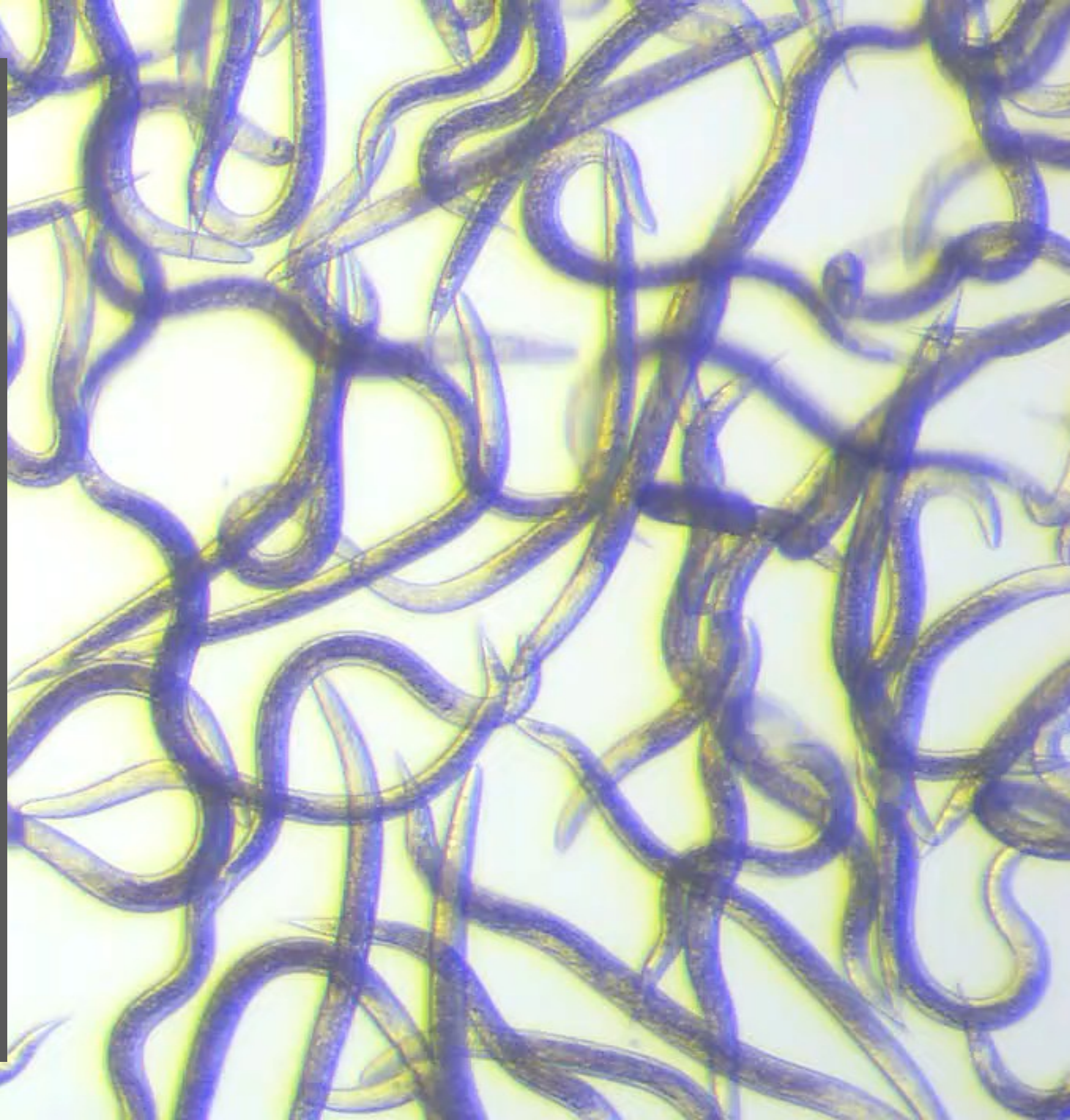


Selection of a South African *Heterorhabditis bacteriophora* isolate for in vitro liquid mass production for the biocontrol of *Thaumatotibia leucotreta*

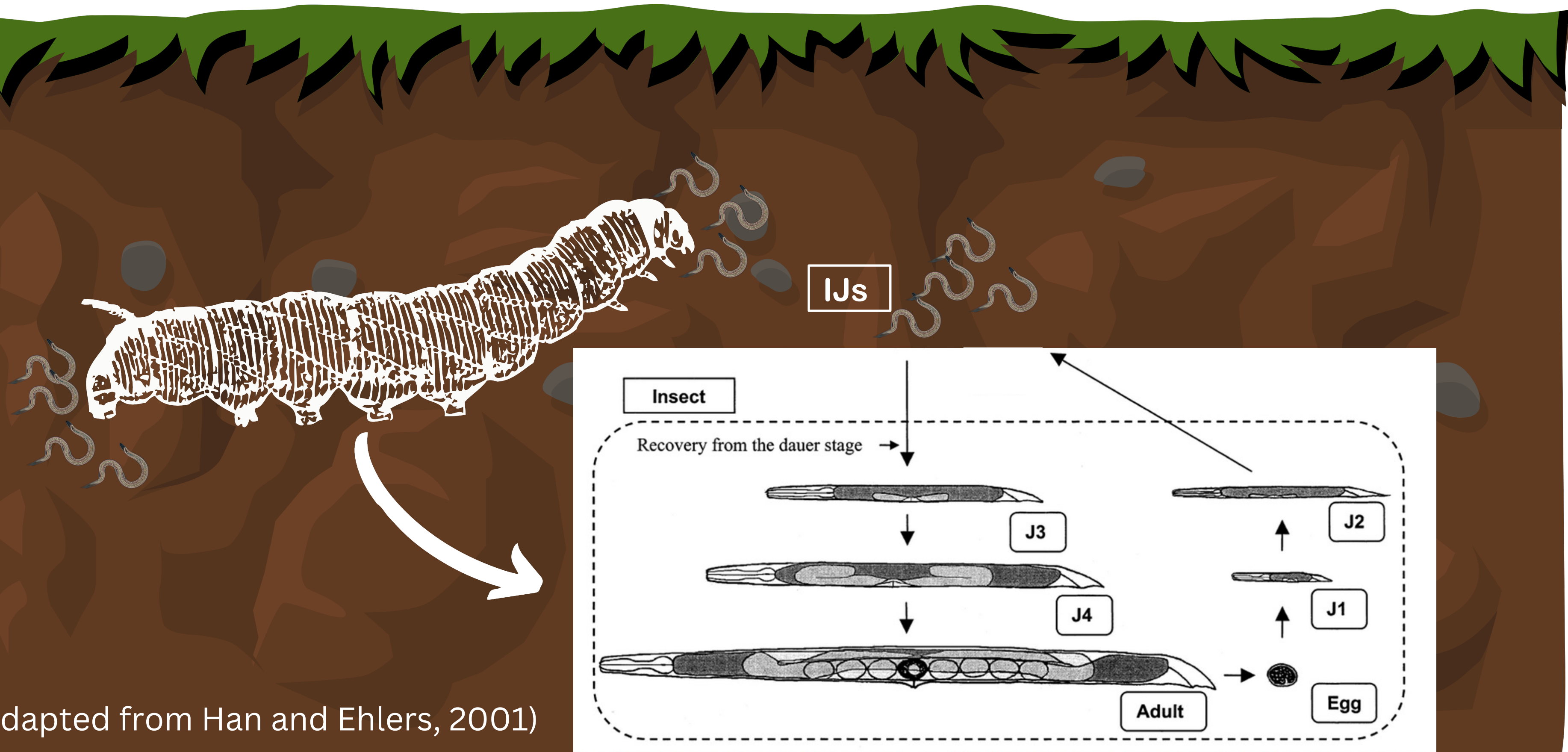
Nicholle Claasen, Prof. Antoinette Malan, Dr. Murray Dunn
Department of Conservation Ecology & Entomology
Stellenbosch University

