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DecliNe project

Report n°3

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Research contract for the reconstruction of historical population sizes of insects and birds

Between

Bundesamt für Umwelt BAFU
Office fédéral de l'environnement OFEV

and

muséum
Genève

INTRODUCTION

On a global scale, numerous studies have highlighted a population decline of the entomofauna and avifauna potentially associated to various factors such as the intensification of land use, the introduction of pesticides and the habitat fragmentation, just to cite a few (Outhwaite et al. 2020; van Klink et al. 2020; Goulson 2019). Neighboring countries such as Germany have produced abundance data across the last 30 years, and have shown a mean decrease of 75% in insect biomass in protected areas (Hallmann et al. 2017).
In Switzerland, a recent report by the Swiss Academy of Sciences (SCNAT) summarizes the observed decline of the more than 30 000 species known to date, at least partly due to agricultural practices, and emphasizing the critical situation of Swiss insect populations, based on red lists, monitoring programs and other scientific studies -sources of information which are highly impacted by time series and reference point bias. The report stresses (i) the urgent need to move beyond the beginning of biodiversity monitoring programs, in order to address the long and middle term tendencies, and (ii) the need to provide faster and more effective tools for species and within-species diversity monitoring at present. The "decliNe" project builds on cutting-edge museum genomics (Suchan et al. 2016; Schmid et al. 2018; Gauthier et al. 2020) to study local and global population size fluctuation in Switzerland since the begging of the 20th century, using population genetic estimators as proxies, notably the genetic diversity, the inbreeding coefficient and the effective population size. It will thus be possible to assess the dynamics of population sizes in many populations of insect in agricultural environments. In addition, other estimators obtained from museum genomics data, and in particular genetic diversity factors, will make it possible to estimate the potential resilience of populations to environmental disturbances. The nationwide scale of the project coupled with the back-in-time research make the decliNe project a unique opportunity to track the massive genetic erosion in insects, providing a deep retrospective of human impact in insect populations along the 20th century and to suggest key guidelines for future environment management.

In the first phase of the project, we pre-selected ten species out of a list of 33 candidate species after extensive consultation with the main Swiss museum institutions hosting entomological collections (Basel, Bern, ETHZ, Geneva, Lausanne) and experts of the Swiss Biological Records Center (CSCF). To match our selection criteria, species should be widely distributed in Switzerland, highly represented in national collections and somehow related to anthropic environments. The goals of the second phase were to complete and gather the databases of collection specimens of those species, to identify the localities that were abundantly sampled in the past for the selected species (hereafter referred to as historical populations) and to perform most of the required fieldwork to achieve the aims of the decliNe project. In the third phase of the project, summarized in this report, our goals were (i) performing the sampling of the collection specimens constituting the historical populations, (ii) concluding the field work and (iii) starting the laboratory work (i.e. DNA extraction) and set up the significant next phase of the project, the library preparation.

HISTORICAL POPULATION IDENTIFICATION

Aiming at monitoring the status of swiss insect populations at different points in time and space, in the previous phases of the project we built up an extensive database of all individuals of the targeted species conserved in the main Swiss collections. The detailed analysis of this database allowed us to identify the species and historical population to be included in the project. The initial species selection was *Harpalus rufipes, Cetonia aurata, Polyommatus icarus, Pieris napi, Vanessa atalanta, Chorthippus parallelus, Omocestus viridulus, Miramella alpina, Baetis alpinus* and *Serratella ignita.* The analysis applied broadly consisted in the assembly of all individuals sampled within an area of 10x10 km (the same grid used by the CSCF) in a period of 5 years. If the total number of individuals was higher than 6 at any time before 1950, the grid cell was classified as containing a historical population and was subsequently selected in this study (Figure 1). Given the differences of life cycle, dispersion abilities and habitat between terrestrial and aquatic insects, for *Baetis alpinus* and *Serratella ignita* the individuals were assembled according to the river they were sampled.

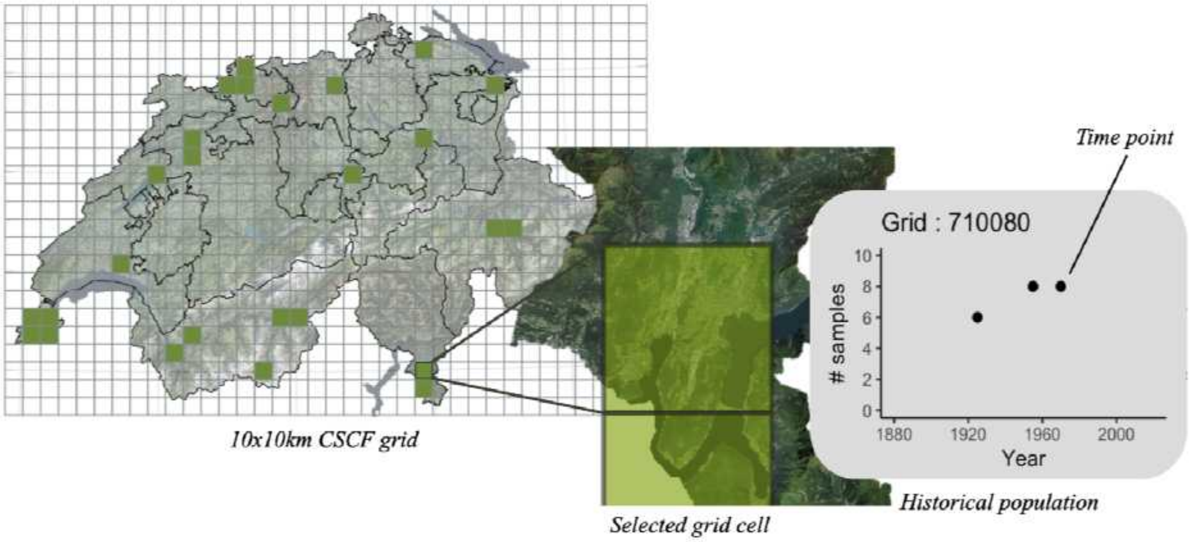

Figure 1. Representation of the 10x10 km CSCF grid cell used, the selected grid cells and an example of historical population and time points for the species Polyommatus icarus. After the addition of the specimens sampled in the same grid cell in a 5-year period basis, only those grid cells having more than 6 individuals at any time before 1950 were selected. Those collection specimens form an historical population composed of different time points.

