



**49<sup>th</sup> Annual Conference  
of the  
Parasitological Society of  
Southern Africa**

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**14-16 September 2021**  
**[www.parsa.ac.za](http://www.parsa.ac.za)**



 **#PARSA2021Con**

## TABLE OF CONTENTS

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TABLE OF CONTENTS .....	2
ORGANISING COMMITTEE .....	3
LIST OF AUTHORS IN ALPABETICAL ORDER .....	4
LIST OF ABSTRACTS IN ALPHABETICAL ORDER.....	5
PROGRAMME .....	8
Day 1 – 14 September 2021 .....	8
Day 2 - 15 September 2021 .....	9
Day 3 - 16 September 2021 .....	10
KEYNOTE PRESENTATION .....	12
Tuesday 14 September 2021 .....	12
Oral Presentations – Tuesday 14 September.....	13
Session 1 .....	13
Session 2 .....	16
Poster Presentation Session 1 .....	19
Session 3.....	23
Oral Presentations – Wednesday 15 September.....	26
Session 4.....	26
Session 5.....	32
Poster Presentation Session 2 .....	38
Session 6 .....	41
Oral Presentations – Thursday 16 September .....	47
Session 7 .....	47
Session 8.....	56

## ORGANISING COMMITTEE

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### PARSA Executive Committee

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## LIST OF AUTHORS IN ALPABETICAL ORDER

---

### A

Acosta, Aline - 38 & 39  
Adebayo, Muideen - 29  
Agarwal, Priyanka - 51  
Austin, Lucinda - 33  
Ayawei, Precious - 55

### C

Conrad, Patricia - 12  
Cruz-Laufer, Armando - 35  
Custers, Jonas - 19

### D

Dos Santos, Quinton - 47  
Du Buisson, Joretha - 25  
Dzemo, William - 30

### E

Emsley, Emily - 22  
Erasmus, Anja - 49

### G

Gaorekwe, Masego - 52  
Garido, Mario - 13  
Gilbert, Beric - 56 & 57  
Grobbelaar, Andri - 35

### H

Harnoster, Florence - 60

### J

Jacobs, Ané - 24  
Jordaan, Bernard - 37

### K

Khumalo, Nozipho - 50  
Kmentova, Nikol - 48

### L

Latief, Lutfiyya - 17  
Lukhele, Linda - 18

### M

Maduenyane, Mpho - 53  
Mahlangu, Jabu - 42  
Mahlobo, Sibongihlanhla - 31  
Makgabo, Sekgota - 28  
Matshotshi, Asiashu - 26  
Mofokeng, Lehlohonolo - 61  
Moons, Tanisha - 20  
Mulaudzi, Fhulufhelo - 16

### N

Nel, Teneal - 23  
Netherlands, Edward - 59  
Nofal, Ashlee - 31

### O

Olaogun, Sunday - 27

### P

Pretorius, Marilie - 58

### R

Rants'o, Thankhoe - 43

### S

Schoeman, Anneke - 44  
Schols, Ruben - 45  
Seetsi, Anna - 41  
Smit, Amber - 14  
Stevens, Llyle - 15  
Syrota, Yaroslav - 46

### T

Tawana, Mpho - 21  
Truter, Marliese - 34

### V

Van der Spuy, Linda - 38  
Vermaak, Anja - 54

## LIST OF ABSTRACTS IN ALPHABETICAL ORDER

---

A can of worms: high diversity of Opecoelidae (Trematoda: Digenea) from the South African intertidal super klipfish, <i>Clinus superciliosus</i> .....	54
A descriptive study of parasites detected in ticks of domestic animals in Lesotho .....	31
A Journey full of amazing parasites and one health lessons learned.....	12
A new <i>Dactylogyrus</i> sp. (Monogenea: Dactylogyridae) from the gills of the smallmouth yellowfish <i>Labeobarbus aeneus</i> (Burchell, 1822) from the Vaal River, South Africa.....	40
A planned taxonomical study and a twenty-year period, statistical overview of Sessilida Kahl, 1933 (Oligohymenophorea: Peritrichia) found on chiton species along the South African coastline.....	36
A study of parasites of three imported ornamental fishes .....	15
A systematic review and meta-analysis of ticks and tick-borne diseases in the Southern African Development Community (SADC) region .....	21
Adult nematode parasites of <i>Clarias gariepinus</i> (Burchell, 1822) from Lake Heritage, in the Crocodile River, South Africa using light and scanning electron microscopy .....	32
An integrative taxonomy approach to document the rectal endociliates of the globally invasive amphibian <i>Xenopus laevis</i> .....	24
Anthelmintic resistance in small ruminants of North-West Province, South Africa .....	22
Aspects of the pathology on the skin of <i>Clarias gariepinus</i> (Burchell, 1822) infected with a gyrodactylid monogenean <i>Macrogyrodactylus congolensis</i> (Prudhoe, 1957) .....	53
Assessment of <i>in vitro</i> anti-trypanosomal activity of nitrofurantoin derivatives against sleeping sickness trypanosomes.....	41
Biomarker responses and parasite infections of <i>Clarias gariepinus</i> from Lake Heritage, Crocodile River .....	17
Clinico-haematological and biochemical features of natural Babesiosis in Nigerian breeds of cattle .....	27
Development of resistance to acaricides by natural tick populations on cattle: A global systematic review and meta-analysis .....	30
Discordant drivers of parasitism from the micro-habitat to global scale in a frog host.....	44
Diversity and distribution of ectoparasite taxa associated with <i>Micaelamys namaquensis</i> , an opportunistic commensal rodent species.....	15
Diversity and infestation of ectoparasites associated with the Bushveld gerbil ( <i>Gerbilliscus leucogaster</i> ) across a wildlife-human/domestic animal interface in the South African Savanna .....	14
First record of <i>Ligophorus minimus</i> Euzet et Suriano, 1977 (Monogenea: Dactylogyridae) from the endemic South African mullet, <i>Chelon richardsonni</i> (Smith, 1846) (Mugiliformes: Mugilidae) .....	39
Gill maggots: new host and locality records for marine lernaeopodids (Lernaeopodidae) from South Africa, including the first molecular characterisation of species from this region.....	49
Hatchability and survival of <i>Lamproglana clariae</i> exposed to increasing concentrations of aqueous aluminium.....	58

Hiding out in trees won't keep you safe: blood parasites of southern African tree frogs.....	59
Identification of sesquiterpene alcohols with larvicidal and anticholinesterase effects on <i>Anopheles funestus</i> .....	43
Identification of ticks using morphological traits.....	50
<i>In silico</i> insights to optimise naphthoquinone analogues as lead inhibitors of the <i>Plasmodium</i> cytochrome <i>bc1</i> complex .....	51
Intralacustrine morphological variation of <i>Dolicirroplectanum lacustre</i> infecting Nile perch ( <i>Lates niloticus</i> ) in African freshwater systems.....	19
Invasive caryophyllidean cestodes of common carp colonising yellowfish in South Africa...	47
Invasive snails, parasite spillback and potential parasite spill over drive parasitic diseases of <i>Hippopotamus amphibius</i> in artificial lakes of Zimbabwe.....	45
Metallothionein levels in the ectoparasite, <i>Lamproglana clariae</i> (Copepoda: Lernaecidae) correspond to water quality .....	57
Metazoan parasites of anurans from the Vhembe area, Limpopo, South Africa .....	23
Metazoan parasites of Tank goby <i>Glossogobius giuris</i> (Hamilton, 1822) from the Komati River, South Africa .....	16
Molecular and morphological characterisation of <i>Argulus japonicus</i> (Japanese fish louse) from the Orange-Vaal River system .....	55
Molecular characterization of the economically important South African <i>Rhipicephalus</i> tick species of domestic animals and their bacterial communities.....	26
Molecular survey of reptiles and associated ticks from South Africa for bacterial and protozoal pathogens .....	61
Monogeneans as indicators of water quality.....	56
Morphological and phylogenetic analysis of Cosmocercidae from amphibians in South Africa .....	60
Occurrence and diversity of avian haemosporidian parasites in South African captive birds	52
ParasiteDB – the instrument for shared managing of parasitological collections .....	46
Parasite population structure in the pelagic ecosystem of Lake Tanganyika: morphology and mitogenomics.....	48
Parasites to the rescue? Three new species of <i>Acanthobothrium</i> van Beneden, 1849, (Cestoda: Onchoproteocephalidea) from the endangered white skate <i>Rostroraja alba</i> (Lacepède), off the Western Cape of South Africa .....	38
Prevalence of gastrointestinal parasites in small ruminants from selected farms in Akinyele Local Government Area of Oyo State, Nigeria .....	29
Occurrence and diversity of avian haemosporidian parasites in South African captive birds.....	43
29	
Somewhere I belong: using phylogenetic comparative methods and machine learning to investigate the evolution of a species-rich lineage of parasites .....	35
<i>Spinitectus petterae</i> Boomker, 1993 infecting <i>Clarias gariepinus</i> (Burchell, 1822) from the Vaal River system: a scanning electron and light microscopical study of aspects of the pathology .....	33

Temporal dynamics of *Anaplasma marginale* infection in calves at the wildlife-livestock interface in the Mnisi communal area, Mpumalanga, South Africa ..... 28

The biodiversity of frog blood parasites from the Vhembe Biosphere, Limpopo, South Africa ..... 25

The metazoan parasite community of invasive *Clarias gariepinus* (Burchell, 1822) in South Africa: testing the co-invasive, spillback and enemy release hypotheses ..... 34

The dilution effect behind the scenes: testing the underlying assumptions of its mechanisms ..... 13

Taxonomic re-evaluation of African anuran trypanosomes with the redescription and molecular diagnosis of *Trypanosoma nelspruitense* Laveran, 1904..... 37

The mosquitocidal and toxicological properties of novel pyrimidine-phenyl hybrid derivatives ..... 42

Unravelling the parasite diversity on the gills of chromidotilapiine cichlid fishes in West and Central Africa ..... 20

## PROGRAMME

### Day 1 – 14 September 2021

Time	Title	Speaker
08h45	Online Platform opens	
09h00	Introduction & Housekeeping	Corné Engelbrecht
<b>Session 1</b> <b>Chair: Florence Harnoster</b>		
09h05	Welcome & Opening	Prof. Sonja Matthee (President, PARSA)
09h15	A Journey Full of Amazing Parasites and One Health Lessons Learned	Prof. Patricia Conrad (University of California, Davis)
10h00	The dilution effect behind the scenes: testing the underlying assumptions of its mechanisms	Mario Garrido
10h10	Diversity and infestation of ectoparasites associated with the Bushveld gerbil ( <i>Gerbilliscus leucogaster</i> ) across a wildlife-human/domestic animal interface in the South African Savanna	Amber Smith
10h20	Diversity and distribution of ectoparasite taxa associated with <i>Micaelamys namaquensis</i> , an opportunistic commensal rodent species	Llyle Stevens
10h30	Question & Answer session	
10h45	Break	
<b>Session 2</b> <b>Chair: Florence Harnoster</b>		
11h00	Metazoan parasites of Tank goby <i>Glossogobius giuris</i> (Hamilton, 1822) from the Komati River, South Africa	Mulaudzi Fhulufhelo
11h10	Biomarker responses and parasite infections of <i>Clarias gariepinus</i> from Lake Heritage, Crocodile River	Lutfiyya Latief
11h20	A study of parasites of three imported ornamental fishes	Linda Lukhele
11h30	Question & Answer session	
<b>Poster Presentations Speed Session 1</b>		
11h45	Intralacustrine morphological variation of <i>Dolicirroplectanum lacustre</i> infecting Nile perch ( <i>Lates niloticus</i> ) in African freshwater systems	Jonas Custers
	Unravelling the parasite diversity on the gills of chromidotilapiine cichlid fishes in West and Central Africa	Tanisha Moons
	A systematic review and meta-analysis of ticks and tick-borne diseases in Southern African Development Community (SADC) region	Mpho Tawana
	Anthelmintic resistance in small ruminants of North-West Province, South Africa	Emily Emsley
12h00	Lunch Break	
<b>Session 3</b> <b>Chair: Ashlee Nofal</b>		
12h30	Metazoan parasites of anurans from the Vhembe area, Limpopo, South Africa	Teneal Nel
12h40	An integrative taxonomy approach to document the rectal endociliates of the globally invasive amphibian <i>Xenopus laevis</i>	Ané Jacobs
12h50	The biodiversity of frog blood parasites from the Vhembe Biosphere, Limpopo, South Africa	Joretha du Buisson
13h00	Question & Answer session	
13h15	Close of Day 1	



Day 2 - 15 September 2021

Time	Title	Speaker
07h45	Online Platform opens	
08h00	Introduction & Housekeeping	Corné Engelbrecht
<b>Session 3</b> <b>Chair: Andri Grobbelaar</b>		
08h05	Molecular characterization of the economically important South African <i>Rhipicephalus</i> tick species of domestic animals and their bacterial communities	Asiashu Matshotshi
08h15	Clinico-haematological and biochemical features of natural babesiosis in Nigerian breeds of cattle	Sunday Olaogun
08h25	Temporal dynamics of <i>Anaplasma marginale</i> infection in calves at the wildlife-livestock interface in the Mnisi communal area, Mpumalanga, South Africa	Marcus Makgabo
08h35	Question & Answer session	
08h50	Prevalence of gastrointestinal parasites in small ruminants from selected farms in Akinyele Local Government Area of Oyo State, Nigeria	Muideen Adebayo
09h00	Development of resistance to acaricides by field tick populations on cattle: A global systematic review and meta-analysis	William Dzemo
09h10	A descriptive study of parasites detected in ticks of domestic animals in Lesotho	Sibonginhlanhla Mahlobo
09h20	Question & Answer session	
09h35	Break	
<b>Session 4</b> <b>Chair: Anja Vermaak</b>		
09h50	Adult nematode parasites of <i>Clarias gariepinus</i> (Burchell, 1822) from Lake Heritage in the Crocodile River, South Africa using light and scanning electron microscopy	Ashlee Nofal
10h00	<i>Spinitectus petterae</i> Boomker, 1993 infecting <i>Clarias gariepinus</i> (Burchell, 1822) from the Vaal River system: a scanning electron and light microscopical study of aspects of the pathology	Lucinda Austin
10h10	The metazoan parasite community of invasive <i>Clarias gariepinus</i> (Burchell, 1822) in South Africa: Testing the co-invasive, spillback and enemy release hypotheses	Marliese Truter
10h20	Question & Answer session	
10h35	Somewhere I belong: Using phylogenetic comparative methods and machine learning to investigate the evolution of a species-rich lineage of parasites	Armando Cruz-Laufer
10h45	A planned taxonomical study and a twenty-year period, statistical overview of Sessilida Kahl, 1933 (Oligohymenophorea: Peritrichia) found on chiton species along the South African coastline	Andri Grobbelaar
10h55	Taxonomic re-evaluation of African anuran trypanosomes with the redescription and molecular diagnosis of <i>Trypanosoma nelspruitense</i> Laveran, 1904	Bernard Jordaan
11h05	Question & Answer session	

