Parasitological Society of Southern Africa

The following are abstracts of papers and posters presented at the 38th Annual Congress of the Parasitological Society of Southern Africa (PARSA), 20–23 September 2009, Magaliesberg Conference Centre, Broederstroom, South Africa

Keynote addresses

Underestimation of skin-dwelling filarial worm diversity and prevalence in wild ungulates: lessons from 20 years of investigations in Japan

O Bain[1], S Uni[2] and M Casiraghi[3]
[1]*Muséum National d'Histoire Naturelle, Origine, Structure et Evolution de la Biodiversité, Paris, UMR 7205, CP52, 61, rue Buffon, 75231, Paris, France*
E-mail: bain@mnhn.fr
[2]*Osaka City University Medical School, Osaka, 545-8585, Japan*
[3]*Università degli Studi di Milano Bicocca, Piazza della Scienza 2, 20126, Milano, Italy*

Our investigations into filarial worms in wild ungulates in Japan began with a case of onchocerciasis at a school in the city of Oita, Kyushu Island, South Japan, in 1989. Since the human parasite *Onchocerca volvulus* is not present outside Africa, its region of origin, and South and Central America, this could only be a zoonosis. Several cases were subsequently recorded. *Onchocerca* species are parasites of ungulates and are located in subcutaneous or dermal tissues. The present Japanese mammalian fauna is the result of waves of migration from North and South China during the last 5 Ma and extinctions during the Quaternary Ice age. Thus, ungulates are few: a suid *Sus scrofa leucomystax*, a cervid *Cervus nippon* (sika deer) and a caprine bovid *Capricornis crispus* (Japanese serow). Skins of large animals are difficult to examine, but the search for filariae proved rewarding. Worms recovered did not only belong to *Onchocerca*, but to other poorly known onchocercid genera as well. (1) *Loxodontofilaria*: known from Africa and India as parasites of elephants; a new species was discovered in the serow. (2) *Mansonella*: ungulates were not previously in the host range of this large and complex lineage; a new species was discovered in the sika. (3) *Cercopithifilaria*: the type species is a parasite of a cercopithecid from Kenya and in South Africa another species was reported from monkeys; the known host range was expanded immensely and now includes bovids, murids etc. In Japan, 5 congeneric species are found in the serow and 2 species in sika deer, each with a defined niche. Incidentally, this genus is also represented in the Japanese black bear. *Cercopithifilaria* species are transmitted by hard ticks (Ixodidae). It is supposed that its zoologically incoherent host range is the result of the ticks' particular feeding behaviour (several mammalian hosts) and mode of dispersal (attached to hosts). (4) *Onchocerca*: represented by 1 species in the serow, 1 species in the sika deer, 1 species common to serow, sika deer and the European deer, and a 4th species in wild boar. The latter was proven to be the agent of human onchocerciasis in the area of Kyushu. It is transmitted by *Simulium* spp. The endosymbiont *Wolbachia* has been studied in these species resulting in a new scenario regarding the worm/bacteria relations.

Charles Darwin 200 and symbiosis

J G van As
Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa. E-mail: vanasjg.sci@ufs.ac.za

The year 2009 is the 200th anniversary of the birth of Charles Darwin and 150 years since the publication of his famous book *On the origin of species.* This book brought about the most monumental paradigm shift in scientific thinking of all times and unleashed a controversy which still today reverberates in pious communities.

In this paper I will address 4 questions: is Charles Darwin's work after 150 years still relevant; has modern science vindicated his ideas; what did insight into the concept of symbiosis or parasitology contribute to the understanding of evolution; and what would Charles Darwin change in his book if he had the benefit of hindsight into modern science?

Although most people believe Darwin to be the father of evolution, the concept, then known as transmutation, existed even before he left on his epic journey on the HMS Beagle. His contribution was that he explained it by recognising the role of natural selection in the process of speciation. He also conceived the concept of the tree of life and the interrelatedness of all living creatures. This was a truly remarkable conclusion almost 100 years before the structure of DNA was explained.

At the time when Charles Darwin published *On the origin of species* the science of Genetics was still in its infancy and there was no way that Darwin or anyone understood the mechanisms of inheritance. Despite this and some minor misinterpretations, Darwin got it right. His work has now been vindicated beyond any doubt by modern science.

In total Darwin published 15 books and a number of scientific papers on a broad spectrum of fields within natural science. He did, however, not cover any topics within the broad spectrum of symbioses in any detail. In *On the origin of species* he used the word parasites in only 2 places, albeit in explaining 2 very pivotal aspects of his theses, *i.e.* the struggle for existence in the early part of the book and later in explaining instinctive behaviour.

If Darwin had the benefit of hindsight into what we know today, he would not need to make major changes but he would probably redraw his tree of life to include branch swapping on a horizontal level. This would be to accommodate the different processes where symbiosis has resulted in the origin of eukaryotic organisms and later complex sexually replicating organisms.

Oral Presentations

Hypotheses on mobiline peritrich transmission

L Basson[1] and J G van As[1]
[1]*Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa. E-mail: bassonl.sci@ufs.ac.za*

Mobiline peritrichs are among the most frequently encountered symbiotic ciliophorans, either as ecto- or endosymbionts from a wide variety of hosts. These hosts are found in marine, euryhaline, freshwater and terrestrial environments. Despite this widespread almost universal occurrence, very few answers exist to basic questions, such as how most of these symbionts spread from 1 host to another. Mobiline peritrichs are placed in 3 families, the Urceolariidae, Trichodinopsidae and Trichodinidae. Representatives of the former 2 families have simple plate-like denticles and are found associated with invertebrate hosts, while members of the largest family, the Trichodinidae, are found associated with both vertebrates and invertebrates. Ectosymbiotic species are encountered on the body surface of hydras, turbellarians, crustaceans and certain echinoderms such as brittle starfish and urchins; the gills of polychaetes; the gills and mantle cavity of aquatic and terrestrial molluscs, as well as the skin and gills of fish and amphibian tadpoles. In these instances, transmission seems to be a simple, direct process, whether aquatic or terrestrial hosts are involved. In the case of endosymbionts, transmission seems to be far more complicated. Endosymbiontic mobilines utilise specific positions, such as the gut of echiurid worms, the digestive tract of sea cucumbers, molluscs and fish, the reproductive organs of molluscs and fish, or the urinary tract and bladder of amphibians and fish. In some instances transmission theories exist and seem feasible and logical, whilst in other cases mere conjecture is the order of the day.

The detection of *Babesia* spp. in domestic felids (*Felis domesticus*) using DNA probes and phylogenetic analysis

A Bosman[1], M C Oosthuizen[1], E H Venter[1] and B L Penzhorn[1]
[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Private Bag X04, Onderstepoort, 0110 South Africa*
E-mail: bossie.bosman@up.ac.za

Babesia is an intracellular erythrocytic haemoprotozoan of

mammals and it has also been reported in reptiles and birds. The 2 most frequently reported *Babesia* species in felids are *B. felis*, which causes clinical babesiosis in domestic cats, and *B. leo*, primarily reported from asymptomatic lions. In this study, DNA was extracted from blood collected from 480 domestic cats (*Felis domesticus)* and the hypervariable region of the 18S rRNA gene was amplified. The PCR products were analysed using the reverse line blot (RLB) hybridization assay, a technique that simultaneously detects and differentiates between *Babesia* and *Theileria* spp. RLB probes to detect *B. felis, B. leo* and *Babesia* sp. (cheetah) were designed, using the 18S rRNA gene sequence data, and used to screen samples collected from domestic cats. Results showed that *B. felis, B. leo* and *Babesia* sp. (cheetah) occur in domestic cats either as single or as mixed infections. However, some samples tested positive only with the genus-specific *Babesia/Theileria* probe. This suggested the presence of a novel species or variant of a species. The full-length 18S rRNA gene of these unknown samples was subsequently amplified, cloned and sequenced. Sequence and phylogenetic analysis confirmed that a novel *Babesia* spp. was present.

Molecular characterization of *Theileria* species of the African buffalo (*Syncerus caffer*) by 18S rRNA gene sequence analysis

M E Chaisi[1,2], N E Collins[1] and M C Oosthuizen[1]
[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa*
E-mail: s27130712@tuks.co.za
[2]*Department of Biology, Faculty of Science and Technology, National University of Lesotho, Roma 180, Lesotho*

The African buffalo (*Syncerus caffer*) is the natural reservoir host of both pathogenic and non-pathogenic *Theileria* species. Corridor disease, caused by *Theileria parva,* is a controlled disease in South Africa. *Theileria* parasites usually occur as mixed infections in infected animals, and although the non-pathogenic forms do not have any significant economic importance, their presence interferes with the diagnosis of *T. parva.* In this study, the phylogenetic relationship of pathogenic and non-pathogenic *Theileria* species obtained from buffalo blood samples originating from different geographical regions in South Africa were investigated using 18S rRNA gene sequences analysis. DNA was extracted, the V4 hypervariable region of the 18S rRNA gene was amplified and subjected to the reverse line blot (RLB) hybridization assay using *Babesia* and *Theileria* genus- and species-specific probes. Results of the RLB revealed the presence of the pathogenic *T. parva*, benign *T. mutans*, and the non-pathogenic *T. velifera, T. buffeli* and *Theileria* sp. (buffalo). In some samples, the PCR products hybridized only with the genus-specific probes, and not with any of the species-specific probes, suggesting the presence of novel species or genotypes. The full length 18S rRNA gene of selected samples was amplified, cloned and the recombinants sequenced. Sequence and phylogenetic analyses indicated that novel *T. mutans, T. velifera* and *Theileria* sp. (buffalo) genotypes occur in buffalo. This could have serious implications, since such sequence variants could compromise the specificity of the real-time PCR test currently used to detect *T. parva* infections in buffalo and cattle in South Africa.