Despite the good results obtained for the near majority of the species, the identification of only two historical populations for *H. rufipes* resulted in its exclusion from the project. Despite the elimination of one species, the number of samples considered still exceeded 3 500 (i.e., 3 732). Subsequently, we chose to perform a sub-selection of the samples while maintaining a comprehensive sampling and maximizing the quality of the produced data. A set of criteria was considered to restrict the number of samples:

- When more than 3 consecutive time points for one historical population were found, we removed the middle time point.
- Historical populations composed by only one time point were eliminated if the adjacent historical populations displayed a richer time series going further back in time.

- In the particular case of *C. parallelus,* whose historical populations create a large solid cluster in the canton of Grisons/Graubunden, we chose the historical populations with the richest time series and homogeneously dispersed, covering the maximum of the region.

Table 1. Number of total cell grids with historical populations, number of time points and historical individuals (=collections specimens) for the terrestrial species.

Species	**Nb grid cells**	**Nb time points**	**Nb historical individuals**
Polyommatus icarus	25	54	462
Pieris napi	11	20	179
Chorthippus parallelus	14	31	262
Omocestus viridulus	12	12	96
Miramella alpina	7	9	73
Cetonia aurata	14	33	289
Vanessa atalanta	1	4	40
Total historical	84	163	1 401
Total fresh	84		840
TOTAL	**840**	**247**	**2 241**

For the aquatic species *Baetis alpinus* and *Serratella ignita* (Ephemeroptera)*,* the sub-selection was determined by the hydrological features on which we based the definition of historical populations. We evaluated the distance between the time points of an historical population as well as their distribution along the stream. Ten kilometers was considered as the maximum distance accepted between time points.

Table 2. Number of waterbodies with sampling stations and historical specimens considered for the two targeted aquatic species.

Species	**Nb water bodies**	**Nb time points**	**Nb historical individuals**
Baetis alpinus	8	20	109
Serratella ignita	11	41	372
Total historical	15	61	481
Total fresh			210
TOTAL	**15**	**82**	**691**

In the case of *Vanessa atalanta,* a migratory species, the analyses were performed at the national scale, considering all Swiss individuals as belonging to one single population. Subsequently, the number of historical time points found (i.e., 14) was substantially higher compared to the other species (see Figure 2). We selected those time periods whose samples were homogeneously distributed in the Swiss territory: 1910, 1950, 1960 and 1970 (Figure 3).

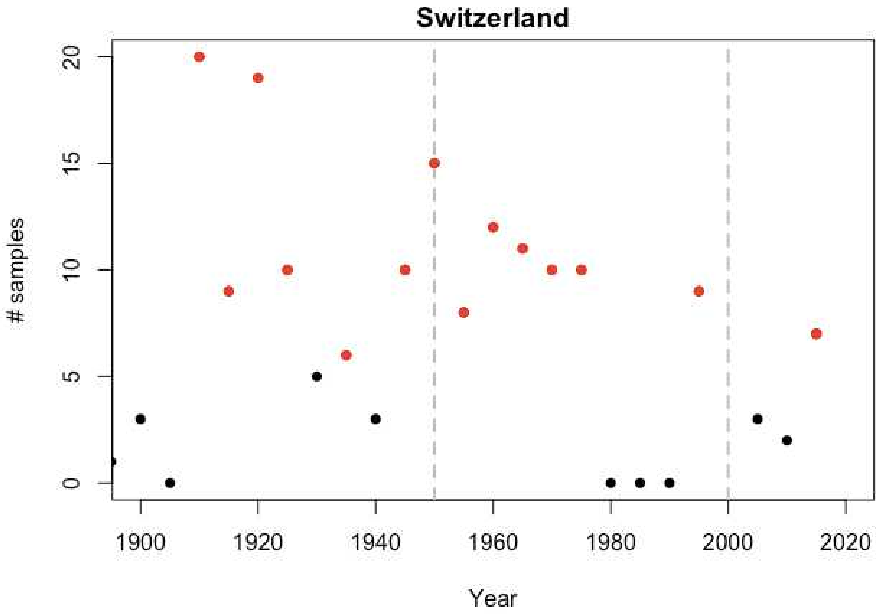

Figure 2. Number of samples per 5-year period for Vanessa atalanta *(red dots = above 5, black dots = 5 or less individuals).*

