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SHORT COMMUNICATION

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# Further spread of the invasive apricot aphid *Myzus mumecola* (Hemiptera: Aphididae) in Central Europe and first insights into its phylogeny

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## Abstract

A new aphid species infesting apricots (*Prunus armeniaca* L.) was identified for the first time in 2016 in Europe. The invasive aphid was assigned to the species *Myzus mumecola* (Matsumura, 1917). The species is native to India and East Asia, and it was found to be highly invasive and is currently spreading throughout Europe. Records were confirmed for Italy, Hungary, Serbia, and Germany. For a phylogenetic analysis, wingless (apterae) adults were sampled in spring 2022 in Italy, as well as in Austria and Germany in spring 2023. Our study reports the first record of *M. mumecola* for Austria. In all three countries, the new pest caused heavy infestations overall in organically managed apricot orchards. Aphids were identified morphologically based on external characteristics, and damages were compared to those reported in the literature. COI and 12S barcoding analyses for aphid samples from Italy, Germany, and Austria were performed to attain a molecular characterization of sampled specimens and to confirm the morphological identification. Only a few COI sequences of *M. mumecola* were present in NCBI GenBank or BOLD Systems, and no sequences were available for 12S. Therefore, the results provide a deeper insight into the phylogeny of this spreading pest insect. The results of the COI barcoding showed no differences between the sampled populations and other *M. mumecola* populations from Europe and Japan, while reports from China showed significant differences. All 12S sequences showed no variability between populations from Austria, Germany, and Italy. A phylogenetic analysis of COI and 12S sequences with sequences from other aphid species from NCBI GenBank or sampled in Italy revealed a close relationship of this species with the damson-hope aphid, *Phorodon humuli*.

**KEYWORDS**
Austria, first report, identification, invasive species, *Prunus*

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## 1 | INTRODUCTION

In 2016, an invasive apricot aphid, was first identified in Europe, in Emilia-Romagna region of Italy precisely (Panini et al., 2017). Subsequent occurrences were reported from Hungary in 2020 (Borbély et al., 2021), Serbia in 2021 (Petrović-Obradović, 2021) and Germany in 2022 (EPPO, 2022). Panini et al. (2017) identified this species as *Myzus mumecola* (Matsumura, 1917) (Hemiptera: Aphididae). Further first reports for European countries (Borbély et al., 2021; EPPO, 2022; Petrović-Obradović, 2021) and preliminary studies on the control of this new pest in apricot cultivation (Tabet et al., 2023) used available identification keys and the mitochondrial cytochrome c oxidase I (COI) sequences from Japan (AB738876, AB738877) for the identification of this species. The species' host range seems to be narrow and known host plants of *M. mumecola* are reported to be apricots (*Prunus armeniaca* L.), Japanese apricots (*Prunus mume* (Siebold)) (Kimura et al., 2016; Panini et al., 2017), and *Prunus cornuta* (Basu & Raychaudhuri, 1976). Unlike other aphid species present on apricot in Europe, which are overwintering on apricot and producing small colonies like *Myzus persicae* (Jevremović et al., 2016), *Phorodon humuli* (Knoll et al., 2004), *Brachycaudus helichrysi*, *Brachycaudus cardui* and *Hyalopterus pruni* (Özgökçe & Atlıhan, 2005), a heavy infestation with the invasive species results in economically important damage (Panini et al., 2017; Tabet et al., 2023). On apricot, this species was described to cause curled and discoloured leaves on young shoots (Panini et al., 2017). Moreover, it was shown that the invasive aphid acts as vector of the *Plum pox virus* (PPV) (Borbély et al., 2021; Kimura et al., 2016). Therefore, this invasive aphid species might also increase the risk of yield losses due to its potential as vector of this disease in European apricot cultivation. With a yearly apricot production of almost 700 thousand tons in Europe (FAOSTAT, 2020), a rapid expansion of this species could negatively impact the future cultivation prospects of apricots in Europe. The aim of our study was to reveal the current spread of *M. mumecola* in Central Europe and to provide additional insight into the phylogeny of this invasive aphid. For molecular identification, the COI gene region, commonly used as standard barcode to distinguish aphid species, was used (Suganthi et al., 2023). In addition, fragments of the 12S rRNA region were considered for the identification of the phylogenetic relationship among aphids (Kim & Lee, 2008; Papasotiropoulos et al., 2013).