Time	Title	Speaker
<b>Poster Presentations Speed Session 2</b>		
11h20	Parasites to the rescue? - Three new species of <i>Acanthobothrium</i> van Beneden, 1849, (Cestoda: Onchoproteocephalidea) from the endangered white skate <i>Rostroraja alba</i> (Lacepède), off the Western Cape of South Africa	Linda Van Der Spuy
	First record of <i>Ligophorus minimus</i> Euzet et Suriano, 1977 (Monogenea: Dactylogyridae) from the endemic South African mullet, <i>Chelon richardsonni</i> (Smith, 1846) (Mugiliformes: Mugilidae)	Aline Acosta
	A new <i>Dactylogyryus</i> sp. (Monogenea: Dactylogyridae) from the gills of the smallmouth yellowfish <i>Labeobarbus aeneus</i> (Burchell, 1822) from the Vaal River, South Africa	Aline Acosta
11h35	Lunch Break	
<b>Session 5</b> <b>Chair: Marliese Truter</b>		
12h00	Assessment of <i>in vitro</i> anti-trypanosomal activity of nitrofurantoin derivatives against sleeping sickness trypanosomes	Anna Seetsi
12h10	The mosquitocidal and toxicological properties of novel pyrimidine-phenyl hybrid derivatives	Jabu Mahlangu
12h20	Identification of sesquiterpene alcohols with larvicidal and anticholinesterase effects on <i>Anopheles funestus</i>	Thankhoe Rants'o
12h30	Question & Answer session	
12h45	Discordant drivers of parasitism from the micro-habitat to global scale in a frog host	Anneke Schoeman
12h55	Invasive snails, parasite spillback and potential parasite spillover drive parasitic diseases of <i>Hippopotamus amphibius</i> in artificial lakes of Zimbabwe	Ruben Schols
13h05	ParasiteDB – the instrument for shared managing of parasitological collections	Yaroslav Syrota
13h15	Question & Answer session	
13h30	Close of Day 2	

### Day 3 - 16 September 2021

Time	Title	Speaker
07h45	Online Platform opens	
08h00	Introduction & Housekeeping	Corné Engelbrecht
<b>Session 6</b> <b>Chair: Joretha du Buisson</b>		
08h05	Invasive caryophyllidean cestodes of common carp colonising yellowfish in South Africa	Quinton Dos Santos
08h15	Parasite population structure in the pelagic ecosystem of Lake Tanganyika: morphology and mitogenomics	Nikol Kmentová
08h25	Gill maggots: New host and locality records for marine <i>Lernaeopodids</i> (Lernaeopodidae) from South Africa, including the first molecular characterisation of species from this region	Anja Erasmus
08h35	Question & Answer session	
08h50	Identification of ticks using morphological traits	Nozipho Khumalo
09h00	<i>In silico</i> Insights to Optimise Naphthoquinone Analogues as Lead Inhibitors of the <i>Plasmodium</i> Cytochrome bc1 Complex	Priyanka Agarwal
09h10	Occurrence and diversity of Avian Haemosporidian parasites in South African captive birds	Masego Gaorekwe
09h20	Question & Answer session	
09h35	Break	

Time	Title	Speaker
09h50	Aspects of the pathology on the skin of <i>Clarias gariepinus</i> (Burchell, 1822) infected with a Gyrodactylid Monogenean <i>Macrogyrodactylus congolensis</i> (Prudhoe, 1957)	Mpho Maduenyane
10h00	A can of worms: high diversity of Opecoelidae (Trematoda: Digenea) from the South African intertidal super Klipfish, <i>Clinus superciliosus</i>	Anja Vermaak
10h10	Molecular and morphological characterisation of <i>Argulus japonicus</i> (Japanese fish louse) from the Orange-Vaal River system	Precious Ayawei
10h20	Question & Answer session	
<b>Session 7</b> <b>Chair: Nozipho Khumalo</b>		
10h35	Monogeneans as indicators of water quality	Beric Gilbert
10h45	Metallothionein levels in the ectoparasite, <i>Lamproglana clariae</i> (Copepoda: Lernaeidae) correspond to water quality	Beric Gilbert
10h55	Hatchability and survival of <i>Lamproglana clariae</i> exposed to increasing concentrations of aqueous aluminium	Marilie Pretorius
11h05	Question & Answer session	
11h20	Hiding out in trees won't keep you safe: Blood parasites of southern African tree frogs	Edward Netherlands
11h30	Morphological and phylogenetic analysis of Cosmoceridae from amphibians in South Africa	Florence Harnoster
11h40	Molecular survey of reptiles and associated ticks from South Africa, for bacterial and protozoal pathogens	Lehlohonolo Mofokeng
11h50	Question & Answer session	
12h05	Close of Presentations & Lunch	
13h00	PARSA AGM & Prize giving	
14h00	Close of Conference	

## SAVE THE DATE



# 2022

# ICPOW

4<sup>th</sup>

INTERNATIONAL CONGRESS  
ON PARASITES OF WILDLIFE

4-8 September 2022 | Kruger National Park, South Africa

## IMPORTANT DATES

- 15 January 2022 Special sessions deadline
- 5 March 2022 Abstract submission deadline
- 8 April 2022 Abstract confirmation notices
- 1 June 2022 Early registration & payment
- 6 July 2022 Regular registration & payment
- 6 July 2022 Accommodation payment






hosted by the Parasitological Society of Southern Africa (PARSA)

1<sup>st</sup> Announcement to follow | [www.savetcon.co.za](http://www.savetcon.co.za)

## KEYNOTE PRESENTATION

Tuesday 14 September 2021

### **A Journey full of amazing parasites and One Health lessons learned**

Patricia Conrad DVM, PhD

*Distinguished Professor, Associate Dean for Global Programs, School of Veterinary Medicine, University of California, Davis, Co-Director, University of California Global Health Institute - [paconrad@ucdavis.edu](mailto:paconrad@ucdavis.edu)*

As parasitologists, we delight in pursuing exciting research questions; making fascinating and often important biological discoveries about the parasites that intrigue us. The parasites we study may infect humans and/or animals, and their life cycles frequently involve development or transmission mechanisms that take place in the environment. Therefore, as parasitologists we are often working at the human-animal-environmental interface which is ideal for the application of a One Health approach to our investigation. In this keynote presentation Dr Conrad will share highlights from her 40-year journey as a veterinary protozoologist and scientist in Scotland, Kenya and the USA. She will explain how a One Health approach transformed her research and resulted in surprising discoveries of both new parasite species and the impact of environmental factors on protozoal pathogen transmission. Lessons learned in challenging the dogma in protozoology, working with productive teams, and making parasitological research fun will be shared.



Patricia Conrad DVM, PhD is the Associate Dean for Global Programs at the School of Veterinary Medicine, University of California, Davis and Co-Director of the system-wide (10 campus) UC Global Health Institute. Dr Conrad is a veterinarian and Distinguished Professor of Parasitology whose research is focused on the transmission of protozoal parasites between wildlife, humans and domestic animals. She received her veterinary professional degree from Colorado State University and PhD from the University of Edinburgh, Scotland. After doing post-doctoral research on the molecular epidemiology of East Coast fever at the International Laboratory for Research on Animal Diseases (now ILRI) in

Nairobi, Kenya, she joined the faculty of the School of Veterinary Medicine at the University of California, Davis. Dr Conrad has published over 235 scientific papers (61 h-index) and book chapters in the fields of emerging infectious diseases, parasitology, ecology of waterborne pathogens and One Health. Dr Conrad was elected to the National Academy of Medicine in 2011, awarded a Fellowship in the American Academy of Microbiology in 2012 and selected as an American Association of the Advancement of Science Fellow in 2018. In 2017 she received the American Veterinary Medical Association Lifetime Excellence in Research Award for her research and contributions to the veterinary profession; taking a One Health approach that addresses the interconnectedness of animals, plants, people and the environment. In 2018 she was awarded the UC Davis Chancellor's Award for International Engagement and in 2019 received the Lifetime Achievement Award for Babesiosis Research.

## Oral Presentations – Tuesday 14 September

### Session 1

#### **The dilution effect behind the scenes: testing the underlying assumptions of its mechanisms**

M.G. Garrido<sup>1</sup>, S. Halle<sup>1</sup> & H. Hawlena<sup>1</sup>

<sup>1</sup>Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev Midreshet Ben-Gurion 84990 Israel - [gaiarrido@gmail.com](mailto:gaiarrido@gmail.com); [snirhalle@mail.tau.ac.il](mailto:snirhalle@mail.tau.ac.il); [hadashaw@bqu.ac.il](mailto:hadashaw@bqu.ac.il)

Lately, we are facing an increase in emerging infectious diseases and a significant loss of biodiversity, leading the scientific community to posit a significant relationship between biodiversity and parasite prevalence. However, mechanisms underlying such a relationship are not clear and focus mainly on the response of parasites to host diversity, whereas the host's responses are often neglected. For example, different mechanisms explain how diversity can lead to change in the prevalence of a target parasite in its target host population. Yet, studies focusing on the role that background hosts play in the diversity-parasite prevalence patterns are rare, even though it is crucial to identifying their operating mechanisms and their direction (parasite amplification or dilution). Here, the reciprocal responses of the main players in the rodent-*Mycoplasma* system were simultaneously studied. A previous study of this system identified a negative diversity-prevalence relationship: one of the rodent species (*Gerbillus andersoni*) was identified as the amplification host and two background species, (*G. gerbillus* and *G. pyramidum*) as the diluter hosts. Under laboratory conditions, individuals of these three rodent species were inoculated with *Mycoplasma*-positive blood and the infection dynamics (i.e., parasite response) were followed, together with behavioural and physiological responses of the host. Results revealed a complex picture. *Mycoplasma* achieved better success in the amplification host (higher infection load, longer infection periods and a lower probability to clear the infection), than in background species. However, the background species became infected for a relatively long period, revealing amplification capacity that was unpredictable in the field patterns. Host preliminary results reveal that the amplification host has tolerance to infection, but again, response of the background species is complex. In general, results suggest that in natural communities, species should be viewed, along an amplification-dilution continuum and, furthermore, demonstrate the importance of exploring diversity-parasite relationships simultaneously from both host and parasite perspectives, as well as under both field and laboratory conditions.

## **Diversity and infestation of ectoparasites associated with the Bushveld gerbil (*Gerbilliscus leucogaster*) across a wildlife-human/domestic animal interface in the South African Savanna**

A.T. Smith<sup>1</sup>, E.A Ueckermann<sup>2</sup>, I.G Horak<sup>3</sup> & S. Matthee<sup>1</sup>

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Parasites represent a colossal component of biological diversity and are especially universal in the lives of wild animals. However, in South Africa, information on parasite-host relationships of small mammals is limited to historic monographs and records. As a result, a huge gap exists in parasite profiles associated with natural occurring rodent species. By focusing on a wildlife-human/domestic animal interface in Mpumalanga, the study aims were to record the ectoparasite diversity and infestations associated with *Gerbilliscus leucogaster* and to establish the importance of the host in harbouring vector species. Rodents (n=127) were trapped in 3 habitat types (natural, agricultural, and urban) during 2014-2020. More than 2500 ectoparasites from 5 taxonomic groups: fleas, lice, ticks, mesostigmatid and trombiculid mites were recorded. A total of 26 ectoparasite taxa were present on *G. leucogaster*. Fleas and lice were the most prevalent and abundant taxa [87.40%, 6.89 ( $\pm 0.69$ ) and 76.38%, 14.32 ( $\pm 1.93$ ), respectively]. Mesostigmatid and trombiculid mites were the most species rich taxa, both represented by nine species. Vector species included fleas (*Xenopsylla brasiliensis* and *X. frayi*) and ticks (*Hyalomma truncatum*). The present study constitutes the first systematic long-term assessment of the ectoparasite species associated with *G. leucogaster* at a local scale.

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## Diversity and distribution of ectoparasite taxa associated with *Micaelamys namaquensis*, an opportunistic commensal rodent species

L. Stevens<sup>1</sup>, E. A. Ueckermann<sup>2</sup>, L. van der Mescht<sup>1</sup>, I. G. Horak<sup>3</sup> & S. Matthee<sup>1</sup>

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South Africa boasts a rich diversity of small mammals of which several are commensal and harbour parasites of zoonotic importance. However, limited information is available on the parasite diversity associated with rodents in South Africa. The Namaqua rock mouse (*Micaelamys namaquensis*) is a regionally widespread and locally abundant species that is often commensal. To address the paucity of available data the aim of the study was to record the diversity of ectoparasites associated with *M. namaquensis* at multiple localities in South Africa. Rodents (n=216) were obtained from 12 localities: Savanna (7), Grassland (3), Succulent Karoo (1) and Fynbos (1) biomes during 2017 – 2018. A total of 5591 ectoparasites representing 5 taxonomic groups: fleas (415), sucking lice (4076), gamasid mites (585), ticks (515) and trombiculid mites (only prevalence data) were recorded. At least 54 ectoparasite taxa were recorded: 10 flea, 2 louse, 11 mesostigmatid mite, 20 tick, and 11 trombiculid mites. Several known vector species were recorded and included ticks (*Haemaphysalis elliptica*, *H. leachi*, *Rhipicephalus simus* and *R. appendiculatus*) and fleas (*Ctenocephalides felis*, *Xenopsylla brasiliensis* and *Dinopsyllus ellobius*). Locality records indicate variable geographic ranges for the two louse species and the three most abundant mite species. It is clear that *M. namaquensis* hosts a rich diversity of ectoparasite taxa and, as such, is an important species to monitor in environments where it occurs in close proximity to humans and domestic animals.

