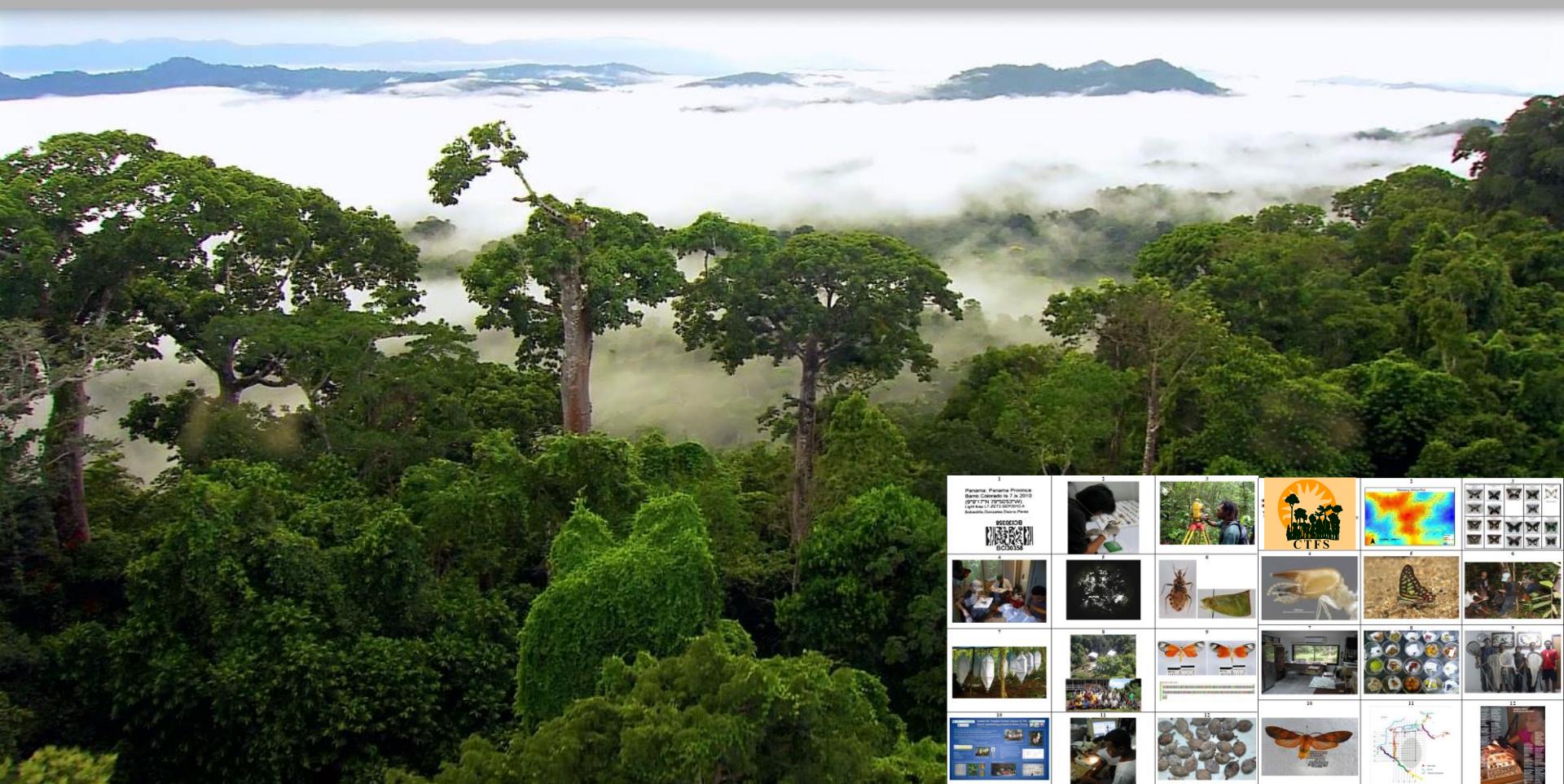


CTFS-ForestGEO ARTHROPOD INITIATIVE 2008-2015:

# Monitoring barcodes?

## Long-term monitoring of tropical arthropods



Yves Basset, Smithsonian Tropical Research Institute, [bassety@si.edu](mailto:bassety@si.edu)

# ForestGEO ®



Currently: 61 sites in 24 countries

6 million of trees monitored, representing 10,000 species

10 science initiatives: Arthropod monitoring: 9 sites



# **CTFS-ForestGEO ARTHROPOD INITIATIVE**

## **Aims:**

**to monitor key insect assemblages over the long-term at CTFS sites**

**and**

**to study insect-plant interactions across the CTFS network**

**At each CTFS site, 3 phases:**

- baseline survey to identify common species**
- monitoring (modeled on baseline survey)**
- interaction studies (different set of protocols)**

**Focus on a priority set of assemblages chosen for their**

- ecological relevance**
- taxonomic tractability**
- ease of sampling**

# Priority assemblages

**Litter ants:** key organisms in tropical forests and often key predators

[Formicidae]



**Selected moths and butterflies:** caterpillars leaf-chewers, adults often pollinators

[Rhopalocera, Geometridae, Arctiinae & Pyraloidea]



**Bees:** important pollinators of many tropical trees

[Apidae Euglossini]



**Termites:** important decomposers in tropical forests

[Isoptera]



**Tephritid fruit-flies:** seed (fruit) predators

[Tephritidae]



**Seed predators:** important influence on fruit/seed survival (whole guild)

[Varia]



Full suite of 15 taxa studied at BCI, Panama

# Methods: baseline survey & monitoring

**Litter ants:** extraction from litter with Winkler



**Bees:** attraction to chemical baits,  
(only Neotropical sites)



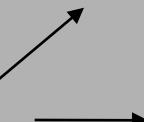
**Tephritid fruit-flies:** baited McPhail traps  
(not in the Neotropics)



**Moths and other taxa:** light traps



**Termites:** light traps & hand search in quadrats



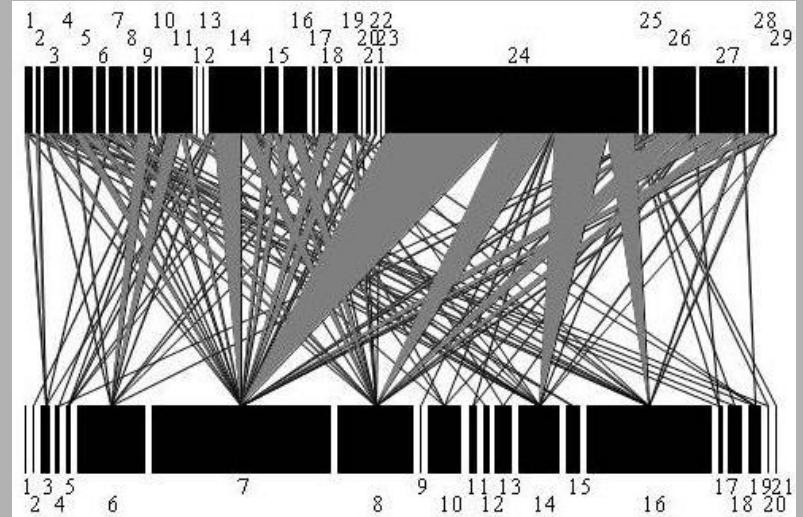
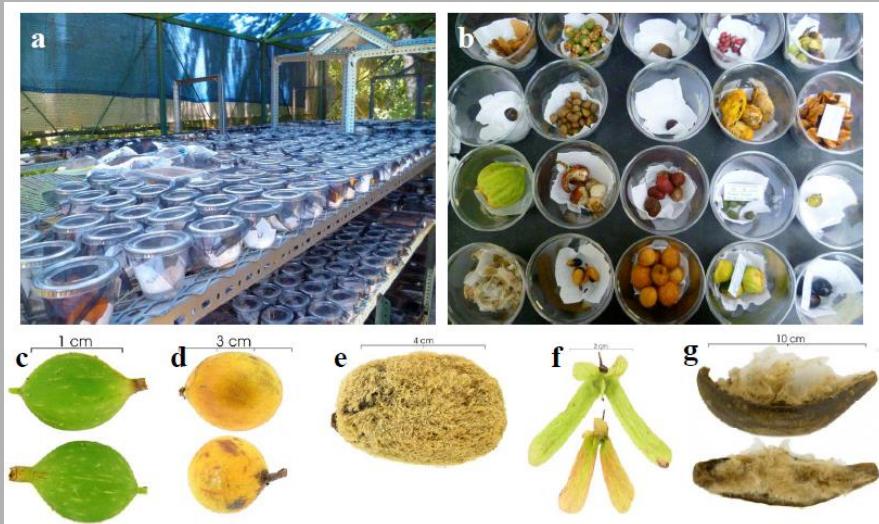
**Butterflies:** walking transects