Fungi for controlling malaria-transmitting mosquitoes

M Coetzee[1]
[1]*NRF Research Chair in Medical Entomology and Vector Control, School of Pathology of the University of the Witwatersrand and the National Health Laboratory Service, Johannesburg, South Africa*
E-mail: maureen.coetzee@wits.ac.za / maureenc@nicd.ac.za

Insecticide resistance in malaria vector mosquitoes is becoming a huge problem in many African countries and is compromising efforts to control this disease that kills almost a million people annually. The search for new chemicals and novel methods has resulted in some groups focusing on the use of entomopathogenic fungi for killing mosquitoes. We have conducted research at the NICD showing that fungi will kill our local vectors, will play a role in reversing conventional insecticide resistance and could possibly be delivered in African clay pots.

Fish eye flukes: not large but possibly in charge?

A Erasmus[1], J G van As[1] and H J B Butler[1]
[1]*Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa. E-mail: vanasjg.sci@ufs.ac.za*

Behavioural changes have been reported from a variety of parasitised organisms and range from decreases or increases in activity, to altered responses to environmental stimuli. Certain parasite-induced behavioural changes can be regarded as simply the symptoms of the pathology associated with infection, but it is possible that some of these changes are more likely to be evolutionary adaptations to increase the parasites' transmission success. Digenetic trematodes, belonging to the family Diplostomidae Poirer, 1886, are endoparasites which need to be transmitted from an intermediate fish host to a final bird host during the completion of the life cycle. Previous studies reported on the formation of cataracts in the lenses of primarily experimentally infected and aquaculture fish. This could result in predisposing these individuals to avian predation by impairing their escape response. During 2008 and 2009 fishes of different families were collected from the Okavango and Orange River Systems and the prevalence of eye flukes was determined by dissection of the eyes. In order to determine the effect of eye fluke infection on the anti-predator behaviour of the fish, specimens were individually held in a small aquarium. An artificial aerial predator was flown overhead and the escape response of the host was noted. Preliminary results indicate that infection with eye flukes, in natural freshwater systems, is too low in number to have a conspicuous altering effect on the intermediate hosts' behaviour. Therefore it is hypothesised that eye flukes might only be notably in charge of their fish hosts' behaviour in artificial mass gatherings, such as aquaculture.

Aspects of copulation in *Argulus japonicus*

L Everts[1] and A Avenant-Oldewage[1]
[1]*Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, Johannesburg, 2006 South Africa*
E-mail: evertslalicia@gmail.com / aoldewage@uj.ac.za

The mechanical process involved in sperm transfer during copulation in *Argulus japonicus* (Thiele, 1900) was first studied in 1806 by Jurine. Since then a debate has arisen; one argument is that the accessory structures of the male, borne on the swimming legs, transfer sperm during copulation. A 2nd argument is that these structures serve only to grip the female's swimming legs and thus hold her in place, while sperm is transferred directly from aperture to aperture. This idea was later devoped further when it was said that the female's spermathecal spines puncture the male's genital aperture membrane to facilitate sperm transfer. It was never observed. This study has been dedicated to studying specimens *in copula.* Pairs seen *in copula* were collected with the use of a cryo freeze aerosol. Some of the pairs were embedded in resin and studied histologically with AZAN, while the rest were freeze-dried for scanning electronmicroscopy studies. The hypothesis for the study was that the spermathecal spines of the female play an active role in sperm transfer. This study recorded that in all females sectioned from copulating pairs both spermathecae appear to be filled with sperm. Furthermore, as sperm has been found on the accessory copulatory structures of the male, the legs of the male may in fact play an active role in sperm transfer.

Identification of taxonomically important characteristics in fish parasitic gnathiid isopod juveniles

C Farquharson[1], N J Smit[1] and A J Davies[1,2]
[1]*Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park 2006 South Africa. E-mail: aquarium@uj.ac.za / nicos@uj.ac.za*
[2]*School of Life Sciences, Faculty of Science, Kingston University, Kingston upon Thames, KT1 2EE, United Kingdom*

Gnathiids are marine isopods with a worldwide distribution, and the majority of species are described from temperate and tropical waters. These organisms are unique among isopods in being protelian parasites with only 5 pairs of walking legs. The parasitic juvenile stages feed on a wide variety of fish hosts, including elasmobranchs and teleosts. Three juvenile stages exist with 2 forms

at each stage, the unfed zupheae (Z1, Z2 and Z3) and fed pranizae (P1, P2 and P3). Mechanical effects resulting from gnathiid blood feeding are reported in aquaria, in fish farming and in the wild. Aquarium and caged fishes are particularly susceptible to attack owing to the confines of their surroundings; deaths among fishes exposed to large numbers of gnathiid juveniles under such conditions have been reported. Since gnathiid taxonomy is based on the morphology of the male, it is unfortunate that the economically important gnathiid juveniles cannot be identified to species level. Furthermore, the majority of gnathiid descriptions unfortunately lack information about juvenile and female morphology, since available museum material, often collected during benthic surveys or dredge sampling, may contain mixed populations of gnathiids. The aim of this paper is thus to compare all described juvenile gnathiids to determine any distinctive morphological characteristics that could be used to identify them to species level when they are found in the absence of adult males. For the purpose of this paper, 20 species from 5 different genera, 6 localities, and both teleost and elasmobranch hosts are compared. Results indicate that the most important discriminating taxonomic characteristics of the juveniles are mouthpart morphology and pleotelson morphometrics. Results from this work will help parasitologists to identify gnathiid juveniles correctly when collected from fish hosts, or in the absence of fishes.

Mobiline peritrich transmission between fish and tadpoles

H Groenewald[1], L Basson[1] and J G van As[1]
[1]*Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa*
E-mail: bassonl.sci@ufs.ac.za / groenewaldh.sci@ufs.ac.za

Trichodinids are mobiline unicellular organisms that are found worldwide on both freshwater and marine fish. Some of these fish trichodinids such as *T. domerguei* f. *latispina* Dogiel, 1940, *T. nobillis* Chen, 1963, *T. reticulata* Hirschmann & Partsch, 1955, *T. heterodentata* Duncan, 1977 and *T. nigra* Lom, 1961 have also been reported from the skin and gills of different tadpole species, mostly from European countries. By contrast, only a single trichodinid has been found from tadpoles in southern Africa, *i.e. T. heterodentata.* Transmission experiments by various workers have shown that the European fish trichodinids utilise tadpoles mostly as facultative hosts during the spring and early summer months. The present study suggested 2 possibilities for the occurrence of *T. heterodentata* on both fish and amphibian hosts, *i.e.* that this species also uses tadpoles as facultative hosts during warmer seasons or that it is in fact a different species occurring on southern African tadpoles. Experiments were undertaken to determine which of the above hypotheses is correct. Cross-transmission experiments were conducted in the Okavango Delta using *Barbus barnardi* as the fish host and *Bufo powerii* as the tadpole host. This study established that *T. heterodentata* can in fact be transferred from infested fish to uninfested tadpoles, and back to uninfested fish. It seems as if this is indeed a fish trichodinid, using tadpoles as facultative hosts. However, this experiment was not conducted with the preferred host of *T. heterodentata*, as infested cichlid species could not be found at that stage. Therefore, this experiment will be repeated in September/October 2009 with *Pseudocrenilabrus philander* and *Xenopus laevis* tadpoles. The 2nd part of the study involves examining the skin and gill smears that have been collected over the last 28 years from various cichlid and tadpole species in southern Africa. These were examined using a compound microscope. The standard methods for describing trichodinid species of Lom (1958) and Van As and Basson (1989) were used to statistically compare the various trichodinid populations. Statistical analysis of these data is still under review.

Buffalo babesiosis caused by *Babesia orientalis* in China

L He[1,2], J Zhao[1,2], Z Liu[2], B Yao[2] and Y Zhou[1]
[1]*State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei Wuhan 430070, China. E-mail: helan0710@yahoo.com.cn*
[2]*College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China 430070*

Buffalo babesiosis was 1st reported in 1984 in China in water buffalo (*Bubalus babalis*) . It had been reported in 11 provinces in south China with a high prevalence in endemic areas. The pathogen of buffalo babesiosis is different from *Babesia* parasites in cattle in morphology, vector, host and other biological characters, so it was named *Babesia orientalis* in 1997. *Rhipicephalus haemaphysaloides* is the only vector of *B. orientalis.* The vector transmits *B. orientalis* by transovarian transmission and the infective stage is the adult ticks. *B. orientalis* occurs at the end of March, reaches its peak in April/May, subsides gradually in June and is not seen after July. Microscopic detection is still a common method for diagnosing acute babesiosis; however, semi-nested PCR and loop-mediated isothermal amplification (LAMP) had been established recently; these methods are more sensitive and specific than the previous indirect fluorescent antibody test (IFAT) and the latex agglutination test (LAT). Chemical treatment is still the dominant method for controlling this disease. Exoantigens of *B. orientalis* had been used as a vaccine to prevent this disease; the results are encouraging, but more work is needed before it will become available as commercial vaccine. A cDNA expression library of *B. orientalis* merozoties was constructed. An expression sequence tag (EST) library was established from the cDNA library, 5 potential metabolic pathways of *B. orientalis* were obtained by analysis of the EST library. Fifteen immuno-related genes were cloned by immunoscreening with anti-*B. orientalis* buffalo serum. Further studies of BoHsp70 and BoP29 indicated that they could be candidate vaccine antigens.

