Assessment of subterranean termite symbiotic fauna under different diets

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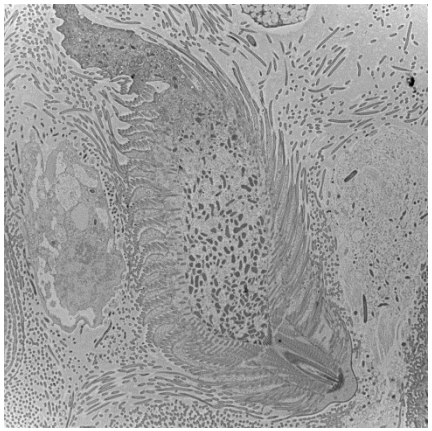
COST-STSM-FP1303-20909



1 LABORATÓRIO NACIONAL DE ENGENHARIA CIVIL

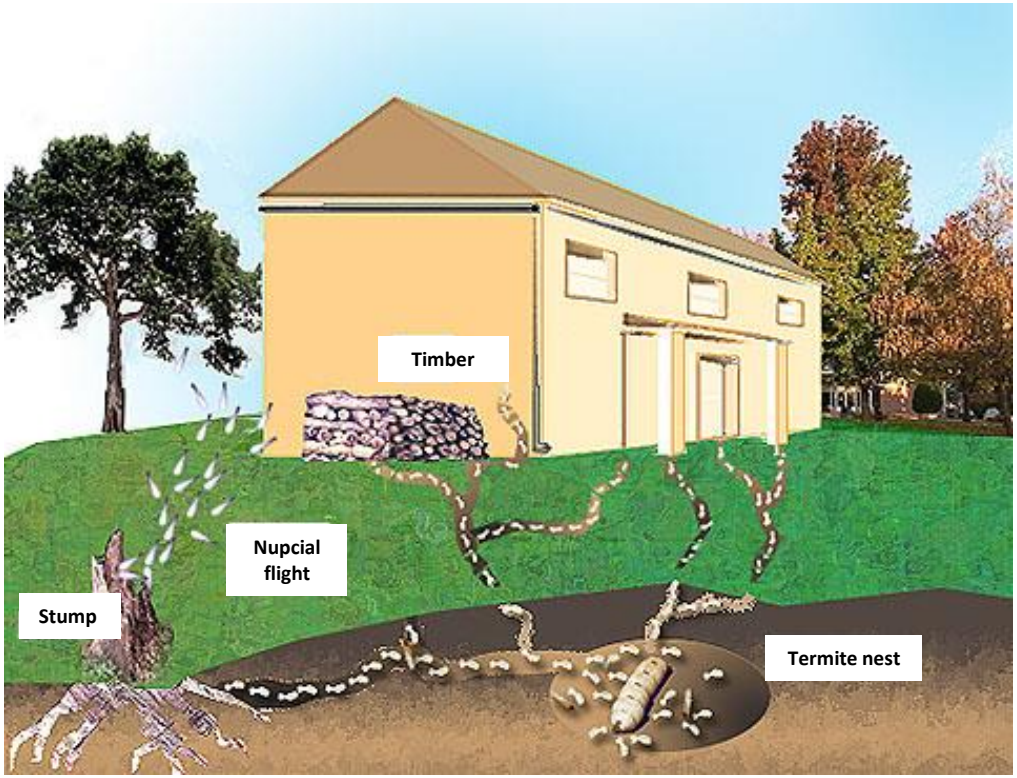
2 cE3c centre for ecology, evolution and environmental changes

azorean biodiversity group Universidade dos Açores

3 skog+ landskap NORWEGIAN FOREST AND LANDSCAPE INSTITUTE

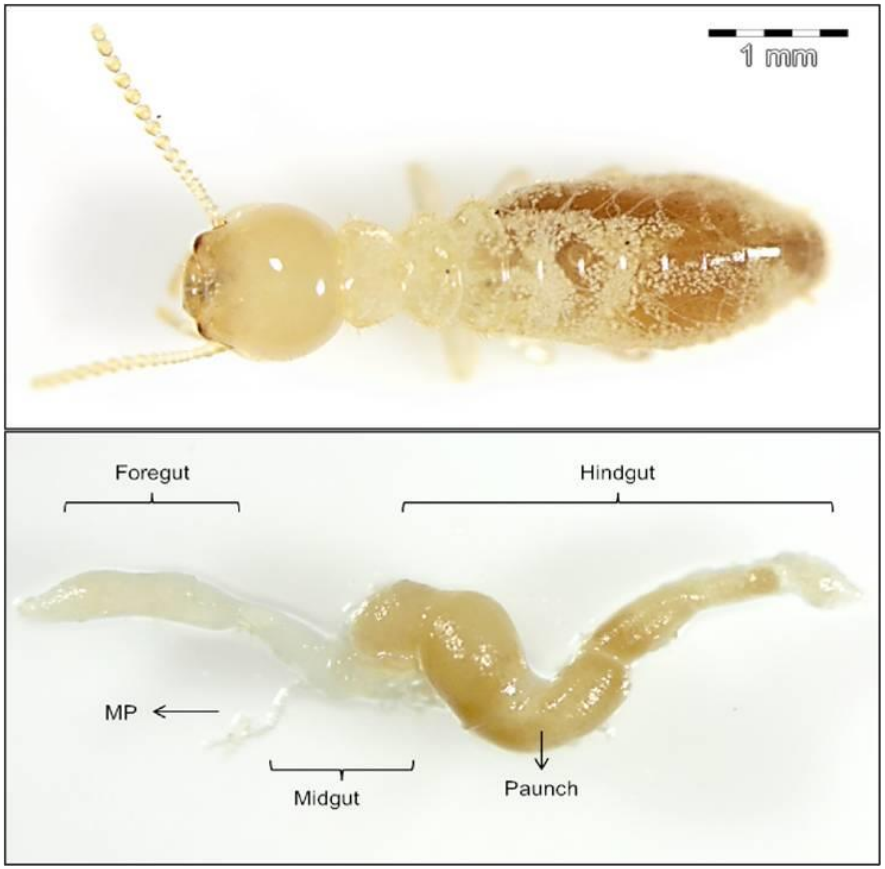
Why termites?