# INTERACTION STUDIES

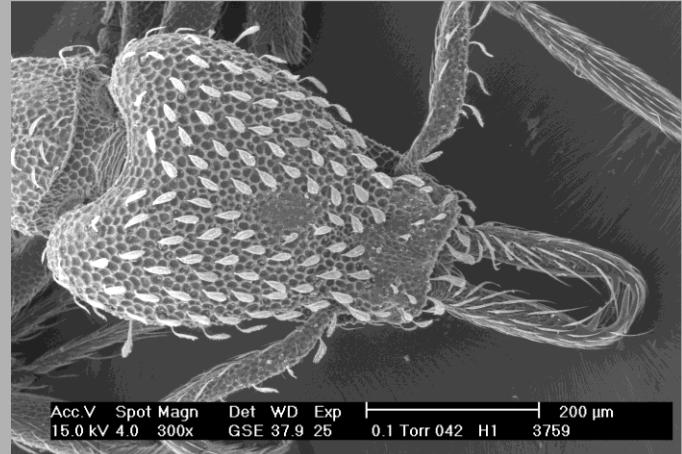
- KHC (2010): Effects of litter composition on ants
- BCI (2010), KHC (2013), WAN (2013):

Insect seed predation: quantitative food webs



# TAXONOMY

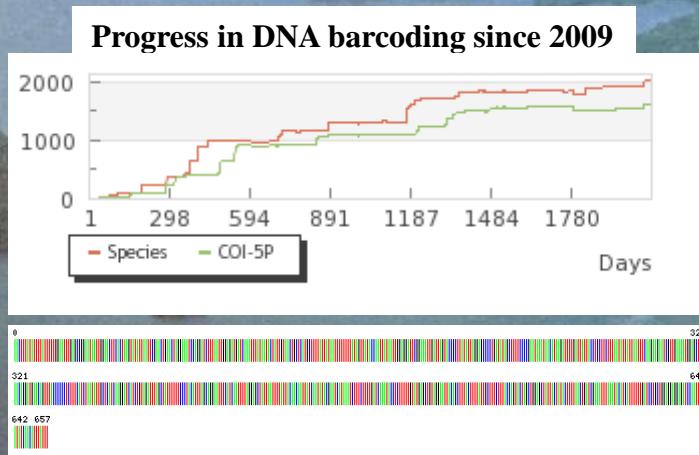
- Local reference collections often managed by “parataxonomists”
- DNA barcoding
- Collaborating experts (in-country or abroad)



**But taxonomic impediment high in the tropics!**

# Example: BARRO COLORADO ISLAND

## PANAMA (BCI)



## STATUS

**2008: baseline survey**

**2009-2015: on-going monitoring (7th year)**

**Collections: 36,388 pinned specimens; 1,864 spp.**

**68% of spp. with Barcode Index Numbers (BINs)**

**Four full-time research assistants, based at STRI**

## MAIN USE OF DNA BARCODING

- Used to refine delineation of insect morphospecies and identifications of common, abundant species, likely to be tractable in the long-term
- Currently > 10,000 sequences:  
**Panama (7,414), Thailand (1,053), Papua New Guinea (1,567)**
- Some of these areas have extensive barcode libraries  
(PNG 25,000 barcodes for Lepidoptera), others not (Thailand):  
**approach for barcoding needs to be different for each site**



# INTEREST OF DNA BARCODING

- Refinement of reference collections

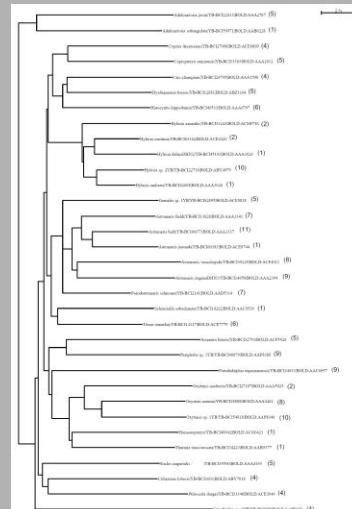
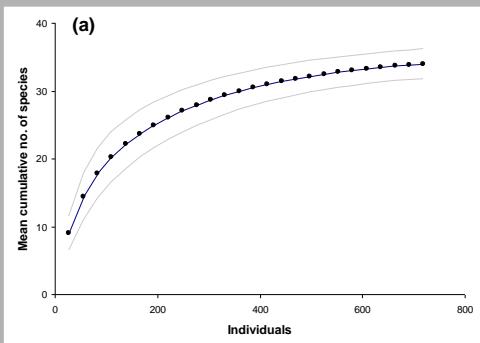
BCI: Arctiinae, Geometridae, etc: best collections in Panama



- Baseline and ecological surveys (Guanacaste: Janzen *et al.*)

Of interest when monitoring protocols are similar to the most efficient

collecting methods for focal taxa. For example: Saturniidae collected at light on BCI:



Representative tree



Documentation of local fauna

# INTEREST OF DNA BARCODING

DNA barcoding allows:

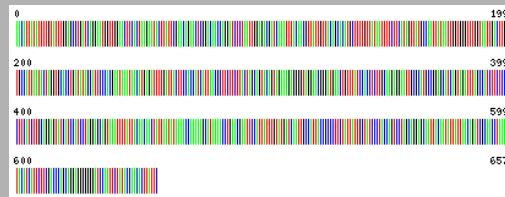
- Matching sexes in case of sexual dimorphism
- Identification of caterpillars, pupae and reared parasitoids
- Matching casts of social insects (sometimes across different protocols)

*Cornitermes walkeri*

BOLD:AAP9751



Soldier,  
termite transect

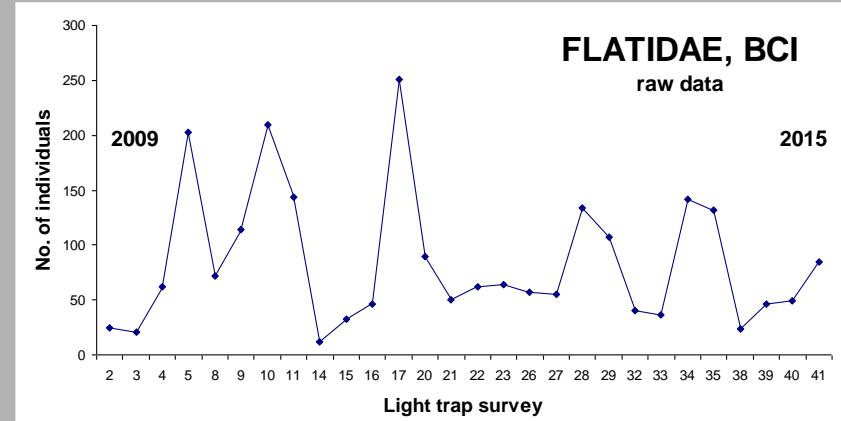
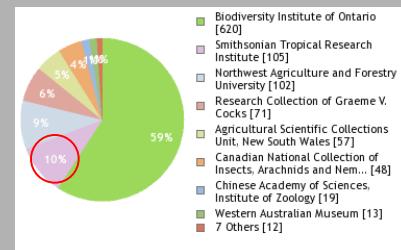
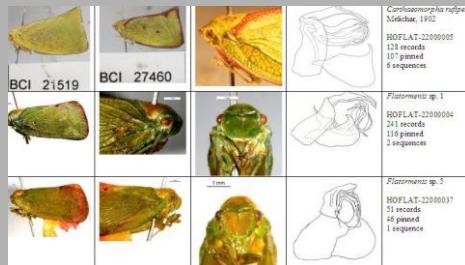
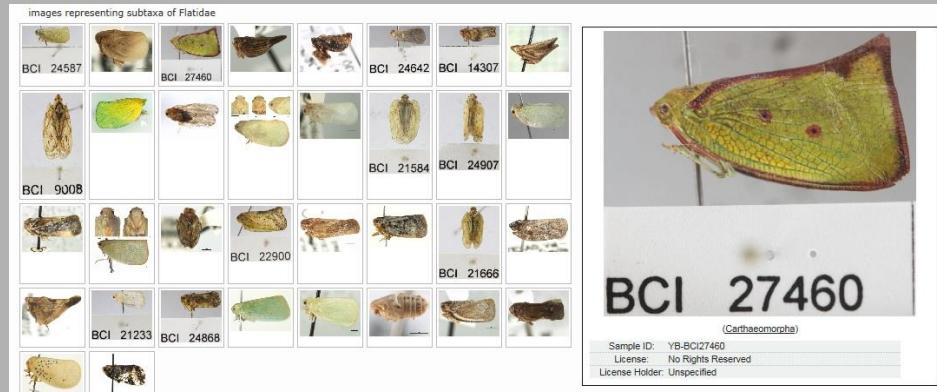


