

Metabarcoding applied to biological control involving microarthropods: early steps and prospects



Denise Navia

Embrapa

Recursos Genéticos e
Biotecnologia



Empresa Brasileira de Pesquisa Agropecuária
Brazilian Agriculture Research Corporation
L'Entreprise Brésilienne de Recherche Agricole



- ✓ 43 research units
- ✓ mission- provide feasible solutions for the sustainable development of Brazilian agribusiness through knowledge and technology generation and transfer





Embrapa
*Recursos Genéticos e
Biotecnologia*



- ✓ Collection, characterization and conservation of genetic resources—
micro-organisms, animals and plants
- ✓ **Adding value to the genetic resources**



Services



**Research and
Development**



Foto: Pedro Neves

Colônia de *Beauveria bassiana* em



Plant Germplasm Quarantine Station – Level 1

General Manager from 2008 to 2010
Responsible for the Acarology Unit
on the Ministry of Agriculture, since 2001



**international
exchange of
plant
germplasm**



**breeding
programs,
genetic banks**

more than 500.000 germplasm samples introduced since 1978
29 exotic intercepted pests

Research Groups

- ✓ Plant genetic resources (GRs) *in situ* conservation and management
- ✓ Wild relatives and native plants
- ✓ Characterization of GR focusing on plant breeding and conservation
- ✓ Conservation and characterization of animal GR
- ✓ Development biology and animal reproduction
- ✓ Plant development and reproduction
- ✓ Biotechnology applied to biotic and abiotic stresses
- ✓ Synthetic biology and bioinformatics
- ✓ Bioactive compounds and nanomaterials
- ✓ **Plant protection and quarantine**
- ✓ **Biological control**

Plant Protection and Quarantine

- ✓ origin of invasive pests
- ✓ systematics of pests of quarantine importance
- ✓ 'Omics' to understanding and managing pathosystem relationships
- ✓ bioecology of invasive pests
- ✓ niche modelling for risk assessment
- ✓ plant genetic resistance to minimizing impact of quarantine pests

Origin of Invasive Species

Bulletin of Entomological Research (2005) 95, 505–516

DOI: 10.1079/BER2005382

The invasive coconut mite *Aceria guerreronis* (Acari: Eriophyidae): origin and invasion sources inferred from mitochondrial (16S) and nuclear (ITS) sequences

D. Navia¹, G.J. de Moraes², G. Roderick³ and M. Navajas^{4*}



29 samples, 9 countries from Africa (Benin, Tanzania), America (Brazil, Cuba, USA, México, Venezuela) and Asia (India and Sri Lanka)

DNA sequences 16S, COI and ITS & morphometrics

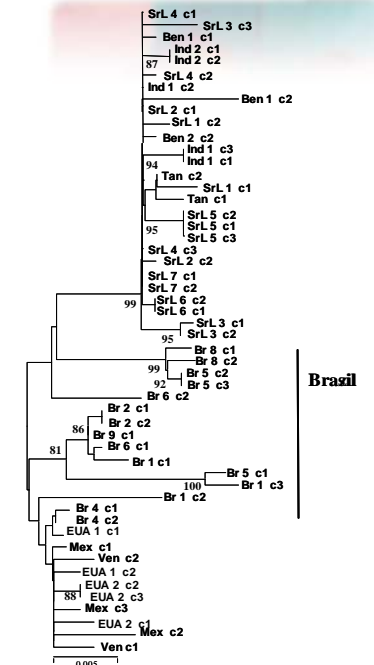


High genetic and morphometric diversity in the Americas & very low diversity in Africa and Asia

Support to an American origin for the coconut mite

Biological Control Program

ITS



Asia & África

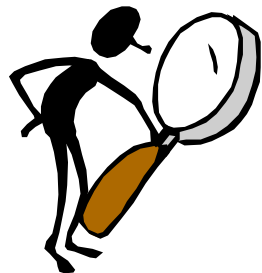
Brazil

Américas

Systematics of Pests of Quarantine Importance

- ✓ On the three most important superfamilies of phytophagous mites

Eriophyoidea



gall mites
bud mites
rusting mites



**important group of phytovirus
vectors**

Tetranychoidea

Tetranychidae

spider mites



Tenuipalpidae

false spider mites
flat mites

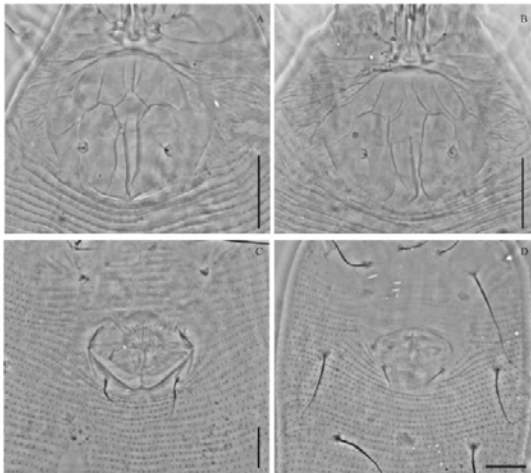


***Brevipalpus*
phytovirus vectors**

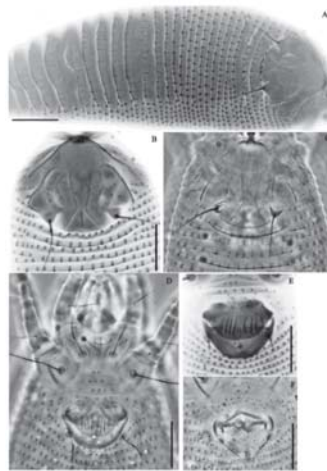
Systematics of Eriophyoidea Mites

✓ 67 new taxa described - 54 spp. and 13 genera- cultivated plants

Diptacus rubuscolum Trinidad,
Duarte & Navia , 2018
vectoring the emanavirus
Blackberry leaf mottle virus on
blackberry



Paraphytoptus serenus sp. nov.
Duarte, Chetverikov & Navia on
Lippia alba leaves



Procalacarus giustolini Damasceno &
Navia, 2009 on cassava leaves



Current activities

- ✓ new taxa on Solanaceae
- ✓ cryptic species on *Colomerus vitis*?
collaboration with Italy

The Pathosystem *Aceria tosichella* Keifer and Transmitted Virus- Wheat streak mosaic virus (WSMV) and High Plain virus (HPV), a new threat to cereal crops in South America- mapping, host plants, characterization, and cultivars susceptibility



PROSUL- Programa Sul-Americano de Apoio às Atividades de Cooperação em Ciência e Tecnologia- 2006 to 2010



WSMV firstly reported in South America, Argentina in 2004



Systematics of Eriophyoidea Mites

- integrative taxonomy revealing cryptic species

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

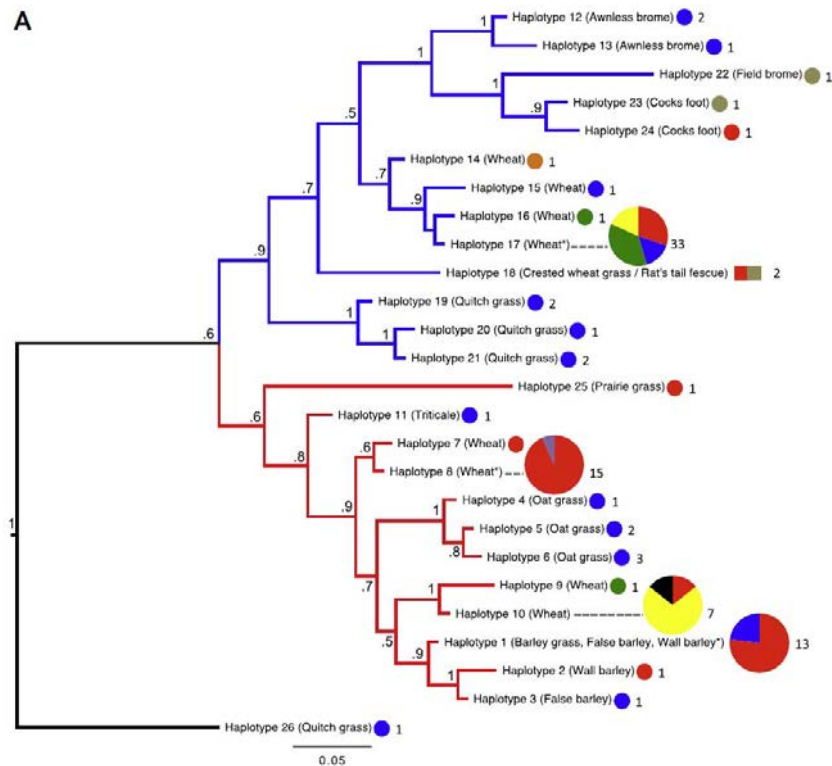
journal homepage: www.elsevier.com/locate/ympev

Molecular Phylogenetics and Evolution 66 (2013) 928–940

Phylogenetic analyses reveal extensive cryptic speciation and host specialization in an economically important mite taxon

Adam D. Miller^{a,b,*}, Anna Skoracka^c, Denise Navia^d, Renata Santos de Mendonca^d, Wiktoria Szydło^c, Mark B. Schultz^e, C. Michael Smith^f, Graciela Truol^g, Ary A. Hoffmann^{a,e}

The Wheat Curl Mite- a complex of species, well supported lineages



Implications for control of WCM & associated pathogens

- identifying plants that form 'green bridge' refuges
- assessing disease transmission risk
- identifying resistance in cereal genotypes to WCM and viruses

Fig. 1. BI phylogenies representing each gene dataset, **(A) 346 bp of the mitochondrial 16S gene**. Clades 1 and 2 are differentiated by red and blue coloration, respectively. Haplotype frequency is indicated and graphical depiction of haplotype origins are provided (**red = Australia, green = Argentina, blue = Poland, yellow = Brazil, brown = Turkey, black = France**).