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Session 2

**Metazoan parasites of Tank goby *Glossogobius giuris* (Hamilton, 1822) from the Komati River, South Africa**

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*Glossogobius giuris* (Hamilton, 1822) has a wide distribution ranging from the Pacific Ocean and Red Sea to East Africa, South Asia and Australia and can be found in fresh, marine and brackish waters. Only two records of metazoan parasites from *G. giuris* are known from Africa. The aim of the study was to determine the metazoan parasite diversity of tank goby from the Komati River. Standard methods were used for collection of hosts (n = 15) in March 2020 and for preservation of parasites. Nine fish (60%) were infected by at least one parasite and 22 metazoan parasites of four parasitic groups were recovered in this parasitological survey. Nematodes (*Camallanus* sp., *Paracamallanus* sp. and *Procamallanus* sp.) were recorded from seven fish with mean intensity of infection (MI) = 2. The highest prevalence among the nematodes was recorded for *Paracamallanus* sp. (26.66%), followed by *Procamallanus* sp. (20%) and *Camallanus* sp. (13.33%). The branchiuran, *Dolops ranarum*, had a prevalence of 13.33% and MI = 1; the prevalence of metacestodes, *Parvitaenia* sp., was 13.33% with MI = 2. The lowest prevalence was observed for acanthocephalan larvae (6.66%) with MI = 2. All the parasites recorded from this study represent new host and locality records. Further studies, including molecular work, are needed to verify the possibility of new nematode species.

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## **Biomarker responses and parasite infections of *Clarias gariepinus* from Lake Heritage, Crocodile River**

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Deteriorating water quality, due to elevated levels of pollutants, negatively affects organisms living in aquatic systems. Biomonitoring is the use of organisms to assess ecosystem health based on deviations of biological organisation. Parasites are important components of host ecosystems and changes in parasite infection dynamics have been proposed as a means of assessing the health of aquatic ecosystems. Fish are generally used as biomarkers; however, parasites affect their hosts' physiology and thus the biomarker responses. Lake Heritage, situated along the Crocodile River in Muldersdrift, Gauteng, is subjected to anthropogenic activities which alters the natural state of the dam. *Clarias gariepinus* is a well-studied bioindicator species of aquatic environment and is host to many different endoparasite and ectoparasite taxa. The aims of the study were (1) to determine the status of Lake Heritage in terms of the water quality, pollution types and resulting effect on fish through assessment of biomarker responses; (2) to determine effects of parasites on biomarker responses in *C. gariepinus*, and (3) determine the effect of water quality on parasites. Physical water quality parameters were determined with a handheld probe and chemical variables were analysed using test kits. For biomarkers gill, liver and muscle tissue from *C. gariepinus* were assessed for total protein concentrations, and activities of acetylcholinesterase, catalase, superoxide dismutase and reduced glutathione. Results obtained for water quality variables during this study show higher pH, nitrate and calcium carbonate levels compared to the South African Target Water Quality Guidelines. Catalase, superoxide dismutase and reduced glutathione showed a physiological response in *C. gariepinus* to pollutants. Comparison of prevalence, mean intensity and mean abundance of endoparasites and ectoparasites showed lower infection levels by ectoparasites compared to endoparasites. No differences in physiological responses of *C. gariepinus* were seen when comparing fish infected with endoparasites compared to those infected with ectoparasites as well as those infected with both endoparasites and ectoparasites.

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## A study of parasites of three imported ornamental fishes

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Invasive alien species pose a significant threat to biodiversity in the area where they have been introduced. In South Africa, most of the ornamental fish species in the market are imported from other countries. The diseases and parasites of alien species can be transported along with their hosts and become co-invasive. In the current study, three ornamental fish species, Red rainbowfish, *Glossolepis incisus* (n = 21); Pearl gourami, *Trichopodus leerii* (n = 15) and Clown loach, *Chromobotia macracanthus* (n = 15), which were imported from Indonesia using an established trading company, were received, and examined for the presence of parasites. Standard methods were used for the dissection of fish, examination, fixation and preservation of parasites. The prevalence and mean intensity of different parasites of each fish species were determined and they were identified to genus and species level where possible. Red rainbowfish were infected by *Camallanus cotti*, *Lernaea cyprinacea* and two new species of monogeneans (named *Ancyrocephalidae* sp.1 and *Ancyrocephalidae* sp.2). Pearl gourami were infected by *Procamallanus* sp., two species of gryporhynchid cestode larvae, *Neogryporhynchus cheilancristrotus* and *Dendrouterina herodiae* and cestode larvae of bothriocephalids. The Clown loach had no parasites. The highest prevalence and mean intensity for monogeneans and nematodes were observed in Red rainbowfish. The present findings confirm the need for awareness of possible translocation of non-native parasites together with their hosts.

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Poster Presentation Session 1

**Intralacustrine morphological variation of *Dolicirroplectanum lacustre* infecting Nile perch (*Lates niloticus*) in African freshwater systems**

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*Dolicirroplectanum lacustre* (Monogenea, Diplectanidae) is a gill parasite of latid fishes (*Lates* spp.), which inhabit most major African freshwater systems. The species presumably experiences a failure to diverge, meaning that the lineage diversifies at a slower rate than expected when compared to its host, or other monogeneans. We have evaluated both intra- and interlacustrine diversification of *D. lacustre* by characterising populations in three of the East African Great Lakes: Lake Victoria, Lake Albert and Lake Kyoga. Parasites from preserved specimens of Nile perches (*Lates niloticus*) originating from each of these three lakes were collected. Morphometric analysis of sclerotised structures revealed clear intralacustrine differences reflecting two morphotypes among the population of Lake Albert. In contrast, a lack of morphological differentiation has been observed between the lakes. Population structure based on sequence analyses of four gene portions (nuclear 28S, 18S + ITS1, rDNA and mitochondrial COI) did not reveal patterns coinciding with the distinct morphotypes within Lake Albert. No interlacustrine patterns were detected. Low genetic differentiation together with the observed morphological variability suggest a high degree of phenotypic plasticity in *D. lacustre*, thus, confirming a lack of speciation within this monogenean lineage. Low variability between populations of *D. lacustre* is in accordance with the limited variation observed among the host, *L. niloticus*. Microhabitat distributions on gills among the examined populations of *D. lacustre* suggest non-random microhabitat selection. In addition to these distributions, sympatric occurrence of the two different morphotypes from Lake Albert on the same host indicates possible ongoing speciation processes resulting in the observed phenotypic variation. This could be a result of a possible niche shift among the *D. lacustre* population of Lake Albert. Alternatively, the possible existence of different ecotypes of *L. niloticus* could account for the observed intralacustrine differences among the parasite morphotypes. Future research regarding the population structure of *L. niloticus* in the investigated lakes based on molecular differences could clarify the observed intralacustrine morphological variability. Additionally, other genetic markers could be used to investigate genetic differences between the two parasite morphotypes from Lake Albert reflected in the observed morphological differentiation.

## Unravelling the parasite diversity on the gills of chromidotilapiine cichlid fishes in West and Central Africa

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Cichlid fishes and their gill parasites belonging to *Cichlidogyrus* Paperna, 1960 (Platyhelminthes: Monogenea) are one of the most extensively studied host-parasite species networks in Africa. This network has been proposed as a model system for studying the macroevolutionary processes shaping host-parasite interactions. Yet only a small proportion of the cichlid hosts are screened for parasites. In order to close a part of this knowledge gap, we investigated the parasite fauna of cichlid species belonging to the tribe Chromidotilapiini. Despite being the most species rich tribe in West and Central Africa, only five species have been examined for gill parasites so far. In this study, gills of 23 different host species were screened. The sclerotised parts of the attachment and reproductive organs were measured and drawn using interference microscopy. Potential novel species have been found belonging to *Cichlidogyrus* and *Onchobdella* Paperna, 1968, with host specificity ranging from specialist to generalist. *Onchobdella* n. sp. infects species from Chromidotilapiini. From previous records this genus infects two hemichromine cichlids. Hemichromini together with Chromidotilapiini is one of the basal lineages. *Cichlidogyrus* n. sp. show morphological similarities with species infecting the sister taxa of Chromidotilapiini, which could be explained by the similar ecology and physiology of the hosts. These similarities might result in a non-differential selection pressure on the morphology of the haptor of their parasites. Resemblances in haptor morphology are also observed with species infecting tropheines, a tribe endemic to Lake Tanganyika. Here, the pattern might be explained by convergent evolution. These results give additional information on the diversity of chromidotilapiine cichlids in West and Central Africa.

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## **A systematic review and meta-analysis of ticks and tick-borne diseases in the Southern African Development Community (SADC) region**

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Ticks are haematophagous arthropods of veterinary and public concern resulting in major economical suffering due to damage they cause to livestock hides, pathogens they are transmitting and the cost of their control. This study is a systematic review and meta-analysis of articles published on ticks and tick-borne diseases (TBDs) in the SADC region following PRISMA 2020 guidelines. A total search of 347 articles were initially identified through PubMed, ScienceDirect, Google Scholar, AJOL and Springer Link. After assessment of titles, abstracts and eligibility, only 61 articles that reported the prevalence of ticks and tick-borne pathogens (TBPs) fulfilled criteria for inclusion. Different ticks and TBPs were detected from genera *Amblyomma*, *Boophilus*, and *Rhipicephalus* which reported 18355 tick samples from 21 studies. Overall pooled prevalence estimate (PPE) of TBPs in animals was 52.2% (95%CI: 43.9 - 60.3%) with highest PPE in cattle (51.2%), followed by sheep (45.4%) and goats (29.9%). For TBPs, *Anaplasma marginale* had a PPE of 45.9% followed by *A. centrale* (14.7%), *A. phagocytophilum* (2.52%) and *A. bovis* (0.88%) whilst the *Ehrlichia ruminantium* had a PPE of 4.2%. For piroplasmids, *Babesia bigemina* and *B. bovis* had a PPE of 20.8% and 20.3%, respectively. For *Theileria*, *T. velifera* had the highest PPE of 43.0% followed by *T. mutans* (29.1%); *T. parva* (25.0%) and other *Theileria* spp. (14.06%). Our analysis revealed the highest PPE of TBPs in Mozambique (62.9%) followed by Tanzania (57.8%), Angola (54.3%), South Africa (52.2%), Zambia (41.7%), Zimbabwe (35.11%) and Botswana (19.43%), with most studies recorded from South Africa (n=18) and the least in Zimbabwe (n=1). Data analysed in this study has showed that other SADC countries lack published scientific information on ticks and TBDs. There is therefore a need for consolidated scientific studies on ticks and TBDs in the SADC region in order to formulate effective control studies in the region.

## **Anthelmintic resistance in small ruminants of North-West Province, South Africa**

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The continuous development of anthelmintic resistance (AR) threatens the maximum output of livestock production, particularly for resource challenged farmers in rural areas (Mphahlele 2020). This study aimed to assess the level of AR in small stock in the North-West province of South Africa. A questionnaire survey was conducted to assess treatment strategy and farm management practices to 86 small-scale farmers in the North-West province. Results indicated that small-scale farmers (89%) relied solely on the use of anthelmintics, while 11% did not practice any form of worm control practice. Most farms that used anthelmintics preferred Benzimidazoles (BZD) (89%), Oxytetracycline (78.14%), Levamisole (LEV) (18%) and Macrocyclic lactones (ML) (3.44%). The most common risk factor associated with the occurrence of AR in this study was the use of visual appraisal to determine dosage of treatment drugs and treating livestock based on clinical signs rather than parasite epidemiology. The faecal egg count reduction test (FECRT%) was used to assess the development of AR of BZD, LEV, and ML in sheep and goats of small holder farmers. Anthelmintic efficacy of 50% was considered as the threshold for development of AR. No significant difference was shown in nematode egg count after 14 days ( $p=0.380$ ). High levels of AR development, particularly against BZD, was detected. Egg hatch assay (EHA) and larval mortality assay (LMA) was used to determine AR at a discriminating dose (DD) of 0.1  $\mu\text{g/ml}$  TBZ. The EHA and LMA results showed development of AR against TBZ in all districts. A strong correlation existed between FECRT, EHA, and LMA as tests confirmed the occurrence of AR in all the districts identified. Resistant gastrointestinal nematodes (GIN) after treatment were confirmed using polymerase chain reaction (PCR) targeting the Internal transcribed spacer 2 (ITS2) gene using genus specific primer pairs. PCR detected the presence of *Haemonchus* spp. and *Oesophagostomum* spp. post AR tests. These findings reveal the presence of AR, highlighting the necessity for resource-poor farmers to be trained/educated on how to prevent AR development in small ruminants.

### Session 3

## Metazoan parasites of anurans from the Vhembe area, Limpopo, South Africa

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Amphibians were the first vertebrate group to appear on land about 350 m.y.a and diversified and colonised all convivial continents and islands since. Along with the amphibians, their parasites diversified and speciated and today amphibians are hosts to vast numbers and an astonishing diversity of parasites representing all major parasitic groups. These include protozoans, nematodes, acanthocephalans, monogenic flukes, digenetic flukes, cestodes, leeches and mites. This impressive parasite diversity can be explained by the fact that amphibians are strongly associated with water, which facilitates parasite transmission. The parasite fauna of amphibians across the globe is in general poorly understood and understudied. This is also the situation for southern Africa where more than 170 amphibian species are currently known. In the present study, we aimed to shed light on the diversity of metazoan parasites from anurans, within the Vhembe Biosphere Reserve that includes the Soutpansberg Mountain range, Limpopo Province, South Africa. A total of 314 specimens of frogs representing 25 species were collected, dissected and all parasites were collected. We documented 36 species of metazoan parasites during this survey, including 22 species of nematodes, four cestodes, four trematodes, three monogeneans, one acanthocephalan, one mite and one annelid species. *Amietia delalandii* (common river frog) and *Xenopus leavis* (African clawed frog) harboured the biggest parasite diversity with six and five helminths, respectively. In a relatively small study area, and in only 25 species of anurans, we found seven nematodes species that are morphologically and genetically different from all previously known species. Understanding the interaction patterns and determinants of the host-parasite association is necessary to fill the knowledge gaps in both community and disease ecology. Parasites are everywhere, diverse, creative and we believe that their integration into fundamental biological studies is of utmost importance and will be a major step forward.