Attacking wood in service… and also biobased materials of cellulosic base



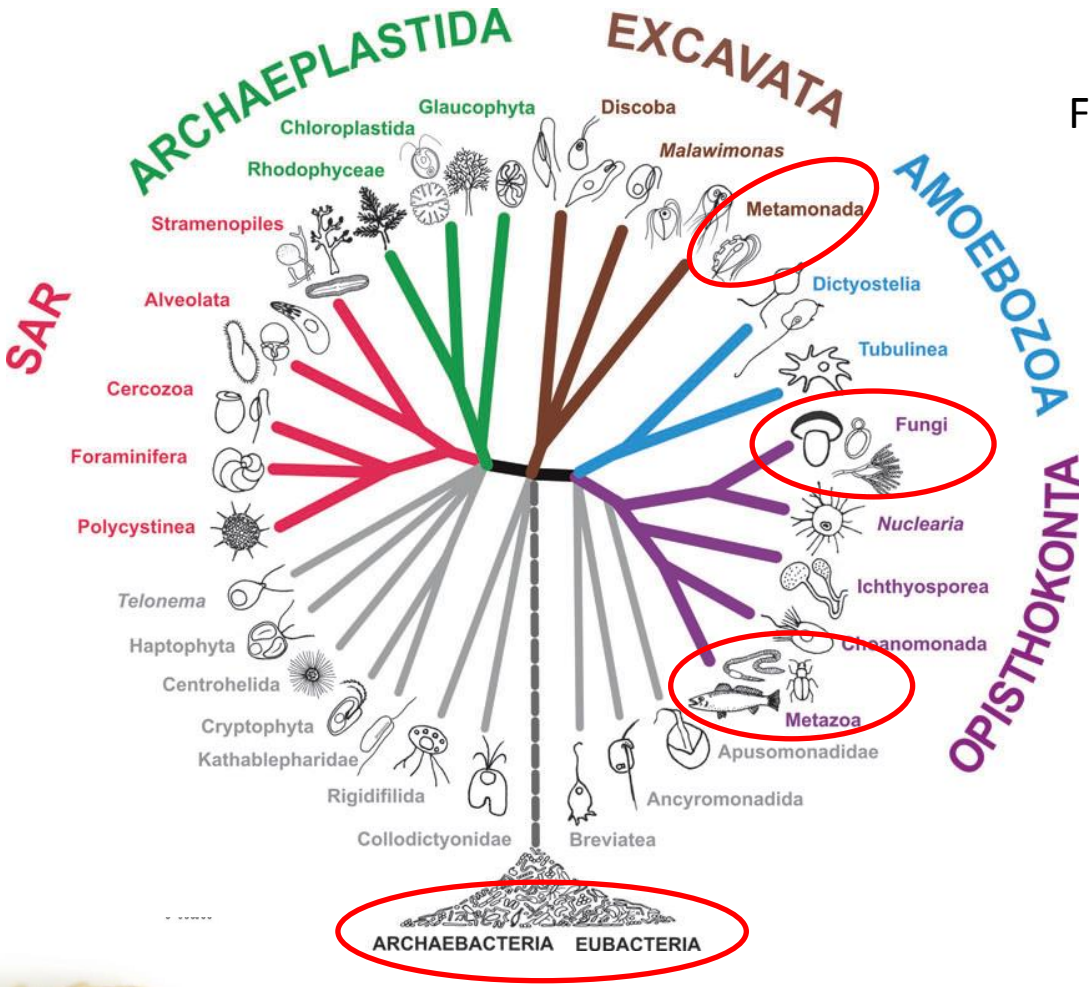
Important in natural ecosystems, worrying in human made environments

How do subterranean termites digest lignocellulose?



Mechanical Physical-chemical

Who are part of the subterranean termite holobiont?



Adl et al. 2012

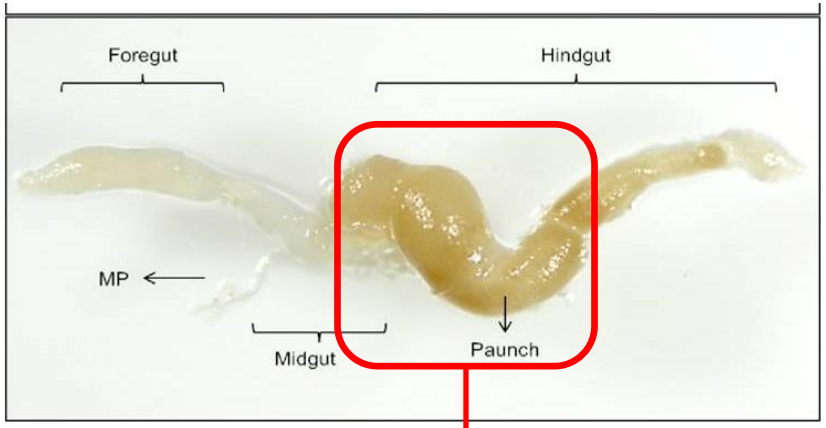
Flagellate protists

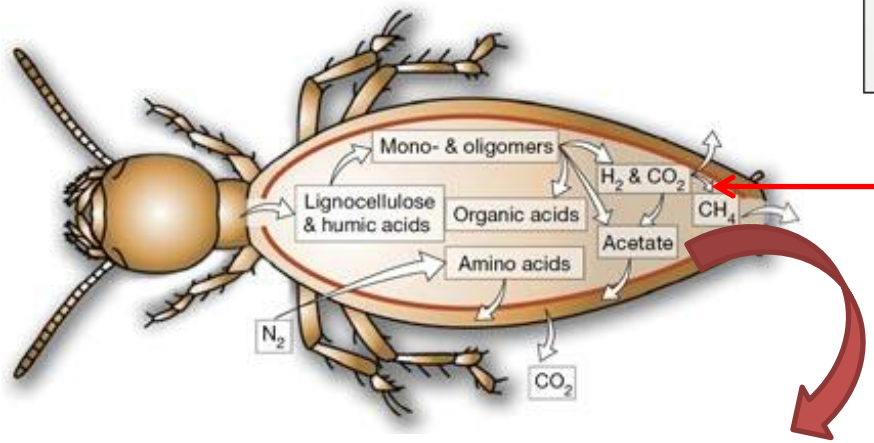
Yeasts

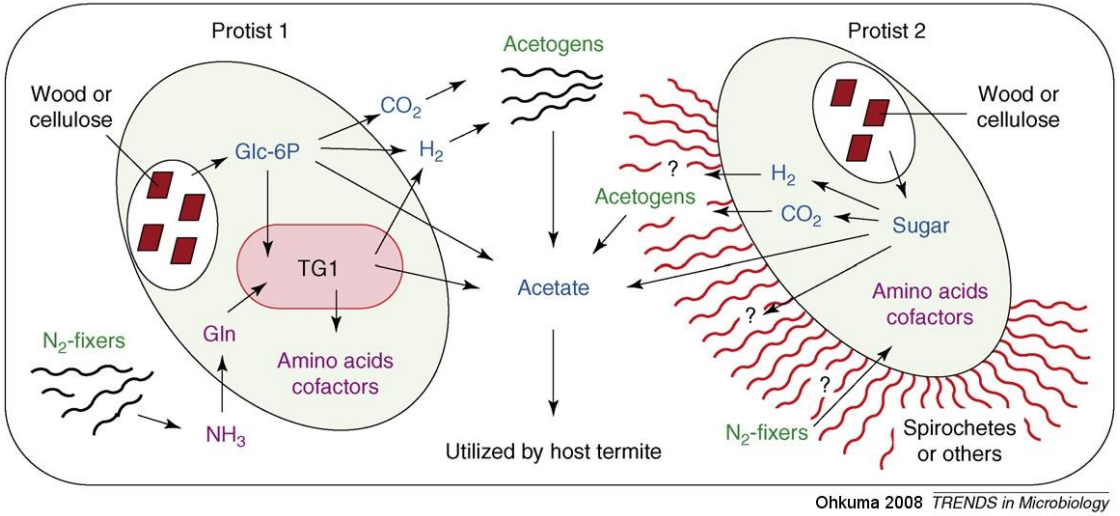
Termite

Bacteria

How do subterranean termites digest wood?

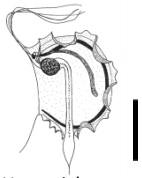






Ohkuma 2008 *TRENDS in Microbiology*

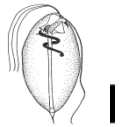
Who are the symbiotic flagellate protists?



