Early detection of invasive species using metabarcoding

LifeWatch ERIC NIS-IJI workflows (LW ES - LW PT)

Emilio de León-Cárdenas
Francisco Manuel Sánchez-Cano
Filipa MS Martins

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PART I. Validation case: NIS Metabarcoding

Key scientific questions

- Metabarcoding is a potentially useful tool for detecting non-native species (NIS) using eDNA samples.
- There is a the lack of standardized bioinformatic protocols and easy-to-use platforms.
- Can we rapidly detect the presence of NIS with metabarcoding?
- What is the error margin associated to NIS detection using eDNA regarding false negatives, i.e. failing to detect?

Data resources available

- Laboratory and bioinformatic procedures related with producing raw and filtered data sequences are being developed at CIBIO-InBIO.
- LIFEWATCH Portugal through CIBIO-InBIO and CESAM can facilitate access to national species lists regarding the presence of invasive alien species in aquatic environments for testing the protocols along the freshwater.

PART I. Validation case: NIS Metabarcoding

How?

- The Metabarcoding validation case describes research carried out in assessing populations of freshwater fish in the Douro Basin in Portugal.
- Collects fish eDNA sequences from metabarcoding water samples, which are processed through a bioinformatic pipeline wrapped
- Bioinformatic workflow conducts a quality check, assigns the DNA sequences to samples and produces a list of taxa.
- The taxa identified might include indigenous organisms as well as newly identified taxa within a certain geographical region.
- The national checklists of introduced and invasive species (GRISS) from GBIF are consulted to check if the organisms detected are recognised as NIS or if previously unrecorded NIS have been detected through eDNA metabarcoding analysis.

PART II. Workflow technical implementation

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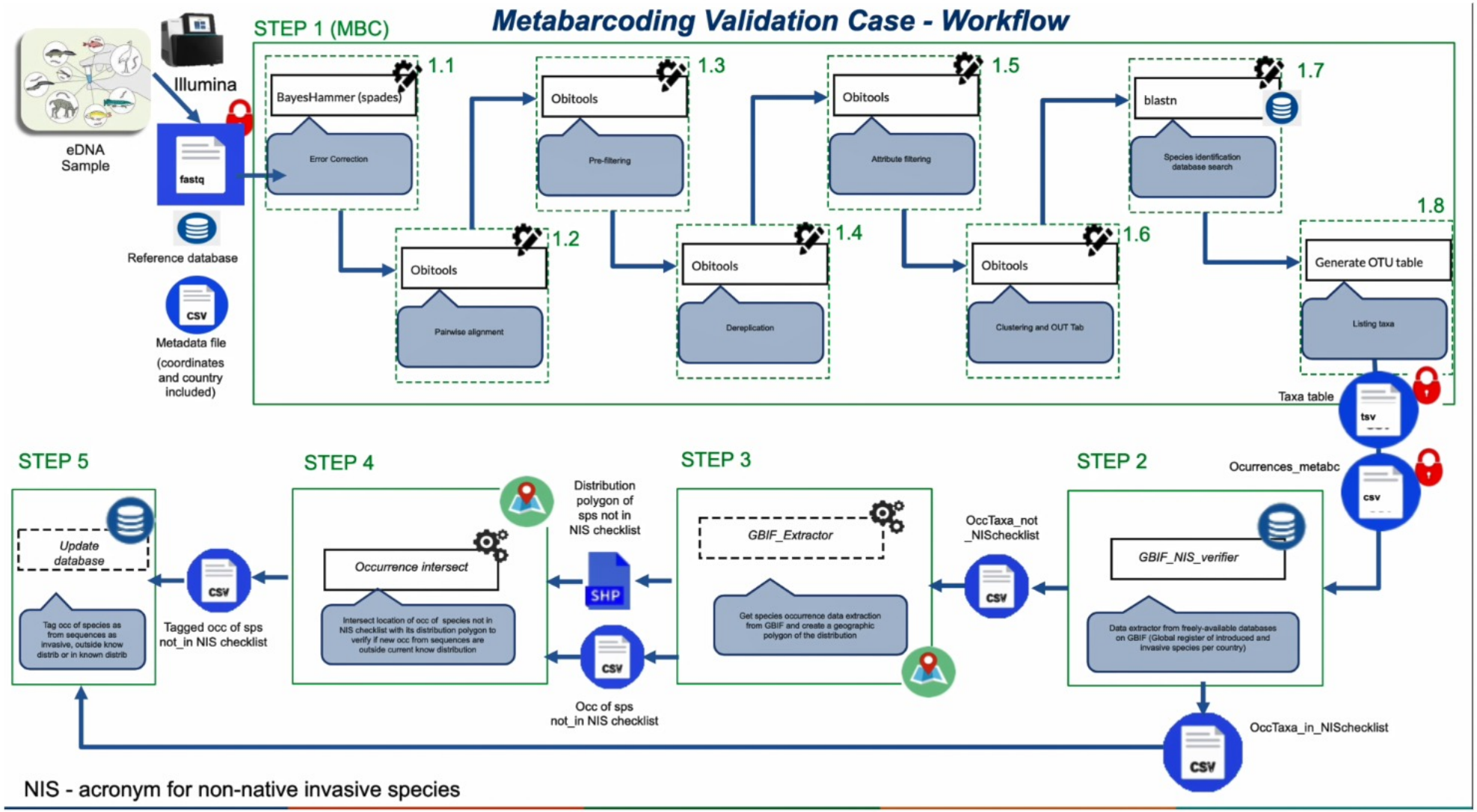
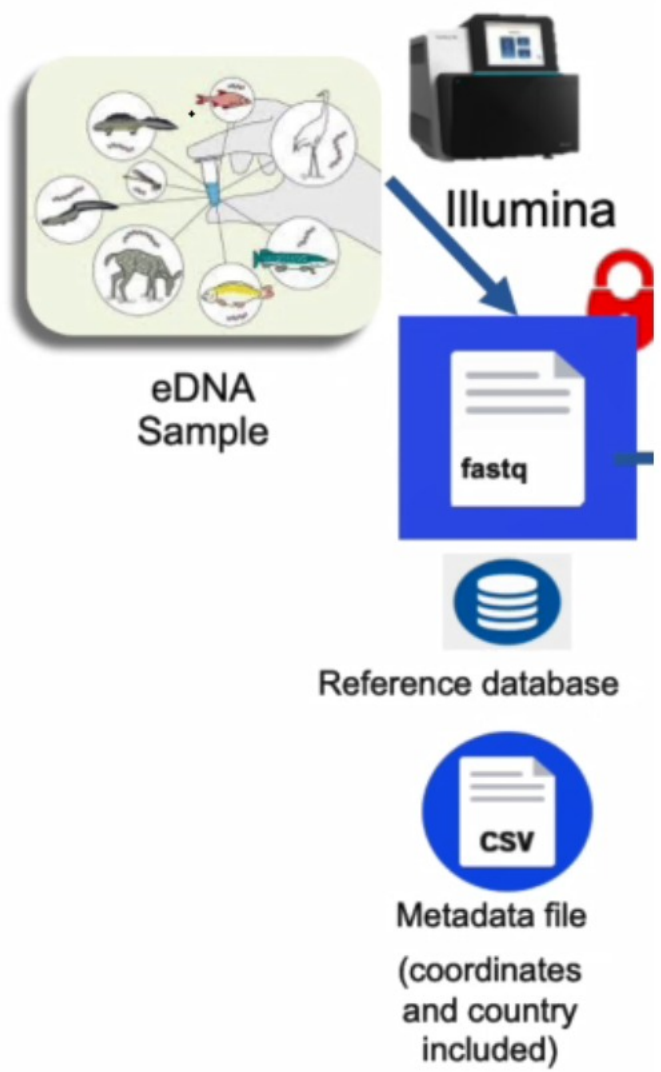
General schema of the Workflow