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## **An integrative taxonomy approach to document the rectal endociliates of the globally invasive amphibian *Xenopus laevis***

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Amphibians act as hosts to a variety of parasite groups including cestodes, nematodes, acanthocephalans, monogenetic flukes, digenetic flukes, acarids and protozoans. However, of all parasite groups infecting amphibians, the protozoan gut ciliates are perhaps the most abundant, yet understudied group. The latter is clearly illustrated in one of the world's most widely distributed amphibian invaders and well-known model animal, the African clawed frog *Xenopus laevis* (Daudin, 1802) (Anura: Pipidae), where the study of protozoan symbionts has fallen behind that of its metazoan counterparts. Previous studies documenting intestinal ciliates of amphibians from South Africa are limited and employ outdated taxonomic techniques. These studies primarily dealt with the systematics of ciliate groups, rarely identifying ciliates below the genus level. Consequently, difficulties arise when rectal endociliate assemblages are compared among populations of the host, in their native range as well as elsewhere. Therefore, the aim of this study was to document the gut ciliate diversity of *X. laevis* by using an integrated taxonomic approach. For the first time, the ciliates of this African anuran were studied both morphologically and molecularly through modern as well as classical techniques. These techniques included differential interference contrast advanced microscopy, scanning electron microscopy, confocal microscopy, silver impregnation, haematoxylin staining and DNA barcoding. Ultimately, this integrated approach will ease future ciliate identification in other anuran hosts and allow a better understanding of the host-parasite relationships, ecological role and diversity of ciliates in a globally invasive species.

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## The biodiversity of frog blood parasites from the Vhembe Biosphere, Limpopo, South Africa

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The Soutpansberg mountain range is part of the Vhembe Biosphere Reserve, an area known to harbour at least 35 species of anurans. Additionally, anurans are the suited host for a variety of blood parasites consisting of intra- and extracellular blood parasites. Intracellular blood parasites include haemogregarines, haemococcidia, intraerythrocytic viruses, rickettsiae intraerythrocytic bacteria, and intrathrombocytic parasites. Extracellular parasites include trypanosomes and microfilariae nematodes. However, currently no data is available for anuran blood parasites from within the study area. In the present study, we show that the rich anuran diversity found within the Vhembe Biosphere serves as hosts for a number of blood parasite species. Blood samples from over 400 individual anurans, and across 22 species, were collected and screened for blood parasites. Preliminary results indicated that 10/22 anuran species from within the study were found infected by several protozoan blood parasites including species of *Trypanosoma*, *Hepatozoon*, and *Schellackia*, and one species of filarial nematode *Neofoleyellides steyni*. These results demonstrate a diverse parasite community across the Vhembe Biosphere providing insight to understanding the diversity and distribution of South African anuran blood parasites.

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## Oral Presentations – Wednesday 15 September

### Session 4

#### **Molecular characterization of the economically important South African *Rhipicephalus* tick species of domestic animals and their bacterial communities**

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Tick infestation and tick-borne diseases are a major concern to domestic animals. Accurate identification and characterization of ticks and their associated microbial communities may lead to development of improved control strategies targeting known tick-bacterial interactions. Current technologies allow us to simultaneously detect microbiota of ticks. These include microbial communities of ecological, commensal, symbiotic or parasitic in nature. Various *Rhipicephalus* tick species have been collected from domestic animals including cattle, sheep, goats, horses and dogs as well as from the vegetation. The ticks were morphologically identified as *R. appendiculatus*, *R. evertsi evertsi*, *R. sanguineus* and *R. simus*. Colony reared ticks including, *R. evertsi evertsi*, *R. microplus* and *R. decoloratus* were provided by ClinVet. These ticks were further characterized by PCR and sequencing of the CO1, ITS2, 16S and 18S genes. The phylogenetic analysis of the above-mentioned ticks indicated that the collected species were accurately identified as they clustered with their respective reference species from the GenBank database. The next generation sequencing has been conducted on the Illumina MiSeq platform to characterize the microbiota harboured by these ticks. Data analysis of the generated reads is still in progress. Results from this study will provide baseline knowledge of the microbiome of *Rhipicephalus* tick species infesting domestic animals in South Africa.

## **Clinico-haematological and biochemical features of natural Babesiosis in Nigerian breeds of cattle**

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The importance of Babesiosis as a clinical condition in cattle cannot be overemphasized, especially in the tropics. This study evaluates the haematological and biochemical changes associated with bovine Babesiosis in Nigerian cattle breeds. A total of 50 samples, confirmed positive for *Babesia* spp., from 305 sera collected from clinically suspected cases of Babesiosis from cattle farms in Oyo state, Southwest Nigeria were analysed. Breeds were identifiable by their morphological features; age was estimated using rostral dentition and sexes morphologically determined. Haematological and biochemical analysis were done by standard procedures and the use of specific kits respectively. Cattle breeds comprised 32 (64%) White Fulani breed, 8 (16%) Sokoto Gudali, 8 (16%) Red Bororo and 2 (4%) Kuri. Of the female animals, 43 (86%), displayed susceptibility while 7 (14%) of males were susceptible. Of the older cattle ( $\geq 4$  yrs), 27 (54%) were susceptible, 17 cattle (34%) from the 3-3½ yrs-old group displayed susceptibility, while 6 of the 2-2½ yrs-old group had the least susceptibility of 6 (12%). Hematological analysis revealed the best parameters in the White Fulani breed with a mean  $\pm$  SD of PCV 28.6  $\pm$  8.76, HB 9.3  $\pm$  2.98 and RBC 7.5  $\pm$  2.44, while the lowest values were found for the Sokoto Gudali breed with the following parameters; PCV 24.6  $\pm$  6.89, HB 8.1  $\pm$  2.29, RBC 7.8  $\pm$  1.59. Biochemical analysis revealed White Fulani to have the most optimal values as follows; Total protein 5.5  $\pm$  1.87, Albumin 1.3  $\pm$  0.20, Globulin 4.2  $\pm$  1.73, Glucose 58.1  $\pm$  18.0, AST 60.3  $\pm$  15.68, ALT 50.7  $\pm$  14.80. The most optimal parameters were observed in the 3-3½ yrs-old group as follows; PCV 29.2  $\pm$  7.76, HB 9.4  $\pm$  2.72, RBC 7.6  $\pm$  2.52, Total protein 5.5  $\pm$  2.11, Glucose 56.9  $\pm$  20.31, AST 61.1  $\pm$  17.15 compared to the 2-2½ yrs with the following values; PCV 26.6  $\pm$  9.81, HB 8.3  $\pm$  3.28, RBC 7.1  $\pm$  2.27, Total protein 4.5  $\pm$  1.64, Glucose 49.3  $\pm$  20.85, AST 54  $\pm$  11.01. No significant difference in their indices were observed, except in blood urea nitrogen value which was significant among the breeds sampled. This investigation reveals variation in the susceptibility to natural Babesiosis among Nigerian cattle breeds, ages and sexes with generalise anaemia, lymphocytopenia, neutrophilia, hypoproteinaemia, hypoalbuminaemia and extremely low blood urea nitrogen.

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## Temporal dynamics of *Anaplasma marginale* infection in calves at the wildlife-livestock interface in the Mnisi communal area, Mpumalanga, South Africa

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Bovine anaplasmosis, caused by *Anaplasma marginale*, is one of the most important tick-borne diseases of cattle in South Africa, with an estimated cost due to mortality of R115 million per year and further costs attributable to morbidity and tick control. Through the Health and Demographic Surveillance System in Livestock in the study area of the Mnisi One Health Platform, Mpumalanga, information has been collected on the cattle in the area, indicating the presence of *A. marginale*. This study aimed to investigate the infection dynamics in calves (n=10) in two areas of the Mnisi community during a one-year period, and the diversity of circulating *A. marginale* strains. Blood samples were collected monthly from five calves each in a peri-urban area and at a wildlife-livestock interface. A duplex qPCR assay confirmed the presence of *A. marginale* in all five calves in the peri-urban area but in only two calves at the wildlife-livestock interface; *A. centrale* was not detected. Preliminary results of *A. marginale* strain diversity as determined by *msp1a* genotype analysis revealed more than 50 genotypes (more than 10 genotypes from the wildlife-livestock interface and more than 40 genotypes in the peri-urban area) circulating in the calves during the one-year study period, with five *msp1a* repeats that have not been previously reported. Our results suggest that calves in the peri-urban area were more likely to be exposed to and infected with *A. marginale* than calves in the wildlife-livestock interface, resulting in endemic stability in the peri-urban area. This finding correlates with the occasional bovine anaplasmosis cases that have been observed at the wildlife-livestock interface. Methods of cattle management, acaricide treatment and cattle density could explain differences in exposure to *A. marginale* in the two areas. Our results revealed that most calves in the area were superinfected by distinct *A. marginale* strains within the 12-month study period, indicating a continuous challenge with multiple strains that should lead to robust immunity in the calves and endemic stability in the area.

## Prevalence of gastrointestinal parasites in small ruminants from selected farms in Akinyele Local Government Area of Oyo State, Nigeria

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Gastrointestinal parasites pose serious health challenges to food producing animals and such unchecked parasitism has led to huge economic losses in the livestock industry. The prevalence of gastrointestinal parasites in small ruminants reared on three selected farms in Akinyele Local Government Area, Oyo state, Nigeria, was determined between the months of April and June 2019. Faecal samples were obtained using standard field and laboratory techniques from 121 randomly selected, apparently healthy, small ruminants consisting of 53 goats and 68 sheep. An overall prevalence of 95.87% accounting for 116 positive small ruminants was recorded. The four species of parasites isolated were *Strongyle* spp, *Eimeria* spp, *Moniezia* spp and *Strongyloides* spp in single parasite infection with respective prevalences of 12.4%, 3.31%, 5.79% and 0.83% in goats and 14.88%, 12.40%, 5.75% and 0% in sheep. Similarly, mixed parasites infections of *Strongyle* + *Strongyloides* spp, *Strongyle* + *Eimeria* spp, *Moniezia* + *Eimeria* spp, *Strongyle* + *Moniezia* spp, *Strongyle* + *Moniezia* + *Eimeria* spp and *Strongyle* + *Strongyloides* + *Eimeria* spp had prevalences of 1.65%, 10.74%, 0.83%, 1.65%, 2.48% and 2.48% in goats and 1.65%, 14.88%, 2.48%, 0%, 1.65% and 0% in sheep, respectively. Age-specific prevalence showed 97.78% and 90.32% in small ruminants younger than two years old and those older than two years old respectively, which was statistically significant ( $P < 0.05$ ). Also, the study revealed that females had a higher prevalence of 98.77% while males had a prevalence of 90.0% which was statistically significant ( $P < 0.05$ ). This study provides valuable information on the gastrointestinal parasitic status of small ruminants reared at the farm settlements at Akinyele Local Government Area, Oyo state, Nigeria, and calls for a more concerted effort in preventing and controlling intestinal parasites to enhance productivity in these food animals.

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## Development of resistance to acaricides by natural tick populations on cattle: A global systematic review and meta-analysis

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The development of resistance by field ticks to acaricides is a major problem in the livestock industry in tropical and subtropical regions of the worldwide. Although prior traditional review articles have described the nature of the acaricide resistance problem, no study seem to have systematically integrated quantified findings from published reports to provide an overall magnitude of the acaricide resistance problem. To determine the current global trends and prevalence of acaricide resistance development (ARD) in natural tick populations on cattle, a systematic review and meta-analysis with emphasis on *Rhipicephalus (Boophilus) microplus* was conducted. Data searches from five English electronic databases, provided 88 journal articles that were published between 1992 to 2020. In total, 218 in-vitro bioassays investigated 3939 field tick populations on cattle, of which 57.6% exhibited ARD, largely limited to South America (Brazil), Central America (Mexico) and Asia (India). A total of 3391 of these field populations were *R. (B.) microplus*, of which 2013 exhibited ARD. Random effects meta-analyses indicated that the exhibition of ARD was higher in *R. (B.) microplus* (66.2%) than in other tick species. Global prevalence estimates of ARD in *R. (B.) microplus* varied as a function of geography, detection methods and acaricide compounds. Generally, high heterogeneity levels were noted among studies. Conversely, homogeneity was observed among studies from Asia (India), suggesting the establishment of acaricide resistance in the Indian *R. (B.) microplus* field populations. Current tick control interventions are urgently needed to limit the evolution and implications of resistance development.

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## A descriptive study of parasites detected in ticks of domestic animals in Lesotho

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Ticks are medically important and significant vectors of diseases affecting livestock, humans, and companion animals more than any other arthropod vectors. In the absence of information on the relationship of tick species and piroplasms parasites in Lesotho, the current study was aimed at detecting piroplasms parasites of economic importance from ticks of domestic animals. A total of 322 pooled tick DNA samples were subjected to PCR screening for the presence of piroplasms. The overall infection rate of piroplasms was 7% with *Babesia bigemina* at 3.4% (11/322), *B. bovis* 0.3% (1/322), *B. ovis* 2.8% (9/322) and 0.6% (2/322) for *B. motasi*. DNA extracted from the Lesotho *Rhipicephalus decoloratus* and *R. evertsi evertsi* tested positive for the presence of *B. bigemina* with a 15% and 3% infection rate, respectively. *Otobius megnini* tested positive for only *B. bovis* at a 12.5% infection rate. *Rhipicephalus e. evertsi* was the only tick species PCR positive for ovine babesiosis with 3.2% for *B. ovis* and 0.7% for *B. motasi*. Equine piroplasm (*Theileria equi* and *B. caballii*) and *Theileria* (*T. parva* and *T. ovis*) parasites were not detected in the current study. The PCR-positive samples were confirmed by direct sequencing of the product. This study is the first to report on a relationship of *Babesia* parasites with tick species in Lesotho and it is evident that vector-borne diseases are present in ticks of domestic animals in this country. Research findings in this study require a joint effort from both veterinary and medical sectors to unite and conduct more epidemiological studies of tick-borne diseases in both animals and humans and to also determine the role played by tick species in the transmission of the detected parasites in domestic animals of Lesotho. This information provides a baseline knowledge of important piroplasms parasites and raising awareness of their prevalence in Lesotho.