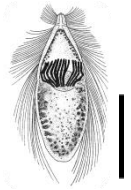
Hypotrichomonadea

Trichomonadea

Triitrichomonadea



Cristamonadea



Trichonymphea

Spirotrichonymphea

Oxymonadida

50µm

					Flagellar apparatus/Mastigont system			Cytoskeletal arrangement			
	Closed mitosis	Golgi body	Hydroge-nosomes	Nuclei	Flagellar system	Basal bodies arrangement	Microtubular fibers/sheets	Axostyle type	Undulating membrane	Costa type	Comb-like structure
Parabasalia	external spindle	+	+		Basically: 4 flagella	Basically: 4 basal bodies, 3 anterior; 1 recurrent	Pelta - covers the anterior part of the cell				
Class Hypotrichomonadea		+	+		4	biramous parabasal body		cone-like, usually stout	lamelliform	A	+
Class Trichomonadea		+	+		2 - 6	single karyomastigont		cone-like or reduced	lamelliform, if present	B, if present	-
Class Tritrichomonadea		+	+	1 or 2	0 - 5	single karyomastigont	pelta-axostyle complex may be reduced or absent	tube or cone-like, if present	rail-form, if present	A, if present	+/ -
Class Cristamonadea		+	+	1 or more	2 to thousands per mastigont	single to multiple mastigonts	often spiralised or ramified axostyle	Stout rod or reduced	transformed into cresta (in some)	type A; reduced or absent	+ (ancestral) or absent
Class Trichonymphea		+	+	1	hundreds to thousands	cell body divided into anterior rostrum and post-rostral area		thin and numerous, do not protude outside the cell	-	-	-
Class Spirotrichonymphea		+	+	1		kinetosomes arranged into spiraled rows		tube-like and stout; multiple thin bands; or reduced	-	-	-
Preaxostyla	internal spindle	-	-	1 or more	4, or a multiple of 4, flagella arranged in pairs	2 to several karyomastigonts	Preaxostylar lamina linking the 2 basal body pairs; Pelta (caps the nucleus)	crystalline axostyle; microtubular rod extending through the entire length of the cell	-	-	-
Order Oxymonadida											

In Duarte et al. submitted

Objectives

H0 (null hypothesis)

"there are no differences among the symbiotic flagellate protists fauna living in the hindgut of termites which feed on different substrates"

- comparison of flagellate protists communities in terms of diversity and abundances
- high quality microscopy images of flagellate protists
- 1st assessment of possible methods to perform a molecular analysis of flagellate protists

From: http://sostermitas.angra.uac.pt/termitas/

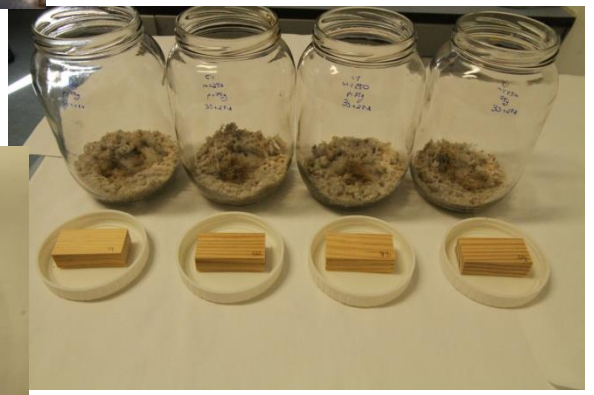
Methods

Termite collection

Laboratory trials

Termite dissection

Protist counting



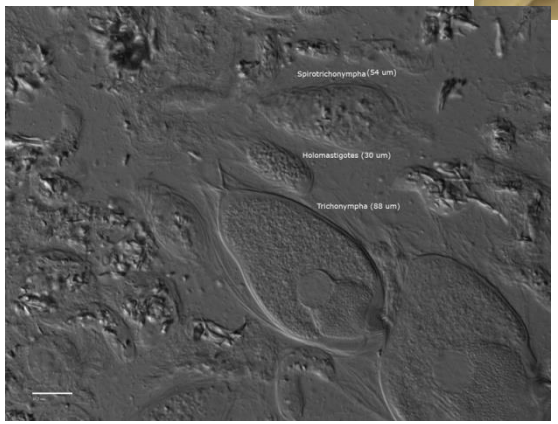
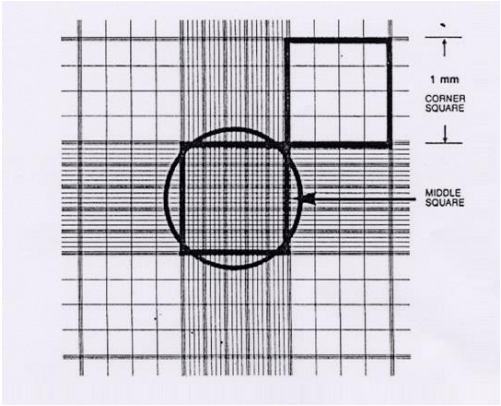


Image (DIC) by Dan Gage



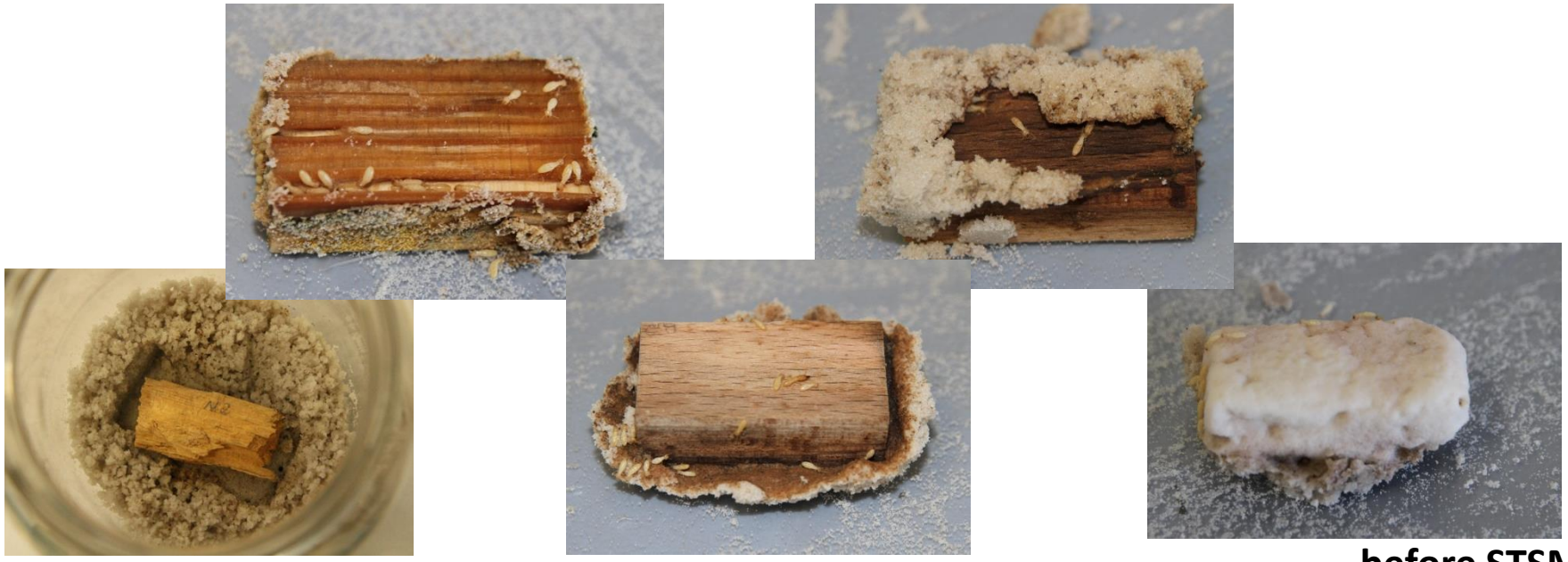
Haemacytometer

…before STSM.

Methods

Diets

- **Natural diet** – pieces of the wood from where termites were collected
- **Pine** – *Pinus pinaster* Ait.
- **Beech** – *Fagus sylvatica* L.
- **Beech TMT** – beech submitted to a thermal treatment of 180ºC for 4h
- **Cellulose** – powder cellulose plus deionized water
- **Starving** – no cellulose source offered to the termites

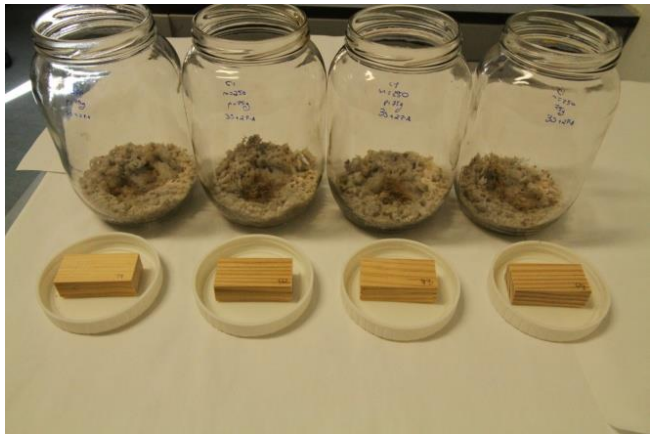


…before STSM.