PART II. Workflow technical implementation

Input for metabarcoding WF:

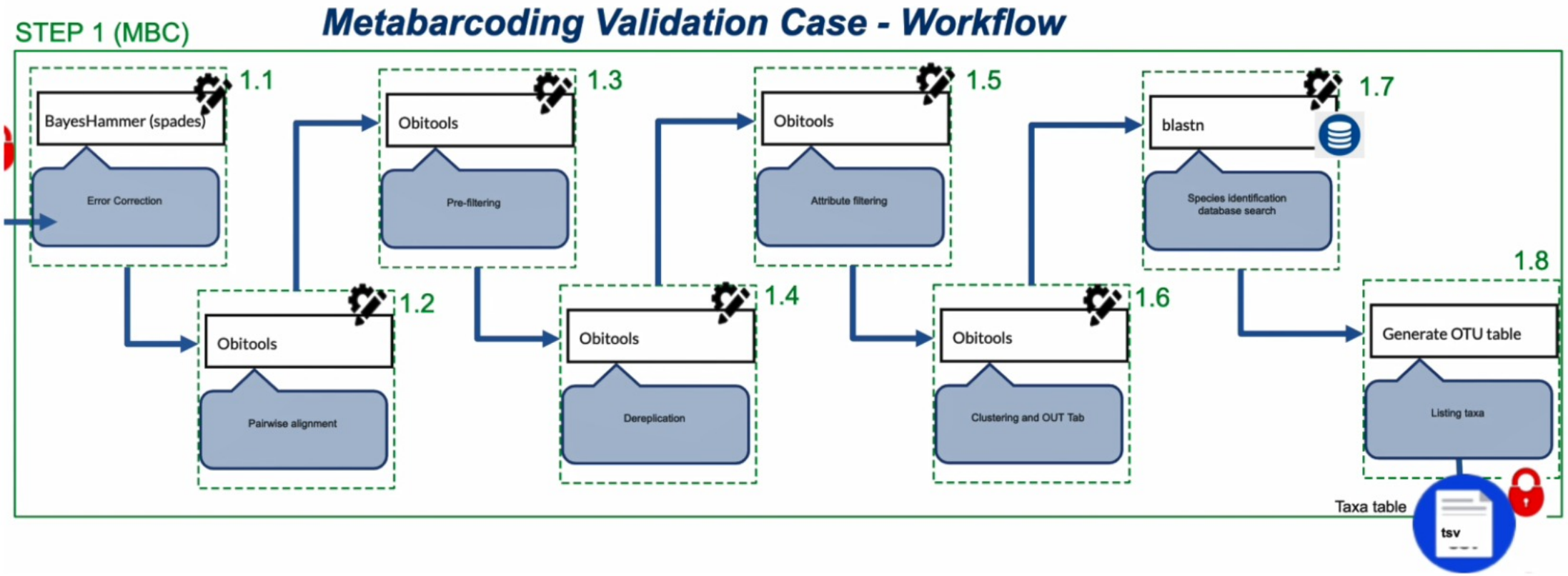
- Collect eDNA samples (water)
- Target eDNA fragments using group-specific amplification (PCR) protocols
- Tag samples using unique molecular identifiers ("barcodes")
- Generate molecular data (fastq) through Illumina sequencing
- Produce a metadata file with information of samples (including sampling and processing conditions)