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Session 5

**Adult nematode parasites of *Clarias gariepinus* (Burchell, 1822) from Lake Heritage, in the Crocodile River, South Africa using light and scanning electron microscopy**

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The African sharptooth catfish, *Clarias gariepinus*, is host to many endoparasitic nematodes. Presently, ten families have been recorded from this host in Africa, including both larval and adult forms. The present study focused on the adult nematode biodiversity of sharptooth catfish in Lake Heritage in Gauteng, South Africa, which forms part of the Crocodile River within the Limpopo River system. Boomker (1982) conducted a study in Hartbeespoort Dam, downstream of Lake Heritage, and identified a high prevalence of two camallanid nematodes, *Procamallanus (Procamallanus) laeviconchus* and *Paracamallanus cyathopharynx*, in the intestine of *C. gariepinus*, as well as an erratic occurrence of *Chordocephalus* sp. However, *Procamallanus (Procamallanus) pseudolaeviconchus* (Moravec and van As, 2015) has since been described from *C. gariepinus*, requiring confirmation of the identification of *Procamallanus* species in historical studies. In the present study, *C. gariepinus* were collected from Lake Heritage in the summers of 2019 and 2020, and the gastrointestinal nematodes removed. Light microscopy, both brightfield and fluorescence, as well as scanning electron microscopy (SEM), was used for morphometric identification of the nematodes. This included soft tissue digestions and microdissections of buccal capsules and spiculae to allow for their study using SEM, the latter of which was described for the first time for these camallanid species. Additionally, DNA barcoding based on 18S and 28S rDNA, and CO1 mtDNA was used to identify the nematodes. Two camallanid species, *P. (P.) pseudolaeviconchus* and *P. cyathopharynx*, were identified based on both morphometric and genetic characterisation. However, two morphotypes of *P. cyathopharynx* were recorded upon further examination, with distinct differences observed in their buccal capsule morphometry. Other than the camallanids, an unknown specimen, which lacked a buccal capsule, was recovered, corroborating the identification of *Chordocephalus* sp. by Boomker. In terms of nematode biodiversity, the adult endoparasitic diversity of *C. gariepinus* has not deteriorated in this river system since Boomker's study, but the use of modern techniques provided additional taxonomic information and revealed additional morphological observations. Additionally, it was confirmed that *P. pseudolaeviconchus* and not *P. laeviconchus* occurs at this site.



***Spinitectus petterae* Boomker, 1993 infecting *Clarias gariepinus* (Burchell, 1822) from the Vaal River system: a scanning electron and light microscopical study of aspects of the pathology**

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Histopathological studies on *Spinitectus* are limited, focusing mostly on North and South American species, with no African species studied. The aim of this study is to determine the histopathological effect of *Spinitectus petterae* in the stomach of *Clarius gariepinus*. Stomach tissue samples were collected from infected hosts from the Vaal Dam, Vaal River Barrage near Parys, and below Grootdraai Dam near Standerton. Samples were fixed in either 10% neutral buffered formalin (NBF) or 70% ethanol. Thereafter, 10% NBF samples were washed and dehydrated with ethanol and stored in 70% ethanol. For scanning electron microscopy (SEM), specimens were further dehydrated with ethanol and hexamethyldisilazane (HMDS) and studied with the aid of a VEGA 3 LMH SEM. Light microscopy samples were dehydrated and embedded in resin. Resin blocks were sectioned at 5-7 µm and stained with heamatoxylin and eosin (H&E). Light photomicrographs were taken with the aid of a Zeiss Axioplan 2 Imaging Light Microscope with Axiovision 4.7.2 software. Leukocyte counts of normal and infected tissues were done according to the physical dissector principle, using 10 serial sections. The anterior of the parasite was embedded into the stomach mucosa and covered by mucus and leukocytes. At the penetration site, host cells displayed structure and membrane disorder and were covered in blebs. *Spinitectus* tunnelled through the mucosa and gastric glands, the annular spines inserted into the surrounding tissue. The epithelial layer was mechanically disrupted, and gastric glands torn from the lamina muscularis mucosa, resulting in ulceration. Nematodes penetrated to the level of the submucosa; the anterior became encapsulated by a chronic foreign body granuloma, indicative of an extended period of association. Bacteria and host leukocytes were present inside the vestibule of the nematodes. All leukocyte counts, except for basophils, were significantly elevated in infected lamina muscularis mucosa, indicating a strong cellular response. It is concluded that the impact of *S. petterae* on the health of *C. gariepinus* is severe. In aquaculture environments, in the presence of a suitable intermediate host, high intensities of *S. petterae* could occur and have a serious impact.

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## The metazoan parasite community of invasive *Clarias gariepinus* (Burchell, 1822) in South Africa: testing the co-invasive, spillback and enemy release hypotheses

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Africa is home to one of the most widely distributed freshwater fish, the African sharptooth catfish *Clarias gariepinus* (Burchell, 1822). Native in almost all countries on the continent, it is one of the best studied freshwater fish species. This includes its parasitic community that is considered to be the most diverse on the continent. *Clarias gariepinus* has also been translocated to various freshwater systems for aquaculture or sustaining subsistence fisheries. Although fishery activities are not primary practice in South Africa, these freshwater systems did not escape the invasion of this hardy apex predator. In the Western Cape (WC) and Eastern Cape (EC) provinces, *C. gariepinus* has been translocated mainly for recreational activities and attempts at aquaculture. In addition, the construction of inter-basin water transfer schemes largely contributed to their invasion, by serving as an introduction pathway from their southernmost native distribution in the Orange River into the Great Fish River (EC) and Riviersonderend (WC). As in most invaded freshwater systems, few to no studies are conducted on invasive hosts' parasitic communities and the potential for co-invasion, parasite spill-over and spillback. Furthermore, the enemy release hypothesis is not frequently assessed. During the present study, the parasite communities of 15 and 11 individuals of invasive *C. gariepinus* in the Riviersonderend (WC) and Great Fish River (EC), respectively, were investigated. In general, metazoan parasite prevalence was 100% (WC) and 91% (EC), with an overall intensity of infection of 1–303 (WC) and 1–305 (EC) individuals per host. Ecto- and endoparasitic communities at both localities were represented by taxa known from *C. gariepinus* and included cestodes (*Barsonella lafoni* and *Tetracampos ciliotheca*), crustaceans (*Ergasilus* sp.), monogeneans (*Quadriacanthus* spp.), nematodes (*Contraecaecum* sp. and *Paracamallanus cyathopharynx*), and trematodes (*Tylodelphys mashonensis* and *Orientocreadium batrachoides*). Our findings support that co-introduction did occur, that there is no parasite spillback at either of the two localities, and that enemy release occurred at both localities in the host having a low parasite richness when compared to native parasite communities. Future studies should focus on potential spill-over of any of these co-introduced parasites to native hosts.

## **Somewhere I belong: using phylogenetic comparative methods and machine learning to investigate the evolution of a species-rich lineage of parasites**

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A substantial portion of species on the planet have a parasitic lifestyle, and these parasites can have an extraordinarily intimate relationship with their host. Some of these host species evolved through explosive radiation, which might have impacted the associated parasite fauna too. We investigated the character evolution of a lineage of gill parasites (Monogenea: Dactylogyridae: *Cichlidogyrus*) infecting cichlid fishes, which is an established model system of evolutionary research due to several adaptive radiation events in the African Great Lakes. To investigate the character evolution of the parasites, we applied multivariate phylogenetic comparative methods (PCMs) and machine learning algorithms (support vector machines – SVMs) to morphometric and host range data widely inferred from the 137 flatworm species. For PCMs, we modelled the attachment organ and host range evolution using the data of 135 species and an updated multi-marker (28S and 18S rDNA, ITS1, COI mtDNA) phylogenetic reconstruction of 58/137 described species. Through a cluster analysis, SVM-based classification, and taxonomic literature survey, we inferred the systematic informativeness of discretised and continuous characters. Regarding the attachment organ evolution, PCMs suggest a pattern associated with multiple selective regimes across the phylogenetic tree. Host and environmental parameters might put this structure under stabilising selection as indicated by a limited morphological variation. The multicollinearity in most measurements, a moderate 73% accordance rate of taxonomic approach and SVMs, and a low systematic informativeness of reproductive organ data suggest an overall limited systematic value of the measurements included in most species characterisations. Furthermore, we concluded that PCMs and SVM-based approaches are suitable tools to investigate character evolution in parasitology.

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## **A planned taxonomical study and a twenty-year period, statistical overview of Sessilida Kahl, 1933 (Oligohymenophorea: Peritrichia) found on chiton species along the South African coastline**

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The scientific literature on sessiline peritrichs (Order Sessilida Kahl, 1933) is comprehensive but focuses mainly on the species that cause fish diseases in aquaculture. Limited research has been done on natural host populations and even less data is available on the taxonomic descriptions and ecology of these symbionts in an African context. Previous surveys along the South African coastline have resulted in the description of *Mantoscyphidia branchi* Van As, Basson and Van As, 1998 from the gills of 19 limpet species, *Mantoscyphidia fanthami* Basson, Botha and Van As, 1999, an ectosymbiont on the gills of five marine topshell species, as well as *Mantoscyphidia spadiceae* Botes, Basson and Van As, 2001 and *Mantoscyphidia midae* Botes, Basson and Van As, 2001 collected from two *Haliotis* Linnaeus, 1758 species, respectively. Worldwide only eight *Mantoscyphidia* (Jankowski, 1980) species are known to be associated with marine gastropod hosts (Class Gastropoda Cuvier, 1795). No research has, however, been done on the occurrence of sessiline peritrichs on other molluscan classes, such as chitons (Class Polyplacophora Blainville, 1816). The current study aims to provide insight into the taxonomic status, distribution, biodiversity and host specificity of sessiline peritrichs occurring on the gills of more than 10 chiton species in the South African intertidal zone. Collection of the chiton material will provide information of the hosts themselves, such as their ecology, distribution, and behaviour. Standard techniques will be used for collection of sessiline peritrichs. Morphometric and comparative analyses will be conducted amongst the possibly different sessiline species. Gene sequencing and molecular analyses will be attempted, to increase the understanding of the evolutionary history of these symbionts, of which almost nothing is known to science. Additionally, data collected by the Aquatic Parasitology group since 1982, will also be analysed and incorporated in the current study. This will provide the first results on more than 600 chiton specimens from various collection sites along the west, south and east coasts of South Africa and symbionts found. In this presentation, a statistical overview of the historic data will be discussed and interpreted by making use of standard parasitology ecological terms.

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## Taxonomic re-evaluation of African anuran trypanosomes with the redescription and molecular diagnosis of *Trypanosoma nelspruitense* Laveran, 1904

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*Trypanosoma* is a genus of extracellular haemoparasitic protozoa, known to infect all vertebrate classes and is globally present. Several trypanosome species cause debilitating diseases in humans and livestock. The aquatic and terrestrial clades of *Trypanosoma* could provide insight into the evolutionary history of the genus, as well as complimentary information for biomedical studies of medically and economically important species of *Trypanosoma*. The ecological interactions and phylogeny of aquatic trypanosomes is currently not well-understood, mostly due to their complex life cycles and a deficiency of data. The anuran *Trypanosoma* of Africa, of which there are 13 recognised species, are one of the least understood groups. *Trypanosoma nelspruitense* (Laveran 1904) is the only anuran trypanosome species described from South Africa. Blood samples from *Amietia delalandii* (Common River Frog) specimens were collected in South Africa. Microscopic screening of the stained blood smears found that nine specimens were infected with trypanosomes. Two overlapping fragments of the 18S rRNA gene were targeted for phylogenetic analysis. *T. nelspruitense* is redescribed in this study with morphological, morphometric, and molecular data. Two new species of *Trypanosoma* are also described in this study. This is the first study to provide molecular data for species of *Trypanosoma* from South African anurans. A platform for future research on South African anuran trypanosomes is created by this study.

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Poster Presentation Session 2

**Parasites to the rescue? Three new species of *Acanthobothrium* van Beneden, 1849, (Cestoda: Onchoproteocephalidea) from the endangered white skate *Rostroraja alba* (Lacepède), off the Western Cape of South Africa**

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The white skate, *Rostroraja alba* (Lacepède), is listed as an endangered species, the second-highest category in the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species. This species is heavily affected by anthropogenic impacts such as capture induced stress by overfishing and by-catch, habitat degradation and pollution (amongst others) that caused a drastic decline in populations in recent years. As part of a larger study on elasmobranch parasites in southern Africa, two specimens of *R. alba* were screened for parasites. In these, tapeworms of the genus *Acanthobothrium* van Beneden, 1849 (Cestoda) were discovered. Application of Caira and Ghoshroy's classification system facilitated the differentiation of congeners through a combination of specific morphological characteristics. Consequently, three species new to science were discovered (manuscript in preparation). Considering these new discoveries from an endangered host, it is important to also start addressing the conservation status of its parasites. Helminth parasites such as cestodes have co-evolved with their elasmobranch hosts for millions of years, thereby creating a unique and intimate host-parasite interrelationship. Currently, altering environmental conditions caused by anthropogenic pressures have direct impacts on this host-parasite system, increasing the extinction risks. Merely 9% of elasmobranchs (18 of 204 species) in South African waters have been examined for parasites, which leaves an enormous hidden species diversity. Given the decline in host populations and steady increase in the number of threatened species, co-extinction events are likely, leading to the loss of many parasite species including undescribed species. Parasites provide ecosystems with fundamental functions and services, occupying key positions in food webs and form vital indicators of ecosystem health. These co-extinctions might therefore trigger a series of long-term indirect negative effects, leaving detrimental repercussions, which are currently not fully understood. Extensive studies on elasmobranch parasites and their hosts implementing multisource approaches (i.e. biogeography, ecology, ecotoxicology) are needed. This will provide a much better understanding on the intimate nature of host-parasite systems that may lead to new prospects in conservation science and the preservation of threatened host species, such as *R. alba*, together with their unique parasite fauna.

**First record of *Ligophorus minimus* Euzet et Suriano, 1977 (Monogenea: Dactylogyridae) from the endemic South African mullet, *Chelon richardsonii* (Smith, 1846) (Mugiliformes: Mugilidae)**

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The class Monogenea is composed of hermaphroditic organisms with the majority being ectoparasites found on the gills, nasal nostrils, and external surface of marine and freshwater fishes. The genus *Ligophorus* presents strict specificity to mugilid fishes, containing more than 50 valid species reported mostly from the Mediterranean Sea, Black Sea, and Red Sea; with some records from the coast of South America, Japan, China and the Sea of Malaysia. There has been a recent increase in phylogenetic (ribosomal genes) and morphometric studies for species of *Ligophorus*. *Chelon richardsonii*, the South African mullet, is endemic to the South African coast, dwelling in both marine and brackish environments. In March 2020, five individuals of *C. richardsonii* were sampled with a seine net in the Groot River estuary within the Garden Route National Park, South Africa. Fish were euthanised according to ethics protocols (percussive stunning and severance of the spinal cord). Gills were removed and screened for ectoparasites under a stereomicroscope. Monogeneans were removed from the gills using fine needles and flattened in slides with glycerine ammonium picrate (GAP) for morphological analyses while some specimens were placed in sterilised tubes with 96% grade ethanol for molecular analyses. Sequences of the partial 28S rDNA (large subunit) gene of four isolates, and sequences of the partial 18S rDNA (small subunit) gene of two isolates were newly generated. The dactylogyrid specimens found were identified as *Ligophorus minimus* based on the morphology of the sclerotized structures of the haptor (bars and anchors) and reproductive organs (vagina and male copulatory complex). Basic Local Alignment Search Tool (BLAST) confirmed the morphological identification of the specimens found. The newly generated 28S and 18S sequences presented 99% similarity with *L. minimus* from the Mediterranean Sea. This is the first record of *L. minimus* from South Africa, as well as the first host record for *C. richardsonii*. *Ligophorus minimus* has only been recorded from *Liza saliens* from the Mediterranean Sea and the Black Sea. This study expands the distribution of *L. minimus* as well as its mugilid host range, contributing to the knowledge of helminth parasites of endemic fishes in South African coastal waters.

