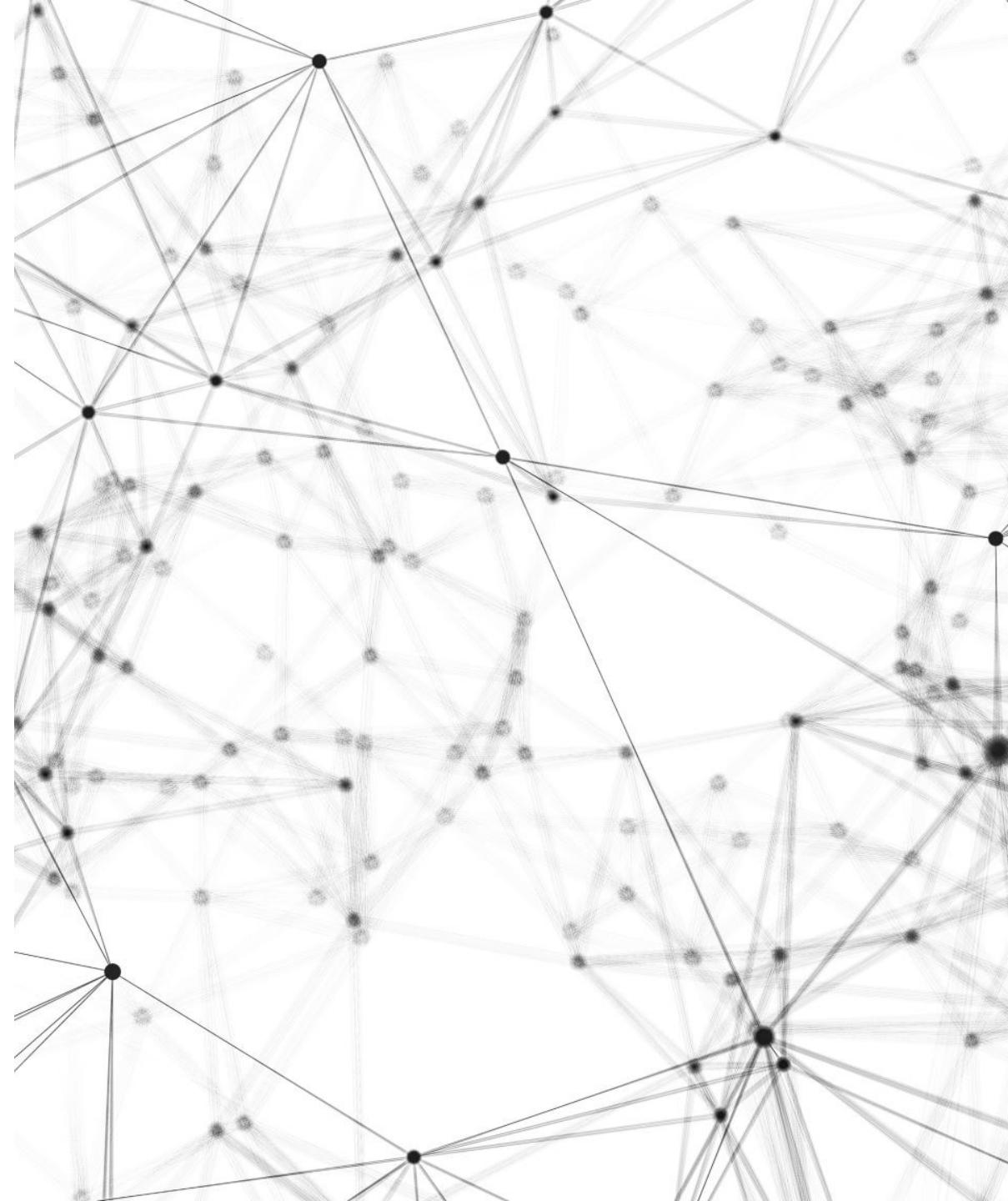

ECOLOGICAL APPLICATIONS OF DNA BARCODING

Brad Reil

Insect Systematics and Biodiversity Lab



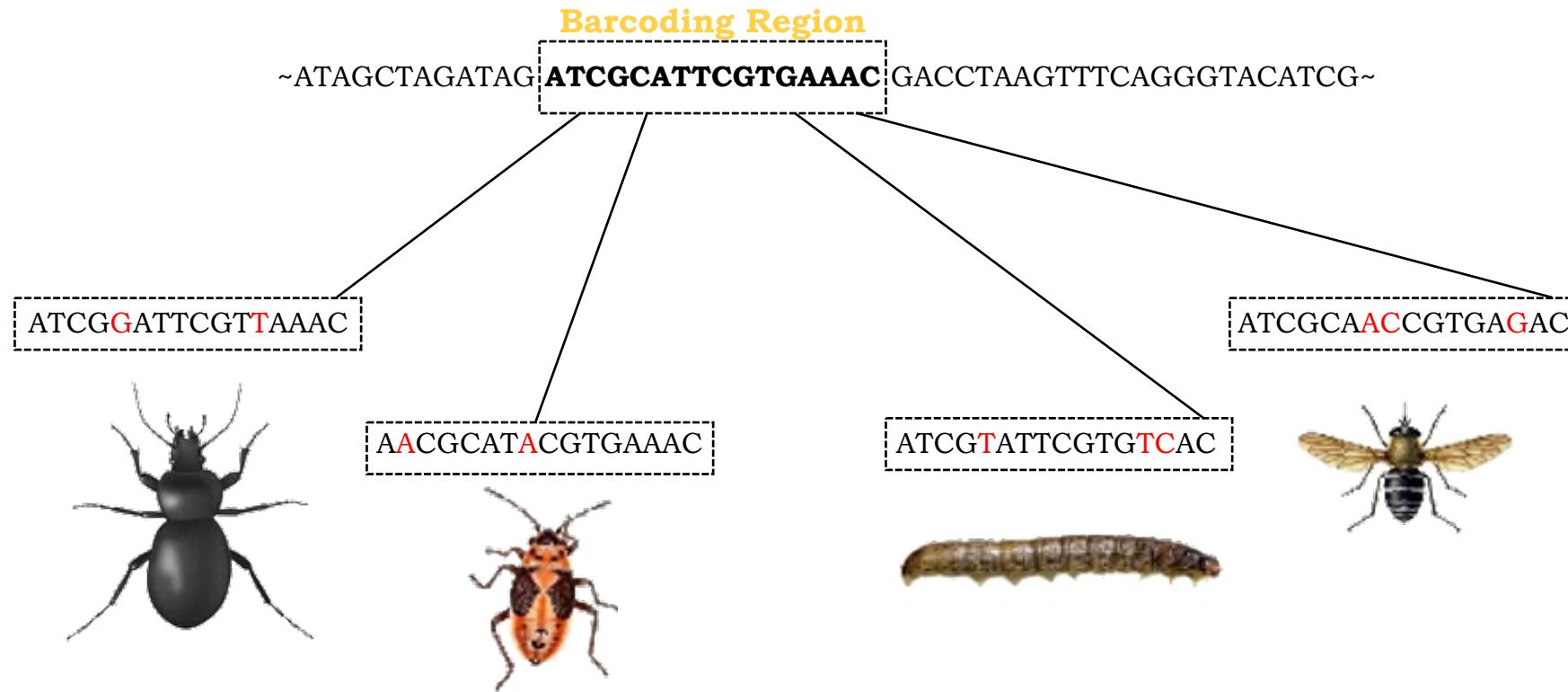
QUICK REVIEW: WHAT IS DNA BARCODING?

