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Short Paper

First record of *Donus intermedius* (Coleoptera: Curculionoidae) as a pest on *Melissa officinalis* in South Tyrol and its identification by molecular methods

Erster Nachweis und molekularbiologische Bestimmung von *Donus intermedius* (Coleoptera: Curculionoidae) als Schädling bei Zitronenmelisse in Südtirol

Primo rilevamento e identificazione molecolare di *Donus intermedius* (Coleoptera: Curculionoidae) come parassita di *Melissa officinalis* in Alto Adige

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ABSTRACT

Lemon balm (*Melissa officinalis*) occurs natively in the Mediterranean Area and is widely cultivated as a medicinal and aromatic plant (MAP). In South Tyrol (Northern Italy), lemon balm is among the most cultivated species of MAP. During spring 2019, two field sites in South Tyrol with cultivations of lemon balm showed extensive damage on the leaves. Injured samples taken from these locations were visually inspected and the presence of larvae and pupae of a Curculionidae species detected, which were kept under laboratory conditions until completion of their lifecycle. The adults were identified morphologically as *Donus intermedius* (Coleoptera: Curculionoidae). This species until now was not known to cause damage to lemon balm cultivations. The partial sequence of the cytochrome oxidase subunit I made it possible to differentiate it from other species of the genus *Donus* and can be used as a tool for the identification of larval stadiums.

KEYWORDS
Medicinal and aromatic plants, Melissa officinalis L., Coleoptera, Donus, morphological identification, molecular identification

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INTRODUCTION

Donus intermedius (Boheman, 1842), has a predominantly central European distribution and can be found in Germany, Poland, the Czech Republic, Slovakia, Ukraine, Romania, Bulgaria, Hungary, Croatia, Slovenia, Switzerland, France, and Italy [1].

Sprick (2003) [2] defined the species as a mountain fauna element colonizing preferably herbaceous perennial vegetation. By conducting feeding studies, Sprick (2003) characterized *D. intermedius* as oligophag on many plant species within the plant families of Asteraceae, Lamiaceae, Apiaceae, and Rosaceae.

There are only a few reports on the distribution of *D. intermedius* in South Tyrol [3]. The three reported records are all from subalpine or alpine habitats. The species is not well presented in entomofaunistic surveys, and their distribution in South Tyrol is not well documented (M. Kahlen, pers. communication).

Here we report for the first time the presence of *D. intermedius* as a pest on *Melissa officinalis*. Lemon balm (*Melissa officinalis* L.) is a perennial plant; for commercial production, it is cultivated for 3-5 years. In May 2019 in two field sites for the production of medicinal and aromatic plants located in the Province of South Tyrol, Italy, an extensive damage of lemon balm leaves was noticed (fig. 1). The leaves presented nibbled holes and curled tips and the presence of larvae on both sides of the leaves. The larvae were collected and brought to the Laimburg Research Centre for species identification by morphological and molecular methods.

MATERIALS AND METHODS

COLLECTION OF THE INJURED PLANTS

Insect larvae from injured plants of *M. officinalis* were collected in May 2019 at the field site "Landesbetrieb Gachhof" in Meran/Labers (Province of South Tyrol, Italy) at an altitude of 620 m a.s.l. with the geographic coordinates: 46°40'01.74''N; 11°11'48,02''E.

Larvae were also collected on a second field site (Laurein, Province of South Tyrol, Italy, 1100 m a.s.l.: 46°27'35.6"N; 11°02'39.9"E) from injured plants of *M. officinalis*. Shoots of the injured plants with insect larvae were collected and transported in plastic bags to the Laimburg Research Centre.

CULTIVATION OF LARVAE UNDER LABORATORY CONDITIONS

The morphological identification of larval stadiums of Coleoptera is difficult, therefore a rearing of the collected larvae under standardized conditions was performed. The larvae were cultivated on steady conditions in a climate chamber at 20 °C with 19 daylight hours (lamp Osram T8 FLUORA 58W 77) and 65-70% air humidity. The larvae pupated after 8-12 days. The imago hatched out after 6-8 days (fig. 2). Two imago individuals were sent to Naturmuseum Südtirol to perform the morphological identification.

DNA EXTRACTION FROM LARVAE

Total DNAs from three larvae was extracted by homogenizing the material in 400 µL of CTAB buffer (CTAB 2.5%, Tris pH 8 100 mM, NaCl 1.4 M, EDTA 50 mM pH 8, PVP-40 1%, Proteinase K 10 mg/mL) in a microcentrifuge

Fig. 1: Larval stadium of *Donus intermedius* feeding on lemon balm (*Melissa officinalis*) leaves.

Fig. 2: Adult of *Donus Intermedius* reared in the laboratory on lemon balm.

tube containing a 5 mm tungsten carbide bead (Qiagen). Samples were disrupted using a *Retsch Mixer Mill MM 400*, at 30 Hz for 3 minutes. After disruption, DNA was extracted using the *DNeasy Plant Mini kit* (Qiagen) following the instructions of the provider.