Entomopathogenic Nematodes

- Families: *Heterorhabditidae* & *Steinernematidae*
- Widely distributed
- Natural enemies to key agricultural pest insects
- Reduce the use of pesticides = environmentally friendly
- Eg. *Heterorhabditis bacteriophora*, registered biopesticide in SA
- No local EPN biopesticide available
- **AIM:** Mass culture a local isolate



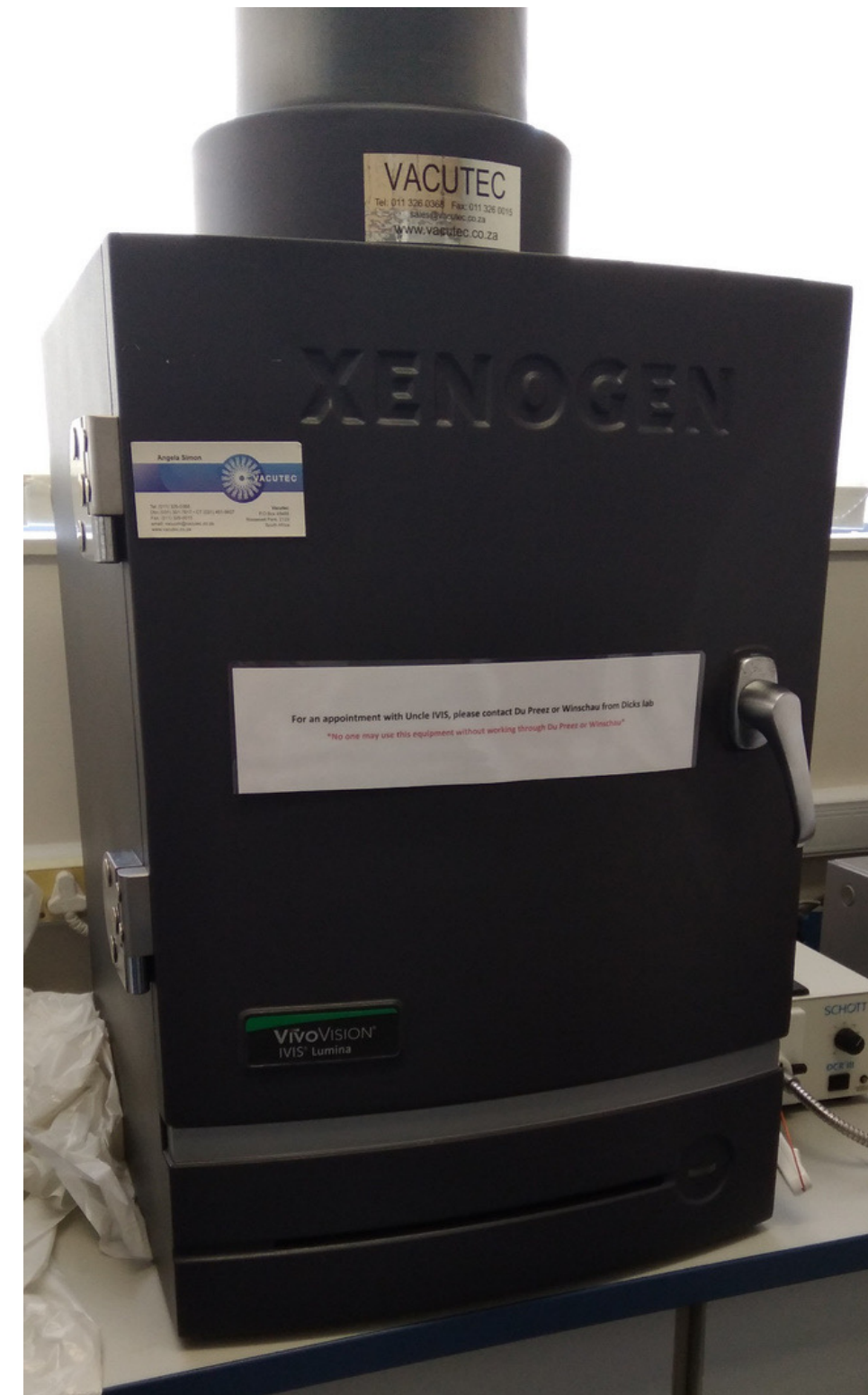
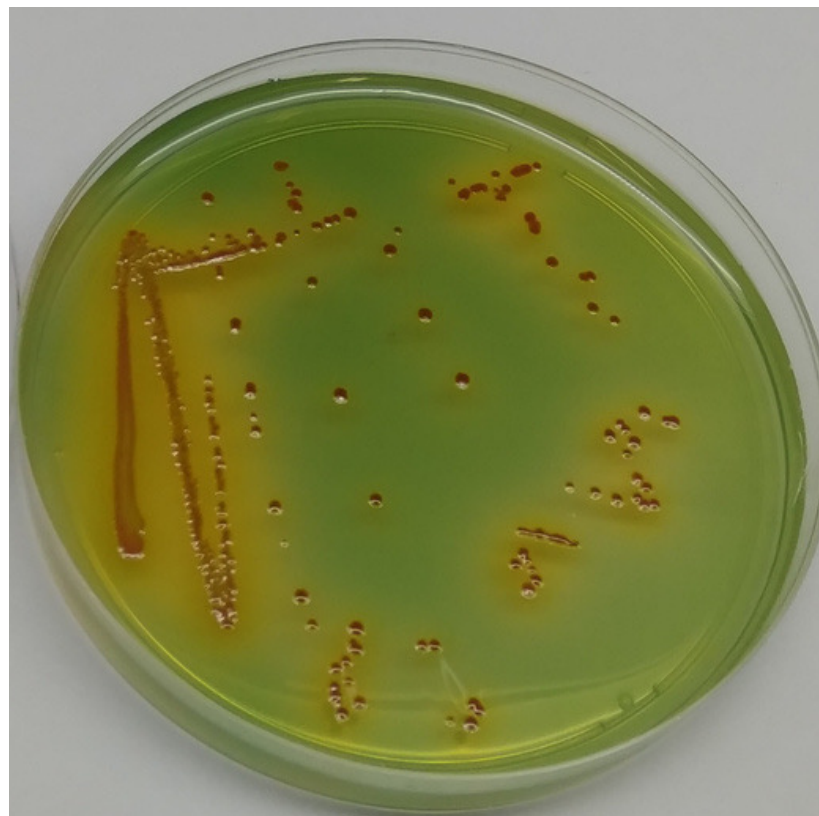
Life cycle of *Heterorhabditis bacteriophora*



