

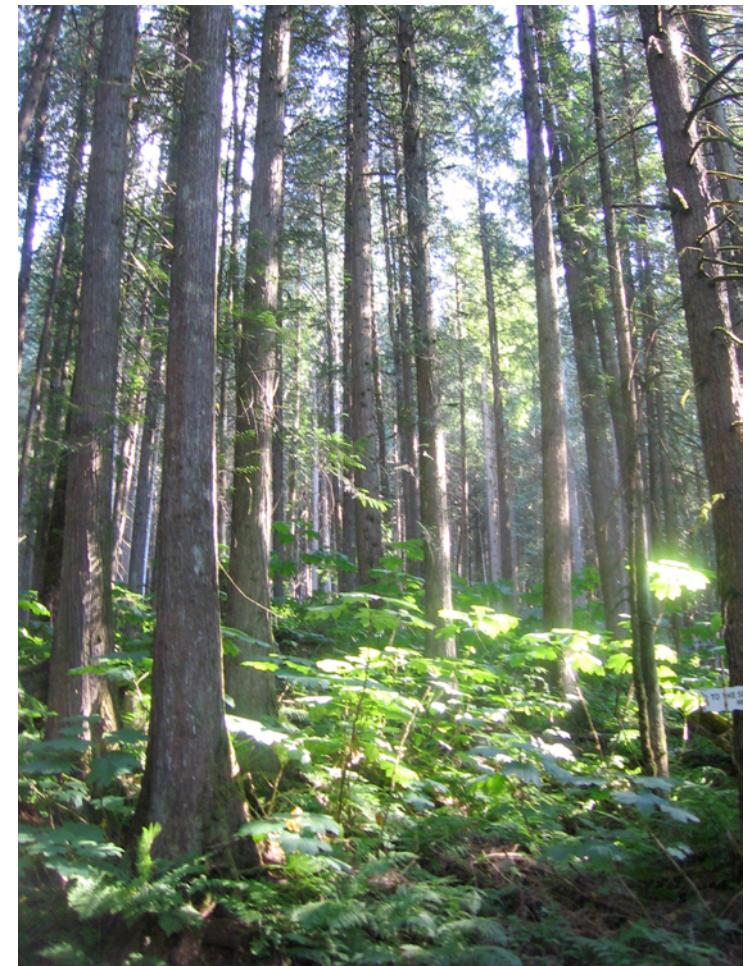


Forest biomonitoring, biosecurity and DNA barcoding

Jeremy deWaard and Leland Humble

DNAB and forest health

1. detecting non-indigenous species
2. conducting baseline inventories
3. measuring effects of disturbance on diversity



1) Non-indigenous species (NIS)

Table 4. Effects of nonindigenous plant pathogens and insects
(after Office of Technology and Assessment 1993).

Organism	% harmful	% neutral	% beneficial
Plant pathogens ($n = 54$)	91	6	4
Insects ($n = 1059$)	35	33	31

(Allen & Humble 2002)



Significant forest insect pests introduced into Canada:
brown spruce longhorn beetle, European gypsy moth, and Asian long-horned beetle (NRC)

Detection of NIS

- inspectors have a difficult job
 - enormous diversity
 - all life stages
 - vectored organisms
- low identification rate
 - e.g. 12.3% of 559 arthropods identified
(Brockhoff et al. 2006)



Wood dunnage and wood packaging arriving by boat (CFS)

Monitoring for NIS

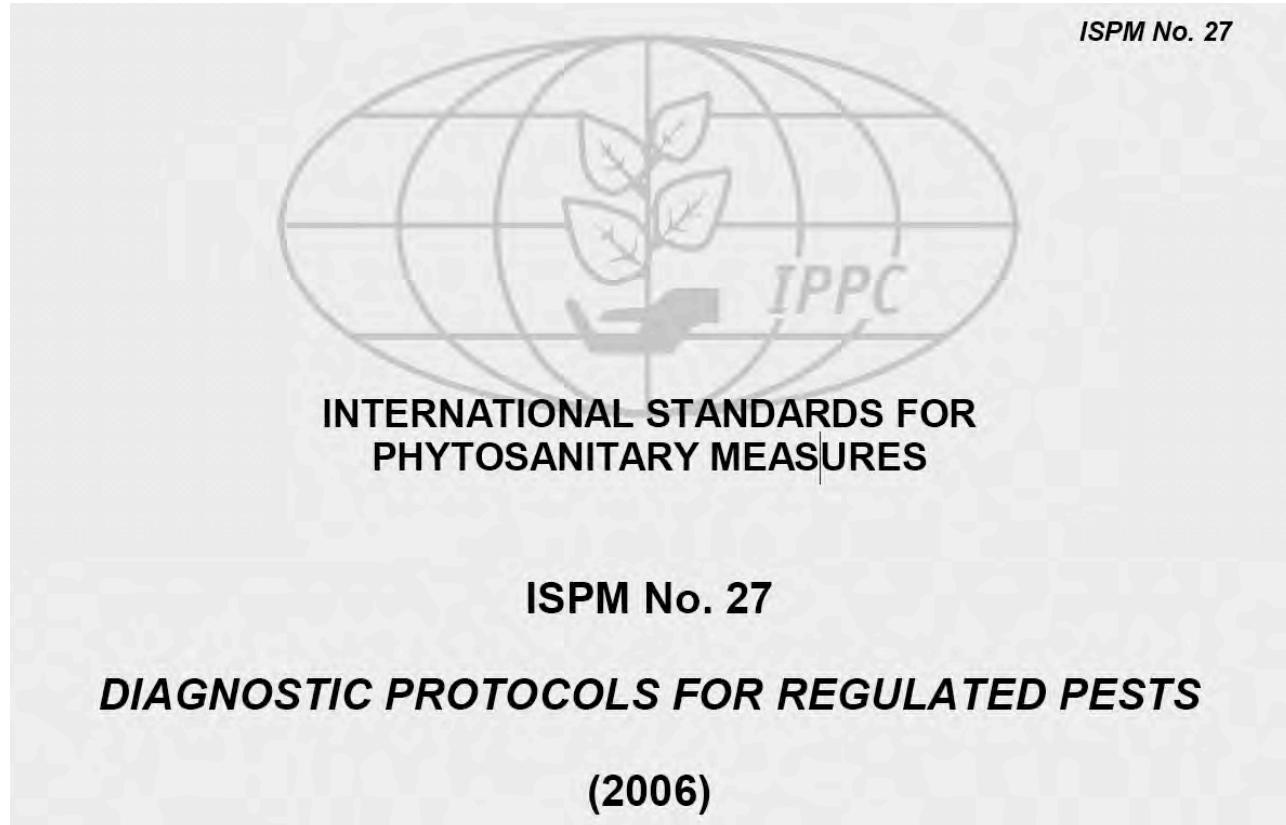
- monitoring for NIS also problematic
 - large samples needed to detect NIS at low density
 - immature life stages might be preferable if rearing could be avoided
 - often poor knowledge of native fauna
- * inadequate identification tools



pheromone trap
& sample (CFS)

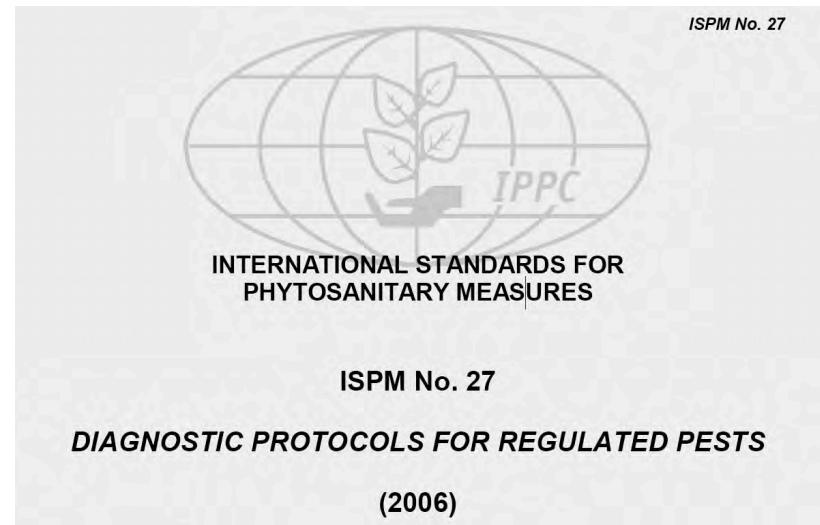
Detection & monitoring of NIS

- International Plant Protection Convention describes several molecular approaches in ISPM No. 27