Alate,  
light trap

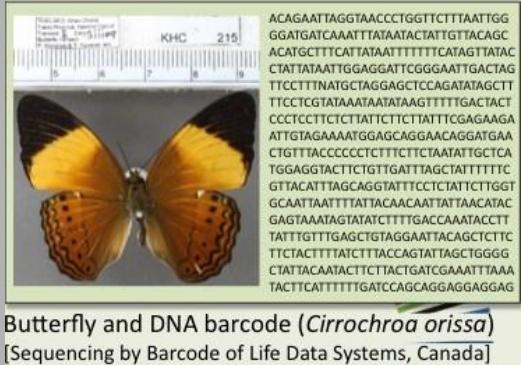
# INTEREST OF DNA BARCODING

DNA barcoding allows monitoring functional groups important to the forest ecosystem but not well known taxonomically

Flatidae (sap-sucking bugs): 10% of BOLD sequences from BCI



# RESULTS: SELECTED EXAMPLES



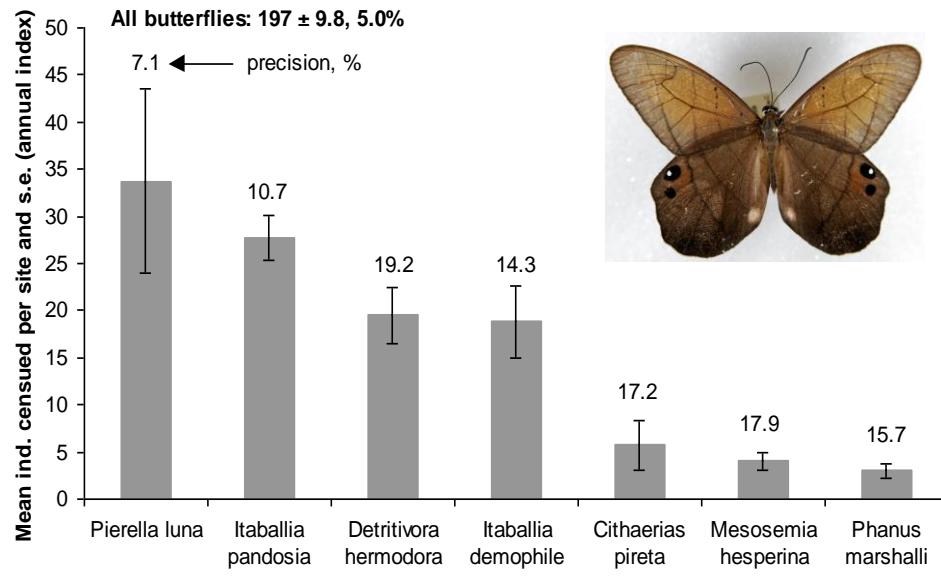
- Yearly results: annual indices (butterflies & ants)
- Population dynamics (saturniid moths and butterflies)
- Yearly changes in assemblages (several taxa)

Immediate significance

vs.

Interpretation of long chronosequences

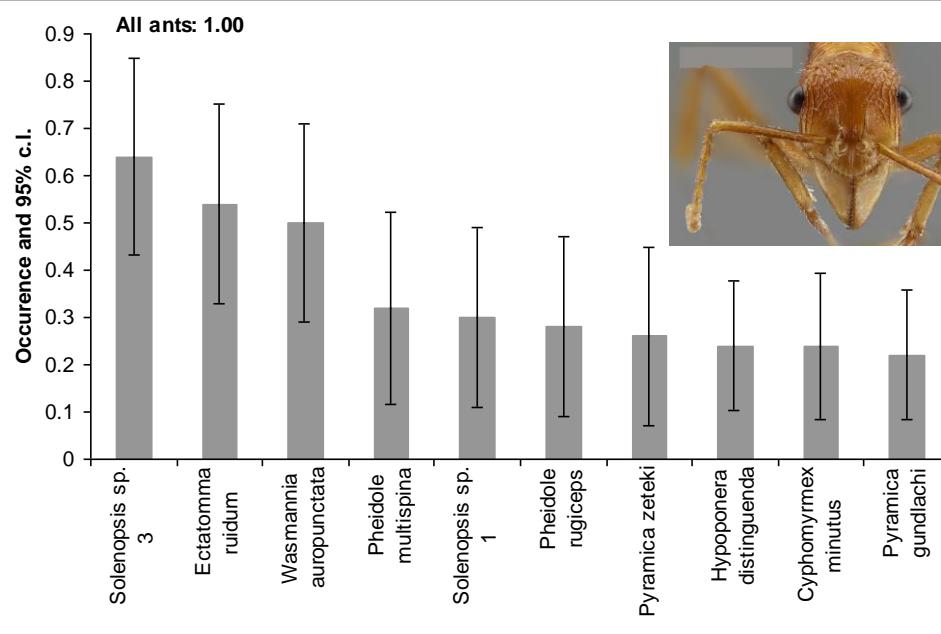
# Annual indices, BCI, Year 2011



60,000 insects collected:  
17,000 focal individuals (910 spp.)

For 56 spp. we can estimate annual indices with good precision

56 spp. = 6% of total spp. but  
55% of total abundance of focal taxa



Annual indices:  
Non-social insects: mean per site (n=10)  
Precision = s.e./mean  
(< 20% very good, economic entomology)

Social insects: occurrence in samples, transects or quadrats  
Precision = 95% c.l. on occurrence data, assuming a binomial distribution

## Annual indices

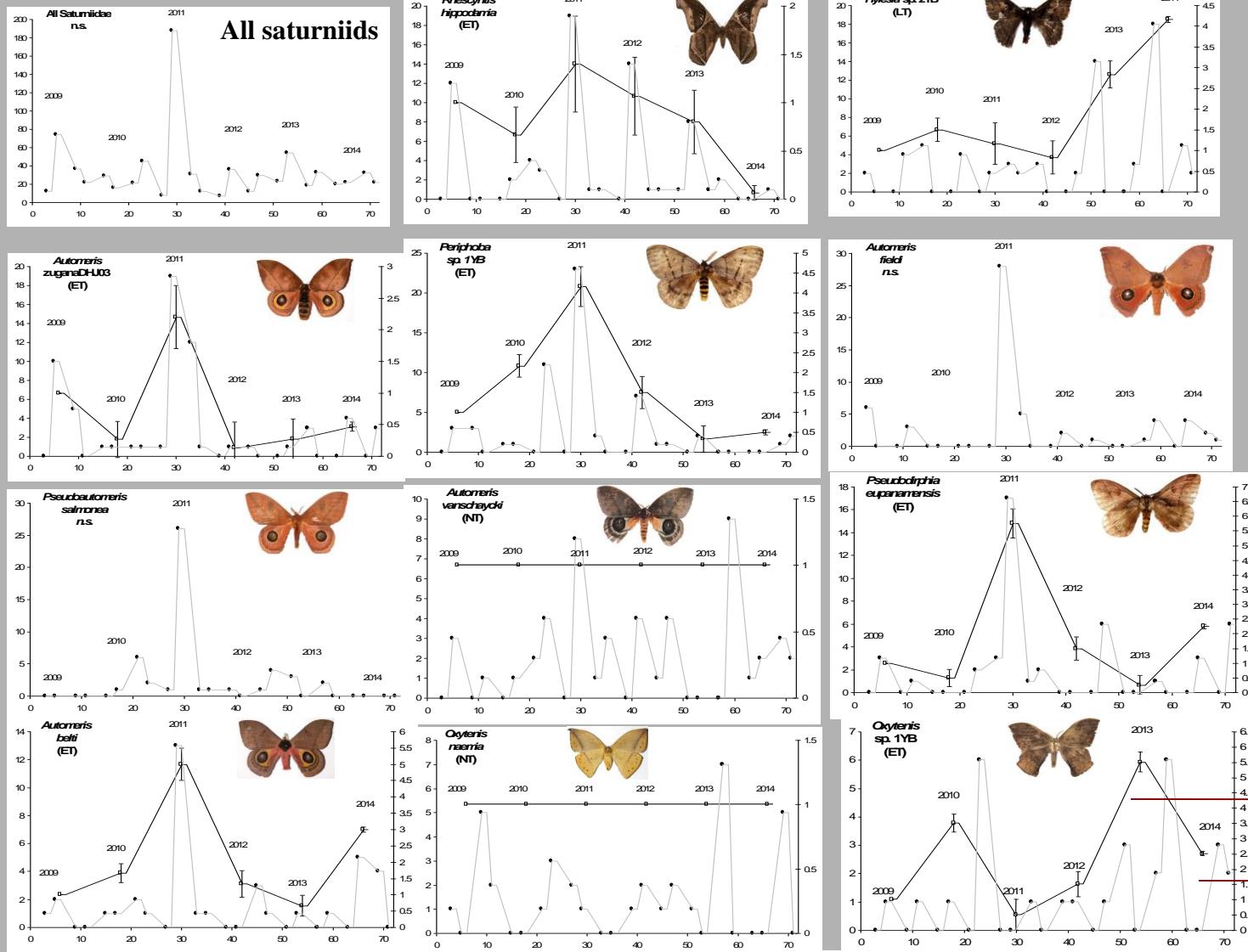
### Immediate significance:



- Common spp. can be monitored with relative precision, even in tropical rainforests
- Few long-term monitoring programs in the tropics  
(butterflies: 10-11 years: Leidner *et al.* 2010, Grøtan *et al.* 2012)
- Indices for social insects need to be reported differently than for non-social insects  
(refinements needed for social insects; geometric mean for non-social insects)
- BCI: all common spp. (with annual indices) have BINs

# Population dynamics, BCI, 2009-2014, saturniid moths

Number of individuals collected per survey



Months since January 2009

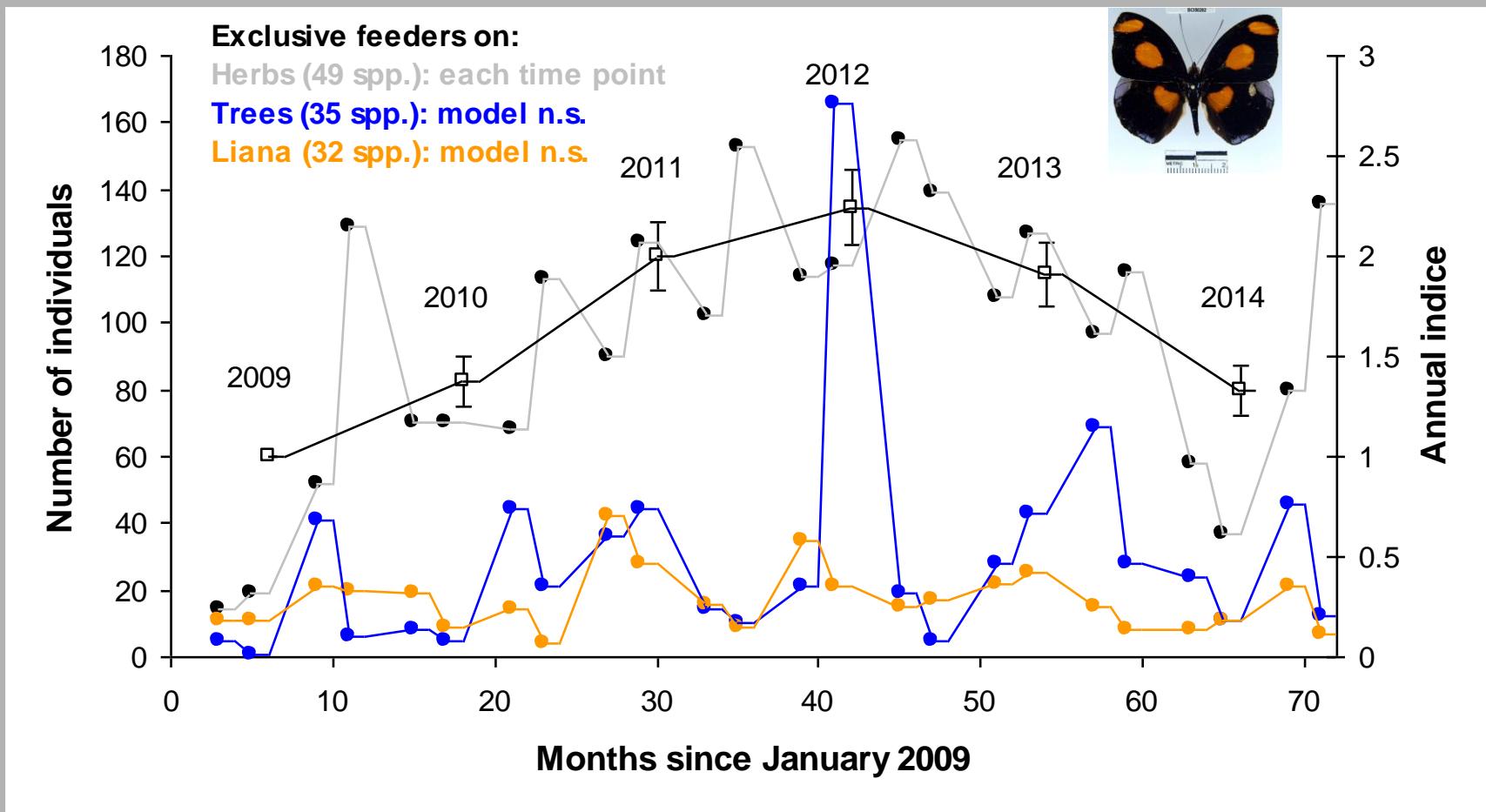
Time-series models (TRIM):  
 (1) No time effects  
 (2) Linear trend  
 (3) Effect for each time point  
 (separate parameters for each time-point)

Annual index

Model

Raw data

# Population dynamics, BCI, 2009-2014, butterflies

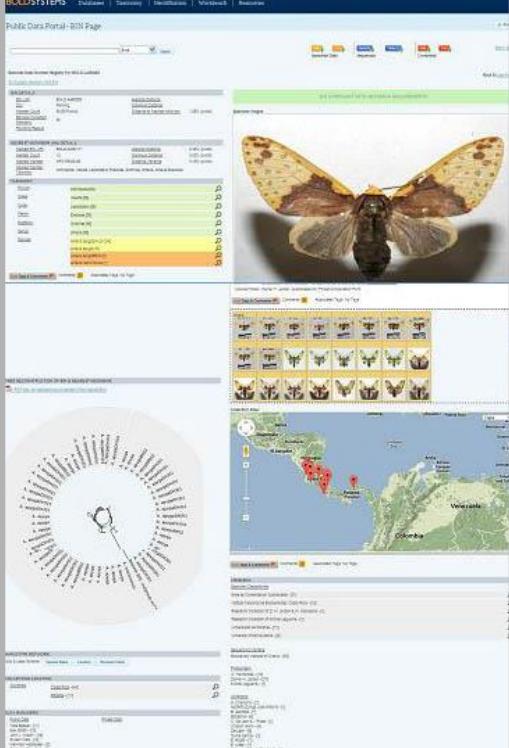


Different feeding guilds, moderate increase for herb feeders since 2009 ( $p<0.01$ )

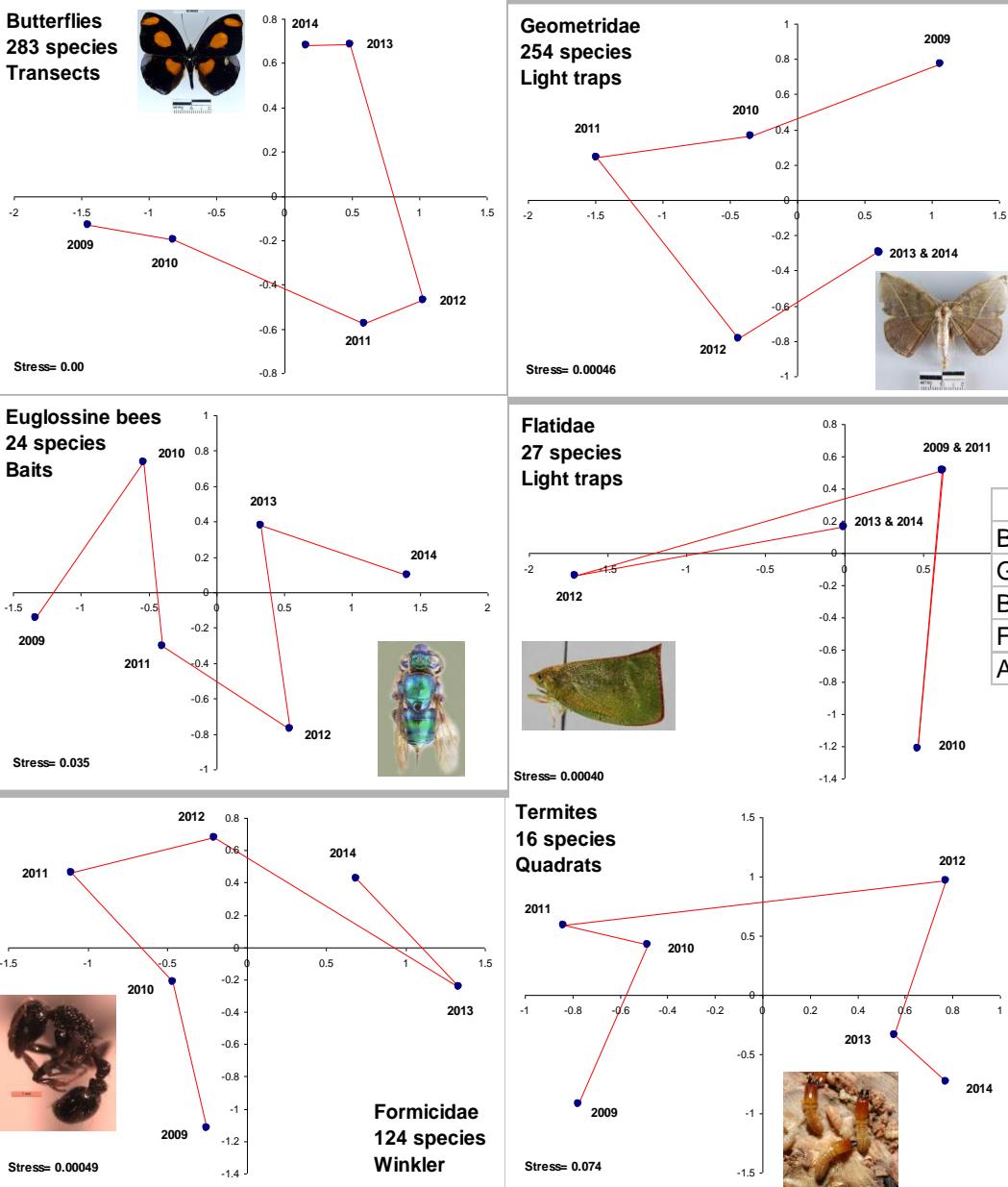
# Population dynamics

## Immediate significance:

- We can detect significant (short-term) trends
- Nearly a quarter of species show significant changes with time (different groups tested)
- Population dynamics may depend on the ecology of species
- Species identity is crucial, therefore BINs are essential