“Genomic approaches to taxon diagnosis exploit diversity among DNA sequences to identify organisms (Kurtzman 1994; Wilson 1995). In a very real sense, these sequences can be viewed as genetic ‘barcodes’ that are embedded in every cell.”

“Genomic barcodes have only four alternate nucleotides at each position, but the string of sites available for inspection is huge. The survey of just 15 of these nucleotide positions creates the possibility of 4^{15} (1 billion) codes, 100 times the number that would be required to discriminate life if each taxon was uniquely branded.”

Hebert, P. D., Cywinski, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1512), 313-321. (Over 12000 citations according to Google Scholar)

QUICK REVIEW: WHAT IS DNA BARCODING?



TECHNICAL LIMITATIONS OF BARCODES

- “mtDNA is not adequate as a sole source of species-defining data due to the following factors:
 - Reduced effective population size [$1/4 N_e$] and introgression [*interspecies gene flow*]
 - Maternal inheritance [*evolutionary processes can affect the sexes differently*]
 - Recombination [*or lack thereof*]
 - Inconsistent mutation rate [*inconsistent across the mtDNA and location dependent*]
 - Heteroplasmy [*two or more mtDNA haplotypes in the same cell*]
 - Compounding evolutionary processes [*selection, cytoplasmic incompatibility*].”

Rubinoff, D., Cameron, S., & Will, K. (2006). A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. *Journal of heredity*, 97(6), 581-594.

APPROPRIATE APPLICATIONS FOR DNA BARCODES

“Barcodes of mtDNA could be useful for confirmation after hypotheses based on other or more complete sources of data have been gathered and analyzed.”

Rubinoff, D., Cameron, S., & Will, K. (2006). A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. *Journal of heredity*, 97(6), 581-594.

ENTER METABARCODING

“Here, we introduce the term ‘DNA metabarcoding’ to designate high-throughput multispecies (or higher-level taxon) identification using the total and typically degraded DNA extracted from an environmental sample (i.e. soil, water, faeces, etc.)”

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular ecology*, 21(8), 2045-2050.



AN INTRODUCTION TO METABARCODING AND HOW PEOPLE ARE USING IT

THE METABARCODING PIPELINE...



Figure from Compson, Z. G., McClenaghan, B., Singer, G. A., Fahner, N. A., & Hajibabaei, M. (2020). Metabarcoding from microbes to mammals: comprehensive bioassessment on a global scale. *Frontiers in Ecology and Evolution*, 8, 581835.

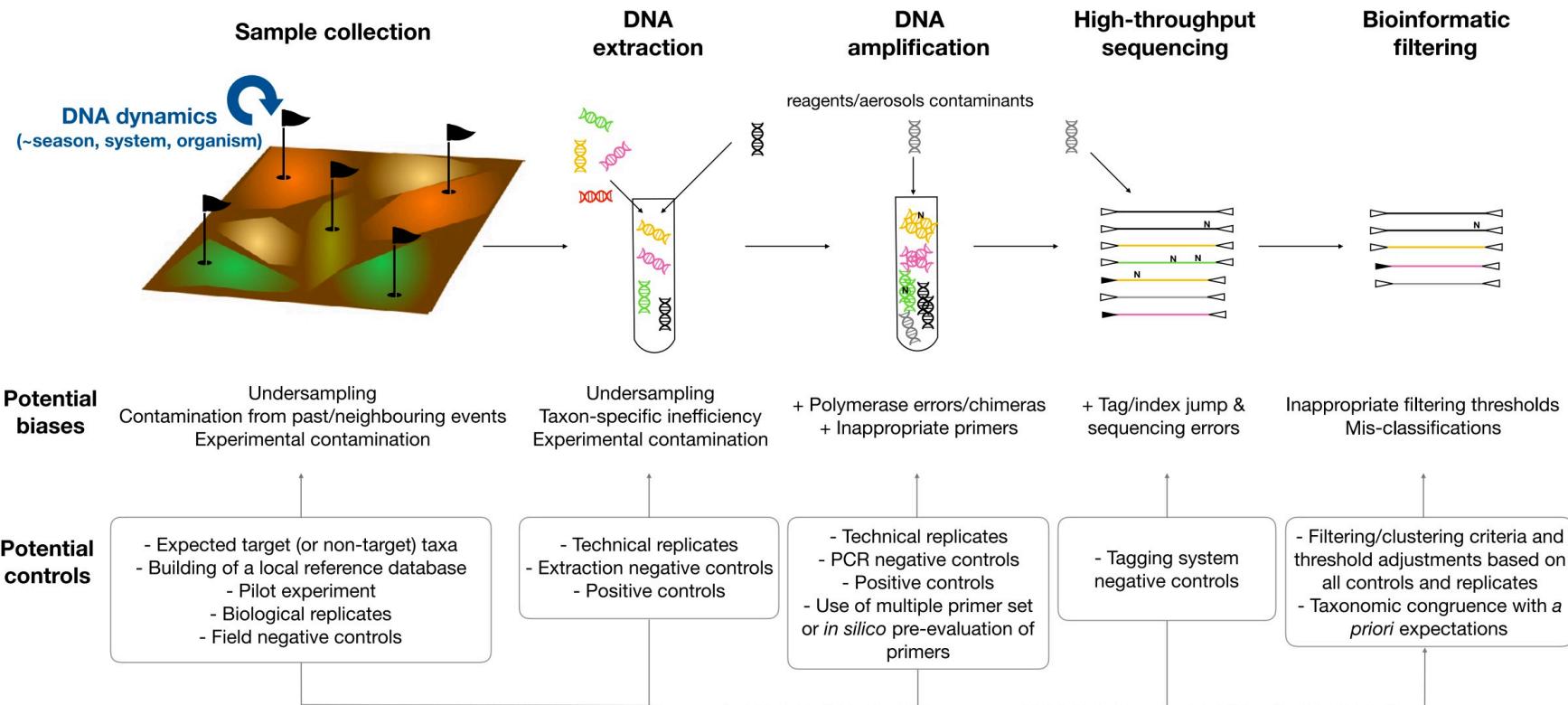
WHY METABARCODING?

Identification method	Taxonomic expertise	Identify specific taxa	Identify broad range of taxa	Throughput level	Time per identification
<u>Morphological</u>					
Microscopic examination	High	High*	High*	Low	Moderate
<u>Molecular</u>					
PCR-restriction fragment length polymorphism	Low	Moderate	Low	Moderate	Moderate
DNA barcoding	Low	High	High	Low	Moderate
Quantitative PCR/droplet digital PCR	Low	High	Low	High	Low
Loop-mediated isothermal amplification	Low	High	Low	Low	Low
Metabarcoding	Low	High	High	Very high	Low

*This morphological identification score assumes a high level of taxonomic knowledge and a low human error rate.

