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Change – The transformative power of citizen science

Then and now: citizen scientists help assess the changing biodiversity of minnows in Austria

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Abstract

The minnows of the *Phoxinus* genus were long thought to be a single species, as even experts found it difficult to distinguish them by external features. In the last 20 years, however, their status has changed dramatically as molecular studies have revealed a high level of genetic diversity within this group of fish. Currently, more than 23 genetic lineages are known in Europe, of which 14 are recognised as valid species. In Austria, instead of one common minnow, studies have revealed at least four species, three of which are presumed native and one introduced. The Citizen Science project "Biodiversity of minnows in Austria", funded by the Federal Ministry of Education, Science and Research as part of the "Sparkling Science 2.0" Programme, aims to collect and analyse the missing data to determine the number of minnow species swimming in Austrian waters. With the help of pupils from six different schools across Austria, fisheries associations and numerous independent fishermen, the minnows are being sampled extensively and their genetic lineages determined. Basic data on their habitat is also being collected using standardised forms. At the same time, the project team is analysing up to 200-year-old specimens from the fish collection of the National History Museum in Vienna to assess the rate of change in the minnow's biodiversity. Our initial results confirm the native distribution of three minnow species in Austria and show several mixing zones between them, which may not be natural. This is therefore a study of change: on the one hand, changes in genetic diversity over time are evaluated. On the other hand, it highlights the changes that new methods are bringing to our scientific and general knowledge about biodiversity. One of the most dramatic consequences of human impact on our planet is the continuing loss of global biodiversity. What better way to experience these changes than by actively participating in a study designed to assess them?

Keywords: *Phoxinus* minnows, Citizen Science, museum collections.

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Introduction

The minnows of the *Phoxinus* genus are cold loving species inhabiting different kinds of oxygen rich waterbodies (Frost 1943). At the end of the 18th and up to the beginning of the 20th century, several species of *Phoxinus* were described, e.g., *P. marsilii* (Heckel, 1836) and *P. csikii* Hanko, 1922. However, as even experts found it difficult to distinguish them by external features, they were all synonymised under one species, *Phoxinus phoxinus* (Linneaus, 1758), the common minnow, whose distribution range included Europe and Asia. In the last 20 years, however, their status has changed dramatically as first some morphological studies (Bogutskaya et al. 2004; Kottelat 2007) and later molecular studies (Geiger et al. 2014; Palandačić et al. 2017; Palandačić et al. 2020) have revealed a high level of genetic diversity within this group of fishes. Currently, more than 23 genetic lineages are known in Europe, of which 14 are recognised as valid species (Palandačić et al. 2017; Denys et al. 2020; Bogutskaya et al. 2023).

In Austria, instead of one common minnow, studies have revealed at least four species, three of which are presumed native (*P. marsilii*, *P. csikii* and *P. lumaireul* (Schinz, 1840) and one introduced (*P. phoixinus*; Palandačić et al. 2020). However, a denser sampling and a more detailed genetic analysis are needed to determine the number of *Phoxinus* species in Austria, their distribution and the state the populations are in. Finally, when analysed, museum specimens up to 200 years old can help determine the changes in biodiversity of Austrian minnows.

The Citizen Science project "Biodiversity of minnows in Austria", funded by the Federal Ministry of Education, Science and Research as part of the "Sparkling Science 2.0" Programme, aims to collect and analyse the missing data to determine the number of minnow species swimming in Austrian waters. In addition to scientific goals, the project also foresees the transfer of modern methods and procedures of biodiversity research into the educational system. Among objectives is also raising awareness of issues such as biodiversity loss and the problem of introduced species.

Methods

With the help of pupils from six different schools across Austria, several cover fisheries associations and numerous independent fishermen, the minnows are being extensively sampled (Figure 1A) and their genetic lineages determined. For this purpose, sampling kits have been prepared, containing information on minnows,

Figure 1: Pupils working on different activities within the project; 1A collecting minnows at Purkersdorf, Lower Austria in June 2023; 1B counting the number of vertebrae and fin rays on a stained minnow.

standardised forms to collect basic data about the habitat, instructions for sampling and swabs to take DNA samples. At the same time, the project team is analysing the historical minnow specimens.

Upon receipt of the collected DNA samples, DNA is extracted using the Qiagen QIAmp DNeasy Blood and Tissue Kit (QIAGEN, Germany) according to the manufacturer's protocol. Cytochrome oxidase I (COI; so-called barcoding fragment) was then amplified by polymerase chain reaction according to the protocol described in Palandačić et al. (2017) and sequenced in one direction at Microsynth (Vienna, Austria). The raw sequences were visually inspected and aligned using MEGA 6.0 software (Tamura et al. 2013). To determine species, sequences were compared using a simple phylogenetic tree reconstruction analysis with the same software.

In addition to the scientific results, several field trips and workshops were organised for the pupils involved. After a theoretical introduction to minnows and their biodiversity, the students went out collecting, accompanied by the scientists involved in the project. They then learnt and carried out DNA extraction and analysis methods, with pupils in the last two years of the secondary school programme carrying out the species identification analysis and presenting the results in the form of a scientific talk or poster (Figure 2). Workshops for younger students included a more creative approach, preparing posters of the minnow food chain and colouring the minnow models to represent their hidden biodiversity. Finally, all students were able to work with stained minnows, which allowed them to count different skeletal features such as the number of vertebrae and fin rays (Figure 1B). To accompany the workshops, all materials were prepared also for the teachers and made available online.