# Changes in assemblages, BCI, 2009-2014



Matrices Spp. x Years

NDMS

Different protocols, different taxa

6 out of 16 assemblages

(different guilds)

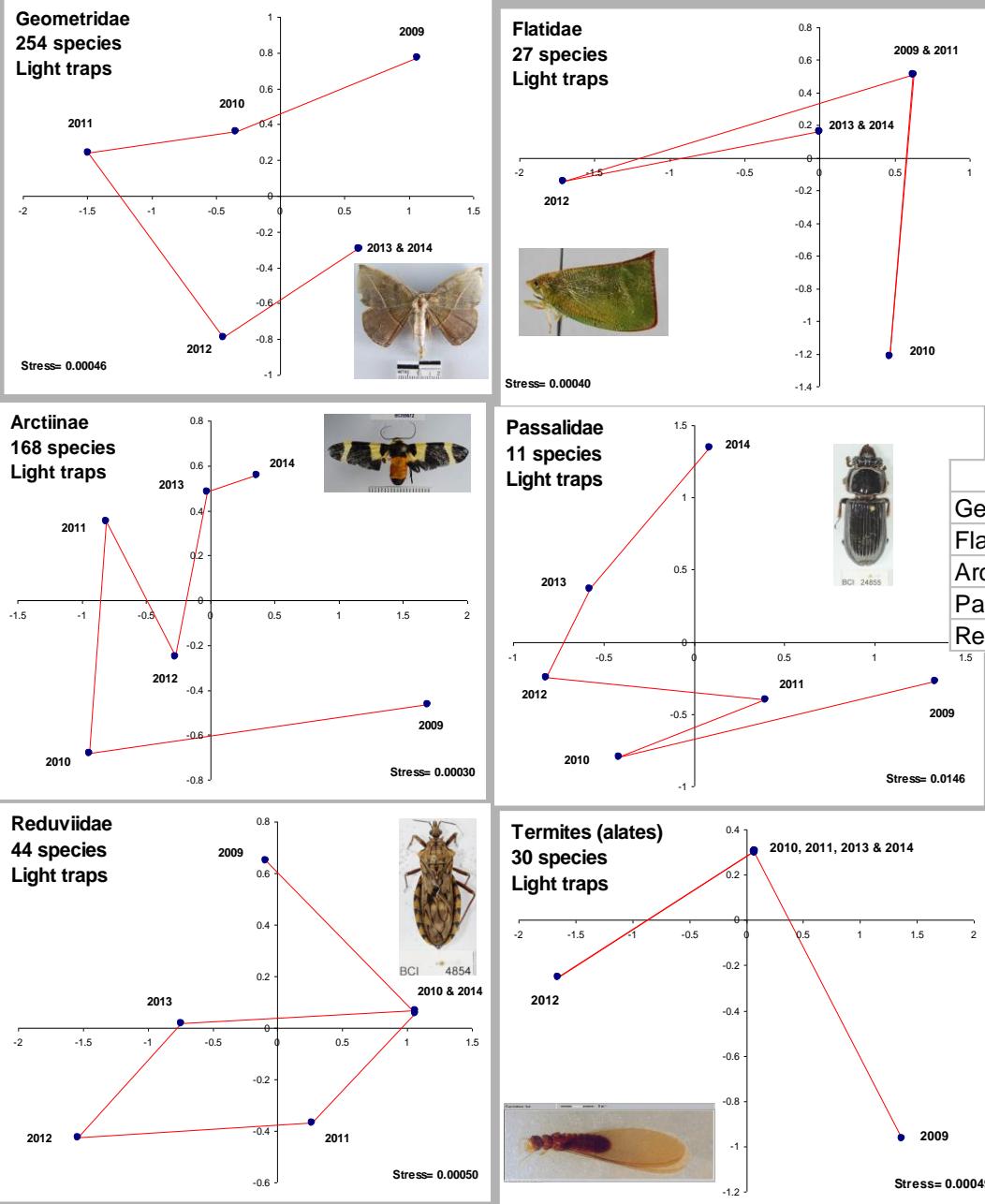
Mantel tests, p-values:

	Geometridae	Bees	Flatidae	Ants	Termites
Butterflies	0.152	0.482	0.815	0.044	0.617
Geometridae	***	0.240	0.593	0.432	0.043
Bees		***	0.094	0.987	0.221
Flatidae			***	0.989	0.210
Ants				***	0.849

Trajectories are mostly independent

Directional changes are few

# Changes in assemblages, BCI, 2009-2014



Matrices Spp. x Years

NDMS

Same protocol (light trap), different taxa  
6 out of 12 taxa (different guilds)

Mantel tests, p-values:

	Flatidae	Arctiinae	Passalidae	Reduviidae	Termites
Geometridae	0.593	0.834	0.320	0.135	0.151
Flatidae	***	0.225	0.981	0.635	0.875
Arctiinae		***	0.665	0.624	0.189
Passalidae			***	0.320	0.306
Reduviidae				***	0.103

Trajectories are independent

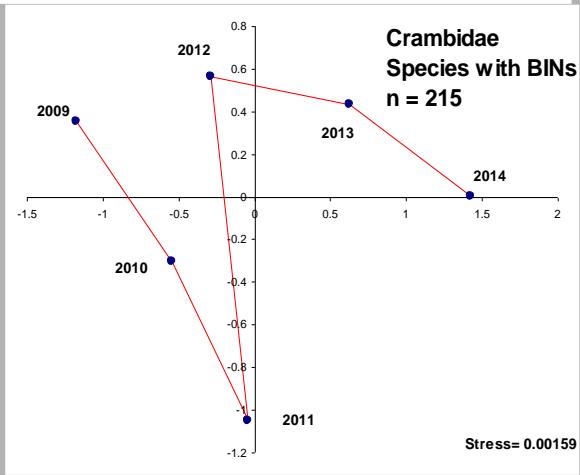
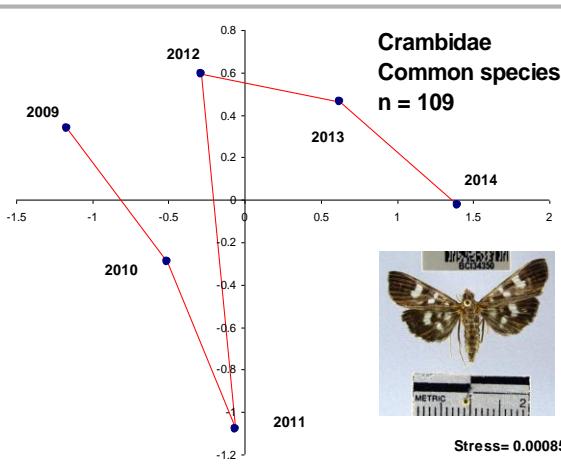
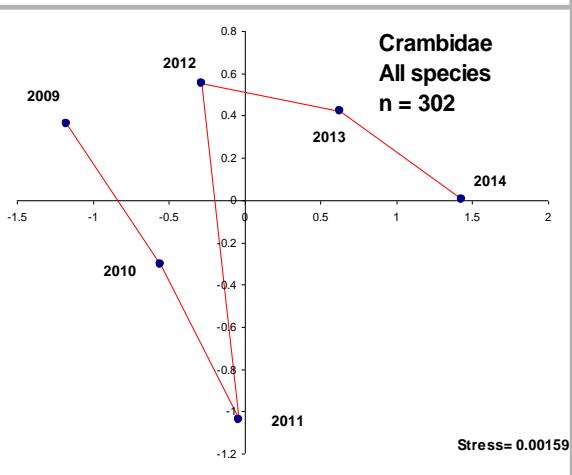
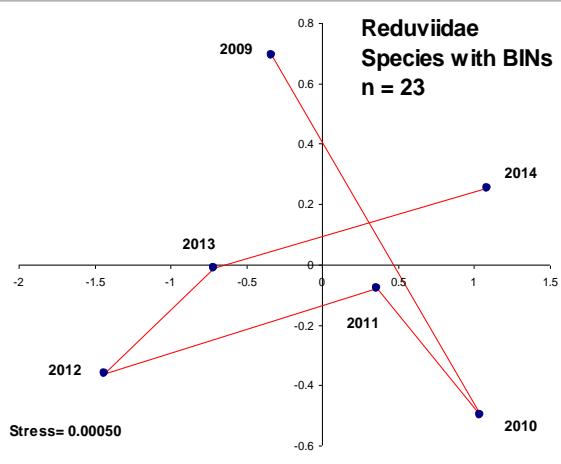
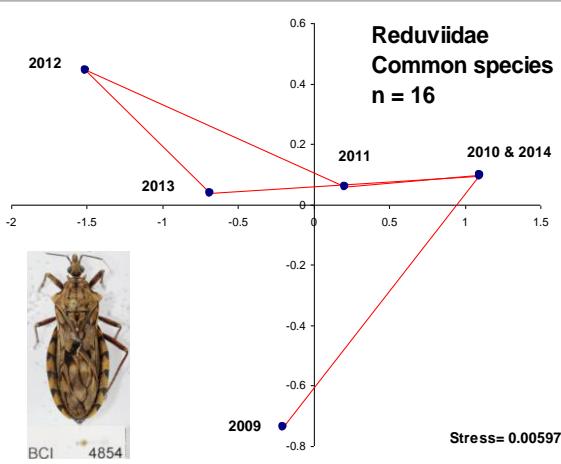
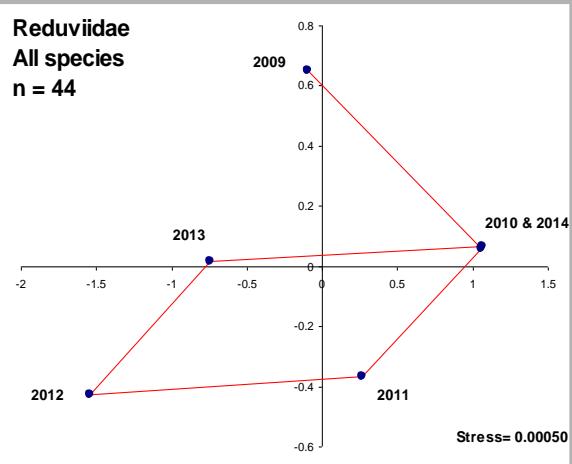
Directional changes are few