Plant Genetic Resistance to Minimizing Impact of Quarantine Pests

Journal of Plant Pathology
<https://doi.org/10.1007/s42161-018-0156-1>

ORIGINAL ARTICLE



Reaction of South American wheat genotypes to wheat streak mosaic virus

Received: 2 February 2018 / Accepted: 10 August 2018

Vanina Alemandri¹ · Carlos Tomas Bainotti² · Douglas Lau³ · Denise Navia⁴ · Sandra Monica Rodriguez¹ · Paola Lopez Lambertini¹ · Graciela Truol¹

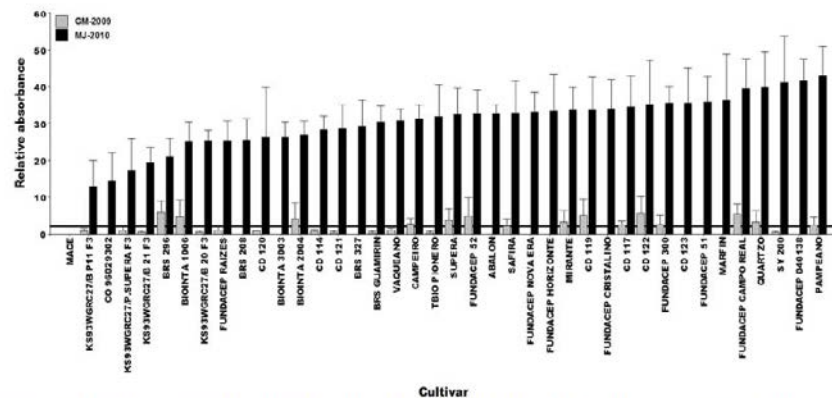


Fig. 2 Reaction of 40 wheat cultivars to WSMV isolates GM-2009 and MJ-2010 under field conditions. Cultivars with relative absorbance values above 2 are considered susceptible

- ✓ evaluation of the susceptibility of 40 Argentine and Brazilian wheat cultivars to two WSMV isolates
- ✓ differential susceptibility levels – WSMV isolate GM-2009
- ✓ 9 cultivars tolerant to infection or uninfected



Systematics of Tetranychidae Mites

- ✓ contributions to molecular systematics

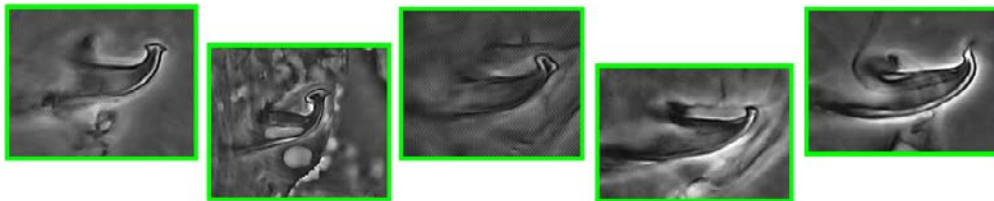
Exp Appl Acarol
DOI 10.1007/s10493-011-9453-5

2011

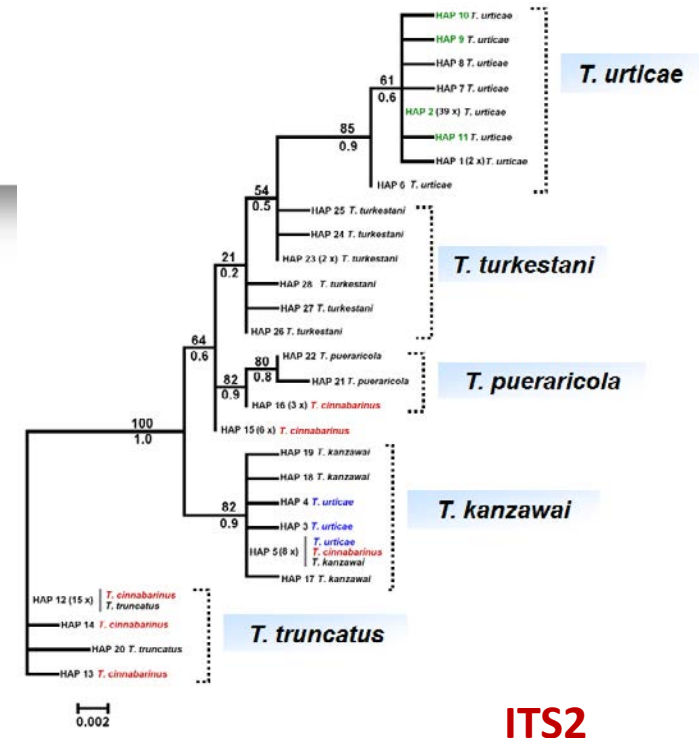
A critical review on some closely related species of *Tetranychus* sensu stricto (Acari: Tetranychidae) in the public DNA sequences databases

Renata S. de Mendonça · Denise Navia · Ivone R. Diniz ·
Philippe Auger · Maria Navajas

- based on ITS2 and *COI* sequences of *Tetranychus* spp..
- around 30% of sequences erroneously identified- *T. urticae*, *T. cinnabarinus*, *T. pueraricola*, *T. kanzawai* and *T. truncatus*



edeago of *Tetranychus* mites erroneously identified



ITS2

Systematics of Tenuipalpidae Mites
***Brevipalpus* mites & BTVs** (*Brevipalpus* Transmitted Virus)
Citrus leprosis virus - CiLV

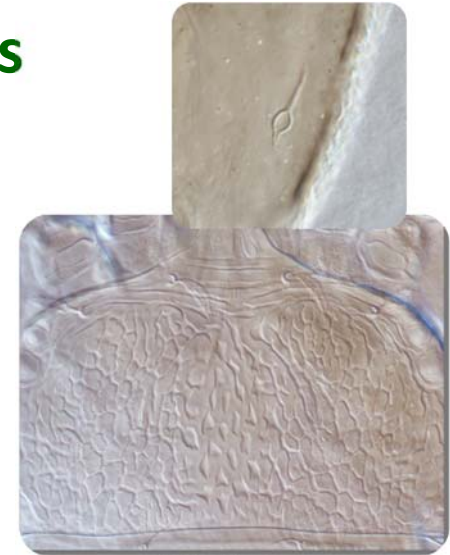
- ❖ increase in production costs
(pruning, anticipated renewal of the orchard...)
- ❖ Acaricides
 - US\$ 60 to 100 millions/ year
 - environmental cost



Systematics of Tenuipalpidae Mites

Zoologica Scripta

2013



Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae)

DENISE NAVIA, RENATA S. MENDONÇA, FRANCISCO FERRAGUT, LETÍCIA C. MIRANDA, ROBERTO C. TRINCADO, JOHAN MICHAUX & MARIA NAVAJAS

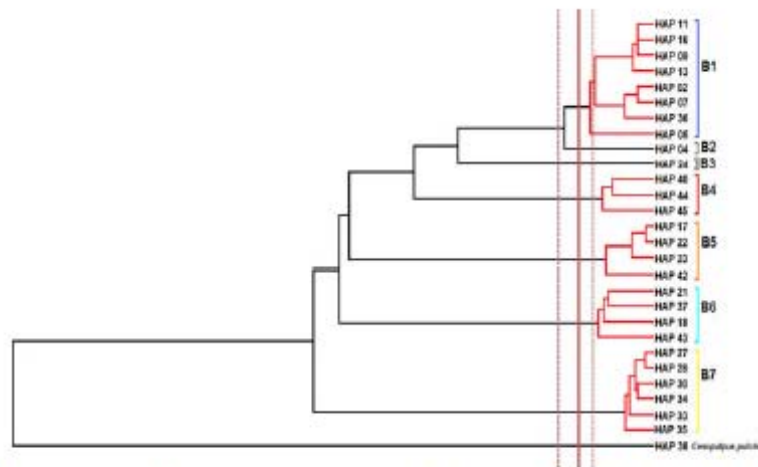


Fig. 2 *Brevipalpus* ultrametric tree and clusters of specimens recognised as putative species by the method of Pons *et al.* (2006). Genetic clusters recognised as a putative species are highlighted in red and separated by longer black branches. The solid vertical red bar indicates the threshold (Test Pons: 93.54 likelihood) which identify seven valid clusters plus the outgroup. The vertical hatched red bars indicate the incertitude zone which spans between six and eight clusters plus the outgroup.