Litomosa chiropterorum (Nematoda: Filarioidea) from *Miniopterus natalensis* in South Africa: *Wolbachia* screening and phylogenetic relationships with *Litomosoides*

K Junker[1], M Barbuto[2], M Casiraghi[2], C Martin[3], S Uni[4], J Boomker[1] and O Bain[5]
[1]*Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.* Present address: *Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa*
E-mail: junkerk@arc.agric.za
[2]*Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano Bicocca, P.zza della Scienza 2, 20126, Milano, Italy*
[3]*Muséum National d'Histoire Naturelle, Parasitologie comparée et Modèles expérimentaux, USM 307, CP52, 61 rue Buffon, 75231, Paris, Cedex 05, France*
[4]*Department of Medical Zoology, Osaka City University Medical School, Osaka 545-8585, Japan*
[5]*Muséum National d'Histoire Naturelle, Origine, Structure et Evolution de la Biodiversité, UMR 7205, CP52, 61 rue Buffon, 75231, Paris, Cedex 05, France*

Sixty-nine *Miniopterus natalensis* (Chiroptera), type host of *Litomosa chiropterorum*, were collected in caves in the Western Cape and Gauteng Provinces in 2006 and 2007 respectively. Filariae were present at both localities in about 50 % of the hosts. *Litomosa chiropterorum* was redescribed and the presence of the bacterial endosymbiont *Wolbachia* was investigated using immunohistological staining as well as PCR screening. The molecular characterisation of *L. chiropterorum*, the 1st done with *Litomosa* from bats, was based on the *cox1* and 12S rDNA gene sequences. These were then used to construct the phylogeny of several representatives of the *Litomosa* and *Litomosoides* group for which molecular data are available. Both these genera are well represented in Microchiroptera, but, whereas *Litomosoides* is largely diversified in Neotropical rodents and marsupials as well, only 2 species of *Litomosa* are known from rodents. In the phylogenetic tree the position of *L. chiropterorum* is at the base of the *Litomosa* and *Litomosoides* group, with *Litomosoides brasiliensis* positioned between it and its congener, *Litomosa westi*, from North American rodents. Earlier studies had pointed out morphological peculiarities in *Li. brasiliensis* that confused its systematic position. Unfortunately, the newly generated molecular phylogeny was equally unable to solve this species' positioning. Clearly, more data on additional species, but also on their intraspecific molecular diversity are needed to elucidate the relationships between these 2 filarioid genera. Contrary to *L. westi*, the only other *Litomosa* studied for the presence of *Wolbachia* and which proved positive, *Wolbachia* could not be detected in *L. chiropterorum.* The current picture of *Wolbachia* distribution is complicated, with the endosymbiont being present and absent in closely related species of both genera.

Monogeneans on the gills of marine fish in the Tsitsikamma National Park, South Africa

P H King[1], N J Smit [2] and C Baker[3]
[1]*Department of Biology, PO Box 139, University of Limpopo: Medunsa Campus, 0204 South Africa. E-mail: pking@ul.ac.za*
[2]*Department of Zoology, PO Box 524, University of Johannesburg, Auckland Park, 2006 South Africa*
[3]*Electron Microscope Unit, PO Box 84, University of Limpopo: Medunsa Campus, 0204 South Africa*

Monogeneans are hermaphroditic ectoparasitic flatworms found on the skin, fins and gills of freshwater and marine fish. They attach with a unique attachment structure called the opisthaptor, which consists of hooks, clamps and/or suckers. The aim of this study was to study the monogenean parasites on the gills of selected marine fish species in the Tsitsikamma National Park, Western Cape Province, South Africa. Fish were collected in rock pools and lagoons in the park using hand nets and line baited with red-bait. After they were anaesthetised with clove oil, the gills were removed and examined for the presence of monogeneans. They were fixed in 70 % EtOH and stained for light microscopy using ammonium picrate. For scanning electron microscopy monogeneans were fixed in glutaraldehyde and studied in a Leica Stereoscan SEM at the Electron Microscope Unit of the Medunsa Campus at 8–12 kV. *Diplodus capensis* hosts were found to be infested with 3 different monogenean species. On the other hand, *Sparodon durbanensis, Amblyrhynchotes honckenii, Liza richardsonii, Diplodus hottentotus* and *Rhabdosargus holubi* hosts were infested with only 1 monogenean species. Although the infestation rates were relatively low, these parasites showed a high degree of host specificity. Future studies are essential in order to verify the diverse spectrum of monogeneans infesting the gills of many marine fish species in this area.

Aspects of the pathology of *Cichlidogyrus philander* (Monogenea) on the gills of *Pseudocrenilabrus philander philander* collected from Padda Dam

L E le Roux and A Avenant-Oldewage
Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: aoldewage@uj.ac.za / lerouxlouise@yahoo.com

Representatives of the genus *Cichlidogyrus* are parasites on the gills of mostly Cichlidae fishes. During the present study *Cichlidogyrus philander* was collected from the gills of *Pseudocrenilabrus philander philander* in the Padda Dam, Gauteng. In general the monogeneans cause damage to fish hosts thereby impacting negatively on profits in aquaculture. In this study some of the pathological effects induced by *C. philander* were studied by fixing several sets of gill arches in 10 % neutral phosphate-buffered formalin, while some samples were placed in Bouin's fixative. Thereafter the gill arches were embedded in resin, and a few samples in wax, and sectioned at 2 to 5 micrometres. Serial sections were subsequently stained with Azan or HE and PAS and studied with the aid of light microscopy. Additional specimens attached to the gill filaments were prepared for scanning electron microscopy, studied and photographed. The condition factor of host fish was determined based on weight and length. Observation of infected fish revealed that even when the intensity of infection was high the gills were not pale, no apparent macro lesions occurred and the fish appeared healthy overall. Behaviour patterns were also not affected. The gills sections revealed that *C. philander* attaches between the secondary lamellae, utilising the 2 pairs of large gripi, which penetrate into the primary epithelial tissue. The haptor bears 7 pairs of ucinuli, which further enhance attachment. Furthermore, the presence of *C. philander* induces hyperplasia of the primary epithelium interspersed between the secondary lamellae. During the SEM study observations of areas of attachment showed evidence of epithelial penetration and lifting. The condition factor fails to indicate a correlation between prevalence, mean intensity or abundance of *C. philander* and the condition of the host. However, a positive correlation between the total number of worms and the condition factor was found, indicating that under natural conditions more parasites would be found on fish with higher condition factors. This is the 1st study conducted on the pathological effects of *C. philander* on *P. p. philander.*

Validation of the molecular technique developed for diminazene-resistance testing using *Trypanosoma congolense* isolates circulating in KwaZulu-Natal

J Masumu[1], R Nkuna[1], I Vorster[1], B L Penzhorn[1], F du Plessis[1], V Delespaux[2], A Latif[3], M Makhosazana[3] and P Van den Bossche[1,2]
[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa E-mail: rhulani.nkuna@up.ac.za*
[2]*Institute of Tropical Medicine, Animal Health Department, Nationalestraat 155, B-2000 Antwerp, Belgium*
[3]*ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa*

Molecular techniques for assessing drug resistance in trypanosomes infecting livestock and humans in Africa have not been validated in the field. Two tools have been developed to detect *Trypanosoma congolense* isolates resistant to diminazene aceturate (DA) and isometamidium chloride (ISM). The aim of this study was to validate the molecular techniques developed for DA-resistance testing using South African isolates. Eleven *T. congolense* Savannah isolates from Hluhluwe in KwaZulu-Natal (KZN) was used: 7 originated from cattle and 4 from African buffalo (*Syncerus caffer*). For each isolate, 2 groups of 6 mice were inoculated intraperitoneally with 10^5 trypanosomes. One group was treated 24 hours later with 20 mg/kg of DA; the other group served as control. Mice were monitored for 2 months for the development of parasitaemia. Parasitaemic blood was collected from the control groups for molecular tests using the polymerase chain reaction primers Ade2F and AdeR and restriction fragment length polymorphism using enzyme *Mbo*II for the detection of drug resistance for DA. Results indicated that all 11 isolates were susceptible to DA. Using the molecular tool, however, all the cattle isolates ($n = 7$) but only 2/4 buffalo isolates displayed a resistant profile. The remaining 2 buffalo isolates had a mixed profile. To ascertain whether the resistance profiles obtained could be related to low doses of DA, multi-dose testing using the mouse model was performed using 3 of the isolates (TC/KZN Cattle 15, TC/KZN Buffalo 2 and TC/KZN Buffalo 3) that had a resistant profile. After 2 months, more than 2 relapses (indicating resistance profile of the isolate used) had been observed only in the 3 groups of mice treated with 1 mg/kg. Relapses at this dosage do not indicate development of DA-resistance. At dosages of 10 and 5 mg/kg, however, all 3 isolates were susceptible to DA. These results indicate that the resistance profile obtained using molecular tools is not associated with development of drug resistance in *T. congolense* circulating in South Africa. Consequently, this tool cannot be used for the characterisation of DA-resistance in *T. congolense* in this area.

