VII International Congress on Cactus & Cochineal
Agadir, Morocco, October 22th 2010

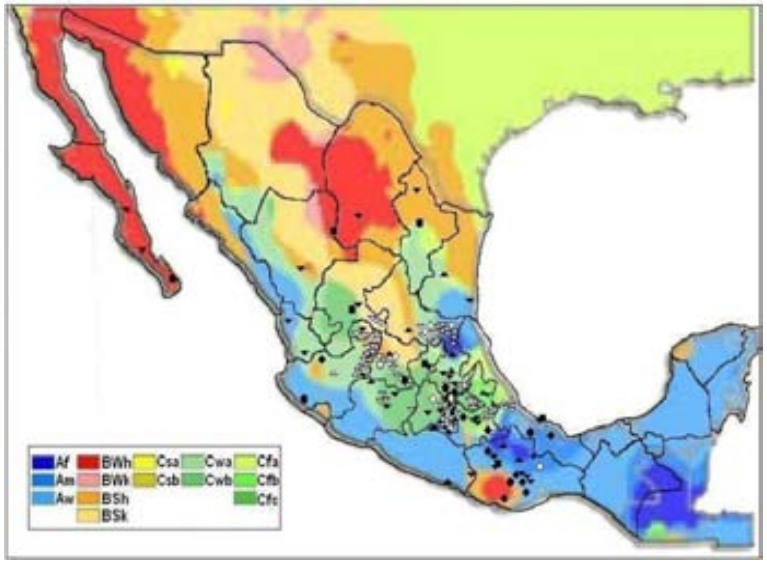

# "The *Opuntia* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution with particular emphasis on chemical and phylogenetic aspects of the *Dactylopius* species"

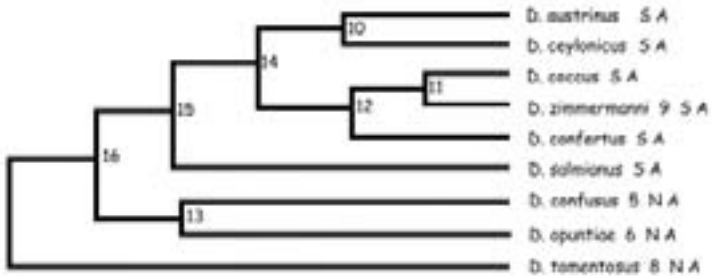
**Dr. Carla Karina Chávez Moreno**
**UNAM - UMSNH**

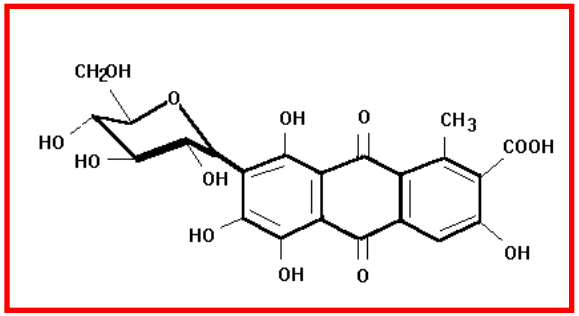
Plant-insect: *Opuntia* – *Dactylopius* represents a **great challenge study because morphological complexity.**

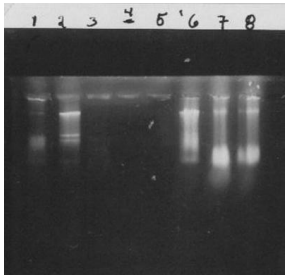
The integration anthropological, geographical, ecological, chemical and molecular analysis, represent important information for understanding biological system.

Combination of different knowledge contribute to establish policies of conservation and strategies for a sustentable use of these natural genetic resources, great significance to Mexico.

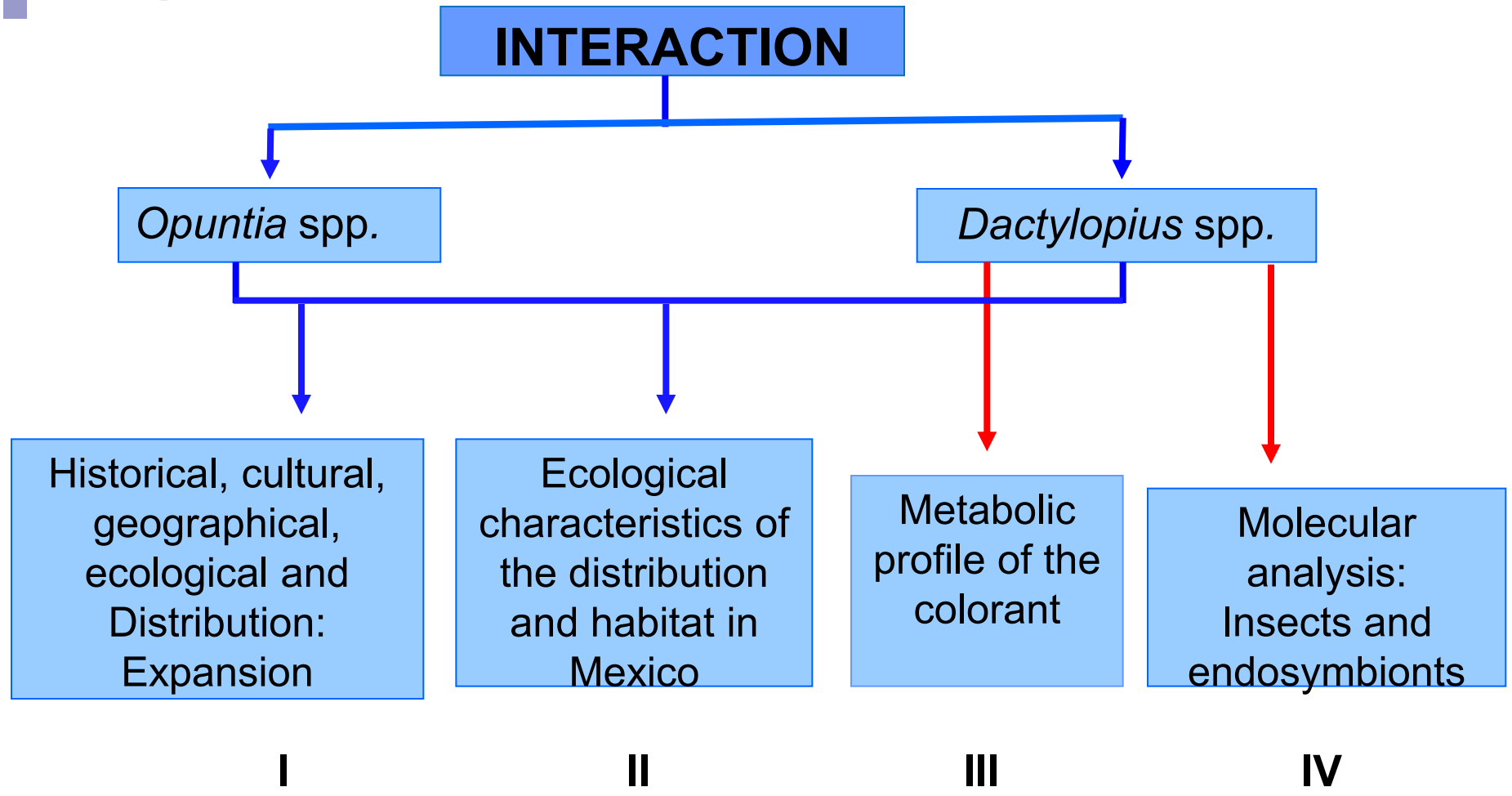


# Objective




Specific measures for protection of such biodiversity and generic resources in Mexico.

Strategies for *in situ* conservation combined with re-established use and cochineal production may enhance conservation policies.

Biodivers Conserv (2009) 18:3337–3355
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REVIEW PAPER

# The *Opuntia* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution

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# Introduction

***Opuntia* (L.) Miller** (Opuntioideae: Cactaceae)

American continent 200 species

Mexico 83-104 species

- 50% endemic
- 20 domesticated species, management and artificial selection was focus on

1) optimize their use for edible stems & fruits.

2) cultivation of *Dactylopius.*

- More than 900 names.





## ***Dactylopius* Costa** (Hemiptera: Dactylopiidae)

*D. confusus* Cockerell
*D. opuntiae* Cockerell
*D. tomentosus* Lamarck
*D. coccus* Costa
*D. ceylonicus* Green
*D. austrinus* De Lotto
*D. confertus* De Lotto
*D. salmianus* De Lotto
*D. zimmermanni* De Lotto
*D. bassi* Targioni Tozzetti

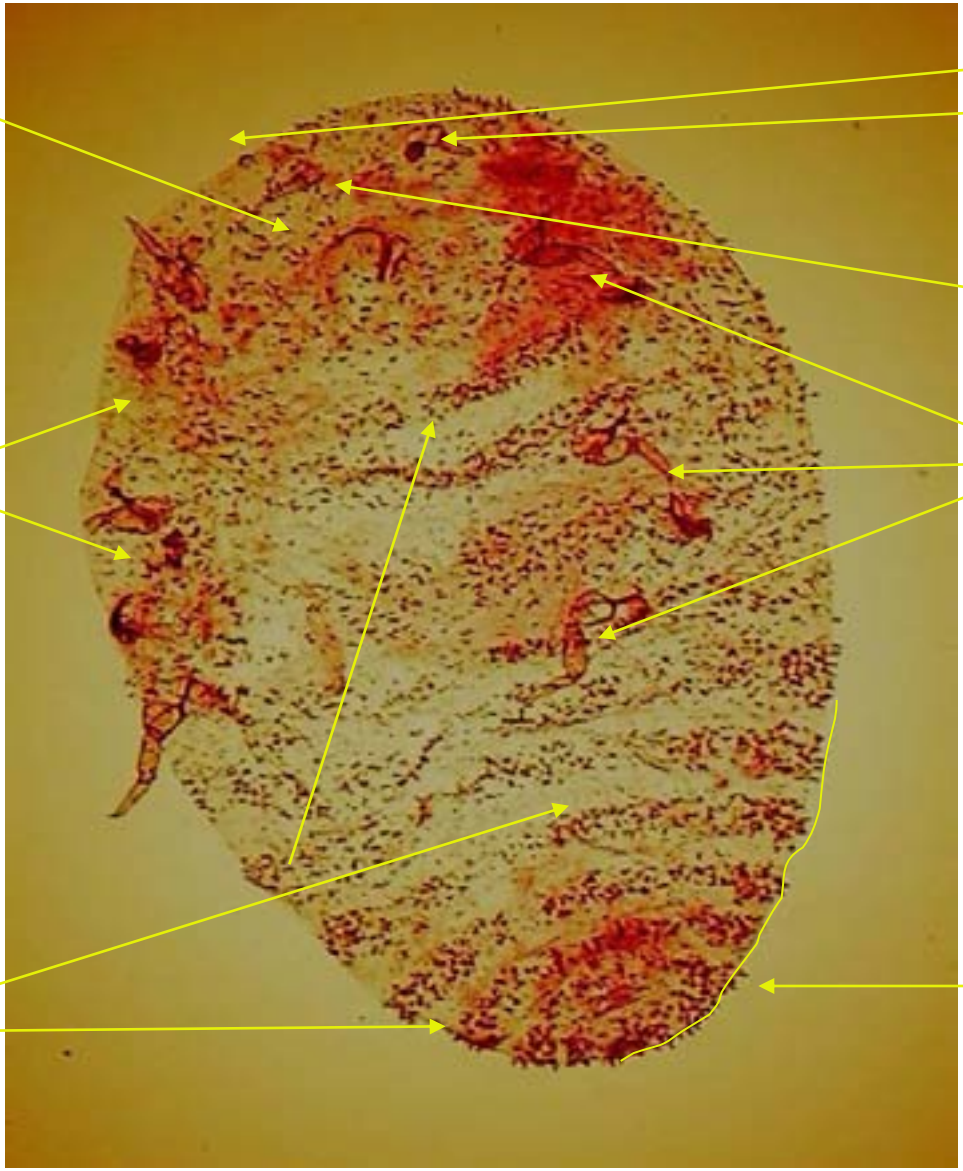
→ Mexico

→ South America



Feeding exclusively on cacti
~80 species hosts worldwide
22 in Mexico.