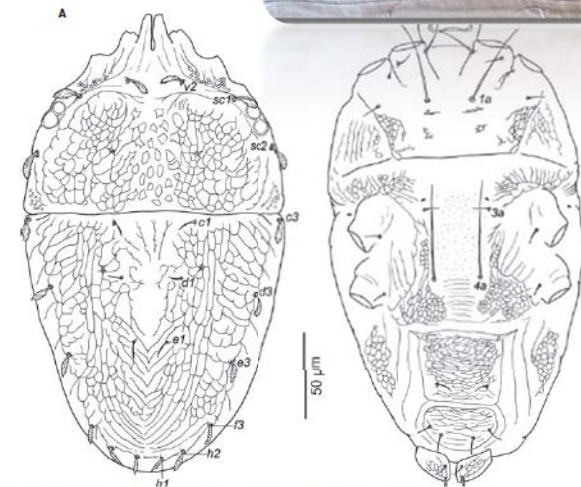


Fig. 4 —A-B. *Brevipalpus incognitus* Ferragut & Navia sp. n. female. —A. Dorsal view. —B. Ventral view.

Brevipalpus incognitus Ferragut & Navia, 2013

Current activities

- ✓ taxonomic revision through an integrative approach
- ✓ detailed characterization of *Brevipalpus* vector species

'Omics' to understanding and managing pathosystem relationships - vector & plant pathogen & host plant



Microbiology
Resource Announcements

GENOME SEQUENCES



Draft Genome Assembly of the False Spider Mite *Brevipalpus yothersi*

Denise Navia,^a Valdenice M. Novelli,^b Stephane Rombauts,^{c,d} Juliana Freitas-Astúa,^e Renata Santos de Mendonça,^f Maria Andreia Nunes,^b Marcos A. Machado,^b Yao-Cheng Lin,^{c,d} Phuong Le,^{c,d} Zaichao Zhang,^{c,d} Miodrag Grbić,^g Nicky Wybouw,^h Johannes A. J. Breeuwer,ⁱ Thomas Van Leeuwen,^h Yves Van de Peer^{c,d,j}



Brevipalpus yothersi, *B. papayensis*, *B. californicus*

- ✓ evolution in Tetranychoida mites
- ✓ endosymbionts host functional relationships
- ✓ managing acaricide resistance- xenobiotics
- ✓ molecular ecology of virus vector relationship
- ✓ exploring target membrane receptors
- ✓ silencing genes- RNA i

CNPq/FWO Project No.
490294/2009-0 Assembly and
Annotation of the haploid flat mite
Brevipalpus phoenicis genome,
International Cooperation



Spreading and Bioecology of Invasive Pests



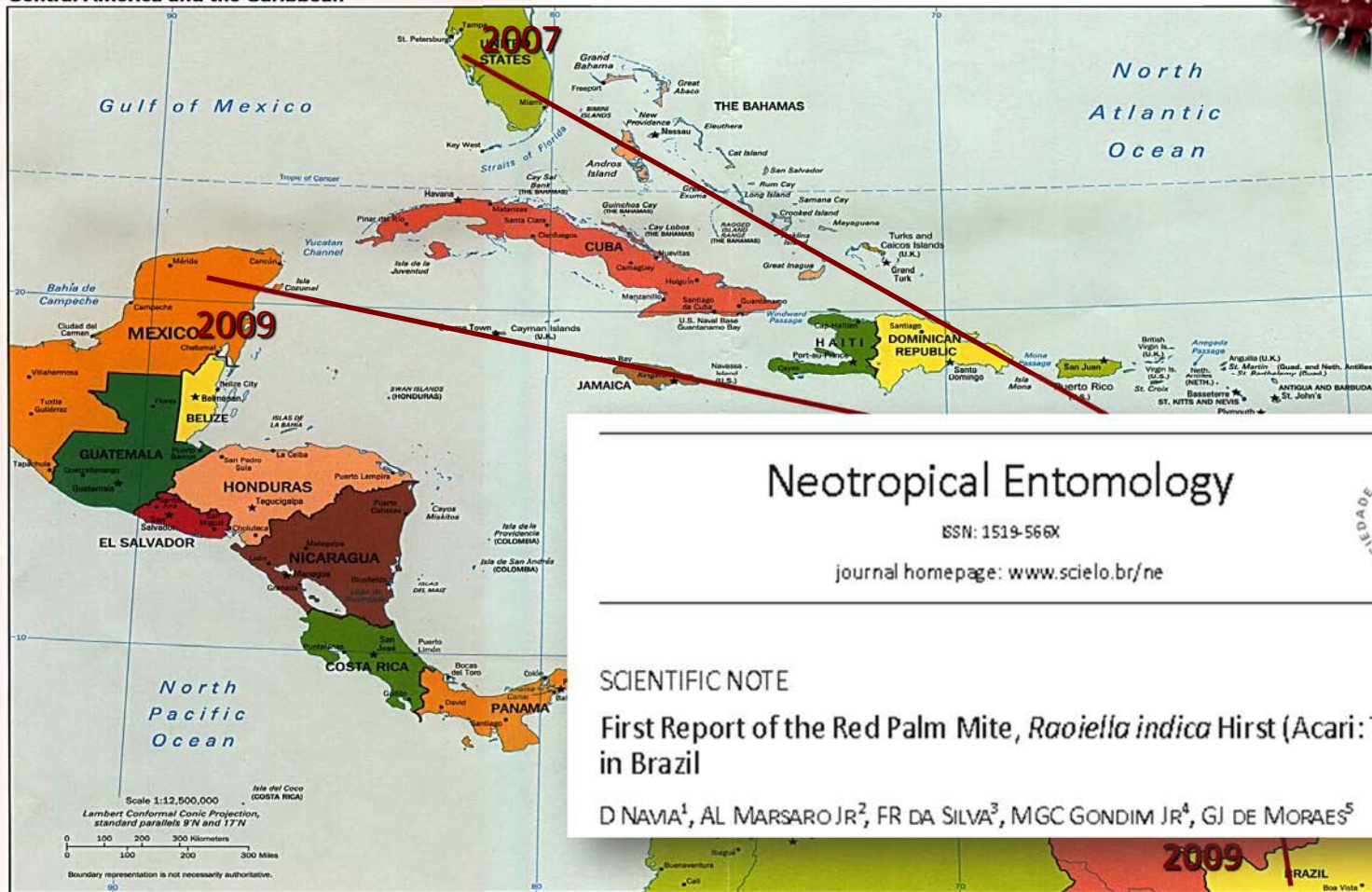
The Red Palm Mite, *Raoiella indica* Hirst, in the Americas

India, 1924, Hirst 1924

Martinica 2004 Flechtmann & Etienne 2004



Central America and the Caribbean



Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne



2009

SCIENTIFIC NOTE

First Report of the Red Palm Mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), in Brazil

D NAVIA¹, AL MARSARO JR², FR DA SILVA³, MGC GONDIM JR⁴, GJ DE MORAES⁵

2009
BRAZIL



Systematics and Biodiversity

Publication details, including instructions for authors and subscription information
<http://www.tandfonline.com/loi/tsab20>



Can the red palm mite threaten the Amazon vegetation?