Methods

EN117 (adapted)

14 days of exposure (except for starving = 6 days)



3 replicates for each diet (except for natural and starvation – 1 replicate)

250 termite workers (*Reticulitermes grassei* Clément) per trial

…before STSM.

Methods

Preparation methods for microscopy analysis

	Step	Solution	Duration	Date
1	Fixation	1.25% glutaraldehyde, 2% paraformaldehyde in 0.05% Pipes buffer	fixed in Lisbon (4); overnight (1; 3)	19-08-2014
2	Wash	Passende buffer - 0.1M CaCodylat buffer, pH7.4	10 min (x3)	20-08-2014
3	Post Fixation	1% OsO4 in0.1M CaCo	90 min	20-08-2014
	Wash	0.1M CaCo, pH7.4	10 min (x3)	20-08-2014
4	Dehydration	70% EtOH	10 min	20-08-2014
		90% EtOH	10 min	20-08-2014
		96% EtOH	10 min	20-08-2014
		100% EtOH	10 min (x4)	20-08-2014
5	Infiltration	1 LR White : 3 100% EtOH	overnight	20-08-2014
		1 LR White : 1 100% EtOH	3h	21-08-2014
		3 LR White : 1 100% EtOH	3h	21-08-2014
		100% LR White	overnight	21-08-2014
6	Embedding	100% LR White, at 60ºC	overnight	22-08-2014

Preparation and embedding in resin of samples for TEM analysis

For each variable – 30 termites hindguts prepared

@Norwegian Forest and Landscape Institute

DNA and RNA tentative extraction - X

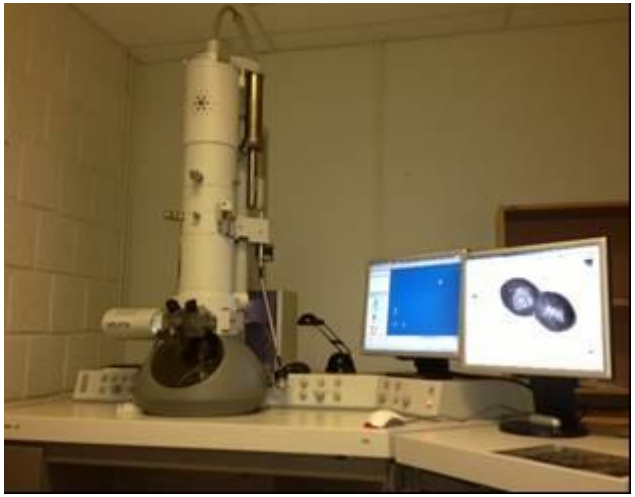
…during STSM.

Methods

Ultramicrotome

Leica EM UC6

Transmission Electron Microscope



FEI Morgagni 268

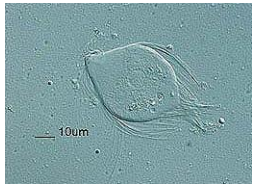
@Imaging Centre of the Norwegian University of Life Sciences, NMBU

…during STSM.

Results

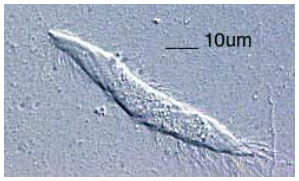
Morphotypes ID

	Phyllum	Class	Order	Family	Genus	Species
n1	Parabasalia	Trychonymphea	Trichonymphida	Trichonymphidae	*Trichonympha*	*Trichonympha agilis*
n2	Preaxostyla	-	Oxymonadida	-	*Pyrsonympha*	
n3	Preaxostyla	-	Oxymonadida	-	*Dinenympha*	*Dinenympha gracilis*
n4	Parabasalia	Spirotrichonymphea	Spirotrichonymphida	Holomastigotoididae	*Holomastigotes*	*Holomastigotes elongatum*
n5	Preaxostyla	-	Oxymonadida	-	*Dinenympha*	*Dinenympha fimbriata*
n6	Parabasalia	Hypotrichomonadea	Hypotrichomonadida	Hypotrichomonadidae	*Trichomitus or Trichomitopsis*	
n7	Preaxostyla	-	Oxymonadida	-	*Pyrsonympha*	
n8	Parabasalia	Spirotrichonymphea	Spirotrichonymphida	Holomastigotoididae	*Microjoenia*	
n9	Parabasalia	Trychonymphea	Trichonymphida	Teranymphidae	*Pseudotrichonympha*	
n12	Parabasalia	Spirotrichonymphea	Spirotrichonymphida	Holomastigotoididae		
n13	Parabasalia	Trichomonadea	Honigbergiellida	Tricercomitidae	*Tricercomitus*	
n16	Preaxostyla	-	Oxymonadida	-		
n18	Parabasalia					

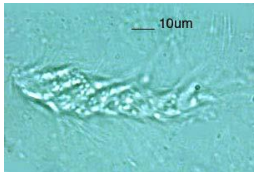


T. agilis

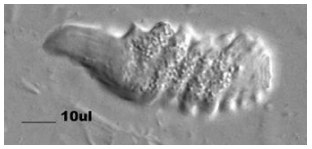
Microjoenia



D. fimbriata



H. elongatum

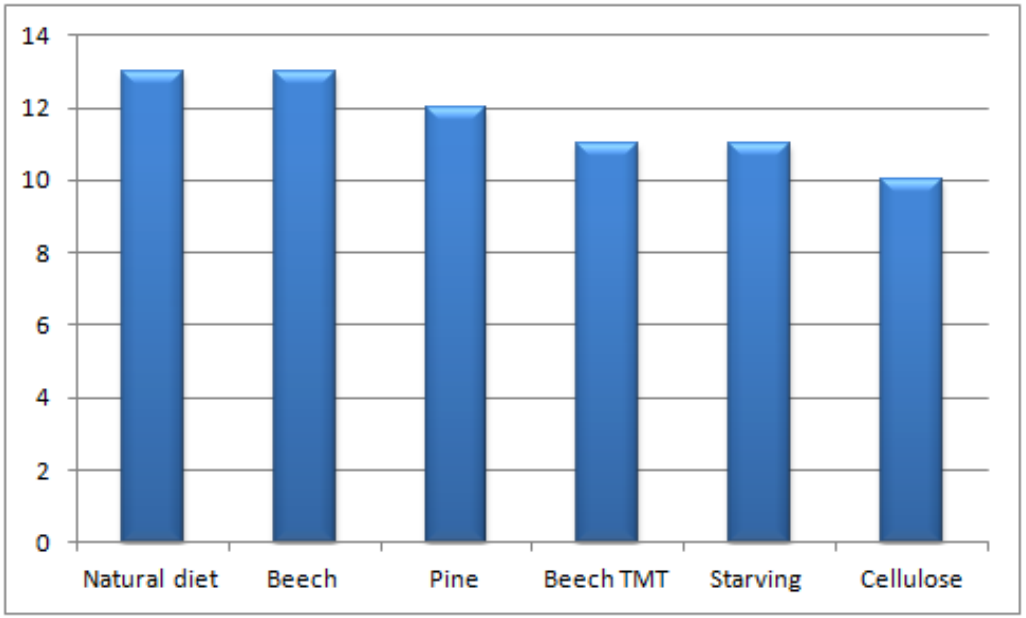


Pyrsonympha spp.