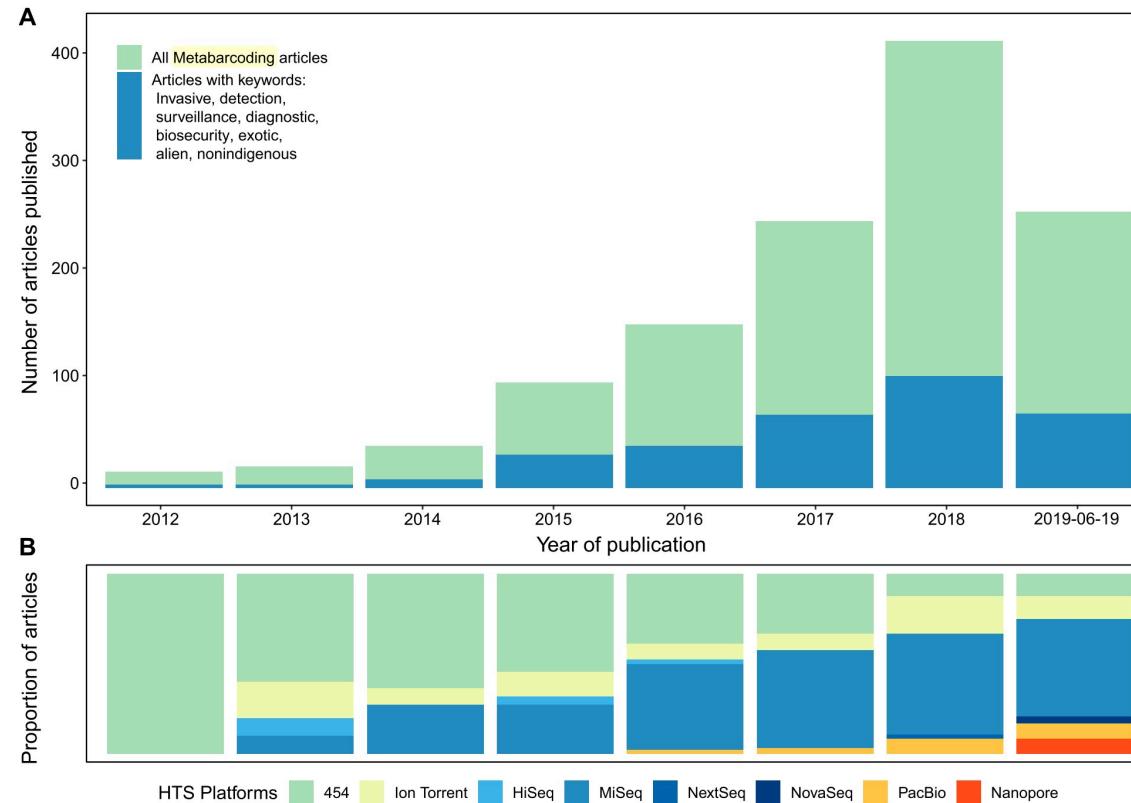
Piper, A. M., Batovska, J., Cogan, N. O., Weiss, J., Cunningham, J. P., Rodoni, B. C., & Blacket, M. J. (2019). Prospects and challenges of implementing DNA metabarcoding for high-throughput insect surveillance. *GigaScience*, 8(8), giz092.

WHAT ARE SOME METABARCODING LIMITATIONS?



Zinger, L., Bonin, A., Alsos, I. G., Bálint, M., Bik, H., Boyer, F., ... & Taberlet, P. (2019). DNA metabarcoding—Need for robust experimental designs to draw sound ecological conclusions. *Molecular ecology*, 28(8), 1857-1862.

THE RISE OF METABARCODING

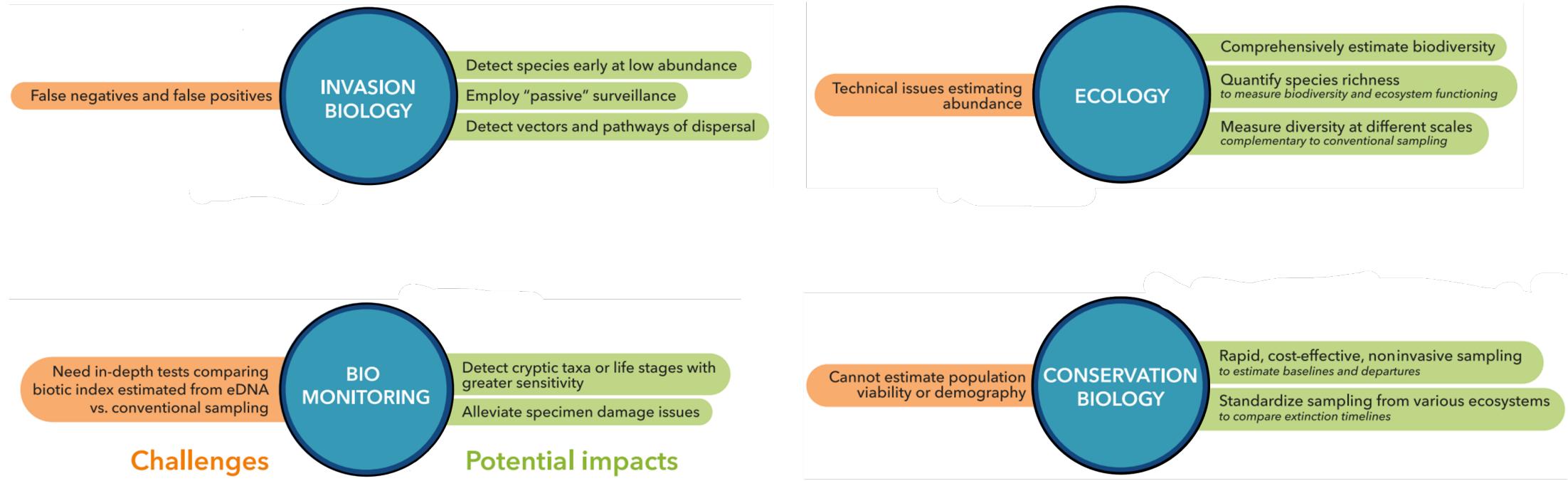


Piper, A. M., Batovska, J., Cogan, N. O., Weiss, J., Cunningham, J. P., Rodoni, B. C., & Blacket, M. J. (2019). Prospects and challenges of implementing DNA metabarcoding for high-throughput insect surveillance. *GigaScience*, 8(8), giz092.

HARNESS ALTERNATIVE DNA SOURCES



METABARCODING APPLICATIONS



Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., ... & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular ecology*, 26(21), 5872-5895.