Manoel G. C. Gondim Jr.^a, Tatiane M. M. G. Castro^b, Alberto L. Marsaro Jr.^c, Denise Navia^d, José W. S. Melo^a, Peterson R. Demite^e & Gilberto J. de Moraes^e

532

M. G. C. Gondim Jr. et al.

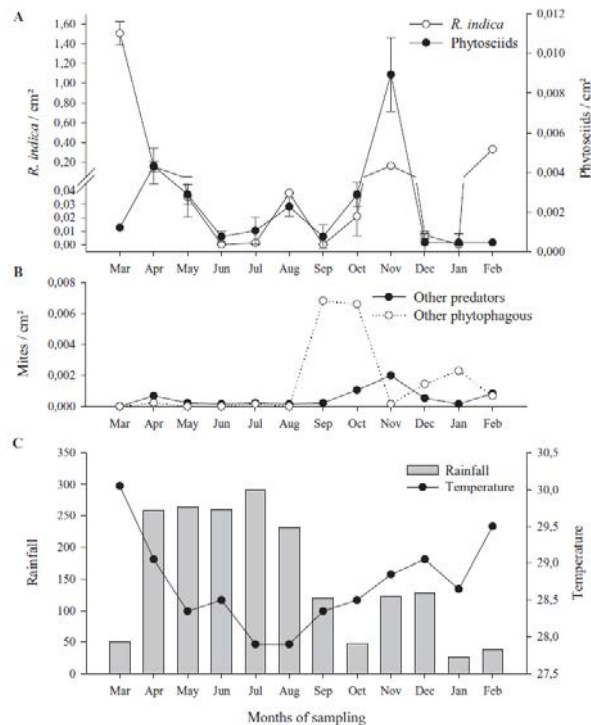
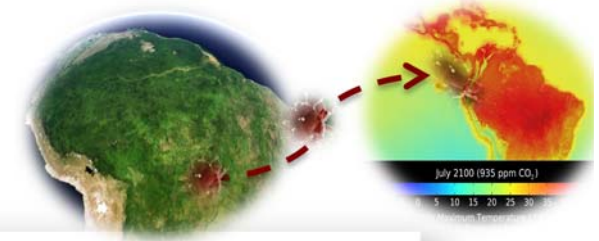


Fig. 2. Mean numbers (and corresponding standard errors) of *Raviella indica* and predaceous Phytoseiidae mites per cm² of coconut leaflet, as well as monthly precipitation (mm) and average temperature (°C) in Mucujai, Roraima state, Brazil between March 2010 and February 2011.



Niche Modelling for Risk Assessment- under current and climate change scenarios



Spatial forecasting of red palm mite in Brazil under current and future climate change scenarios

Denise Navia⁽¹⁾, Emília Hamada⁽²⁾, Manoel Guedes Correa Gondim Jr.⁽³⁾ and Norton Polo Benito⁽¹⁾

Pesq. agropec. bras., Brasília, v.51, n.5, p.586-598, maio 2016
DOI: 10.1590/S0100-204X2016000500020

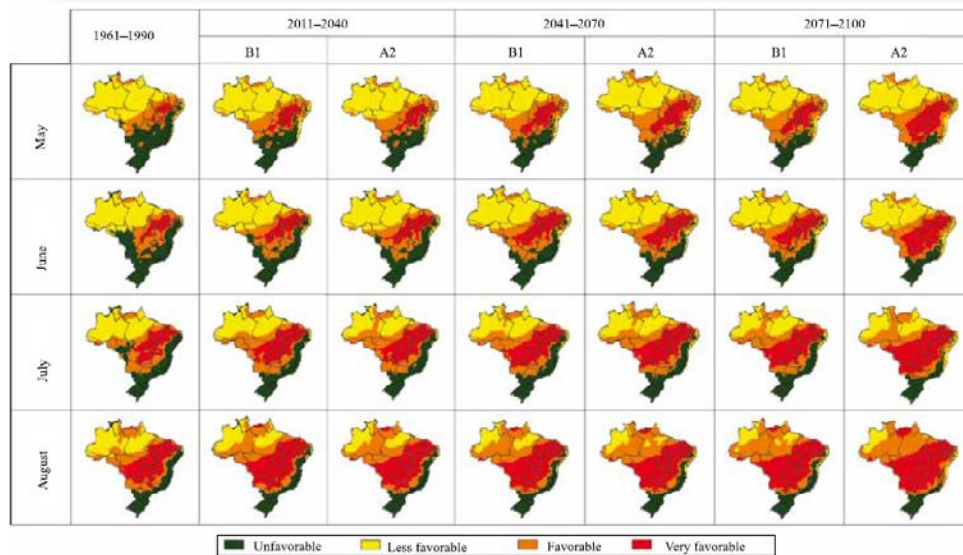


Figure 2. Maps of favorability for the red palm mite (*Raoiella mdica*) in Brazil, from May to August, for the climate normal from 1961–1990 and for future climates (2011–2040, 2041–2070, and 2071–2100) in the B1 and A2 scenarios.

Spatial forecasting of red palm mite in Brazil

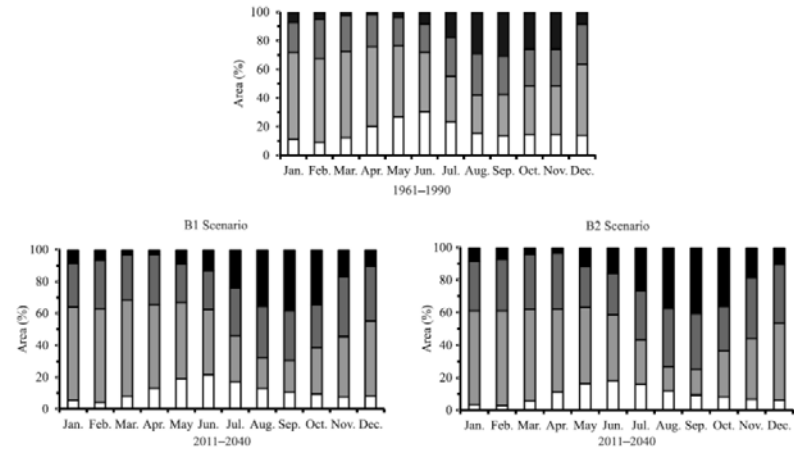


Figure 4. Graphs showing variations in the favorability levels for the red palm mite (*Raoiella indica*) in Brazil, along the year, for the climate normal from 1961–1990 and for future climates (2011–2040, 2041–2070, and 2071–2100) in the B1 and A2 scenarios.

Biological Control

- ✓ integrative taxonomy for biological control agents
- ✓ effect of the natural vegetation on the mite fauna in agroecosystems - an agroecological approach
- ✓ prospecting Phytoseiidae predatory mites in unexplored areas
- ✓ metabarcoding applied to biological control involving predatory mites



Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon



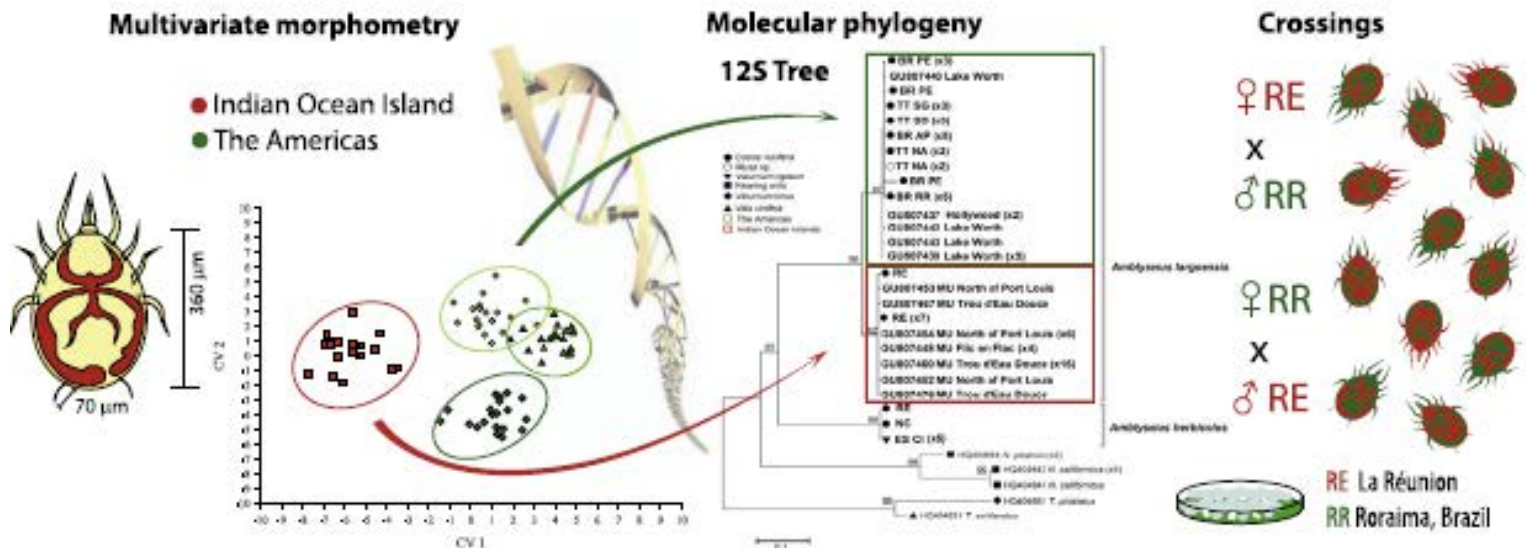
Reproductive compatibility and genetic and morphometric variability among populations of the predatory mite, *Amblyseius largoensis* (Acari: Phytoseiidae), from Indian Ocean Islands and the Americas




