Identifying various aphid species from bulk insect samples using Mitochondrial Metabarcoding

Virgile Ballandras[1,2,*], Louise McNamara[1], James Carolan[2], Marta Niedzicka[1] and Stephen Byrne[1]

[1] Crop Science Department, Teagasc, Oak Park, Carlow R93 XE12, Ireland. [2] Biology department, Maynooth University, Maynooth W23 F2H6, Co. Kildare, Ireland.
*virgile.ballandras@teagasc.ie

Key Message

- We are developing a **non-destructive**, **DNA metabarcoding** assay that will enable the rapid identification of key aphid pests in Ireland from heterogeneous insect samples.
- This assay will be deployed in aphid monitoring programmes, especially for analysing the samples of **6 and 12 meter suction traps** which can catch **100s of aphids each day.**

Introduction

- Insect identification in monitoring programs is slow and laborious, and depends on taxonomic expertise.
- Molecular based methods can accelerate species detections and enable the identification of challenging specimens.
- We are currently developing a DNA metabarcoding assay for species identification in samples with high aphid abundance and diversity.
- We are initially focusing on the detection of 19 pest aphid species in Ireland, for which we have sequenced and assembled the mitochondrial genomes.
- We also validated a non-destructive DNA extraction method that allows follow up morphological verification for each specimen.

Methods

➢ Reference library

- For each species, we identified 10 - 15 aphids from our traps in order to create a HD picture library that confirms the accuracy of the morphological identifications.