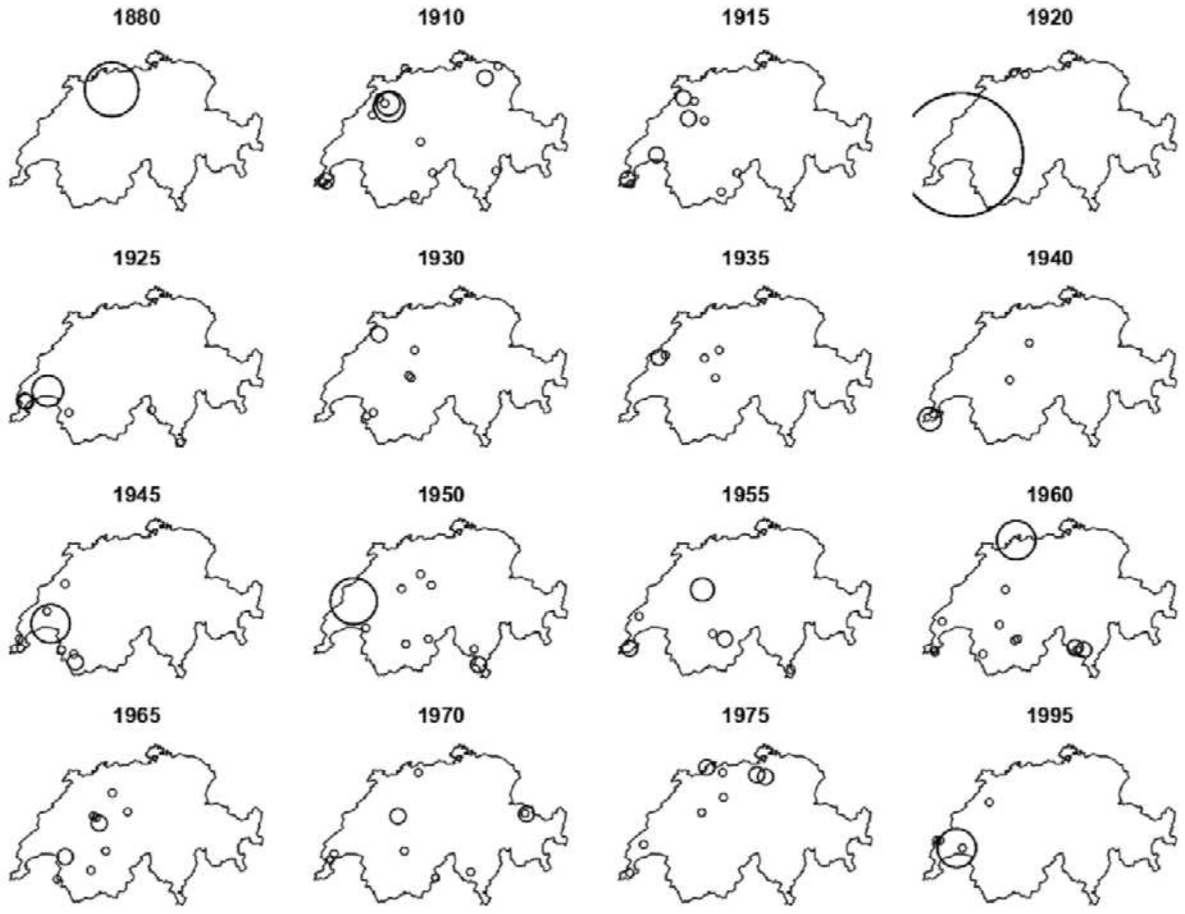

Figure 3. Distribution of the collection specimens of Vanessa atalanta for those 5-year period with more than 6 individuals. The circle size corresponds to the number of individuals.

With 1 401 specimens for the terrestrial species, and 481 specimens for the two aquatic species, altogether, **the number of historical specimens reaches 1 882 individuals.** Moreover, for each species at each grid cell (terrestrial non-migratory species) or sampling station (aquatic species), 10 individuals will be sampled following a standardized protocol, thus resulting in 1 050 fresh specimen*s*. Therefore, **the total number of historical (1 882) and fresh (1 050) individuals to be included in this study rises to 2 932.**

SAMPLING OF MUSEUM COLLECTION SPECIMENS

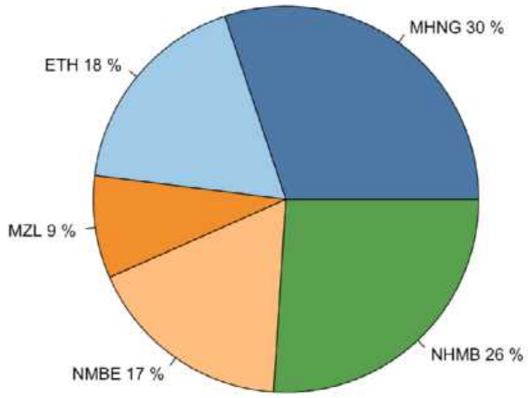

Figure 4. Distribution of samples in the collections. Percentage of historical specimens to sample in each Swiss collection. ETHZ: Eidgenössische Technische Hochschule Zürich; MHNG: Muséum d'histoire naturelle de la Ville de Genève; MZL: Musée de Zoologie de Lausanne; NHMB: Naturhistorisches Museum Basel ; NMBE: Naturhistorisches Museum Bern.

The 1 882 specimens from the selected historical populations were sampled in the five main Swiss collections (Figure 4). We sampled only one leg of each specimen to reduce the impact of the project on collections. We applied an adapted protocol which minimizes contamination between specimens (Figure 5). It consists of cleaning of surfaces using bleach, wearing gloves and sterilizing the forceps between individuals for 1 minute at 200 °C. Because the location of the samples were indicated on the previous phase of the project, the retrieval of the samples in the collections was significantly easier.

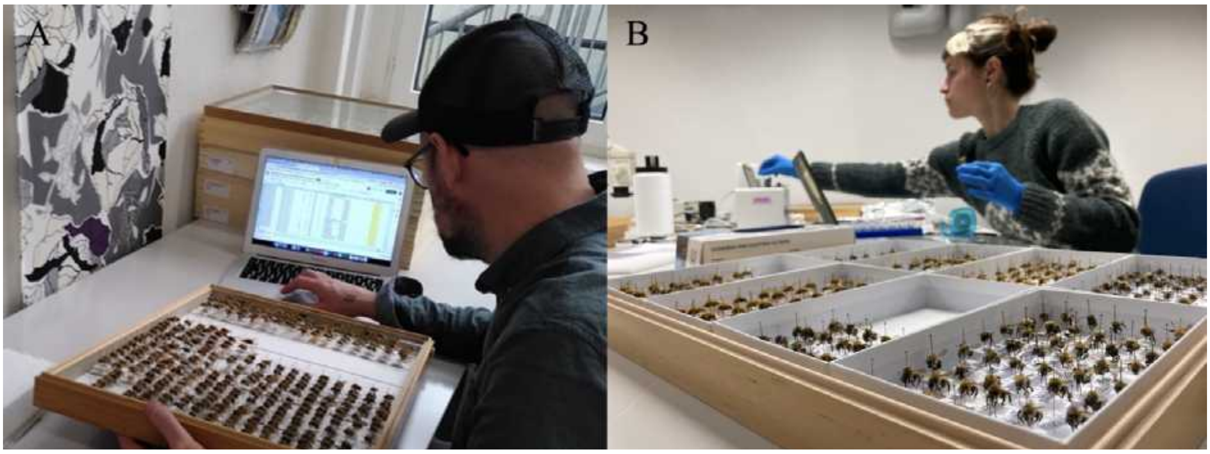

Figure 5. Work at the collections. 5A. During the database of the specimens their location was indicated so that they can be easily re-found during the sampling. 5B The sampling in the collections involved the transport of the specialized equipment to ensure sterile conditions and avoid cross-contamination.