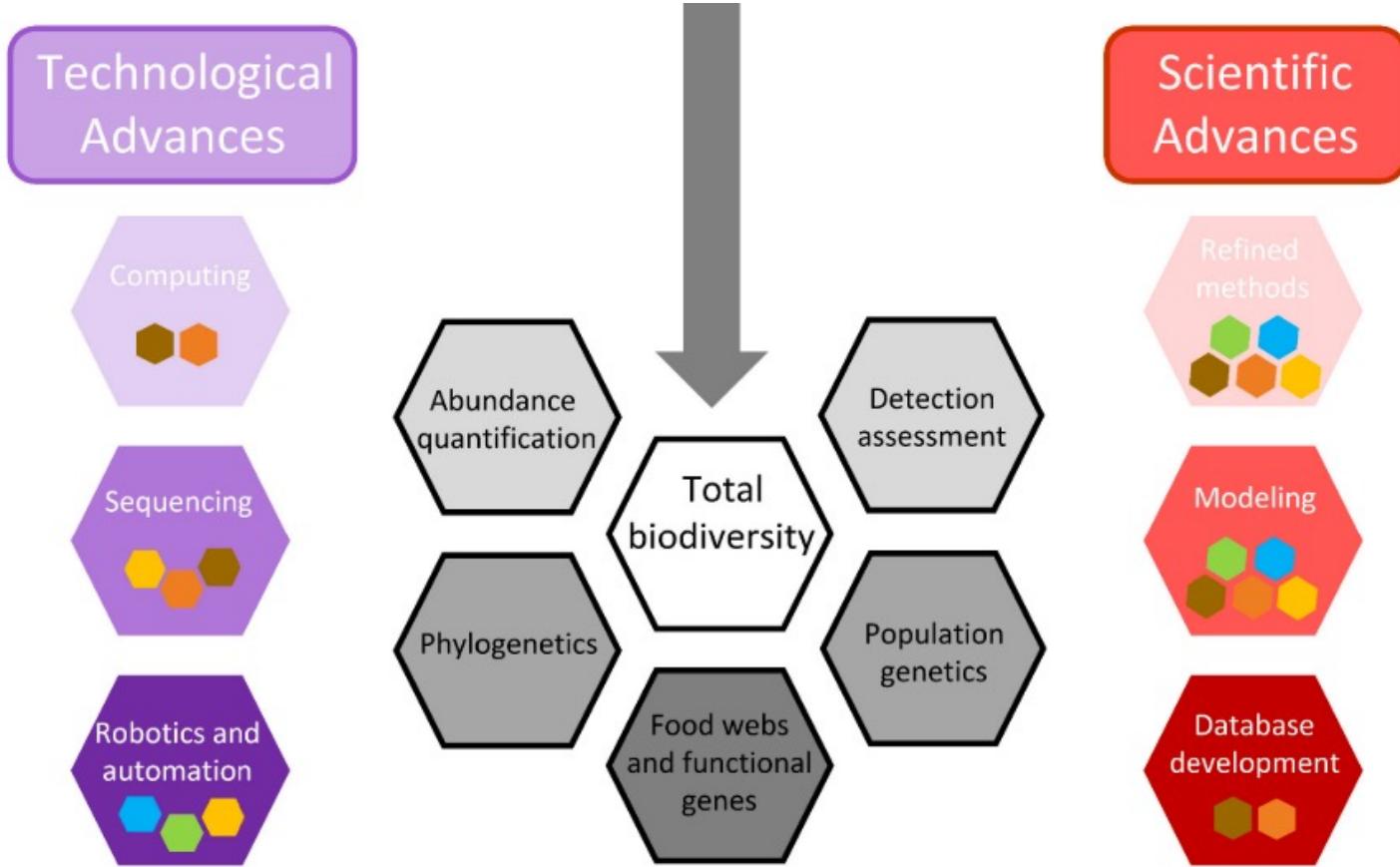


FIGURE 3 | Conceptual model outlining the DNA metabarcoding pipeline, including (1) sample collection, (2) DNA isolation and extraction, (3) high throughput sequencing, (4) bioinformatics, and (5) biodiversity analysis. Purple and red hexagons depict technological and scientific advances that will improve this workflow, with inset colored symbols reflecting the steps of the pipeline that will most benefit from these advances. The gray arrow indicates progress toward total biodiversity assessment (white) and other related advances (gray). Shape shading depicts advances and applications that range from those that are currently receiving a lot of attention and are achievable in the short term (light colors) to those that will require more research and development to achieve in the long-term (dark colors).

METABARCODING AND BIO MONITORING

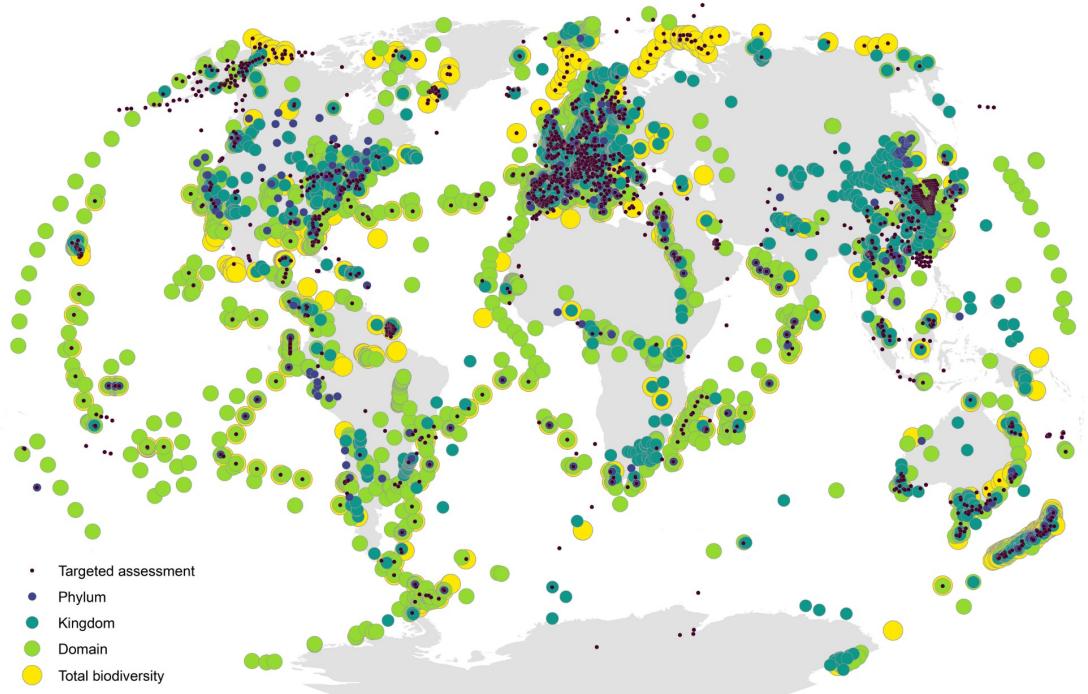


FIGURE 2 | Global map of metabarcoding sampling locations. Points are scaled and colored by the breadth of biodiversity covered, ranging from targeted assessment (class level or below) to total biodiversity assessment (samples using broad-coverage primers covering two or more domains). Data come from a systematic literature search of 1,563 published articles on DNA metabarcoding (**Supplementary Material 1**); for methodological details, see **Supplementary Materials 2, 3**.

