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ORIGINAL ARTICLE

First records of *Ceroplastes ceriferus* (Fabricius) (Hemiptera: Coccidae) and *Ceroplastes japonicus* (Gray) in Switzerland identified by DNA barcoding

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Abstract

Wax soft scales (Hemiptera: Coccidae) include many important pest species of agricultural and ornamental plants which spread along international trading networks of plants and plant material. Here we report the first findings of the wax scales *Ceroplastes japonicus* and *Ceroplastes ceriferus* for Switzerland. The two new findings of *Ceroplastes* were collected on *Laurus nobilis* and *Vaccinium myrtillus*, respectively, and identified by DNA barcoding.

KEYWORDS
Ceroplastes, Coccidae, DNA barcoding, first record, wax scale

Premiers signalements de *Ceroplastes ceriferus* (Fabricius) (Hemiptera: Coccidae) et *Ceroplastes japonicus* (Gray) en Suisse, identifiés par barcoding moléculaire

Les cochenilles lécanides (Hemiptera: Coccidae) comprennent de nombreuses espèces d'organismes nuisibles d'importance sur les plantes agricoles et ornementales et se disséminent via les réseaux internationaux de commerce de plantes et de matériel végétal. Les premiers signalements en Suisse des cochenilles lécanides *Ceroplastes japonicus* et *Ceroplastes ceriferus* sont présentés dans cet article. Les spécimens ont été collectés respectivement sur *Laurus nobilis* et *Vaccinium myrtillus* et identifiés par barcoding moléculaire.

Первые *сообщения* о ложнощитовках *Ceroplastes ceriferus* (Fabricius) (Hemiptera: Coccidae) и *Ceroplastes japonicus* (Gray) в Швейцарии, идентифицированных с помощью ДНК-штрихкодирования

Восковые ложнощитовки (Hemiptera: Coccidae) включают многие важные виды организмов, вредные для сельскохозяйственных и декоративных растений и распространяющиеся по международным сетям торговли растениями и растительным материалом. Здесь мы сообщаем о первых обнаружениях восковых ложнощитовиток *Ceroplastes japonicus* и *Ceroplastes ceriferus* в Швейцарии. Эти две новые находки *Ceroplastes* были собраны на *Laurus nobilis* и *Vaccinium myrtillus* соответственно и идентифицированы с помощью ДНК-штрихкодирования.

1 | INTRODUCTION

The unintended dispersal of invasive insect pests by global trade represents an increasing threat for agricultural and horticultural production that can cause serious economic damage (Bacon et al., 2012; Ali et al., 2020). These insects include species from the family Coccidae (Miller & Miller, 2003), which has more than 1200 described species to date (García Morales et al., 2016). The subfamily Ceroplastinae is characterized by a thick waxy layer covering the entire insect (Qin & Gullan, 1994). One important genus within this subfamily is *Ceroplastes* Gray, which contains many agricultural pest species that have been introduced to the EPPO region (García Morales et al., 2016). The EPPO Global Database contains six *Ceroplastes* species with confirmed records from Europe. Of all members of the genus *Ceroplastes*, only *Ceroplastes rusci* is regulated in the EU and Switzerland, classified as regulated non-quarantine pest.

Although Papadopoulou et al. (2020) provide a very helpful key to the morphological identification of the *Ceroplastes* species occurring in Europe, their identification with classical taxonomy remains challenging. Accurate and fast species identification is an important prerequisite for many research disciplines and a key factor for the implementation of stringent management strategies against invasive insect pests (Floyd et al., 2010; Hodgetts et al., 2016). Traditional identification based on morphological characteristics is often time-consuming and relies on expert knowledge of skilled taxonomic specialists (Hebert et al., 2003; Hodgetts et al., 2016). The identification of insects can be especially challenging as they are often found as immature stages lacking distinct morphological characters (Hodgetts et al., 2016; Saccaggi et al., 2016; Blaser et al., 2018). Further problems arise if specimens are damaged or no taxonomic key is available (Hodgetts et al., 2016). To circumvent these drawbacks, species identification can alternatively be performed using molecular methods such as antibody-based, protein-based or molecular genetic-based approaches (Armstrong & Ball, 2005; Saccaggi et al., 2016). Among them, DNA barcoding represents a powerful and generic tool for the identification of species from a wide taxonomic range that can be easily standardized between different laboratories (Hebert et al., 2003). DNA barcoding relies on PCR amplification and sequencing of a conserved signature sequence that is subsequently queried against a database (e.g. Bold, GenBank or Q-bank) containing reference sequences from previously identified specimens (Sujeevan & Hebert, 2007; Benson et al., 2008; Floyd et al., 2010; Bonants et al., 2013; Blaser et al., 2018).