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## **A new *Dactylogyrus* sp. (Monogenea: Dactylogyridae) from the gills of the smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822) from the Vaal River, South Africa**

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*Dactylogyrus* is considered the most species-rich genus of helminths, with more than 900 nominal species that exhibit strict host-specificity to cyprinids, reflecting the evolutionary history of their fish hosts. *Dactylogyrus* spp. occurring in Africa are, based on the morphology of their sclerotized structures, divided into three groups, namely: *D. afrobarbae*-like group, *D. pseudoanchoratus*-like group, and *D. varicorhini*-like group. Species of the *D. varicorhini*-like group are parasites of yellowfishes of the genus *Labeobarbus*, mostly occurring in Africa. The smallmouth yellowfish, *Labeobarbus aeneus*, is endemic to South Africa's Orange-Vaal River system. In May 2019, five specimens of *L. aeneus* were collected from the Vaal River as part of a larger fish parasitological survey. Fish were euthanised by percussive stunning and severance of the spinal cord and the gills were screened for parasites under a stereomicroscope. Monogeneans were removed from the gills using fine needles and flattened on slides with glycerine ammonium picrate (GAP) in order to study the sclerotized structures. Some specimens were placed in sterilised tubes with 96% grade ethanol for molecular analyses. The morphology of the sclerotized structures of the specimens found correspond to that of the *D. varicorhini*-like group: haptor composed of two transverse bars – dorsal bar butterfly-like shaped, ventral bar V-shaped; anchor base possessing a depression in its middle; presence of a spiral male copulatory organ, accessory piece gutter-like shaped presenting parallel ramifications forming folds. *Dactylogyrus* n. sp. closely resembles *Dactylogyrus oumiensis*, differing mainly by the shape of the ventral bar and accessory piece. A single sequence of the partial 28S rDNA (large subunit) gene and one sequence of the partial 18S rDNA (small subunit) gene were generated for this new *Dactylogyrus* species. Using the Basic Local Alignment Search Tool (BLAST) the new 28S sequence showed 95% similarity with the congeners *D. kulindrii*, *D. volutus*, and *D. zatensis*, which are also part of the *D. varicorhini*-like group, and over 90% of similarity with the same congeners for the 18S sequence. This study contributes to unravelling the diversity of dactylogyrids from fish endemic to South African river systems.



Session 6

## Assessment of *in vitro* anti-trypanosomal activity of nitrofurantoin derivatives against sleeping sickness trypanosomes

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Human African trypanosomiasis (HAT) also known as sleeping sickness is caused by *Trypanosoma brucei gambiense* that is endemic in western and central Africa, and *T. b. rhodesiense* which is endemic in eastern and southern Africa. HAT develops in two stages hence its stage specific treatment. There is currently a trend of resistance to drugs by trypanosome parasites hence the need to continuously develop and assess new therapeutic agents. The aim of this study was to assess the *in vitro* anti-trypanosomal activity of novel nitrofurantoin derivatives against *T. b. gambiense* and *T. b. rhodesiense* parasites. Anti-trypanosomal activity was evaluated against trypanosomes of *T. b. gambiense* IL1922 and *T. b. rhodesiense* IL1501 strains. Cytotoxicity of the nitrofurantoin derivatives was evaluated on the Madin-Darby bovine kidney (MDBK) cells. Anti-trypanosomal activity differed between the *T. b. gambiense* and *T. b. rhodesiense* but both species were resistant to most of the NFT derivatives. Furthermore, the activity was structure specific. The *para*-phenyl fluoro-substituent containing NFT analogue displayed the strongest activity against *T. b. gambiense* and *T. b. rhodesiense* with IC<sub>50</sub> values of 0.08 ± 0.04; 0.11 ± 0.06 µM, respectively. The compound also has log*P* value of 1.72 indicating balanced hydrophilicity and lipophilicity properties which ultimately enable its effective permeation through biological membranes and tissues. The nitrofurantoin derivatives have the potential to be used as anti-trypanocidal agents but require further *in vivo* experiments to assess the efficacy and toxicity in mammalian animal models.

## The mosquitocidal and toxicological properties of novel pyrimidine-phenyl hybrid derivatives

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*Plasmodium falciparum* is the most prevalent species accounting for more than 90% of the world's malaria mortality. While the current eradication programmes against the *Anopheles* vector and *Plasmodium* parasite have been effective, there is a growing concern about toxicity, and the emergence of resistant strains of *Anopheles* and *Plasmodium*. Hence, there is a need for novel insecticidal and antimalarial drugs to prevent transmission and manage infected patients. Hybridisation of two active molecules by a biologically active linker can help overcome the toxicity and emergence of resistance while increasing antimalarial potency. Hybrid compounds undergo pharmacokinetic processes at a single rate, thereby reducing the risk of drug interactions and this offers an advantageous effect compared to combination therapy. Pyrimidine hybrid compounds have shown to possess potent *in vitro* antimalarial, anticancer and insecticidal activity with minimum toxicity. This study is investigating the *in vitro* inhibitory effects of novel pyrimidine-phenyl hybrids on the mosquito larvae and malaria parasite, along with determining a preliminary toxicological profile. The twelve-novel pyrimidine-phenyl hybrid derivatives exhibited moderate mosquitocidal properties (100µM) against *Anopheles arabiensis* 3<sup>rd</sup> instar larvae over 48hrs (5.0-57.5% death) compared to DDT (100% death); with derivatives T2 and T11 the most inhibitory. The pyrimidine-phenyl hybrid derivatives showed minimal toxicity to *Artemia* after 48hrs (2.1-34.9% death), except for T2, T9, T10, T11 that showed toxicity comparable to potassium dichromate (100% death). The pyrimidine-phenyl hybrids exhibited minimal haemolysis of uninfected human red blood cells (<0.1% haemolysis); whilst there was cytotoxicity directed to the human chronic myelogenous leukaemia (K562) cells (IC<sub>50</sub>: 8.1-69.1µM) and the normalised human epithelial kidney (HEK293) cells (IC<sub>50</sub>: 5.0-76.1µM). The antimalarial activity of the pyrimidine-phenyl hybrid derivatives against the NF54 chloroquine-sensitive strain of *P. falciparum* is still to be verified. Computationally determined pharmacokinetic properties of the derivatives indicated that all the derivatives showed drug-like properties with favourable oral absorption and bioavailability according to the Lipinski Rule of Five. This set of pyrimidine-phenyl hybrid derivatives have shown some interesting preliminary pharmacokinetic properties and activity against the *Anopheles* larvae with a varied toxicity profile and warrants further investigation.

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## Identification of sesquiterpene alcohols with larvicidal and anticholinesterase effects on *Anopheles funestus*

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Malaria is a life-threatening disease caused by the *Plasmodium falciparum* parasites transmitted to humans by female *Anopheles* vectors. The main challenge in malaria transmission is the escalating resistance to antimalarial drugs and insecticides signalling an urgent need for novel compounds. Inhibition of the acetylcholinesterase (AChE), the enzyme needed for normal neuronal impulse transmission, has been the mainstay target site for insecticides. Among the plant-derived natural products, terpenoids have shown to possess larvicidal, insecticidal, repellent and antiplasmodial activities. This study investigated the larvicidal effects of terpenoids associated with anticholinesterase activity. To assess the potential non-selectivity, the active terpenoids were further screened for activity against electric eel AChE, representing human AChE. This study reports on the screening of terpenoids for larvicidal effects on *Anopheles funestus* in which four sesquiterpene alcohols exhibited potent larvicidal effects with LC50 values lower than that of a positive control, propoxur. Further, the sesquiterpene alcohols induced morphological changes different from that observed with a positive control. Additionally, this research reports, for the first time, on the potential anticholinesterase effects of four closely related aliphatic terpenoids, farnesol, *cis*-nerolidol, *trans*-nerolidol and bisabolol using the modified Ellman/Lowry assays. These sesquiterpene alcohols showed potent *An. funestus* AChE inhibitory activity compared to propoxur. Among these, farnesol was the only terpenoid that also showed activity against electric eel AChE suggesting a risk for nonselective activity. This study suggests an AChE inhibition as a potential mechanism of action for the identified sesquiterpene alcohols with larvicidal activity. Additionally, *cis*-nerolidol, *trans*-nerolidol and bisabolol have potential *Anopheles*-selective AChE inhibition.

## Discordant drivers of parasitism from the micro-habitat to global scale in a frog host

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The drivers of novel host-parasite associations have fluctuating influence across different ecological scales. In the Anthropocene, the question of spatial heterogeneity in parasitism is imperative if we consider that parasites are undergoing translocation in ever-altering habitats at unprecedented rates. Nonetheless, there is a paucity of studies where invasion biology and parasitology meet. The globally invasive amphibian *Xenopus laevis* (Anura: Pipidae), its accompanying metazoan parasite assemblage and its two most highly associated parasites across its range, the cestode *Cephalochlamys namaquensis* and the monogenean *Protopolystoma xenopodis*, emerge as the ideal model to examine the drivers of parasitism across multiple replicates. The metazoan parasite infracommunities were documented from 186 adult *X. laevis* from 31 water bodies in native South Africa and 114 *X. laevis* from 11 water bodies in invasive France. Variability in parasite infracommunity crowding and cestode and monogenean abundance was modelled through Bayesian generalised linear mixed models in response to predictors at five scales – host, population, habitat, bioclimatic region, and range. Frog body size, but not body condition, strongly influences cestode abundance and parasite crowding in both the native and invasive frog populations. Lower, yet more variable cestode abundance can be expected at highly disturbed habitats across the native range. Likewise, frogs on the core of the invasive range are more likely to harbour high cestode loads than their counterparts that participate in range expansion towards the periphery. At the biogeographic level, milder, wetter regions in the native range have frogs with more crowded metazoan parasite communities with higher tapeworm abundance when compared to the more arid regions. In contrast, monogenean, not cestode, infection levels respond to large scale range shifts, with lower prevalence of this parasite in the invasive than in the native range, hinting at enemy release. Thus, the parasite dynamics of this global invader depend upon the complex interplay of factors that elicit species-specific and scale-dependent responses. This highlights the unpredictable nature of parasitism amidst global change. Moreover, we underscore the need for species-specific studies with multiple geographic replicates across various ecological scales in the emerging field of invasion parasitology.

## **Invasive snails, parasite spillback and potential parasite spill over drive parasitic diseases of *Hippopotamus amphibius* in artificial lakes of Zimbabwe**

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Humans impose a significant pressure on large herbivore populations, such as hippopotami, through hunting, poaching and habitat destruction. Anthropogenic pressures can also occur indirectly, such as artificial lake creation and the subsequent introduction of invasive species that alter the ecosystem. These events can lead to drastic changes in parasite diversity and transmission, but generally receive little scientific attention. To document and identify trematode parasites of the common hippopotamus (*Hippopotamus amphibius*) in artificial water systems of Zimbabwe we applied an integrative taxonomic approach, combining molecular diagnostics and morphometrics on archived and new samples. In doing so, we provide DNA reference sequences of the hippopotamus liver fluke *Fasciola nyanzae*, enabling us to construct the first complete *Fasciola* phylogeny. We describe parasite spillback of *F. nyanzae* by the invasive freshwater snail *Pseudosuccinea columella*, because of a cascade of biological invasions in Lake Kariba, one of the biggest artificial lakes in the world. Additionally, we report an unknown stomach fluke of the hippopotamus transmitted by the non-endemic snail *Radix* aff. *plicatula*, an Asian snail species that has not been found in Africa before, and the stomach fluke *Carmyerius cruciformis* transmitted by the native snail *Bulinus truncatus*. Finally, *Biomphalaria pfeifferi* and two *Bulinus* species were found as new snail hosts for the poorly documented hippopotamus blood fluke *Schistosoma edwardiense*. Our findings indicate that artificial lakes are breeding grounds for endemic and non-endemic snails that transmit trematode parasites of the common hippopotamus. This has important implications, as existing research links trematode parasite infections combined with other stressors to declining wild herbivore populations. Therefore, we argue that monitoring the anthropogenic impact on parasite transmission should become an integral part of wildlife conservation efforts.

## ParasiteDB – the instrument for shared managing of parasitological collections

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The African Amphibian Conservation Research Group (AACRG) accumulated a diverse collection of frog and reptile parasites representing all major parasite groups. In order to manage this collection, the AACRG needed an instrument to catalogue the collection. Modern technology provides a range of possibilities for managing and storing data. However, we were unable not find any ready-to-use solution that addressed our needs. Therefore, we developed a custom application base on web technologies. The main objective was to develop a robust but user-friendly application that will allow multiple users access on almost any modern device but with built-in security measures to protect the integrity of the database. Furthermore, the idea was to design the application in a generic way to allow any parasite collection facility to make use of this free application. The database includes numerous fields commonly used by parasitologists and includes many useful features, including remote access, complex searches, visualisation of data on a map, exporting datasets, and multi-row import of data. The application's source code is free for usage or modification and available in this Github repository: <https://github.com/goobar4/aacrg>. Despite the application being developed for the need of AACRG, we believe that it can be helpful to other parasitologist groups. Demonstration videos are available at <https://syrota.info/wormbase/>.