Denise Navia^{a,*}, Cleiton A. Domingos^b, Renata S. Mendonça^a, Francisco Ferragut^c,
 Maria Angélica N. Rodrigues^a, Elisângela G.F. de Moraes^d, Marie-Stéphane Tixier^e,
 Manoel G.C. Gondim Jr.^b



GRAPHICAL ABSTRACT



Molecular and morphological characterization of the predatory mite *Amblyseius largoensis* (Acari: Phytoseiidae): surprising similarity between an Asian and American populations

Debora B. Lima¹ · Daniela Rezende-Puker¹ · Renata S. Mendonça²  · Marie-Stephane Tixier³ · Manoel G. C. Gondim Jr.¹ · José W. S. Melo⁴ · Daniel C. Oliveira⁵ · Denise Navia⁶

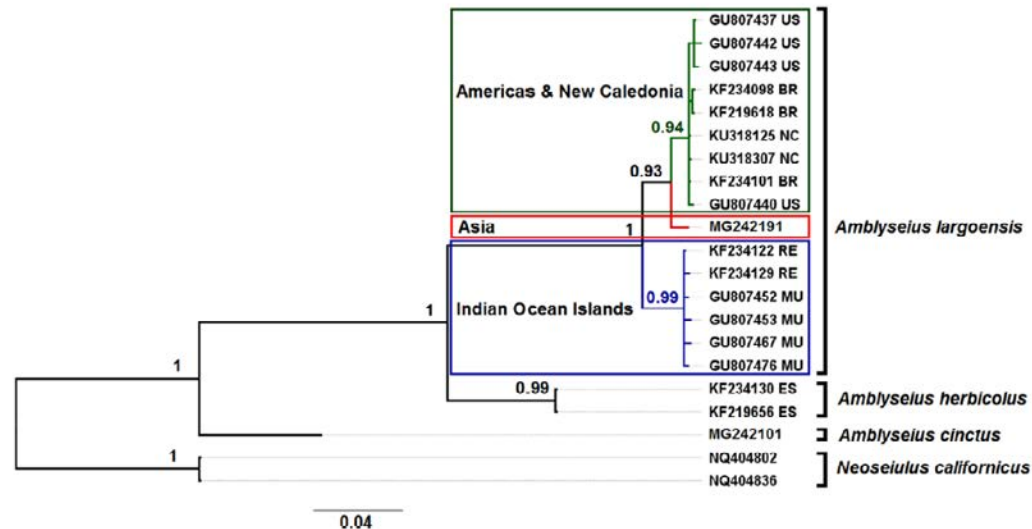


Fig. 4 Combined Bayesian inference (BI) analysis tree for *Amblyseius* species on coconut plants calculated from the ribosomal region ITSS and 12S rRNA sequences. Statistical support indicates Bayesian posterior probabilities; only probabilities > 0.6 are indicated above branches. *Amblyseius largoensis* populations from different geographic locations are highlighted in colored squares. The species names based on morphological identification are to the right of the tree

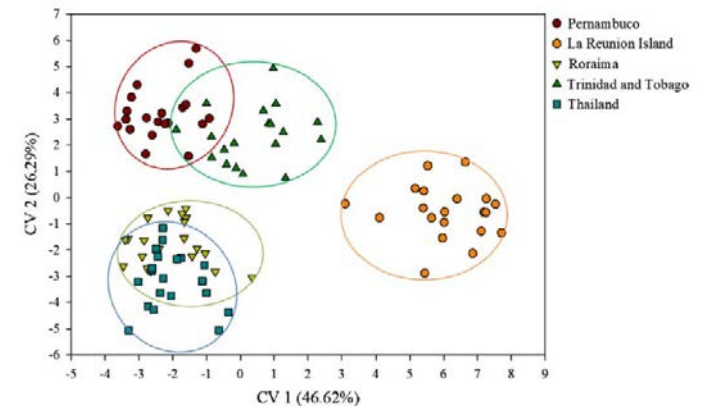


Fig. 2 Canonical variable analysis of 36 morphological characters of females from five populations of *Amblyseius largoensis*. Ovals were formed based on the projection of individuals from each population to CV 1 and 2 (variation explained by the two canonical variables is shown in parentheses)

Prospecting Phytoseiidae Predatory Mites in Unexplored Areas



Zootaxa 2997: 37–53 (2011)
www.mapress.com/zootaxa/

Copyright © 2011 · Magnolia Press

Article

ISSN 1175-5326 (print edition)

ZOOTAXA

ISSN 1175-5334 (online edition)

Phytoseiid mites (Acari: Phytoseiidae) of the Dominican Republic, with a re-definition of the genus *Typhloseiopsis* De Leon

FRANCISCO FERRAGUT^{1,4}, GILBERTO JOSE de MORAES² & DENISE NAVIA³



Zootaxa 3990 (4): 525–550
www.mapress.com/zootaxa/

Copyright © 2015 Magnolia Press

Article

ISSN 1175-5326 (print edition)

ZOOTAXA

ISSN 1175-5334 (online edition)

<http://dx.doi.org/10.11646/zootaxa.3990.4.3>

<http://zoobank.org/urn:lsid:zoobank.org:pub:04B6F8A3-671D-4EE2-8271-2CA9E3BA333F>

Phytoseiid mites (Acari: Phytoseiidae) from Patagonia and Tierra del Fuego

FRANCISCO FERRAGUT¹ & DENISE NAVIA²

Systematic & Applied Acarology 22(10): 1585–1621 (2017)

<http://doi.org/10.11158/saa.22.10.4>

ISSN 1362-1971 (print)

ISSN 2056-6069 (online)

Article

<http://zoobank.org/urn:lsid:zoobank.org:pub:E98B0007-190C-44CB-8694-CDCE832CBDA0>

Phytoseiid Mites (Acari: Mesostigmata) of the Azores Islands

FRANCISCO FERRAGUT¹ & DENISE NAVIA²

Effect of the Natural Vegetation on the Mite Fauna in Agroecosystems - an agroecological approach

Exp Appl Acarol (2014) 64:501–518
DOI 10.1007/s10493-014-9844-5

Mites from Cerrado fragments and adjacent soybean crops: does the native vegetation help or harm the plantation?

José M. Rezende · Antonio C. Lofego ·
Felipe M. Nuvoloni · Denise Navia



Unveiling the Diet of Predatory Mites through DNA Metabarcoding

Team

since 2015



Marie-Stéphane Tixier



Jean-François Martin



Francisco Ferragut

Special issue on

“Metagenomics and the Science of Biological Control”

May 2018



BC success depends on the knowledge of ecological relationships, specially trophic interactions

Evolutionary Applications

Open Access

Evolutionary Applications ISSN 1752-4571

REVIEWS AND SYNTHESIS

Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications

Elizabeth L. Clare

MOLECULAR ECOLOGY

Molecular Ecology (2012) 21, 1931–1950

doi: 10.1111/j.1365-294X.2012.02688.x

INVITED REVIEW

Who is eating what: diet assessment using next generation sequencing

FRANCOIS POMPANON,^{*1} BRUCE E. DEAGLE,^{†1} WILLIAM O. C. SYMONDSON,^{§1} DAVID S. BROWN,[§] SIMON N. JARMAN[†] and PIERRE TABERLET^{*}

MOLECULAR ECOLOGY RESOURCES

Molecular Ecology Resources (2013)

doi: 10.1111/1755-0998.12188

DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet

M. DE BARBA, C. MIQUEL, F. BOYER, C. MERCIER, D. RIOUX, E. COISSAC and P. TABERLET

Metabarcoding Applied to the Study of Trophic Interactions in Agroecosystems

OPEN ACCESS Freely available online

PLOS ONE

Cover Cropping Alters the Diet of Arthropods in a Banana Plantation: A Metabarcoding Approach

Gregory Mollet^{1,2}, Pierre-François Duyck^{1,3*}, Pierre Lefevre³, Françoise Lescouret², Jean-François Martin⁴, Sylvain Piry⁵, Elsa Canard^{1,6}, Philippe Tixier^{1,7}