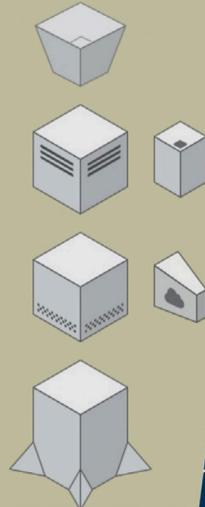
Compson, Z. G., McClenaghan, B., Singer, G. A., Fahner, N. A., & Hajibabaei, M. (2020). Metabarcoding from microbes to mammals: comprehensive bioassessment on a global scale. *Frontiers in Ecology and Evolution*, 8, 581835.

METABARCODING AND BIO MONITORING

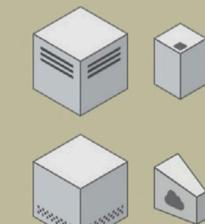
(A) Automated sampler and sequencing

Schematic of the key elements of an automated sampler and sequencer to be distributed across a global array of sample points

Sample mechanism



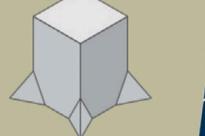
DNA extractor and reagents pack



Sequencer and communication pack

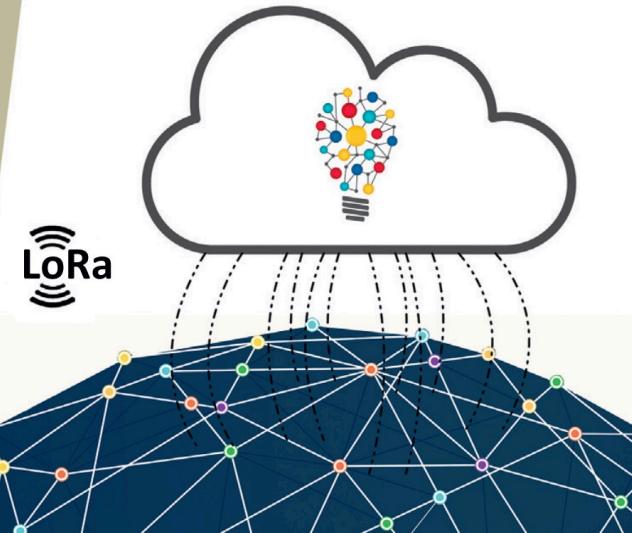


Battery/solar pack and processor



(B) Global array of samplers and in-cloud network reconstruction

Sequences in all uploaded samples are identified and the implicit interactions reconstructed into networks using machine learning in the cloud



(C) Analysis across highly-replicated networks

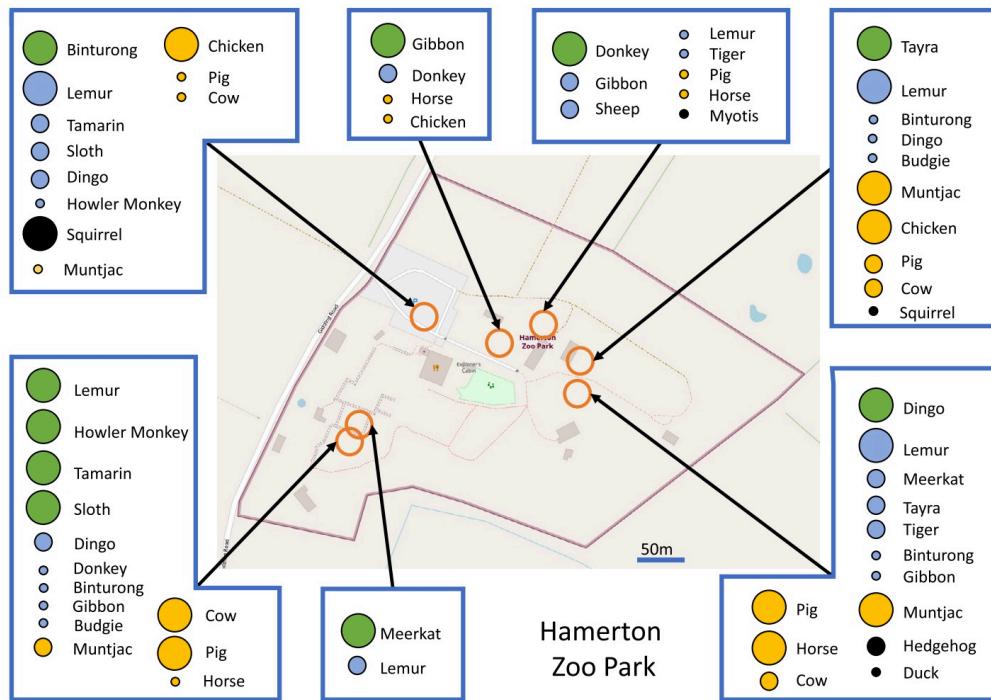
Detection of change in network structure, from analysis of variation between networks, across the sample array



Bohan, D. A., Vacher, C., Tamaddoni-Nezhad, A., Raybould, A., Dumbrell, A. J., & Woodward, G. (2017). Next-generation global biomonitoring: large-scale, automated reconstruction of ecological networks. *Trends in Ecology & Evolution*, 32(7), 477-487.

METABARCODING AND BIO MONITORING

● >10,000 reads ● >1,000 reads • >100 reads ● Target Species ● Other Zoo Resident ● Local Wildlife ● Dietary Items



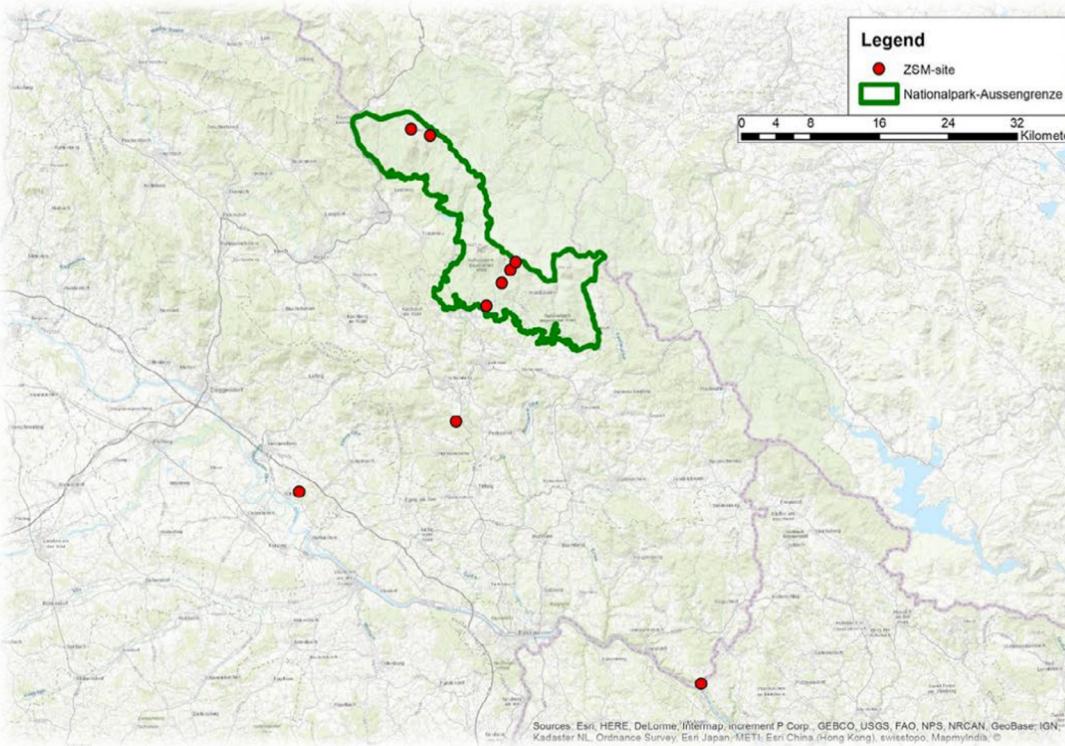
Clare, E. L., Economou, C. K., Bennett, F. J., Dyer, C. E., Adams, K., McRobie, B., ... & Littlefair, J. E. (2022). Measuring biodiversity from DNA in the air. *Current Biology*.