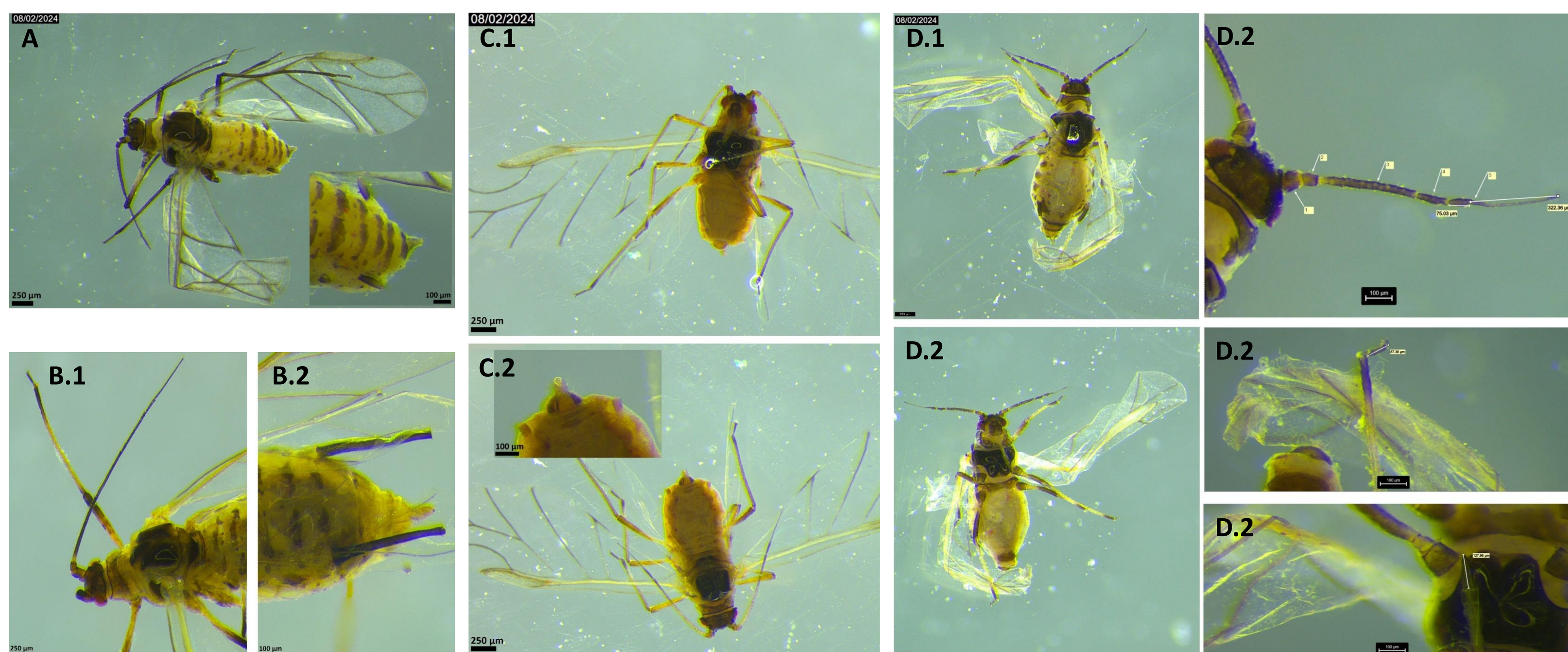


Fig 1: Sample of the picture reference library. For each individual, we focused on the identification criteria. All the pictures were realised using a Flexacam C3 mounted on a Leica M125 Stereoscope. All the pictures of the library are stack images generated with the Leica LAS X Software. The individuals displayed are belonging to the species: A. Brevicoryne brassicae, B. Sitobion fragariae, C. Hialopteris pruni, D. Rhopalosiphum insertum

- Whole genome sequencing of each of the 19 species was carried out using the vouched specimens.