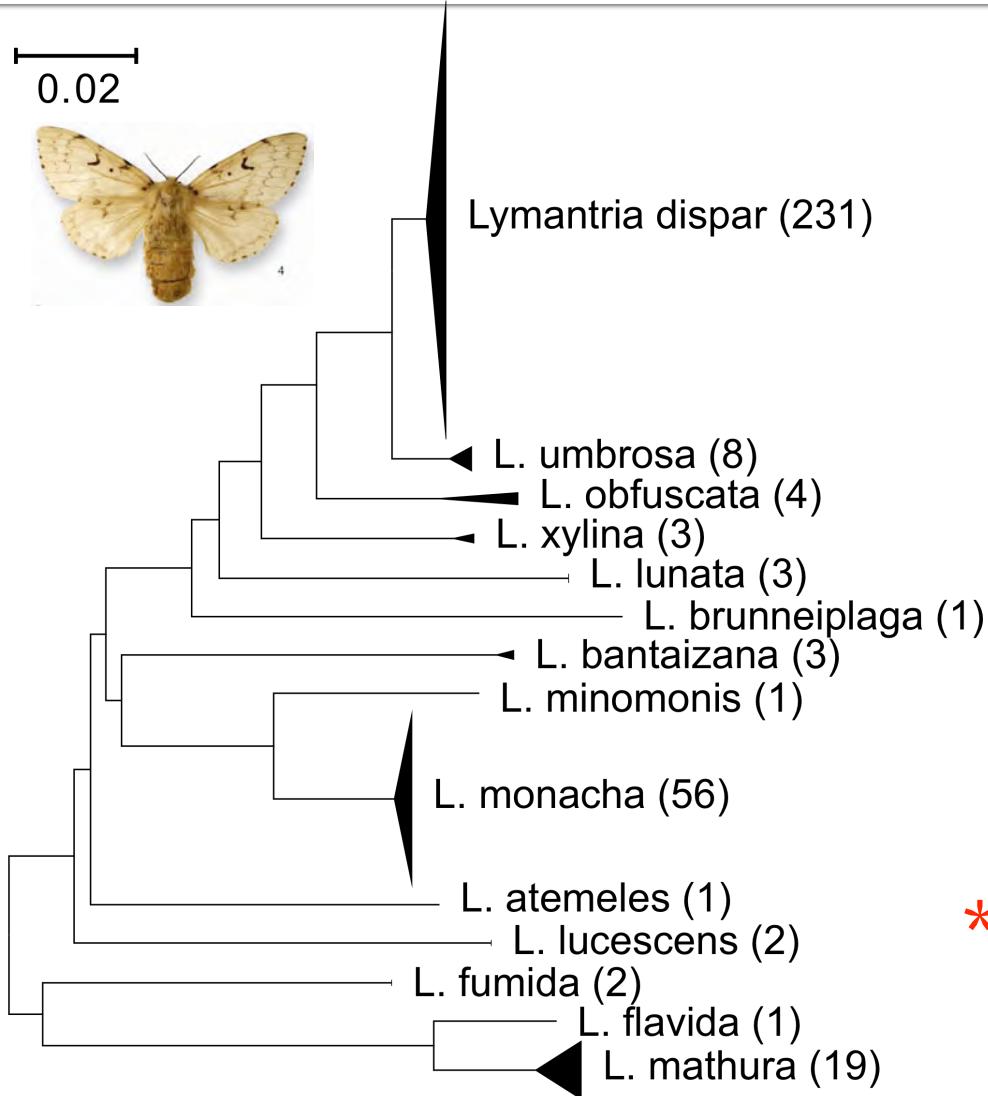
Detection & monitoring of NIS

- DNA barcoding (DNAB) recognized in ISPM No. 27
 - other molecular approaches ad hoc, taxonomically-limited
 - DNAB universal, yet meets and exceeds rigorous standards



Protocol: Floyd et al., *in revision*

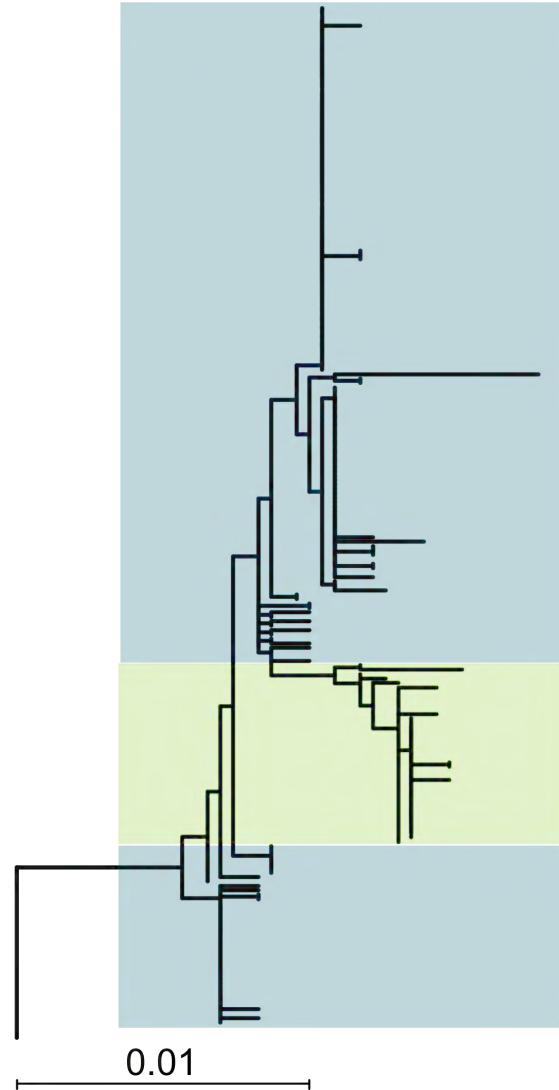
Case study 1: *Lymantria* spp.



- 335 sequences, 13 species of *Lymantria* tussuck moths
- previous studies (e.g. Ball and Armstrong 2006), field collections and regulatory interceptions

* can differentiate all spp.

Results: Gypsy moth (*L. dispar*)



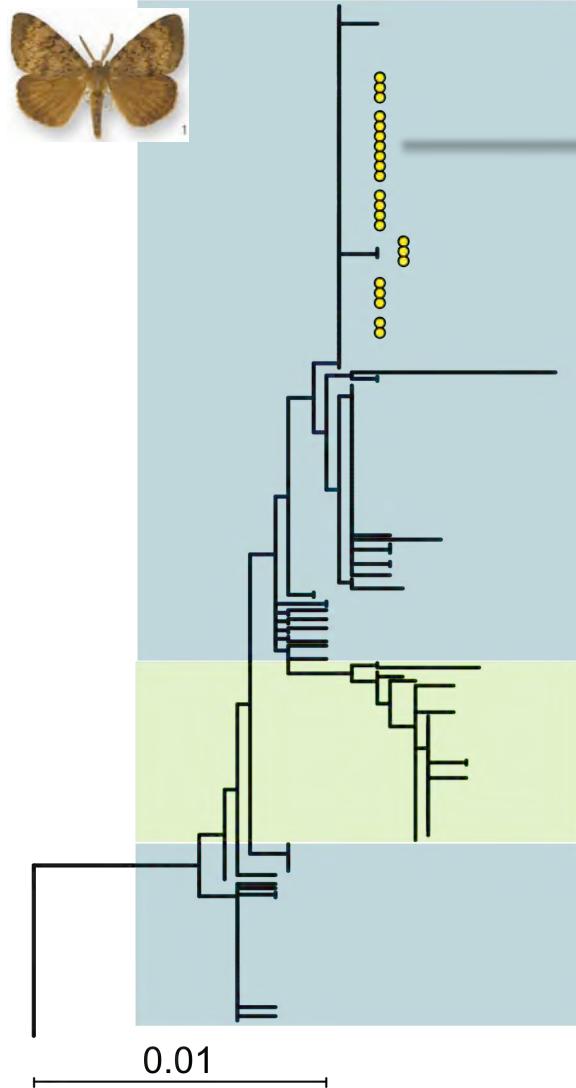
- two Asian subspecies form a monophyletic cluster
- female flight ability relates to dispersal and establishment capability