(Adapted from Han and Ehlers, 2001)

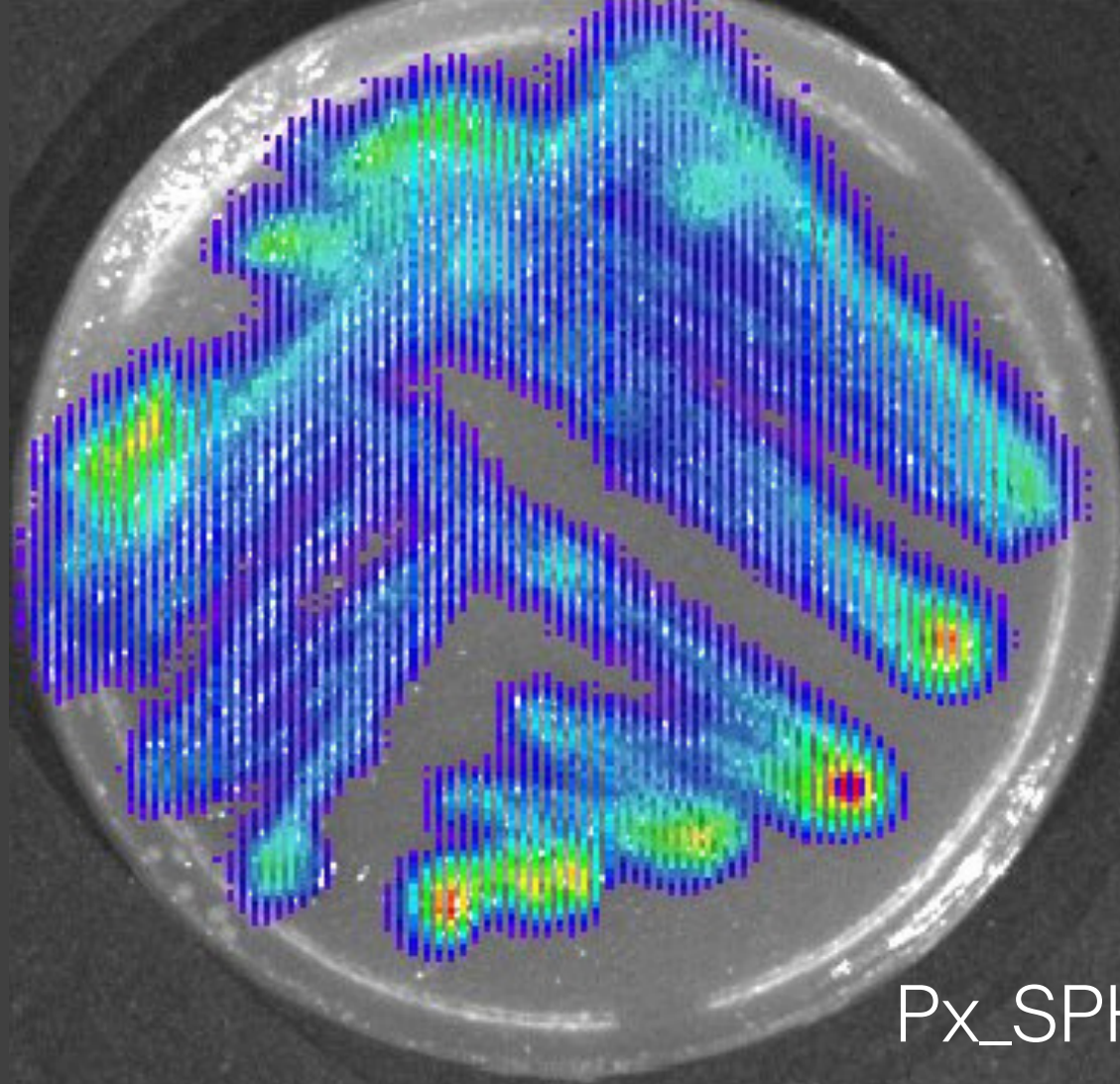
Selection of *H. bacteriophora* isolates & identifying their symbiotic bacteria

| Isolate | Location | Province | Orchard type | Collected from |
|---------------|--------------------------|--------------|--------------|-------------------------------------|
| SGI_170 | <u>Fouriesburg</u> | Free State | Apple | soil |
| CRI_LC | Sunday's River Valley | Eastern Cape | Citrus | soil |
| <u>Px SPH</u> | <u>Witzenberg Valley</u> | Western Cape | Apple | <u><i>Phlyctinus xerophilus</i></u> |
| LLM | <u>Grabouw</u> | Western Cape | Apple | soil |

