hyaena sequences formed a monophyletic group with *B. lengau, B. conradae* and sequences previously isolated from humans and wildlife in the western USA. Within the *B. lengau* clade, the obtained sequences and the published *B. lengau* sequences grouped into four distinct groups, of which groups I, II and III each represented a novel *B. lengau* genotype. We suggest that these genotypes cannot be classified as new *Babesia* species, but rather as variants of *B. lengau.* The study confirms that hyaenas are susceptible to infection by a *Babesia* sp similar to *B. lengau*, but also demonstrates that they are not clinically affected by the infection. Their role as carriers of this organism and their ability to carry this infection over to other species still needs elucidation.

Field Studies on Aestivation in Umkhanyekude District of South Africa

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Schistosomiasis transmitting intermediate host snails survive drought periods through aestivation, a survival strategy whereby they lower their metabolic activities to survive seasonally adverse conditions with limited or no food and burrowing into the ground. It is envisaged that with climate change dry/wet seasons may become shorter or longer, thereby reducing or increasing their chances of survival. This study investigated the ecology of the snails in three rivers in Umkhanyakude district to determine the influence of climatic change on snail survival and breeding. Aestivation patterns in *Biomphalaria pfeifferi* and *Bulinus globosus* were studied by digging transects across the floor of their dried habitats and by monitoring changes in snail population structure during the period when the habitats were flooded. Dried up riverbeds were scrapped to 10 cm at specified points in Luthobane and Sunduza rivers to determine spatial distribution. Soil was also scrapped to different depths at Ngezabafazi River to determine effect of depth on aestivation. A wide size range of both species of snails were found to aestivate. Successfully aestivated snail numbers in Luthobane and Ngezabafazi decreased from 17 and 9 to 2 and 1 respectively (June 2014 to March 2015). In these 10 months, soil moisture levels decreased to less than 14 % from the time the river dried up. In Sunduza River no snails were observed in the first month after the onset of rains. However in the second month, 92% (n = 13) of *B. pfeifferi* snails recovered by scooping were more than 10 mm in size, suggesting they could have been aestivating. More snails were found at the river banks compared to the centre of the riverbeds suggesting they preferred sheltering themselves among the vegetation and did not necessarily follow the receding waters. This study showed that aestivation ability declines with time and occurred in the first 5 cm from the surface.

Prevalence and genetic relatedness of *Besnoitia besnoiti* isolates from different geographical regions of South Africa

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Bovine besnoitiosis is a protozoan disease caused by an apicomplexan parasite *Besnoitia besnoiti*, re-emerging in Europe and occurring in many other countries including South Africa. The disease have been one of the neglected disease of domestic animals, it was until recently that it have received attention due to its increasing geographical distribution. This parasite cause significant economic losses due to its dramatically effect on the body condition, declined milk production, irreversible sterility in male and mortality. This study was designed to determine the prevalence of *Besnoitia besnoiti* infection in cattle, and establish the phylogenetic relationship of the parasite isolates in different geographical regions of South Africa, where the disease was previously reported. A total of 395 cattle were randomly selected from three (3) provinces (Limpopo, Gauteng and Eastern Cape) of South Africa between September 2014 and February 2015. Thirty nine (39) out of 395 (9.87%) blood samples, and 18 out of 118 (15.25%) skin samples were positive by polymerase chain reaction (PCR), while 104 of the total of 166 (62.65%) sera screened were positive by enzyme-linked immunosorbent assay (ELISA). The prevalence results will also indicate the current geographical distribution of the disease in South Africa.

Afrodiplozoon polycotyleus (Monogenea: Diplozoidae) recorded from cyprinids from the Luvuvhu River: Morphological analysis and molecular characterisation

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The genus *Afrodiplozoon* was proposed by Khotenovsky in 1981 when *Afrodiplozoon polycotyleus* (Paperna, 1963) was excluded from the genus *Neodiplozoon* Tripathi, 1959 based on the number of clamps on the attachment apparatus. Since then, the inconsistency in the use of the name for this parasite can be seen throughout the literature. Specimens of *A. polycotyleus* were collected during a fish survey carried out in April and July 2014 from tributaries of the Luvuvhu River in the Venda region, Limpopo Province, South Africa, from *Labeobarbus maraquensis* (n = 36; mean total length = 7.9 cm) and *Barbus paludinosus* (n = 1; total length = 6.6 cm). A prevalence of 64% and 59% were recorded for *A. polycotyleus* during April and July, respectively. Morphological analysis of the composition of the internal organs and attachment clamps using different microscopic methods i.e., light microscopy of stained/unstained specimens and scanning electron microscopy (SEM) provided details for the redescription of the genus. The type material was also studied. Molecular characterisation based on the second internal transcribed spacer (ITS2) rDNA revealed the taxonomic relationship to other representatives of Diplozoidae. Parasites can bear asymmetrically from seven up to ten clamps in one row on each side of the attachment apparatus, with the first clamp significantly smaller. The connection sclerite of the posterior end of the central plate is wide while the anterior end has short processes. SEM observations showed the presence of numerous papilae around the mouth of the worm. Molecular characterisation and subsequent analysis revealed *A. polycotyleus* to be a sister species of the recently described *Paradiplozoon bingolensis* Civáňová, Koyun & Kobková, 2013, recorded a far distance from African representatives of the genus *Paradiplozoon* (Achmerov, 1974).