*L. dispar
asiatica /
japonica (41)*



Females of *L. dispar asiatica* & *japonica* (Pogue & Schaefer 2007)

Results: Gypsy moth surveillance



L. dispar
dispar (190)

- library evaluated using
30 surveillance specimens
 - conclusively diagnosed as European gypsy moth

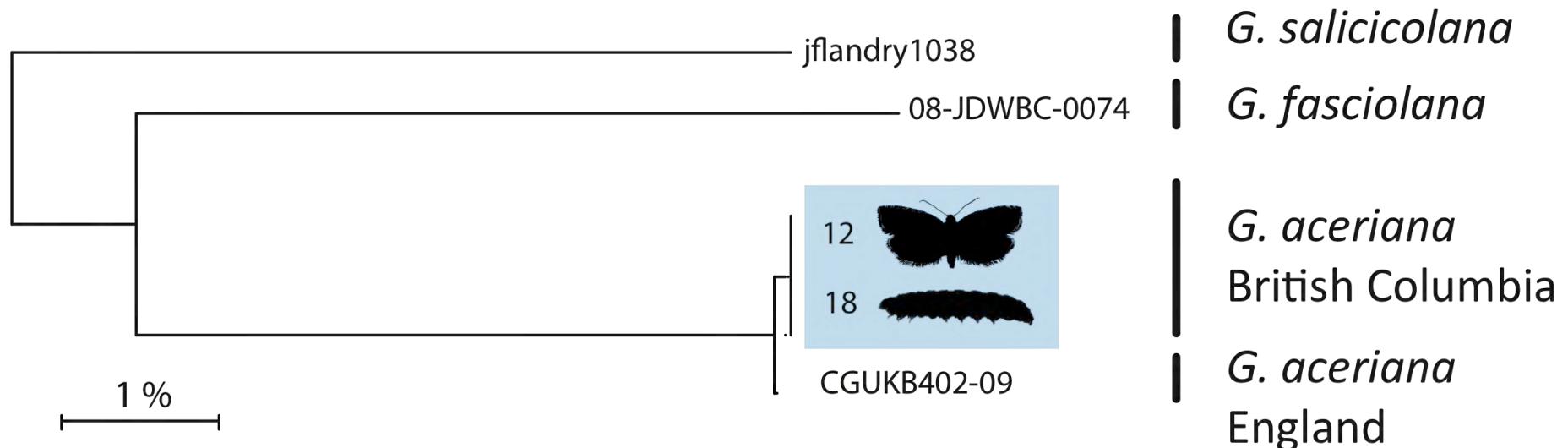
Case study 2: *Gypsonoma aceriana* (Duponchel)

- European Poplar shoot borer
 - reported in Washington State (LaGassa et al. 2001)
 - mine leaves and bore into shoots



adult male & damage (Humble et al. 2009, LaGassa et al. 2001)

Case study 2: *Gypsonoma aceriana* (Duponchel)



- 1981 specimens originally identified as 'probably *Epiblema* sp.'
- barcodes confirm long-term presence in BC, now established

2) Baseline inventories

- require substantial specialist time
 - sorting/prep time 40X longer than sampling time (Marshall et al. 1994)
 - identification time usually longer
- can integrate DNAB into both sorting (Caesar et al. 2006) and identification



unsorted trap sample (BIO)

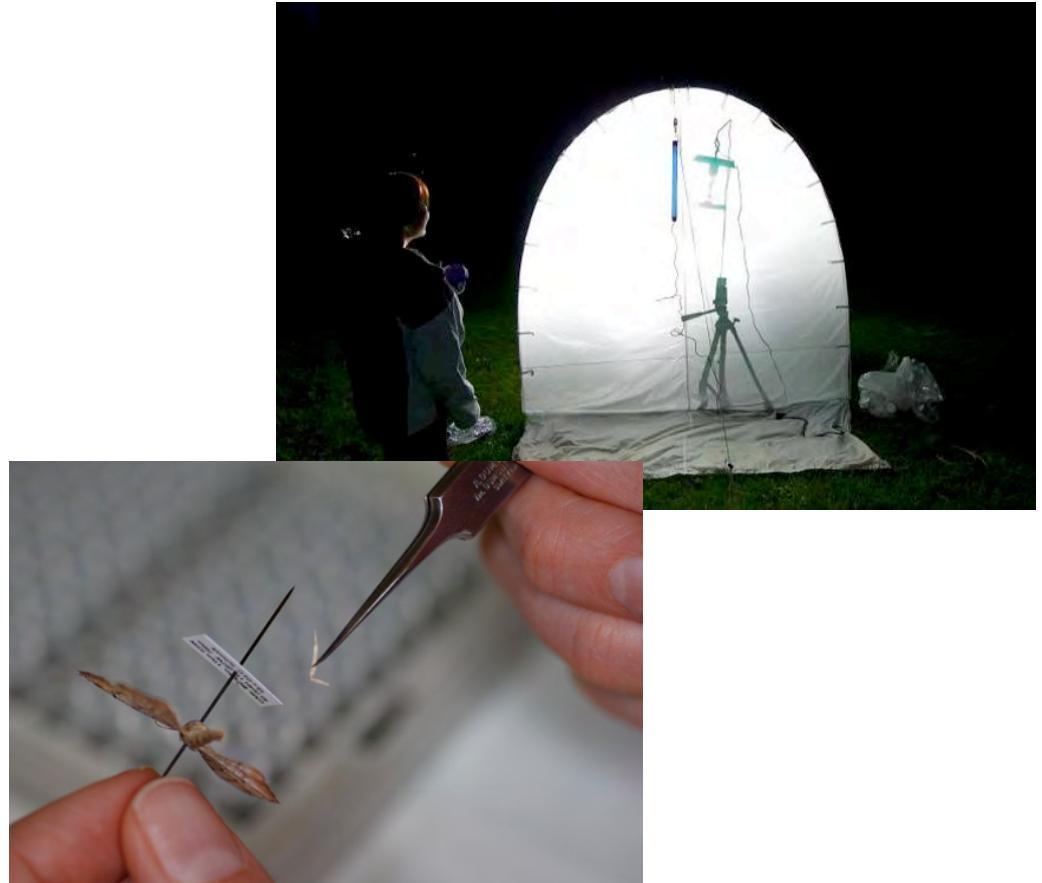
Case study 3: Stanley Park nocturnal Lepidoptera survey

- baseline inventory initiated
 - barcoding for rough sorting & tentative species assignment



Methods: collection & analysis

- MV light / sheet collecting
- synoptic collection (1-5 individuals per morphospecies) each night
- DNAB following standard procedures



Technician collecting moths at MV light sheet; sampling tissue (P. Colangelo, S. Kirk)

Methods: species assignment

- BOLD-ID for IDs (where possible)
- confirmed morphologically



http://www.barcodinglife.org – BOLD Systems – Identification Result

BOLDSYSTEMS | Management & Analysis

Specimen Identification Request

Search Request:
Type : Full Database Search

Search Result:
Identification Summary :

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Arthropoda	100
Class	Insecta	100
Order	Lepidoptera	100
Family	Geometridae	100
Genus	Campaea	100
Species	<i>Campaea perlata</i>	100

A species level match has been made. This identification is solid unless there is a very closely allied congeneric species that has not yet been analyzed. Such cases are rare.