Photos of JL Lewis

Results

Number of morphotypes



	Survival rate (%)	Mass loss (%)
Natural diet	74.4	5.2
Pine	39.3	3.7
Beech	45.9	3.6
Beech TMT	39.5	3.7
Cellulose	39.6	11.4
Starving	33.2	-

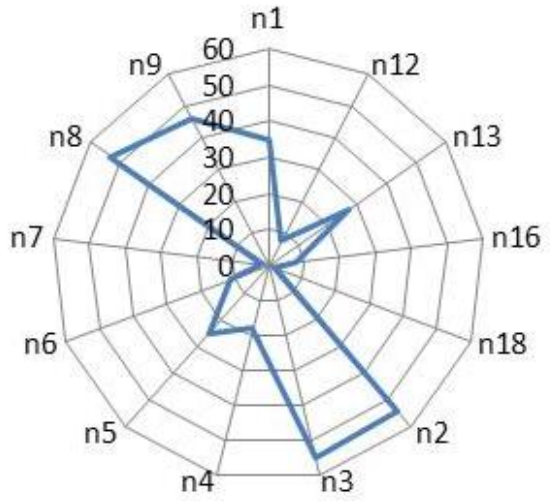
Results of paired T-test (p<0.05)

H0?

	Natural diet	Pine	Beech	Beech TMT	Cellulose
Natural diet					
Pine	✓				
Beech	✓	✓			
Beech TMT	✗	✗	✗		
Cellulose	✗	✗	✗	✓	
Starving	✗	✗	✗	✓	✓