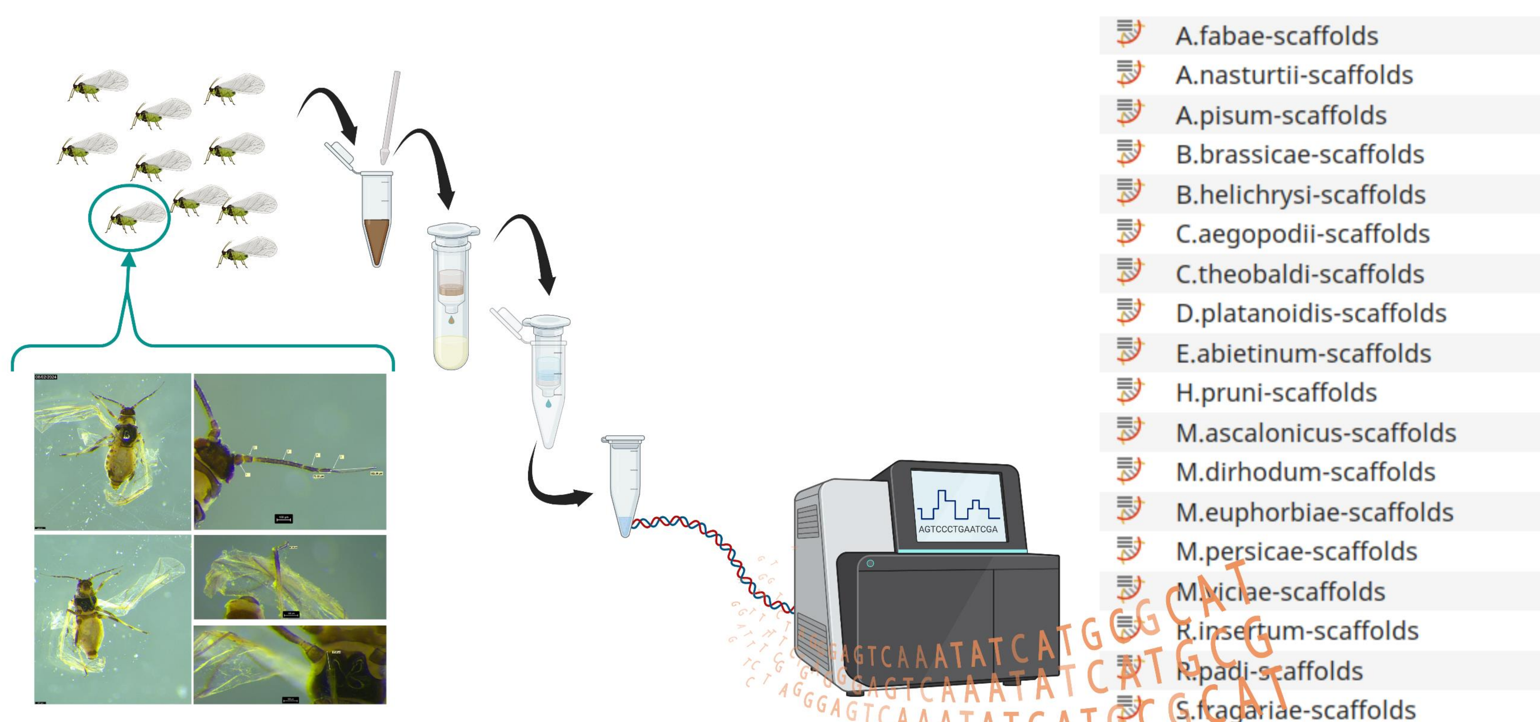


Fig 2: Process of the image/genome reference library generation

➢ Non-destructive DNA extraction

A protocol was validated by comparing the performances of two gDNA extraction kits:

- DNeasy Blood and Tissue Kits (QIAGEN)
- Monarch® genomic DNA purification kit (New England Biolabs)