# Possess very limited morphological characteristics



# Expansion

## *Opuntia – Dactylopius*

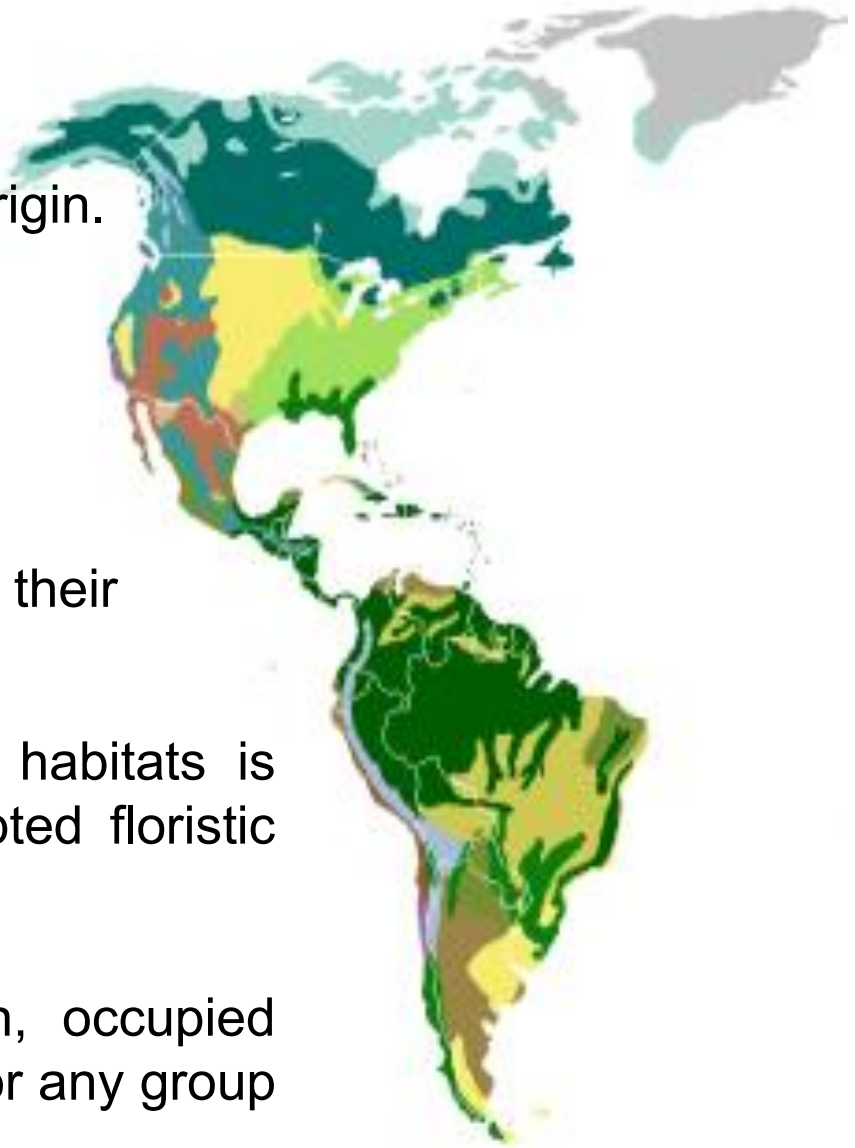
Cactus ancestor South American center origin.

Molecular evidence 2 lineages:
Cylindrical-stemmed
Flat-stemmed prickly pears,

Diverged in South America prior to their migration North, East & South America.

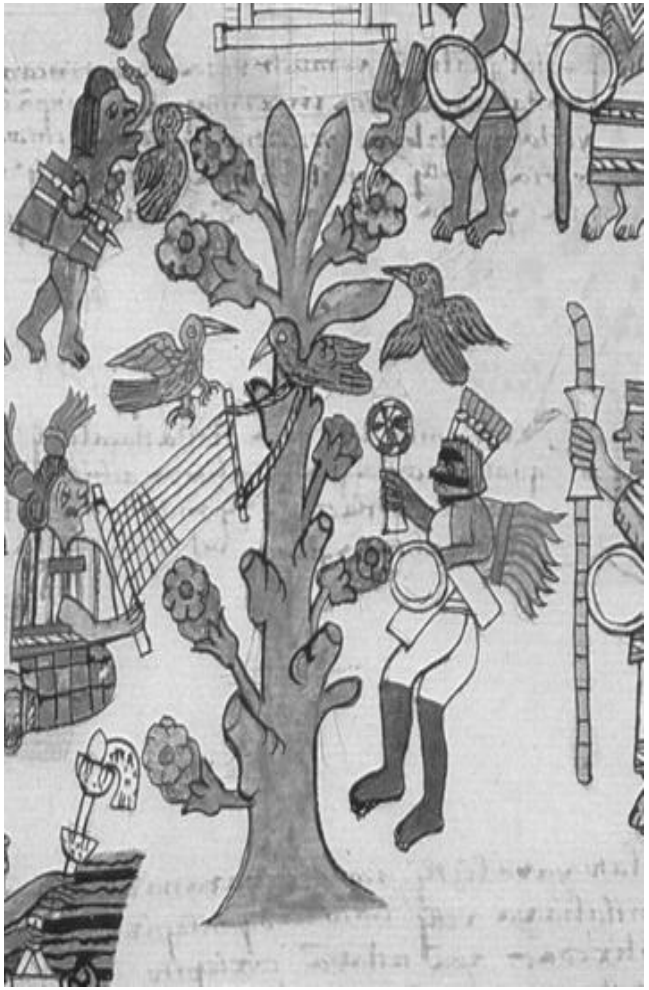
North-American desert and semi-desert habitats is result of parallel migration as xeric-adapted floristic cohort, later for *Dactylopius*.

*Opuntia* extensive evolutionary radiation, occupied the most widespread geographic region for any group within the Cactaceae (Anderson, 2001).



# II. Historical use review

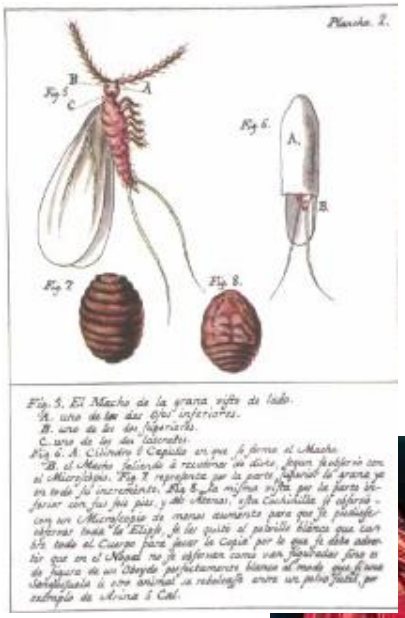
***Opuntia*** main components of human diet during preagricultural times. Gathering *Opuntia* data12,000–14,000 years ago.



Cultivation more than 700 years, exhibited botanical gardens or iconographically represented on building walls.

***Dactylopius.*** Rearing of cochineal, used and cultivated at least from X century, Toltec period, *amatl.*

# *Opuntia* and *Dactylopius* *production and* marketing





Tribute to Aztecs, 394 comunities Mixtec/4,400 kg/year

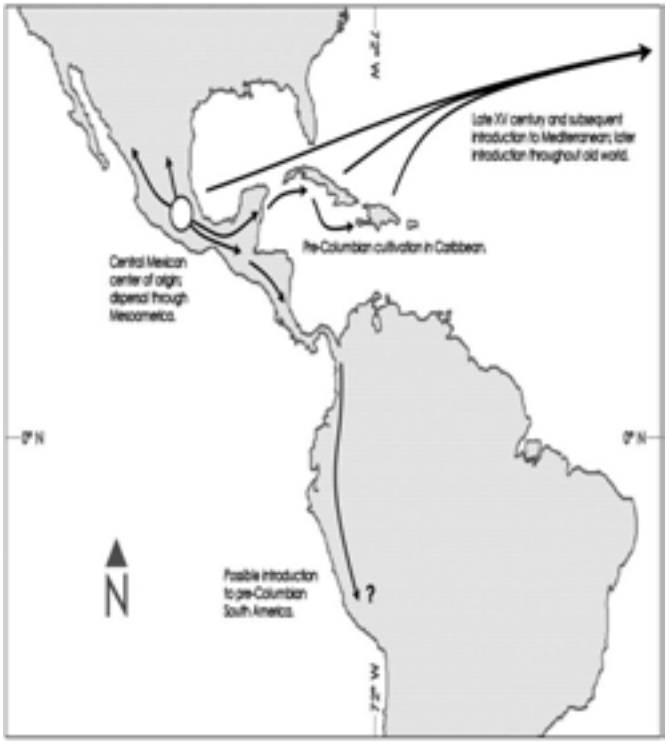
# Prehispánic period

Mixtec production best quality dye, greatest commercial distribution.

Possible commercial and cultural links Mesoamerican and Andean exchange and propagation *Opuntia-D. coccus*.

Mexico and Peru shared use of dye from X-XII.

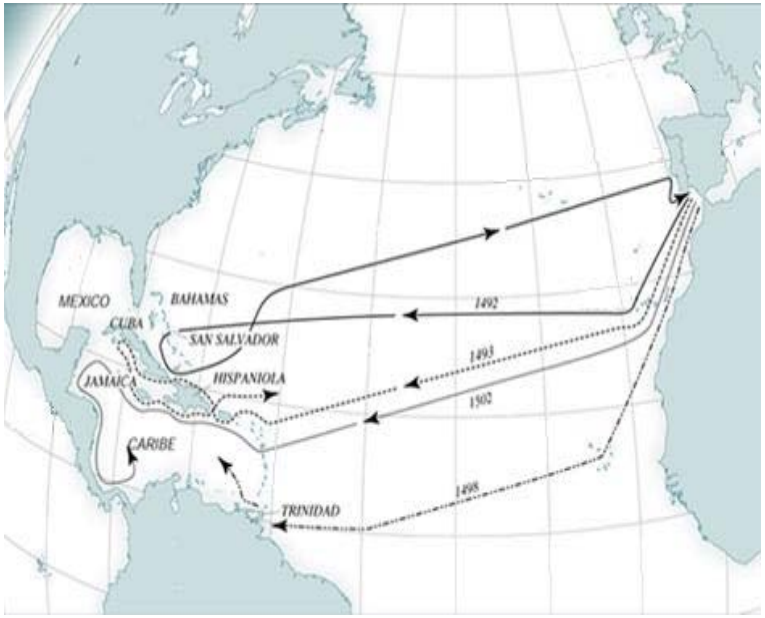
Chemical analyses Andean textiles (Peru, pre-Inca times) colored with *D. ceylonicus, D. confusus,* and *D. coccus* possible interchange materials & techniques.




# Conquest & Colony

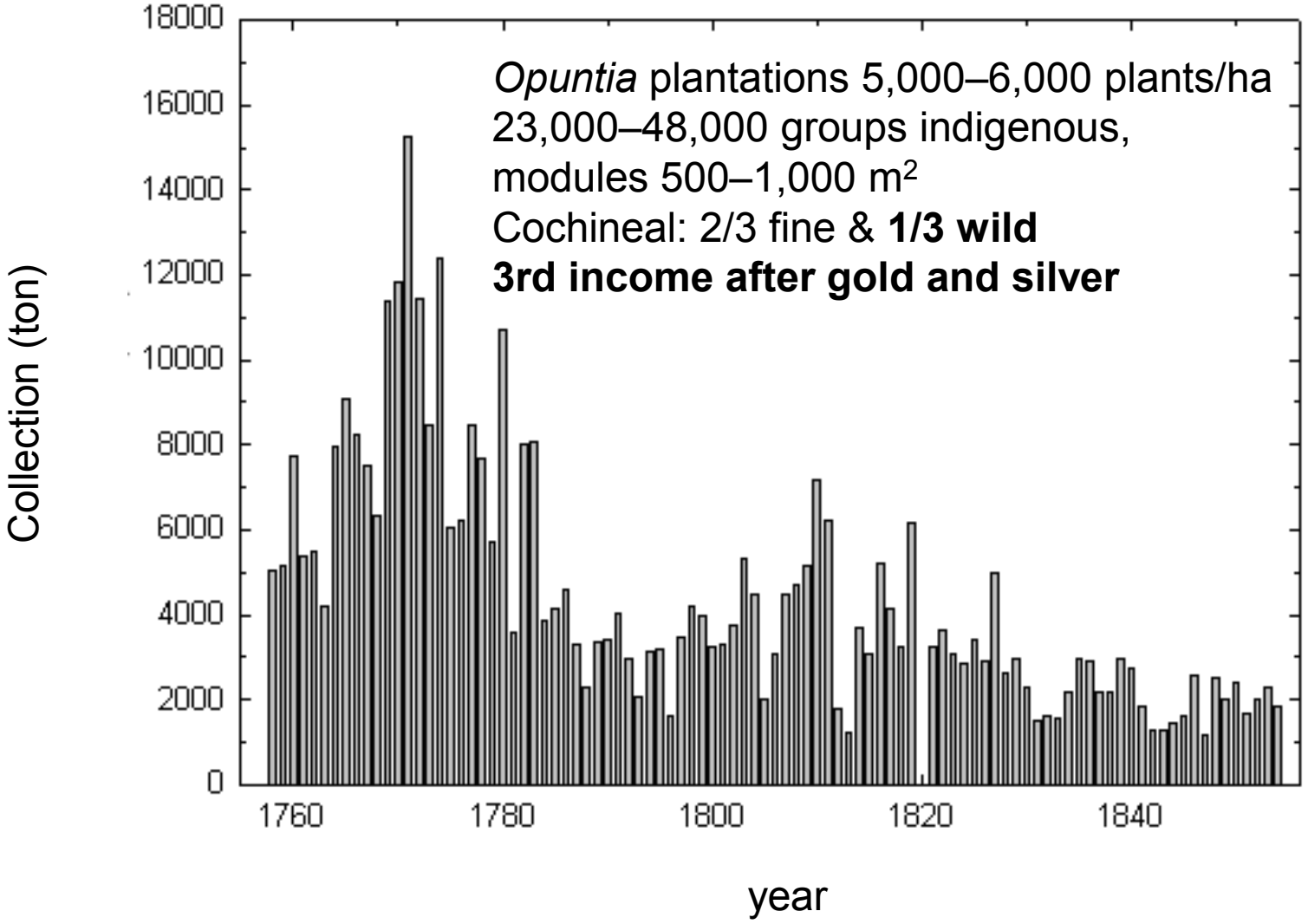
1493 ***Opuntia***,
Christopher Columbus
Lisbon