Distance Summary :

Similarity scores of the top 100 matches

Tree Based Identification | Species Page

TOP 20 Matches :

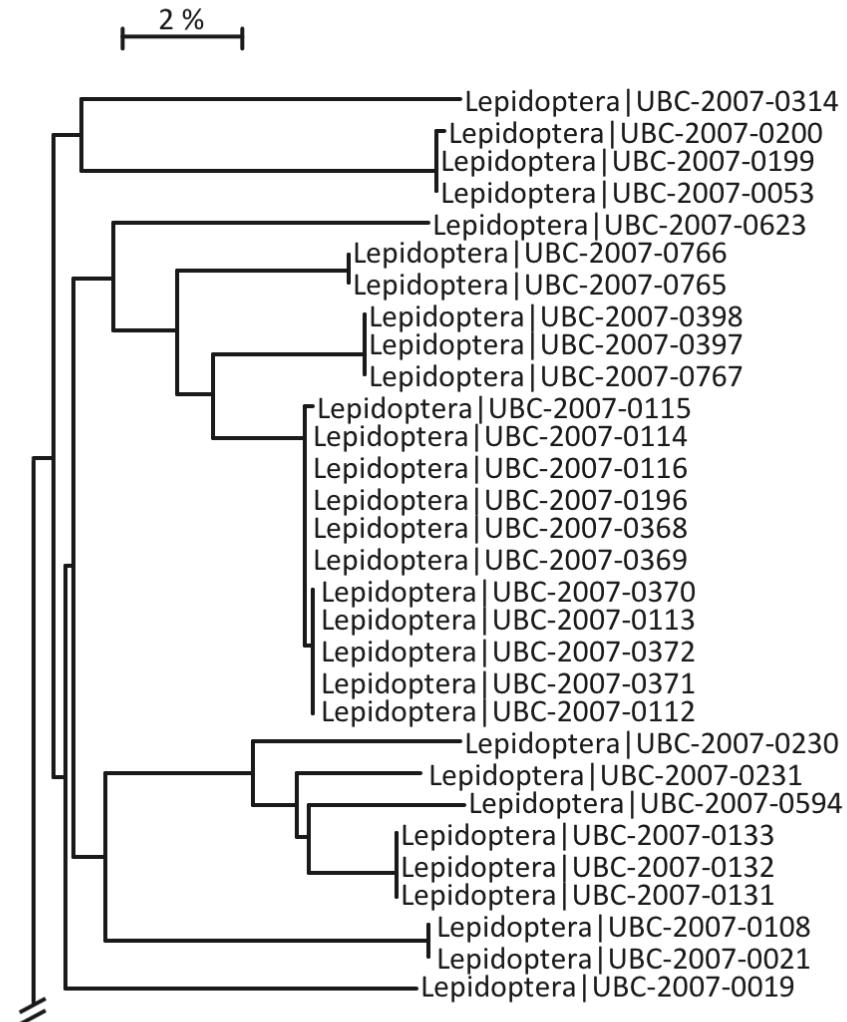
Phylum	Class	Order	Family	Genus	Species	Specimen Similarity (%)
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	100
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	100
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	100
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	99.84
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	99.84
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	99.69
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	99.69
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	99.61
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.89
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.78
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.78
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.78
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.77
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.74
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.7
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.69
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.68
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.67
Arthropoda	Insecta	Lepidoptera	Geometridae	Campaea	<i>perlata</i>	98.62

Display option: default ▾

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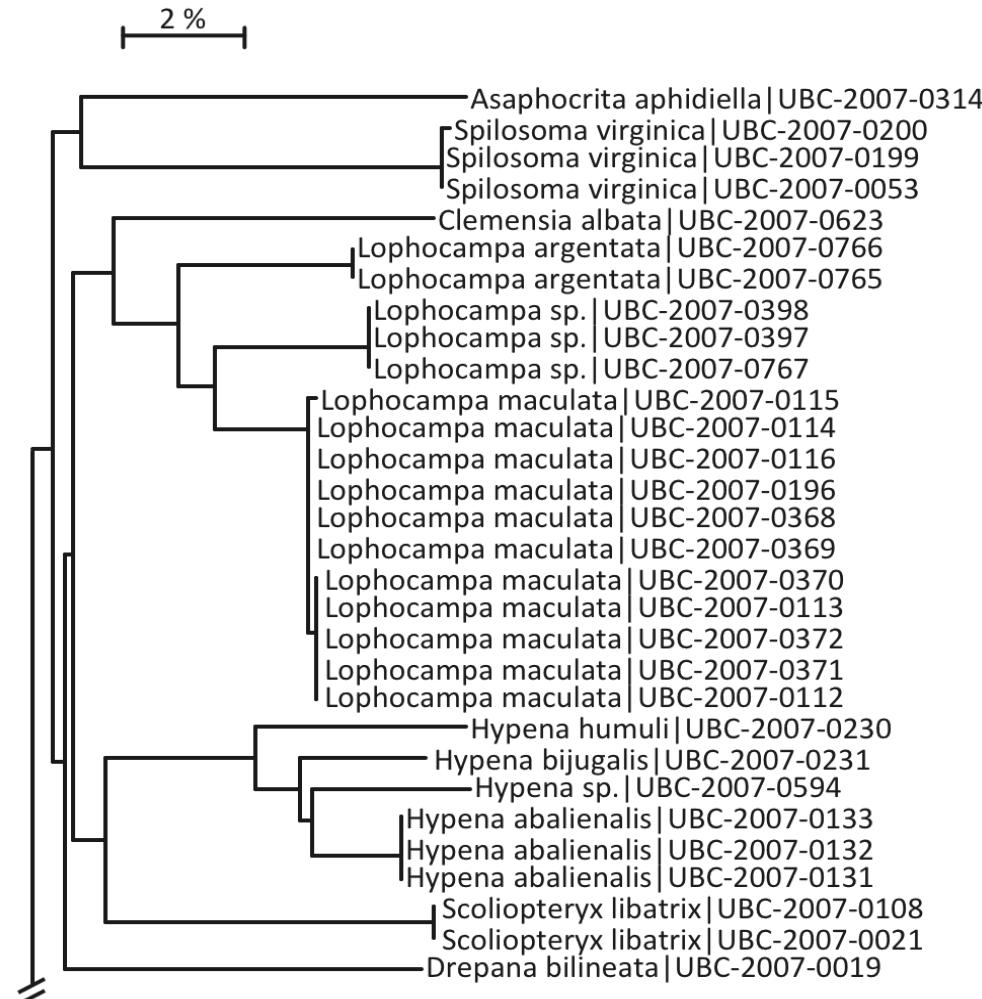
Done

Results: barcode analysis



- 925 specimens
- ~190 species clusters with 3% sequence divergence cutoff (Hebert *et al.* 2003)

Results: species assignment



- 124 clusters assigned to species, 61 to genus using BOLD-ID
- all species assignments confirmed as correct