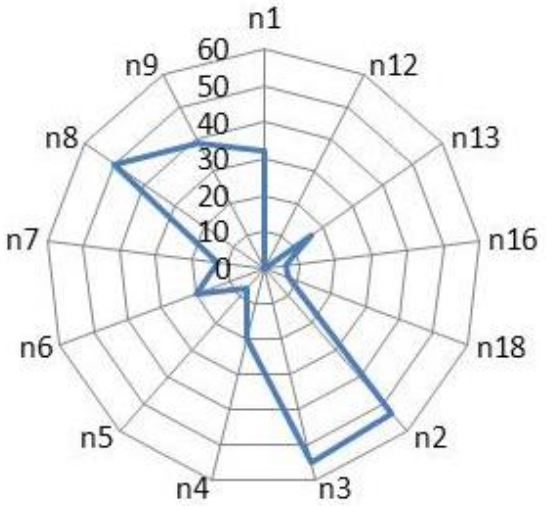
Natural diet

↑*Dinenympha fimbriata – n5*

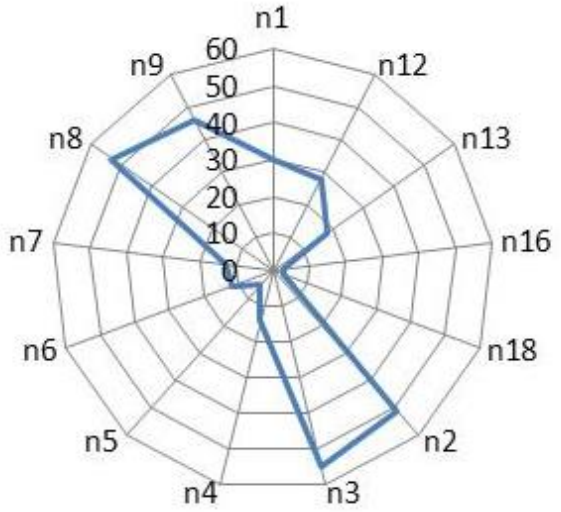


Pine

↑Hypotrichomonadidae – n6

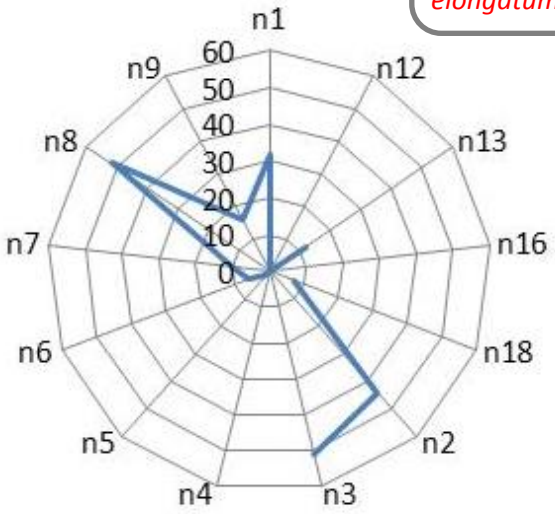


Beech



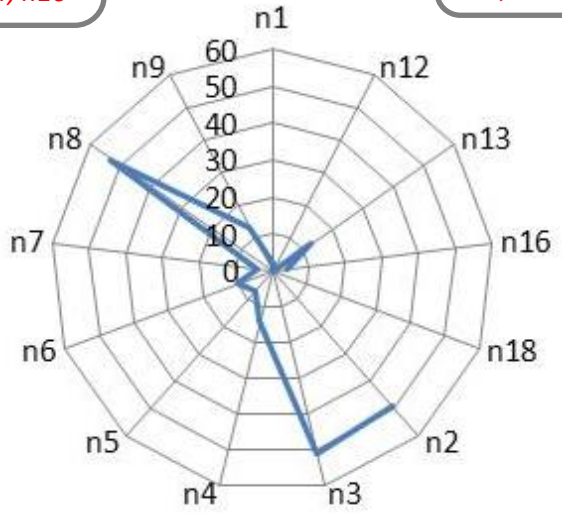
Cellulose

↓*Pseudotrichonympha spp. – n9*
Holomastigotes elongatum – n4; n16



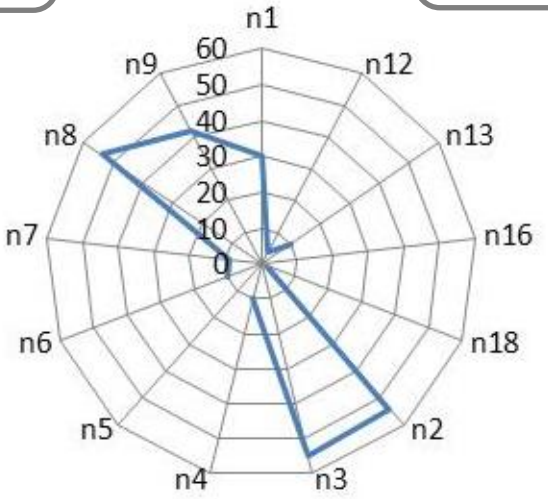
Starving

↓*Trichonympha agilis– n1*
n16; n18



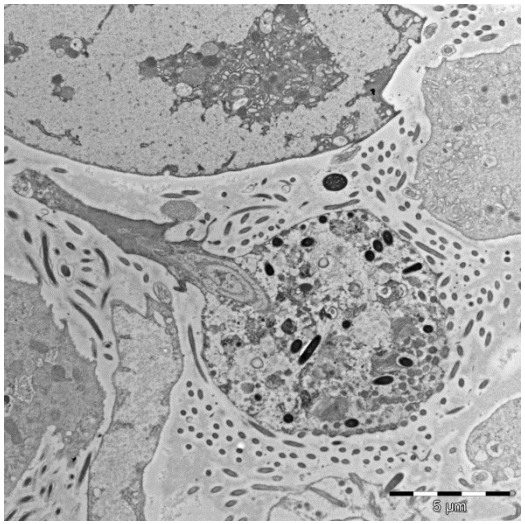
Beech TMT

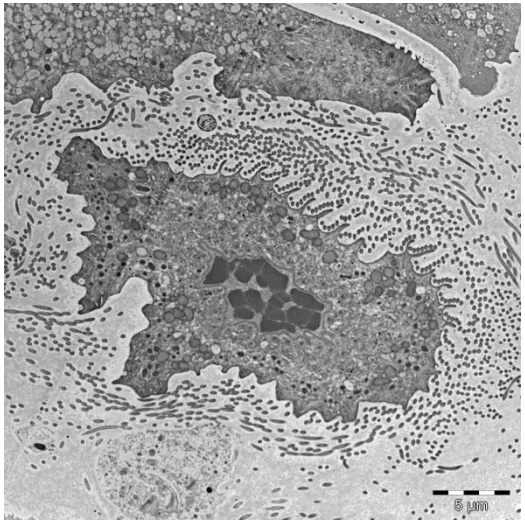
Dinenympha fimbriata – n5
n16; n18

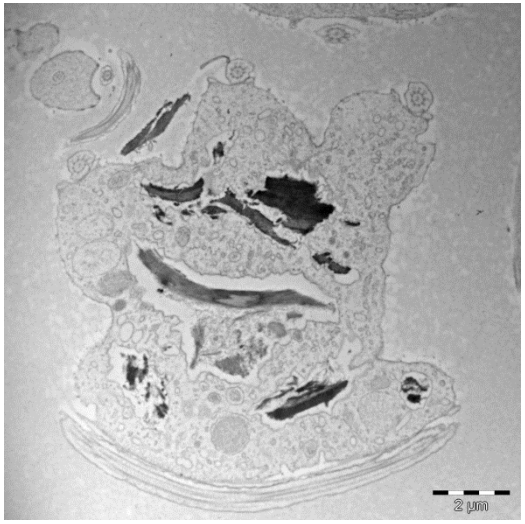


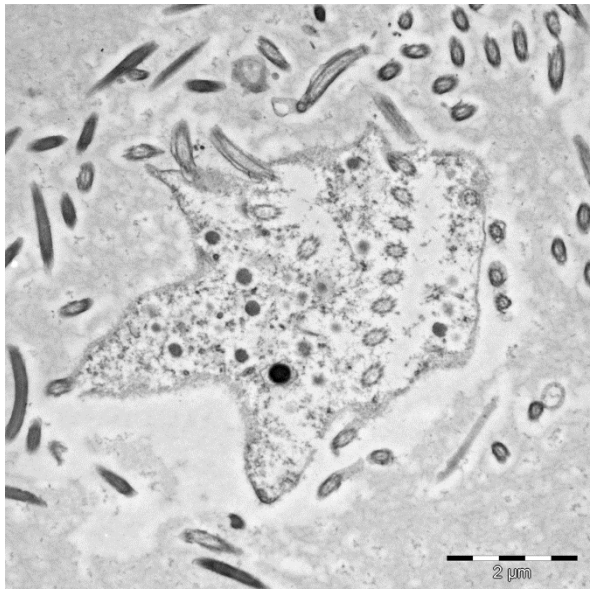
Results

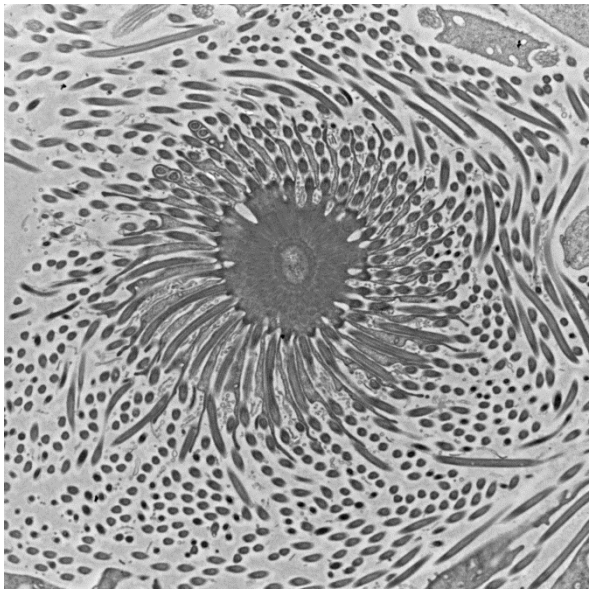
Parabasalia

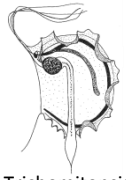








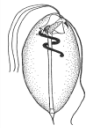




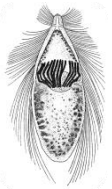
Trichomitopsis spp.

Trichomitus spp.

Triitrichomonadidea



Devescovina spp.

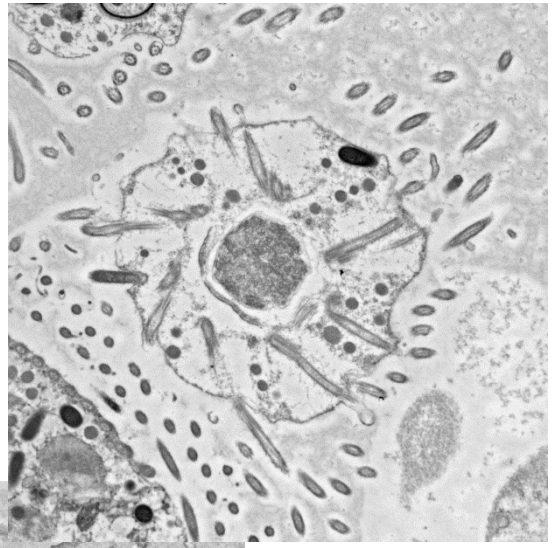


Trichonympha spp.

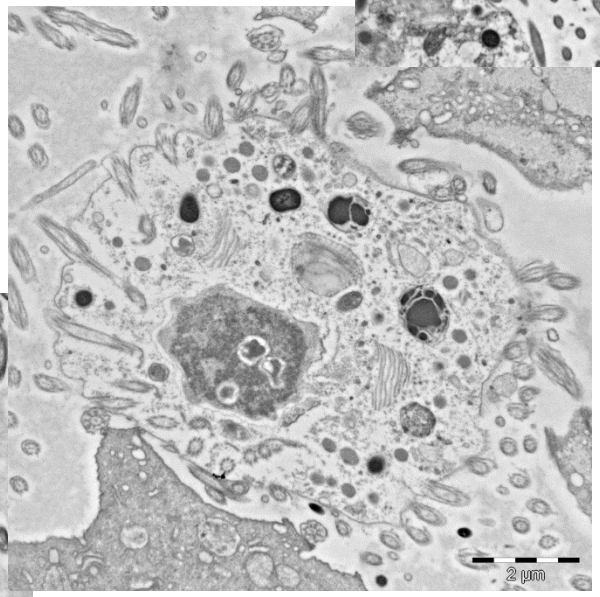
Holomastigotes spp.