FIELD CAMPAIGN 2022

In this phase of the project, we completed the fieldwork started the previous year aiming at obtaining a point of comparison and a snapshot of the genetic settings of the current populations. From mid-May to mid-August, eleven long- and middle-distance field trips including more than 40 days of work were carried out by the decliNe team. As a result, we sampled 566 individuals, following the standardized sample protocol used for the previous field campaign aiming to standardize the field work and minimize its impact on insect populations.

For each species, we sampled one complete individual and only one leg for the nine other individuals in each of the defined grid cells. Several studies (Holehouse et al 2003; Koscinski et al 2011; Marschalek et al 2013) have outlighted the null impact of this kind of sampling on insect survival and fitness. Unfortunately, this was not possible for Ephemeroptera and 10 complete individuals were collected due to reasons of reduced individual size or difficulties in species identification. Samples were stored individually in 100% ethanol for latter treatment at the laboratory. The field campaign was successful and we were able to finish the sampling of all the species.

Polyommatus icarus

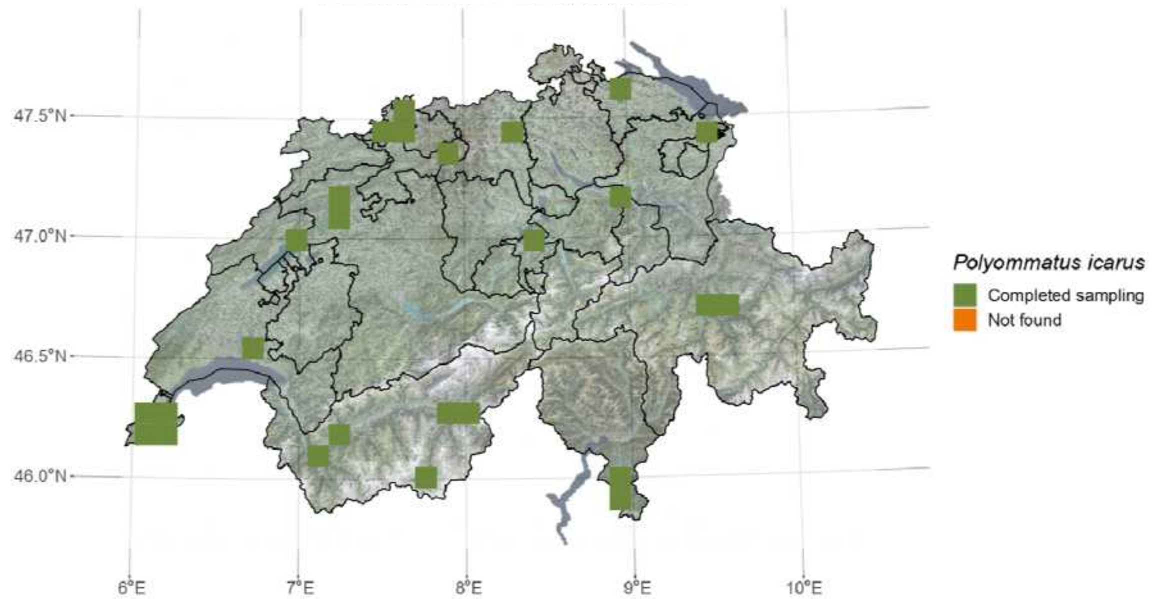

Figure 6. Map of grid cells containing historical populations in Swiss museum collections for Polyommatus icarus.

P. icarus is undeniably the species with the most historical populations identified. Interestingly, the 25 populations are homogeneously distributed throughout Switzerland (Figure 6), a feature that will allow us to compare different biogeographic regions in future steps of the project. In this field campaign, we sampled the remaining historical populations unsampled and, thus, we completed the field work for this species.

Pieris napi

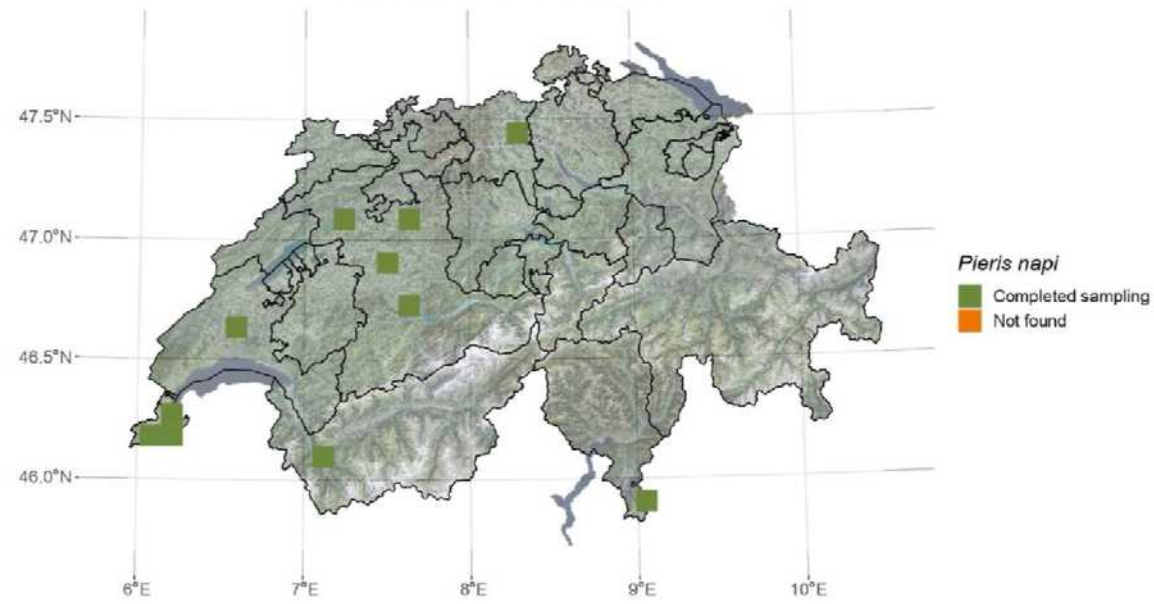

Figure 7. Map of grid cells containing historical populations in the Swiss museum collections for Pieris napi*.*

In the case of *P. napi*, we found 11 historical populations mainly located in the cantons of Geneva and Bern (Figure 7). The sampling of this species was successfully completed during the second field campaign. This species is found throughout Switzerland in the lowlands at edges, hedges, clearings and open forests.