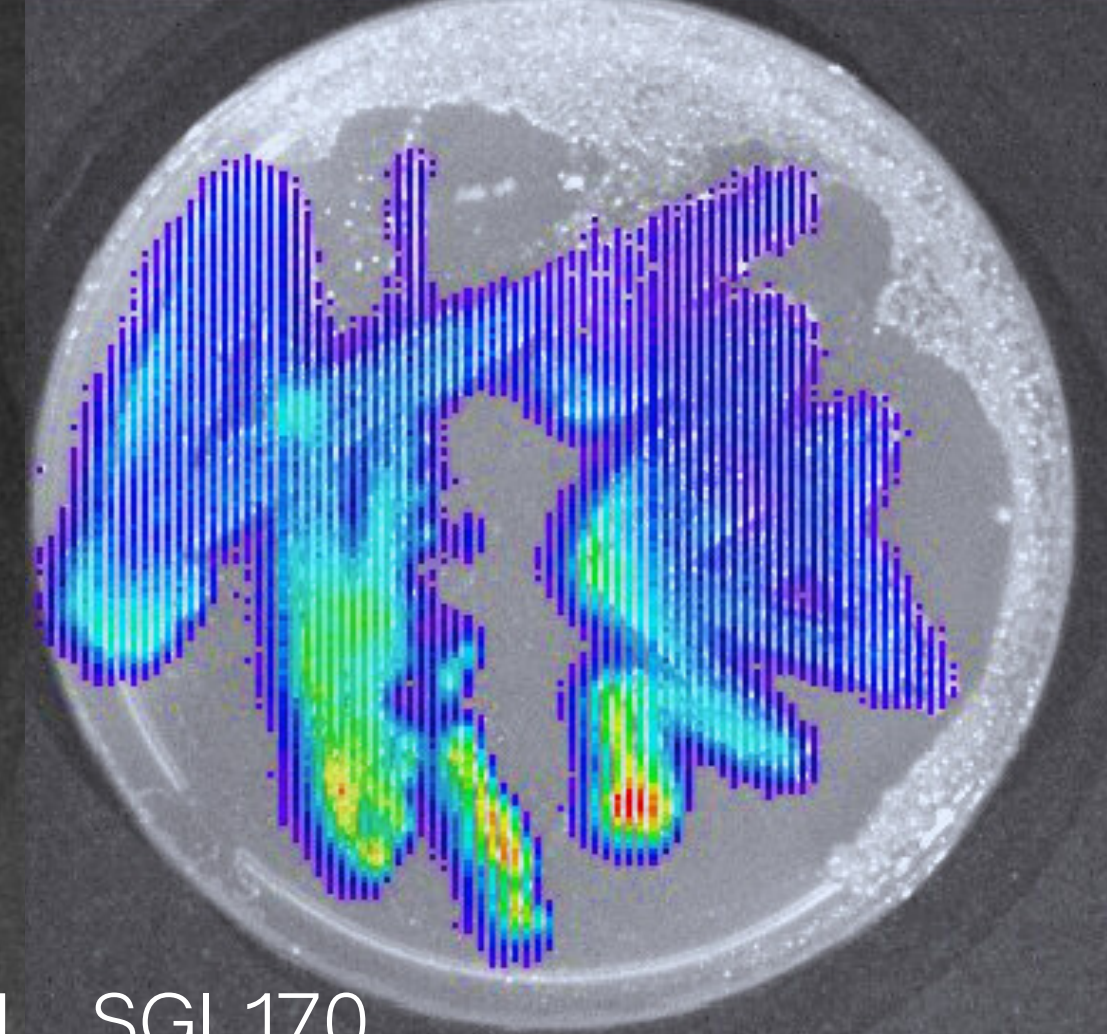


Bioluminescence

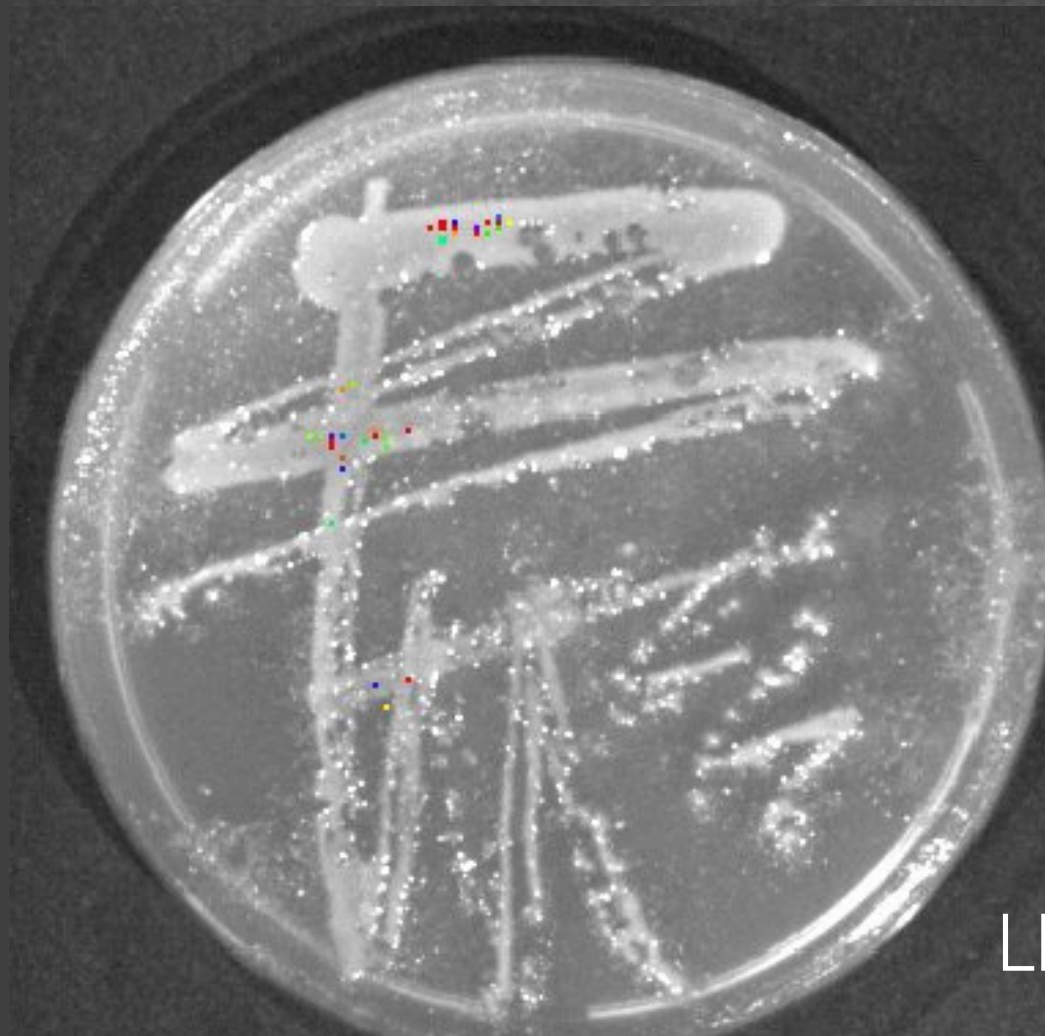
- *Photorhabdus* bacteria known to be bioluminescent.
- Symbiotic bacteria identified as:
 - SGI_170 & Px_SPH: spp. close to *P. namnaonensis*
 - LLM: *P. laumondii* subsp. *laumondii*
 - CRI_LC: *P. laumondii* spp.



