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OIE SYMPOSIUM ON
EMERGING INFECTIOUS AGENTS IN HONEY BEES AND OIE-LISTED DISEASES
to be held during the
45th APIMONDIA International Apicultural Congress
Istanbul (Turkey), Monday 2 October 2017

CRITHIDIA SPP. AND LOTMARIA SPP. POSSIBLE EMERGING PATHOGENS IN HONEY BEES

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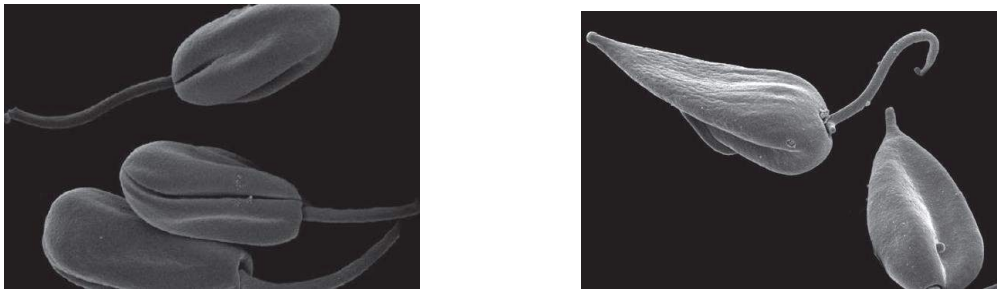
Introduction

Crithidia mellificae and ***Lotmaria passim*** are enteric, unicellular, protozoan **parasites of *Apis mellifera***.
They belong to the **Family *Trypanosomatidae,*** with two different Genera: ***Crithidia*** and ***Lotmaria***.
Of the 19 trypanosomatid genera currently recognized, 14 are **monoxenous**, parasitizing one or more insect hosts.



SEM image (Schwarz et al. 2015)

Family: *Trypanosomatidae*

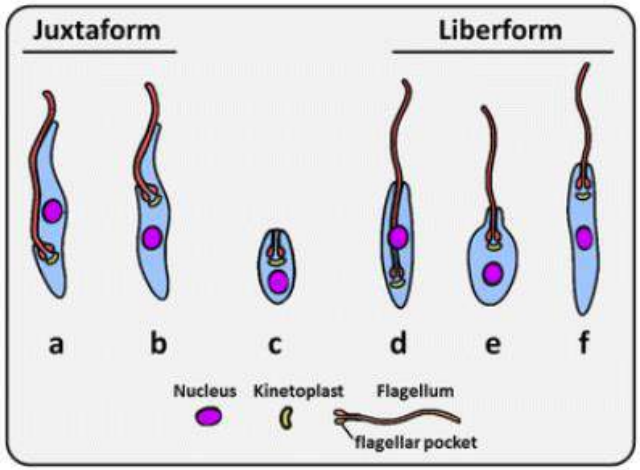
Genus *Crithidia*:
Crithidia mellificae

Genus Lotmaria:
Lotmaria passim

Trypanosomatids have been known to infect *Apis mellifera* since at least 1912 *(Fanthan and Porter, 1912)* characterized by a **global distribution** (reports from EU, Turkey, Switzerland, Australia, America and Asia).

Trypanosomatid systematic was classically based on:

- **host preferences** (one-insect-one-parasite rule);
- **morphotypes** (amastigote, chanomastigote, promastigote, etc.)



Kaufer *et al.*, 2017

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Crithidia mellificae was characterized and denominated in **1967** by **Langridge and mcGhee**, and it was **the only one trypanosomatid species** found in honey bee until 2014.

In 2014 a **new trypanosomatid genus and species** in the honey bees was identified and described by **Schwarz: *Lotmaria passim.***

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ORIGINAL ARTICLE

Characterization of Two Species of Trypanosomatidae from the Honey Bee *Apis mellifera*: *Crithidia mellificae* Langridge and McGhee, 1967 and *Lotmaria passim* n. gen., n. sp.

