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

2 espèces de Cychrus.
5 » de Feronia.
1 » de Patropus nouv. planiusculus N.
1 . d’Amara, et 1 espèce de Nebra.
Classical DNA Barcoding Workflow
Next-Gen DNA Barcoding Workflow
Methods: field

3 samples, all from Headquarters Lake, Soldotna

- 2 sweep net (one 100 m² circular plot split in half), 16.Sept.2013
  [KNWR:Ento:10656, KNWR:Ento:10657]

  [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"
[4] "Cicadellidae"
[7] "Colladonus"
[10] "Diptera"
[13] "Incestophantes"
[16] "Mydaea furtiva"
[19] "Psocoptera"

"Boletina"
"Clepsis persicana"
"Diastata"
"Elasmostethus interstinctus"
"Lepthyphantes alpinus"
"Pemphigus populiglobuli"
"Tanytarsus"

"Cecidomyiidae"
"Coenosia comita"
"Dicrotendipes"
"Helophora reducta"
"Lucilia"
"Phoridae"
"Valenzuela flavidus"
Results: sweep 1 species

- Araneae (3)
- Diptera (2)
- Hemiptera (2)
- Lepidoptera (1)
- Psocoptera (1)
Results: sweep 2
n = 21 names

[1] "Arthropoda"          "Balclutha"
[4] "Calvia quatuordecimguttata" "Chironomidae"
[7] "Estrandia grandaeva"   "Helophora reducta"
[10] "Insecta"              "Megaselia"
[13] "Mycetophilidae"       "Mycomya"
[16] "Phoridae"             "Rugathodes aurantius"
[19] "Thripidae"            "Xestia c-nigrum"

"Boletina"
"Diptera"
"Ichneumonidae"
"Musoidea"
"Nematocera"
"Sciaridae"
"Zoogenetes harpa"
Results: sweep 2 species

- Araneae (3)
- Coleoptera (1)
- Stylommatophora (1)
- Lepidoptera (1)
<table>
<thead>
<tr>
<th></th>
<th>Scientific Name</th>
<th>Taxonomic Order</th>
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<tr>
<td>1</td>
<td>&quot;Actia&quot;</td>
<td>Anthomyiidae</td>
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<td>4</td>
<td>&quot;Boletina&quot;</td>
<td>Chironomidae</td>
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<tr>
<td>7</td>
<td>&quot;Coenosia&quot;</td>
<td>Coenosia comita</td>
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<tr>
<td>10</td>
<td>&quot;Cosmetopus longus&quot;</td>
<td>Cucujiformia</td>
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<td>13</td>
<td>&quot;Diptera&quot;</td>
<td>Dolichopus genualis</td>
</tr>
<tr>
<td>16</td>
<td>&quot;Fannia spathiophora&quot;</td>
<td>Insecta</td>
</tr>
<tr>
<td>19</td>
<td>&quot;Limnephilus argenteus&quot;</td>
<td>Lonchaea</td>
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<td>22</td>
<td>&quot;Meliscaeva cinctella&quot;</td>
<td>Muscidae</td>
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<td>25</td>
<td>&quot;Mycomya&quot;</td>
<td>Mydaea</td>
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<td>&quot;Platyccheirus holarcticus&quot;</td>
<td>Polycentropus flavus</td>
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<td>&quot;Psychoda&quot;</td>
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<td>&quot;Spilogona&quot;</td>
<td>Spilogona sororcula</td>
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<tr>
<td>46</td>
<td>&quot;Thricops&quot;</td>
<td>Zaphne</td>
</tr>
</tbody>
</table>
Results: malaise species

- Diptera (15)
- Trichoptera (2)

Total: 17 species
Results: comparison

Names identified (n = 76)

sweep 1    sweep 2    malaise

Red = non-detection; Buff = detection
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.