1523 ***Dactylopius,***
Hernán Cortés
Spain

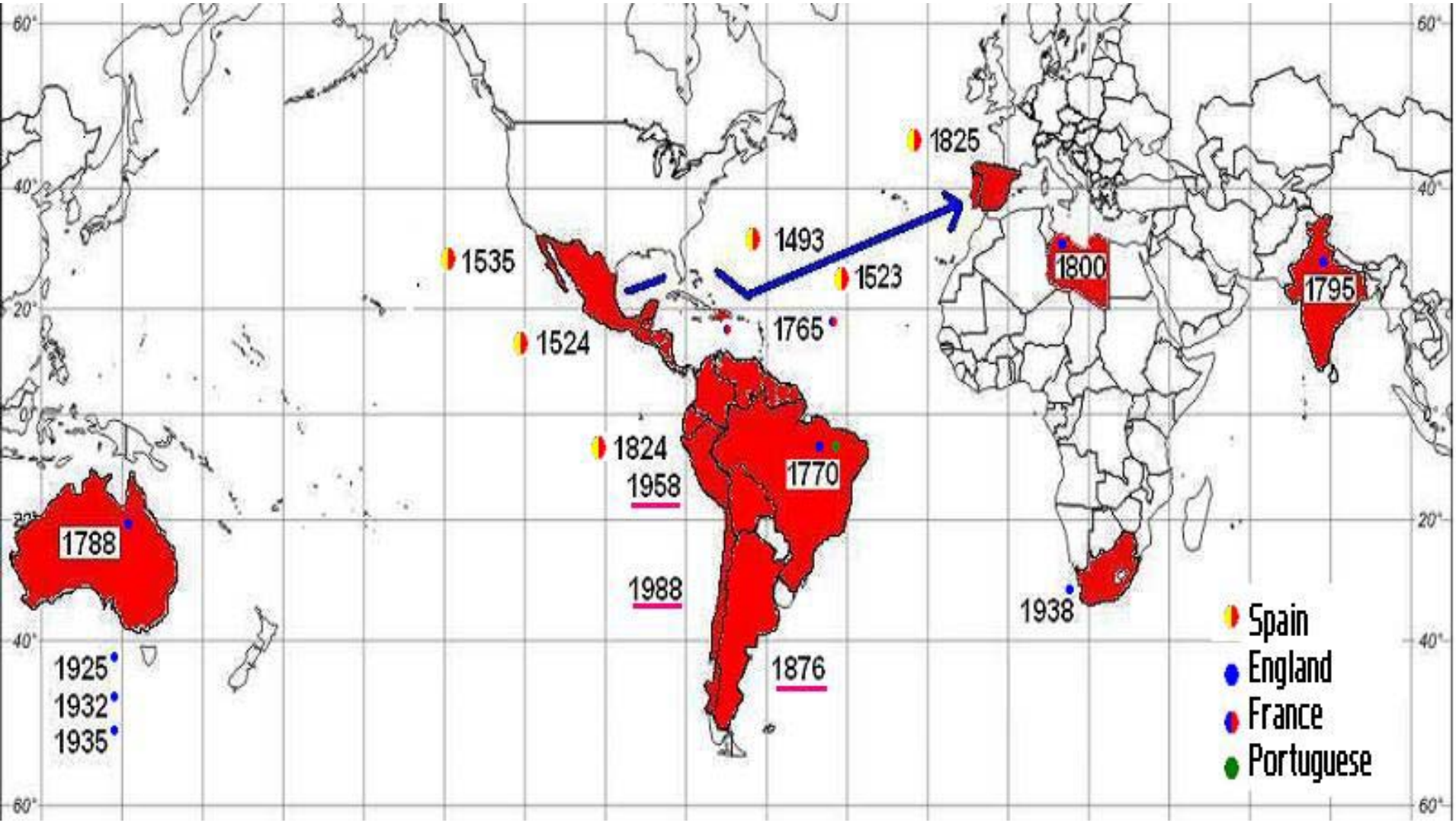



XVII-XVIII
America
Europe

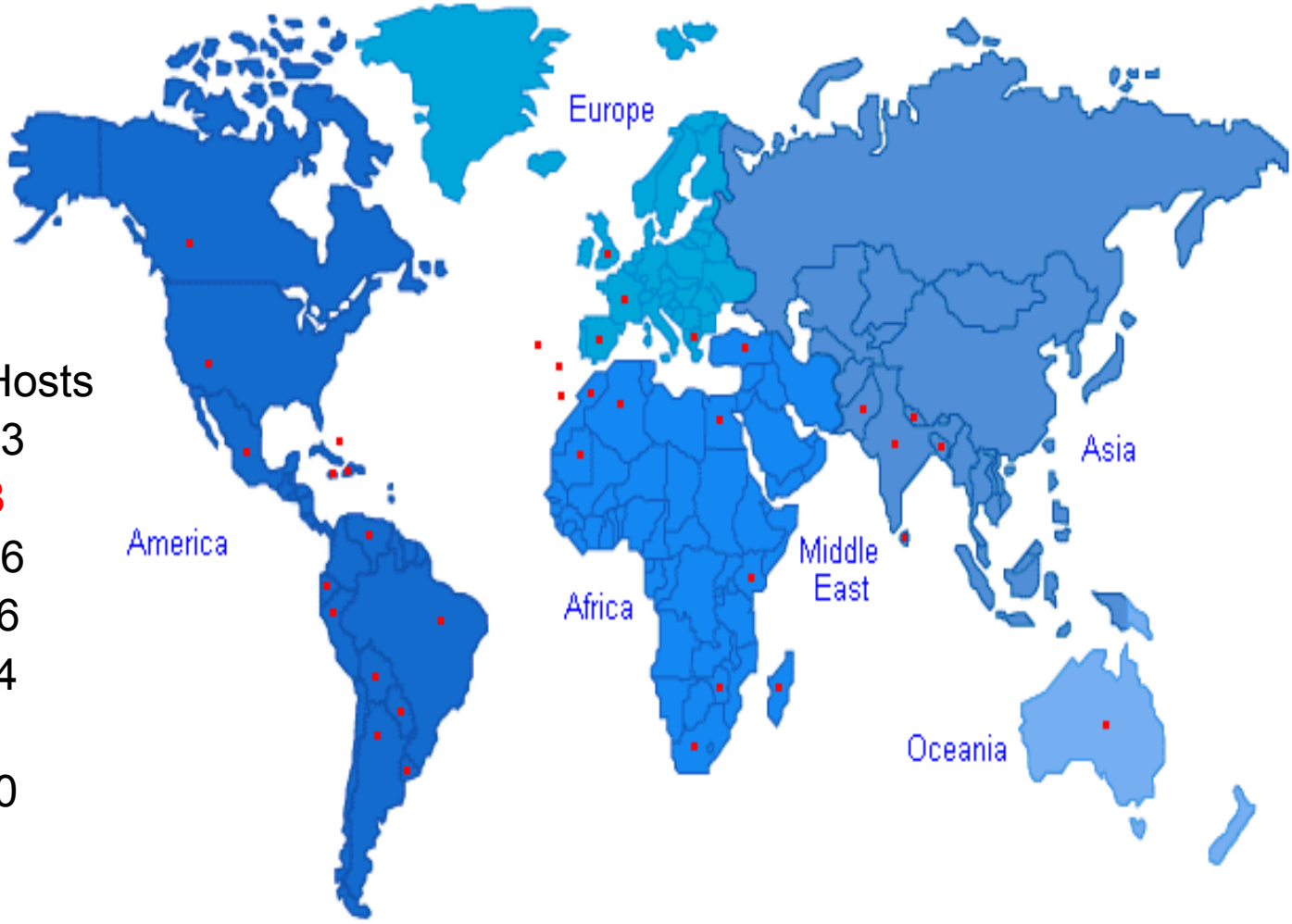
# Collection grana cochineal Oaxaca (1760 to 1850)
# 1700 T/year



# Expansion of *Dactylopius* and *Opuntia*



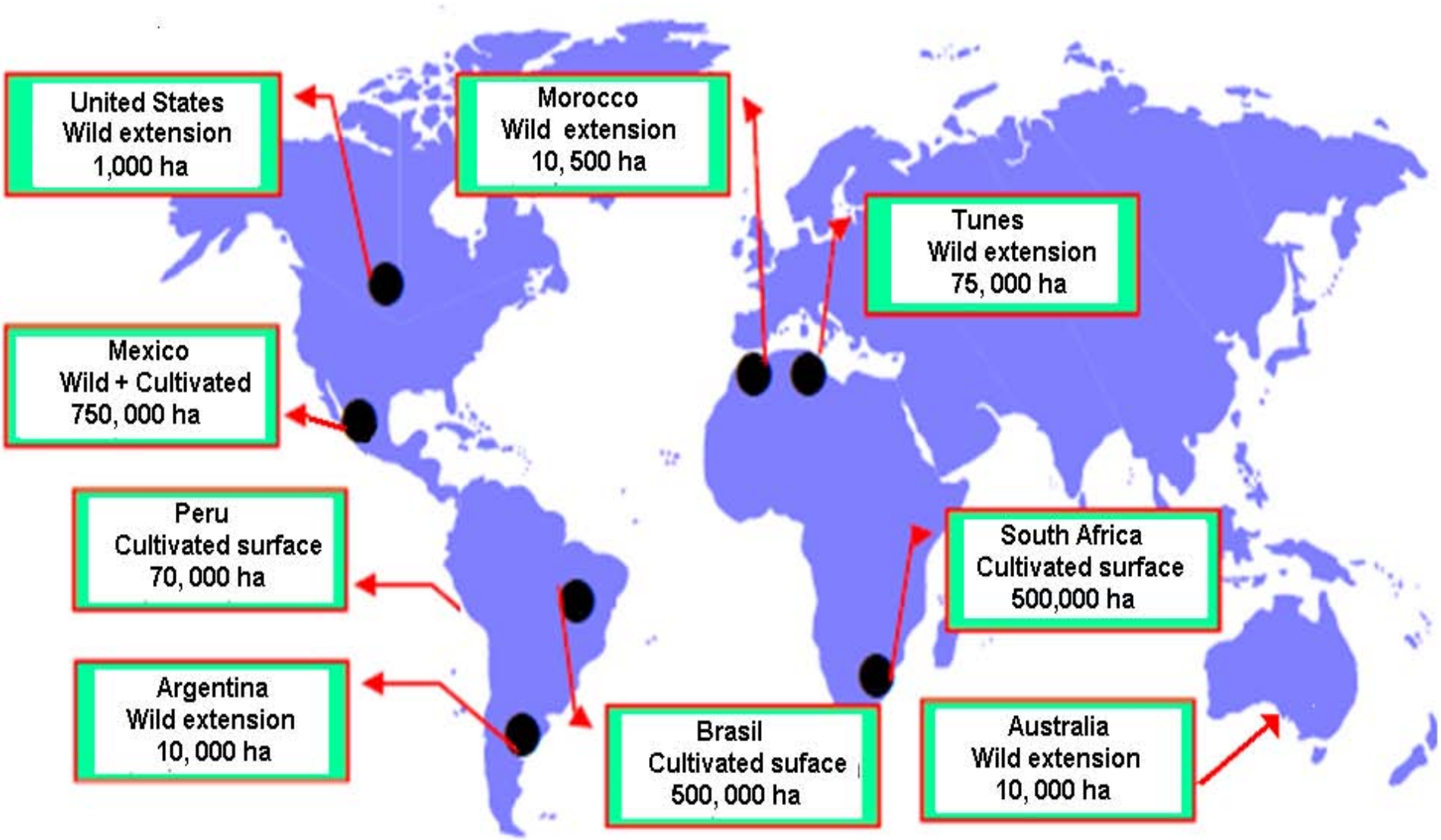
# Current distribution




| | Countries / Hosts |
|---|---|
| *D. opuntiae* | 16/33 |
| *D. coccus* | 15/8 |
| *D. ceylonicus* | 13/16 |
| *D. confusus* | 12/36 |
| *D. tomentosus* | 7/14 |
| *D. austrinus* | 3/8 |
| *D. confertus* | 3/10 |
| *D. salmianus* | 3/1 |
| *D. zimmermanni* | 3/7 |

**Opuntioideae** *Cylindropuntia, Grusonia, Maihueniopsis,* ***Opuntia*** **60%** *Tacinga, Tephrocactus, Tunilla.* **Other genus:** *Cereus, Cleistocactus, Denmoza, Echinopsis, Gymnocalcium, Harrisa, Maihuenia, Mammillaria, Pilosocereus, Selicereus.*

# *Opuntia*




Market: Local markets, Mexico, United States, Japan

# Uses in Mexico

**Cochineal culture (14 species/ 10 ha)**

Prickly pear (24 species/ 170,000 ha)

Vegetable (9 species / 9,000 ha)

Pharmaceutical industry
Fiber: mucilage and pectine

Fodder for cattle (13 species/ 130,000 ha)