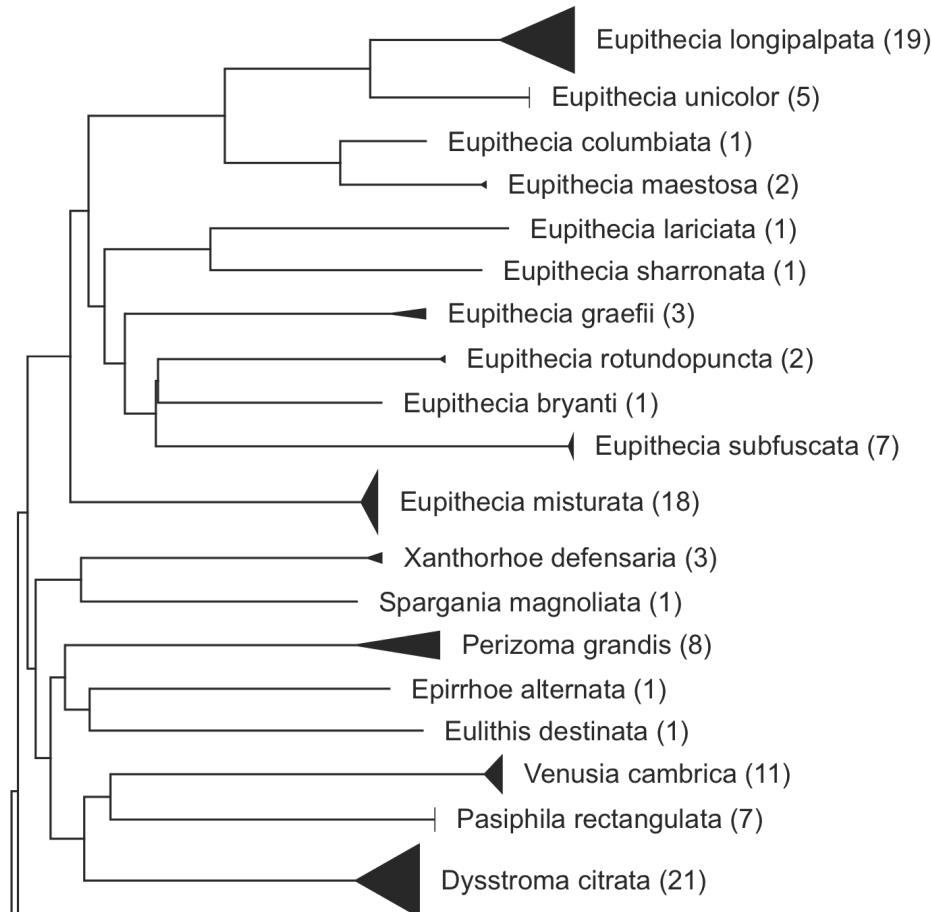
Results: specialist time

- 66 remaining clusters identified (61 with 'guidance')
 - 25 with brief comparison
 - 41 with genitalic dissections
- * first (and only) step for specialist



Several difficult looper moth species now entered into barcode library

Results: highlights of inventory



- 190 species
- 21 families
- 71 singletons
- 93 uniques
- * taxonomically diverse; many at low density

Results: NIS detection



Prays fraxinella (Donovan, 1793)



Dichelia histriionana (Frölich, 1828)



Paraswammerdamia lutarea
(Haworth, 1828)



Argyresthia pruniella (Clerck, 1759)

Results: inventory progress

- >120 species still to sample
 - only nocturnal, phototactic Lepidoptera
- * not complete, but solid start

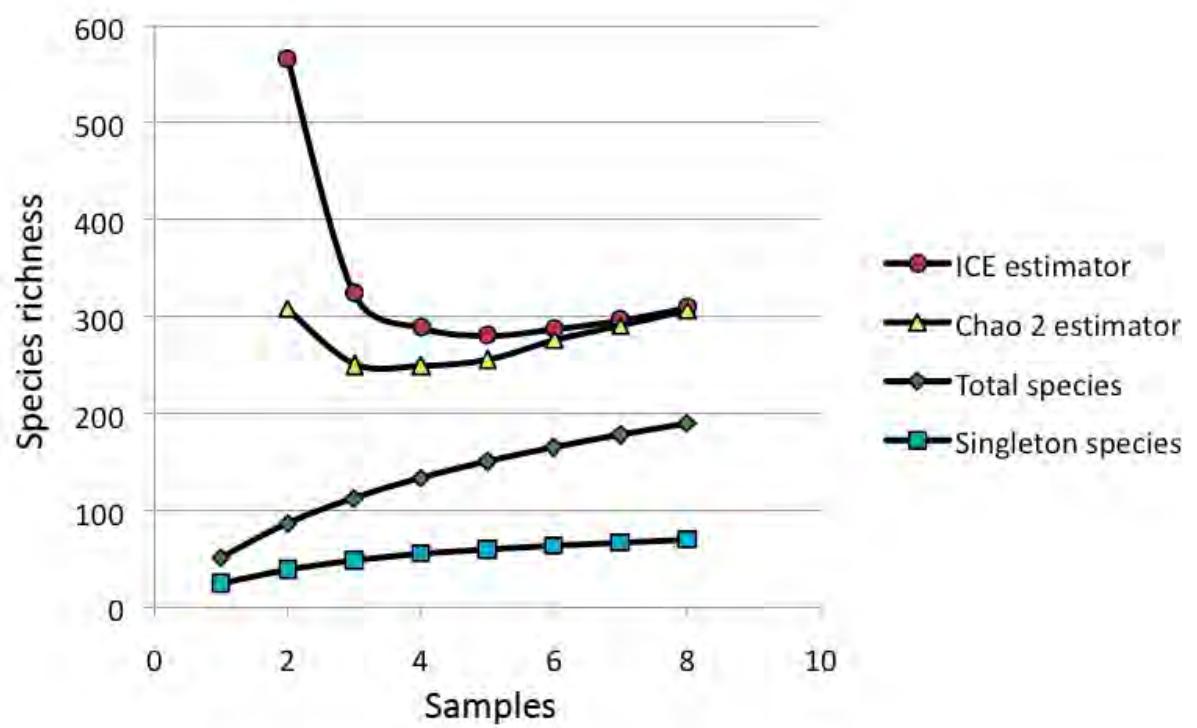
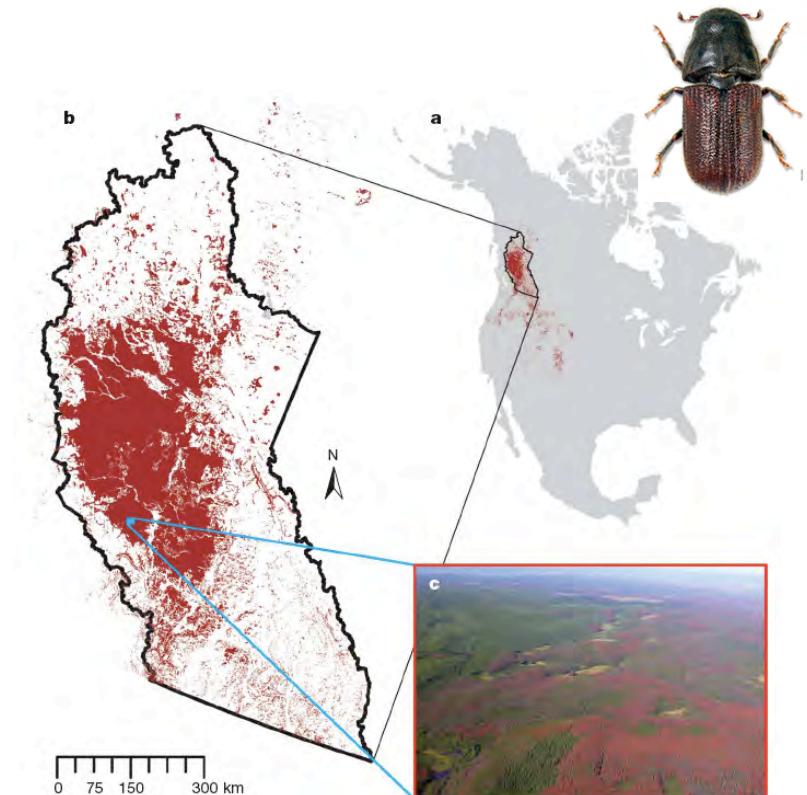


Fig.3, deWaard et al 2009. Biodivers Conserv. *In press.*

3) Diversity effects of disturbance

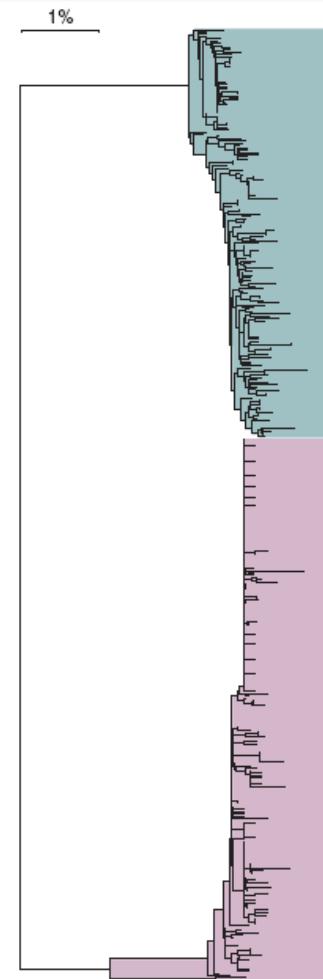
- how do anthropogenic and natural disturbances affect biodiversity?
 - we can now rapidly assess diversity changes in hyper-diverse assemblages