PART II. Workflow technical implementation

Step 1 Process and filter the input (raw) molecular data.

Eliminate redundancies, correct errors based on quality, divide into samples, detect meaningful sequences, assign to taxonomy.



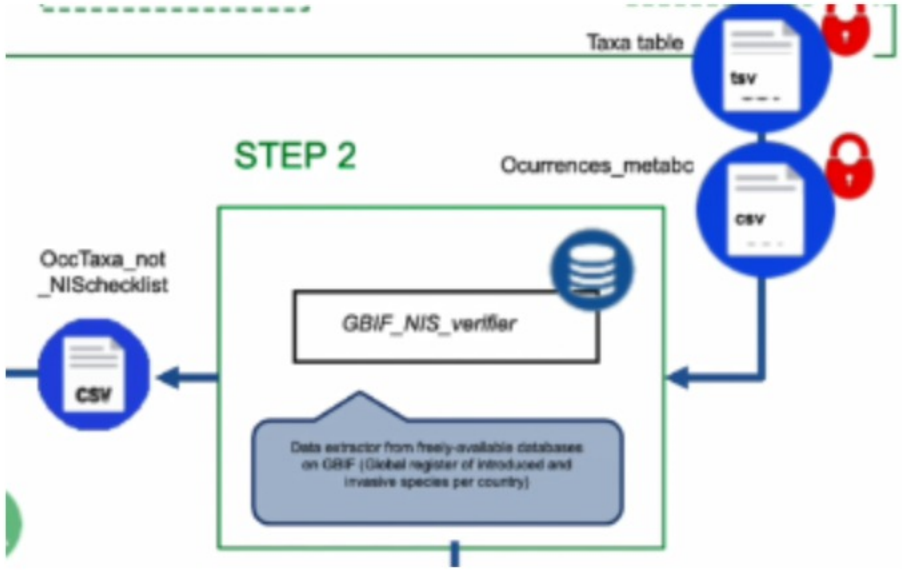
PART II. Workflow technical implementation

Step 2 Verify/detect NIS in the produced taxa list using GBIF:

- Identify which contain invasive species (NIS) and which are not.

- For samples with NIS, further analysis are conducted to know their geographical occurrence.

- Samples without NIS are ignored and sent the final step.



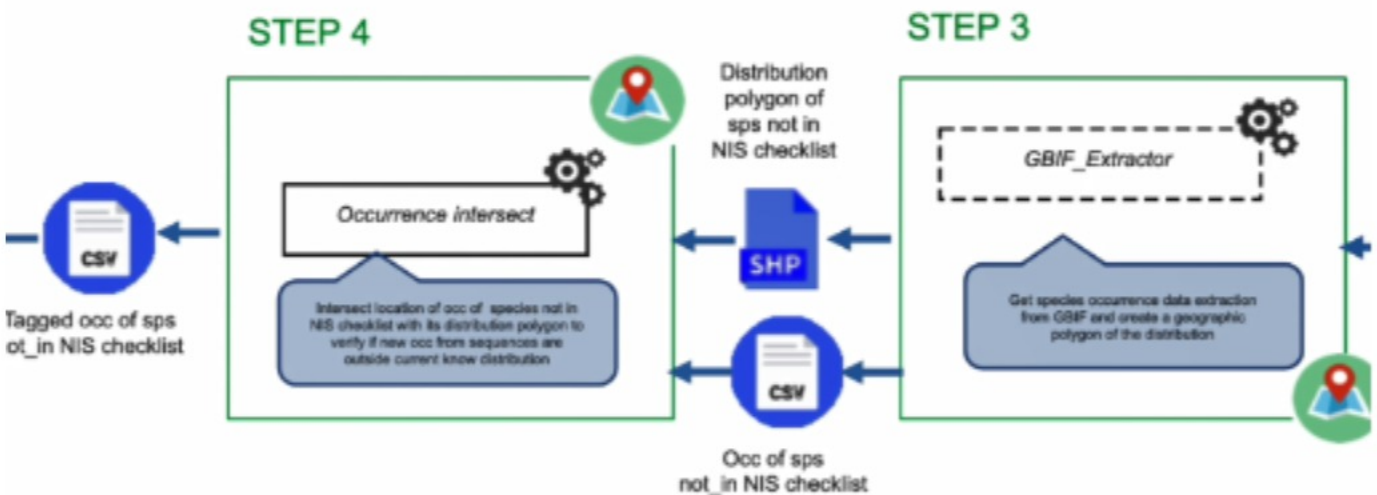
PART II. Workflow technical implementation