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## Oral Presentations – Thursday 16 September

### Session 7

#### **Invasive caryophyllidean cestodes of common carp colonising yellowfish in South Africa**

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The presence and spread of invasive organisms in freshwater systems of southern Africa has received well-needed attention lately. Common carp, *Cyprinus carpio*, is an important host species in this regard, as it has been linked to the co-introduction and co-invasion of several parasite species. One such parasite, the cestode *Atractolytocestus huronensis*, was recorded from South Africa for the first time in 2015, expanding its range from the Olifants River System to the middle reaches of the Vaal River system by 2017. During standard parasitological surveys in November 2020, unidentified cestodes were collected from *Labeobarbus aeneus* in the Vaal River, just below the Vaal Dam (Gauteng), while others were collected from *Labeobarbus marequensis* in the Crocodile River upstream of Mbombela (Mpumalanga). The identity of the cestodes were studied using both scanning electron and light microscopy, aided by genetic characterisation. The cestodes from *L. aeneus* were identified genetically and morphologically as the caryophyllidean cestode *Atractolytocestus huronensis*, previously reported in Africa only from common carp. This parasite is known to occur in other hosts outside of Africa, but such infections are considered accidental or auxiliary. The infection in *L. aeneus* followed this trend as no gravid individuals were collected. However, the presence of this infection at a relatively high prevalence (20%) is concerning as yellowfish from this site showed no sign of infection the previous year. The cestodes from *L. marequensis* were identified as the caryophyllidean cestode *Archigetes sieboldi* based on both morphology and genetic characterisation. This species is also considered specific to carp, with limited records of infecting other hosts. This cestode has not yet been recorded from Africa, raising questions about its origin and spread. The cestodes collected from *L. marequensis* were gravid, but species of this genus are known to reach maturity and become gravid in the intermediate host and as such the infection in yellowfish may not be definitive. The confirmation of host switching of invasive caryophyllidean cestodes from common carp to native yellowfish species in South Africa should be monitored as some yellowfish species are protected (*Labeobarbus kimberleyensis*) and their populations possibly impacted.

## Parasite population structure in the pelagic ecosystem of Lake Tanganyika: morphology and mitogenomics

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Species richness in open water areas is generally lower compared to nearshore habitats. Little phylogeographic structure is presumed for highly mobile species in the pelagic realm. Fish parasites have been targeted as biological tags as they might, due to their shorter generation times and presumed higher substitution rate, magnify biological patterns of their hosts including hardly traceable long-distance migration. Lake Tanganyika is an ideal place to study general mechanisms of host-parasite interactions in an open water environment because it comprises a large but still monitorable pelagic zone. *Cichlidogyrus*, a monogenean flatworm lineage, has been proposed as a model system to study parasite-host relationships. In bathybatine cichlid fishes, predators inhabiting the eu- and bathypelagic zone of Lake Tanganyika, the pattern of lake-wide population differentiation differs amongst species. We investigated the geographic population structure of *Cichlidogyrus casuarinus* (Monogenea, Dactylogyridae) infecting bathybatine hosts in Lake Tanganyika to test the link between its low level of host-specificity and its lake-wide occurrence and assess its magnifying potential. Lake-wide population structure of *C. casuarinus* ex *Hemibates stenosoma* was assessed based on a portion of the mtCOI gene combined with (geo)morphometrics of parasite sclerotised structures. Additionally, intraspecific mitogenomic variation among 80 individuals within one spatially constrained parasite metapopulation sample was assessed using shotgun NGS (PoolSeq, NovaSeq6000). While no clear phylogeographic genetic structure was detected across the lake (~ 600 km), both geographic and host-related phenotypic variation was apparent. The results indicate independence of host driven morphological and genetic variation in *C. casuarinus* and highlight the importance of an integrate approach to the parasites' potential as "tags" for their hosts. The lack of clear geographical genetic structure of *C. casuarinus* contrasts with the previously reported north-south gradient in *H. stenosoma*. Our results, therefore, show a limitation of the parasite's magnifying potential by the most mobile host species (eupelagic *Bathybates* spp.), as these may be even in low densities sufficient to maintain a wide parasite distribution. We provide the first parasite mitogenome from Lake Tanganyika and demonstrate the feasibility of PoolSeq for studying intraspecific mitogenomic variation of dactylogyrid monogeneans.



## Gill maggots: new host and locality records for marine lernaeopodids (Lernaeopodidae) from South Africa, including the first molecular characterisation of species from this region

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The Lernaeopodidae Milne Edwards, 1840, commonly known as gill maggots or gill lice, is an ecological and economically important fish parasitic family of copepods (Crustacea: Copepoda) and consists of 48 genera with 334 valid species. To date, 14 genera have been documented from both teleost and elasmobranch hosts from South African marine waters. As part of parasitological surveys targeting parasites of the super klipfish *Clinus superciliosus* (L., 1758) (Clinidae) and the blacktail *Diplodus capensis* (Smith, 1844) (Sparidae) along the South African coast, various species of Lernaeopodidae were discovered on the gills of these hosts. A prevalence of 40% and mean intensity of 2.9 (1–13) was found in *C. superciliosus* at the Langebaan marina in Saldanha Bay on the west coast, 25% *D. capensis* from Chintsa East (in the Eastern Cape) were infected with a mean intensity of 2.5 (2–3), and 73% *D. capensis* from the Garden Route National Park (Storms River Mouth section) were infected with a mean intensity of 2 (1–3). The specimens were studied morphologically using a stereo microscope in the field, as well as light and scanning electron microscopy in the laboratory. Morphometric measurements of the specimens were taken, and detailed drawings were made. Morphological comparisons were made between collected specimens and other representatives of this family, revealing various morphological differences between these specimens. DNA was extracted and amplified (*cox1* mtDNA) from excised pieces of the egg sacs. Molecular analyses confirmed that the specimens collected from *C. superciliosus* and *D. capensis* were not conspecific as they differed by 24.4–24.7% (144–146 nt). Both species grouped with other members of the Lernaeopodidae in the phylogenetic analyses. This is the first report of Lernaeopodidae from *C. superciliosus*, or in fact any member of the Clinidae, and *D. capensis*, as well as the first molecular characterisation of any marine lernaeopodid from South Africa. This study contributes valuable genetic and morphological data for this copepod family, as well as significant new host and distribution records.

## Identification of ticks using morphological traits

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Ticks are ectoparasites that depend on their host for feeding and growth. They are classified as arthropods of medical and veterinary importance as they are known to transmit bacteria, viruses and protozoa with infections reported in both humans and animals. Globally, they come second, following mosquitos, as vectors of human infections. However, the diversity of ticks of wild animals and their role in the epidemiology of zoonotic diseases in South Africa is not well documented. Ticks have a wide geographical distribution and diversity - about 896 species in 3 families (Nuttalliellidae, Argasidae and Ixodidae) are recognised globally. The exact number of described tick species is uncertain and further studies are required to resolve taxonomic issues between and within species. The current study is aimed at identifying archived ticks collected from 14 host species of captive wild animals from different parts of South Africa using morphological traits and molecular methods (DNA barcoding). The ticks were stored at the SANBI Wildlife Conservation Biobank. A total of 39 individual engorged adult ticks and nymphs were submitted to the Acarology Diagnostic laboratory at the Agricultural Research Council (ARC) for morphological identification by a taxonomist. The following tick species were identified from various hosts: *Amblyomma marmoreum* (leopard tortoise, roan antelope, brown eagle snake, African black footed cat); *Haemaphysalis elliptica* (cheetah); *Amblyomma hebraeum* (African black footed cat, vervet monkey, roan antelope, cheetah); *Amblyomma nuttalli* (leopard tortoise); *Ixodes* spp. (banded mongoose); *Hyalomma truncatum* (white rhino, feral goat, tankwa goat, leopard) *Rhipicephalus follis* (feral goat); *Hyalomma rufipes* (cheetah); *Otobius megnini* (hog deer); *Rhipicentor nuttalli* (leopard); *Rhipicephalus simus* (porcupine, cheetah, feral goat); *Rhipicephalus evertsi evertsi* (cheetah, leopard, roan antelope); *Amblyomma (Aponomma) exornatum* (Bosc's monitor lizard); *Rhipicephalus evertsi mimeticus* (leopard); and *Rhipicephalus* spp. cf. sp. nr. *pravus* (roan antelope). The study indicates that wild animals in South Africa are infected by diverse ticks of medical and veterinary importance. Molecular characterisation of the 16S, 12S and COI genes of the ticks is ongoing.

## ***In silico* insights to optimise naphthoquinone analogues as lead inhibitors of the *Plasmodium* cytochrome *bc1* complex**

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Hydroxynaphthoquinone compounds, including atovaquone are effective antimicrobial agents, as well as antimalarial when combined with proguanil. Based on the effectiveness of atovaquone in targeting the cytochrome *bc1* complex of *Plasmodium*, the research question of this study was to determine if there were additional hydroxynaphthoquinones that could improve upon the selectivity, pharmacological efficacy, and pharmacokinetic profile of atovaquone. As such, the effect of 29 different naphthoquinone compounds compared to atovaquone were assessed on the *Plasmodium* cytochrome *bc1* complex using various computational models. These compounds were evaluated against the *Plasmodium* cytochrome *bc1* complex (PDB 4PD4) to compare the docking scores with atovaquone. *In silico* modelling was done using the Schrödinger software suite to evaluate the naphthoquinone compounds using Glide XP, Prime MM-GBSA and QikProp programs. Docking studies revealed that compounds NPHQ-4, NPHQ-16, NPHQ-15 and NPHQ-18 displayed higher ranking docking scores than atovaquone, with -12.26, -11.70, -11.62 and -11.37kcal/mol, respectively, compared to -11.22kcal/mol for atovaquone. Prime MM-GBSA analysis yielded the relative binding energies (MMGBSA\_dG\_Bind) of NPHQ-4, NPHQ-16, NPHQ-15, NPHQ-18 and atovaquone with -57.23, -56.33, -65.95, -56.63 and -51.30kcal/mol, respectively. These results suggest that select compounds bind to the target site more strongly than atovaquone. QikProp analysis indicated atovaquone, NPHQ-4 and NPHQ-16 complied with all Lipinski Rule of 5 “drug-like” properties. Whilst NPHQ-15 did not comply with 2, contributing to a 94.5% predicted oral absorption compared to 100% for atovaquone and being too polar to cross the blood brain barrier. Swiss-ADME drug-likeness analysis also indicated that compounds NPHQ-4 and NPHQ-16 showed favourable properties, where compound NPHQ-18 did not comply with 1 rule (MlogP >4.15). Preliminary data indicated that compounds NPHQ-4 and NPHQ-16 have the potential to serve as lead antimalarial compounds for designing inhibitors of the cytochrome *bc1* complex. Although NPHQ-17 and NPHQ-14 showed lower ranked docking score (-10.59 and -8.78 kcal/mol) compared to atovaquone, their predicted relative binding energies were better (-68.84 and -56.59 kcal/mol). When analysed for drug-likeness, compounds NPHQ-14 and NPHQ-17 did not comply with 1 Lipinski’s rule of 5 (i.e. MlogP >4.15). These compounds can also be studied further for their potential to design new ligands.

## Occurrence and diversity of avian haemosporidian parasites in South African captive birds

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Avian malaria parasites (*Plasmodium* spp.) and related species (*Haemoproteus* and *Leucocytozoon* spp.) are transmitted by blood-sucking insects and infect a wide range of bird species globally. Infection can result in severe disease and sometimes death. However, the severity of infection mainly depends on the host species, age, fitness and parasite lineage. The study aimed to determine the prevalence and genetic diversity of avian haemosporidian parasites in 14 species of captive birds from captive facilities in South Africa. One hundred and eighty-three (183) blood samples from captive flamingos, vultures, owls, ibises and parrots were analysed by a nested PCR assay using mitochondrial primers targeting the cytochrome b (*cytb*) gene of the parasites. Infections were characterised by sequencing DNA from selected positive samples. Of all screened samples, 64 (35%) were positive for infection by haemosporidian parasites, 119 (65%) tested negative, 8.7% of the samples had mixed infections. Infections by *Leucocytozoon* spp. were more prevalent (31.7%) than those of *Plasmodium* and/or *Haemoproteus* spp. (14.2%), and the observed difference was significant ( $p < 0.0001$ ). Overall, four lineages of *Leucocytozoon* spp. and one *Haemoproteus* spp. lineage were identified by sequencing. The *Leucocytozoon* spp. lineages were considered as broad generalist as they were identified from multiple species of birds, whilst the *Haemoproteus* spp. lineage was only identified from Barn Owls (*Tyto alba*) and is therefore considered as host specific. This study indicates that various species of captive birds in South Africa are exposed to parasitic infections that are transmitted by insects, consequently posing a health concern to the conservation of these birds. Regular monitoring of infections by these pathogens in the NZG collection and other aviaries in South Africa, particularly in threatened bird species, is recommended to avoid disease outbreaks.

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## **Aspects of the pathology on the skin of *Clarias gariepinus* (Burchell, 1822) infected with a gyrodactylid monogenean *Macrogryodactylus congolensis* (Prudhoe, 1957)**

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*Clarias gariepinus* is one of the two most actively cultured freshwater fishes in Africa and therefore economically important. This catfish species is parasitized by *Macrogryodactylus congolensis*, an ectoparasitic gyrodactylid monogenean which is viviparous and uses polyembryony to reproduce. This allows it to reach extremely large numbers in a short time, potentially affecting the host. It attaches to its host with anterior adhesive discs and a posterior haptor supported with sclerotised structures which may cause harm to the host's skin. *Macrogryodactylus congolensis* was found on the skin of catfish purchased from a fish farm in southern Africa and was observed to reproduce profusely in the favourable conditions of the aquarium tanks in the Department of Zoology at the University of Johannesburg. This study examined the isolated haptoral sclerites of the parasite using scanning electron microscopy alongside the pathological effects caused by this infection with light microscopy. Haptoral sclerites were isolated by removing soft tissue with a digestion buffer and studied using scanning electron microscopy (SEM). Histopathological analysis involved light and scanning electron microscopy of parasites on the host's skin. Skin samples with attached parasites were processed for SEM by dehydration through a graded ethanol series, increasing hexamethyldisilazane concentrations, drying in a desiccator, and sputter-coating with gold. Light microscopy involved examination of haematoxylin and eosin-stained sections (epoxy embedded) of infected skin. SEM of infected skin displayed excessive mucus production, corroborated by light micrographs showing an increased number of mucus cells alongside proliferation of cells and abnormally enlarged club cells, which resulted in thickening of the epidermal layer. At the site of attachment, the basement membrane became detached from the dermis because of vacuum applied by the haptor. Moreover, hamulus points and marginal hooks of the parasite pierce through the skin, resulting in tearing. Epidermal cells and melanin granules were observed in the parasites' intestinal lumen and also absorbed into the intestinal epithelium. These results corroborated previous studies that concluded that the parasite feeds on host skin tissue. This study was the first to examine isolated haptoral sclerites of *Macrogryodactylus* with scanning electron microscopy, which, in conjunction with the pathological results may contribute to control of these parasites in the aquaculture industry.