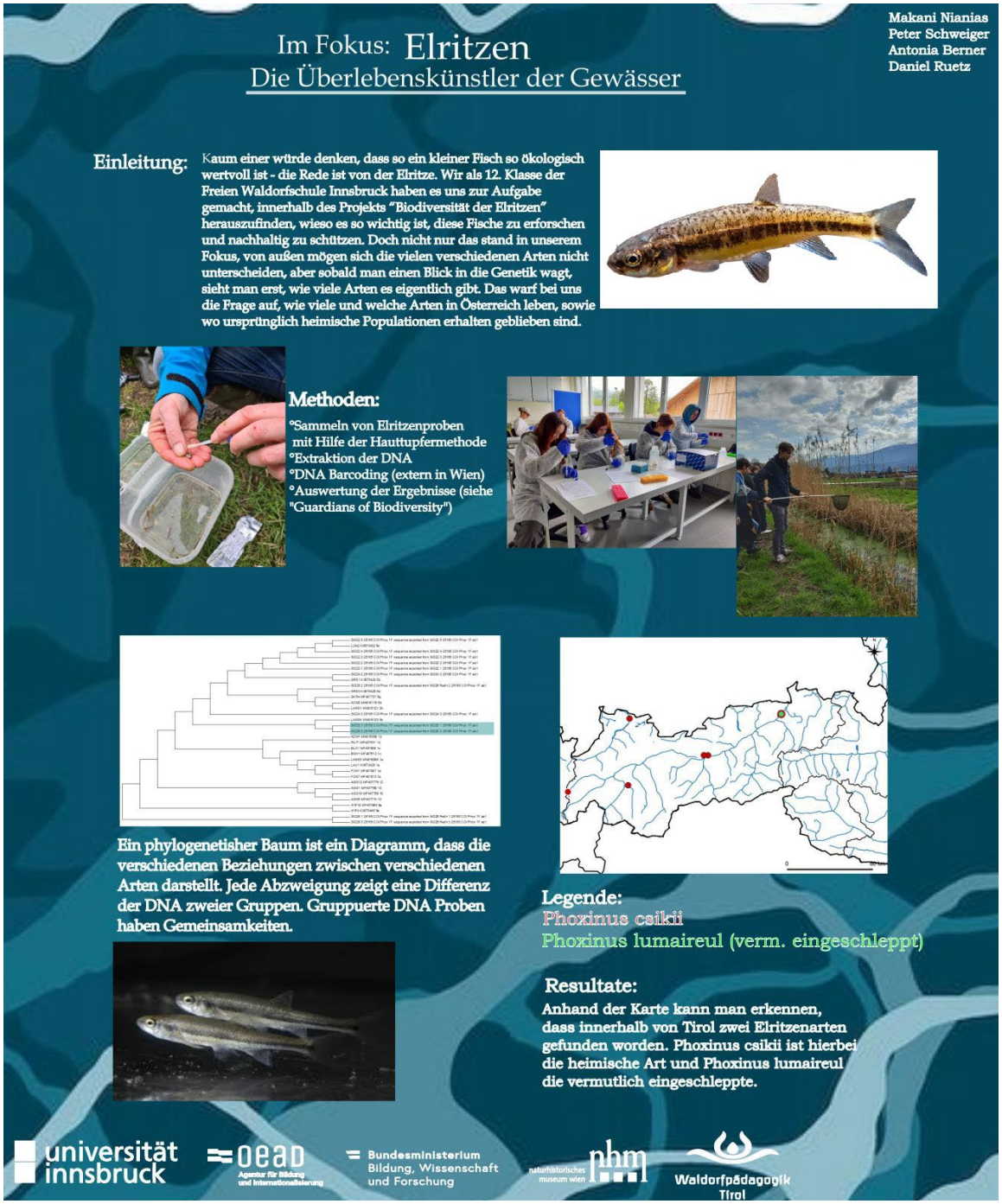

Figure 2: A scientific poster prepared by the pupils about their work on *Phoxinus* minnows. The poster includes all chapters relevant for presenting scientific study.

Results

In the first 1.5 years of the project, 174 fin clips, 91 swabs, 84 whole fish were collected and analysed. Of these 349 specimens, the pupils (accompanied by project scientists) collected 70 (20%) and the fishermen collected 155 (44%). The rest, 124 (36%) was collected by scientists and field biologists not directly involved with the project. This was another positive effect of the project, as even in scientific circles the biodiversity of the minnows in Austria was not known. The results confirmed the presence of three native and one introduced species in Austria. So far, the introduced species appears to be present only at two sites in Styria and at Lake Constance. Two of these populations, one in Styria and the one in Lake Constance, are a mixture of two species - one native and the other introduced. Further, mixtures of two native minnow species were also detected in several other areas, however these may point to the natural hybridisation zones.

Discussion

As shown by the results, future sampling should focus on the areas with less data (Styria, Carniola) and on possible hybridisation zones. The DNA samples already collected in the hybridisation zones should be analysed in more detail, not only to identify the species, but also to infer possible introgressions between species in these populations.

In summary, this project has enabled us to collect valuable data on the biodiversity of minnows throughout Austria. As all nine different federal states have different rules for obtaining collecting permits, fishermen were a crucial stakeholder in providing us with this data. Together with pupils, these two groups of citizen scientists have supported biodiversity research in Austria, while at the same time experiencing research first-hand. Thus, the project is helping to raise awareness of conservation and reduce scepticism about science. What better way to experience changes in biodiversity than by actively participating in a study designed to assess them?

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