Natural disturbance example: native pest outbreak mountain pine beetle (Kurz et al 2008; NRC)

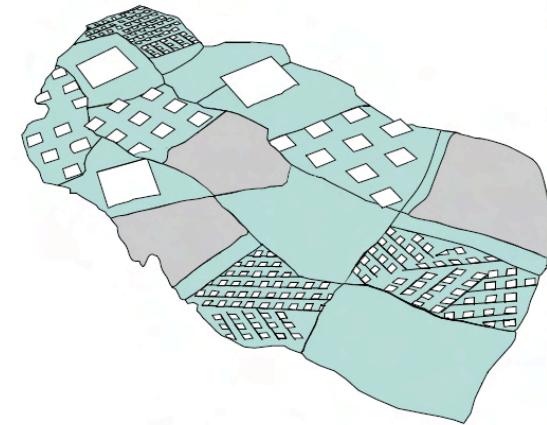
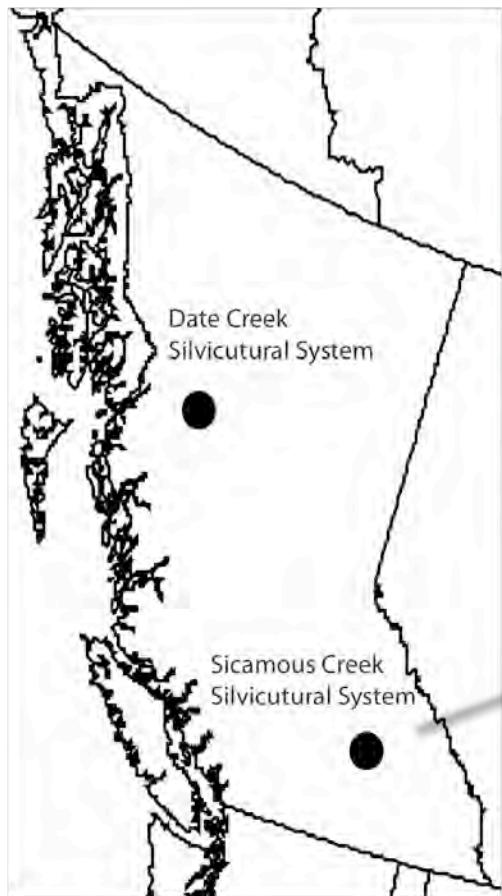
Additional levels of diversity

- genetic diversity (GD)
 - e.g. haplotype diversity
 - can add rapid marker(s) to primary barcode, or not
- phylogenetic diversity (PD)
 - variation in evolutionary history
 - can add phylogeny backbone to primary barcode, or not (see Faith 2007)



COI haplotype diversity in the salmon louse (Boulding et al. 2009)

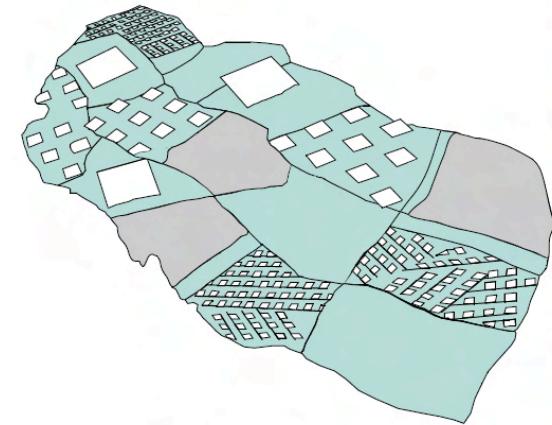
Case study 4: The effect of harvest types on 3 levels of moth diversity



Sicamous Creek Silvicultural System (Huggard & Vyse 2002)

Case study 4: The effect of harvest types on 3 levels of moth diversity

- 3 treatments, 1 control
 - CC – clear-cut
 - HR – heavy removal (patch cut)
 - LR – light removal (individual tree selection)
 - NH – no harvest (350 year old stand)



Sicamous Creek Silvicultural System (Huggard & Vyse 2002)

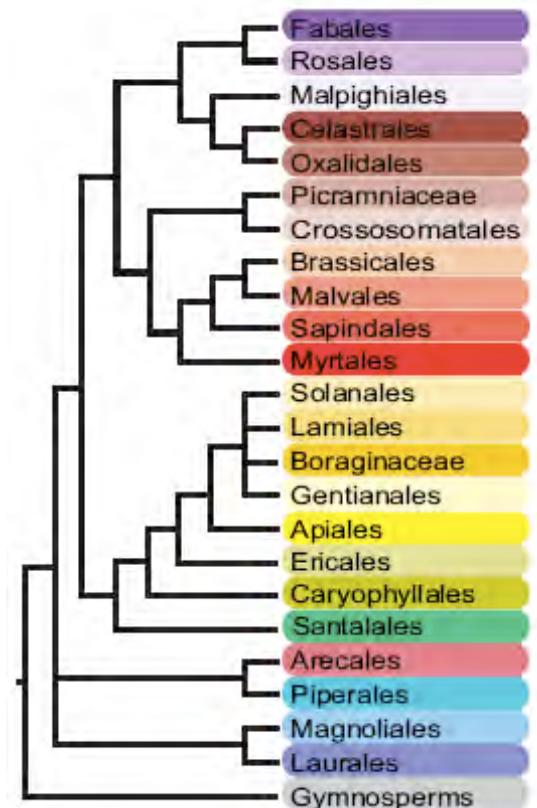
Methods

- procedure follows Stanley Park inventory
 - UV light traps allow standardized effort
 - single trap per treatment
 - 4 or 8 traps per night; ~tri-weekly through adult flight season



Methods

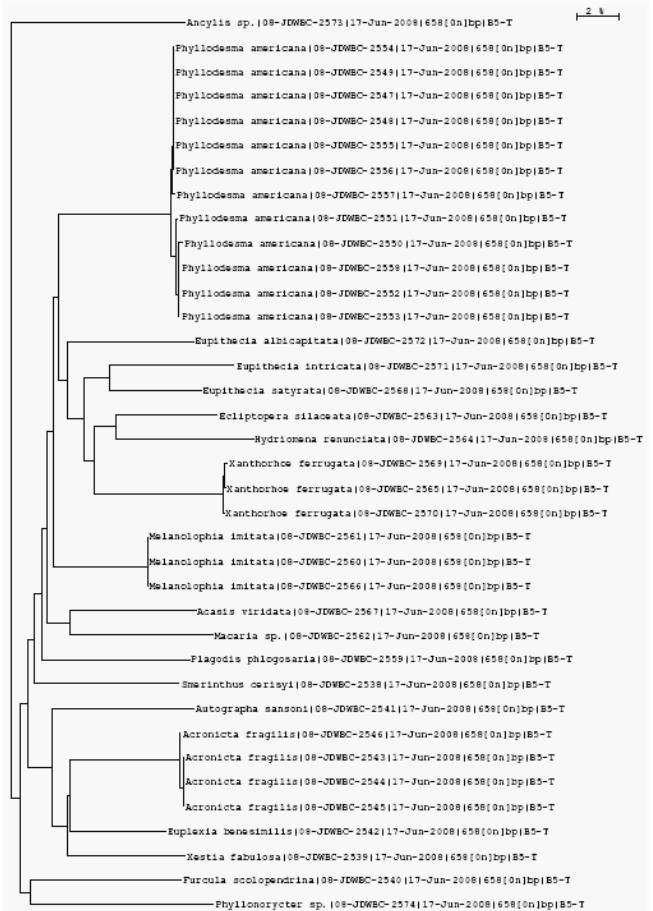
- GD estimated as haplotype variation in COI
 - Stanley Park Leps – 77% of species with 8 or more individuals have >1 COI haplotype (mean = 3.4, new haplotype every 3.9 individuals)
- PD estimated with COI (today)
 - will be reconstructing community phylogenies using LepTree dataset (Regier et al., submitted)



