

# We're getting there: a first look at (cheap!) next-generation barcoding of bulked arthropod samples

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# Outline

- Background: working through the taxonomic impediment
  - Conventional (morphological) workflow
  - Classical DNA barcoding workflow
  - Next-gen DNA barcoding workflow
- A trial of NGS methods on bulked samples
  - Methods
  - Results
  - Discussion



# Conventional Workflow



D e S i t k a .

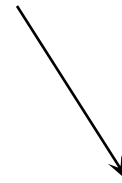
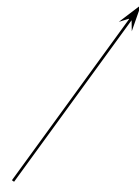
2 espèces de *Cychnus*.  
5 » de *Ferontia*.  
1 » de *Patropus* nouv. *planusculus* N.  
1 . d'*Amara*, et 1 espèce de *Nebria*.

# Classical DNA Barcoding Workflow



```

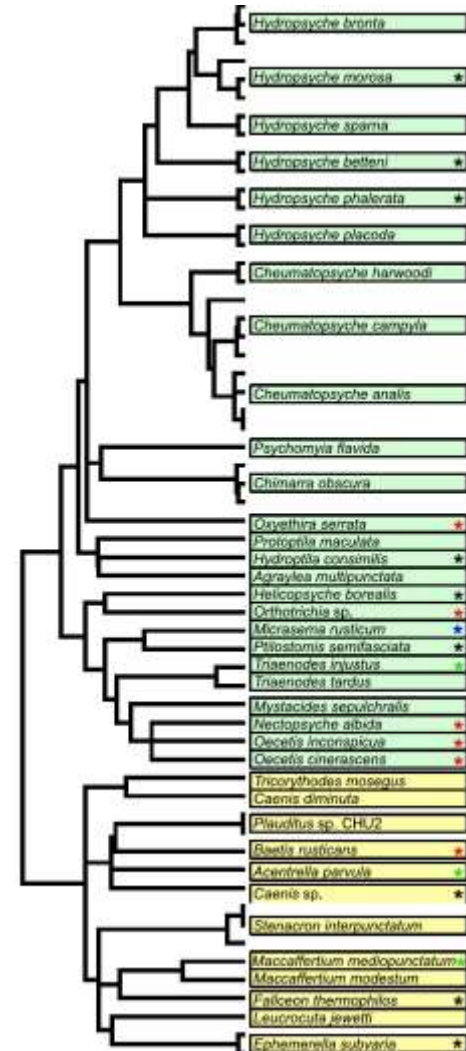
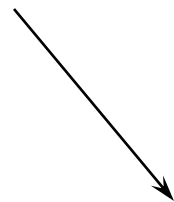
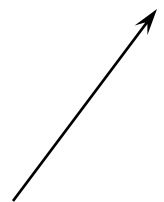
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TATAGTTTG
ATTGGATT
TCCTCC
TTCTCTAA
TGGGGATC
TTCCAGC
AATTTTT
AGGAGCG
TTCTAAGT
TTGATCGT
TTTACCTGTATTGGCTGGAGCAATTACAATATTACTTAC
AGACCGAAATTTAAATACTTCTTTTTTTGACCCCGCTGG
AGGAGGAGATCCAATTTTATACCAACATTTATTT
    
```



D e S i t k a .

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- 5 » de *Ferontia*.
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# Next-Gen DNA Barcoding Workflow



# Methods: field

3 samples, all from Headquarters Lake,  
Soldotna

- 2 sweep net (one 100 m<sup>2</sup> circular plot split in half), 16.Sept.2013

[KNWR:Ento:10656](#), [KNWR:Ento:10657](#)

- 1 malaise (leftovers), 27-28.June.2011

[KNWR:Ento:7114](#)

# Methods: lab

- Vial contents recorded (quick, coarse identifications).  
Arthropod fragments and original preservative ethanol retained.
- Processing by [Research and Testing Laboratory](#) (Lubbock, Texas), mailed out 21.Nov.2014.
- NGS sequencing using *ZBJ-ArtF1c* and *ZBJ-ArtR2c* primers, 157 bp from COI (\$95 / sample).
- Sequences posted 20.Jan.2015, identified using RTL's [boldsystems.org](#) and [NCBI BLAST](#).