A partial region of the cytochrome c oxidase subunit I gene was amplified with primers HCO2198 (5´GGTCAACAAATCATAAAGATATTGG3´) and LCO1490 (5´TAAACTTCAGGGTGACCAAAAAATCA3´) [4].

PCR amplification of samples was performed in a final volume of 20 µL containing one-fold of *Phusion High-Fidelity PCR Master Mix with HF Buffer* (New England BioLabs) and 200 nM final concentration of each primer and 2 µL of insect DNA.

Reactions of samples were performed on a *Verity 96-well Thermal Cycler* (Applied Biosystems) as follow: 30 s at 98 °C followed by 35 cycles of 50 s denaturation at 98 °C and 90 s for annealing at 52 °C, 15 s elongation at 72 °C.

The purified amplicons were sequenced by LGC Genomics GmbH (Berlin Germany).

RESULTS AND DISCUSSION

The adult individuals were identified morphologically as *Donus intermedius* (Coleoptera: Curculionidae; det. M. Kahlen). One of the individuals was inserted in the collection of the Naturmuseum Südtirol.

The sequences generated by the partial amplification of the cytochrome oxidase I gene of several larval individuals were compared using a multiple sequence alignment with hierarchical clustering [5]. All the sequences were identical, and one was deposited into the GenBank, The National Center for Biotechnology Information under the number SUB9778907. The generated consensus and unique sequence of *Donus intermedius* was used as a query to perform a basic local alignment. The results of this BLAST identify as a first retrieved match a *Donus tesselatus* sequence KU915144.1 with percentage of identity of 88.53%, E-value of 7 e-177 and a Query coverage of 98%, confirming that the sequence SUB9778907 is the first reported partial sequence of the cytochrome oxidase I gene for *Donus intermedius*. A survey of the integrated bioinformatics platform Barcode of Life Data System database (http://www.barcodinglife.org) revealed no entry as well for *Donus intermedius*. Other species of the genera *Donus* (*D. ovalis*, *D. globosus*, *D. segnis*, *D. cyrtus* and *D. tesselatus*) are present in one or several copies in the BOLDSYSTEM platform.

The morphological identification of larval stadiums of Coleoptera represents a challenge since the known illustrations, descriptions, and comprehensive keys are not always available or many species are not included in the literature [6]. The molecular

identification through DNA barcoding represents an important tool for the identification of Coleoptera [7] and can be used for larval stadiums. Here we present a unique consensus barcode sequence for the species *Donus intermedius* supported with taxonomic consistency that can be used for identification and discrimination within the genera at species level.

On the field site at Meran/Labers, larval stadiums were found also on spring 2020 and 2021 feeding on lemon balm. Damage on the leaves was noticed only in May before the first harvest. The first cut at the field site is usually done around end of May. Afterwards, larval stadiums and leaf damage were not found. According to Sprick (2003) [2], *D. intermedius* is highly sensitive to mowing, therefore the first harvest may reduce the population density on lemon balm.

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ZUSAMMENFASSUNG

Zitronenmelisse (*Melissa officinalis*) stammt ursprünglich aus dem Mittelmeergebiet und wird verbreitet als Heil- und Gewürzpflanze angebaut. In Südtirol zählt die Zitronenmelisse zu den am häufigsten angebauten Arten der Heil- und Gewürzpflanzen. Im Frühjahr 2019 wurden an zwei Standorten mit Zitronenmelisse Fraßschäden an den Blättern beobachtet. Proben der geschädigten Pflanzen wurden untersucht und es konnte das Vorkommen von Larven und Verpuppungsstadien von Käfern der Familie der Curculionidae festgestellt werden. Die Larven und Puppen konnten im Labor ihre Entwicklung abschließen. Die adulten Käfer wurden morphologisch als *Donus intermedius* (Coleoptera: Curculionoidae) bestimmt. Diese Käferart ist als Schädling im Anbau von Zitronenmelisse bisher nicht bekannt. Die Sequenzierung einer Teilsequenz der Cytochrome Oxidase Untereinheit I ermöglichte die molekularbiologische Unterscheidung von anderen Arten der Gattung Donus und kann zukünftig als Tool zur Bestimmung von Larvenstadien der Gattung Donus verwendet werden.

RIASSUNTO

La melissa (*Melissa officinalis*) è originaria della regione mediterranea e viene ampiamente coltivata come pianta officinale. In Alto Adige la melissa è tra le specie di piante officinali più coltivate. Nella primavera 2019 in due siti sono stati osservati danni sulle foglie di melissa. Sono stati esaminati campioni delle piante danneggiate ed è stata rilevata la presenza di larve e stadi pupali di coleotteri della famiglia Curculionidae. In laboratorio è stato possibile far concludere lo sviluppo delle larve e delle pupe. I coleotteri adulti sono stati determinati morfologicamente come *Donus intermedius* (Coleoptera: Curculionoidae). Questa specie non è stata precedentemente segnalata come specie dannosa nella coltivazione di melissa. Il sequenziamento parziale della subunità I del citocromo ossidasi ha permesso di differenziare *D. intermedius* da altre specie del genere Donus e può essere usato in futuro come strumento per determinare anche gli stadi larvali di questo genere di coleotteri.

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