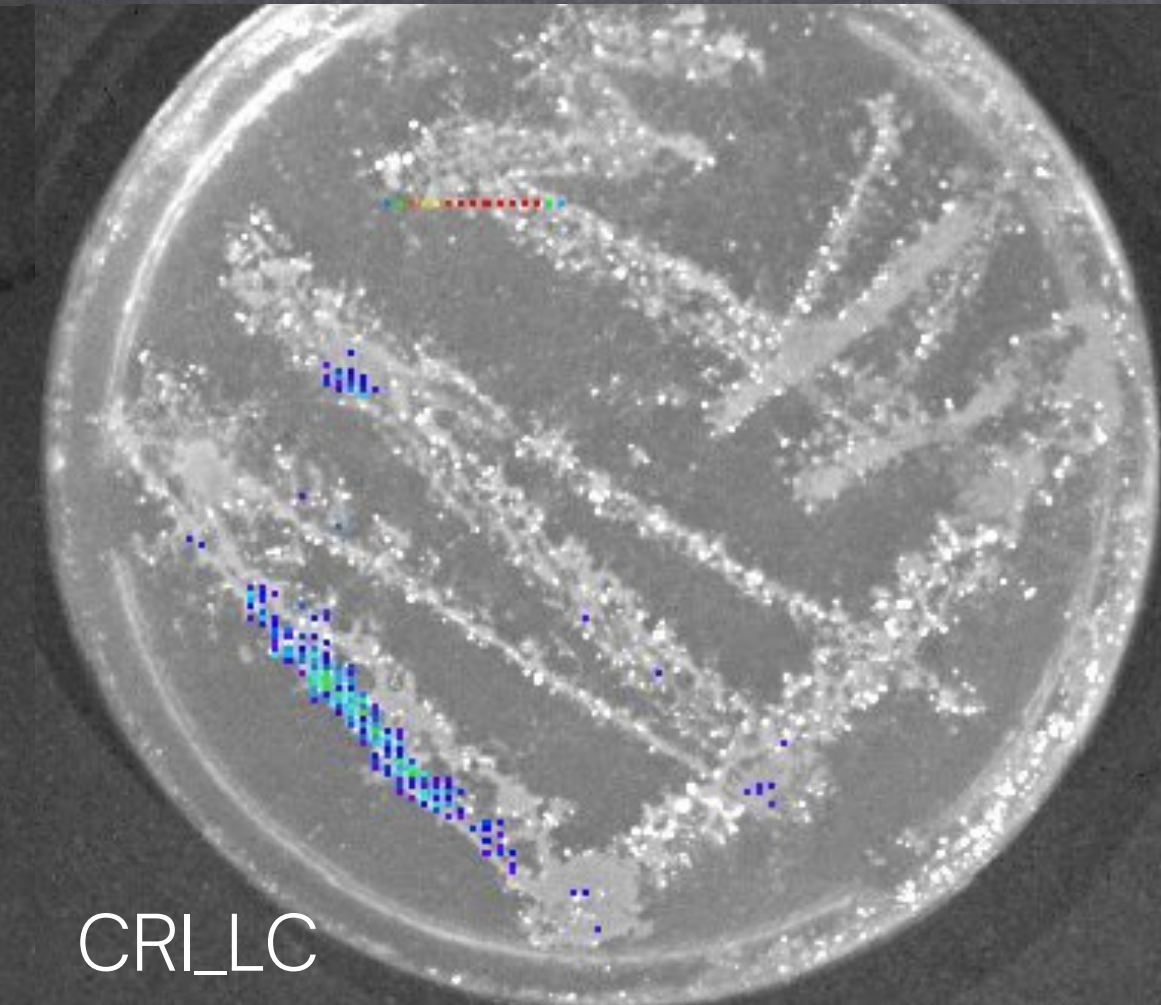
Px_SPH



SGI_170



LLM



CRI_LC

Mortality bioassay of the four isolates

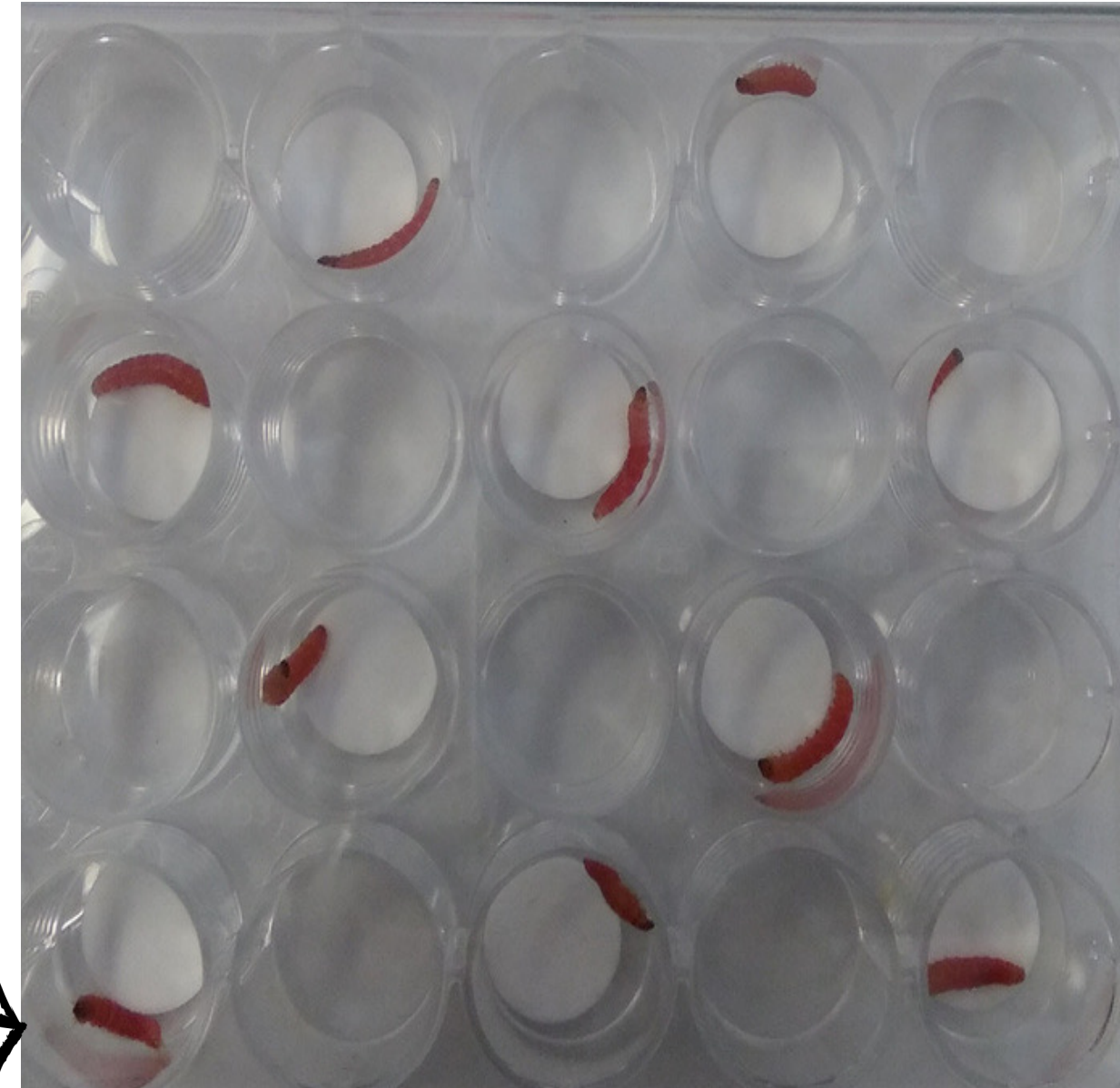