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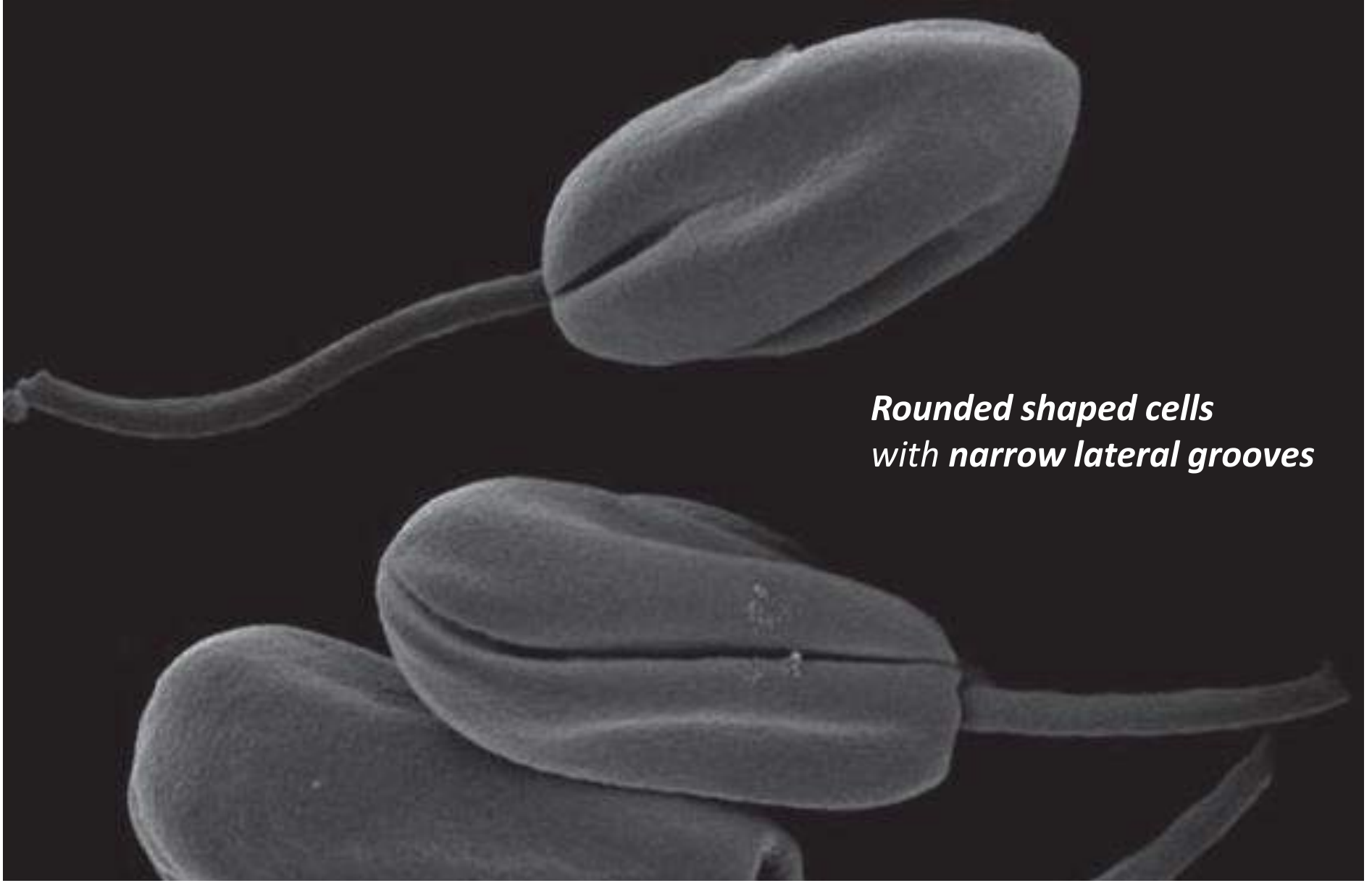
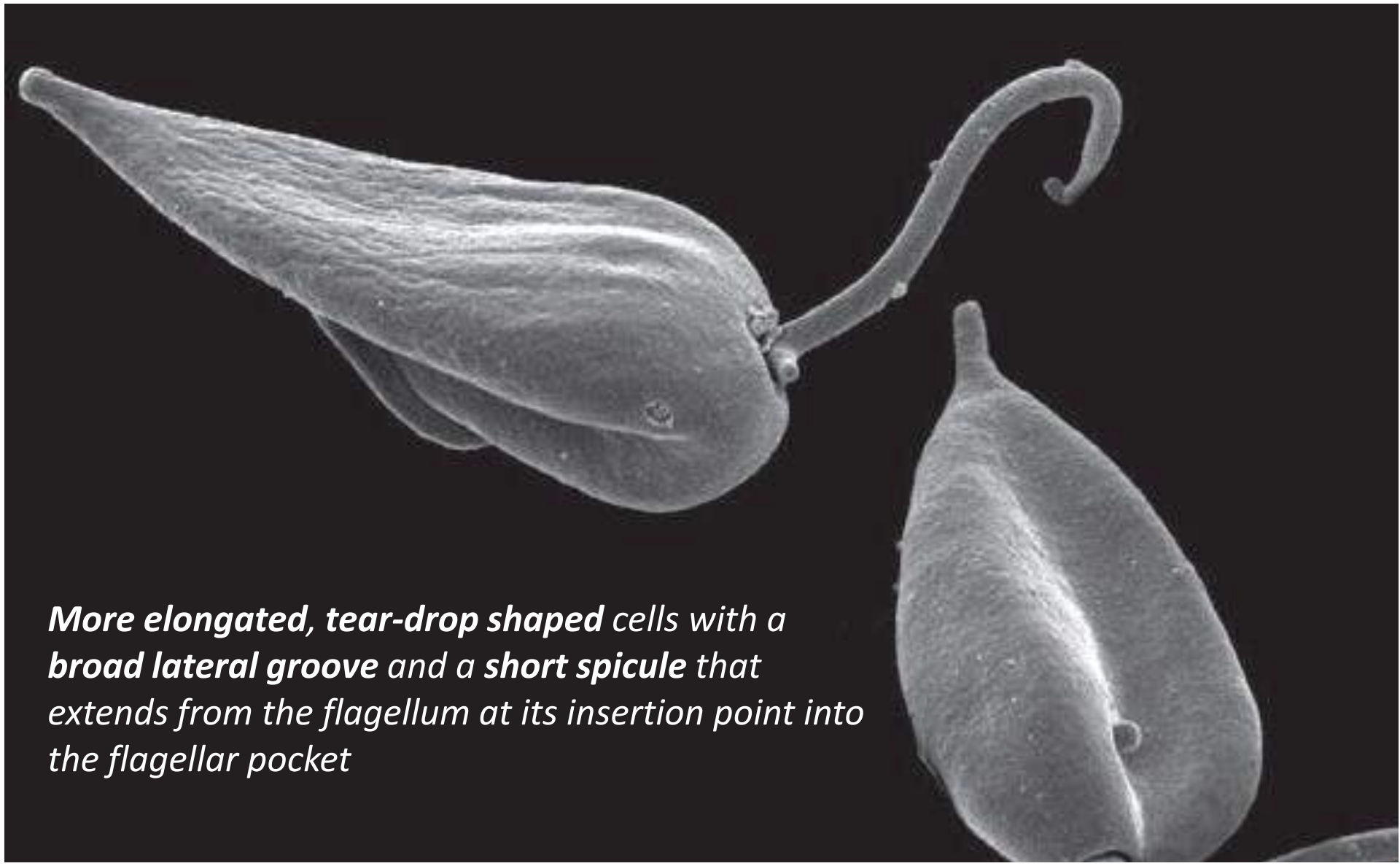
Keywords
Apidae; flagellate; Kinetoplastea; *Leptomonas*; protists; systematics; taxonomy; ultra-