Adhesives for whitewashing and building

Raw material for cosmetics products

Energy
Bio-gas, ethanol and wood

Living fences

Carbon consumers

Barriers for protection against soil erosion

**cladodes**

young

mature


PODER
Avena
Nopal

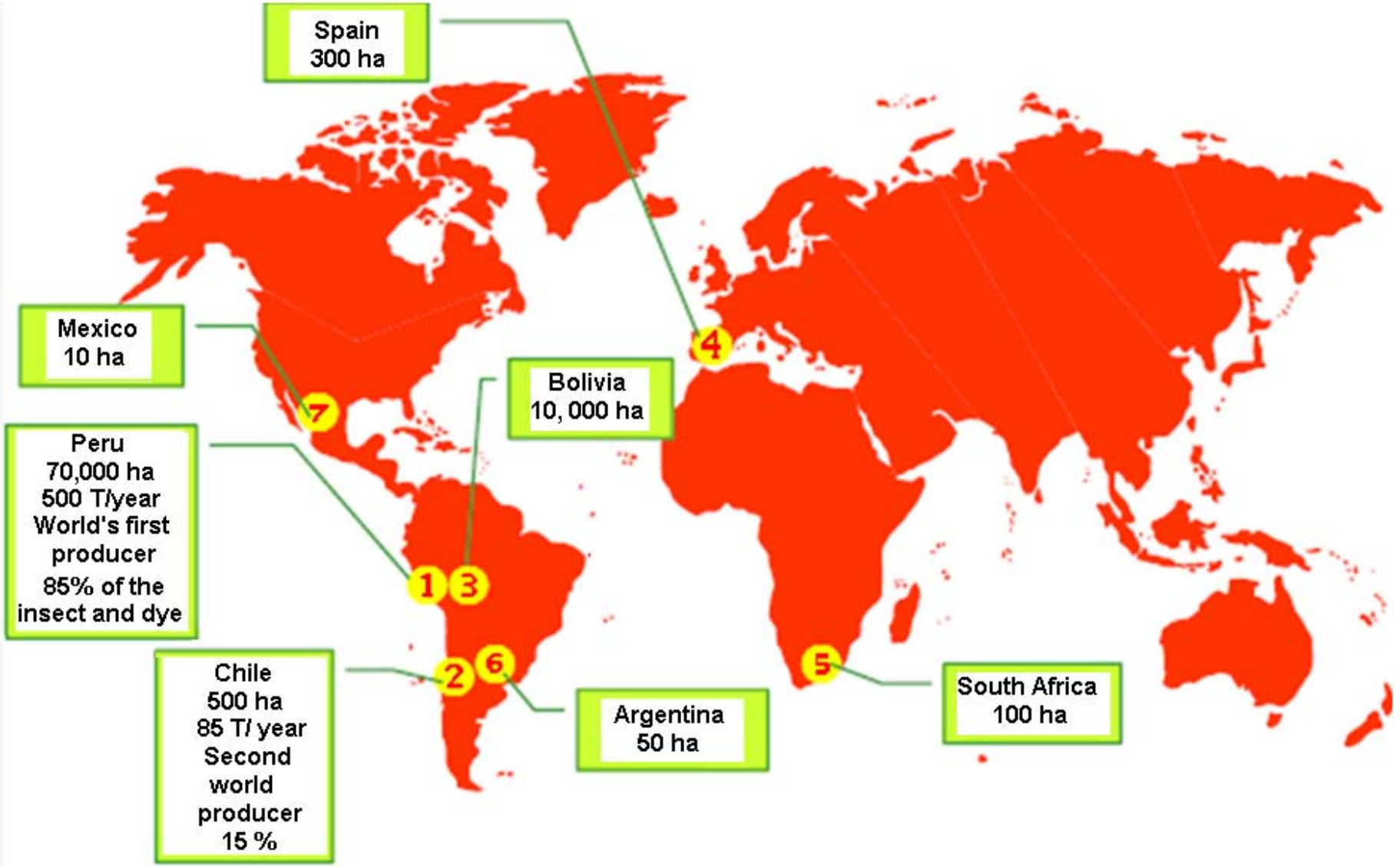

NOPALINA'S
PLUS


Señor Cactus
Tortillas de Nopal


Vivendi
ACTIVIA


Nopalfitz

# *Dactylopius coccus*




Market: Local, France, England, Italy, Japan, United States and Argentina

# Uses in Mexico

Biological control of invasive species (i.e. *D. opuntiae/ Opuntia*)

**Coloring materia**l for food and beverages cosmetics products

Sub-products

- Insects repellent
- prepare fertilizers
- Enrich food for avian species
- Antibacterial, antiviral and insecticidal

**Clothes**: Natural textil fibers: cotton, silk, linen and jute. Feathers

**Artistic works** Textil fibers, paper, fine cardboard, wood, leather, feathers, stone, murals, among others

**Tradicional medicine** stomach, head and heart diseases , stimulating tonic, sudiorific, alexipharmic, febrifuge, and collutory.

**Coloring materia**l for pharmaceutical, cosmetic and food industry

**Applications**

- traditional
- extended

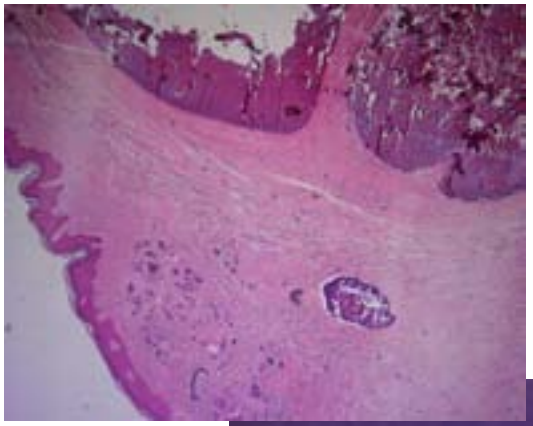
- Food

- Cosmetics

- Medicines

- Textiles

- Antibacterial, antiviral, and insecticidal



# Cconservation strategies

1. Constant fragmentation promoted by the extraction and exploitation of wild species, including *Opuntia*, without restrictions is reducing the possibility of studying the interaction between *Dactylopius* and *Opuntia*.

2. South American cactus-feeding moth, *Cactoblastis cactorum* a serious threat to the high diversity of native *Opuntia* species in Mexico.

51 endemic, 6 cultivated and 18 wild-growing species

Environmental Protection of native species in Mexico of wild flora and fauna, risk categories and specifications inclusion or change. *Opuntia* 5/284. *Dactylopius* is not even considered.







Specific measures for protection of such biodiversity and generic resources, the patron of distribution and the habitat characteristics to maintain these resources.

II. Chávez-Moreno, C.K.[1] Tecante A.[1], Casas A.[2], Claps L.E.[3] Distribution and habitat in Mexico of *Dactylopius* (Hemiptera: Dactylopiidae) and their hosts of the subfamily Opuntioideae (Cactaceae)

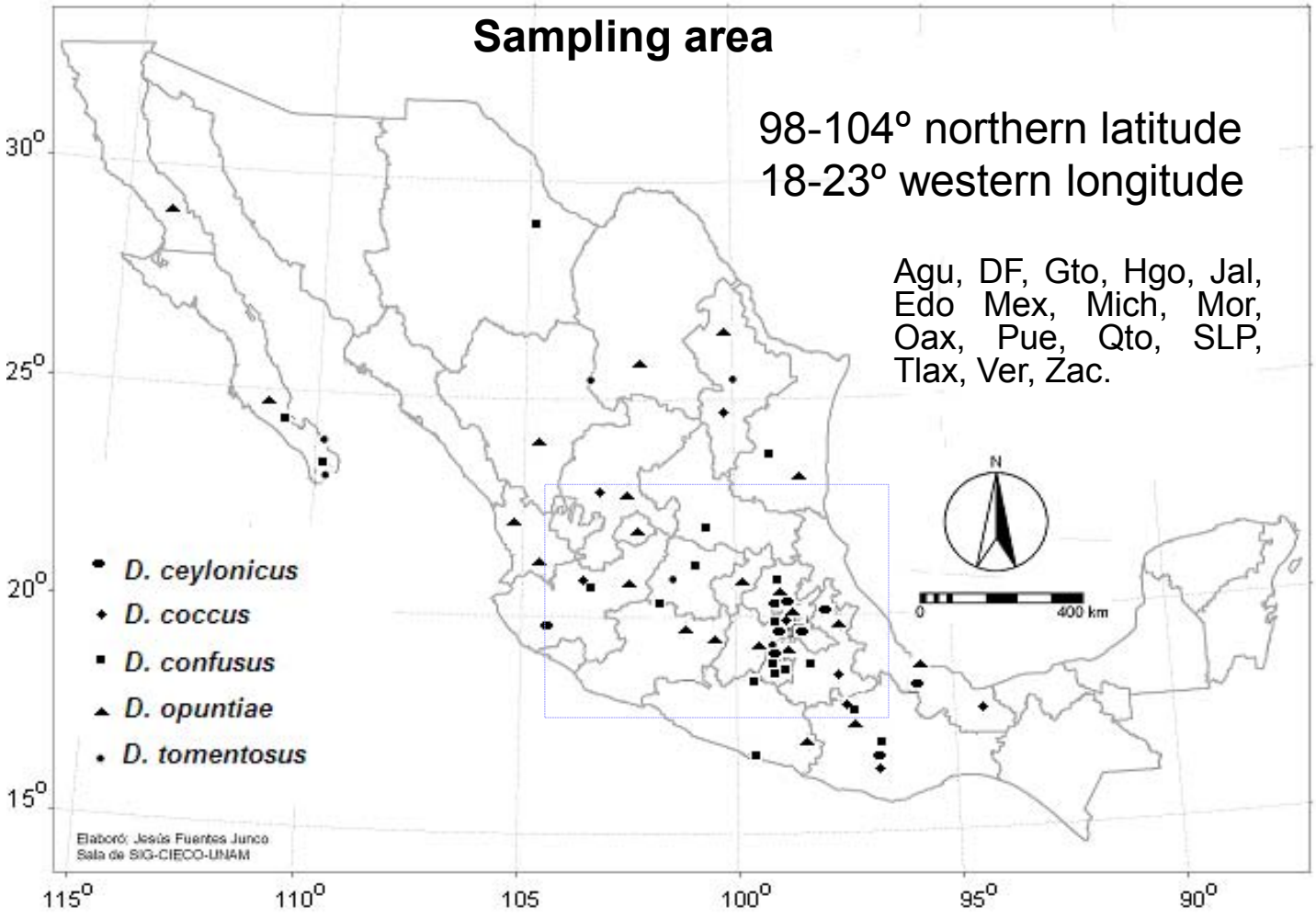
***Neotropical Entomology***

1 Facultad de Química, Departamento de Alimentos y Biotecnología, Universidad Nacional Autónoma de México, Ciudad Universitaria, México, D. F., 04510, MÉXICO

2 Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro, No. 8701, Morelia, Michoacán, 58190, MÉXICO.

3 INSUE - Instituto Superior de Entomología “Dr. Abraham Willink” Facultad de Ciencias 14 Naturales e Instituto Miguel Lillo. Universidad Nacional de Tucumán. Miguel Lillo 205 15 (4000) San Miguel de Tucumán, ARGENTINA.

# II. Distribution maps




**NOTE: Records of distribution of *Dactylopius ceylonicus* and their hosts**. Data compiled Britton & Rose 1963, Bravo-Hollis & Sánchez-Mejorada 1978, González *et al* 2001, Guzmán *et al* 2003, and herbaria MEXU and IBUG. Insect records Mann 1969, De Lotto 1974, Piña 1977, MacGregor & Sampedro 1983, Pérez-Guerra & Kosztarab 1992, Portillo & Vigueras 2003a, b). New records (empty symbols) described in this study . CNI-IB-UNAM, GIS constructed ILWIS 3.3 mapping geographic location *Dactylopius* and hosts.

# Field collection

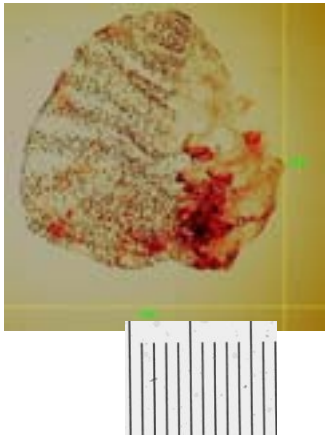
**25-100** specimens **208** populations **120** localities **14** states

**2005** Feb, May –June, Sep; **2006** April-June; **2007** Feb, Nov.

***Dactylopius***
Female
Male

stages of development
different portions host
collected separately
wild populations
production research centers
urban and rural zones

→ a live
preserved
70%, 96%
ethanol
CNI-IBUNAM



**HOSTS**
*Opuntia*
*Nopalea*
*Cylindropuntia*

*C*ollected 3X
Propagation

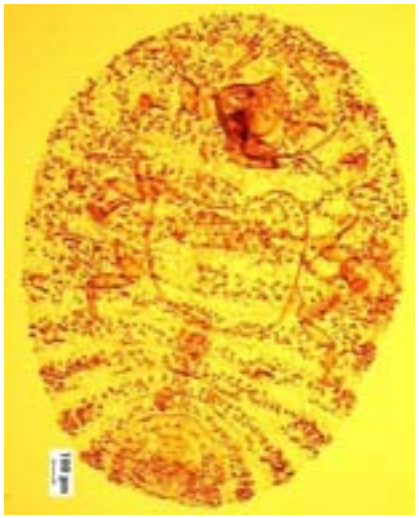
→ living
collection
Botanical
garden
CIEco-UNAM

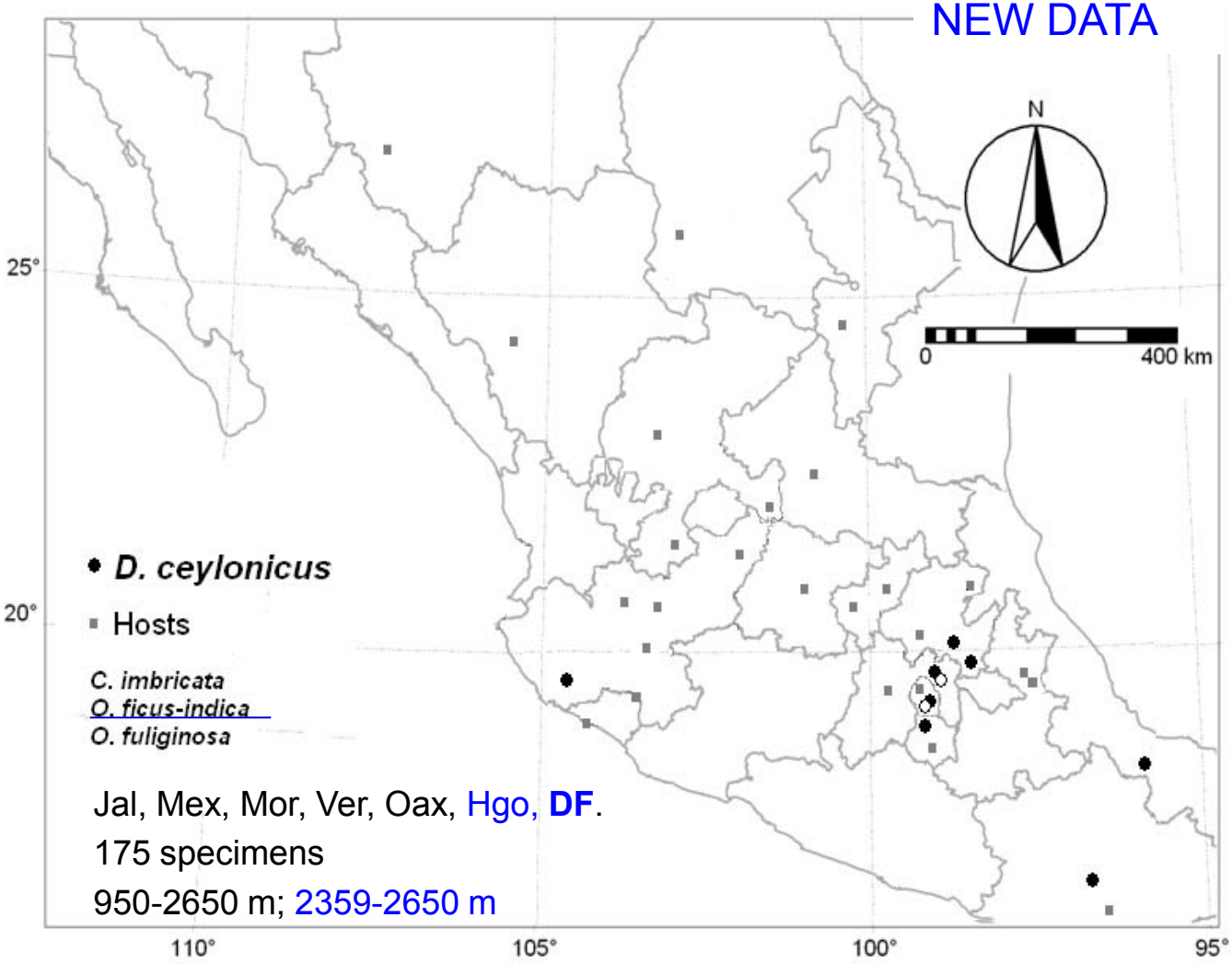
**Identification of species Taxonomic keys Support**
(De Lotto 1974, Pérez-Guerra y Kosztarab 1992).