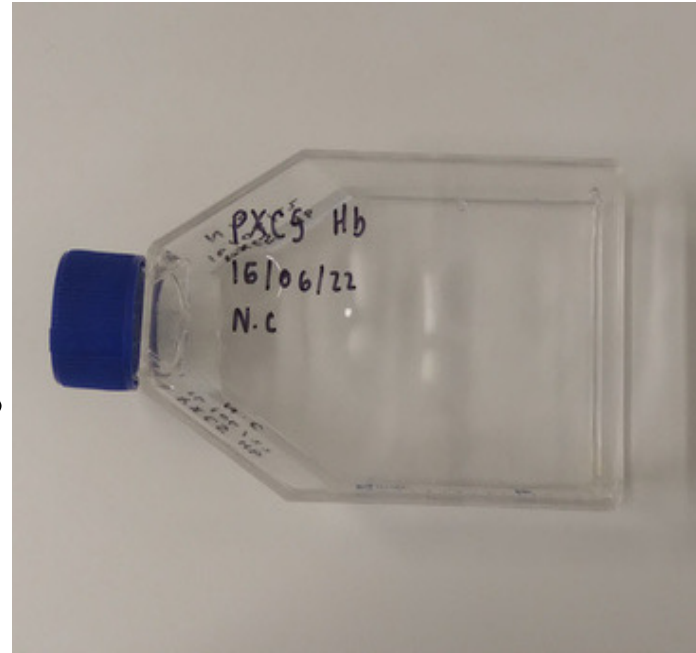
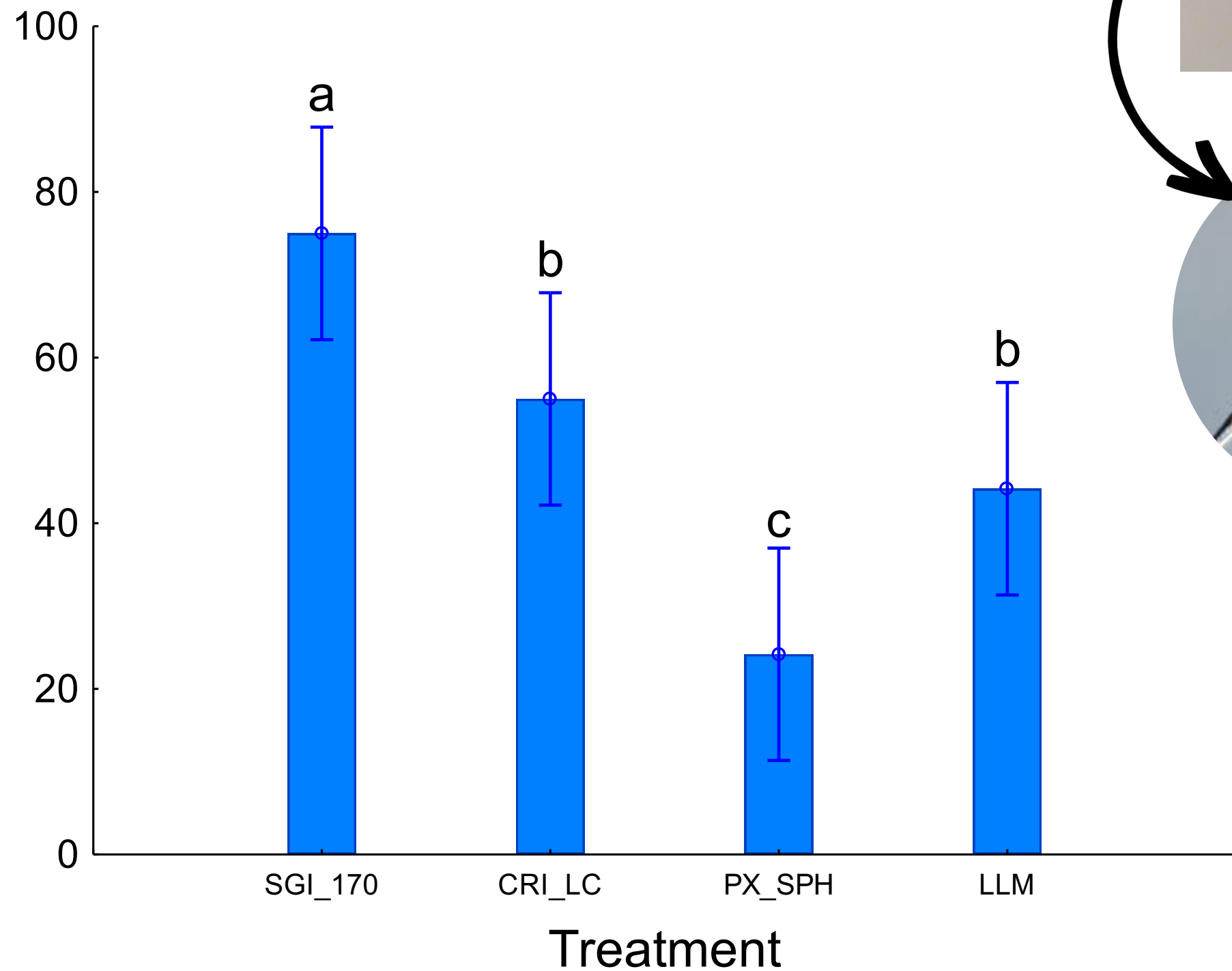
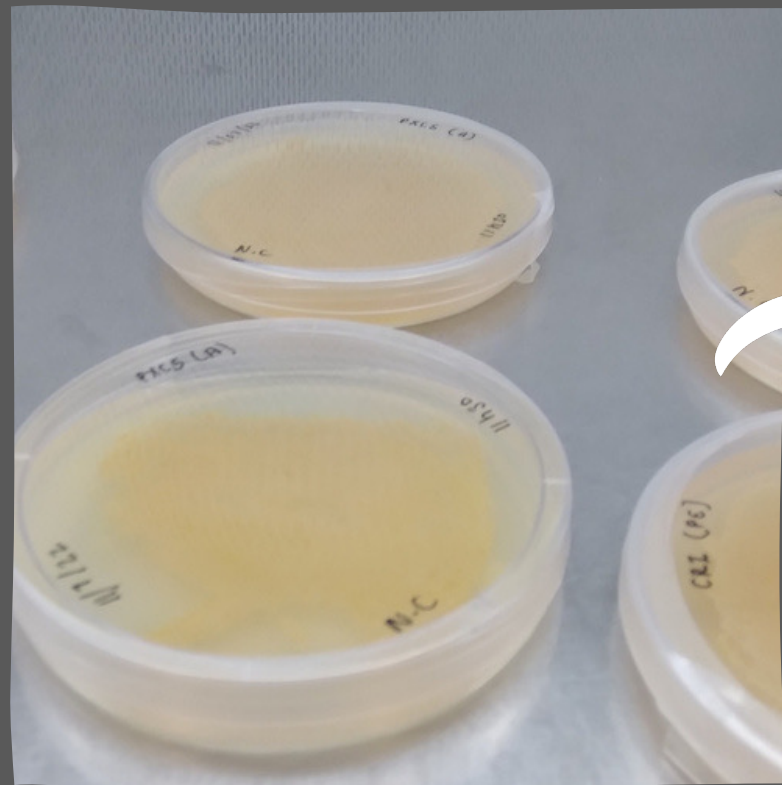