ABSTRACT

Trypanosomatids are increasingly recognized as prevalent in European honey



Chanomastigotes of *Crithidia mellificae* (Scharz et al. 2015)



Promastigotes of *Lotmaria passim* (Schwarz et al. 2015*)*

Lotmaria passim Schwarz, 2014 	***Crithidia mellificae*** Langridge & McGhee, 1967 
Flagellated morphotype	
promastigote (lanceolate)	choanomastigote (ovoid)
broad, shallow groove	narrow, deep grooves
length = 7.4µm ; width = 3.2µm	length = 6.6µm ; width = 3.3µm
Adherent morphotype	
spheroids ; diameter 3-4µm	spheroids ; diameter = est. 4µm*
primary niche = posterior ileum – rectum	primary niche = posterior ileum - rectum

Based on R. Schwarz et al. 2015. *J. Euk. Microbiol.* and D. Langridge and R. McGhee 1967. *J. Protozool.* *Estimate only

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Diagnosis

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Cell line cultures: isolation with «supplemented DS2» medium (from dissected gut of honey bees)

Optical/Confocal laser scanning microscopy

Electron microscopy (scanning or transmission)

Genetic characterization (phylogenetic analyses)

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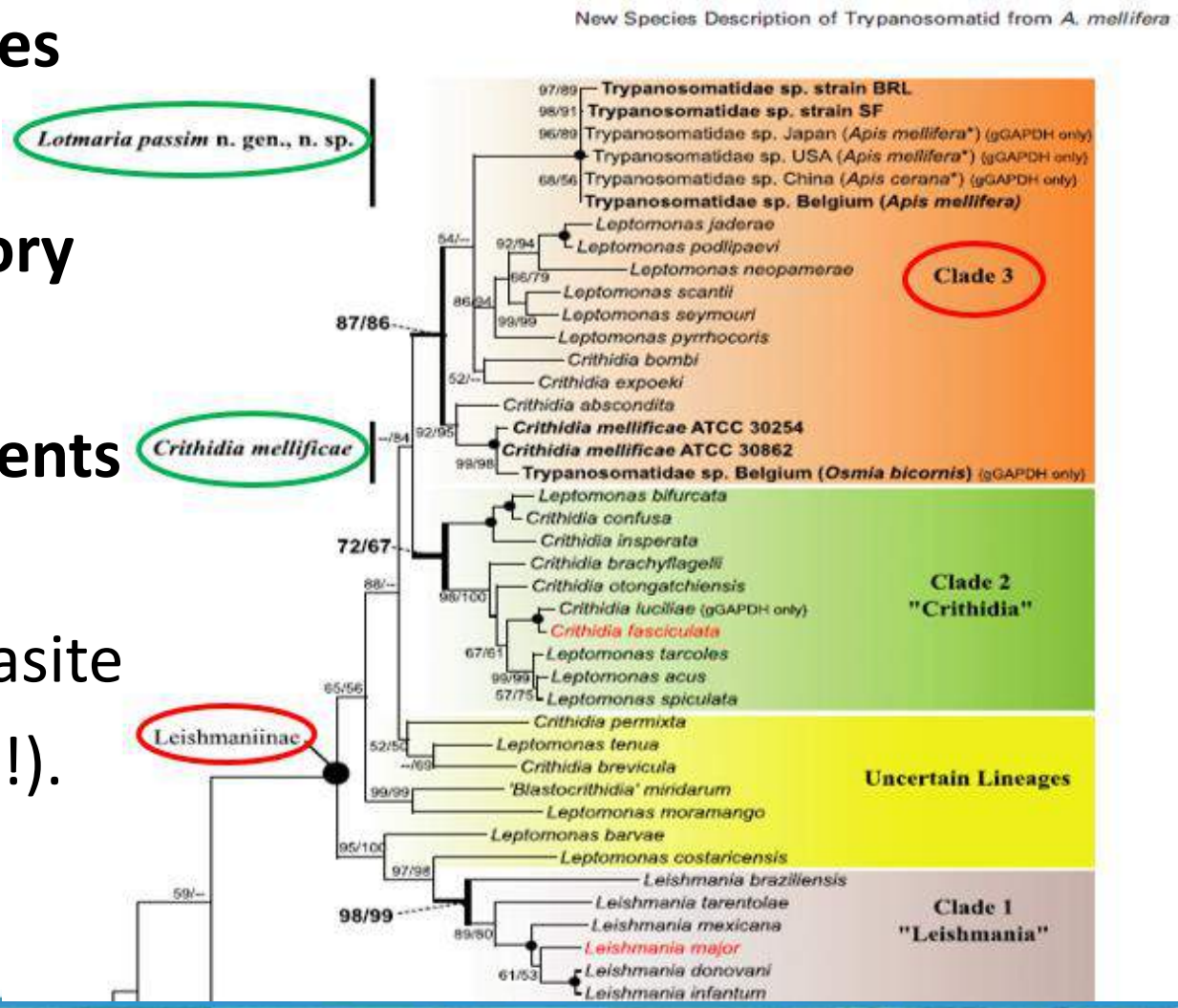
Phylogenetic analyses are now considered **mandatory for making accurate taxonomic assignments** for new isolates (one-insect-one-parasite rule is no more valid!).