Monogenea of the genus *Dactylogyrus* from cyprinids of the genera *Barbus, Labeobarbus* and *Labeo* in Lake Tzaneen, South Africa

M M Matla[1], N M Mokgalong[1] and S N Mashego[2]
[1]*Department of Biodiversity, University of Limpopo, Prinate Bag X1106, Sovenga, 0727 South Africa. E-mail: matlam@ul.ac.za*
[2]*University of North-West, Private Bag X2046, Mmabatho, 2735 South Africa*

Monogenetic parasites are host specialists that naturally occur in intensities that are not very harmful to their fish hosts, but they usually cause epizootics under culture conditions. Their identification is vital for any subsequent ecological studies, their prevention or treatment so as to avoid the fatal effects on hosts when cultured. The hosts ($n = 88$) were collected using gill nets and killed by cutting through the spinal cord. The gills were removed from fish and examined for the worms under a stereo-microscope with both incident and transmitted light sources. The collected specimens were stored in 70 % ethanol, later mounted using glycerine jelly. Identifications were based on morphological analyses with the help of drawings, micrographs and dimensions. Nine *Dactylogyrus* spp. were collected from the various cyprinids sampled. *Dactylogyrus afrolongicornis afrolongicornis, D. allolongionchus* and *Dactylogyrus* sp. 1 co-occurred on some specimens of *Barbus trimaculatus. Barbus radiatus* shared the same *Dactylogyrus* sp. with *B. trimaculatus. Barbus unitaeniatus* hosted *Dactylogyrus* sp. 2. *Labeobarbus marequensis* had only 1 species,

D. spinicirrus. Dactylogyrus brevicirrus and *D. cyclocirrus* specimens co-occurred with *Dogielius dublicornis* on some *Labeo cylindricus* hosts. The 2 other *Dactylogyrus* spp. (species 2 & 3) co-occurred with *Dogielius* sp. on *Labeo molybdinus*. *Dactylogyrus brevicirrus* and *D. cyclocirrus* are the1st records for South Africa. All 4 *Dactylogyrus* spp. are the 1st host records and 1st records for South Africa (Africa!) and may be described as new species. These data contribute to knowledge of the ichthyo-parasitic fauna of Lake Tzaneen.

Corridor disease (*Theileria parva* infection in cattle): Report of outbreaks in the Vryheid district, KwaZulu-Natal, from 2005–2009

S Mbizeni[1], F Potgieter[2] and A A Latif[2]
[1]*State Veterinarian, Pietermaritzburg, KwaZulu-Natal, South Africa*
[2]*Parasites, Vectors and Vector Borne Diseases Program, Onderstepoort Veterinary Institute ARC-OVI, Private Bag X05, Onderstepoort, 0110 South Africa*
E-mail: latifa@arc.agric.za

In South Africa, Corridor disease (*Theileria parva* infection of buffalo origin) within the endemic disease areas in KwaZulu-Natal, has been reported to be on the increase. From 2002–2004, 13 outbreaks were reported in the veterinary districts of Vryheid and Hluwhluwe. Subsequently, the occurrence of Corridor disease outbreaks in the Vryheid district was closely monitored by State Veterinarians and the Onderstepoort Veterinary Institute from 2005–2009. Recorded information included the number of cattle involved, clinically reacting animals coupled with parasitological and *post mortem* examinations. Blood samples were also collected from sick and recovered cattle to screen for the presence of *T. parva* antibodies, as well as for parasite DNA, using the indirect fluorescent antibody test (IFAT) and the real-time PCR, respectively. A total of 27 outbreaks in 8 localities (communal cattle on small-holdings and commercial farms,) were reported from 2005–2009. On 1 farm, the outbreaks occurred every year from 2005–2009 and on another farm from 2007–2009. The most severe outbreak occurred in Nyalisa in 1 herd in which 202 cattle were involved and 57 mortalities were recorded within 30–40 days of the onset of the disease. The diagnosis of Corridor disease was confirmed in the laboratory. During the investigation period, a total of 846 cattle were tested for corridor disease and the prevalence of the infection was found to be 27 %. Infected cattle found to be positive by PCR was 16.5 %, whereas only 10 % tested serologically positive for *T. parva* antibodies. This report supports earlier observations that Corridor disease should be considered an 'emerging disease' if these PCR-test-positive 'carrier' cattle are infective to ticks. More stringent veterinary control is indicated.

Potential human infections with lesser known trematodes in the Tshwane district

E B E Moema[1], P H King[1] and C Baker[2]
[1]*Department of Biology, PO Box 139, University of Limpopo: Medunsa Campus, 0204 South Africa. E-mail: emoema@ul.ac.za*
[2]*Electron Microscope Unit, PO Box 84, University of Limpopo: Medunsa Campus, 0204 South Africa*

It is well-documented that digenetic trematodes exhibit complex life cycles where the definitive hosts are vertebrates – mostly birds, but also mammals and reptiles. It is also known that digenetic trematodes are host specific, but changes in our feeding habits, agricultural and social practices have led to humans being infected with parasites that were previously regarded to be of no or little medical importance. The objective of this study was to identify the lesser known digenetic parasites in the water bodies around Tshwane that may result in possible human infections. Various parasitic stages of different trematode parasites were collected from water bodies in the proximity of Tshwane. Cercariae were harvested from freshwater snail species, metacercariae from various invertebrate and vertebrate hosts and adult parasites from different vertebrate hosts, including experimental infections. The parasites were then identified employing standard light- and electron microscopy techniques. The following parasites were collected and identified: clinostomatid cercaria and metacercariae; echinostomatid cercariae, metacercariae and adults; an avian schistosome cercaria as well as metacercariae and adults of *Fasciola gigantica.* Some of these parasites had similar morphological characterizations to parasites that were found to infect human beings in other parts of the world. The occurrence of several lesser known trematodes in localities adjacent to Tshwane may pose serious health threats to communities living around these natural water bodies. It is therefore imperative to educate these communities as to the potential dangers and to implement necessary control measures.

Siamese monogeneans of southern Africa

J H Möller[1], J G van As[1] and L L van As[1]
[1]*Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa. E-mail: vanasll.sci@ufs.ac.za*

Members of the family Diplozoidae Yamaguti, 1963 are ectoparasites on the gills of freshwater fish. These monogeneans seem to be host specific to members of the family Cyprinidae. Diplozoidae are the only members of the Monogenea where hermaphroditic adults fuse and live in permanent copula. In comparison to Diplozoidae from Europe and Asia, not much is known of the African fauna and even less of southern Africa. Thus far 2 species of *Diplozoon* von Nordman, 1832, are known from Northern Africa, *i.e. D. aegyptensis* Fischtal & Kuntz, 1963 collected from *Labeo* spp. and *D. ghanense* Thomas, 1957 described from *Alestes* spp. *Afrodiplozoon polycotyleus* Paperna, 1973, previously of the genus *Neodiplozoon,* Tripathi 1959, was described from *Labeo victorianus* in Kenya and from *Barbus* spp. in Uganda and South Africa. During fish parasitological surveys over the last 15 years various monogenean species were collected from the Okavango, Orange–Vaal and Zambesi River Systems. Amongst this material were unidentified Diplozoidae that had been collected from 9 cyprinid species *i.e. Barbus afrovernayi, B. multilineatus, B. paludinosus, B. poechii, B. radiatus, Labeo capensis, L. lunatus, L. umbratus* and *Labeobarbus aeneus.* The collected fish were anesthetised using MS222, after which the gills were dissected and examined for the presence of the monogeneans. In order to compile a taxonomic study on the diplozoids found, the material collected was fixed in 70 % ethanol or AFA and stored in 70 % ethanol. Other specimens were fixed in 4 % BNF, 10 % BNF or post-fixed in osmium for further microscopical analysis. The aim of this study is to shed more light on the biology and ecology of these unique Siamese worms found on the gills of freshwater fish species.

Susceptibility of Nguni cattle to experimental *Trypanosoma congolense* infection in South Africa

M Y Motloang[1], J Masumu[2], P Van Den Bossche[2,3] and A A Latif[1]
[1]*ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: motloangm@arc.agric.za*
[2]*Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, 0110 South Africa*
[3]*Institute of Tropical Medicine, Veterinary Department, Nationalestraat 155, 2000 Antwerpen, Belgium*

Nguni cattle are indigenous to eastern and northern South Africa and are known for their ability to perform well under harsh conditions and their resistance to tick infestations. For this reason there are now plans to introduce these cattle into communal farms in South Africa to ensure sustainable agricultural development. A total of 30 Nguni cattle, aged 2 years, were used. They were divided into 3 groups: 2 treatment groups of 12 each and a 3rd group of 6 that served as the negative control. The challenge material comprised of *Trypanosoma congolense* belonging to the Savannah subgroup. Within this subgroup and prior to cattle infection, 2 strains of high virulence (HVS) and low virulence (LVS), previously characterised in mice and cryopreserved as stabilates, were used to infect the 2 groups. The parasites were reactivated in 2 Balb/c mice before use and the challenge dose (10^5) was inoculated intravenously into cattle. The parasitaemia, temperature and PCV of all cattle were monitored daily for the 1st 2 weeks and 3 times a week for the remainder of the observation period (70 days). All of the animals in group 1 (HVS) became parasitaemic on day 6 compared with only 33 % (4/12) in group 2 (LVS). The temperature of all cattle in the 2 groups remained normal during the observation period. Eight per cent of animals in the HVS became anaemic (PCV of 24 % or less) from day 13 while

the same percentage of animals in the LVS group had anaemia by day 20 post-infection (PI). By day 32 PI, 100 % of the animals infected with HVS became anaemic compared to 50 % of animals that received the LVS. Forty-two per cent (5/12) of the animals in the HVS group required treatment (PCV less than 18 % for 3 consecutive days) while no animals in the LVS group were treated. This study using a *T congolense* virulent strain demonstrated the susceptibility of Nguni cattle to infection, although fewer than 50 % required treatment during the observation period.