# Changes in assemblages, BCI, 2009-2014

All species:

Common species:

Species with BINs:



Matrices Spp. x Years

NDMS

Species-poor and -rich assemblages

Trajectories similar,  
especially for species-rich  
assemblages

Mantel tests, p-values:

	Common	BINs
Reduviidae, all	0.0012	0.0017
Reduviidae, common	***	0.0013
	Common	BINs
Crambidae, all	0.0017	0.0016
Crambidae, common	***	0.0014

## Changes in assemblages

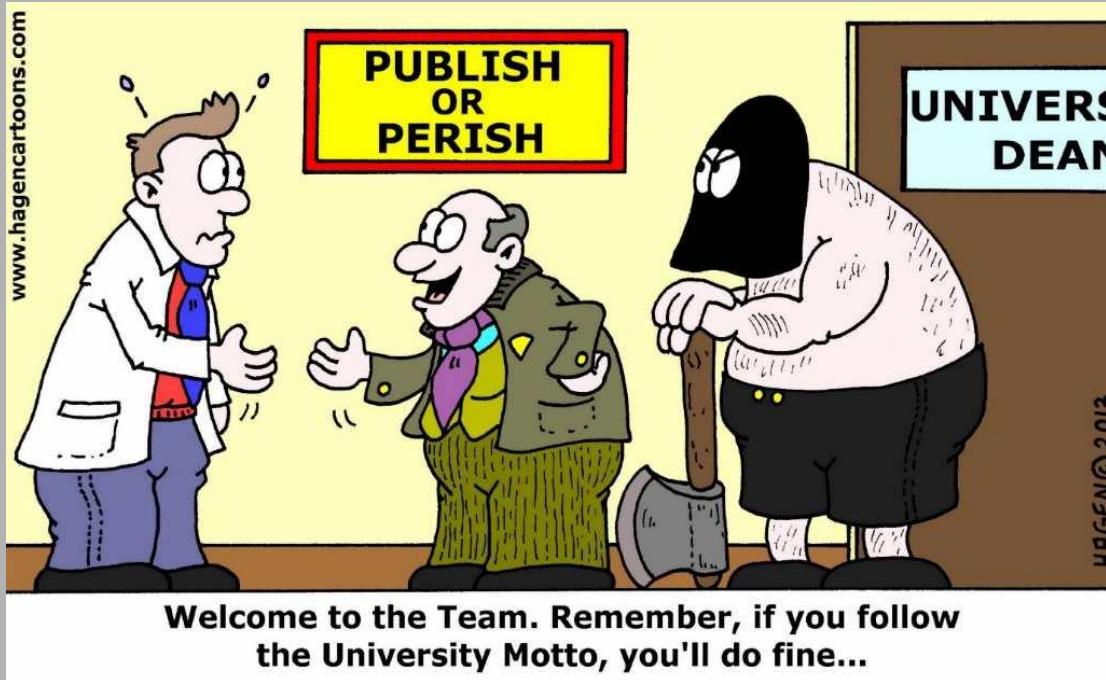
### Immediate significance:



- Trajectories appear largely independent, even when considering a particular protocol: **need to monitor an array of taxa**
- Directional changes appear also to be few
- Species-rich and poorly known assemblages: **their yearly changes may be estimated with common species, especially if they have BINs**

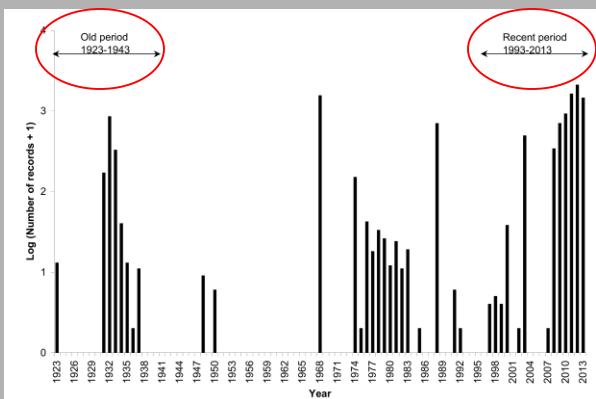
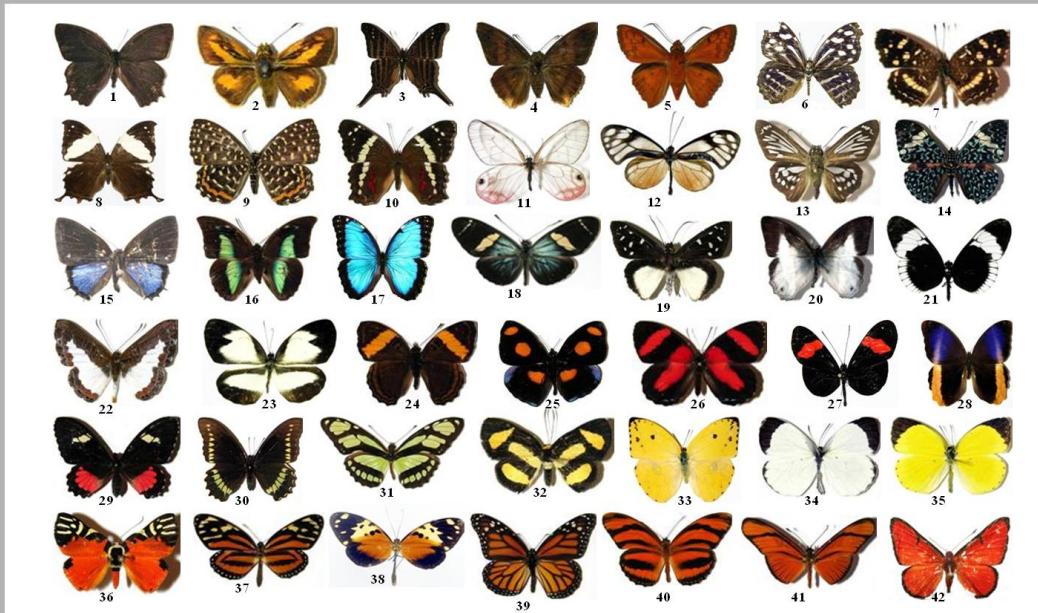
## Challenge: publish or perish