Chorthippus parallelus

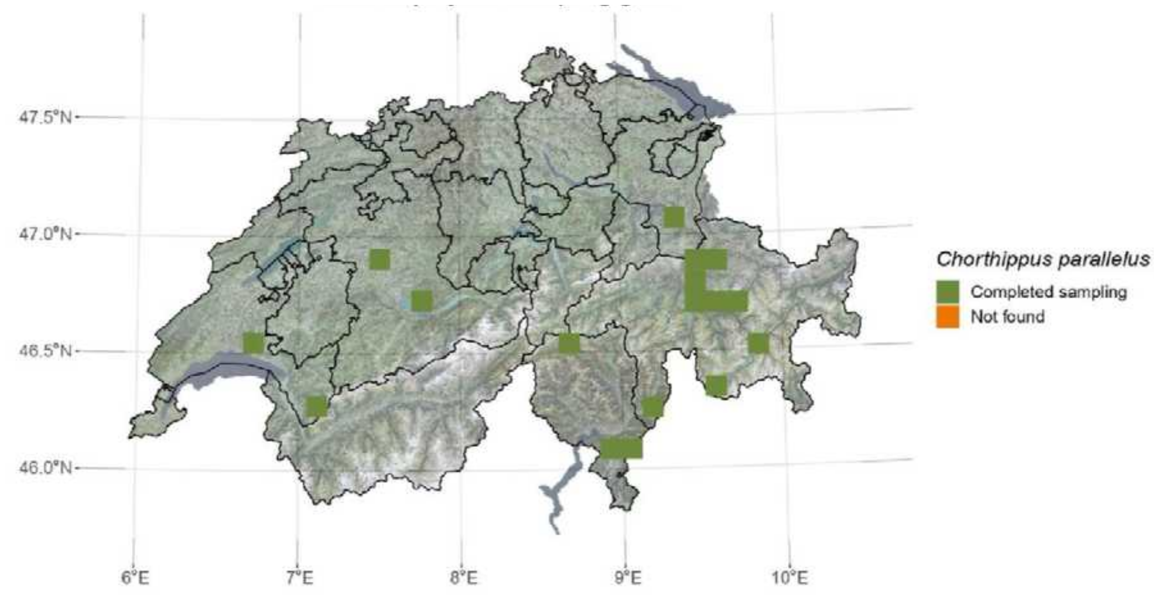

Figure 8. Map of grid cells containing historical populations in the Swiss museum collections for Chorthippus parallelus*.*

For the Orthoptera species *C. parallelus*, we can easily note how the historical populations are closely related to Adolf Nadig's collection, who mostly sampled in the canton of Grisons

(Figure 8). This species, which is especially common in intensive agricultural landscapes, was completely sampled this summer.

Omocestus viridulus

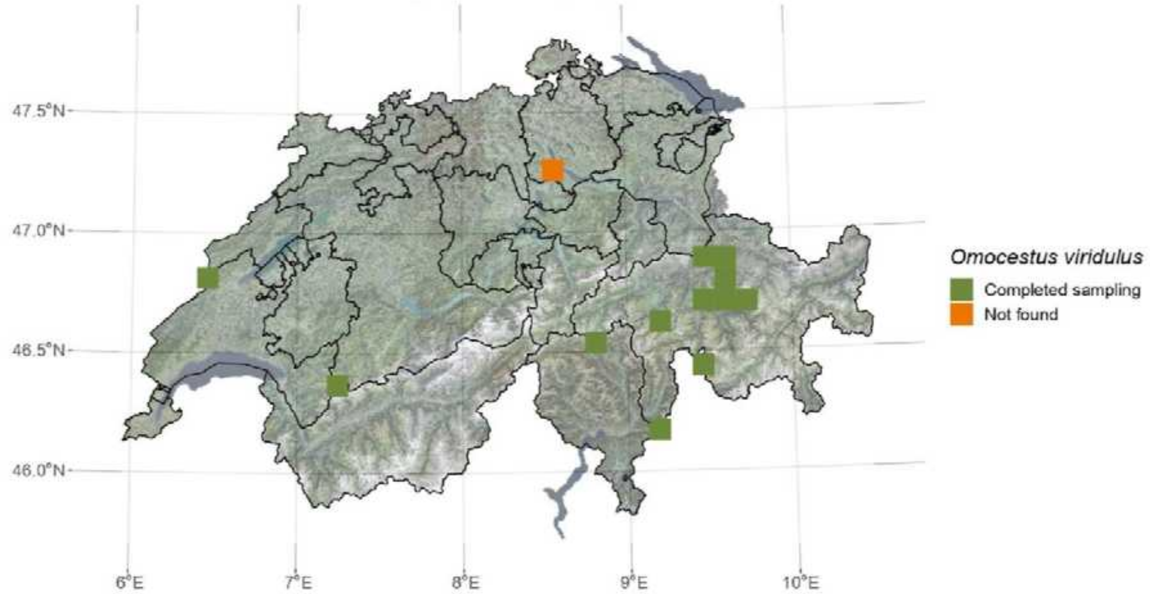

Figure 9. Map of grid cells containing historical populations in the Swiss museum collections for Omocestus viridulus.

Just as observed in the case of *C. parallelus*, the historical populations of *O. viridulus* mostly come from Adolf Nadig's collection and subsequently many of them are located in the canton of Grisons (Figure 9). This species inhabits wet meadows, fens and pastures at moderate altitudes in the Alps. All grid cells excepted one, in which no individuals were found, were successfully sampled.