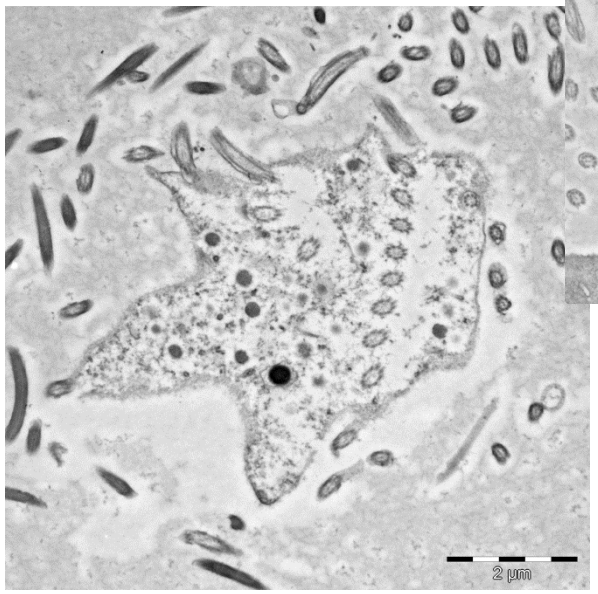
Results

Parabasalia



Spirotrichonymphidae

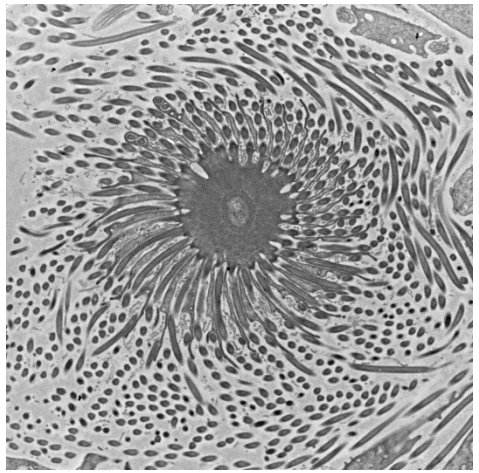


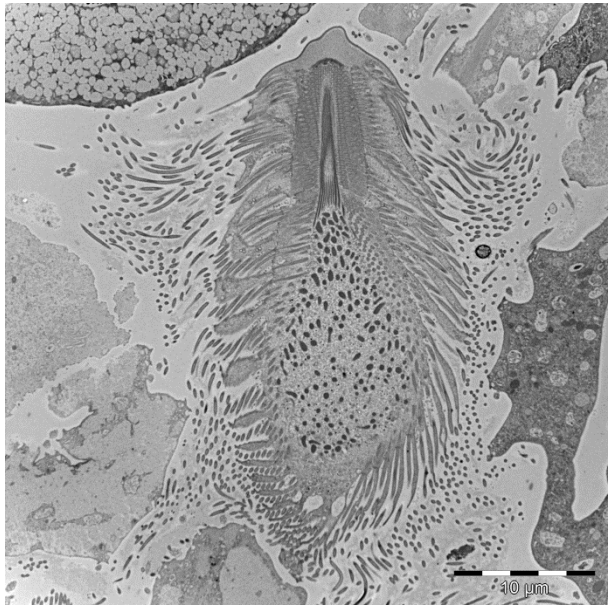


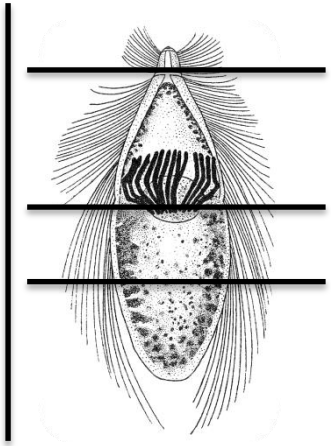
Flagella - spiralized pattern

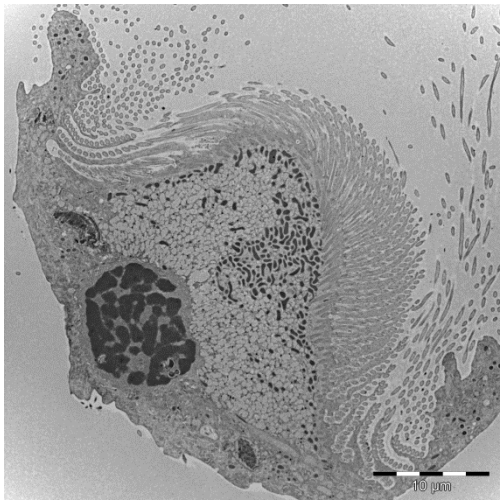
Results

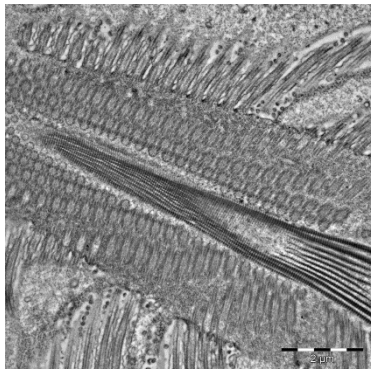
Parabasalia

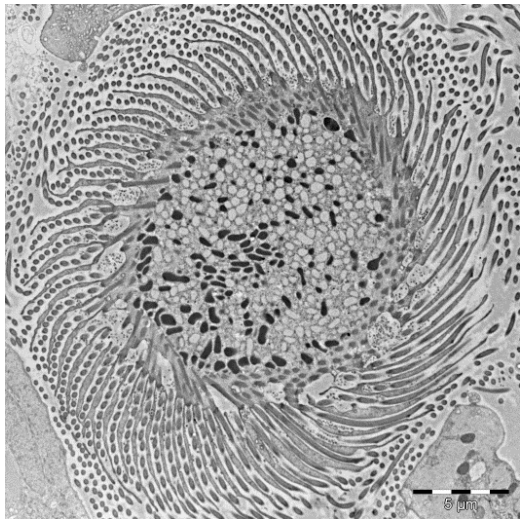








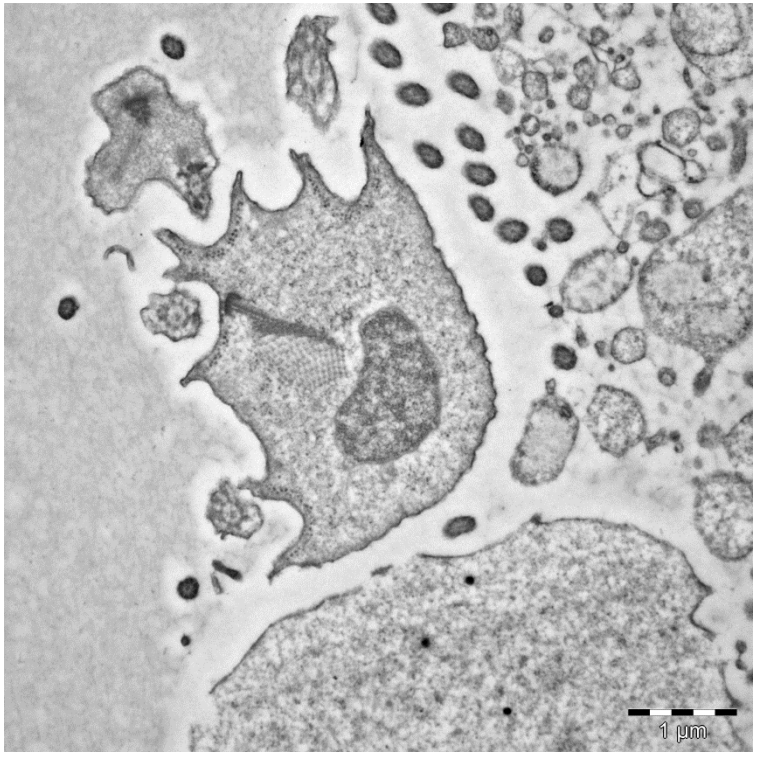


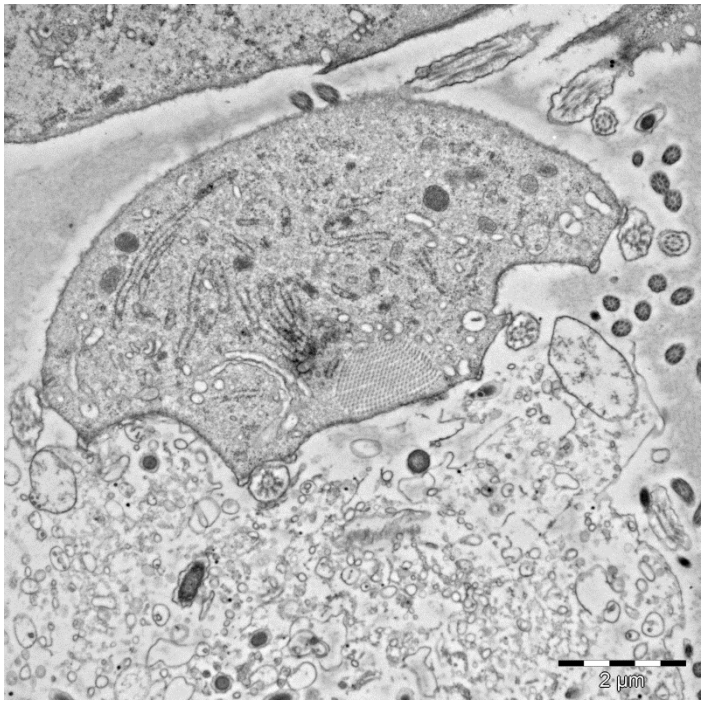


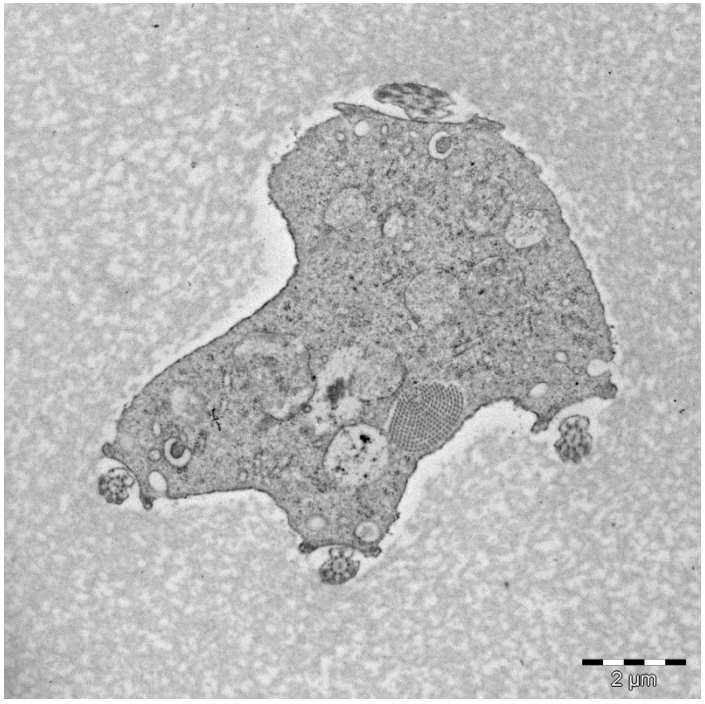
Trichonymphidae

Results

Preaxostyla





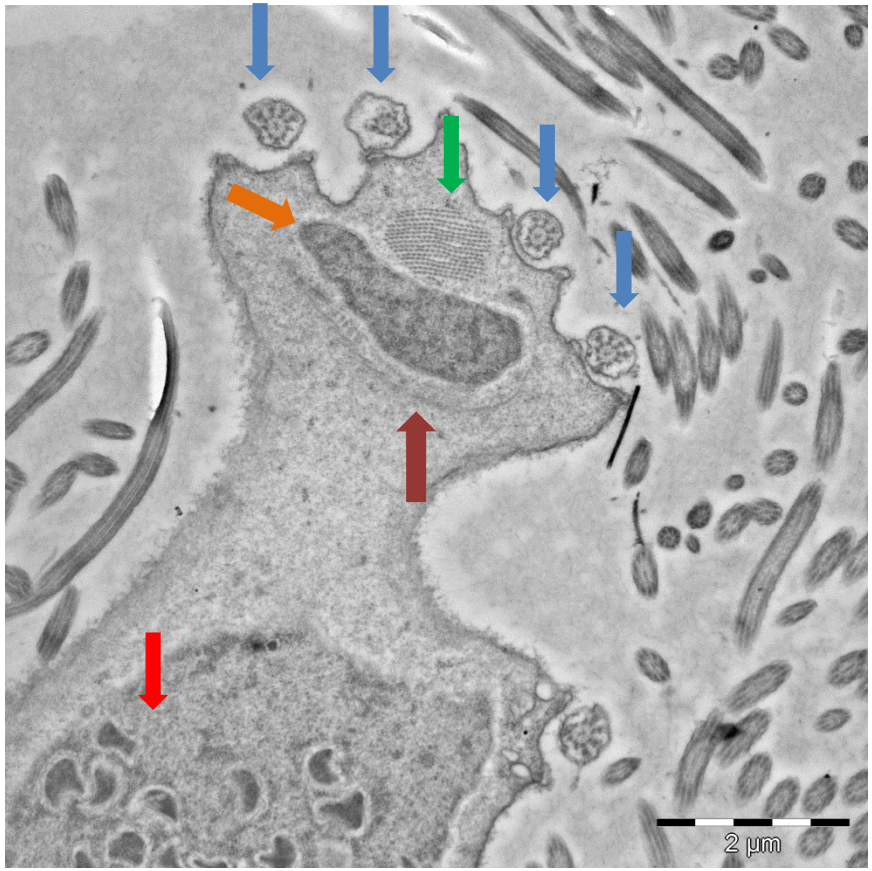


Pyrsonympha spp.

Oxymonas spp.

Results

Preaxostyla



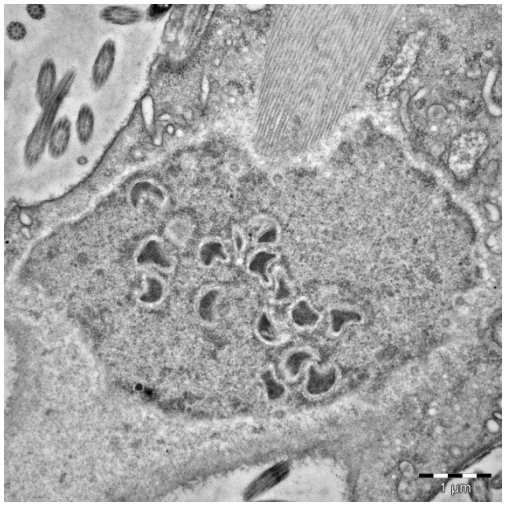
4 Flagella

2nd Nucleus

Axostyle