Polymerase Chain Reaction as a tool to confirm identification of *Taenia saginata* and *T. solium* metacestodes made during meat inspection at abattoirs

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Taenia saginata and *T. solium* are parasitic cestodes of medical, veterinary and economic importance causing taeniosis in humans and cysticercosis in cattle and pigs/humans respectively. Currently meat inspection, a post mortem examination, is the standard diagnostic tool used for bovine and porcine cysticercosis. Mistaken identifications, however do occur during meat inspection due to morphological similarities in taeniid larvae and lesions caused by other tissue parasites such as *Sarcocystis* species. The objective of the study was therefore, to use Polymerase Chain Reaction (PCR), a more specific tool to confirm identifications of cyst samples made during meat inspection. A total of 181 cyst samples identified as *T. saginata* at meat inspection were collected from bovine carcasses in abattoirs, whilst only 2 cyst samples identified as *T. solium* were collected from porcine carcasses. Genomic DNA was extracted from these samples, DNA concentration and purity was measured and conventional PCR assay with primers targeting the mitochondrial *COX* 1 gene of *T. saginata* and *T. solium* was subsequently used. The PCR results respectively confirmed that 95% (171/189) and 100% (2/2) of the cysts were correctly identified as *T. saginata* and *T. solium* during meat inspection. Negative results obtained during the study could be due to misidentification of cysts as it is reported that meat inspection is a subjective method which depends on the skills of the meat inspector. This implies that that the use of meat inspection records alone can overestimate the disease prevalence. There is, however, also a possibility that the negative results obtained could be due to cyst degeneration as the sensitivity of PCR decreases with degeneration of cysts. It is therefore recommended that the cyst status be established and recorded before DNA extractions are done.

Metazoan parasites of *Chetia flaviventris* and *Coptodon rendalli* from Doorndraai Dam, Limpopo River System

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The study of freshwater fish parasites is important, not only to give us a better understanding of the ecosystem but also to provide information on health threats and assisting in the protection of fish populations. The first parasitological study at Doorndraai Dam was carried out during May and April 2015. This dam forms part of the Sterkrivier (Limpopo River System) near Mokopane in the Limpopo province. *Chetia flaviventris* (n=21) and *Coptodon rendalli* (n=20) were sampled using conventional angling gear and examined for endo- and ectoparasites. The parasites were collected, fixed and preserved according to standard methods for each parasite group. The results are presented as prevalence, mean intensity and mean abundance for each species. The following parasites were recorded for *C. flaviventris*: three nematode species, i.e. *Contracaecum* larvae from the body cavity (90.4%; 4.4; 4), *Paracamallanus* sp. (4.7%; 1; 0.04) from the intestine and *Eustrongylides* sp. (9.5%; 3; 0.2) from the body cavity; one cestode, i.e. gryporynchid larvae from the body cavity (80.9%; 27.3; 20.8); two monogenean species, i.e. *Enterogyrus* sp. 1 (28.5%; 6; 2.8) from the stomach wall and *Cichlidogyrus* sp. 1 (95.2%; 15.7; 15) from the gills and one copepod *Ergasilus* sp. (85.7%; 14.7; 12.6) from the fins. The following parasites were recorded for *C. rendalli*: one nematode i.e. *Contracaecum* sp. (65%; 2.0; 1.3) from the body cavity; two monogenean species, *Enterogyrus* sp. 2 (15%; 5.6; 0.8) from the stomach and *Cichlidogyrus* sp. 2 (90%; 7.7; 7.4) from the gills and one copepod, *Ergasilus* sp. (95%; 3.5; 3.3) from the fins. The water quality at the dam was good with oligotrophic conditions recorded which may be the reason why both fish species harbour a wide variety of parasites. These finding represent new host and geographical records.

Fish eye trematodes, metacercariae and adult

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Parasites as one group of pathogens represent an important source of economic losses for aquaculture. Among them, eye fluke infections can also be associated with decreased growth of fish.

In this study, the infection of two species of eye trematodes was investigated, *Diplostomum* sp. (Diplostomatidae) and *Nematobothrium labeonis* (Didymozooidae) from freshwater fish hosts in Limpopo and Olifant River systems, in South Africa. The first species is an ubiquitous parasite while the second one is limited to *Labeo* species hosts, which was mainly collected from *Labeo rosae* in the present work. In addition, the light and SEM micrographs of these two parasite species, are provided.