Step 3-4 Provide information on NIS

- Generates a SHP polygon with the samples where NIS have been found and how many have been found.

- Always using the GBIF databases.

Output: csv file with the generated information and the SHP file with the polygons.

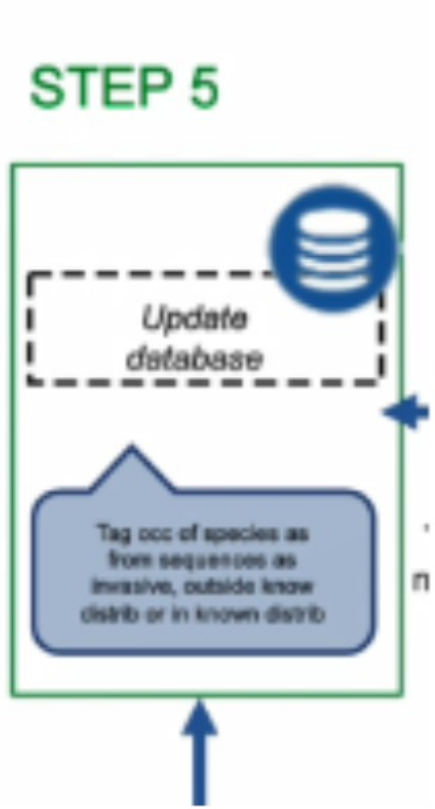


PART II. Workflow technical implementation

Step 5 Compile information of samples with and without NIS (geolocation, occurences)

- The WF Metagenomics database is updated with the results of the analyses.

- It is planned to generate a map viewer that makes use of the generated SHPs.



PART III. LifeWatch ERIC IJI-NIS VRE

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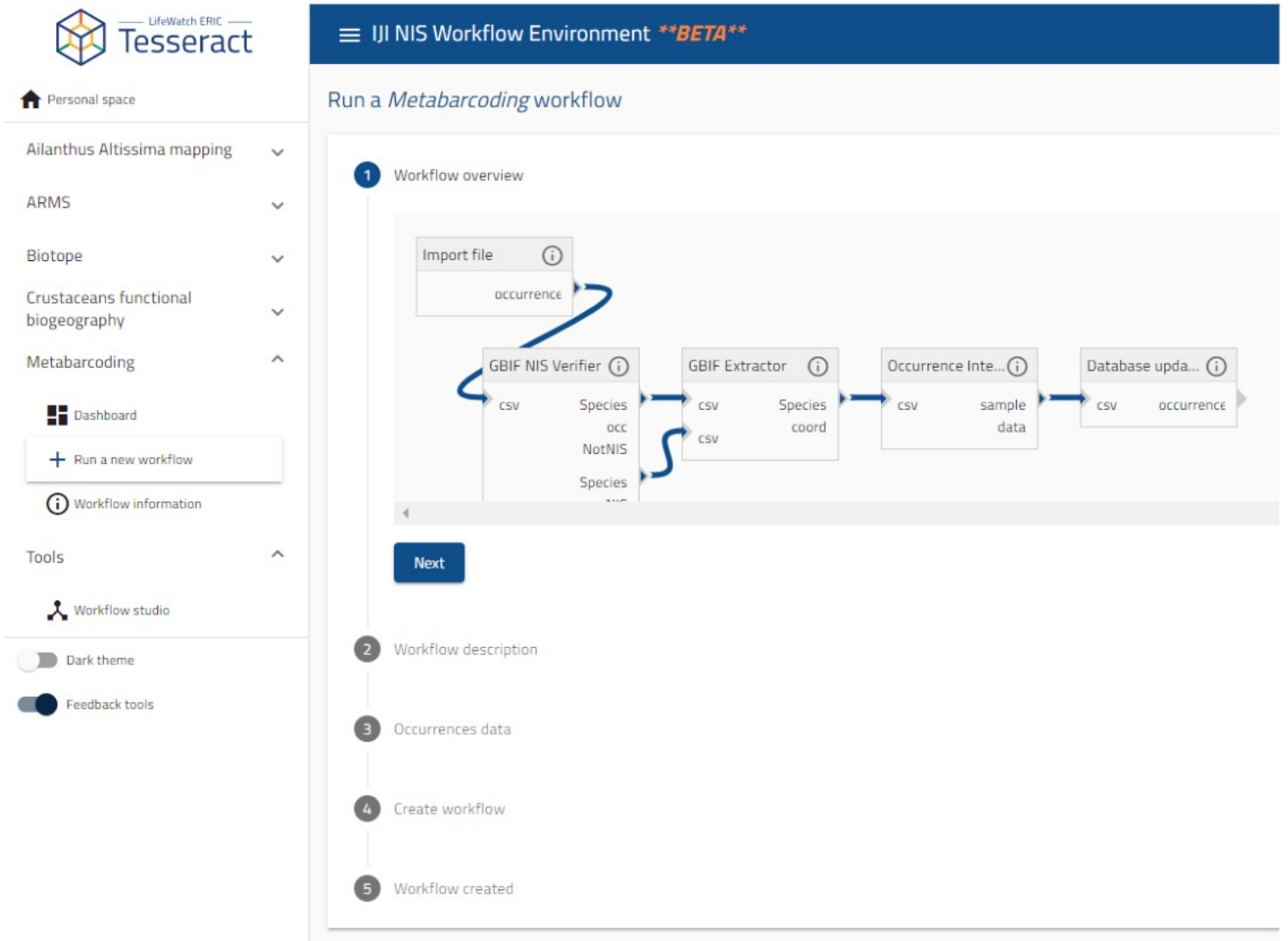
Run a new WF