METABARCODING AND INVASIVE SPECIES



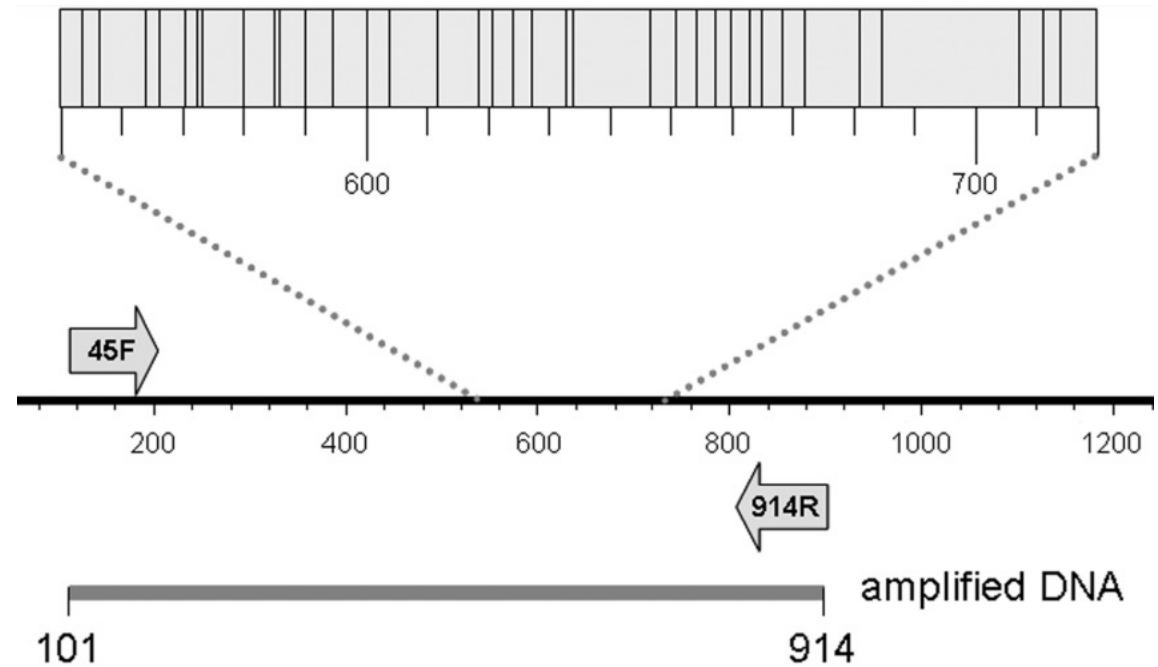
Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology letters*, 4(4), 423-425.

METABARCODING AND INVASION BIOLOGY



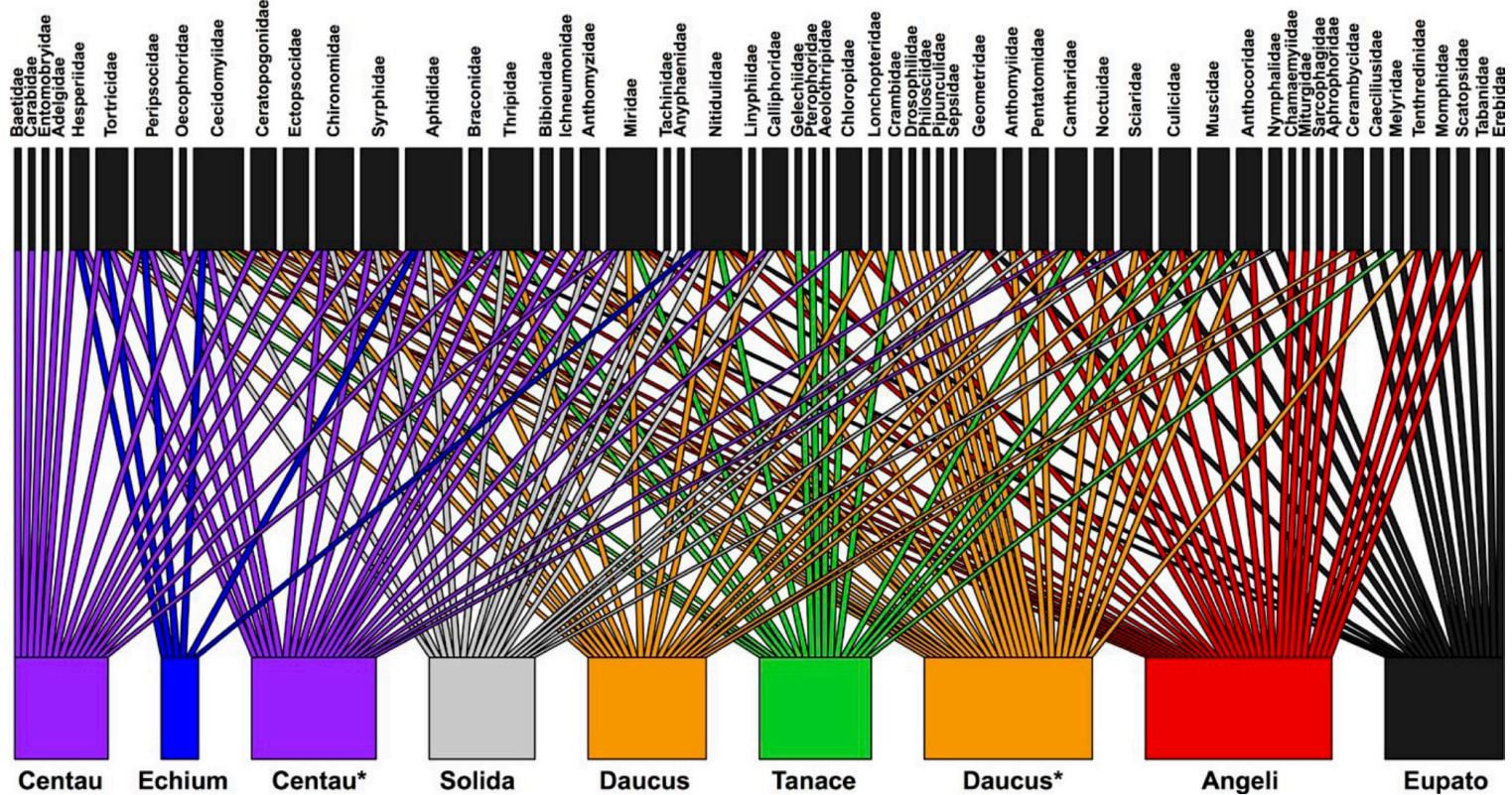
Hardulak, L. A., Morinière, J., Hausmann, A., Hendrich, L., Schmidt, S., Doczkal, D., ... & Haszprunar, G. (2020). DNA metabarcoding for biodiversity monitoring in a national park: screening for invasive and pest species. *Molecular ecology resources*, 20(6), 1542-1557.