Susceptibility of selected arthropods to infection with *Spirocerca lupi* and *Gongylonema ingluvicola*

S Mukaratirwa[1], E Pillay[1] and K Munsammy[1]
[1]*School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, Durban. E-mail: mukaratirwa@ukzn.ac.za*

Gongylonema ingluvicola and *Spirocerca lupi* require arthropod intermediate hosts in order to complete their life cycle. Beetles of the family Scarabaeidae are reported to serve as intermediate hosts for both these parasites. In this study selected species of beetles of the family Scarabaeidae as well as other groups of arthropods were screened for susceptibility to infection with *S. lupi* and *G. ingluvicola.* The arthropods were exposed to infective eggs of both parasites for a determined period and dissected/digested to determine the presence or absence of pre-infective and infective larvae. All 5 species of dung beetles exposed to infection with *S. lupi*, *Pachylomerus femoralis, Scarabaeus rugosus, Gymnopleurus humanus, Kheper nigroaeneus* and *Anachalcos convexus* were susceptible, and of the 2 exposed to *G. ingluvicola,* only *Gymnopleurus humanus* was susceptible. *S. lupi* eggs developed in the millipede species *Daratoagonus cristulatus*, and remained as encysted larvae, while in *Orthoporoides kyrhocephalus,* no development was observed. *S. lupi* larvae were not detected in the cricket species, *Gryllus assimilis,* and cockroach species *Periplaneta americana,* and similarly, *G. ingluvicola* larvae were not detected in the millipede species *Orthoporoides kyrhocephalus.* The difference in the susceptibility of these arthropods as intermediate hosts of the 2 parasite species may depend on the feeding habits and structure of their mandibles.

Characterization of resistance mechanisms in the major malaria vector *Anopheles arabiensis* from southern Africa

G Munhenga[1,2], R H Hunt[1,2], K Hargreaves[3], B D Brooke[1,4], M Coetzee[1,4] and L L Koekemoer[1,4]
[1]*Vector Control Reference Unit, National Institute for Communicable Diseases, NHLS, Private Bag X4, Sandringham, Johannesburg, 2131 South Africa*
E-mail: givemorem@nicd.ac.za
[2]*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[3]*Malaria Control Programme, KwaZulu-Natal Department of Health, Jozini, South Africa*
[4]*Division of Virology and Communicable Diseases Surveillance, School of Pathology of the National Health Laboratory Service and the University of the Witwatersrand, Johannesburg, South Africa.*

In southern Africa malaria control programmes depend heavily on insecticides for vector control. Unfortunately, the continual increase in resistance to all the major classes of insecticides is threatening the efficacy and sustainability of insecticides for malaria control due to lack of resistance management programmes. We investigated the susceptibility status and underlying resistance mechanisms in *Anopheles arabiensis* from a malaria-endemic area in Zimbabwe. In addition, this study reports changes in insecticide resistance levels, detoxification enzymes and over-expressed P450 genes following artificial selection of laboratory colonies of *An. arabiensis.* Pyrethroid resistance in *An. arabiensis* from southern Africa is metabolic based with monooxygenases and esterases playing a central role. Two P450 genes *CYP6M2* and *CYP4G16* constitute an important general defense against permethrin resistance. The detection of permethrin and DDT resistances in *An. arabiensis* populations from Gwave has serious implications for malaria vector control in this area, particularly since Zimbabwe has reverted to DDT for indoor house spraying. Use of mosaic insecticide application or rotational use of insecticides is recommended.

Detoxification enzymes associated with DDT resistance in populations of *Anopheles arabiensis* of different geographical origin

L Nardini[1,2], M Coetzee[1,2,3] and L L Koekemoer[1,2]
[1]*Vector Control Reference Unit, National Institute for Communicable Diseases, NHLS, Private Bag X4, Sandringham, Johannesburg, South Africa*
E-mail: luisan@nicd.ac.za
[2]*Department of Virology and Communicable Disease Surveillance, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, Private Bag 3, WITS 2050, South Africa*
[3]*NRF Research Chair in Medical Entomology and Vector Control, School of Pathology of the University of the Witwatersrand and the National Health Laboratory Service, Johannesburg, South Africa.*

Anopheles arabiensis is 1 of the 3 major malaria vectors in Africa. Currently, *An. arabiensis* is controlled in South Africa using indoor residual spraying with insecticides. Insecticide resistance in this species has been reported in a number of countries and, given that insecticide use has been the most successful way of controlling mosquitoes, the development of resistance in target populations has a significant impact on vector control, and ultimately on the prevalence of malaria. The overall aims and objectives of this study were to investigate what detoxification enzymes are involved in *An. arabiensis* resistance to DDT using the *An. gambiae* detoxification chip. In addition, we investigated the different detoxification profiles between 2 DDT-resistant strains, 1 from South Africa and 1 from Sudan. Microarray data suggest that numerous genes are up-regulated in the South African strain, while in the Sudanese population, only 1 gene, *CYPPL1*, was found to be up-regulated. These data suggest that insecticide-resistance data should not be extrapolated for use on different vector populations. Furthermore, we hope that such information might allow scientists to better monitor resistance trends in different populations and to predict resistance development in vector populations of different regions, where similar insecticides are being used for control

A comparison of the McMaster and Pitchford-Visser sieve faecal egg count methods used to monitor helminth infections in indigenous goats

A Spickett[1,2], A F Vatta[1,3], M F Smith[4], J Boomker[2], M O Stenson[1] and E F van Wijk[1]
[1]*Parasites, Vectors and Vector-borne Diseases Programme, ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa*
E-mail: spicketta@arc.agric.za
[2]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa*
[3]*Current Address: Ross University School of Veterinary Medicine, PO Box 334, Basseterre, St Kitts, West Indies*
[4]*Biometry Unit, ARC, Private Bag X519, Silverton, 0127 South Africa*

Faecal egg counts (FECs) are a basic methodology used to assess the level of gastrointestinal nematode infection in live animals. A relatively new method, the Pitchford-Visser Sieve method (FEC-P) has been promoted as an alternative to the modified McMaster method (FEC-McM), but no data have been published comparing their efficacies. The aim of the present investigation was to compare the 2 methods and this was done as part of a project that investigated the effect of administering copper oxide wire particles (COWP) on helminth infection in goats raised under small-scale farming conditions in the Bergville area, KwaZulu-Natal Province, South Africa. A total of 172 animals belonging to 15 farmers was monitored for 12 months at 4-weekly intervals starting during the week of 15 October 2007. At each sampling occasion an individual rectal faecal sample was taken from each animal and placed in a uniquely marked sealable bag. The bags were stored in a refrigerator and transported on ice to the laboratory. In the week following the sampling procedure, the faecal samples were individually subjected to faecal egg counts (in eggs per gram of faeces) by both the McMaster and Pitchford-Visser Sieve methods. Both methods showed an increase in FEC from October 2007 to January 2008. After administration of the COWP in January 2008, the methods both indicated a marked decline in FEC for the COWP-treated goats 2 weeks after treatment (FEC-P = 223; FEC-McM = 391) compared with the controls (FEC-P = 2382; FEC-McM = 1945) followed by an increase in FECs during the next sampling event, 2 weeks later. The

difference in FEC between COWP-treated and control animals 2 weeks after the administration of COWP was statistically very significant ($P < 0.001$) for both methods. Based on mean FECs, the Pitchford-Visser Sieve and McMaster methods correlated well overall ($r = 0.9759$, $P < 0.0001$) as did the mean FECs for the COWP-treated ($r = 0.9631$, $P < 0.0001$) and control ($r = 0.9914$, $P < 0.0001$) groups. The Pitchford-Visser Sieve method, which gives a cleaner sample to examine for eggs than the McMaster method, generally gave higher egg counts in this study, although these differences were not statistically significant.