Figure 1 Percentage mean infection rate of South African *Heterorhabditis bacteriophora* isolates against the last instar larva of *Thaumatotibia leucotreta* (FCM).

In vitro liquid culture protocol

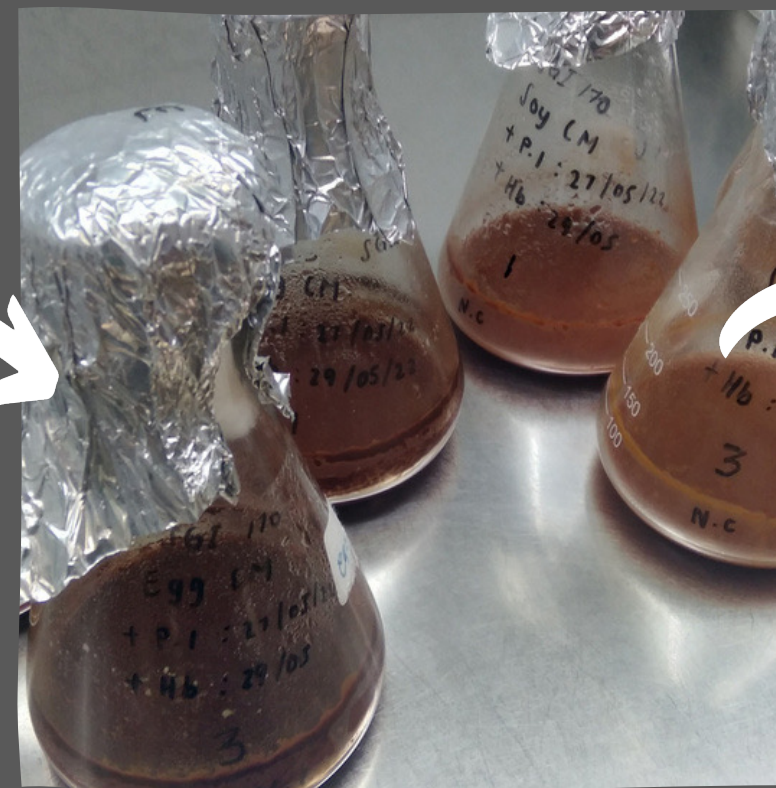
Monoxenic cultures established from modified Lunau et al. (1993) method



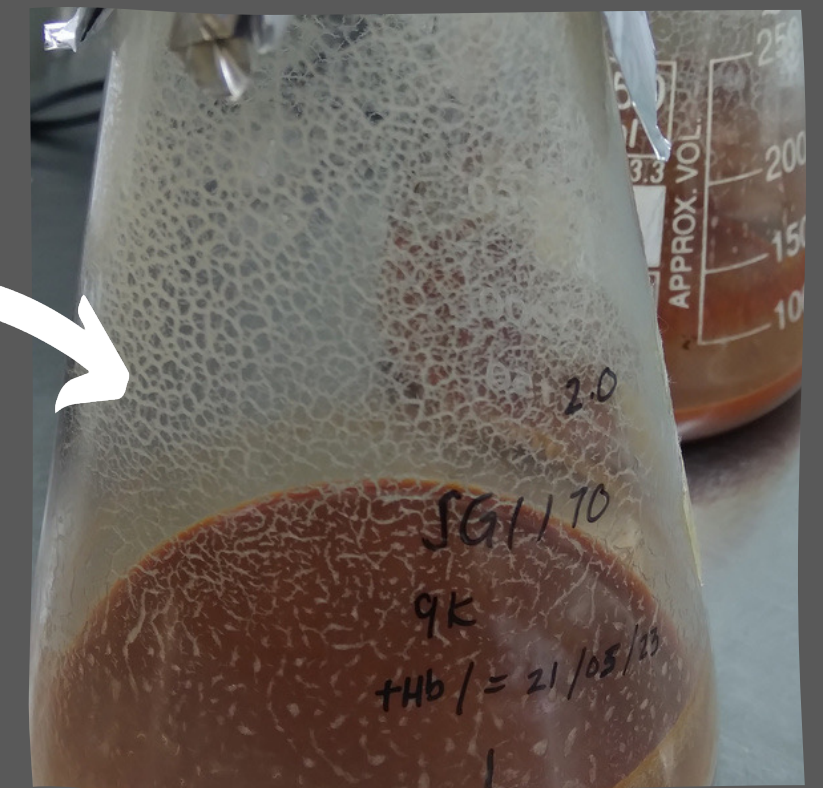
Hermaphroditic females harvested off Wouts plates after 3 – 4 days



Surface sterilised eggs



Two-day old juveniles placed into culture media containing only its symbiotic bacteria