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C. Mellificae and *L. passim* diagnosis by PCR:

Two pairs of primers that are **species-specific** were designed at **polymorphic regions** of the **cytochrome b gene.**
(Jevrosima Stevanovic e*t al.*, 2016)

Lotmaria passim
LpCytb_F1 (5'-cGAAGTgCaCATATATGCTTTAC-3')
LpCytb_R (5'-gcCAaAcACCaATaACtGGtACt-3')

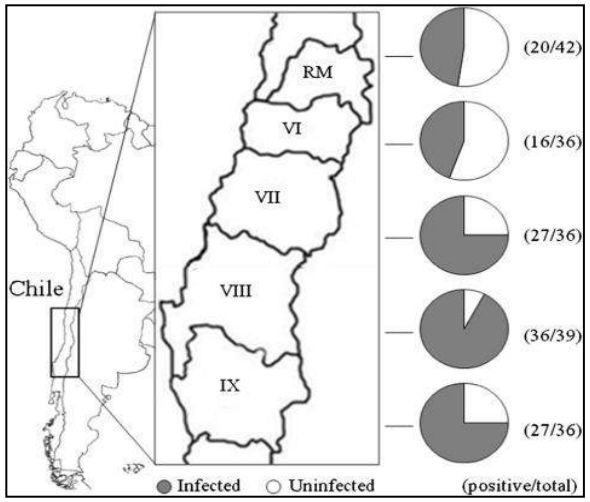
… alternatively, for *L. passim*, see Nolberto Arismendi et al., 2016: primers targeting different loci SSI rRNA (459 bp) and gGAPDH (402 bp)

Crithidia mellificae
CmCytb_F (5'-AGTtTGAgCtGTtGGaTTTgTt-3')
CmCytb_R (5'-AACCtATtACaGGcACaGTTGC-3')

Distribution

Before 2014 *Crithidia mellificae* was identified in many countries, showing a **cosmopolitan distribution**:

- Australia (Langridge & mcGhee, 1967)
- Switzerland (Schmid-Hempel & Tognazzo, 2010)
- USA (Runckel et al., 2011)
- France (Dainat & Neumann, 2012)
- China (Yang et al., 2013)
- Japan (Morimoto et al., 2013)
- Italy (Cersini et al., 2015)



Prevalence of Lotmaria passim in honey bee in Chile
(Arismendi et al. 2015)

Distribution after Lotmaria passim characterization

Previous results concerning Crithidia mellificae diagnosis should all be reconsidered performing a differential diagnosis with *Lotmaria passim*.

According to Schwarz and collegues (2015) that accessioned genetic data, *C. mellificae* still extants in bee populations, however *L. passim* is currently the predominant trypanosomid in *A. mellifera*, globally.