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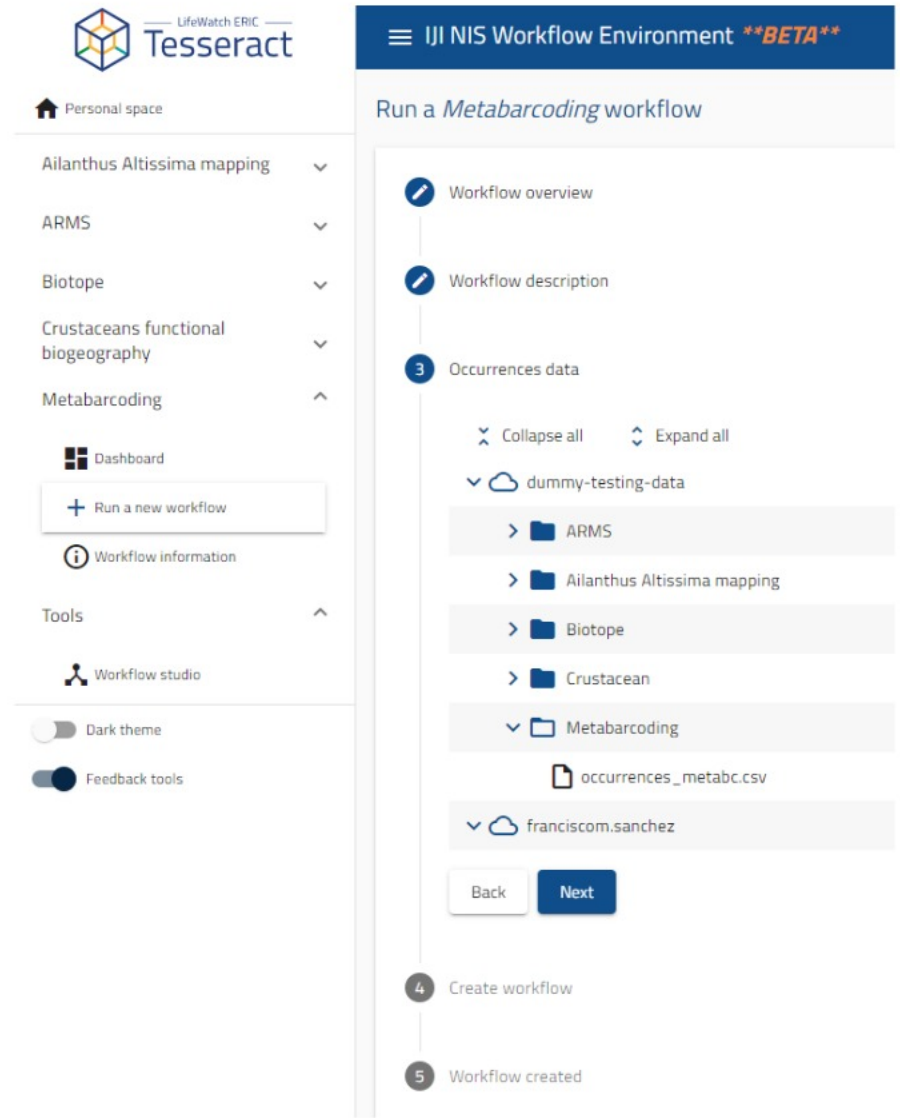
Provision of the input file:

- eDNA samples from Illumina
- Metadata with ocurrences

occurrenceID	scientificName	kingdom	taxonRank	decimalLatitude	decimalLongitude	eventDate	recordedBy	identifiedBy
30483dd2-4f9f-4410-96b5-839a14c9c193	Arundo donax	Plantae	SPECIES	390923	-80384	01/05/2020	A. Sousa	A. Sousa
74dd183b-f7e6-4940-9397-5328bb2e0d9d	Abutilon theophrasti Medik.	Plantae	SPECIES	400923	-70384	02/05/2020	A. Sousa	A. Sousa
5ebc6148-7620-46fa-a4ef-cf54ec792118	Acacia baileyana F.Muell.	Plantae	SPECIES	450923	-20384	03/05/2020	A. Sousa	A. Sousa
763208a8-317e-46ac-b147-2400a4157a50	Acacia cultriformis Cunn. ex Don	Plantae	SPECIES	430923	-40384	04/05/2020	A. Sousa	A. Sousa
c780dcb3-9d61-41a9-bbd0-e08d70cb7e8a	Acacia cyclops A.Cunn. ex Don	Plantae	SPECIES	400923	-70384	05/05/2020	A. Sousa	A. Sousa
0dd3f5da-c41c-4f50-a1b7-f3bf499c1aeb	Acacia dealbata Link	Plantae	SPECIES	400923	-70384	06/05/2020	A. Sousa	A. Sousa
29238892-6dea-407a-ac4d-12b7724a3561	Pinus pinea	Plantae	SPECIES	440923	-30384	07/05/2020	A. Sousa	A. Sousa
6c62ba6f-e386-491a-9586-9efd3775edd8	Pinus pinaster	Plantae	SPECIES	400923	-70384	08/05/2020	A. Sousa	A. Sousa
731d57b1-58e1-446a-973e-80590f57c875	Quercus suber	Plantae	SPECIES	400923	-70384	09/05/2020	A. Sousa	A. Sousa
0568f41c-9774-4e47-9382-8625e8aa478f	Hypnum cupressiforme	Plantae	SPECIES	-73	200	10/05/2020	A. Sousa	A. Sousa



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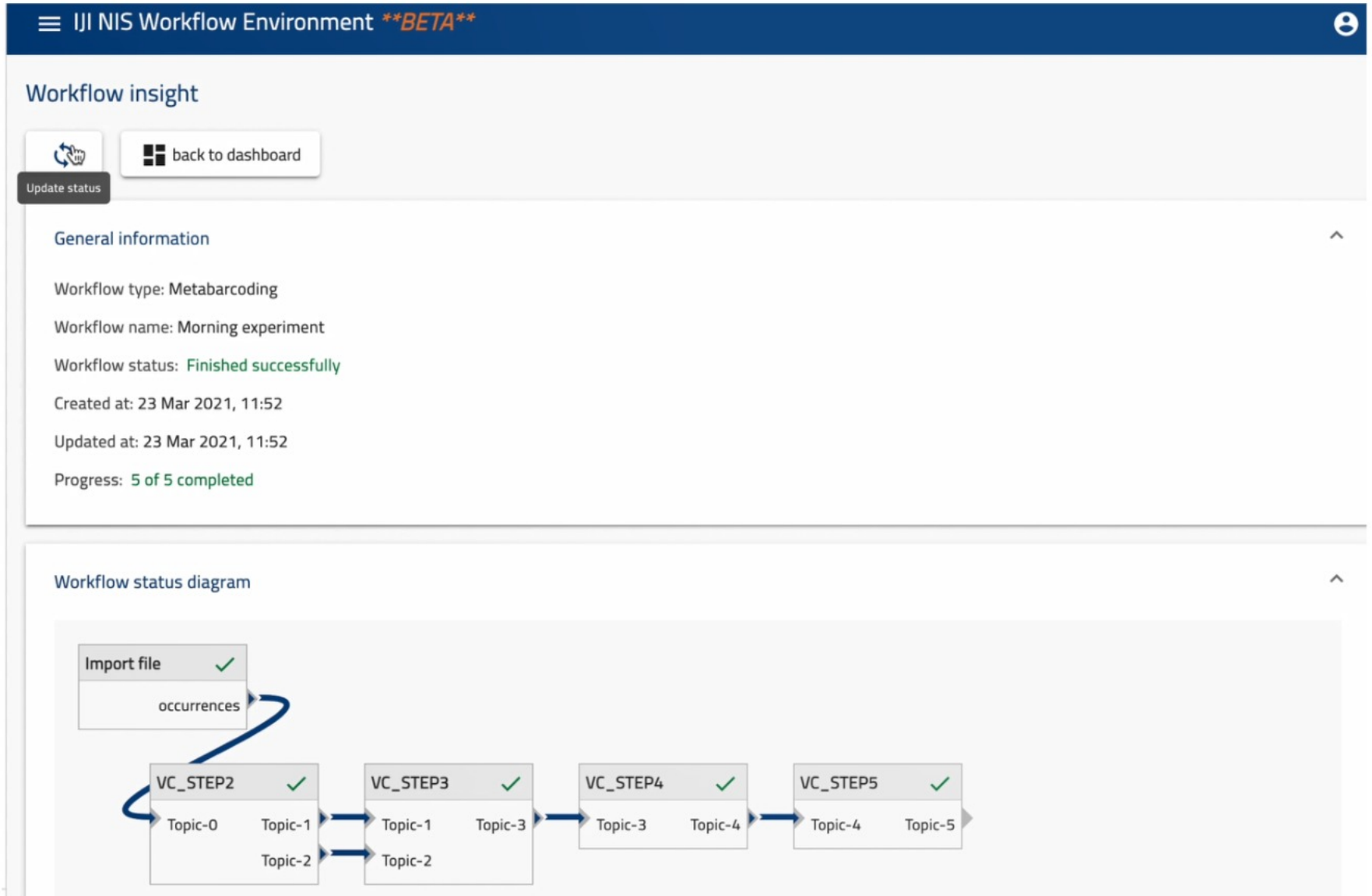
Run the WF