Miramella alpina

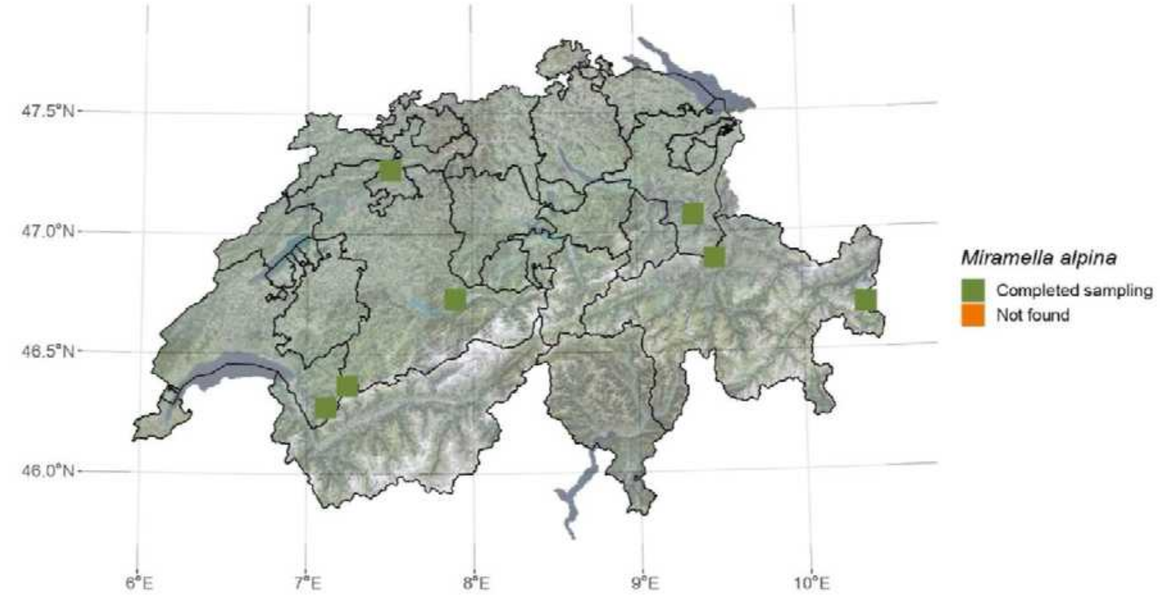

Figure 10. Map of grid cells containing historical populations in the Swiss museum collections for Miramella alpina.

The seven grid cells of *M. alpina* are evenly distributed across the Alps except for the cell corresponding to the Center of the Jura (Figure 10). The species colonizes fresh and moist habitats along mountain streams, sparse forests and high herbaceous meadows. The sampling of all the grid cells was successfully completed this year.

Cetonia aurata

The coleopteran *C. aurata* is a common species, which can be associated with agriculture but also with woodland and dead wood in which the larvae develop and it is often found feeding on rose blossoms and white flowers. We could sample individuals for the majority of the grid cells, except for three located in Basel, Brisago and Scoul that could not be sampled (Figure 11),

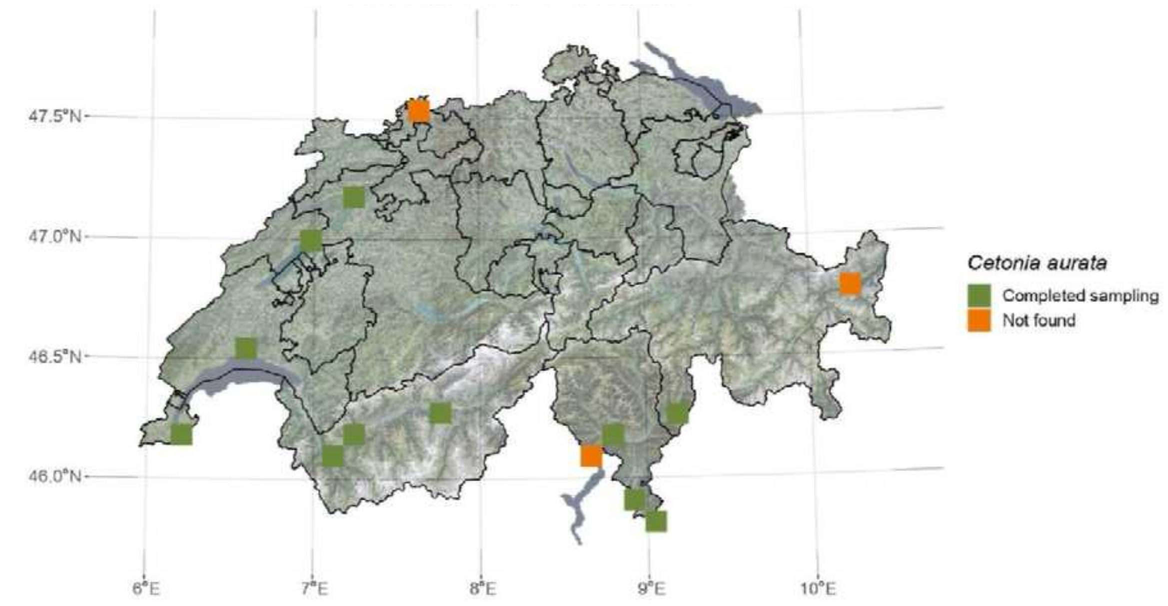

Figure 11. Map of grid cells containing historical populations in the Swiss museum collections for Cetonia aurata.

Vanessa atalanta

While looking for other species at the identified grid cells, we collected the three *Vanessa atalanta* individuals lacking.