Community phylogeny
of Panama forest plot
(Kress et al. 2009)

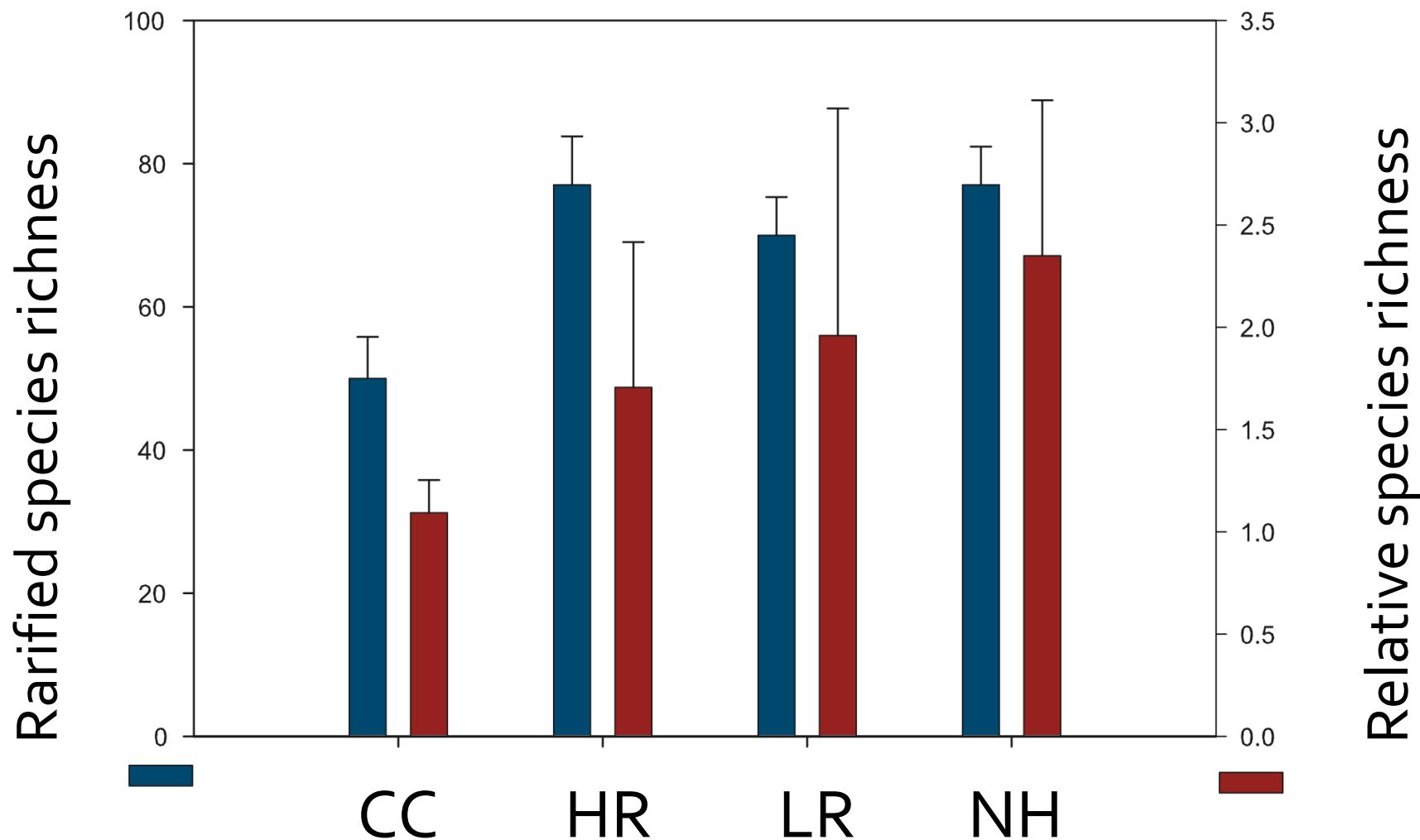
Preliminary results

- barcoded 22 of ~136 traps
 - 1157 specimens
 - 5 complete comparisons
(same collection event)
- preliminary results to follow:
 - observed values (left)
 - relative means from same collection event (right)