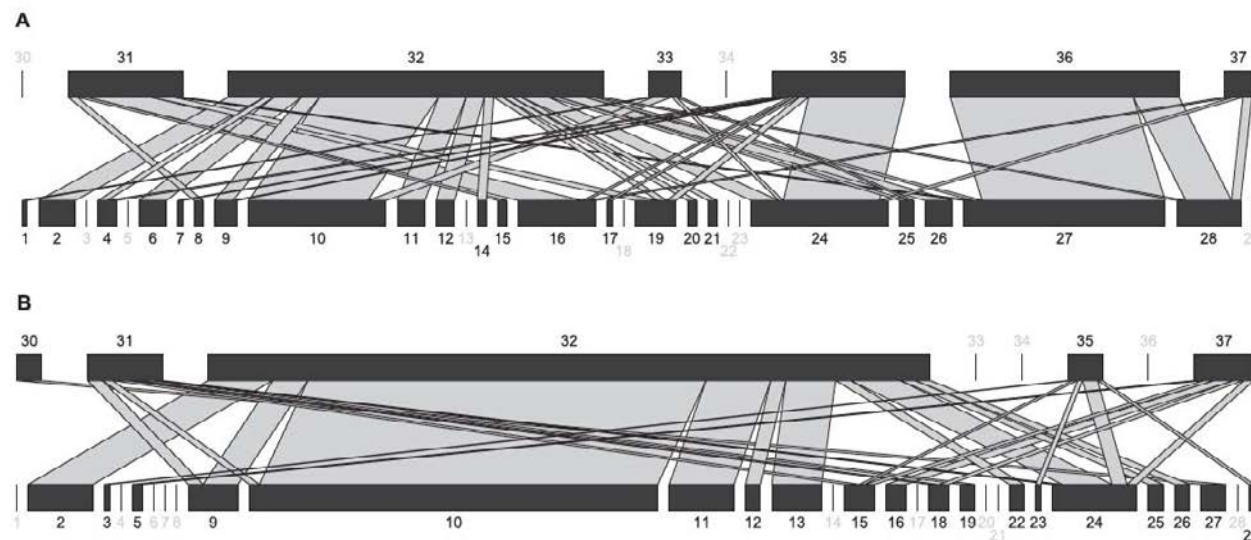


Figure 2. Bipartite food webs of predator-prey interactions on (A) bare soil, and (B) cover cropped banana plantation. For each web, lower bars represent relative abundance of consumed prey, and upper bars represent relative abundance of positive ground-dwelling predators, each drawn at different scale. The width of links between ground-dwelling predators and prey represents the frequency of consumption. Numbers in grey indicate unlinked taxa. Visualization was performed with the R package “bipartite” [50]. 1: *Anopheles claviger*. 2: *Anopheles nimbus*. 3: *Baetis rhodani*. 4: *Blatella germanica*. 5: *Calliphora vomitoria*. 6: *Carabidae* spp. 7: *Codophila varia*. 8: *Coridius chinensis*. 9: *Cosmopolites sordidus*. 10: Diptera. 11: *Drosophila anceps*. 12: *Drosophila melanica*. 13: *Drosophila montana*. 14: *Gryllus*. 15: Hemiptera. 16: *Jalysus spinosus*. 17: *Nebria chinensis*. 18: *Neoneides muticus*. 19: *Nezara viridula*. 20: Oniscidae. 21: *Ophyra spinigera*. 22: *Periplaneta americana*. 23: *Podisus sericeiventris*. 24: *Polytus mellerborgi*. 25: *Resseliella yagoi*. 26: *Sarcophila*. 27: *Scolopendra*. 28: *Scolopendra mutilans*. 29: *Stephensioniella sterrei*. 30: Lycosidae. 31: *Camponotus sexguttatus*. 32: *Euborellia caraibea*. 33: *Odontomachus baurii*. 34: Scolopendridae. 35: *Solenopsis geminata*. 36: Staphilinidae. 37: *Wasmannia auropunctata*. doi:10.1371/journal.pone.0093740.g002

What about trophic interactions involving predatory mites ???

How to determining the diet of predatory mites in the field?



- **no possible visual observations**- minute predatory mites and preys
- **no possible the study of gut content**- pre-oral digestion, no prey morphological traces

What is known about the diet of Phytoseiidae predatory mites???

- Phytoseiidae includes 2,400 species worldwide, 90% are generalist predators, i.e. feeding on mites, small insects, fungi mycelium, pollen and other plant products
- feeding behavior studied in the laboratory for the most widely used and/or commercialized species (**no more than 20 spp.**)
- results of laboratory studies extrapolated to the field... uncertainties!
- *in situ* feeding behavior almost unknown



Diet of Predatory Mites on Citrus orchards in Spain

DNA-based approach



Euseius stipulatus & *Phytoseiulus persimilis*
predating

Tetranychus urticae & *Panonychus citri*

methodology developed to detecting two
tetranychid mite preys

Steps

✓ **methodological development**

✓ **controlled experiments**

✓ ***in situ* evaluations**

M & M

Methodological Development

- ✓ choosing the mitochondrial DNA fragment to be used as barcoding
- ✓ designing group-specific primers
- ✓ *in vitro* screening of designed primers on a wide range of preys and predatory mites



Methodological Development

choosing the mitochondrial DNA fragment to be used as barcoding

Cytochrome Oxidase I (COI) or *Cytochrome B (CYT B)* ?

M & M

- ✓ building datasets (predatory mites & potential preys- Tetranychidae, Tenuipalpidae, Eriophyoidea and Tarsonemidae) and alignment analyses.

Result

- ✓ **COI fragment was chosen**- No. of available sequences in databases and alignment polymorphisms

Methodological Development

designing group specific primers- Why?

using universal DNA mini-barcode primers that would co-amplifying predatory mites and prey DNA with equal efficiency or even better amplify preserved DNA than DNA traces could hamper prey detection

- search for primers with total specificity or highest efficiency to the Acariformes (phytophagous mites) & nule or low efficiency to Parasitiformes (most important predatory mites)



A big challenge !!!!

Predators & Preys very closely related taxa!!!

Methodological Development

designing group specific primers

M & M

- ✓ *COI* dataset containing sequences of 108 mite species/morphospecies belonging to the Trombidiformes (56 Eriophyoidea, 9 Stigmaeidae, 2 Tarsonemidae, 38 Tetranychoidae, 3 Tydeidae); 4 to the Astigmatina (Acaridae); and 21 to Mesostigmata predatory mites in the Phytoseiidae
- ✓ alignment (583bp) using software MEGA 6
- ✓ design of group specific primers (19-22 bp) that could amplify 270-300bp (Mini barcodes) using **ecoPrimers** software implemented in **OBITools**

Result

- ✓ five forward (1F, 2F, 3F, 4F, 5F) and nine reverse (1R, 2R, 3R, 4R, 5R, 6R, 7R, 8R, 9R) potential oligonucleotide primers were designed

***in vitro* validation of Mini-COI group specific primers- 4 stages**

M & M

Methodological Development

in vitro validation of Mini-COI group specific primers

1st stage

adjusting PCR conditions

PCR for fasting predatory mites & TSM from lab rearings
27 primer combinations
1F 2F 3F
&
1R 2R 3R 4R 5R 6R 7R 8R 9R

Amblyseius swirskii
Neoseiulus barkeri
Neoseiulus californicus
Phytoseiulus persimilis
Typhlodromus recki
&
Two Spotted Spider Mite
-TSM

TSM satisfactory amplification
&
nule or poor amplification of
predatory mites
2F 3F & 7R 8R

M & M

Methodological Development

in vitro validation of Mini-COI group specific primers

2nd stage

PCR for a wide range of potential preys field collected four primer combinations
2F 3F & 7R 8R

164 specimens, 40 taxa, 23 genera, 9 families –
Acaridae, Bdellidae, Eupodidae, Eriophyidae, Tarsonemidae, Stigmaeidae, Tenuipalpidae, Tetranychidae, Tydeidae

3F primer combinations-
nule or weak amplification of
Acaridae, Bdelloidea,
Eriophyidae,
Stigmaeidae,
Tarsonemidae and
Tydeidae

uneven efficiency of primers in
amplifying the different families
of potential mite preys

3F primer combinations
better amplification of
Tetranychidae and
Tenuipalpidae than
2F combinations

M & M

Methodological Development

in vitro validation of Mini-COI group specific primers

3rd stage

cocktail of primers
PCR for a wide range of
potential preys using a
2F & 3F & 7R & 8R

67 specimens, 22 taxa,, 9 families –
Acaridae, Bdellidae, Eupodidae,
Eriophyidae, Tarsonemidae,
Stigmaeidae, Tenuipalpidae,
Tetranychidae, Tydeidae

unsatisfactory amplification for
most of potential preys

M & M

Methodological Development

in vitro validation of Mini-COI group specific primers

4th stage

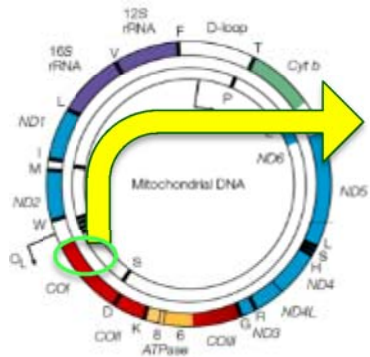
two new more degenerated forward primers - 4F and 5F

PCR for a wide range of potential preys and with predatory mites using 4F & 5F in combination with 7R & 8R