Baetis alpinus

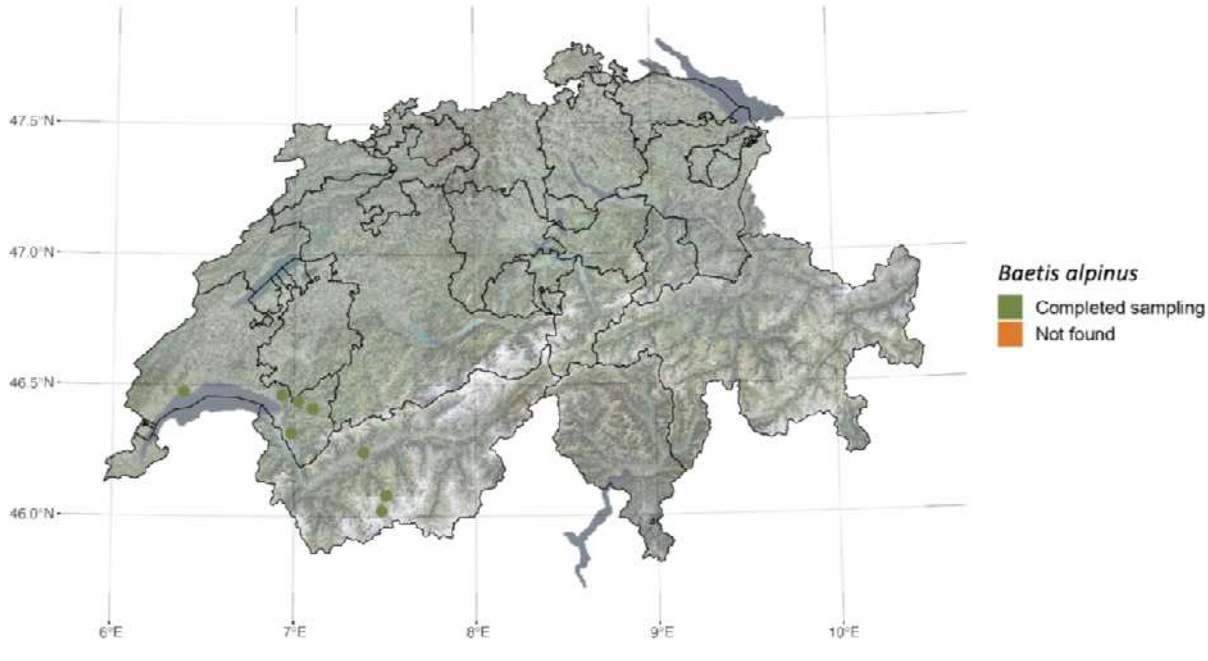

Figure 12. Location of the sampling stations of Baetis alpinus *where historical populations were identified.*

As a reliable identification of ephemeroptera can only be done at the larval stage, more abundant in spring before they emerge, the sampling was successfully completed during the spring 2022 (Figure 12). The specimen's identification in the field turns out to be extremely difficult for non-experts, hopefully we could rely on the Swiss Ephemeroptera expert Andre Wagner, who joined us for the sampling and helped us with the re-identification of all the individuals.

Serratella ignita

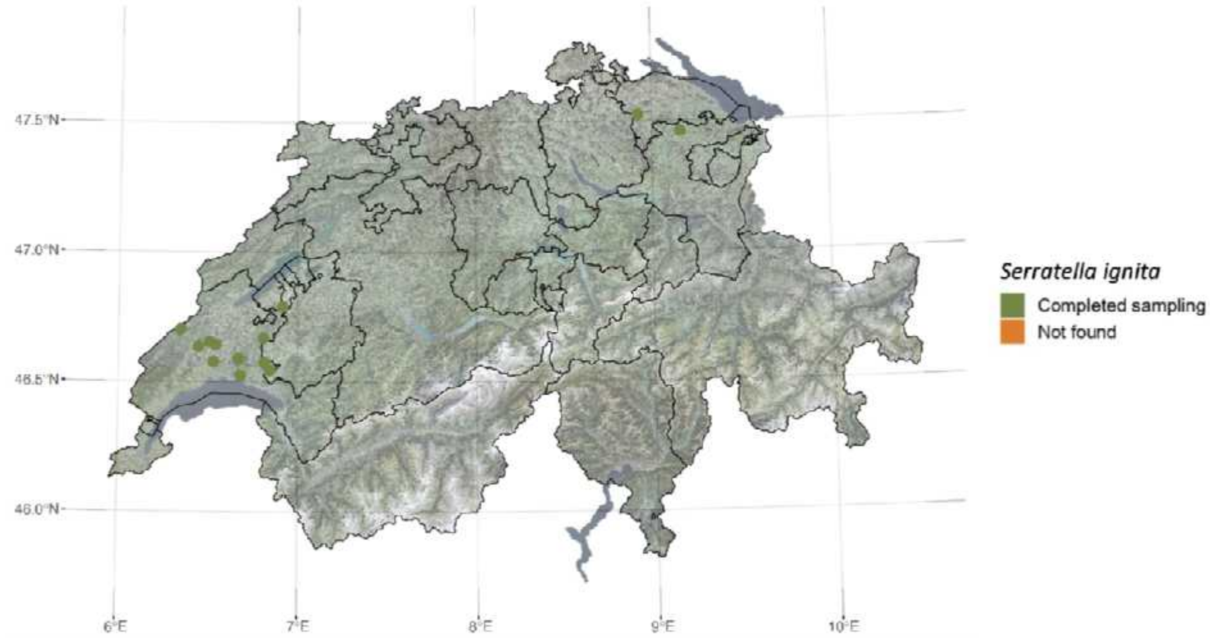

Figure 13. Location of the sampling stations of Serratella ignita *where historical populations were identified.*

As in the case of *B. alpinus*, the sampling of *S. ignita* was fulfilled this spring with the help of André Wagner.

Bird species

The difficulty to obtain the permits and especially the technical and logistical skills to undertake bird captures forced us to put aside the bird species initially envisaged. Various contacts and collaborations with, for example, Gilberto Pasinelli and Pietro Milanesi from the Vogelwarte as well as Pierre Bize from the University of Edimbirgh, enabled us to make some progress on the species to be considered but while the Alpine Swift seems the best candidate, the feasibility of the study on a bird species is not demonstrated due to the very limited numbers of collections specimens available.

LABWORK

Extraction

To extract the DNA of the fresh individual sampled during the campaign 2021, we used the BioSprint available at the Department of Ecology and Evolution of the University of Lausanne allowing the simultaneous extraction of 96 samples at once. The samples collected during the fieldwork of 2022 are currently being extracted. In addition, we have recently hired a lab technician to extract the historical samples using the QIAamp DNA Mini Kit, which provides the most reliability compared to other kits. The extraction of the 1 882 collection specimens will be finished by March 2023.

Library preparation

In this phase of the project, we achieved two important milestones concerning the library preparation step:

1. We adapted the single-strand shotgun library protocol from a strip-based (eight samples) to plate (ninety-six samples) mode. This protocol allows recovery of most of the historical DNA thanks to several reactions, which can take from five to six working days if the pipetting is done by hand.
2. We did a market survey to find the library preparation robot meeting our technical and financial criteria and the ACSIA NGS Libprep was chosen (funded by additional matching funds, see below). As a result of our exchanges with their technician, an adapted workflow has been created and the robot will be put into action in January with the first optimizations.