**Environmental database**: insect, host, place of collection, vegetation and soil types, weather Peel *et al* (2007).

# *D. ceylonicus*

Review
NEW DATA





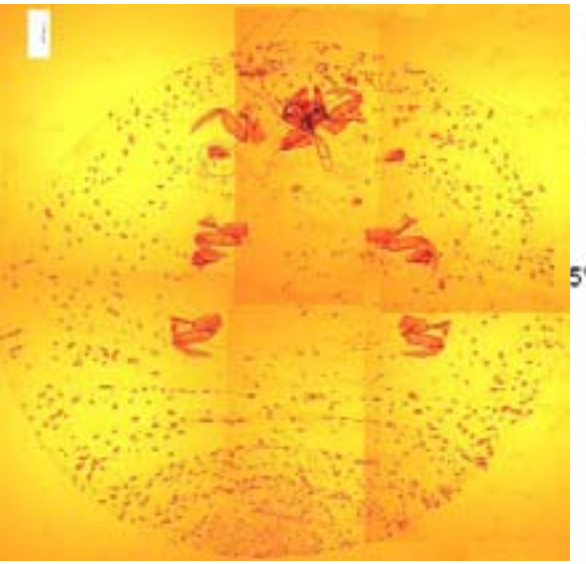

Jal, Mex, Mor, Ver, Oax, Hgo, **DF**.
175 specimens
950-2650 m; 2359-2650 m

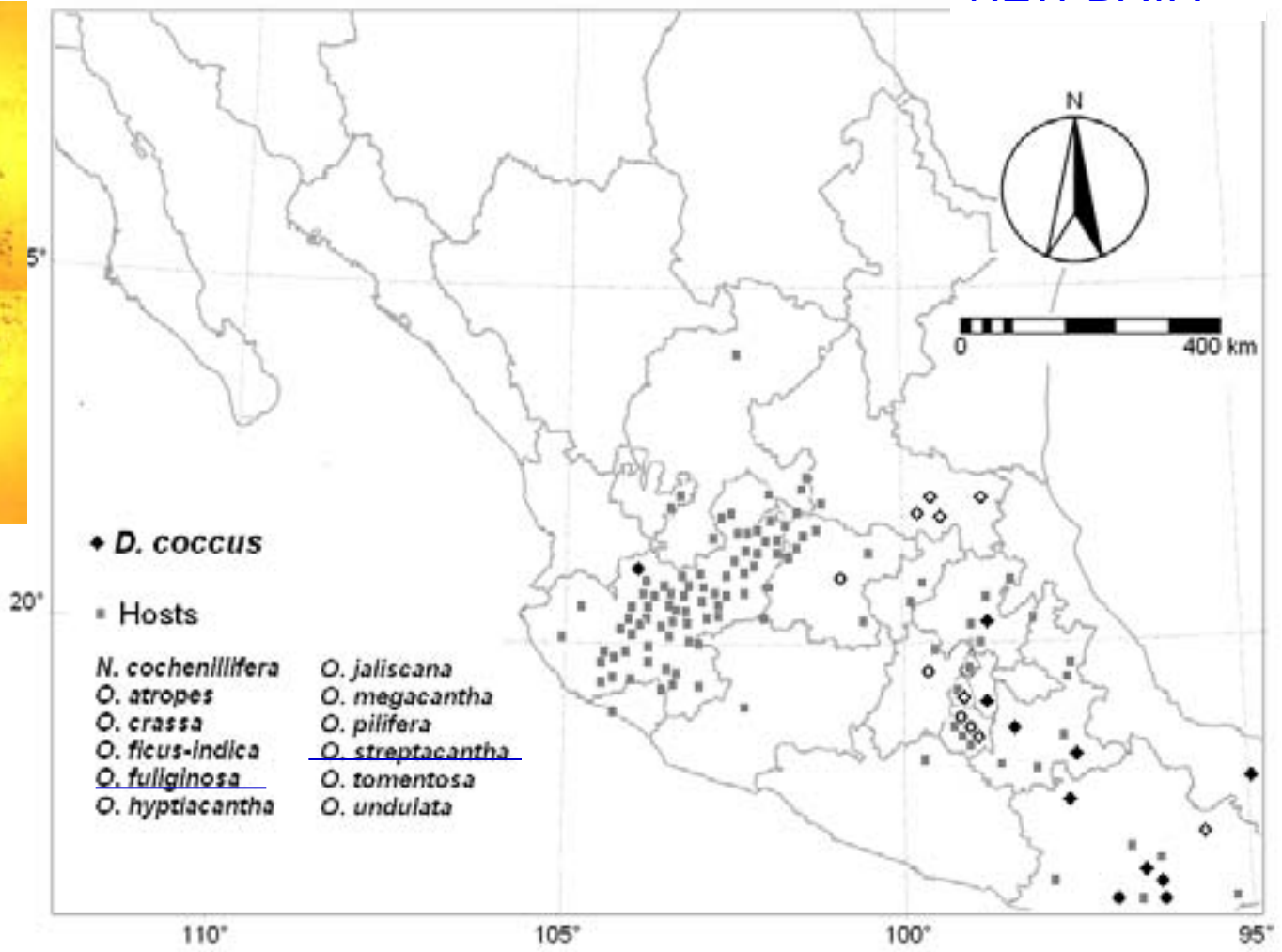
**NOTE**

Root nodules in winter / summer xerophilous thickets & arenosol

# *D. coccus*

Review
NEW DATA

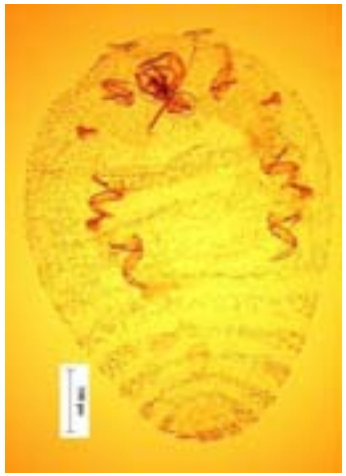




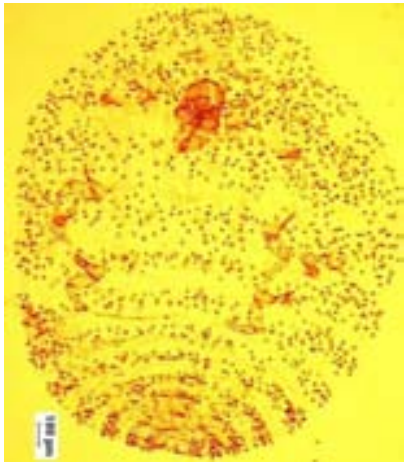

Jal, Hgo, Ver, Pue, Oax ©, **Gto, Mex ©, Mor** © y **SLP**© 575 specimens
1250-2200; 1654-2845 m

Research & production centers, living fences /xerophilous thickets, agricultural fields/calcisol, xerosol, vertisol, regosol, leptosol, foezem

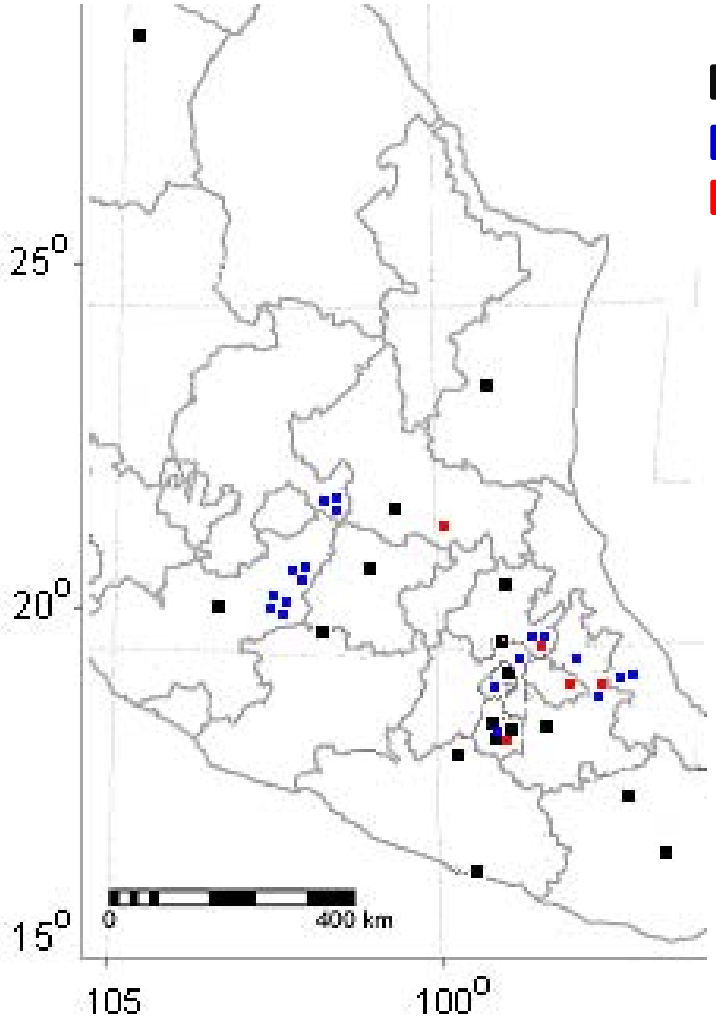
# *D. confusus*



*D. confusus*



*D. salmianus*




■ Review
■ *D. confusus*
■ *D. confusus* biotype1

| | | |
|---|---|---|
| Son | Coah | NL |
| Dgo**?** | Chih | Tam |
| Gto | Gro | Oax |
| Jal | DF | Pue |
| Hgo* | Mor* | **Ver** |
| **Zac** | **SLP** | **Tlx** |

1100-2200 m
1200-547 m
1654-2773 m

*Opuntia y Nopalea O.grahamii, C.imbricata, C.leptocaulis, C.tunicata***, *O.fuliginosa, O.jaliscana, O.joconostle, O.spinulifera, O.streptacantha, O.phaeacantha, O.hyptiacantha, O.ficus-indica**, nopal tuna roja

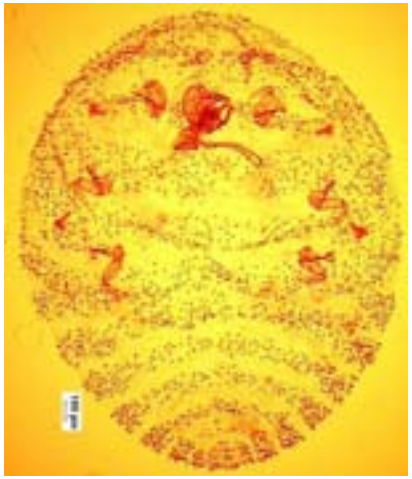
SLP

Morelos

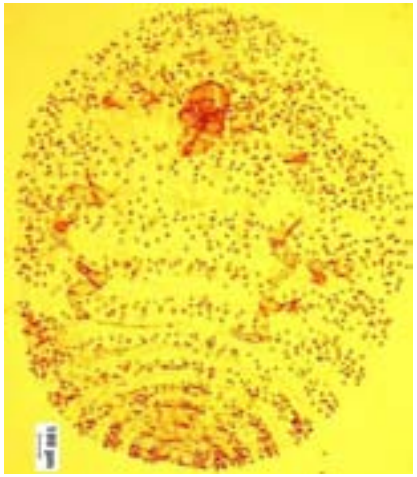
Living fences / urban zones
Production centers foezem y regosol