The metacercariae of *Diplostomum* species are located in the eye lenses causing cataracts, which in the case of heavy infections, can lead to blindness of the fish, while the adults of *N. labeonis* are found within the orbit of fish. This is the first record of *N. labeonis* in South Africa. Although infections by eye trematodes are common in nature, they can also cause outbreaks in cultured fish farms which may have significant implications such as high abundance and prevalence in economic important fishes, thus knowledge of occurrence and their effects on hosts is an essential prerequisite of preventative procedures for the parasite problem in fish farming.

First molecular characterisation of fish parasitic marine cymothoid isopods from southern Africa

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Isopods from the family Cymothoidae are parasitic on numerous marine, brackish and freshwater fishes where they can be found on the host's external surfaces, inside the body cavity, or within the gill or buccal cavity. Although easy to observe with the naked eye, these isopods are not often studied and as a result there are many aspects of their life history and taxonomy that remain unknown. One such area with a paucity of information is their phylogeny and taxonomy based on molecular data. With only a few species of cymothoids having been sequenced, and many genera without any sequences on GenBank, the need for good quality cymothoid sequences is evident. This is especially important due to the difficulty arising in accurately identifying species in this morphologically variable group of parasitic isopods. A number of cymothoid species from *Ceratothoa* Dana, 1852, *Cymothoa* Fabricius, 1793 and *Mothocya* Costa, in Hope, 1851 were collected from the south and east coasts of South Africa as well as from southern Mozambique. DNA was extracted and fragments of two mitochondrial genes (16S rRNA and cytochrome oxidase I) from each species were amplified and sequenced. Resulting sequences were compared to each other as well as selected sequences from GenBank and aligned using the software package Geneious. This preliminary work resulted in a number of new cymothoid sequences being added to GenBank which will aid future research and molecular phylogentic studies in cymothoid isopods, as well as clarifying several morphologically ambiguous species based on their molecular configuration.

Assessment of Sulfur, Calcium and Phosphorus Content in the Cuticle Covering the Cordons of *Desportesius invaginatus* (Nematoda, Acuariidae) Using Energy Dispersive X-Ray Analysis (EDXA)

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The structure of cordons is a principal characteristic in the identification of acuariid nematodes. The cordons of *Desportesius invaginatus* are formed of consecutive structurally elaborated plates of variable size and topography. Energy dispersive X-ray microanalysis (EDXA) is an analytical technique that had been used to evaluate the element composition and crystalline nature of the body surface of many helminthes. It has been also used to measure the concentration of minor (S, P, Ca, K) and trace elements (Fe, Zn, Cu, Ni, and Mn) in the cuticle of some acanthocephalans and free-living nematodes. In the present study, EDXA revealed variations in the concentration of sulphur, calcium and phosphorous in different regions of the cuticular ridges (tip, middle and base) of the three regions (anterior, middle, posterior) of the cordons of *Desportesius invaginatus*. In the anterior region of the cordons, phosphorus was the highest in concentration followed by calcium then sulphur ;in the middle region, sulphur exhibited highest concentration followed by phosphorus then calcium; while in the posterior region, sulphur showed the highest concentration followed by calcium and phosphorus. The study revealed a specific pattern for the distribution and concentration of each element in relation to the region in the cuticular ridges of the cordons. The concentrations of the three elements in the cuticle in different regions of the cordons were relatively higher than in the cuticle of the body specially the area behind the cordons. The use of EDXA in the present study showed that calcium and phosphorous, probably form a calcium phosphorous apatite responsible for hardness in the encountered structures. The detectable increase of the three elements in the cordons elaborations in comparison to the body cuticle of *Desportesius invaginatus* could support the idea that cuticular ridges of nematode cordons are basically spines that function for anchorage and in feeding by abrasing the host mucosa.

Morphological and molecular characterization of an African freshwater fish trypanosome, including its development in a leech vector

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Trypanosomes are ubiquitous blood parasites of fishes and at least 13 species were originally described infecting African freshwater fishes. This number was later reduced to three and in the late 1990's it was proposed that most records of freshwater fish trypanosomes across Africa might be *Trypanosoma mukasai* Hoare, 1932. Recently, results from a molecular analysis of fish trypanosomes from the Okavango Delta, Botswana reported the presence of at least two genotypic groups and concluded that the identification of *T. mukasai* remains problematic. The aims of the present study were thus to elucidate the life cycle of the freshwater fish trypanosome from southern Africa and to do a morphological and molecular characterization of the parasites from both the fish host and leech vector. To locate trypanosome stages, leeches were removed from fishes captured in the Phongolo River, South Africa, and fish blood films and leech squashes were Giemsa-stained and screened. To determine whether trypanosome stages in fishes and leeches were of the same genotype, DNA was extracted and fragments of the 18S rRNA gene were amplified and sequenced. Trypanosomes were detected in the fish families Cichlidae, Clariidae, Mochokidae and Schilbeidae. Sequence data showed that trypanosomes from leeches, identified as *Batracobdelloides tricarinata* (Blanchard, 1897), were identical to those obtained from fish. This paper presents the first completely life cycle of a freshwater fish trypanosome from southern Africa as well as the first study to link the vertebrate hosts and vector of an African freshwater fish trypanosome by molecular means.

Notes

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