METABARCODING AND INVASION BIOLOGY



Nagoshi, R. N., Brambila, J., & Meagher, R. L. (2011). Use of DNA barcodes to identify invasive armyworm *Spodoptera* species in Florida. *Journal of Insect Science*, 11(1), 154.

METABARCODING AND COMMUNITY STRUCTURE



Thomsen, P. F., & Sigsgaard, E. E. (2019). Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. *Ecology and evolution*, 9(4), 1665-1679.

METABARCODING AND ECOLOGY

Predators

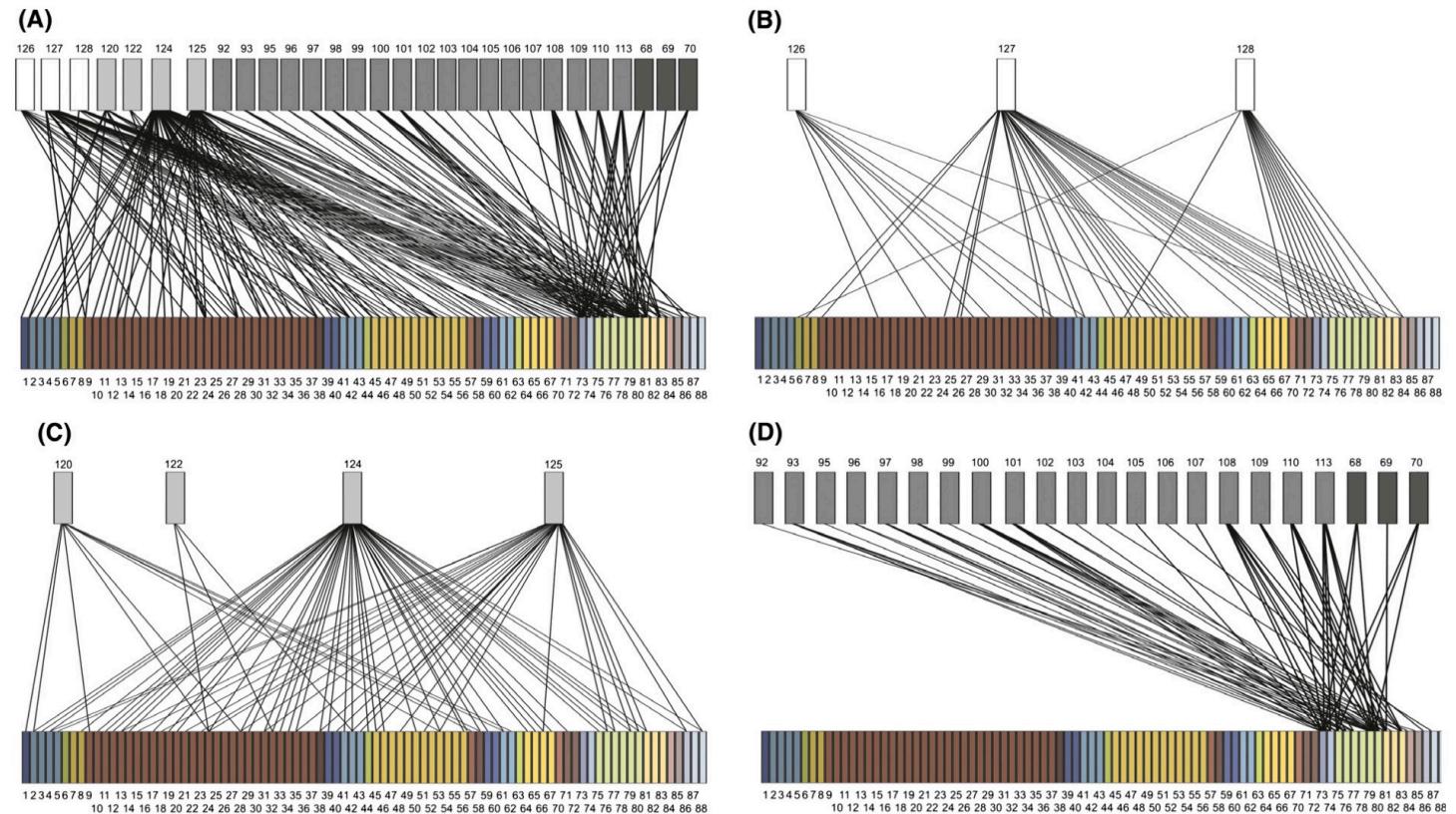
- Birds
- Spiders
- Parasitoid wasps
- Parasitoid flies

Diptera

- Agromyzidae
- Anthomyiidae
- Cecidomyiidae
- Ceratopogonidae
- Chironomidae
- Culicidae
- Dolichopodidae
- Empididae
- Heleomyzidae

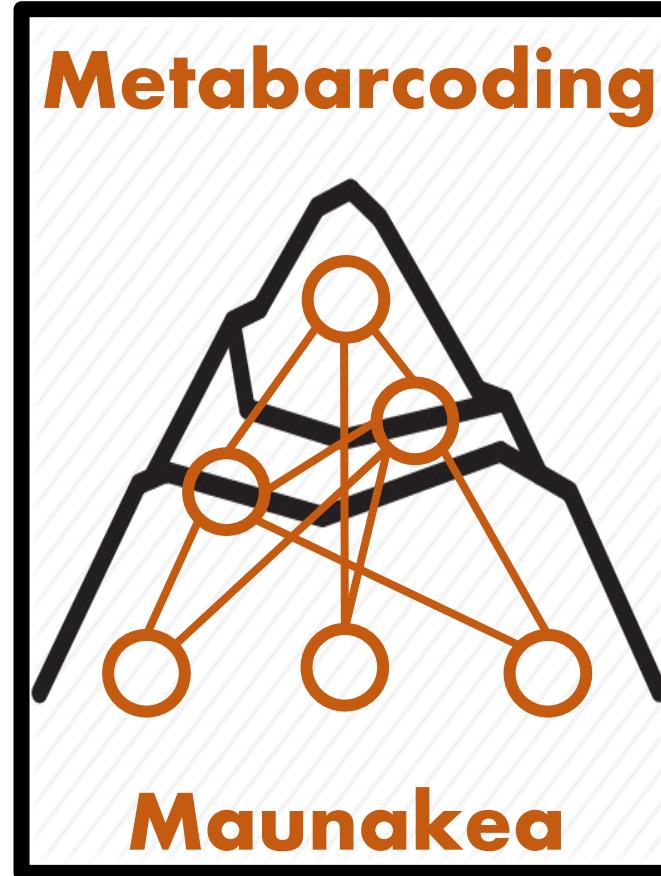
Lepidoptera

- Muscidae
- Mycetophilidae
- Noctuidae
- Nymphalidae
- Scathophagidae
- Sciidae
- Sphaeroceridae
- Syrphidae
- Tachinidae
- Tipulidae



Wirta, H. K., Vesterinen, E. J., Hambäck, P. A., Weingartner, E., Rasmussen, C., Reneerkens, J., ... & Roslin, T. (2015). Exposing the structure of an Arctic food web. *Ecology and Evolution*, 5(17), 3842-3856.