Reesearch centers / xerophilous thickets*/arenosol*, xerosol*

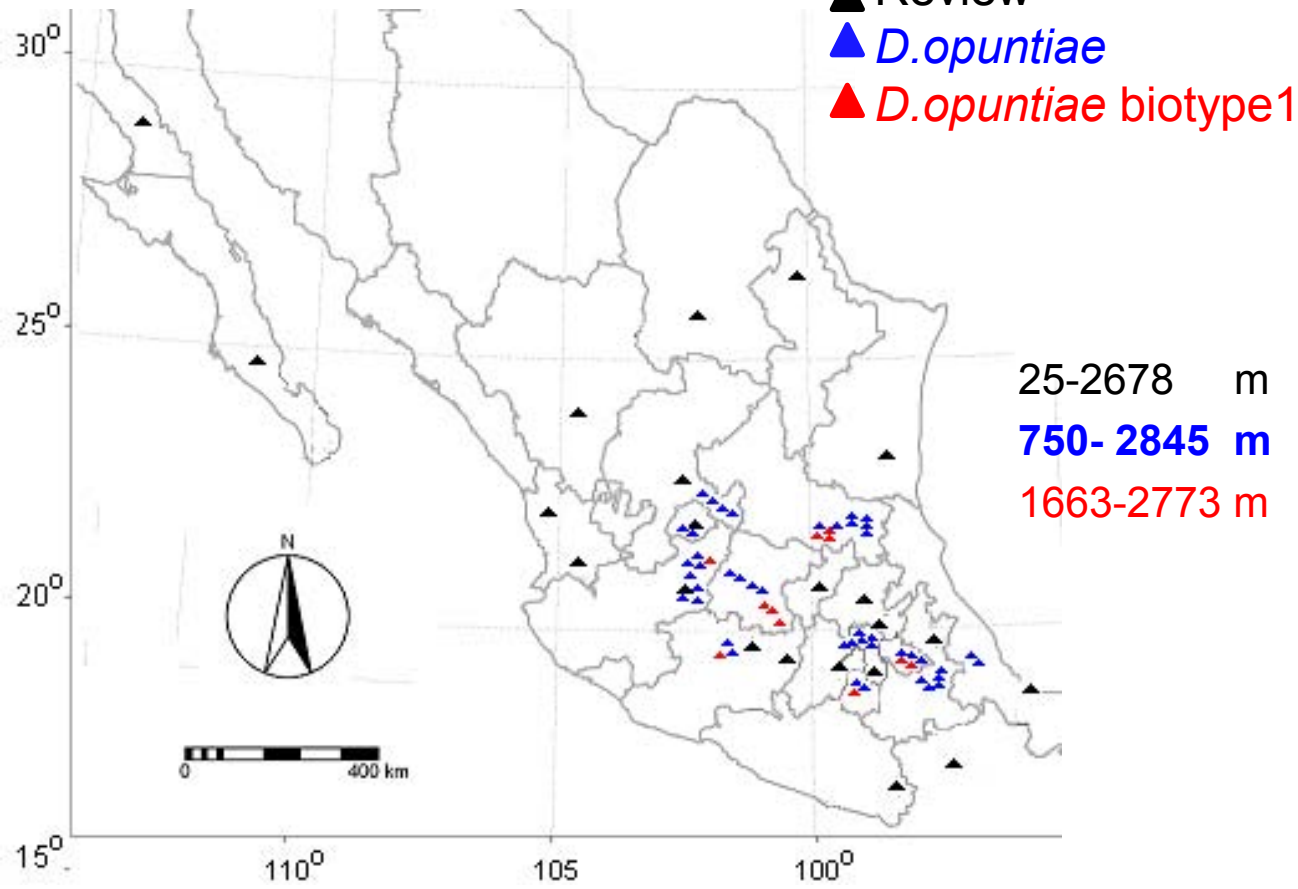
# *D. opuntiae*



*D. opuntiae*



*D. salmianus*




Oax, Gro, BC, Coah, NL, Tam, Dgo, Nay, Qro, Mex, Hgo,Chia Zac, Ags, Jal*, Mich* DF, Pue, Ver, **Gto*, Mor*, SLP*, Tlx***

*O. ficus-indica***, *O. tomentosa**, *O. robusta*, *Opuntia*, *Nopalea* y *Cactus*, *O. hyptiacantha*, *O. megacantha*, *O. joconostle*, *O. phaeacantha*, *O. atropes*, *O. albicarpa*, *C. tunicata*, *O. streptacantha**, variantes * y cultivares *

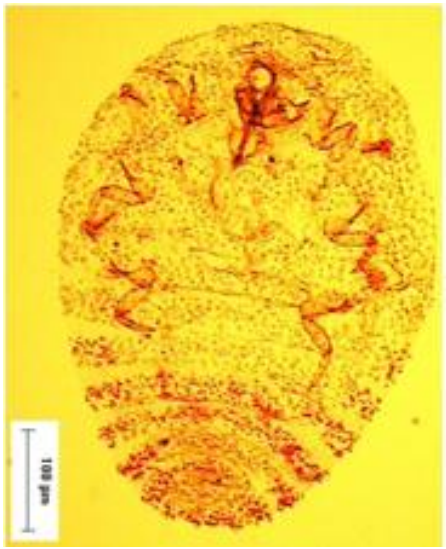
# *D. opuntiae*

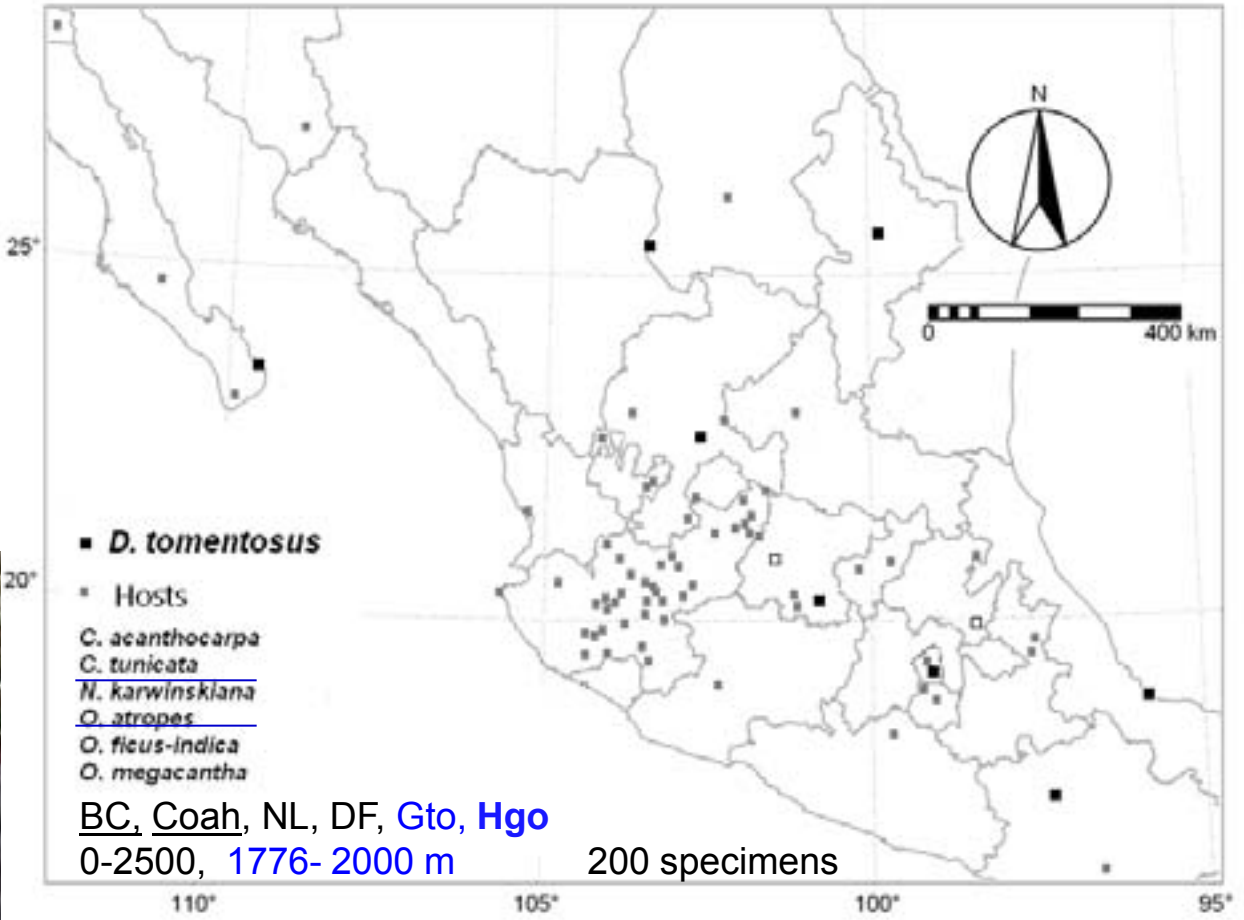
Estado de Mexico

1200 specimens

Living fences,  urban zones Production centers / Cultivated Forest oak-pine / calcisol, regosol, foezem, regosol, vertisol

# *D. tomentosus*



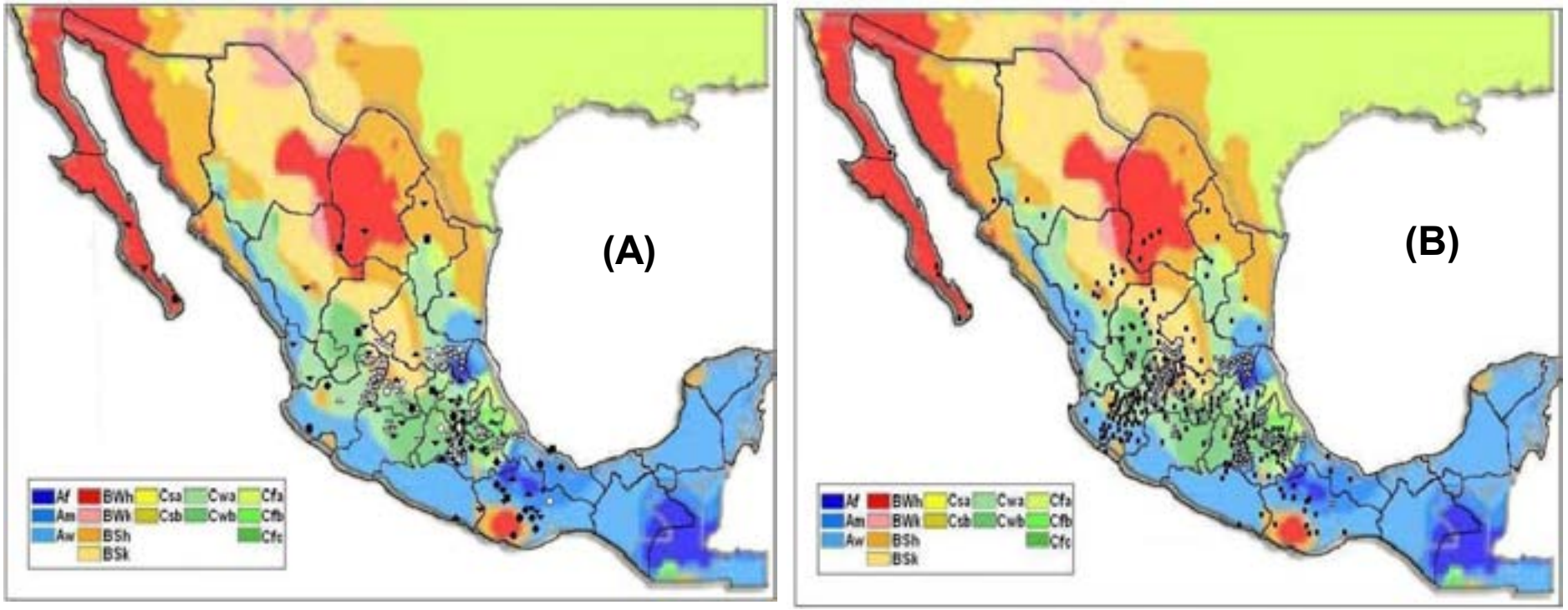



BC, Coah, NL, DF, Gto, **Hgo**

0-2500, 1776- 2000 m 200 specimens

**Areolas of cladodes / living fences xerophilus thickets vertisol, arenosol**

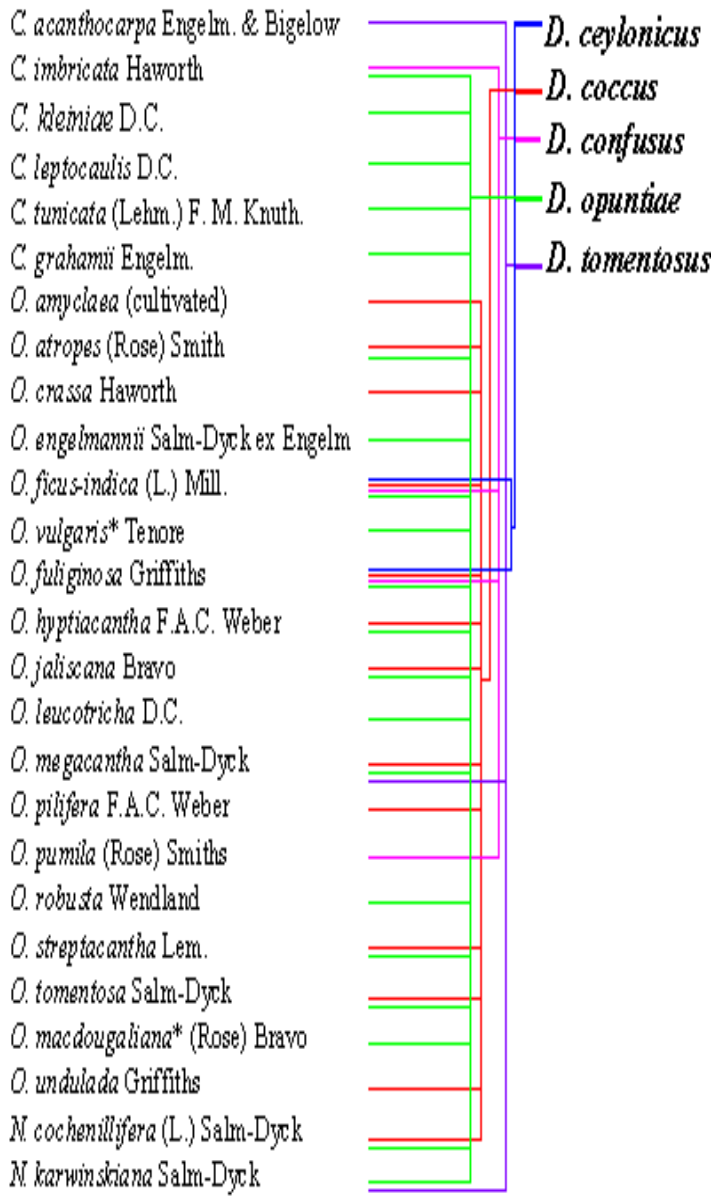
# Biogeographical region




Distribution of **A)** ***Dactylopius*** *D. ceylonicus* (circles), *D. coccus* (diamonds), *D. confusus* (triangles), *D. opuntiae* (inverted triangles), *D. tomentosus* (rectangles) and **B)** ***Opuntia*** over the Köppen-Geiger climate type map of Mexico (North America) extracted from Peel *et al* (2007).