- Challenge: initial wait for quality data may be long (long chronosequences)
- Remedy: (a) comparison of insect data with other sites  
(b) faunistical surveys (if reasonably complete)  
(c) mine barcoding data; data release  
(d) mine historical data



## Example: mining historical data

# The butterflies of Barro Colorado Island, Panama: local extinction since the 1930's



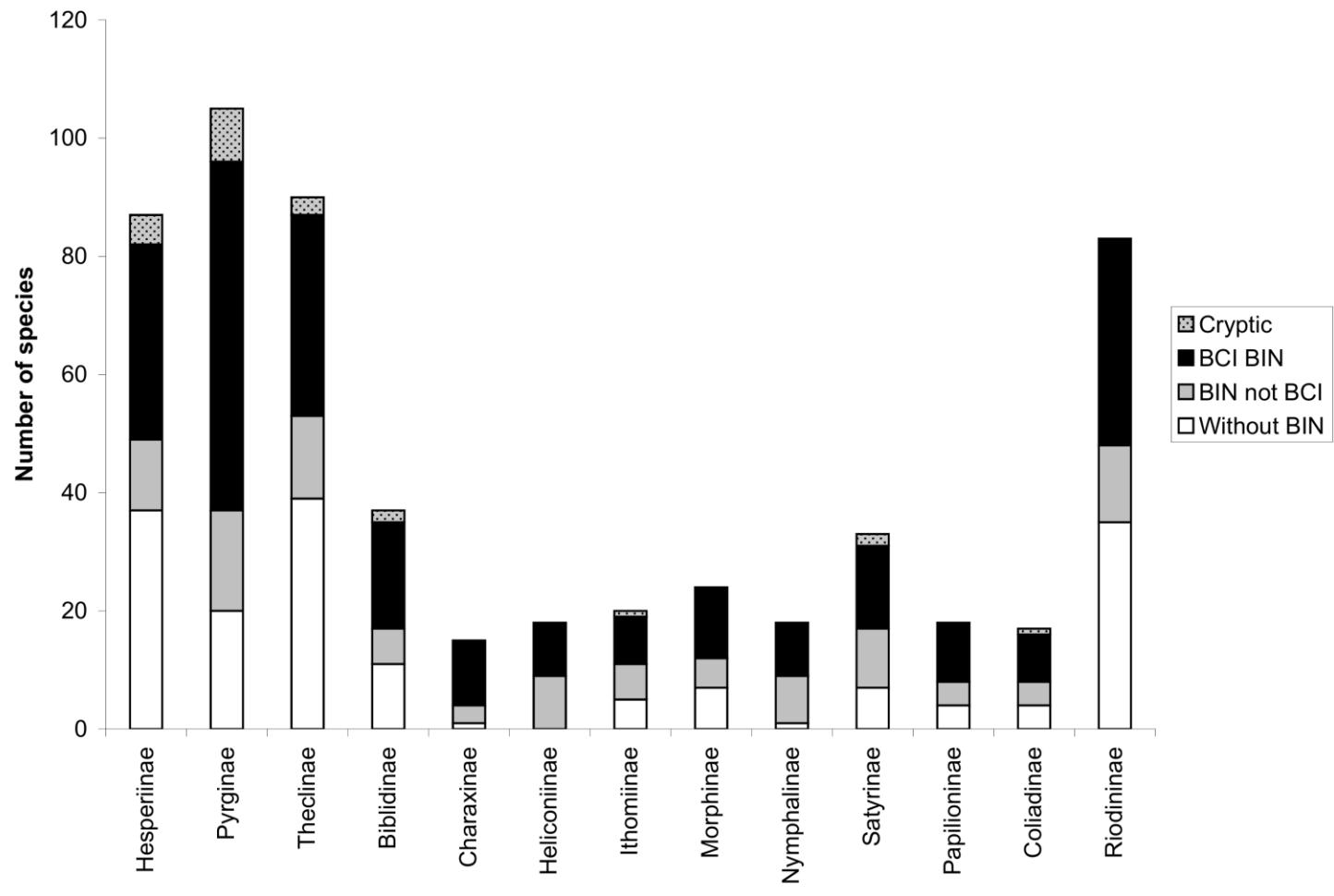
**Compilation of butterfly records on BCI**  
**1923-2013: 601 species**  
**Old period: 1923-1943, 267 species (Huntington, 1932)**  
**Recent period: 1993-2013, 373 species**

## Barro Colorado Island history:

- ca 1880: 45% old growth forest, rest shifting agriculture
- ca 1910: became a 1542 ha island after damming the Chagres river, to create the Panama canal
- ca 1920-1923: 96.7% forested, rest clearing and small agricultural areas
- today: 100% forested

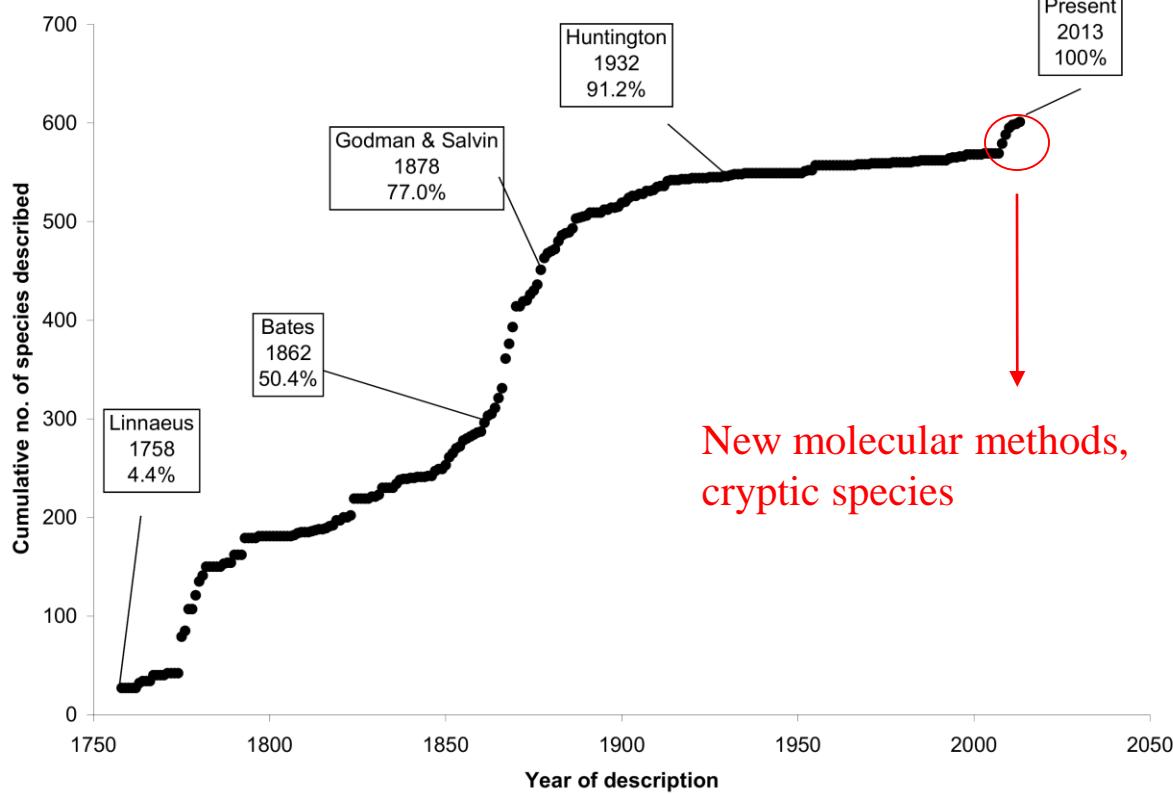
Main vegetational changes: fragmentation/disparition of herbs/crops