This step was made possible by the acquisition of a robot thanks to the "Collembola Swiss diversity" project recently awarded to Nadir Alvarez by the Fondation Alpes Sauvages (awarded on August 24, 2022). This methodological development is a central point because it will allow us to prepare the libraries for the large number of samples we have in a reasonable delay. Indeed, discussions with the technicians allow us to estimate the process time at 2 plates of 96 samples per week. In theory, it would therefore be possible to prepare the libraries of the 2 932 samples in 17 weeks. In addition to the lab technician, two masters students have joined the decliNe team to work specifically on the Ephemeroptera and Lepidoptera orders.

PLANNING AND PERSPECTIVES

Once again, the completion of the previous milestones of the decliNe project have only been possible thanks to the multiple partnerships with Swiss institutions and experts. Now both the samplings of the fresh and the historical specimens are completed. We will be able to move on to the third phase of the project including (i) laboratory (both historical and fresh DNA extraction, hyRAD protocol (Toussaint et al. 2021), (ii) sequencing, (iii) bioinformatic analysis to estimate the genetic erosion and changes in the effective population size and (iv) writing report and scientific articles.

Despite the covid pandemics, sampling in collections and in the field have been completed within a reasonable time frame. However, we consider that the end of the project will be possibly delayed by July 2024. Figure 14 represents in detail the schedule of each milestone. This was mainly due to the inaccessibility of several collections during the partial lockdown that affected Switzerland.

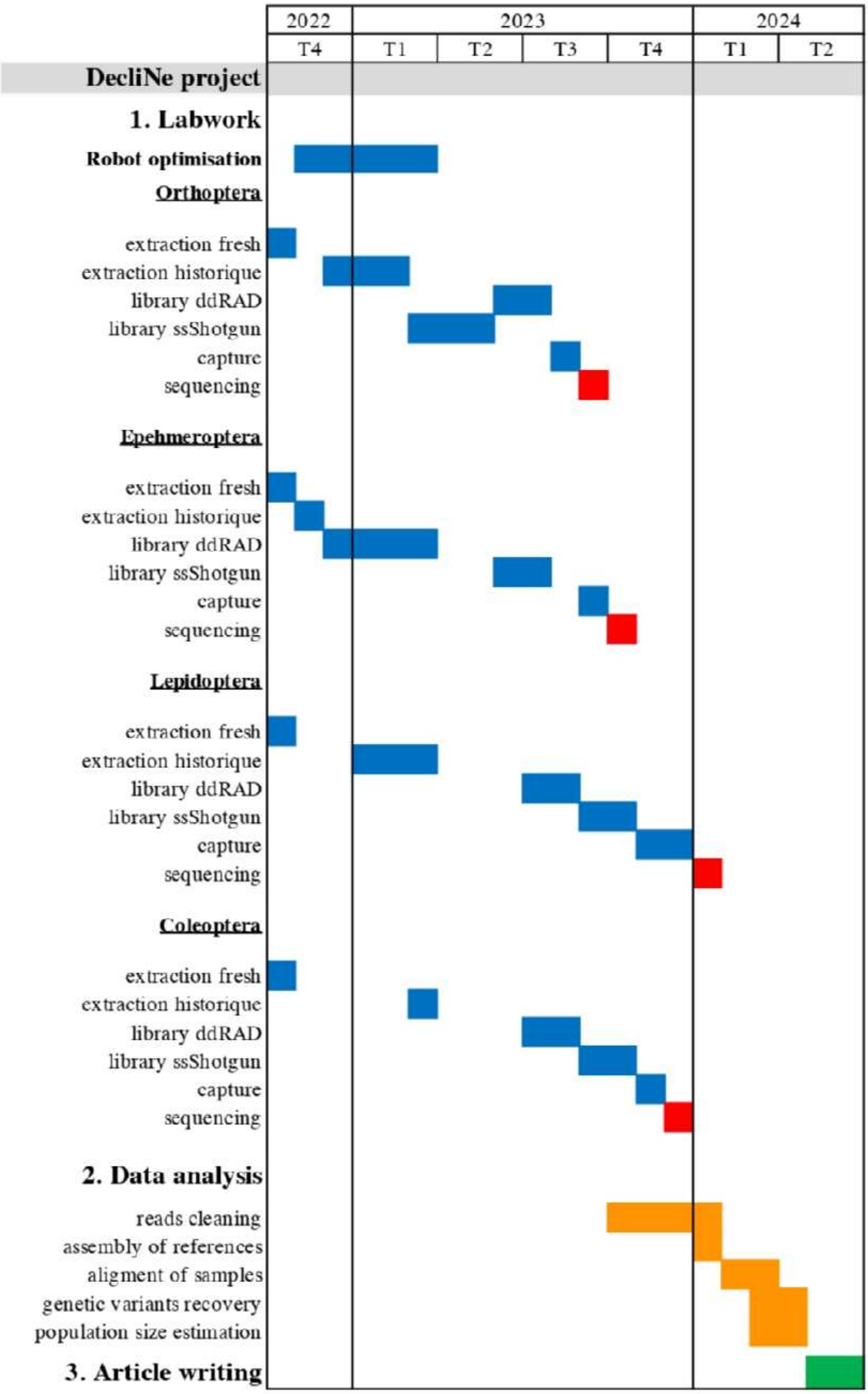

Figure 14. Gantt chart illustrating the decliNe project schedule for the next 22 months.

SCIENCE COMMUNICATION

The results of the decliNe project can play a major role in raising a science-based awareness of the insect decline in the Swiss society, therefore it has been important to establish good communication with the general public since the early phases of the project. We relied on the extensive experience of scientific dissemination carried out by the Natural History Museum of Geneva and we chose to develop two channels of communication.

On the one hand, we created a Twitter account (@Decline_Proj) to establish direct and dynamic communication with the public using short sentences. This account allows us to keep the public updated about our progress and the articles resulting from this project will be posted on it, to be more accessible.

On the other hand, a short documentary about the decliNe project was filmed by LemanBlue, a Swiss television channel based in Geneva. Working together with the journalist Coline Utz offered us the opportunity to disseminate the project outside the scientific environment and to create an appealing presentation of the project which, undoubtedly. The documentary was broadcasted in *Genève Grandeur Nature* the 6th October 2021 and is available in this link.
In addition, we have started to work with Lydie Billaud, communication manager of the Natural History Museum of Geneva, to produce a short video (1'30'') which will be shared in the social media of the institution.

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