Pelta

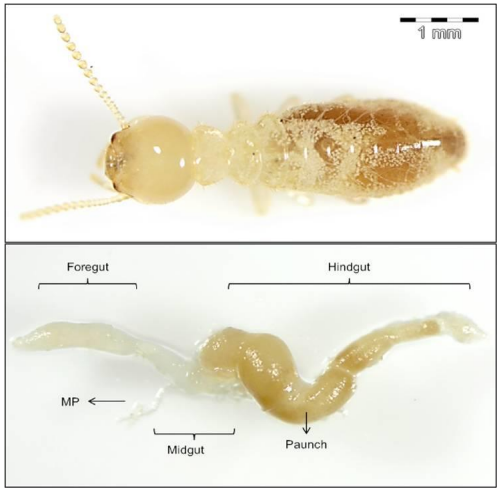
Nucleus w/ intranuclear symbionts

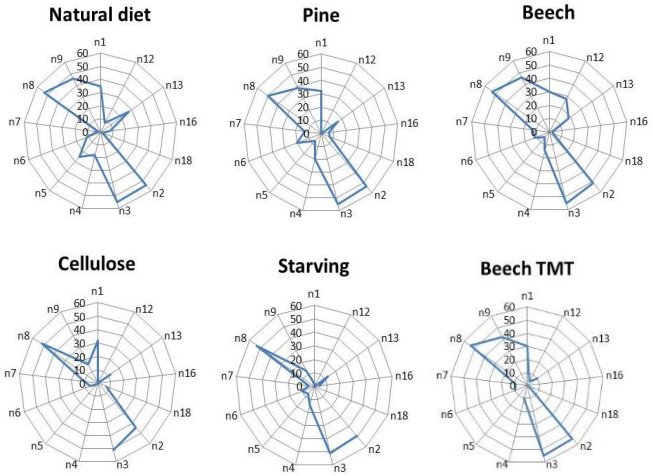


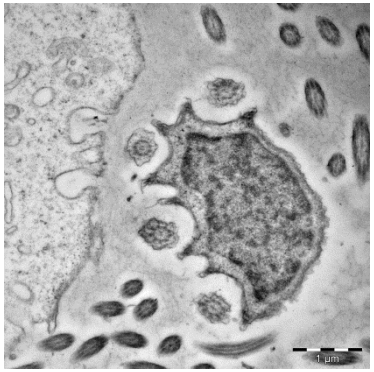
Discussion/Conclusions

There are significant differences in the flagellate protists community of termites according with different type of diets

This work was a first step towards the understanding of the changes occurring inside *Reticulitermes grassei* hindgut due to the ingestion of different diets







The evaluation of the flagelate protist community may be a valuable tool to detect substances which may constrain the termite hindgut "bioreactor" correct functioning

Acknowledgments

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Thank you for your attention