Habitat suitability modelling of South African ticks

A M Spickett[1], I G Horak[2], H Heyne[1] and R Williams[1]
[1]*ARC-Onderstepoort Veterinary Institute, Private Bag X 05, Onderstepoort, 0110 South Africa. E-mail: spickettam@arc.agric.za*
[2]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa*

In the South African context, more than 120 tick species have been identified. Of these, 35 are normally associated with domestic animals, and approximately 10 are considered of major economic importance. They transmit, or are associated with, some 23 diseases/disease syndromes. A national tick survey was conducted in South Africa from 2005–2008. All collected specimens were identified and entered into an electronic database. Historical records of the distribution of the tick species concerned were additionally gleaned from the literature and databases constructed from historical sources. Locality data for each of the major tick vectors were subsequently subjected to maximum entropy analysis (Maxent). Maxent, a recently introduced machine-learning modelling technique uses presence-only data to predict the habitat suitability of a species by estimating the most uniform distribution of points of occurrence across the area of study. A total of 492 variables consisting of satellite data, climate data and physical environmental data were used as prospective variables in the modelling process to establish habitat suitability indices. ArcGIS (ESRI) software was used to convert all digital images to a 1 × 1 km spatial resolution grid format covering the whole of South Africa. Results for the major tick vectors of disease show *Amblyomma hebraeum* to be contained within its historical range, *Rhipicephalus appendiculatus* to have occupied limited foci not previously within its range and both *Rhipicephlaus (Boophilus) decoloratus* and *Rhipicephalus (Boophilus) microplus* to have extended their distributional range beyond previously established boundaries but within those predicted by the habitat suitability models.

New *Anopheles funestus* group species: What does it mean for malaria transmission?

B L Spillings[1,2], B D Brooke[1,2], L L Koekemoer[1,2], J Chiphwanya[5], M Coetzee[3] and R H Hunt[4]
[1]*Vector Control Reference Unit, National Institute for Communicable Diseases, NHLS, Johannesburg, South Africa. E-mail: lizettek@nicd.ac.za*
[2]*Division of Virology and Communicable Disease Surveillance, School of Pathology of the University of the Witwatersrand and the National Health Laboratory Service (NHLS), Johannesburg, South Africa*
[3]*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[4]*NRF Chair in Medical Entomology and Vector Control, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa*
[5]*National Malaria Control Programme, Malawi*

The major malaria vector *Anopheles funestus* belongs to a group of morphologically similar species that are commonly distinguished from one another through the use of chromosomal and molecular techniques. Indoor resting collections of mosquitoes from Malawi were initially identified as *An. funestus* by morphology, but failed to have this confirmed by the species-specific PCR assay. Sequence analysis of the ITS2 region identified variations within the *An. funestus*-specific primer binding site and revealed 4.5 % sequence variation compared with *An. funestus.* D3 analysis showed 1.5 % sequence variation from *An. funestus.* Cytogenetic analysis of the polytene chromosome banding arrangements showed that the specimens were homosequential with *An. funestus*, with fixed inverted arrangements of the 3a, 3b and 5a inversions commonly polymorphic in *An. funestus.* The chromosomes of hybrid females showed levels of asynapsis typical of inter-species crosses. These molecular and cytogenetic observations support the hypothesis that this Malawi population is a new species and has been provisionally named '*An. funestus*-like'. The role of this new species in malaria transmission will be discussed.

Mobiline peritrichs found on African limpets

L L van As[1] and L Basson[1]
[1]*Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa. E-mail: vanasll.sci@ufs.ac.za*

During parasitological surveys conducted along the east coast of South Africa specimens of *Cellana capensis* (Gmelin, 1791) were collected and found to be infested with *Mantoscyphida branchi* Van As, Van As & Basson, 1999 and a mobiline peritrich. Mobiline peritrichs were also found on limpet species collected along the west coast of Africa. Live observations were noted and specimens were photomicrographed for measurements. Wet smears were fixed in Bouin's, transferred to 70 % ethanol and stained with Mayer's haematoxylin for studying the nuclear apparatus and to obtain additional body measurements. In order to study details of the infundibulum and denticle ring, air-dried smears were impregnated with Protargol and $AgNO_3$. A number of Urceolariidae species are found associated with marine invertebrates, with 4 species known from gastropod hosts. Some confusion existed as to how to distinguish *Urceolaria* Stein, 1867 and *Leiotrocha* Fabre-Domergue, 1888 from each other. The most important generic features are the macronucleus and the central adhesive disc cilia (scopula cilia). In *Urceolaria* the absence of scopula cilia is a consistent generic feature, whilst in *Leiotrocha* the presence of these cilia as a generic characteristic must still be confirmed. Features such as the adhesive disc dimensions and all the somatic cilia are used in the generic differentiation. Based on this information, the mobiline peritrichs collected from the African limpets were identified as *Leiotrocha patellae* (Cuénot, 1891). Motivation will be presented why *Urceolaria patellae* (Cuénot, 1891), *U. cellanae* (Suzuki, 1950) and *U. viridis* (Richards, 1971), with the exception of *U. karyolobia* Hirschfield, 1949, must be placed in the genus *Leiotrocha.*

Poster Presentations

First record of *Haemoproteus* spp. (Apicomplexa: Haemoproteidae) infecting grey crowned and blue cranes housed at the Hlatikulu Crane and Wetland Sanctuary, KwaZulu-Natal

C Cook[1], N J Smit[1] and J M Pittman[2]
[1]*Department of Zoology, University of Johannesburg, South Africa*
E-mail: courtneycook11@yahoo.com / nicos@uj.ac.za
[2]*Johannesburg Zoological Gardens, Johannesburg, South Africa*

Southern Africa is home to 3 species of crane: *Anthropoides paradiseus* (blue crane), *Grus carunculatus* (wattled crane) and *Balearica regulorum* (grey crowned crane). Haematozoans described infecting cranes worldwide include *Haemoproteus antigonis*, *Hp. balearicae*, *Leucocytozoon grusi* and a *Plasmodium polare*-like species, along with a number of unidentified *Haemoproteus* spp. Within the southern African grey crowned crane, *B. regulorum*, both *Hp. antigonis* and *Hp. balearicae* have been recorded, transmitted by *Culicoides* spp. Both haemoproteid spp. were identified during routine health checks of captive *B. regulorum* within the Eastern Cape and KwaZulu-Natal provinces. Similar checks were performed in summer 2008 on *A. paradiseus, G. carunculatus* and *B. regulorum* housed at Hlatikulu Crane and Wetland Sanctuary, of which a single *B. regulorum* and *A. paradiseus* was found to be infected with a haemoproteid species. Unprocessed blood smears were fixed in absolute methanol for ~10 min, Giemsa's stained for 20 min and screened along with Leucostat-stained smears at ×1000 magnification and images of haematozoans were captured using a Carl Zeiss Axiocam digital camera attached to a Zeiss Axioplan 2 photomicroscope. Measurements (μm) were taken using AxioVision Release 4.3 software, calibrated to a stage micrometer and the parasitaemia calculated as infection levels per 10^5 erythrocytes. The Student's *t*-test was used to check the validity of the results. Two intraerythrocytic stages were

identified in the peripheral blood of *B. regulorum*, possible microgametocytes measuring 18 ± 2.1 × 4.1 ± 0.8 μm with a circumference of 44.7 ± 7.9 μm (n = 8), and macrogametocytes measuring 15.1 ± 2.2 × 2.9 ± 0.3 μm with a circumference of 33.5 ± 3.6 μm (n = 8) at a parasitaemia of 0.0001 %. Comparisons of these and the main diagnostic characteristics of *Hp. balearicae* suggest that it may be the same species. Young intraerythrocytic stages, too small to be identified to species level, were found in the peripheral blood of *A. paradiseus* at a parasitaemia of 0.0002 %. It would be beneficial in future to seasonally monitor captive populations of cranes for possible transmission of *Hp. balearicae* between crane species and for the presence of unidentified haematozoans.

Copepod parasites from elasmobranchs off the coast of South Africa

M Dikgale and S M Dippenaar
Department of Biodiversity, SMLS, University of Limpopo, Private Bag X1106, Sovenga, 0727 South Africa. E-mail: susand@ul.ac.za

Little is known about the diversity of symbiotic copepods from South African waters even though copepods are symbiotic on animals ranging from sponges to marine mammals. This study formed part of a larger study of the metazoan parasites of elasmobranches along the South African coast and included elasmobranchs caught during commercial prawn trawls off Durban as well as *Squalus* cf. *megalops* caught during a demersal cruise of Marine and Coastal Management along the south coast. Sixty-seven different hosts belonging to 7 different species were investigated for copepod infection. All the collected copepods were fixed and preserved in 70 % ethanol. Collected copepods were studied, after being stained in lignin pink, using the wooden slide technique. Two species of Siphonostomatoida (*Eudactylina* sp. and *Achtheinus oblongus* Wilson, 1908 (*sensu* Ho, 1975)) were found on *S. megalops* collected from the south coast. *Achtheinus oblongus* individuals were found on the fins while *Eudactylina* sp. specimens were collected from between the primary gill filaments attached to the secondary gill filaments. *Eudactylina* sp. exhibited 18 % prevalence, a mean intensity of 5 individuals per host and a mean abundance of 0.921 on *S. megalops*. *Achtheinus oblongus* showed lower prevalence (5 %), mean intensity (4 individuals per host) and mean abundance (0.211) on their hosts. Additionally, *Eudactylina* sp. exhibited a random distribution while too few *A. oblongus* individuals were collected to calculate a meaningful distribution. *Eudactylina* sp. is not similar to *E. vilelai*, previously described from *S. megalops*, since it belongs to the group of eudactylinids with unmodified 2nd leg exopods.