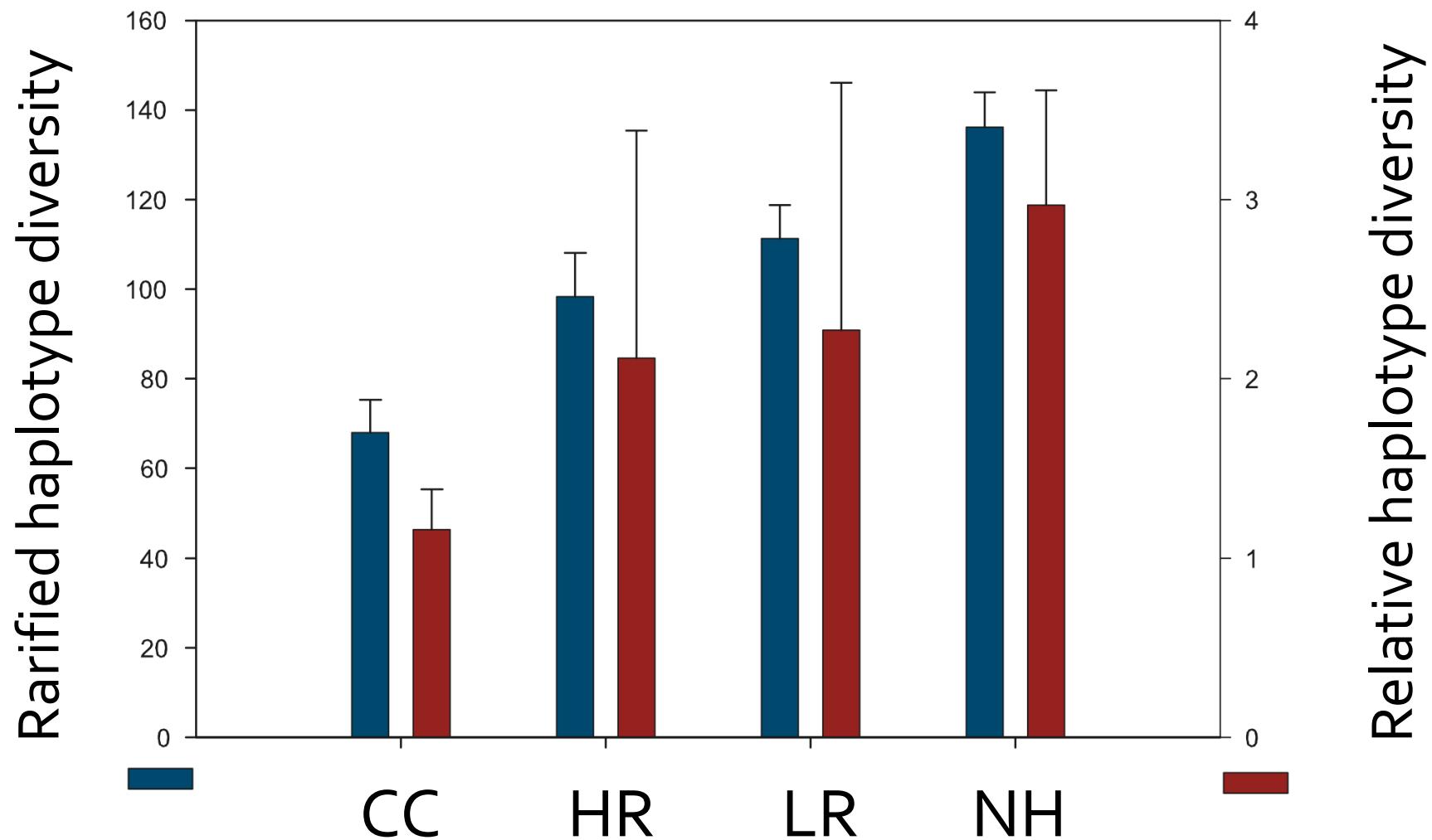


BOLD TaxonID tree for trap LR5

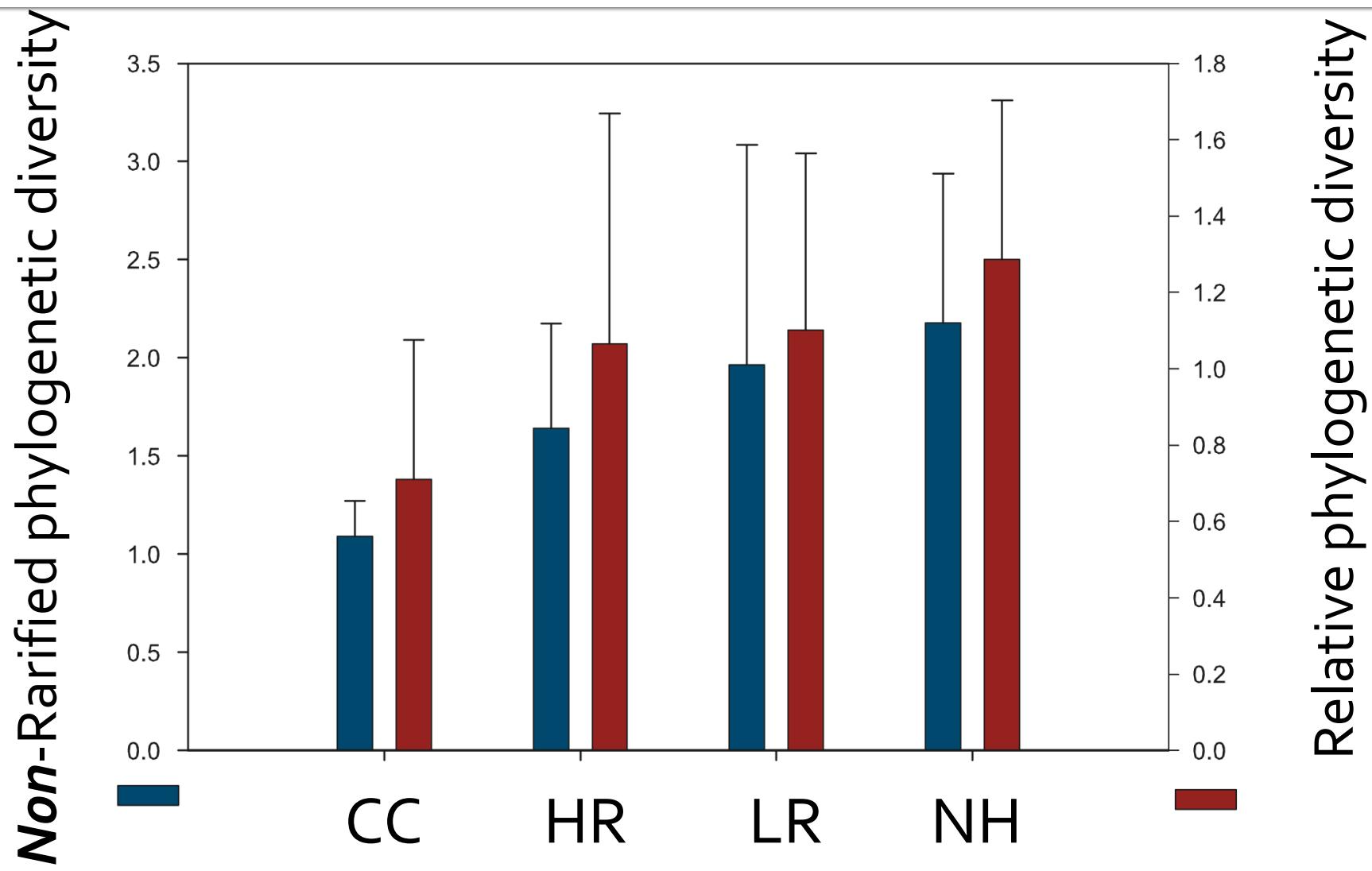
Preliminary results: SD



Preliminary results: GD

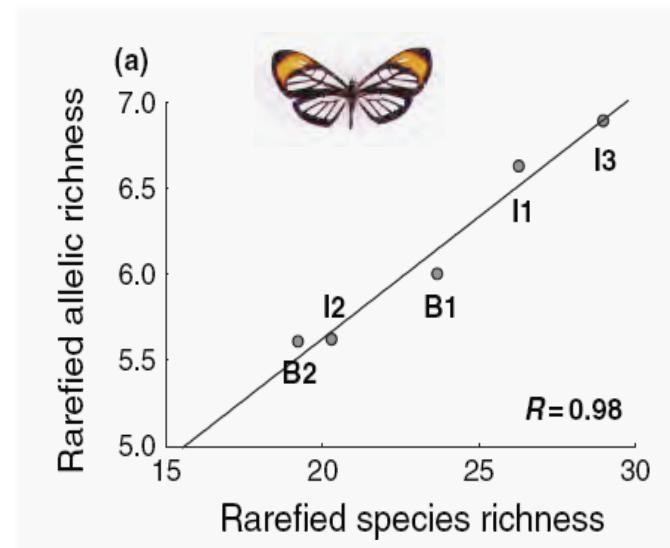


Preliminary results: PD



Preliminary conclusions

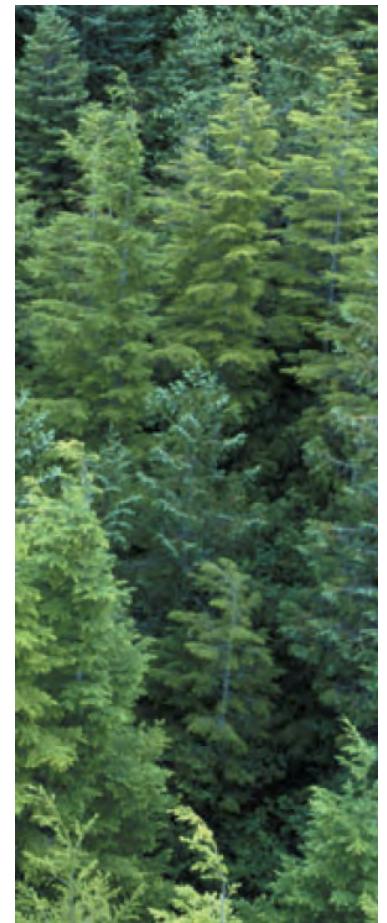
- interesting hypotheses can be addressed
 - intermediate disturbance hypothesis (Connell 1978)
 - species/genetic diversity correlation (SGDC) (Vellend 2003)
 - introduced taxa less related to native species are more invasive (Strauss et al 2006)



Post-disturbance SGDC in butterflies (Cleary et al 2006).

Overall conclusions

- DNA barcoding has proven valuable for NIS detection, baseline inventory work, and post-disturbance assessments
- DNA barcoding provides informed sorting, reduction of specialist time, enhanced sensitivity at low density, and estimates of multiple levels of diversity
- forest health applications discussed are *very* amenable to next generation sequencing



Acknowledgements: co-authors

- ISPM 27 DNAB protocol (in revision)
 - Robin Floyd, João Lima, Robert Hanner (U. of Guelph)
- *Lymantria* tussock moths (in prep.)
 - as above, and Melody Keena (USDA Forest Service)
- Poplar Shoot borer (in press)
 - Meghan Quinn (CFS)
- Stanley Park moth inventory (in press)
 - John McLean & Jennifer Derhousoff (UBC Forestry)
 - Jean-François Landry & Chris Schmidt (CNC)
- Harvest effects on 3 levels of moth diversity
 - Doug Steventon and Alan Vyse (BC Forest Service)
 - Jean-François Landry (CNC)

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Thank you.

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