Although it has many advantages, DNA barcoding is limited by the fact that the method can only identify species for which reference sequences as well as primer information are available (Armstrong & Ball, 2005; Blaser et al., 2018). Further limitations can arise when the degree of genetic differentiation of the barcoding fragment is too low to enable clear differentiation between closely related species (Armstrong & Ball, 2005).

In Switzerland, DNA barcoding is implemented in the regular import control process of plant consignments and represents the standard method for the identification of potential quarantine insect pests (Blaser et al., 2018). In addition to its use for diagnostic purposes, DNA barcoding combined with next-generation sequencing (NGS) was evaluated as a promising method for biodiversity monitoring schemes (Gueuning et al., 2019).

Studying the genetic diversity of the *COI* barcoding fragment within and among six closely related *Ceroplastes* species (*C. floridensis*, *C. japonicus*, *C. ceriferus*, *C. pseudoceriferus*, *C. rubens* and *C. kunmingensis*) demonstrated that DNA barcoding properly resolves the different taxa into six reciprocally monophyletic clades congruent with the taxonomic units derived from morphological characters.

The aim of the present study was to reliably identify two different findings of *Ceroplastes* species in Switzerland, confirming the first record of these two introduced agricultural and ornamental pest species for Switzerland.

2 | MATERIALS & METHODS

2.1 | Insect sampling

The specimens from the Canton Valais were collected on *Vaccinium myrtillus* in March 2021. The finding was not related to an official survey activity, but to a research project performed in collaboration with a farmer. The specimens from the Canton Ticino were collected by a private person on *Laurus nobilis* in December 2020.

Specimens from both collection sites were preserved in 70% ethanol prior to DNA extraction. A map displaying the collection sites of specimens was generated using QGIS version 3.10.8 based on free geodata from the Swiss Federal Office of Topography, swisstopo.

2.2 | DNA extraction and DNA barcoding

DNA was extracted from approximately 1 mm^3 of insect tissue from one adult specimen of each finding following a protocol modified from Kawasaki (1990), as described in Brunner et al. (2002) and Frey and Frey (1995). In brief, insect tissue was placed in 1.2-mL collection microtubes (Qiagen AG, Hilden, Germany), each containing one 2-mm diameter steel bead (Retsch GmbH,

Haan, Germany) and 100 µL of lysis buffer (10 mM Tris-HCL pH 8.0, 1 mM EDTA, 0.5% Tween 20, 100 µg/mL Proteinase K). After grinding the tissue samples twice for 3 min at 30 Hz on a mixer mill MM 300 (Retsch GmbH), the homogenization product was incubated for 30 min at 95°C on a thermomixer comfort (Eppendorf AG, Hamburg, Germany).

To amplify the barcode fragments at the 5′ end of the mitochondrial gene cytochrome *c* oxidase subunit 1 (*COI*), polymerase chain reaction (PCR) was run on a thermal cycler (Senso-Quest GmbH, Göttingen, Germany). For the sample from Valais, the barcode fragment was amplified using the primers C1-1554F (5′-CAGGAATAATAGGAACATCAATAAG-3′) and C1-2342R (5′-ATCAATGTCTAATCCGATAGTAAATA-3′). For the sample from Ticino, the barcode fragment was assembled from two overlapping consensus sequences amplified using the primer combination described above together with the primers C1-J-2183 (5′-CAA CATTTATTTTGATTTTTTGG-3′) and C1-N-2568 (5′-GCWACWACRTAATAKGTATCATG-3′) (Brady et al., 2000). The PCR reaction was carried out in a total volume of 20 µL containing 1 µL of DNA extract, 0.4 µM of each primer and 1 × HotStarTaq (Qiagen AG, Hilden, Germany) using the following cycling conditions: 15 min at 95°C, followed by 35 cycles of 40 s at 95°C, 15 s at 45°C, ramping over 60 s to 60°C and 2 min at 72°C. After a final elongation step for 7 min at 72°C, the amplification product was cleaned with the NucleoFast 96 PCR system (Marcherey-Nagel GmbH, Düren, Germany) following the manufacturer's protocol and the subsequent linear amplification was run in a total reaction volume of 8 µL containing 1 µL of amplification product diluted 1:10 in molecular grade water, 0.2 µM of either forward or reverse primer (see above) and 1 × BigDye Terminator v1.1 Ready Reaction Mix (Applied Biosystems) (Waltham-Massachusetts-United States). After removing unincorporated dye terminators using the DyeEx 96 Kit (Qiagen AG) according to the manufacturer's protocol, the linear amplification product was sequenced on a SeqStudio Genetic Analyzer (Applied Biosystems). Applying the de-novo assembly function implemented in Geneious version 10.0.9 with the Geneious assembler and default settings, consensus sequences of both samples were assembled and blasted for species identification against the publicly accessible databases Bold and GenBank (Sujeevan & Hebert, 2007; Benson et al., 2008; Kearse et al., 2012). Classification of the best species matches on GenBank was performed based on the Max Score values of the BLAST results. The sequences of the specimens from canton Valais and canton Ticino were both uploaded to GenBank with the accession numbers MZ836057 and MZ836034, respectively (Benson et al., 2008).