# Results: pooled

- 169 Operation Taxonomic Units identified by software
- 76 unique names (identifications) at various levels of taxonomic resolution
- 2 phyla (Arthropoda and Mollusca)
- 3 classes
- 12 orders
- 34 families
- 57 genera
- 35 species



# Results: sweep 1

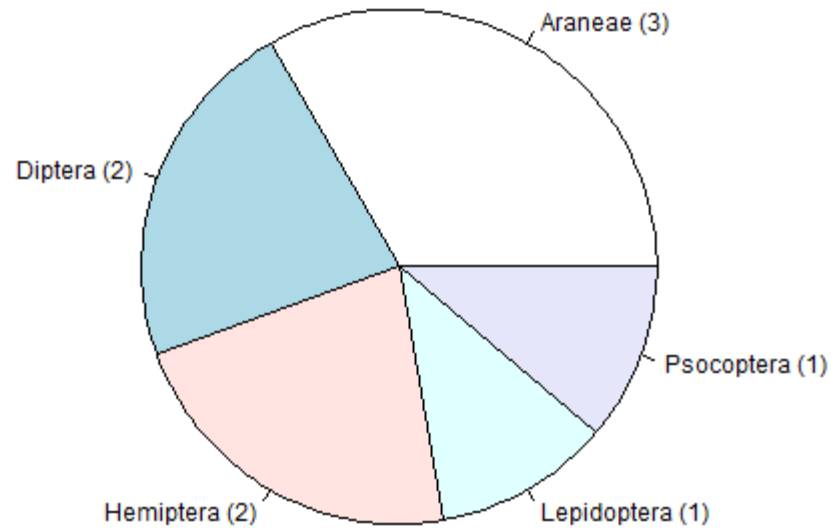
n = 21 names

[1] "Bathyphantes pogonias"	"Boletina"	"Cecidomyiidae"
[4] "Cicadellidae"	"Clepsis persicana"	"Coenosia comita"
[7] "Colladonus"	"Diastata"	"Dicrotendipes"
[10] "Diptera"	"Elasmotethus interstinctus"	"Helophora reducta"
[13] "Incestophantes"	"Lepthyphantes alpinus"	"Lucilia"
[16] "Mydaea furtiva"	"Pemphigus populiglobuli"	"Phoridae"
[19] "Psocoptera"	"Tanytarsus"	"Valenzuela flavidus"



# Results: sweep 1 species

sweep 1 species (n=9)



# Results: sweep 2

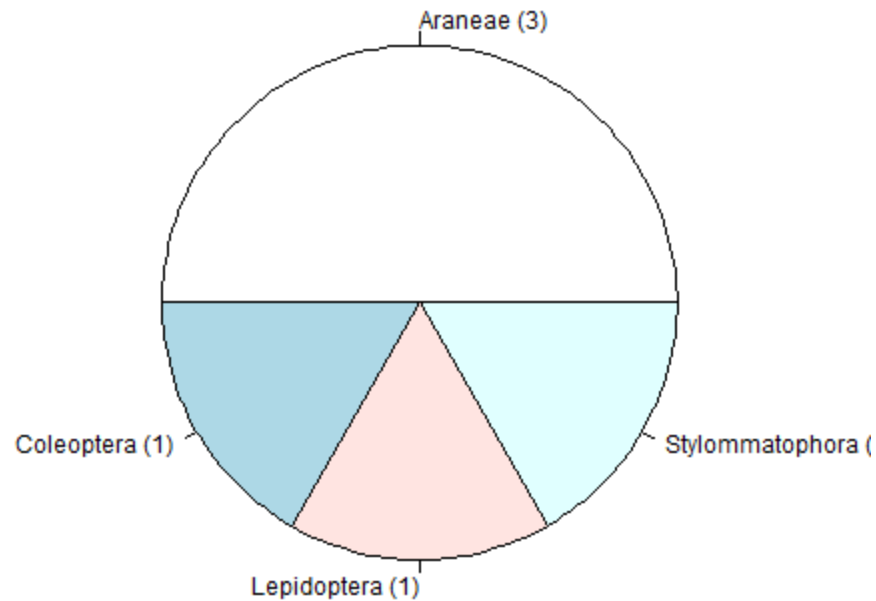
## n = 21 names

[1] "Arthropoda"	"Balclutha"	"Boletina"
[4] "Calvia quatuordecimguttata"	"Chironomidae"	"Diptera"
[7] "Estrandia grandaeva"	"Helophora reducta"	"Ichneumonidae"
[10] "Insecta"	"Megaselia"	"Muscoidea"
[13] "Mycetophilidae"	"Mycomya"	"Nematocera"
[16] "Phoridae"	"Rugathodes aurantius"	"Sciaridae"
[19] "Thripidae"	"Xestia c-nigrum"	"Zoogenetes harpa"