# Conclusion

| DATA | BEFORE | AFTER |
|---|---|---|
| **Region** | 14 | 23 |
| **Species** | 5 | 5 or more |
| **Biotypes** | -- | 2 |
| **Hybrids** | -- | ¿? |
| **Cohabit** | -- | 2, 3, 4 species |
| **Hosts** | 26 | 41+26 = 67 |
| **Vegetation** | Hosts`s | xerophilous thickets, cultivars, collections, urban zones & forest (pine, oak) |
| **Soil** | Hosts`s | foezem, vertisol, xerosol, arenosol, calcisol, regosol, leptosol |
| **Weather** | Hosts`s | Template, dry arid and semiarid, Forest pine-oak |
| **Elevation** | --- | 0-2845 |




Chemical and genetic analysis contribute to stablish interaction specificity

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# Metabolic profiling of *Dactylopius* (Hemiptera: Dactylopiidae) species pigments by geographical origin and hosts using multivariate data analysis

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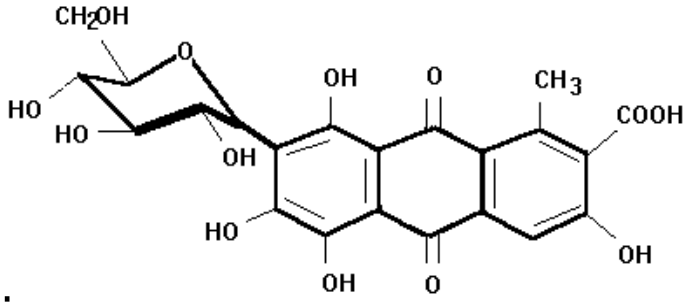
# III. Chemical analysis

*Dactylopius coccus*




- Source of natural colorant

(Food & Drug Administration. 2009).

- Historicaly wild species could serve as alternative.

or additional sources of the colorant and its derivatives. ($C_{22}H_{21}O_{13}$)

- Studies HPLC–MS-NMR
  - Origin, constituents hydroxyanthraquinone (González *et al*., 2002),
  - Chemical structure characteristics (Méndez *et al*., 2004; Maier, 2004),
  - Identify CA: foods, antique textiles, (Yamada *et al*., 1993; González *et al*., 2002; Szosteketal, 2003; Maier *et al*., 2004; Surowiec *et al*., 2007; Peggie *et al*., 2008;Karapanagiotis *et al*., 2008, 2009).
  - Optimize extraction conditions, quality (Wouters,1985; González *et al*., 2002; Méndez *et al*., 2004; Szosteketal, 2003; Peggie *et al*., 2008; Karapanagiotis *et al*., 2009).

**OBJETIVE:** compare the metabolic profile 5 mexican species and the argentinan.

- Material populations
  - 35 Mexico *Dactylopius*
  - 2 Argentina *D. ceylonicus*
  - Outgroup *K. Vermilio*

Reheodyne 117 volt AC, 50-60htz

Binary LC: pump Water 600

Column C18 waters

Dual λ absorbance detector

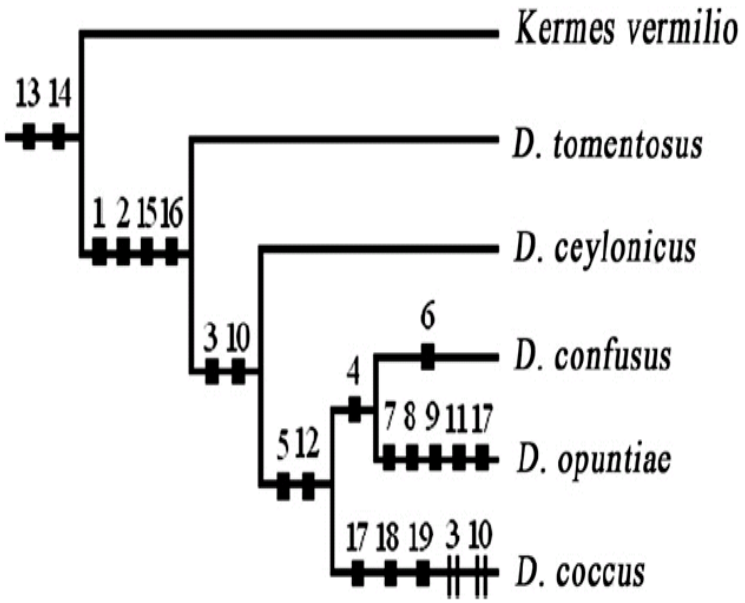
0.5 mL/min / 20 μL

Quintuplicate

(Méndez *et al*., 2004; González *et al*., 2002)

| Peak | tR(min) | D. ceylonicus | D.coccus | D.confusus | D.opuntiae | D.tomentosus |
|---|---|---|---|---|---|---|
| 1 | 0.678 | 1 | 1 | 1 | 1 | 1 |
| CAc | 1.84 | 1 | 1 | 1 | 1 | 1 |
| 3 | 3.29 | 1 | 0 | 1 | 1 | 0 |
| 4 | 4.58 | 0 | 0 | 1 | 1 | 0 |
| 5 | 5.36 | 0 | 1 | 1 | 1 | 0 |
| 6 | 6.05 | 0 | 0 | 1 | 0 | 0 |
| 7 | 8.31 | 0 | 0 | 0 | 1 | 0 |
| 8 | 9.73 | 0 | 0 | 0 | 1 | 0 |
| 9 | 10.6 | 0 | 0 | 0 | 1 | 0 |
| 10 | 12.5 | 1 | 0 | 1 | 1 | 0 |
| 11 | 13.4 | 0 | 0 | 0 | 1 | 0 |
| 12 | 14.3 | 0 | 1 | 1 | 1 | 0 |
| FkAc | 15.4 | 1 | 1 | 1 | 1 | 1 |
| KAc | 16.6 | 1 | 1 | 1 | 1 | 1 |
| 15 | 17.5 | 1 | 1 | 1 | 1 | 1 |
| 16 | 18.8 | 1 | 1 | 1 | 1 | 1 |
| 17 | 19.3 | 0 | 1 | 0 | 1 | 0 |
| 18 | 21.9 | 0 | 1 | 0 | 0 | 0 |
| 19 | 22.1 | 0 | 1 | 0 | 0 | 0 |
| **Total peak** | | **8** | **11** | **12** | **16** | **6** |

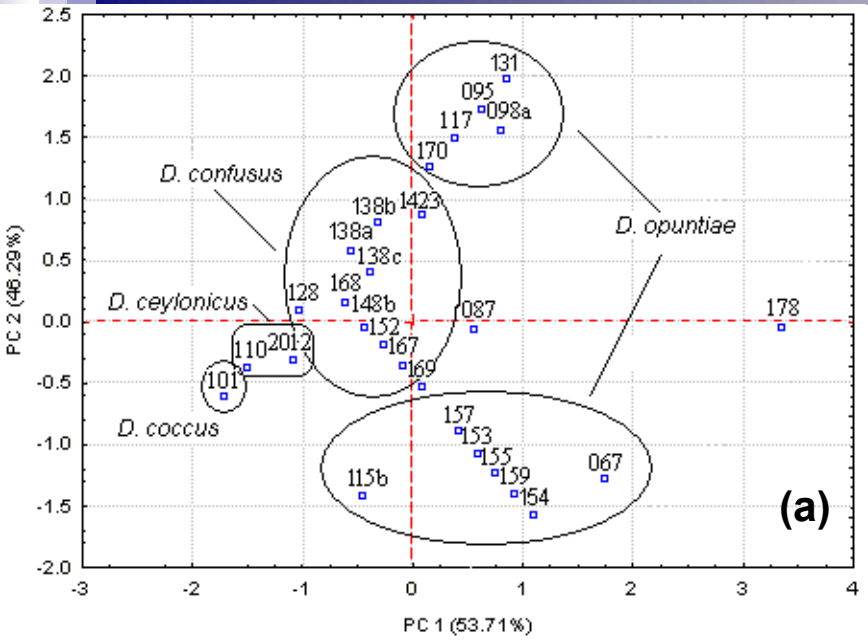
**1** Presence: **unique** or **shared**
**0** Ausence

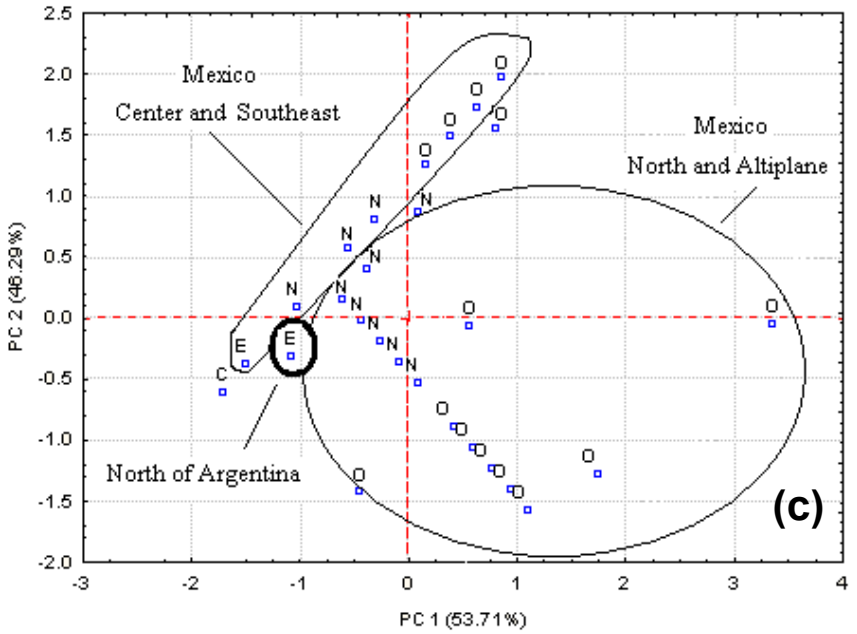


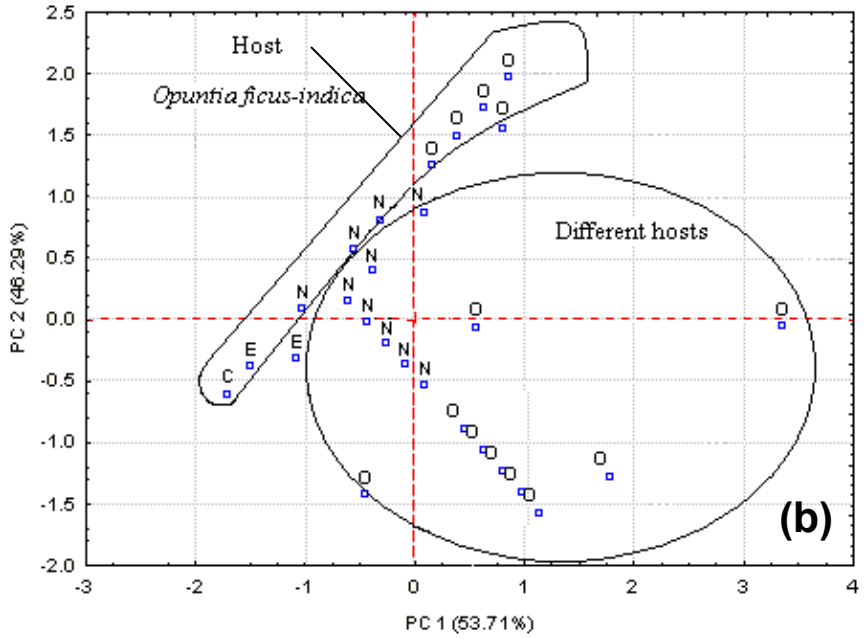

Dendogram (1-0). Resulting from a cluster analysis using principle of parsimony for *Dactylopius* & *K.vermilio*. The numbers above the lines indicate the peaks of the chromatographic profile.