Preliminary results

➢ Genome reference library

The raw sequence data were aligned to a reference mitogenome, this enabled us to characterise the intraspecific and interspecific diversity

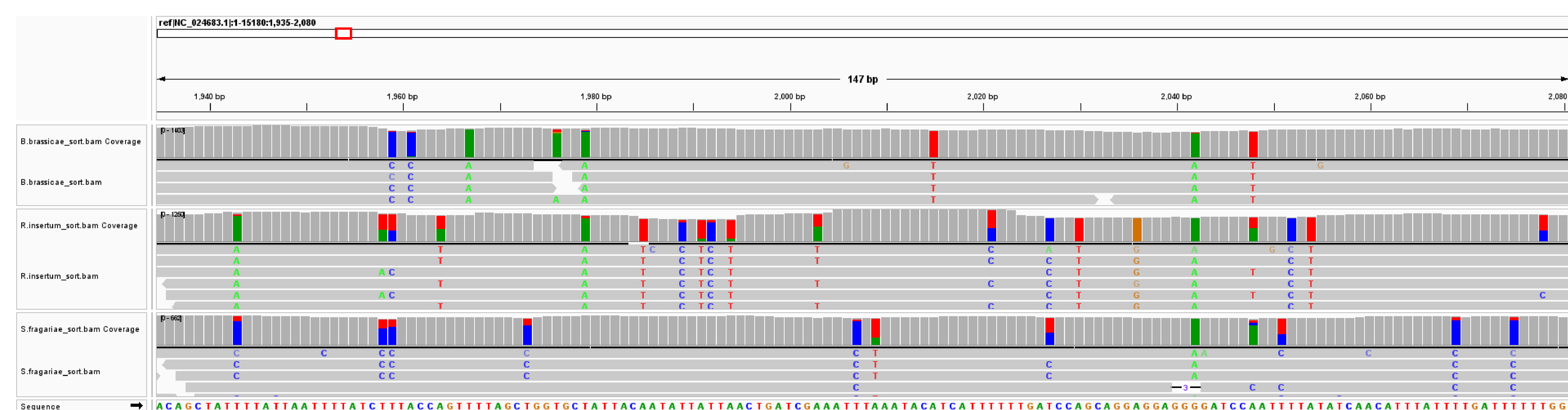
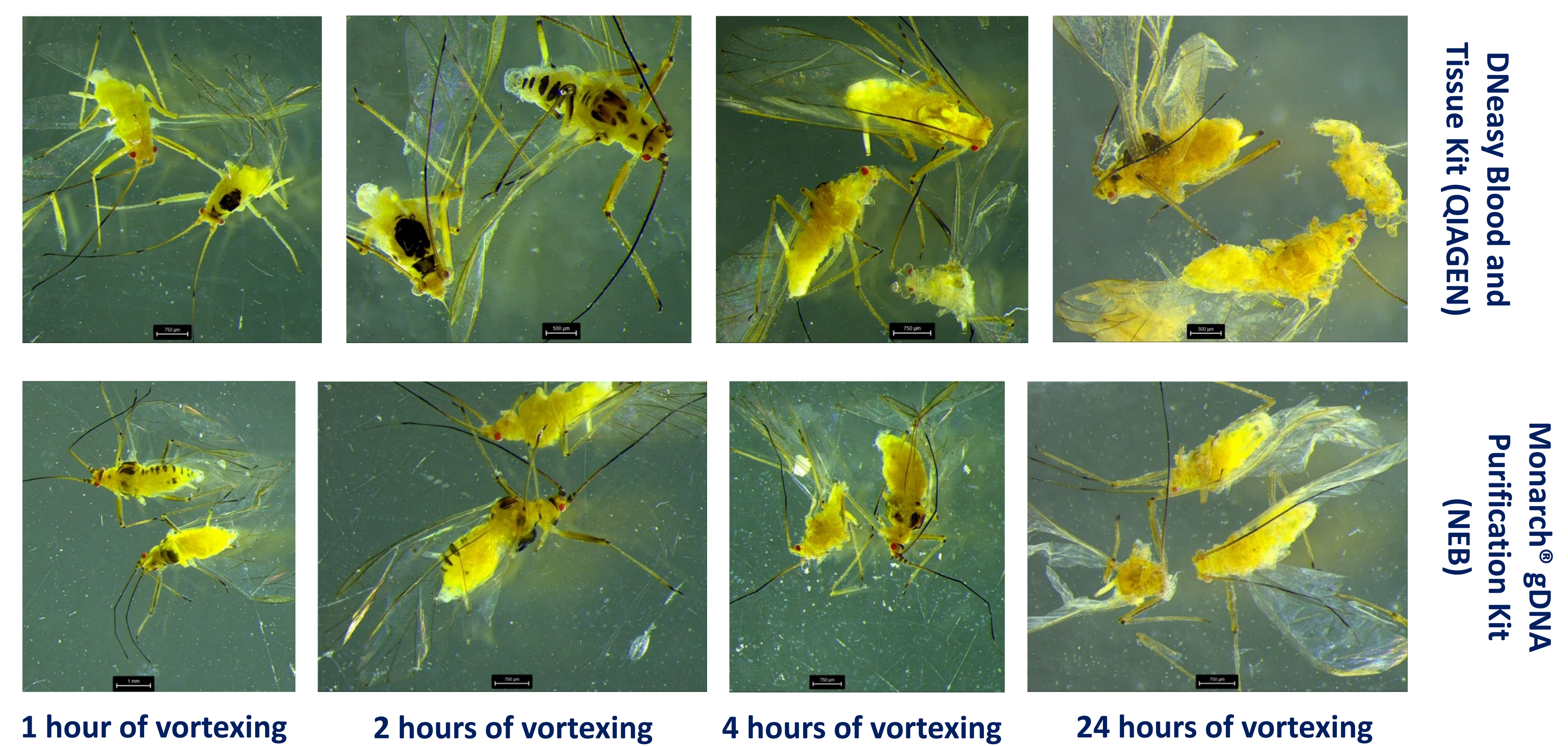