3 | RESULTS AND DISCUSSION

3.1 | Host plant and infestation

The specimens from Valais were collected on *Vaccinium myrtillus* in one plastic tunnel of 0.1 ha. Approximatively 5% of plants were heavily infested. The specimens from Ticino were collected in a private garden on a single *Laurus nobilis* plant, where no other plants were found to be infested (Figures 1 and 2).

3.2 | Species identification by DNA barcoding

After the *de novo* assembly step, trimmed consensus sequences of 654 and 867 bp were retained from the samples from the cantons of Valais and Ticino, respectively. When querying the consensus sequence of the Valais sample against the reference databases, the sample was clearly identified as *C. ceriferus* (GenBank, identity 98.17%, coverage 100%; Bold, similarity 100%). The second-best species match was found for *C. pseudoceriferus* with a substantially lower identity percentage (GenBank, identity 95.41%, coverage 100%; Bold, similarity 95.7%).

FIGURE 1 *Ceroplastes ceriferus* on *Vaccinium myrtillus* in Switzerland [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2 *Ceroplastes japonicus* on *Laurus nobilis* in Switzerland [Colour figure can be viewed at wileyonlinelibrary.com]

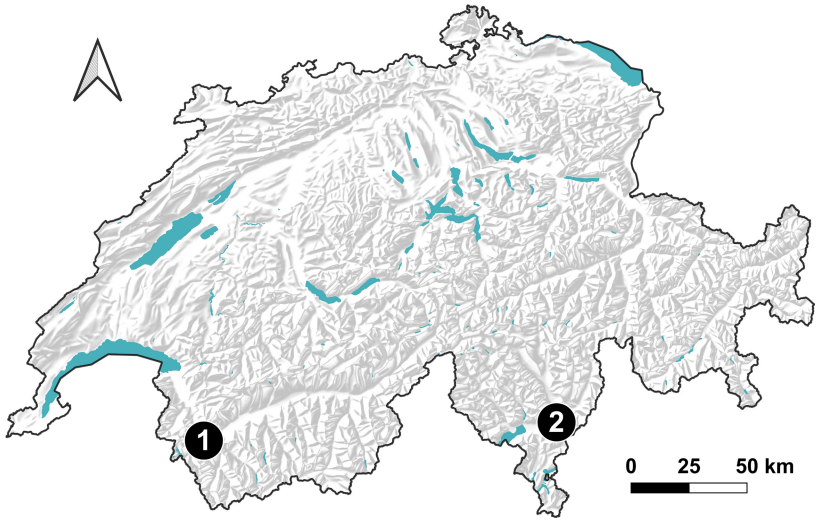

FIGURE 3 Locations of first findings of *C. ceriferus* (1) and *C. japonicus* (2) in Switzerland visualized using free geodata from the Swiss Federal Office of Topography, swisstopo [Colour figure can be viewed at wileyonlinelibrary.com]

Querying the assembled and trimmed consensus sequence of the Ticino sample, a clear match was found for *C. japonicus* (GenBank, identity 99.85%, coverage 75%; Bold, similarity 99.27%). Additionally for this sample, the second-best species match was considerably lower and assigned to *C. kunmingensis* (GenBank, identity 92.23%, coverage 65%; Bold, similarity 97.07%).

3.3 | Localization of findings

The locations of the first findings of *C. ceriferus* and *C. japonicus* in Switzerland are shown in Figure 3.

4 | CONCLUSIONS

The findings of *C. ceriferus* and *C. japonicus* represent the first records of two recently introduced insect pest species for Switzerland. One finding was initiated by a private person, another by an unrelated research project, stressing the fact that occasional findings by vigilant observers are important for pest detection. This fact shows the importance of awareness raising. These findings demonstrate the necessity of official monitoring efforts in the form of systematic surveys conducted on a national level for early detection of foreign pests and pathogens. This paper further emphasizes the importance of molecular methods for the identification of new or cryptic species. Particularly in cases where classical taxonomic knowledge on a group is scarce, these tools may be an important pillar of survey and sampling efforts.

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