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## **A can of worms: high diversity of Opcoelidae (Trematoda: Digenea) from the South African intertidal super klipfish, *Clinus Superciliosus***

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Two digenean species of the family Opcoelidae Ozaki, 1925 have previously been reported from the super klipfish *Clinus superciliosus* (L., 1758) in South Africa: *Coitocaecum capense* Bray, 1987 and *Helicometra fasciata* (Rudolphi, 1819). During a study that explored the diversity of digeneans of this fish host from five localities along the coast of South Africa (Saldanha Bay, Cape Town harbour, Hermanus, Tsitsikamma National Park and Chintsa), we found an astonishing diversity of intestinal digeneans - most of which belonging to the Opcoelidae. This is no surprise, as the Opcoelidae is the largest of all digenean families. Specimens were characterised both molecularly (28S rDNA, ITS/ITS2) and morphologically (morphometric data, detailed illustrations, and descriptions). This revealed the presence of six opcoelid species: the previously reported *C. capense*; three undescribed species of *Coitocaecum* Nicoll, 1915; and two undescribed species of *Helicometra* Odhner, 1902. Based on both morphology and molecular data all undescribed species are new to science. Apart from *C. capense* and *Helicometra* sp. 1, all species were only present in a single respective locality and often not in extremely high numbers. Interestingly, *Helicometra* sp. 2 was found to be conspecific (based on ITS2 data) to unidentified metacercariae collected from the shrimp, *Hippolyte inermis* Leach, 1816 in the Mediterranean. We therefore also provide the partially elucidated life cycle of this species and information on its potential wide distribution. Thus, proving the importance of molecular data not only in the identification of such homoplastic species, but also in life cycle studies that involve larval stages with not many characteristic features. We also highlight the importance of incorporating various sampling localities along the hosts' distributional range, to achieve a better idea of the array of parasites that the host can harbour. This is the first study to molecularly characterise trematodes of the family Opcoelidae from South Africa, consequently providing important molecular, morphological and distribution data of these species.

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## Molecular and morphological characterisation of *Argulus japonicus* (Japanese fish louse) from the Orange-Vaal River system

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Currently there are 35 species of the branchiuran genus *Argulus* in Africa, with six in South Africa including the Asian invasive species, *Argulus japonicus*, reported as the most widely encountered *Argulus* species in the South African freshwater systems. Due to its invasive status globally, *A. japonicus* has been subject to studies on its biology, histology, and morphology but lacks an integrated examination using both molecular and morphological techniques. To date there are also no available sequences on GenBank from a single site for this species for mitochondrial DNA cytochrome c oxidase 1 (COI), 18S ribosomal RNA (rRNA) and 28S rRNA. Additionally, there are no published sequences from these gene regions from Africa. This study aims to add information on the distribution of the parasite across southern Africa, as well as link the morphological and molecular data of this species from southern Africa using COI, 18S, and 28S sequences. As part of a larger ongoing fish parasitological survey, fishes were collected from 2013 to 2020 at seven different localities within the Orange-Vaal River system. Following collection, fishes were humanely euthanised and screened for external and internal parasites. A total of 28 adult females, 43 adult males, and 67 larvae of an *Argulus* sp. were collected from *Clarias gariepinus*, *Cyprinus carpio*, *Labeo umbratus*, and *Labeobarbus aeneus*. All specimens were morphologically identified as *A. japonicus* with the aid of light and scanning electron microscopy. Twenty-five new COI, 18S, and 28S sequences were generated for *Argulus japonicus*. New COI sequences grouped with available *A. japonicus* COI sequences from Japan, confirming morphological identification. However, they differ by more than 22% from sequences of *A. japonicus* from India, raising questions regarding species identification of sequences available on GenBank. Intraspecific genetic variation also existed with Middle Vaal River specimens forming a sub-clade within the South African *A. japonicus*. This study reports on four new localities for *A. japonicus* within the Orange-Vaal River System, provides information on body measurements for all life stages of *A. japonicus*, and records the first molecular characterisation of this species from the African continent.

## Session 8

### Monogeneans as indicators of water quality

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Ectoparasites exposed to the macroenvironment of the host presumably evolved at a similar rate to the host, in order to tolerate and survive environmental alterations. Such changes have been, amongst others, related to either the effect of pollutants on the physiology of hosts which results in increased susceptibility toward infections by parasites or as direct impacts of pollution on parasites. Studies have mostly compared the sensitivity of parasites according to Class with few studies scrutinizing sensitivity at lower taxonomic levels. Monogeneans are recognised as being important and a major group of parasitic platyhelminthes of freshwater and marine fishes, and amphibians. They display high host and infection site specificity and for this reason may be useful indicators of environmental change. This study was divided into two parts, one where a meta-analysis of the effects of different pollutants on the infection biology of monogeneans from studies reporting changes in different environments, and the other where metal concentrations were measured in the tissues of the monogenean, *Paradiplozoon ichthyoxanthon*. The effects were summarised as either having a positive, negative or no effect on infections. Most studies have reported on the effects of pollution in natural conditions with a handful having tested effects under laboratory conditions. Within specific families there was a degree of variability with regards to the responses toward various pollutant types, with some species being more sensitive toward pollution exposure than others. *Paradiplozoon ichthyoxanthon* was found to accumulate metals such as Cr, Ni, Fe and Zn at higher concentrations than the tissues of the host fishes, *Labeobarbus aeneus* and *Labeobarbus kimberleyensis*. Based on the meta-analysis and measurement of metal levels in *P. ichthyoxanthon*, results indicate, in general, that monogeneans are sensitive toward environmental changes and are therefore useful indicators of water quality.

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## **Metallothionein levels in the ectoparasite, *Lamproglena clariae* (Copepoda: Lernaecidae) correspond to water quality**

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Metallothioneins are a family of metal binding proteins which are important in regulating the levels of metals in organisms. Due to their metal binding properties, metallothioneins have been used as biomarkers for monitoring environmental health. *Lamproglena clariae* is an ectoparasite infecting the gills of the African sharp-toothed catfish, *Clarias gariepinus*. In the Vaal River, changes in the infection biology of this parasite result from differences in water quality. The aims of this study were to determine if metallothionein levels in *L. clariae* corresponded to changes in water quality, and if the levels in the parasite were greater than those in the host fish. Water quality variables, and water, sediment and fish samples were collected from two sites in the Vaal River; the Vaal Dam and below the Vaal River Barrage. These sites were chosen due to their distinct water quality differences. Thirty-seven *C. gariepinus* were sampled with gill nets, euthanised by severing the spinal cord and gills, liver and muscle were removed. Gills were examined for *L. clariae* which were removed and along with host tissues, frozen in liquid nitrogen. Levels of metals in sediment and water samples were analysed by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Metallothionein content in host tissues was analysed using a spectrophotometric assay and in *L. clariae*, protein levels were quantified by Size Exclusion Chromatography (SEC). Water quality variables and metal levels between the sampling sites indicated poorer water quality below the Vaal Barrage. Metallothionein levels in *L. clariae* were higher than host muscle tissue, but lower compared to the gills and liver. Differences between sites showed that levels of the protein were higher in both *L. clariae* and *C. gariepinus* from below the Vaal River Barrage compared to the Vaal Dam. Exposure to poor water quality does elicit a physiological response in *L. clariae* and expression of metallothioneins in parasites occurs in a concentration dependant manner. These results support the usefulness of *L. clariae* as a bioindicator for monitoring water quality.

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## Hatchability and survival of *Lamproglena clariae* exposed to increasing concentrations of aqueous aluminium

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*Lamproglena clariae* an ectoparasite on *Clarias gariepinus* in the Vaal River system, shows potential as a sentinel organism. Mean abundance and mean intensity of *L. clariae* is lower at localities with high metal concentrations. In a previous study in the Vaal River, aluminium (Al) concentrations had a negative effect on the survival of *Paradiplozoon ichthyoxanthon* eggs. The effect of Al exposure on the hatchability and survival of larval stages of *L. clariae* is unknown. During October 2019 and October 2020 infected *C. gariepinus* were collected from the Vaal River and transferred to a temperature and daylight-controlled aquarium room at the University of Johannesburg. A parasite infection was established and adult female parasites (F2 and F3 generation) on the gill filaments of live fishes were inspected for egg-strings. Egg-strings were removed carefully, and the females remained attached to produce further eggs. Partially static bioassays were performed in varying concentrations (0 µg/L-120 µg/L) of Al (aluminium nitrate non-hydrate 99.997%) in reverse osmosis water. All exposures were prepared in triplicate and aerated before a minimum of 10 eggs were placed in each beaker, resulting in ± 30 eggs per Al concentration. The eggs were monitored at 24 hours intervals, using a dissection microscope. Hatching, developmental stages and mortality were recorded for each egg. *In situ* physical water quality parameters were measured every 24 hours with a YSI 556 Multi-Probe meter and water samples were collected for metal analysis with inductively coupled mass spectrometry. The experiment was terminated when all juveniles perished. Twenty six percent of eggs did not hatch in 120 µg/L Al, compared to 5% not hatching in the control solution. Maturation was also influenced by increased Al concentration as demonstrated by a higher mortality rate. Hatchability and survival results indicate that *L. clariae* is a sensitive sentinel for Al. Utilising this ectoparasite from live fish eliminates the need to euthanize the host and emphasises the usefulness of *L. clariae* as a sentinel organism for metal pollution.

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## Hiding out in trees won't keep you safe: blood parasites of southern African tree frogs

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Amphibians are regarded as the most threatened vertebrate class, leading to increased efforts in documenting and conserving their biodiversity. However, despite the increase in work on amphibians in recent years, little is known about their blood parasites. The recorded blood parasites from frogs include intraerythrocytic haemogregarines, haemococcidia, viruses and bacterial organisms, and extracellular trypanosomes and microfilariae. Most, if not all, of these parasites make use of hematophagous invertebrate vectors for transmission. Tree frogs of the genus *Leptopelis* (Arthroleptidae) are charismatic species with interesting life history traits and habitat preferences. Three of the 52 known species occur in KwaZulu-Natal, South Africa, namely the brown-backed tree frog (*Leptopelis mossambicus*), the Natal tree frog (*Leptopelis natalensis*) and the endangered long-toed tree frog (*Leptopelis xenodactylus*). In the current study, two species, *L. mossambicus* (n=7) and *L. natalensis* (n=69), were screened for the presence of blood parasites. Intraerythrocytic blood parasites observed included two species of *Hepatozoon* (25/76), one species of *Lankesterella* (1/76), and several viral- or bacterial-like organisms (10/76). Extracellular parasites included two species of *Trypanosoma* (27/76), and a microfilaria nematode *Neofoleyellides* species (11/76). Little is known about the ecology of these host-parasite relationships. As such the biodiversity and phylogenetic placement of these parasites are important as it may provide an indication of the possible invertebrate host/vector responsible for the transmission of the parasitic infection thus aiding in elucidation of these parasites' life cycles.

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## Morphological and phylogenetic analysis of Cosmocercidae from amphibians in South Africa

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Nematodes of the family Cosmocercidae are one of the most common and most neglected groups of parasites found globally. There are about 200 species from about 20 genera commonly parasitising amphibians, fish, snails, and some reptiles. Majority of the species are known to be rather host-specific, except for *Cosmocerca ornata* (Du Jardin, 1845) and *Aplectana macintoshii* (Stewart, 1914) each reported from more than 100 host species from different continents. Due to the absence of molecular data and high morphological similarities, both host species and geographical records continue to increase. However, two recent publications by Chen and co-workers dealt with molecular data on cosmocercid nematodes representing clear genetic differences in morphologically similar species. In the present study, morphological analysis was conducted on male and female specimens of six cosmocercids. We obtained DNA sequences of ITS-28S genetic markers from several species of Cosmocercidae from South Africa and *C. ornata* from its type of host collected in Ukraine. The morphological analysis presented distinct differences in gubernaculum shape in *Cosmocerca* spp. as well as differences in the position and number of caudal papillae in *Aplectana* spp. The phylogenetic analysis indicated closer relationships between *Cosmocerca* and *Aplectana* from South Africa in comparison to the species found in the study by Chen, despite significant morphological differences. Species of *Cosmocercoides* Wilkie, 1930 formed basal branches regardless of the morphological similarities with species of *Cosmocerca*. The resultant tree illustrates a clear distinctness between *C. ornata* and African *Cosmocerca* spp. along with the species reported as *C. ornata* from China. Based on our preliminary results, we hypothesise that such a wide host and geographical distribution of *C. ornata* might be rather doubtful and fauna of this genus might be more diverse than previously supposed.

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## **Molecular survey of reptiles and associated ticks from South Africa for bacterial and protozoal pathogens**

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South Africa has a rich biodiversity of reptiles which includes ~384 species. Most of these reptiles are infected with a wide range of parasites, including blood parasites and tick-borne pathogens (TBPs). However, knowledge on the biodiversity, prevalence and intensity of these parasitic and pathogenic infections in reptiles remains lacking, particularly on their role in harbouring potential zoonotic diseases. Therefore, this study aims at identifying the diversity of blood parasites and TBPs in reptiles and their ectoparasites (particularly ticks) in South Africa. To date, a total of 37 blood samples and 140 ticks collected from Kwa-Zulu Natal and Western Cape from 2014–2017 were screened for bacterial and protozoal pathogens using molecular techniques. Ticks were morphologically identified using diagnostic characters and these confirmed through amplification of the ITS2 region, 16S rRNA and CO1 genes. All PCR results were confirmed by sequencing amplicons of positive samples, comparing them with their respective parasite genes in the NCBI GenBank database. Examined ticks were identified as *Amblyomma marmoreum*, *A. sylvaticum*, *A. latum* and *A. exornatum*. An overall infection rate of blood protozoans of the genus *Hepatozoon* was 18.9% (7/37) and 7.1% (10/140) from reptiles and ticks, respectively. *Rickettsia* spp. and *Borrelia* spp. were detected from 13.6% (19/140) and 7.9% (11/140) ticks, respectively. This study is ongoing with the aim of identifying the potential of reptiles to act as reservoir hosts for TBPs, particularly those of zoonotic importance. It is hoped that these data contribute to more effective management in regulating both the national and international trade in reptiles.

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