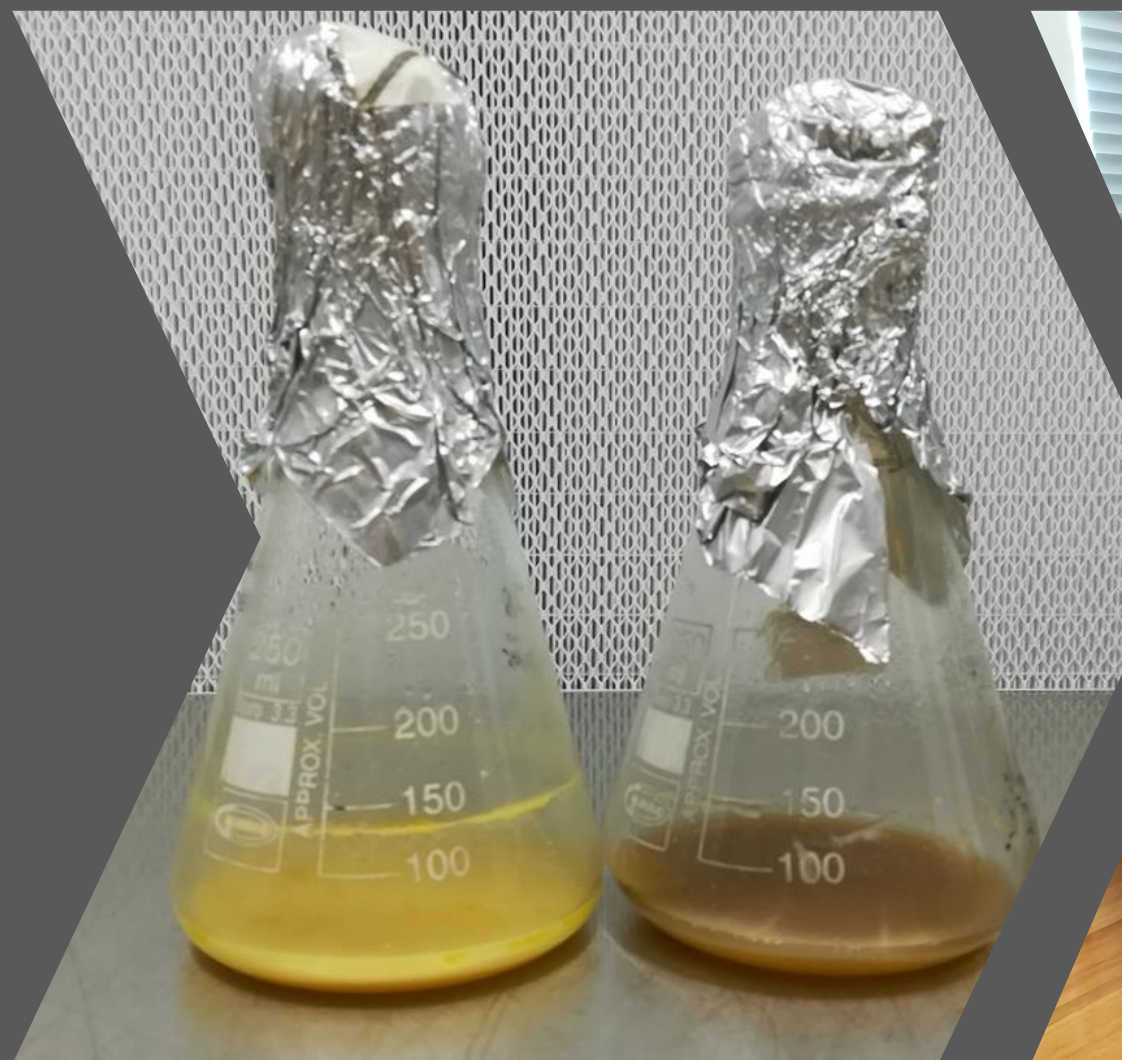


Once IJs present – used as inoculum

In vitro culture protocol

- Standard protocol developed for *Steinernema jeffreyense* & *S. yirgalemense*.
- Successfully mass cultured *H. bacteriophora* under *in vitro* liquid conditions in a 30ml flask (150 000 IJs/ml = 4 500 000 IJs in a 30ml flask)
- More EPNs to be mass cultured (i.e. *H. zealandica*)





Flask SP



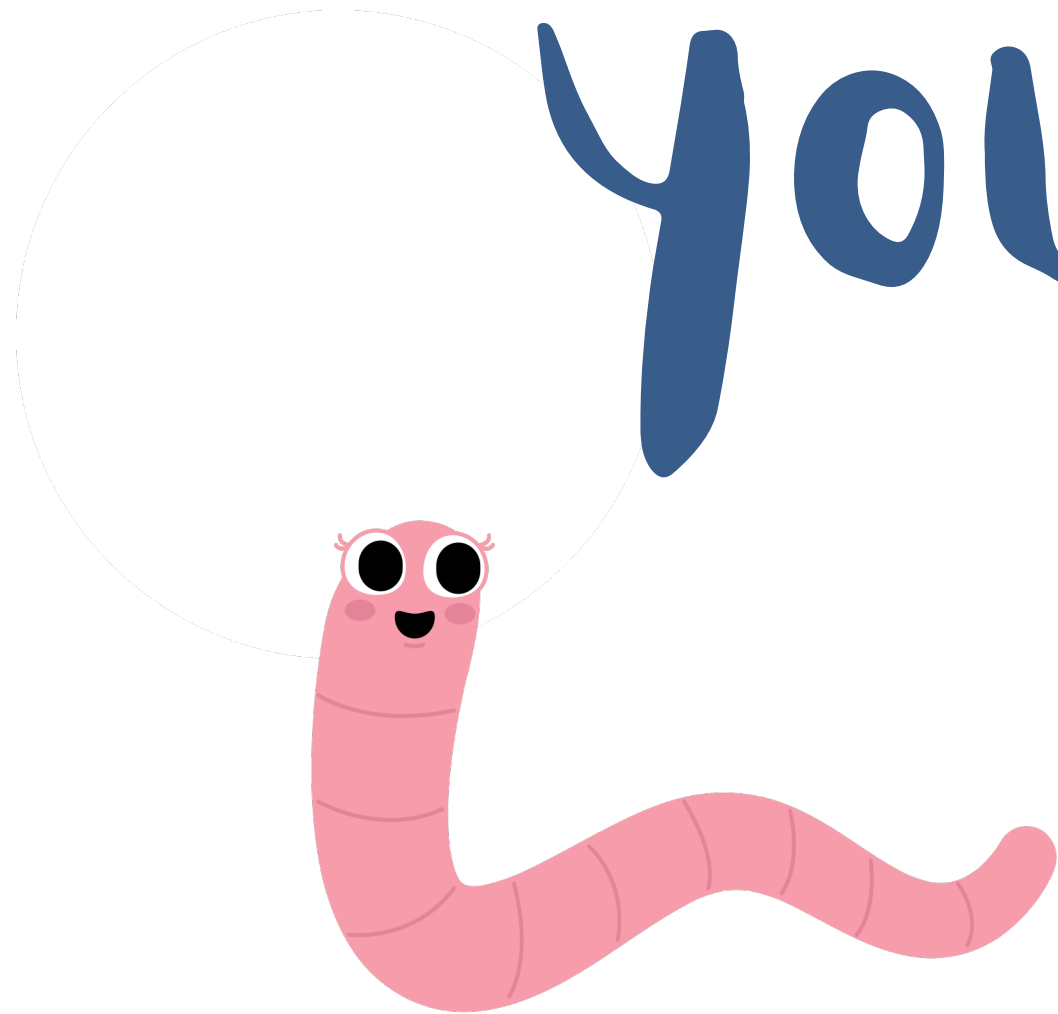
**Mass
production
optimisation**



Bioreactor

Thank

you



njclaasen@sun.ac.za



Stellenbosch

UNIVERSITY
IYUNIVESITHI
UNIVERSITEIT

forward together
sonke siya phambili
saam vorentoe