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Distribution after 2015

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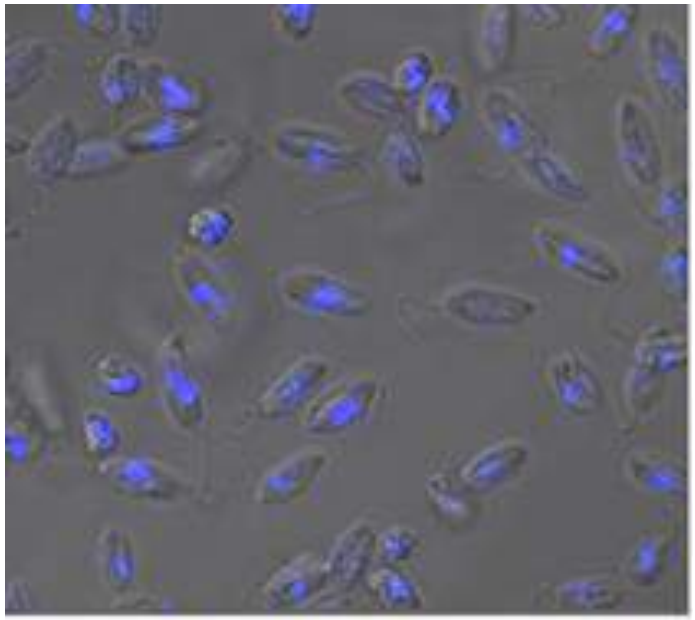
Country	Year	C. mellificae	L. passim	Author
Serbia	2007-2015	0%	39-83%	Stevanovic *et al.*, 2016
Belgium	2015	Yes (rare)	Yes (dominant)	Ravoet *et al., 2015* Schwartz *et al.*, 2015
Japan	2015	No	Yes (dominant)	Ravoet *et al., 2015* Schwartz *et al.*, 2015
Switzerland	2015	No	Yes (dominant)	Ravoet *et al., 2015* Schwartz *et al.*, 2015
Chile	2014-2015	?	Yes (44-92%)	Arismendi *et al., 2016*
USA	2010-2012	?	Yes	Schwartz *et al.*, 2015
China	2013	?	Yes	Schwartz *et al.*, 2015

The relative distribution of these two species largely **remains unknown.** No surveys of honey bee trypanosomatids have been conducted in the majority of countries.

Pathogenesis

Trypanosomatids are **responsible of disease only in adult honey bees** (they have never been detected in bees younger than 6 days old).

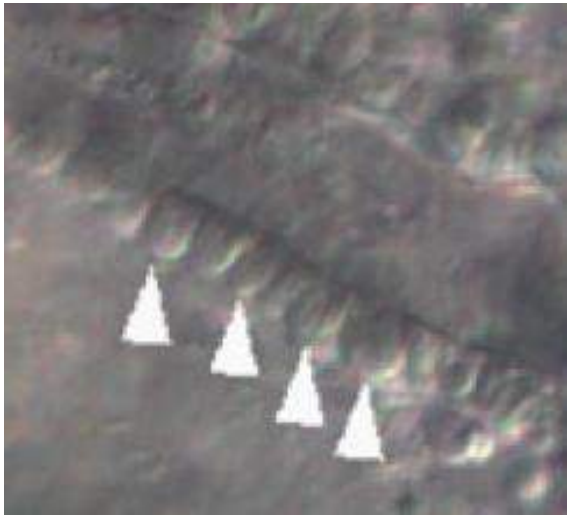
Their **life-cycle is not completely known**.



Schwarz et al. 2015

***C. Mellificae* and *L. passim* interact with the intestinal epithelium using their flagellum.**

They **colonize the rectum and multiply via spherioids attached to the lumenal surface** (epithelium of the gut mucosal), **forming a compact, single layer of cells (spheroids)** surrounding melanized tissue.



Schwarz et al. 2015

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Trasmission

Cysts (cyst-like amastigotes) in Infected feces **can be transmitted along the fecal-oral route.**

Cysts ingestion by healthy bees could happen by **trophallaxis** or **cleaning the hives**, by **robbing**.

Moreover, it should be consider the possibility by foragers to contact cystis during foraging on **flowers**.