Blood protozoans of mullet (Mugilidae) from the East Coast estuaries of South Africa

M L Ferreira[1], N J Smit[1] and A J Davies[1,2].
[1]*Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: mlcoetzee@webmail.co.za / nicos@uj.ac.za*
[2]*School of Life Sciences, Faculty of Science, Kingston University, Kingston upon Thames, KT1 2EE, United Kingdom*

Past research has shown that mullet (Mugilidae) are often hosts to a variety of blood protozoans of the genera *Trypanosoma*, *Desseria* and *Babesiosoma*. The currently known haematozoan species from mullet worldwide are: *Trypanosoma froesi* Lima, 1976, *Trypanosoma mugilicola* Becker and Overstreet, 1979, which is probably also *T. froesi*, and *Trypanosoma platanusi* Ribeiro *et al.* 1992; 1 species of haemogregarine, *Desseria* (*Haemogregarina) mugili*, Carini, 1932; and a species of dactylosomatid, *Babesiosoma* (*Dactylosoma) hannesi* Paperna, 1981, which may be *Babesiosoma mariae* Hoare, 1930. The only blood protozoans known from South African mullet are a *Desseria* sp. from flat head mullet, *Mugil cephalus,* from the Seekoei River Estuary and *B. hannesi* from grey mullets, *Mugil cephalus* L., *Liza richardsonii* (Smith, 1846) and *Liza dumerili* (Steindachner, 1870) from the Swartkops Estuary, both on the South Coast of South Africa. The aim of this study was thus to examine the blood parasite diversity of mullet species along the East Coast of South Africa. Fishes were caught during September 2008 and April 2009, using drag nets, in 3 estuaries (Mvoti, Thukela and Amatikhulu), identified and measured. Blood was taken from the caudal vein, and thin blood smears were made immediately. Slides were air-dried, fixed with methanol and stained with phosphate-buffered Giemsa prior to screening. The fish taxa with the highest abundance collected were *Valamugil cunnesius* Valenciennes, 1836, and *Mugil cephalus.* In 2008, Amatikhulu Estuary *V. cunnesius* showed a 77 % prevalence of *T. froesi*, as well as a 30.7 % prevalence of a *Desseria* sp.. In 2009, *V. cunnesius* in the Mvoti Estuary had a 13.3 % prevalence of *B. hannesi*, whereas, *V. cunnesius* in the Thukela Estuary showed 25 % prevalence of *B. hannesi*, with *Mugil cephalus* from the same estuary having 3.1 % prevalence of *T. froesi.* These are the 1st records of blood protozoans from East Coast estuaries of southern Africa.

Cymothoid isopods collected from museum fishes in the South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa

K A Hadfield[1] and N J Smit[1]
[1]*Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: kerryh26@yahoo.com / nicos@uj.ac.za*

Cymothoid isopods have been found to parasitise a large number of fish species around the world but have rarely been studied in South Africa. There are 42 different genera of cymothoids with 400 known species worldwide. Only 16 of these species, belonging to 8 different genera, are known from southern African waters. Thus, little information is available on the biodiversity of these parasites, their distribution and their hosts. These data are necessary to understand the effects these parasites will have on the South African fish populations and the aquatic environment as a whole. In South Africa, the little that is known is incomplete and limited in both host records and localities. These cymothoid parasites are found externally on the fish host, in the gill chamber or inside the buccal cavity. Owing to their large size, those cymothoids observed on the external surfaces have been collected, but those hidden inside the buccal cavity often go unnoticed and are thus unknown. It was thus hypothesized that these isopods in the buccal cavity might still be preserved along with their fish hosts in the South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, which has an extensive collection of fish from southern African waters. A fish species was chosen and looked at in its entirety to also determine whether host size played a role in the presence of the parasite. As this was a preliminary study only 4 species of fish (which have previously been identified as hosts) were looked at, which included a total of 1398 fish. These hosts included *Sparodon durbanensis* (white musselcracker), *Diplodus sargus capensis* (blacktail), *Spondyliosoma emarginatum* (steentjie), and *Amblyrhynchotes honckenii* (evileye pufferfish). Of these, 116 were found to be infected with a cymothoid isopod. The prevalence for each of these host fish was calculated and found to between 7 and 12 % with *Sparodon durbanensis* having the highest prevalence. Further studies on other host fish will also be done to determine the presence of these isopods in other fish in South African waters.

Loop-mediated isothermal amplification (LAMP) detection of *Babesia orientalis* in water buffalo (*Bubalus babalis*, Linnaeus 1758) in China

L He[1,2], Y-Q Zhou[2], M C Oosthuizen[3] and J-L Zhao[1,2]
[1]*State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei Wuhan 430070, China. E-mail: helan0710@yahoo.com.cn*
[2]*College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China 430070*
[3]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, South Africa*

Babesia orientalis causes water buffalo babesiosis, one of the most important diseases of buffalo in central and south China, annually resulting in enormous economic losses. Timely implementation of effective treatment depends on rapid diagnosis. Loop-mediated isothermal amplification (LAMP) is a rapid method with high specificity and efficiency under isothermal conditions using a set of 4 specifically designed primers that recognize 6 distinct sequences on the target gene. In this study, a LAMP method was developed for specific detection of *Babesia orientalis* in water buffalo (*Bubalus babalis*, Linnaeus, 1758). Four primers were designed from the V4 hyper-

variable region of the 18S rRNA gene of *B. orientalis*. Blood samples were collected from *B. orientalis* experimentally-infected water buffalo as well as 165 water buffalo from 8 different regions of the Hubei province, south China. Genomic DNA was extracted, subjected to the LAMP assay and compared with results obtained using a previously described semi-nested PCR. The LAMP assay proved to be *B. orientalis* specific and more sensitive than the semi-nested PCR. While no *B. orientalis* has been reported before north of the Yangtse River in China, results have shown that *B. orientalis* has spread to the north of the river. This could pose a serious threat to the water buffalo industry.

Metazoan parasites of the angelfish *Brama brama* (Bonnaterre, 1788) (Perciformes, Bramidae)

T Mangena, B P Jordaan and S M Dippenaar
Department of Biodiversity, SMLS, UL, Private Bag X1106, Sovenga, 0727 South Africa. E-mail: susand@ul.ac.za

Brama brama (Bonnaterre, 1788), the angelfish or Atlantic pomfret, is a cosmopolitan species occurring at depths of up to 1000 m and in temperatures varying between 12 and 24 °C. Angelfish were collected during a demersal cruise of Marine and Coastal Management along the west coast of South Africa during January 2008. Specimens were collected from different trawl depths varying between 100 and 300 m. The gills of 11 selected fish were examined for the presence of metazoan parasites. All collected parasites were fixed and preserved in 70 % EtOH. Collected copepods were examined, after staining with lignin pink, using the wooden slide technique, while monogeneans were stained in alum carmine, transferred to xylene and mounted in xylene based mountant on microscope slides. All collected copepods were *Hatschekia conifera* Yamaguti, 1939, while the monogeneans were probably a member of the subclass Heteronchoinea, infrasubclass Oligonchoinea and family Diclobothriidae. Both *H. conifera* and the collected monogeneans had a 100 % prevalence on their hosts. The mean intensity of *H. conifera* was higher (97 parasites per host) than that of the monogeneans (10 parasites per host). The mean intensity of *H. conifera* individuals was higher for hosts caught between 201–300 m (108 individuals per host) than for those caught between 101–200 m (89 individuals per host). However, there was not a significant difference between the total number of hatschekiids collected from hosts caught at the 2 different depth zones ($\chi^2 = 0.0467$, df = 1, $P = 0.83$). Both *H. conifera* and the monogeneans exhibited an aggregated distribution on their hosts.

Evaluation of environmental deterioration by analysing fish parasite biodiversity and community structure

G N Madanire[1], W J Luus-Powell[1], P A S. Olivier[1] and A Jooste[1]
[1]*Department of Biodiversity, Private Bag X1106, University of Limpopo, Sovenga, 0727 South Africa. E-mail: powellw@ul.ac.za*

The upper and lower catchments of the Olifants and Limpopo Rivers are characterised by mining, agricultural, industrial and other anthropogenic activities that adversely impact on the water quality. The effects of such activities may be evident in changes of species diversity and composition of biota as ecosystems adapt to disturbances. To establish the relationship between metazoan parasite communities and water quality levels, the present study examined the fish metazoan parasite communities of 3 dams with varying degrees of pollution: Nwanedi-Luphephe (near pristine), Flag Boshielo (moderately polluted) and Anglo-Platinum Return Water Dam (polluted). The sharp tooth catfish *Clarias gariepinus* was used as a model host. Seasonal sampling of fish for parasitological examination was done at all sites from June 2008 to August 2009. Water samples were taken concurrently and selected variables were determined. The distribution of the branchiuran, *Chonopeltis inermis*, and an unidentified leech was limited to the near pristine site, while the cestode, *Polyonchobothrium clarias*, the digenean, *Diplostomum* sp. and the nematode, *Contracaecum* sp. larvae were present at all the sampled sites. The monogeneans, *Gyrodactylus rysavyi, Macrogyrodactylus clarii, M. congolensis, Quadriacanthus* sp., the nematodes, *Paracamallanus cyathopharynx* and *Procamallanus laevionchus*, the copepod, *Lamproglena clariae*, and the digenean, *Glossidium pedatum*, were limited to the near pristine and moderately polluted sites. The variability of the calculated infection indices (prevalence, mean abundance and mean intensity), the biotic indices (Margalef, Shannon, Evenness, Simpson and Berger-Parker) as well as the degree of interactivity among parasites suggest that the structure of parasite communities is affected by the water contamination levels.