METABARCODING MAUNAKEA



C. Gillett ©

WHY MAUNAKEA?

- Restricted Assemblage of Taxa
- Available Biodiversity Data
- Biodiversity Concentrated in Arthropoda
- Active Efforts to Monitor Biodiversity
- Area of Conservation Concern



PROJECT OVERVIEW

STEP 1

Comprehensive Sampling of Arthropods

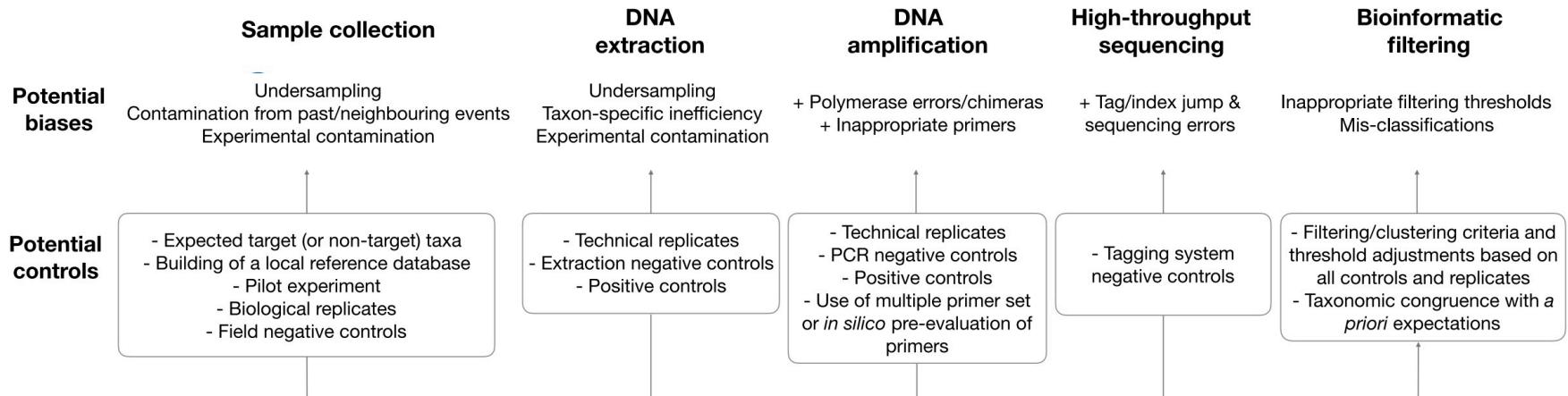
Maunakea Arthropods

STEP 2

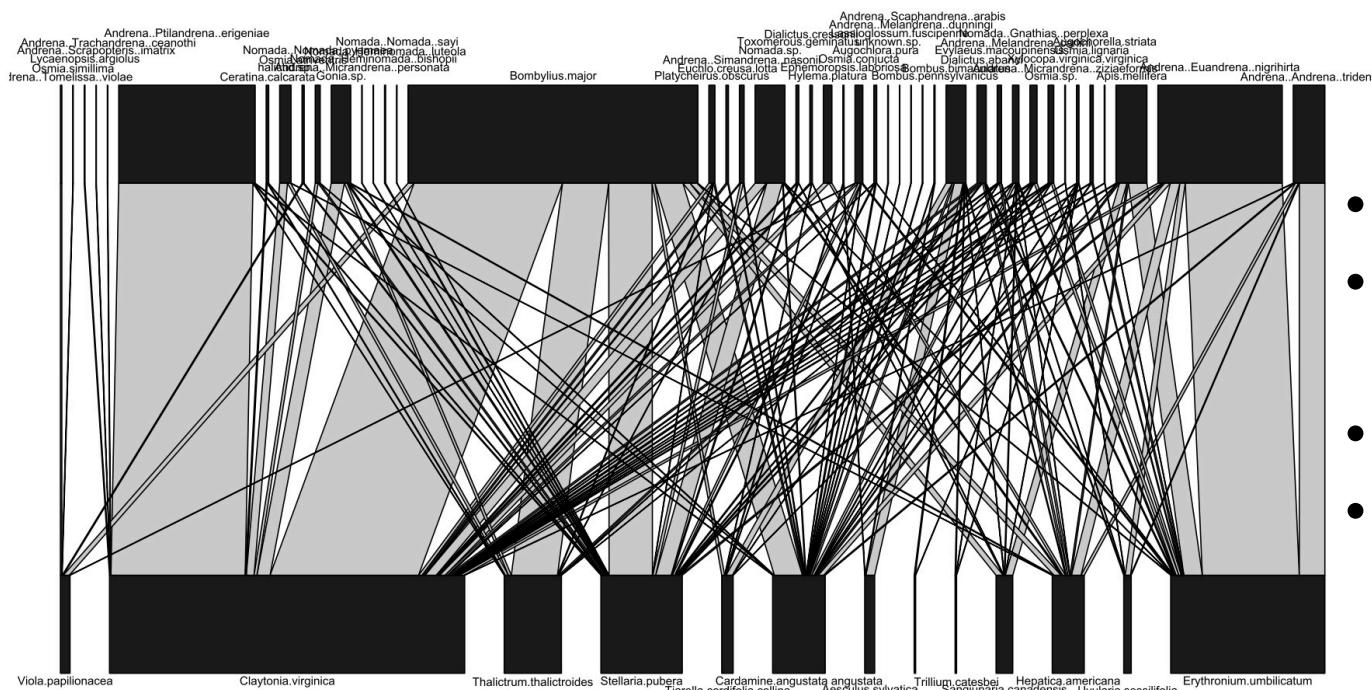
Local Reference Database of Arthropod Barcodes

STEP 3

Metabarcoding Arthropod Predators and Scavengers



ANALYZING METABARCODING DATA



- Connectance (Sensitivity)
- Degree/Degree Distribution (Node Importance)
- Diameter (Propagation)
- Modularity (Containment)

Fig. (1). A network matrix (top) and its bipartite graph (bottom). Shading of matrix entries indicates number of observations. Sequence of species is identical in both network visualizations (minimized crossing of lines). Dataset: Motten (1982).

Delman, E., Beslon, M., Briñez, M., Hogenboom, N., & Grubellier, B. (2009). MinHoots, graphs and Poisson trees (2019). Analyzing ecological networks of species interactions. *The Open Ecology Journal*, 2(1), 16-36.

THANK YOU FOR LISTENING

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