# Results: sweep 2 species

sweep 1 species (n = 6)



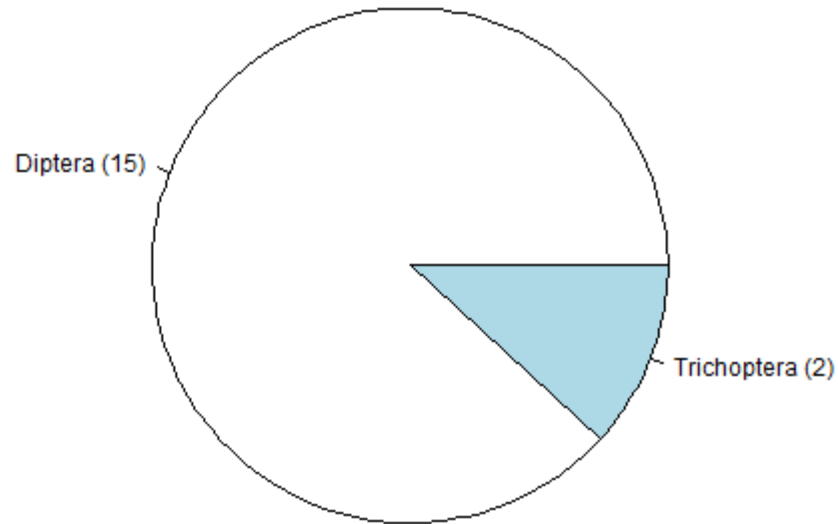
# Results: malaise

## n = 48 names

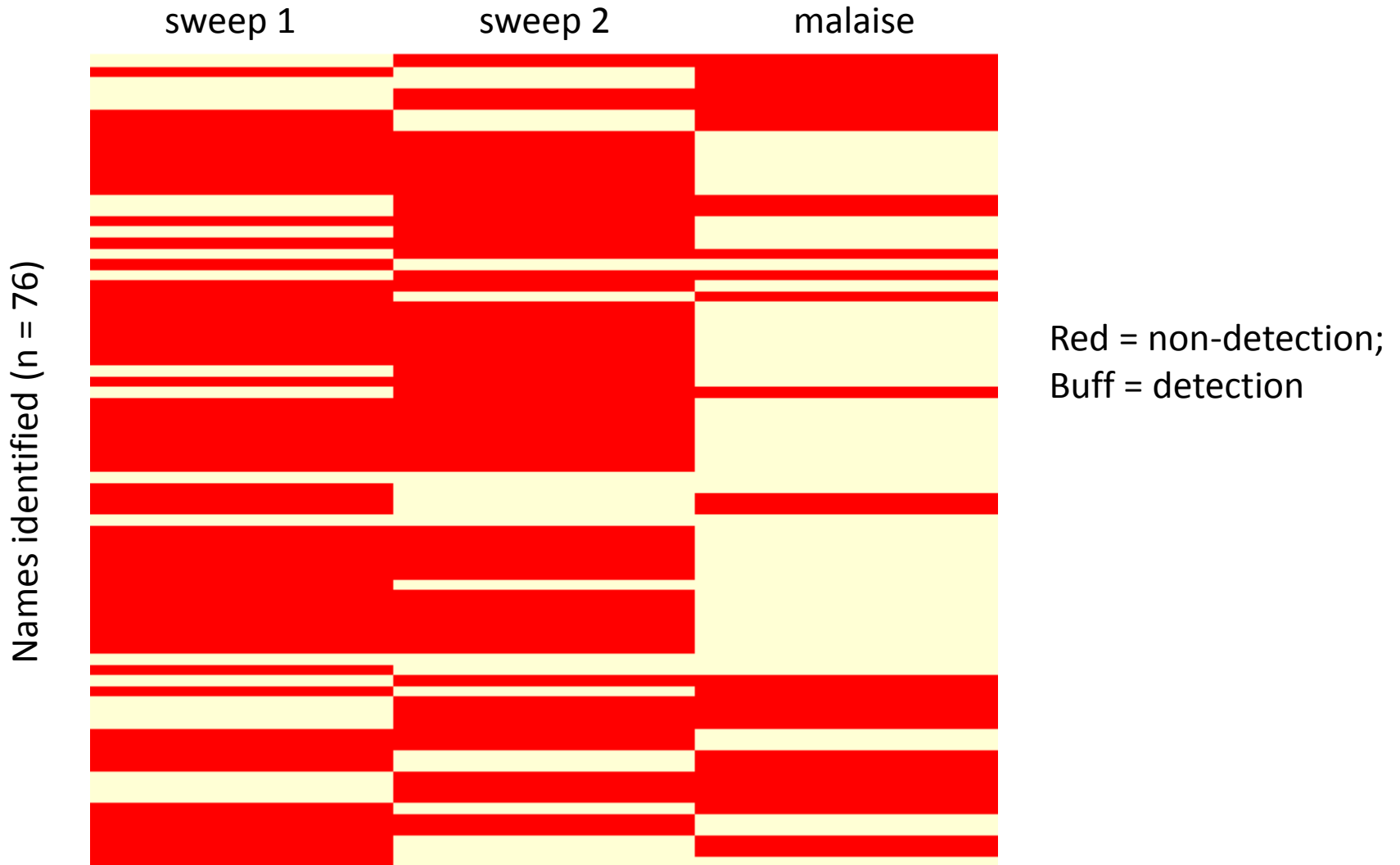
[1]	"Actia"	"Anthomyiidae"	"Baccha elongata"
[4]	"Boletina"	"Chironomidae"	"Cladotanytarsus"
[7]	"Coenosia"	"Coenosia comita"	"Cordilura"
[10]	"Cosmetopus longus"	"Cucujiformia"	"Dicrotendipes"
[13]	"Diptera"	"Dolichopus genualis"	"Fannia fuscula"
[16]	"Fannia spathiophora"	"Insecta"	"Lasiomma cuneicorne"
[19]	"Limnephilus argenteus"	"Lonchaea"	"Lonchoptera"
[22]	"Meliscaeva cinctella"	"Muscidae"	"Muscoidea"
[25]	"Mycomya"	"Mydaea"	"Myospila meditabunda"
[28]	"Pegomya"	"Phaonia"	"Phaonia atrocyanea"
[31]	"Phoridae"	"Pimpla"	"Platycheirus "
[34]	"Platycheirus holarcticus"	"Polycentropus flavus"	"Polypedilum"
[37]	"Psychoda"	"Rhamphomyia"	"Sarcophagidae"
[40]	"Scathophagidae"	"Sciaridae"	"Sphaerophoria novaeangliae"
[43]	"Spilogona"	"Spilogona sororcula"	"Stictopisthus"
[46]	"Thricops"	"Zaphne"	"Zaphne lineatocollis"

# Results: malaise species

malaise species (n = 17)



# Results: comparison



# Discussion

- 157 bp fragment led to ambiguous, multiple matches for many Operation Taxonomic Units (OTU's).
- Taxonomic resolution was often at generic or coarser level, but could be restricted to species given geography (e.g., *Elasmostethus*).
- Primer seemed to amplify Diptera, Araneae, and Hemiptera well; Coleoptera and Hymenoptera present in samples were underrepresented.
- Some OTU's appeared to be valid sequences with no close match in library databases (BOLD, GenBank).



# Discussion

- Sequences from species not obviously present in the sample were amplified (fragment, frass, meal within another arthropod, or internal parasitoid?).
- Less overlap than expected, especially in the adjacent sweep-net samples.
- In future, it would be ideal to sample from preservative fluid, leaving the specimens intact.
- Overall, I would like to be able to obtain more species-resolution identifications / OTU's. I would like to try different primer sets with a longer read length.