Fig 3: Visualisation of the genetic variations within and between three of the target species. The sequence data were aligned on a Sitobion avenae mitogenome from NCBI, (access number:NC_024683.1). The sequence displayed is a fraction of the COI gene, visualised on IGV

➢ Non-destructive DNA extraction



Vortexing time	gDNA (ng/µl) DNeasy kit	gDNA (ng/µl) Monarch kit
1 hour	3.21	5.58
2 hours	3.56	8.8
4 hours	8.56	22.8
24 hours	56	34.8

Fig 4: Non destructive DNA extraction was carried out by immerging 50 aphids from the same specie (Drepanosiphum platanoidis) into the respective kit's lysis buffer. For each kit we used five replicates that we placed in a thermomixer. The replicates varied in the amount of time they were vortexed at 1400 RPM, 56°C. Top : Pictures of the aphids taken after extraction. Bottom left: Picture of the QC gel with each vortexing time (NV: not vortexed). Bottom right: DNA concentration measured using a broad range Qubit assay for each vortexing time (concentrations for the not vortexed replicate were too low to be measured).

Toward the Assay's validation

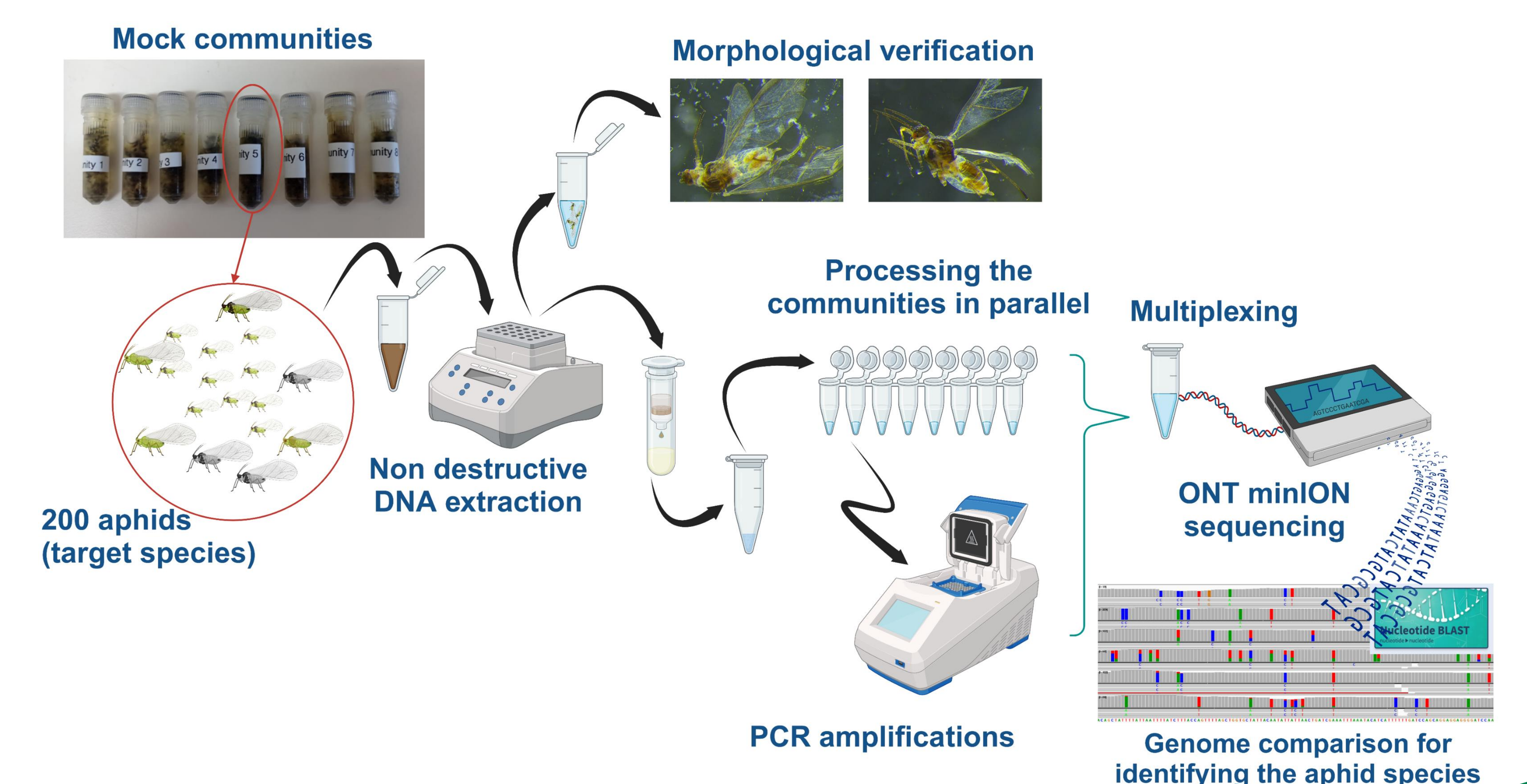


Fig 5: Process of the assay's validation: 8 mock communities have been composed by varying the number of individuals per species. We extracted their DNA using our validated method. We intend to use the Oxford Nanopore Technologies MinION sequencer to take advantage of the long amplicon sequencing capacity and also give full autonomy to our assay.

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