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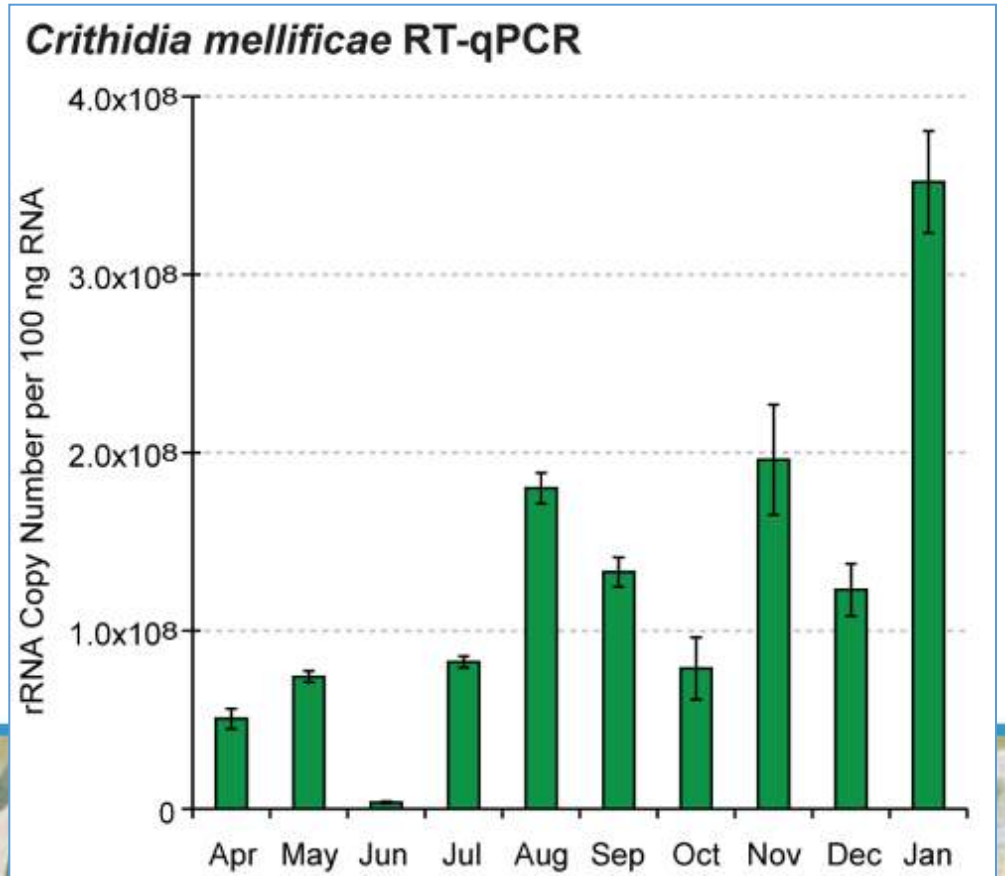
Temporal distribution of the disease

According to Schneeberger (2014), **the infection** in the honey bee **is lower in spring** compared to summer and autumn.
Moreover, Runckel (2011) observed a **peak infection in January.**



Pathological effects on the honey bees

It's still **not clear how *trypanosomatids* affect honey bee health**.

Some researchers report a **positive correlation between trypanosomatids, *Nosema ceranae* and winter losses**, in addition to *Varroa destructor* (Ravoet, 2013).

Pathological effects on the honey bees

More studies are needed to evaluate relationships with:

1. **growth of colonies** during active season;
2. increase of **winter mortality**;
3. **microbiota population** modification;
4. changes in **immuno-responses**;
5. **ovarian development/oviposition** of the queen;
6. **reproductive fitness;**
7. **behavioural changes.**

Transmission to humans

Patiens with **immunocompromised status** (e.g. affected by HIV) may be infected by trypanosomatids that usually infect insects, *Crithidia* included.

In these case **symptoms** may be represented by **skin-lesions, splenomegaly** (Kaufer *et al.*, 2017) or **enteric disease**.

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Conclusions

The recent identification of *Lotmaria passim* will give new possibilities in **better understanding prevalence of trypanosomatids in honey bees** in the different areas of the world, their **interactions with other pathogens** and **relationships with colony losses.**

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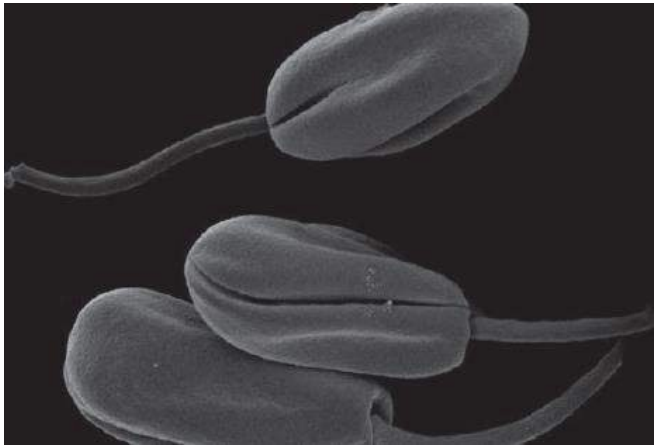
Thanks for your kind attention!



SEM image (Schwarz et al. 2015)