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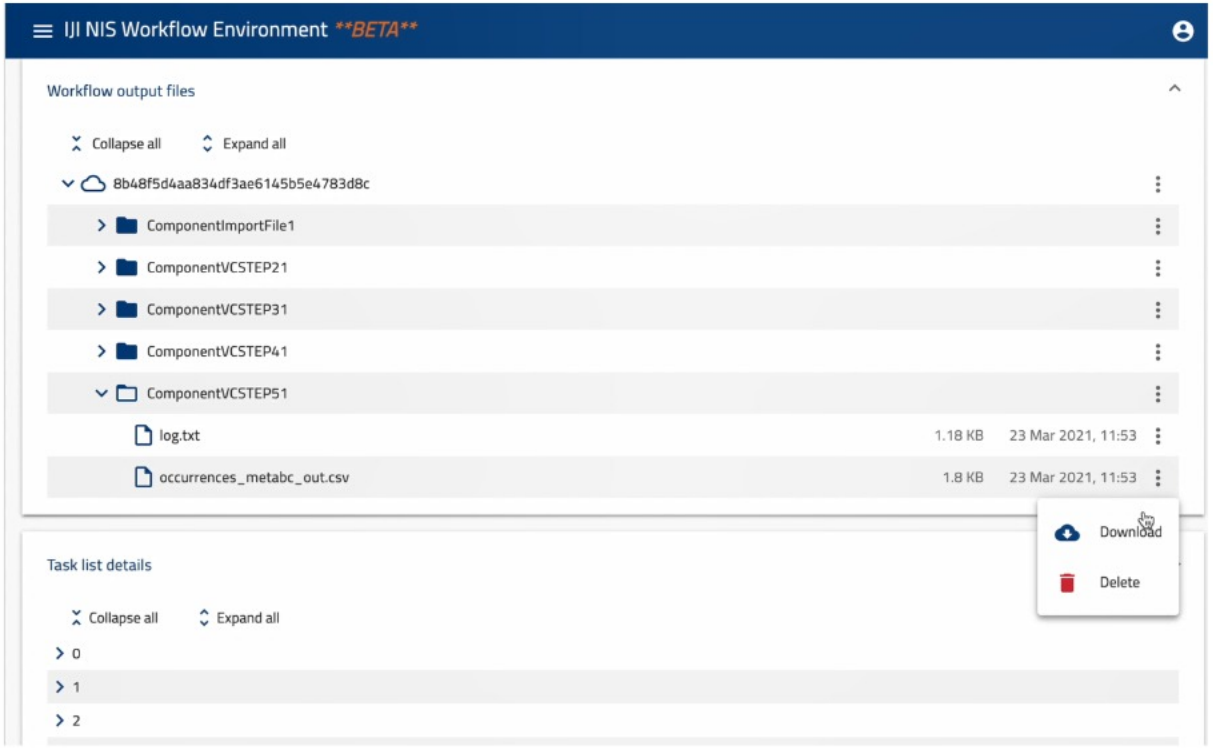
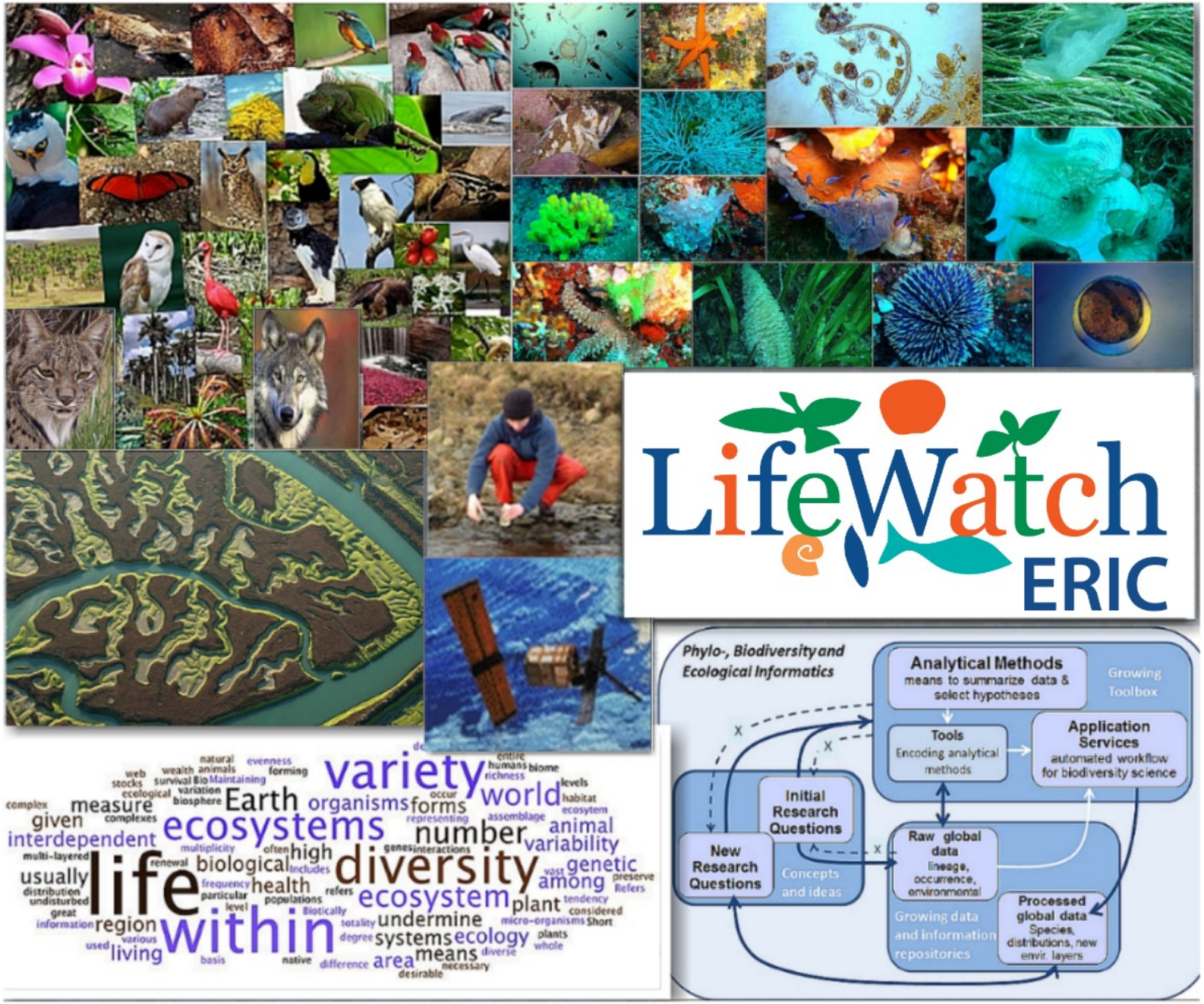
Final Output:
- NIS and ocurrences.
- Indigenous species.



Column1	decimalLatitude	decimalLongitude	eventDate	recordedBy	identifiedBy	urllocaltion	keyword	taxonKey	isInDistribution
1	390923	-80384	01/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=39.0923&lng=-8.0384	country_PT	2703041	1
2	400923	-70384	02/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=40.0923&lng=-7.0384	country_PT	3152614	1
3	450923	-20384	03/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=45.0923&lng=-2.0384	country_FR	2981303	0
4	430923	-40384	04/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=43.0923&lng=-4.0384	country_ES	2979559	1
5	400923	-70384	05/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=40.0923&lng=-7.0384	country_PT	2980425	0
6	400923	-70384	06/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=40.0923&lng=-7.0384	country_PT	2979474	1
7	440923	-30384	07/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=44.0923&lng=-3.0384	country_ES	5285165	1
8	400923	-70384	08/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=40.0923&lng=-7.0384	country_PT	5285565	1
9	400923	-70384	09/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=40.0923&lng=-7.0384	country_PT	2879411	1
10	-73	20	10/05/2020	A. Sousa	A. Sousa	https://api.gbif.org/v1/geocode/reverse?lat=-73&lng=20	country_AQ	2681972	0



Thank you very much !

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