Monogenean parasites of *Clarias gariepinus* from the Limpopo River System

G N Madanire[1], M P Malefo[1], M M Matla[1], H E Hattingh[1] and W J Luus-Powell[1]
Department of Biodiversity, Private Bag X1106, University of Limpopo, Sovenga, 0727 South Africa. E-mail: powellw@ul.ac.za

Monogeneans, widespread throughout freshwater and marine habitats, are in apparent equilibrium with their fish hosts in nature, presenting few if any problems to the host. However, under culture conditions monogeneans build up heavy worm burdens which provoke epizootics. Therefore, a prerequisite for the success of fish breeding is to prevent epidemics and the development of potential infections. This requires a good taxonomic and biological knowledge of pathogenic agents. The aim of this investigation was to identify and study some aspects of the ecology of the monogenean parasites on *Clarias gariepinus* from the Nwanedi-Luphephe Dams of the Limpopo River System. Fish were collected from June 2008 to June 2009 using gill nets of various mesh sizes and killed by severing the spinal cord. A total of 45 host fishes were examined of which 12 (26.7 %) were infested by at least 1 monogenean parasite. In all, 76 monogenean specimens were collected, belonging to 5 different species and 3 genera: *Macrogyrodactylus* (*M. clarii* and *M. congolensis*), *Quadriacanthus* (*Q. aegypticus* and *Q. clariadis*) and *Gyrodactylus rysavyi*. Infracommunity diversity was poor, with only 5 (11.1 %) hosts harbouring 4 of the 5 species observed. *Macrogyrodactylus clarii* (prevalence 26.7 %, mean intensity 1.7, abundance 0.4) was the most dominant species, while *Quadriacanthus clariadis* (prevalence 11.1 %, mean intensity 1.8, mean abundance 0.2) was the least dominant species. The parasite abundance and intensity levels were not influenced by either the sex or the size of the host. Infection values exhibited seasonal fluctuation, reaching peaks in winter and summer. The spatial distribution of each parasite was studied on different regions of the gill, and positive associations among the different species were revealed. *Clarias gariepinus* is infested throughout the year and the recruitment of the monogeneans, although relatively weak, is continuous.

Three dactylogyrids (Dactylogyridae Bychowski 1933: Monogenea) from selected cyprinid species of the Nwanedi-Luphephe Dams in the Limpopo Province, South Africa

E M Mbokane[1], W J Luus-Powell[2], M M Matla[2] and J Theron[1]
[1]*Aquaculture Research Unit, School of Agriculture, University of Limpopo, Private Bag X1106, Sovenga, 0727 South Africa*
[2]*Department of Biodiversity, School of Molecular and Life Sciences, Department of Zoology, University of Limpopo, Private Bag X1106, Sovenga, 0727 South Africa*
E-mail: powellw@ul.ac.za

Dactylogyrids are frequently occurring monogeneans that show strict host and site specificity. There was an opportunity to investigate a total of 162 cyprinids, comprising *Labeobarbus marequensis* (n = 53), *Barbus trimaculatus* (n = 63) and *Barbus radiatus* (n = 46) seasonally from 2008 to 2009 in the Nwanedi-Luphephe Dams (22°39′S, 30°25′E), Limpopo River System. Fish were sampled by means of gill nets and electrofishing or seine netting in accordance with the habitat conditions. The following dactylogyrids were recovered: *Dactylogyrus spinicirrus* was recovered from all 3 hosts, *Dactylogyrus afrolongicornis alberti* and *Dactylogyrus afrolongicornis afrolongicornis* were recovered from *B. trimaculatus*. Parasites were mounted and cleared using either glycerine jelly or ammonium-picrate glycerine solution. The intensity of the parasites varied amongst the different seasons and hosts. *Labeobarbus marequensis* appeared to be the preferred host followed by *B. trimaculatus* and *Barbus radiatus*. Summer and spring appeared to be optimal seasons for the

dactylogryrids. A prevalence of 20 % was recorded for *D. spinicirrus* on *Lb. marequensis* during winter and 100 % during summer. On *B. trimaculatus*, the prevalence was 20 % during winter. A prevalence of 20 % was recorded for *Dactylogyrus afrolongicornis alberti* during winter. There was no correlation between either fish length or condition factor and the number of parasites. The study indicated that the abundance of dactylogyrids on cyprinids is partly influenced by season.

Molecular detection of *Babesia rossi* and *Hepatozoon* sp. in African wild dogs (*Lycaon pictus*) in South Africa

P T Matjila[1], A L Leisewitz[1], F Jongejan[1], H J Bertschinger[1] and B L Penzhorn[1]
[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: tshepo.matjila@up.ac.za*

Blood specimens from wild dogs (n = 301) were obtained from De Wildt Cheetah and Wildlife Centre (Pretoria) and 5 game reserves (4 in the North West Province and 1 in Limpopo Province), South Africa. Specimens were screened for *Babesia, Theileria, Hepatozoon* and *Ehrlichia/Anaplasma* species using PCR and Reverse line blot (RLB) assays. Positive results were obtained in 18 (6 %) wild dogs. Sixteen specimens were found positive for *B. rossi* and 2 dogs were *Hepatozoon* sp. positive. It appears that these tick-borne pathogens are not widely distributed in wild dog populations.

Metazoan parasites of *Schilbe intermedius* from Nwanedi-Luphephe Dams

W J Smit[1], W J Luus-Powell[1], H E Hattingh[1] and M M Matla[1]
[1]*Department of Biodiversity, Private Bag X1106, University of Limpopo, Sovenga, 0727South Africa. E-mail: smitw@ul.ac.za*

Schilbe intermedius (Silver catfish) is a freshwater fish species of the family Schilbeidae and occurs abundantly in most dams and river systems in the Limpopo Province. Parasitological surveys were carried out at the Nwanedi-Luphephe Dams during different seasons in 2007, 2008 and 2009. The hosts (n = 31) were collected using gill nets and killed by cutting through the spinal cord. All endo- and ectoparasites were fixed and preserved using standard methods and stored in 70 % ethanol. The following parasites were recorded: 2 monogenean species (*Schilbetrema*) from the gills (prevalence 71 %); 3 species of trematodes, *i.e.* an unidentified adult digenean in the small intestine (prevalence 22.6 %), *Clinostomum* metacercariae in the body cavity (prevalence 22.6 %) and *Diplostomulum* from the eye (prevalence 3.2 %); 2 species of nematodes, *i.e. Contracaecum* sp. in the body cavity (prevalence 96.8 %) and *Paracamallanus cyathopharynx* from the intestine (prevalence 51.6 %); 1 species of Branchiura, *i.e. Dolops ranarum* from the skin (prevalence 9.7 %). *Schilbe intermedius* bears a high parasitic load and wide range of parasites compared to other freshwater fish species examined at the Dams. A high intensity was recorded for *Schilbetrema* sp. (*e.g.* 317) on the gills of some of the hosts as well as the unidentified adult digenean (*e.g.* 39) from the small intestine. Some of these parasites (*e.g.* the monogeneans) might affect the condition of the host negatively.

Haemogregarines (Apicomplexa: Adeleorina) of *Pseudocordylus* spp. and their possible definitive hosts from the Free State highlands

J van As[1], N J Smit[2], N J L Heideman[3] and A J Davies[4]
[1]*Dept Zoology & Entomology. University of the Free State, Qwaqwa Campus, Phuthaditjaba, South Africa. E-mail: nicos@uj.ac.za*
[2]*Department of Zoology, University of Johannesburg, Auckland Park Campus, Auckland Park, 2006 South Africa*
[3]*Office of the Dean, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa*
[4]*School of Life Sciences, Kingston University, Kingston upon Thames, Surrey, KT1 2EE, United Kingdom*

The study of blood parasites of reptiles is a relatively new and unexplored field of research in South Africa. The purpose of this research project was to explore the haemogregarine fauna of cordylid lizards, *Pseudocordylus melanotus melanotus, Pseudocordylus melanotus subviridus* and *Pseudocordylus langi* and to search for their possible definitive host vectors. Lizards were captured at various altitudinal gradients on the Sentinel Trail area at the top of the Tugela Falls and Namahali Pass, and at the top of Platberg, near Harrismith. A rich diversity of undescribed infections, likely of the genus *Hepatozoon*, was found in the blood of *P. m. melanotus, P. langi* and *P. m. subviridus* from the 2 disjunct study sites, mostly accompanied by other infections including *Sauroplasma*, filarial nematodes and various forms of saurian malaria. Infections were typically over-dispersed at both study sites. Attempts were made to find the life-cycle stages of the presumptive *Hepatozoon* spp. in the internal organs of the lizards as well as in ectoparasitic lizard mites, by means of stained histological sections, and in mites and mosquitoes by means of stained squashes. Thus far, no merogonic or sporogonic development has been seen in sections of lizard or mite tissues, respectively, although haemogregarines have been observed with digested blood in the gut of squashed mites. At this stage, mosquitoes (*Culex* (*Afroculex*) *lineata*) appear the more likely vector and they were collected at night on infected lizards. Mosquito squashes have revealed early oocysts. The next step in investigating the haemogregarine life-cycles will be to attempt experimental transmission in the laboratory, where it is anticipated that merogonic and sporogonic stages will be studied by means of histology, confocal microscopy and TEM.