63 templates, 39 taxa,, 9 families –
Acaridae, Bdellidae, Eupodidae,
Eriophyiidae, Tarsonemidae,
Stigmaeidae, Tenuipalpidae,
Tetranychidae, Tydeidae

4F & 7R

satisfactory amplification for the evaluated prey taxa & none or weak amplification of predatory mites

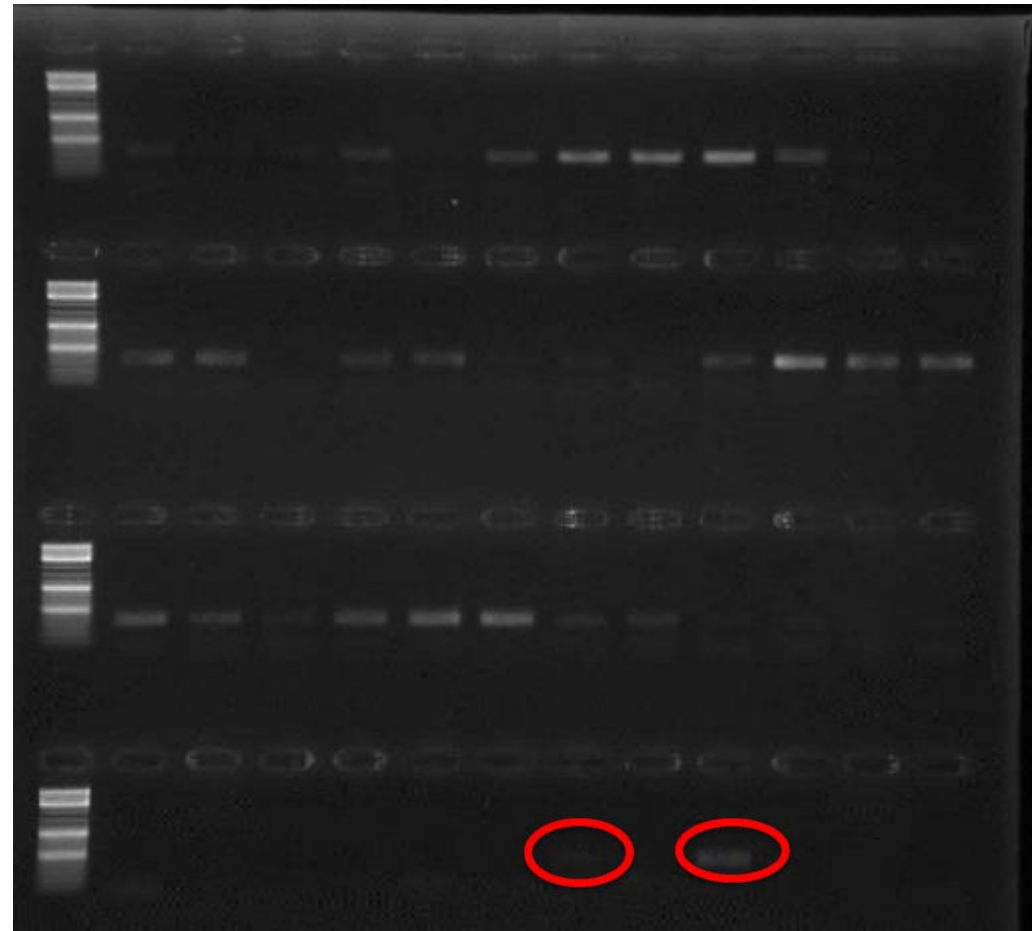


MiniCOI 4F 5' AAAATNGTNGTAATRAARTT 3'
 MiniCOI 7R 5' CATGCTATAATTATAATTTT 3'

280 bp amplicons

phytophagous & no
 Parasitiformes
 mites

Phytoseiidae
 predatory mites



- ✓ not completely group-specific – weak amplification for some predatory mite species
- ✓ DNA amplification of the main families of potential preys

Controlled Experiments – DNA extraction & feeding status essays for prey detection

- two Phytoseiidae mites – *Phytoseiulus persimilis* (Pp) and *Neoseiulus barkeri* (Nb)- both preying on TSM eggs, from laboratory colonies
- two DNA extraction protocols- not crushed (NC) & crushed (C)
- two feeding status
 - ✓ **Partially fed (PF)- half of dairy feeding supply for the predatory mite species**
 - ✓ **Well fed (WF)- full dairy feeding supply for the predatory mites**



DNA extraction & feeding status essays- detectability

Feeding status	Predatory mite species	Crushed	Not crushed	Total specimen detection
Well fed				
	<i>Phytoseiulus persimilis</i>	2 (83), 3(10), 4 (5; 22), 5 (12) [80%]	2 (58, 33), 3 (9), 4 (25), 5 (319, 6) [80%]	4 C, 4 NC specimens
	<i>Neoseiulus barkeri</i>	2 (104), 3 (6, 3) [50%]	no detection [0%]	2 C
Total				10 specimens (5 C, 5 NC) 50% WF
Partially fed				
	<i>Phytoseiulus persimilis</i>	1 (2, 16), 2 (6, 72), 4 (7) [60%]	3 (1933) [20%]	3 C, 1 NC
	<i>Neoseiulus barkeri</i>	3 (8) [20%]	no detection [0%]	1 C
				5 specimens (3 C, 2 NC) 25% PF
Total predatory mites with prey detection		10 C specimens (7 WF, 4 PF) [50%]	5 NC specimens (4 WF, 1 PF) [25%]	

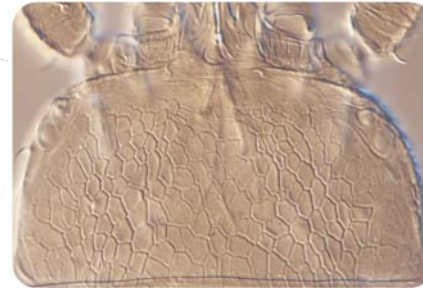
- detectability different between species- higher for *P. persimilis*
- prey detectability was higher when specimens were crushed
- feeding status affected prey detectability for both species, however *N. barkeri* was more affected

In Situ Evaluations

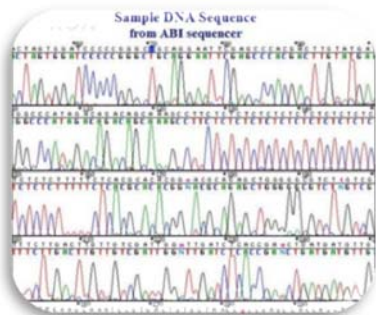
- sampling - **5 host plants, two localities** (Montpellier France Fr and Valencia Spain Sp) - *Malus domestica* (Fr Sp), *Vitis vinifera* (Fr Sp), *Viburnum* (Fr), *Prunus domestica* (Sp), *Cydonia oblonga* (Sp)
- morphological identification of community in the agroecosystems
- enrichment of the reference database of potential preys for use for metabarcoding (MiniCOI sequences, Sanger sequencing)
- DNA extraction (crushed) of 193 predatory mites
- molecular identification of processed predatory mites, CYT B identification (Sanger sequencing)
- libraries preparation for MiSeq Illumina run- 4 plates, 3 technical replicats, including positive and negative controls
- metabarcoding data analyses- R Dada2

In Situ Evaluations

Morphological identification of communities

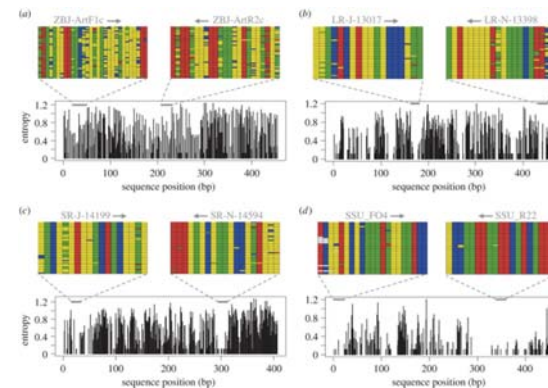


Sanger sequencing



- ✓ Mini-COI sequence reference bank
- ✓ predatory mites ID, Cyt-B

Illumina sequencing



- ✓ prey identification

***In Situ* Evaluations**

- ✓ six Phytoseiidae mites species were identified at species level –
diet information for 4 species

***Euseius gallicus* Kreiter & Tixier (75)**

***Typhlodromus phialatus* Athias-Henriot (44)**

***Amblyseius andersoni* (Chant) (38)**

***Kampimodromus aberrans* (Oudemans) (31)**

***Euseius stipulatus* (Athias-Henriot) (1)**

***Typhlodromus recki* Wainstein (1)**

- ✓ prey amplicons were assigned to 15 taxonomic units

Prey templates were detected in 49.7% of the Phytoseiidae predatory mites, varying from 41.9%, in *K. aberrans*, to 56% in *E. gallicus*