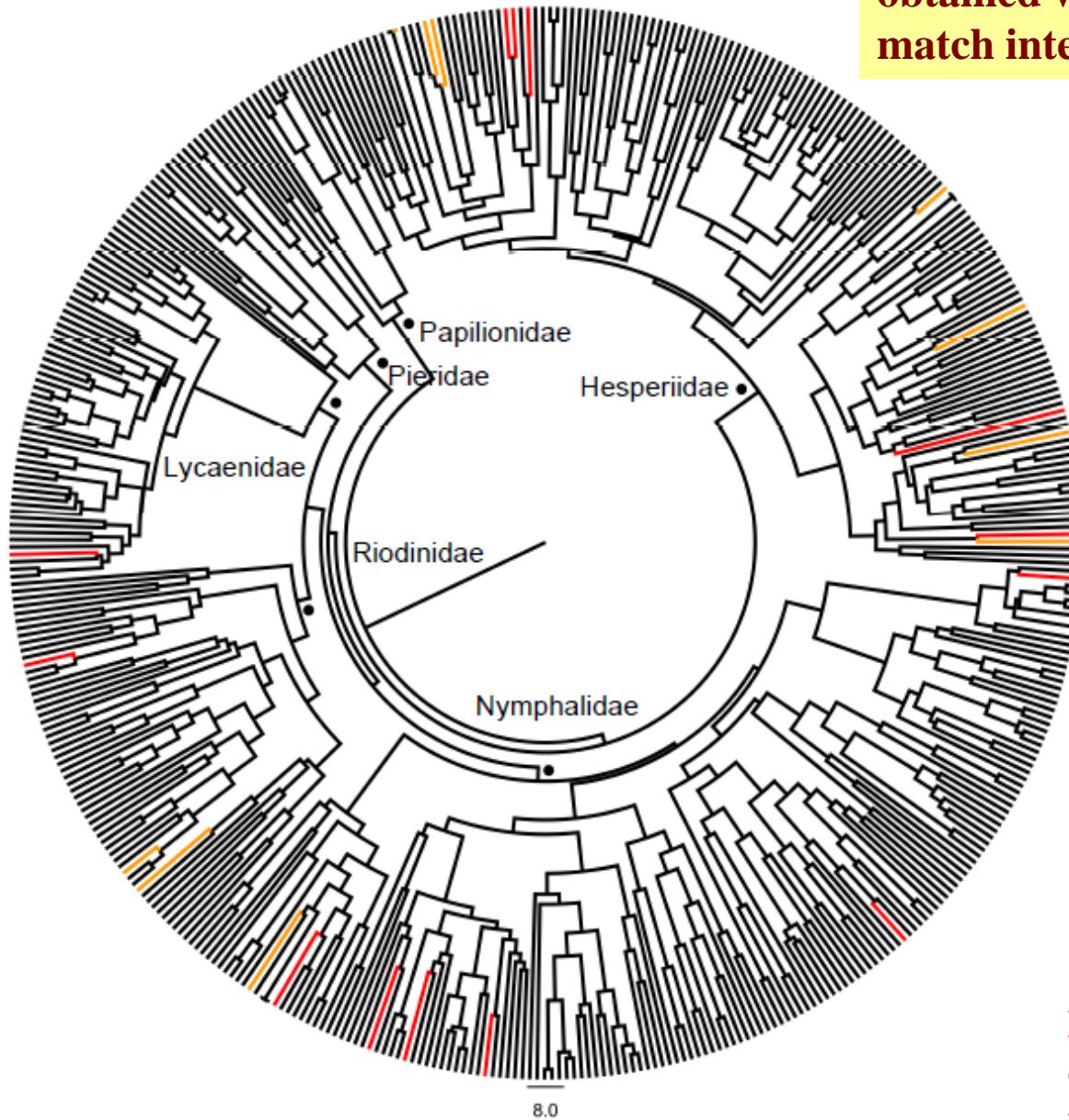
Actualized list with BINs  
BINs for species collected only  
during the old period mined  
elsewhere from BCI

## Historical knowledge of BCI butterflies



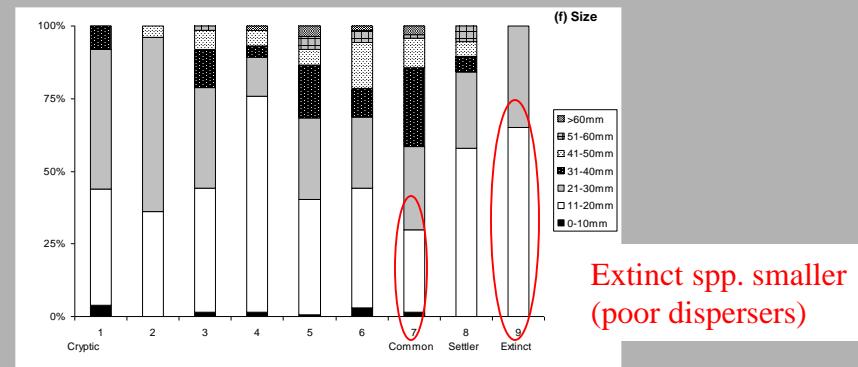
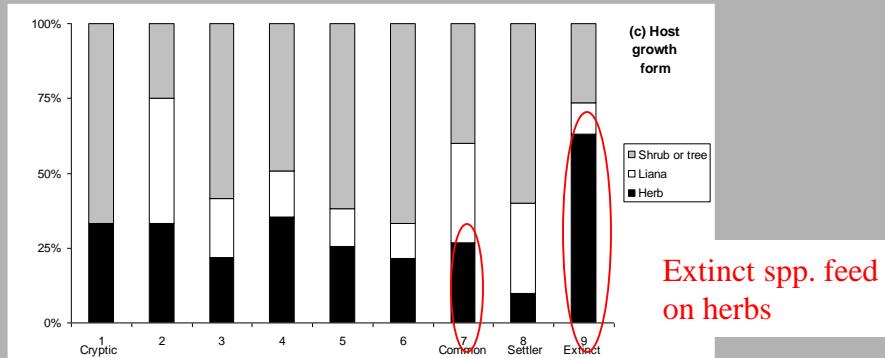
Comparison of old vs recent data: sampling effort old << recent:  
23 abundant species were not recorded during the recent period  
We cannot discount misidentification of species but comparison  
of BINs reduces (but do not avoid) this possibility

**Phylogenetic tree for BCI butterflies,  
obtained with barcodes and constrained to  
match inter-familial relationships**



**Extinct species (in red or orange)  
do not cluster within the tree:  
phylogenetic signal weak**

Host growth form and wing size were significant predictors of probability of extinction, independently of phylogenetic relations



The 23 missing species (6% of recent records) may be extinct locally  
Most likely candidates for extinction were small hesperiids feeding  
on herbs. Poor dispersers may find difficult to locate declining herbs

During the same period many more (50-60, 21%) bird species were lost  
from BCI → small reserves still effective for invertebrate conservation



Locally extinct

BOLD:AAE3730



BOLD:AAB0089

# The future of monitoring: meta-barcoding

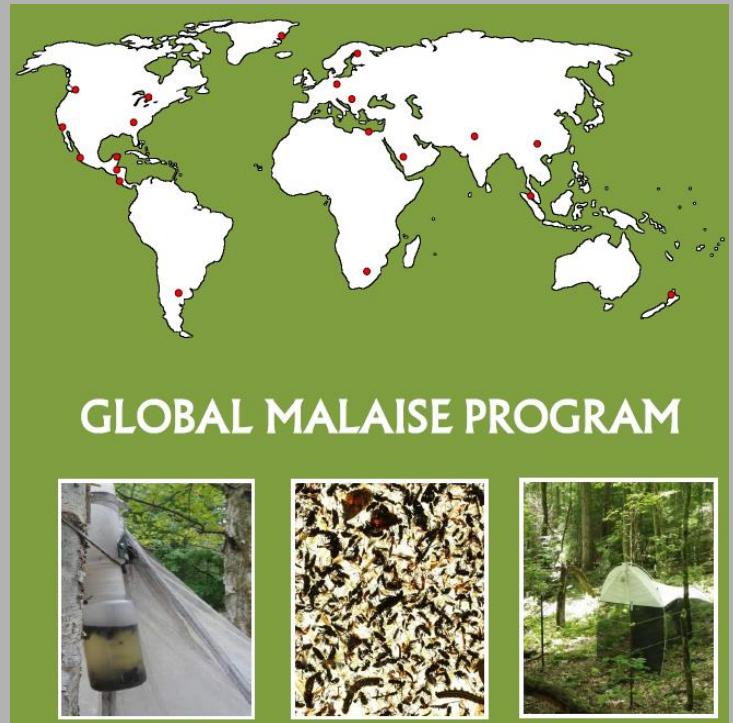
Example: Global Malaise Program (Biodiversity Institute of Ontario)

Abundance data better than presence-absence data for monitoring

Calibrations and test of the methods (with respect to seasonality) in species-rich tropical rainforests, where arthropod BINs are already available: ForestGEO sites...



BCI, Panama



## Conclusions

- Nascent mini-network focused on monitoring arthropods in tropical rainforests
- With adequate protocols (including DNA barcoding) common species of tropical insects can be precisely monitored in the long-term
- Because most insects have short generation times in the tropics, it may be possible to develop efficient warning systems that can yield results within 5-10 years (equivalent to ca. 40-80 insect generations)

## Invitation

- To join the mini-network  
(site; development of databasing and statistical tools)
- To collaborate to the analysis of monitoring and  
barcoding data  
(currently 0.6 M specimens, 6,000 species & 10,000 sequences)



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# Acknowledgments



**Biodiversity Institute of Ontario, Genome Canada,  
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Smithsonian Tropical Research Institute, Czech GACR,  
SENACYT, Universidad de Panama**

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W. Sackchoowong, P. Kongnoo, V. Novotny, G. Weiblen, C. Dahl,  
P. Hebert, A. Borisenco, R. Eastwood, D. Lohman, L. Sam,  
M. Leponce, D. Donoso, D. Roubik, S. Gripenberg, O. Lewis,  
S.J. Wright, T. Bonebrake, A. Nakamura