Quantitative analyses. Absolute area (*10-4) of the HPLC profile of commercial carminic acid and five *Dactylopius* species (0 = absence, CAc = carminic acid, KAc = kermesic acid).

| Pico | Acido carminico | *D. ceylonicus* | *D. coccus* | *D. confusus* | *D. opuntiae* | *D. tomentosus* |
|---|---|---|---|---|---|---|
| Cac | 9.83 ± 3.13 | 3.70 ± 0.241 | 8.60 ± 3.12 | 3.45 ± 0.91 | 4.70 ± 0.41 | 6.37 ± 0.280 |
| 3 | 1.83 ± 3.17 | > 0 ± 0.002 | 0 | 0.50 ± 0.13 | 0.24 ± 0.028 | 0 |
| 4 | 0 | 0 | 0 | 0.30 ± 0.12 | 0.18 ± 0.31 | 0 |
| 5 | 0 | 0 | 1.08 ± 0.38 | 0.30 ± 0.12 | 0.12 ± 0.13 | 0 |
| 6 | 0 | 0 | 0 | 0.020 ± 0.023 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | > 0 ± 0.001 | 0 |
| 10 | 0.010 ± 0.016 | 0.13 ± 0.23 | 0 | 0.06 ± 0.05 | > 0 ± 0.0002 | 0 |
| 12 | 0 | 0 | 2.39 ± 1.21 | 0.62 ± 0.34 | > 0 ± 0.0003 | 0 |
| Kac | 0.83 ± 0.05 | 0.75 ± 0.17 | 0.03 ± 0.05 | 0.07 ± 0.13 | 0.59 ± 0.09 | 1.92 ± 0.286 |




Component analysis the plane formed by the first two principal components.
*D. coccus* (C), *D. ceylonicus* (E), *D. confusus* (N) and *D. opuntiae* (O).
a) species
b) goegraphic distribution **3, 9, 15**
c) host ***O. ficus-indica* 3, 7, 11 CAc**

INSECT–SYMBIONT INTERACTIONS

# Molecular Phylogeny of the Genus *Dactylopius* (Hemiptera: Dactylopiidae) and Identification of the Symbiotic Bacteria

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# Systematic of *Dactylopius*

- Molecular
  - Philogeny of Coccoidea Dactylopiidae-Eriococcidae. Mithocondrial gene secuenciation 12S rARN and nuclear 18S rARN (Cook et al., 2002)
- Morphological analysis
  - Taxonomical keys (De Lotto, 1974; Pérez-Guerra & Kosztarab, 1992), and Rodríguez *et al*. (2001)
- Endosymbionts
  - *Wolbachia* present in *Dactylopius* sp. eggs secuenciated by nuclear 16S rARN gene. (Thao et al., 2002, Baumann, 2005; Moran, 2006, (Gruwell et al., 2007, Pankewitz et al., 2007).

# Metodología

**OBJETIVE.** *Dactylopius* philogeny of 5 mexican species with mithocondrial 12S rARN and nuclear 18S rARN genes for insectos.

Endosymbionts phylogeny of insects molecular gene 16S rARN.

**Sample**
ethanol 96º and alive

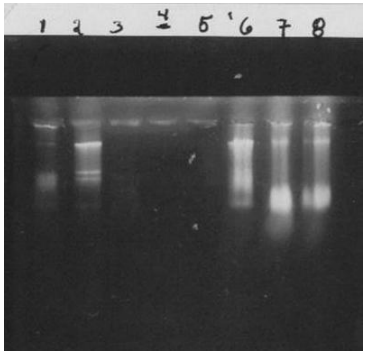
2-8 insects,
5 populations
5 Localities of Mexico
1 from Brasil

↓

**DNA extraction** DNeasy Blood and Tissue Kit (Qiagen)

↓

**RCP**




↓

**Fragments purification**

↓

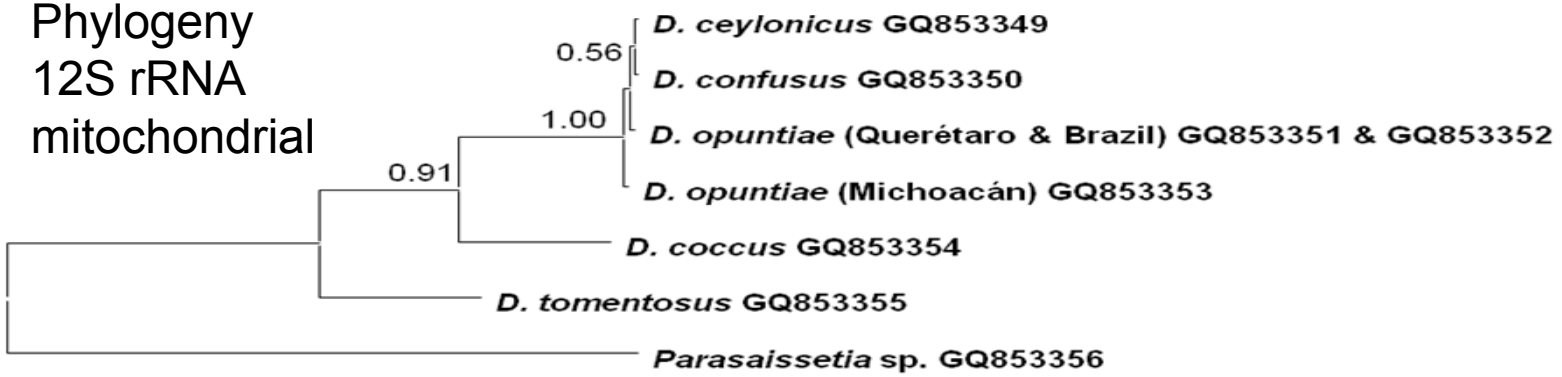
**Secuenciation**
**Dendogram & Computer analysis**

Outgroups
Genetic Bank

(Tautz *et al*., 1988, Weisburg et al., 1991, von Dohlen & Moran, 1995, Thao *et al.*, 2004)

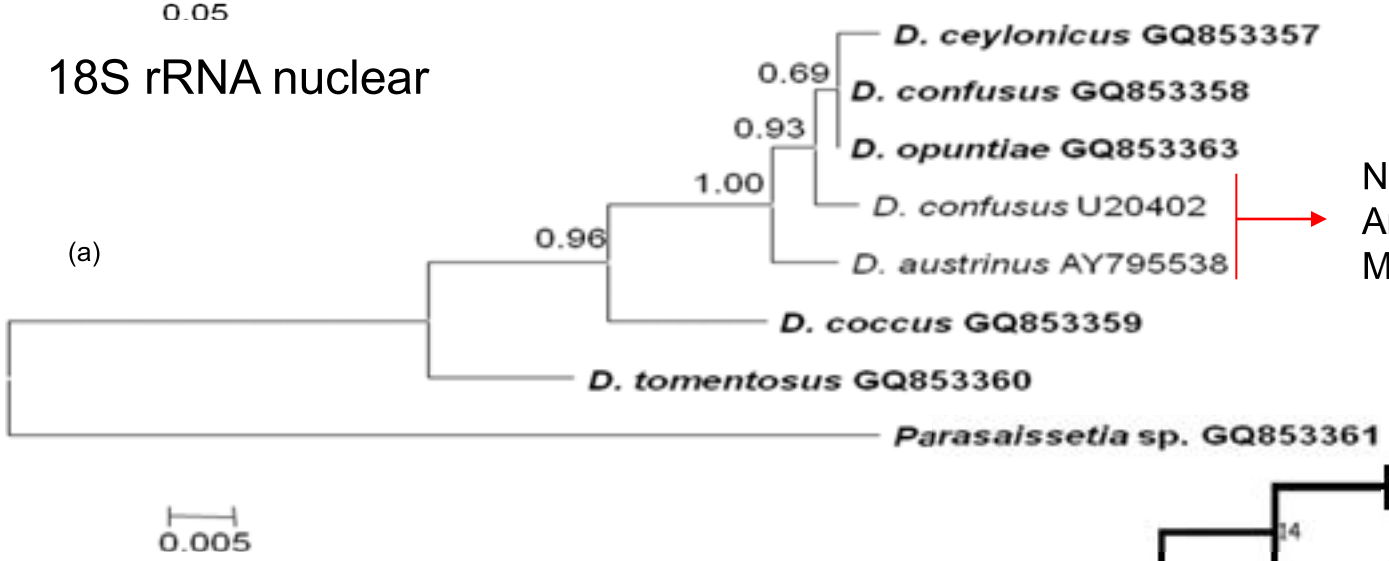
# III. Molecular analysis

Phylogeny
12S rRNA
mitochondrial

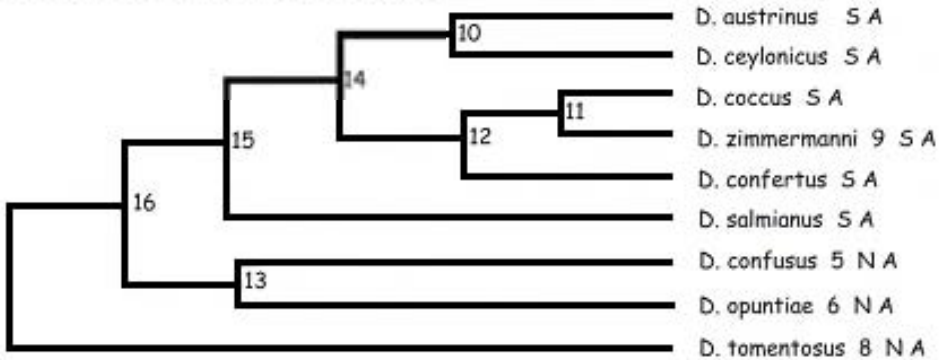



18S rRNA nuclear

(a)

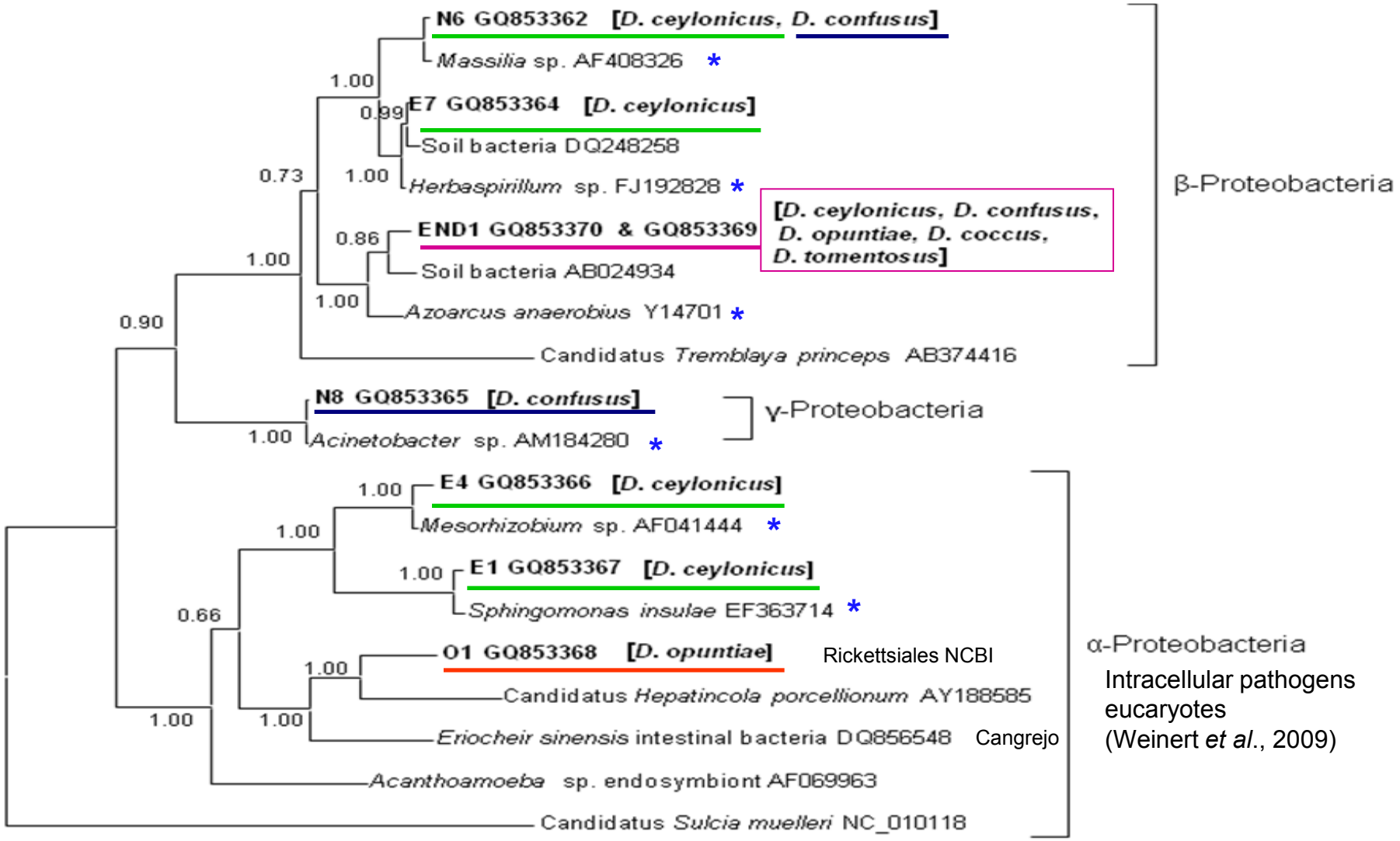



Comparison of molecular phylogeny (18S rRNA nuclear) Morphological phylogeny (Rodríguez *et al*., 2001), resulted in different topographies.



# Molecular analysis

## *Dactylopius* endosymbionts phylogeny 16SrRNA




* Free living bacteria in the intestine similar to those present in plants/soil

# Conclusions

- Mexico Center origen genetic diversity of *Dactylopius* and their hosts
- Real and potencial uses of these genetic resources in Mexico.
- Geographic patrons and habit characteristics, main protected areas.

- Chemical analysis:
  - Metabolic profile Colorant of 5*Dactylopius,* diferentiate according species, geographical origin and host and
  - recognise *Dactylopius* source of colorant.

- Molecular analysis:
  - Molecular phylogeny 5 Mexican *Dactylopius* sequenced mitochondrial genes 12S rRNA and nuclear sequenced 18S rRNA for insects.
  - Comparison between molecular phylogeny and morphology dendogram characters resulted in different topographies.
  - IInsect endosymbiont phylogeny 16S rRNA demonstrated presence:
    - α-protobacteria (E1, E4, O1)
    - β-protobacteria (N6, N7, END1)
    - γ-protobacteria (N8)

    → Bacteria intestine/insects, and free living/plants and soil.

- The Conservation for the interaction plant-insect resources must be the guide to use in a sustentable maner these genetic resourses.

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