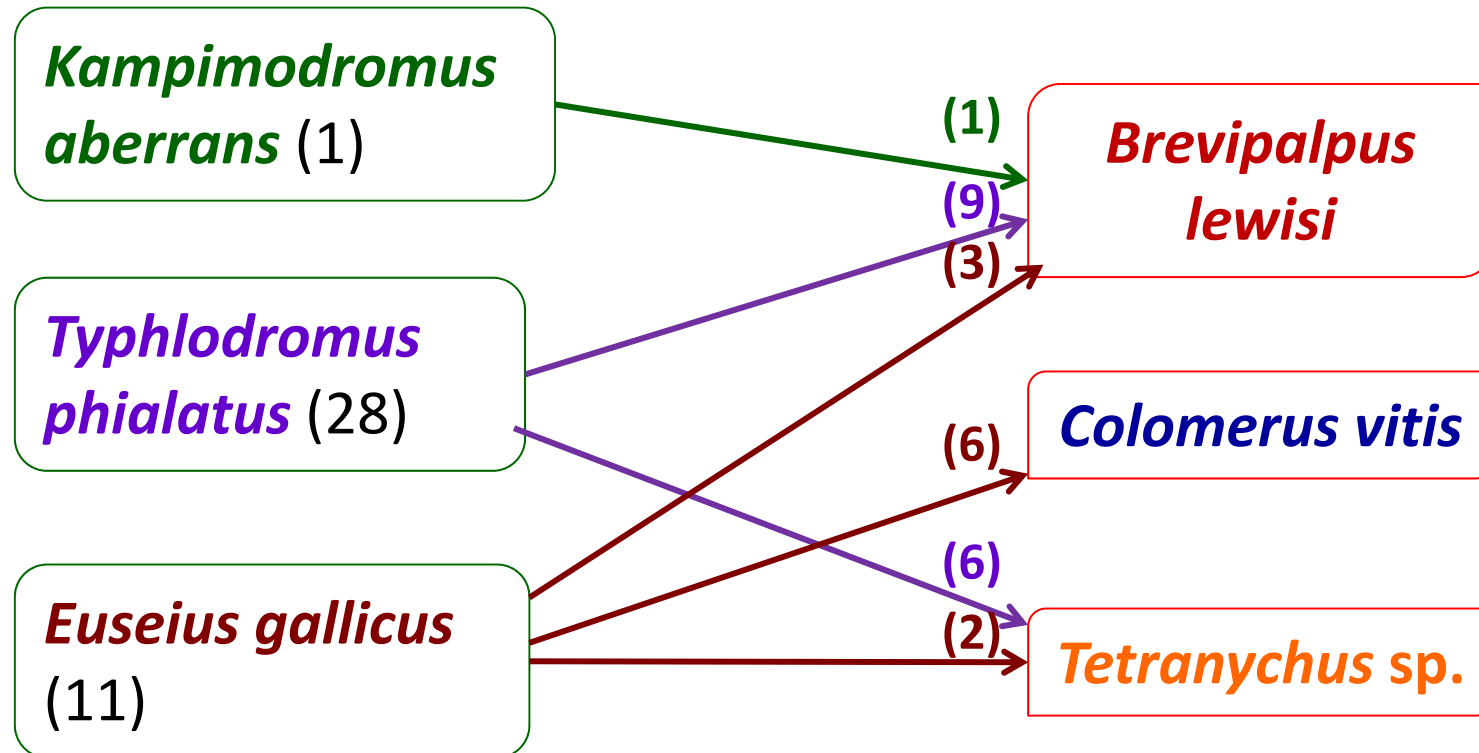
In Situ Evaluations

Euseius gallicus Prey Detection

Predatory mite	Sample code	Host plant	Country	Total No. of predatory mites_ No with prey detection	Prey assignment	No. of predatory mites with prey detection	Predatory mite code (No. amplicons per technical replica)
<i>Euseius gallicus</i>	M1 e M5	<i>Vitis vinifera</i>	France	19_8	<i>Tetranychus urticae</i>	5	M5.9 (6); M5.10 (2979); M5.17 (18944); M5.19 (136); M5.20 (5)
					<i>Brevipalpus lewisi</i>	3	M1.2 (11); M5.9 (6); M5.20 (4033)
					<i>Ectopsocus</i> sp. (Psocoptera)	2	M5.10 (214, 300, 183); M5.19 (3721, 109, 15)
					<i>Brevipalpus</i> sp.	1	M5.20 (51)
	M2	<i>Viburnum</i> sp.	France	3_1	<i>Ectopsocus</i> sp. (Psocoptera)	1	M2.6 (61, 26, 28)
	M3 e M4	<i>Malus domestica</i>	France	38_28	<i>Cenopalpus pulcher</i>	26	M3.1 (51074, 51745, 23706); M4.3 (766, 796, 158); M4.9 (128, 88, 4758); M4.10 (13.288, 12.476, 9.158);.....
					<i>Tetranychus urticae</i>	8	M4.9 (38); M4.12 (3415); M4.14 (33); M4.17 (2); M4.21 (2); M4.28 (405); M4.31 (405); M4.35 (2222)
					<i>Ectopsocus</i> sp. (Psocoptera)	8	M4.14 (4); M4.19 (2); M4.23 (37); M4.26 (257); M4.28 (993, 117); M4.31 (26, 518); M4.35 (70); M4.40 (188, 56, 53)
					<i>Cenopalpus</i> sp.	3	M4.13 (17); M4.25 (3869); M4.31 (384)
					<i>Eotetranychus</i> sp.	2	M4.13 (2); M4.14 (7)

In Situ Evaluations

Vitis vinifera- Spain



In Situ Evaluations

Euseius gallicus Kreiter & Tixier



- ✓ *Brevipalpus lewisi*, *Colomerus vitis* and *Tetranychus* sp. on *Vitis vinifera* from Spain
- ✓ *Cenopalpus pulcher*, *Aculus schlechtendali* and *Brevipalpus* sp. on *Malus domestica* from France
- ✓ *Brevipalpus lewisi*, and *Tetranychus* sp. on *Vitis vinifera* from France

- described from *Prunus cerasus* in 2009 from France
- commercialized since 2014, to the control of whiteflies, thrips, also feeding on Tetranychidae and Tarsonemidae mites
- **new report feeding on Tenuipalpidae and Eriophyid mites and on Psocoptera**

Current Activities

- evaluating the effect of biotic and abiotic parameters on the prey detectability through DNA metabarcoding
- designing primers for detection of other predatory mites diet items- insects, pollen and other plant material traces
- evaluating the effect of cultural practices-weeding & no weeding- on the feeding behavior of predatory mites in the soybean-caupi bean successional crop



2017-2020, Project No. 428092/2016-0
« Uncovering food webs involving predatory mites
in agroecosystems through DNA metabarcoding »

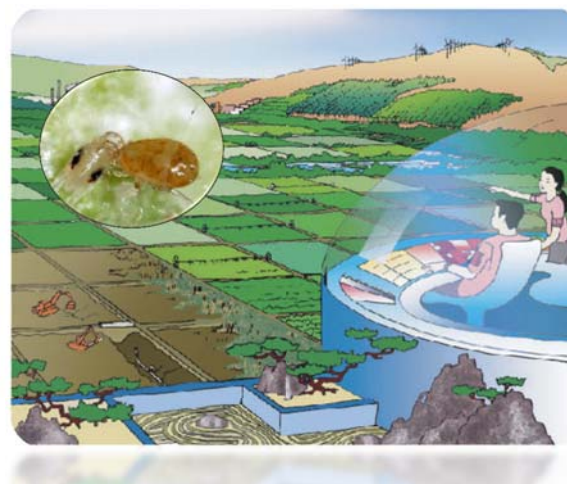


Applications

- evaluating the effect of agricultural measures– e.g. agroecological practices, crop rotation, weeding & no weeding- on target and non-target organisms etc.
- screening for promising BC agents – e.g. along prospections for new biological control agents; dry stress adapted strains/species; soil predatory mites to control of thrips, Sciaridae flies, nematodes
- monitoring feeding behavior of commercialized predatory mites in the field along seasons, different crops

Ecosystem Engineering !!!

info on community relationships supporting management of communities in a way to maximizing the delivery of ecosystem services



A microscopic image showing a mite on a leaf surface. The mite is a small, oval-shaped organism with a reddish-brown body and four pairs of legs. It is positioned in the center-right of the frame. The background is a textured, greenish surface, likely the leaf, with some fine hairs and small droplets visible. The lighting is bright, highlighting the mite's body and the surrounding leaf texture.

Merci beaucoup!

Muito obrigada!

Thank you!

Plataforme Moleculaire
Martial Douin, Alain et
Phillippe au labo Acarologia

denise.navia@embrapa.br