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling

Apricot orchards in various locations across South Tyrol, Italy, were inspected during the spring of 2022, whereas orchards in Austria and Germany were inspected during spring 2023 (Figure 1). Infested apricot trees were identified based on published descriptions (Panini et al., 2017). The aphid *M. mumecola* forms large colonies at the tips of young shoots, which are not reported for other aphid species present on apricot (Figure S1). The affected shoots are reduced in growth, the leaves are curled and often red in colour. High infestation leads to leaf fall, dieback of shoot tips and large quantities of honeydew on infested branches. Young trees are significantly impacted in their development.

Wingless individuals of green colour were collected from the sampling sites. Other aphid species, serving as outgroup, were sampled from plants of the Rosaceae family in South Tyrol during spring 2022. Aphids were preserved in 99% ethanol and stored at 4°C for molecular identification. Sampling sites in detail are outlined in Table S1.

Sampled aphids were identified based on their morphological characteristics using stereo- and light microscopy. Other aphid species found in the past on apricot trees in Europe differ significantly in terms of size and colour (Blackman & Eastop, 1994; Borbély et al., 2021; Panini et al., 2017; Petrović-Obradović, 2021; Tabet et al., 2023).

### 2.2 | Molecular identification

DNA was extracted from five individuals per sample with the Quick-DNA™ Tissue/Insect Miniprep Kit (Zymo Research, CA, USA) according to manufacturer's instructions with minor modifications: sample disruption was performed with a bead mill (TissueLyser II, Qiagen, Hilden and Germany) for 10 minutes at maximum speed (30 hz) and DNA was eluted in 25 µL of ultrapure sterile water. DNA concentration and quality of each extract was valuated with a spectrophotometer (NanoPhotometer® N60, Implen, Munich, Germany).

Identification of aphids was done by molecular DNA analysis of the partial COI and 12S regions. The amplification of the two loci was performed in a 35 µL reaction volume consisting of 3.5 µL extracted DNA (diluted 1:10 in ultra sterile water, v/v), 5×Phusion Green HF Buffer (Thermo Scientific, Vilnius, Lithuania), 0.35 µL of Phusion High-Fidelity DNA polymerase (Thermo Scientific, Vilnius, Lithuania), 0.2 mM dNTP mix, and 0.3 µM of each 5′ and 3′ end primers LCO1490 and HCO2198 (Folmer et al., 1994) for the COI locus or 12SCFR and 12SCRR (Hanner & Fugate, 1997) for the 12S locus. PCR was performed with a thermal cycler (Veriti™ 96-Well Fast Thermal Cycler) under following conditions: initial denaturation at 98°C for 30 s, then 30 (COI) or 35 (12S) cycles of denaturation at 98°C for 10 s, annealing at 49°C for 30 s (COI) or at 54°C for 30 s (12S), extension at 72°C for 45 s (COI) or 40 s (12S) and a final extension for 5 min at 72°C. PCR reactions were electrophoresed on a 1.5% agarose gel.

The obtained PCR products were purified and sequenced by LGC Genomics GmbH (Berlin, Germany). Quality control of sequenced data was performed with Geneious 11.1.5 software (Biomatters Ltd. Auckland, New Zealand) and sequence identity was confirmed by using the BLASTN algorithm at NCBI GenBank.

A multiple alignment including obtained sequences of the present study as well as reference sequences from NCBI GenBank was performed based on the CLUSTAL W algorithm in the MEGA X 10.2.6 software (Kumar et al., 2018). Model test analysis revealed

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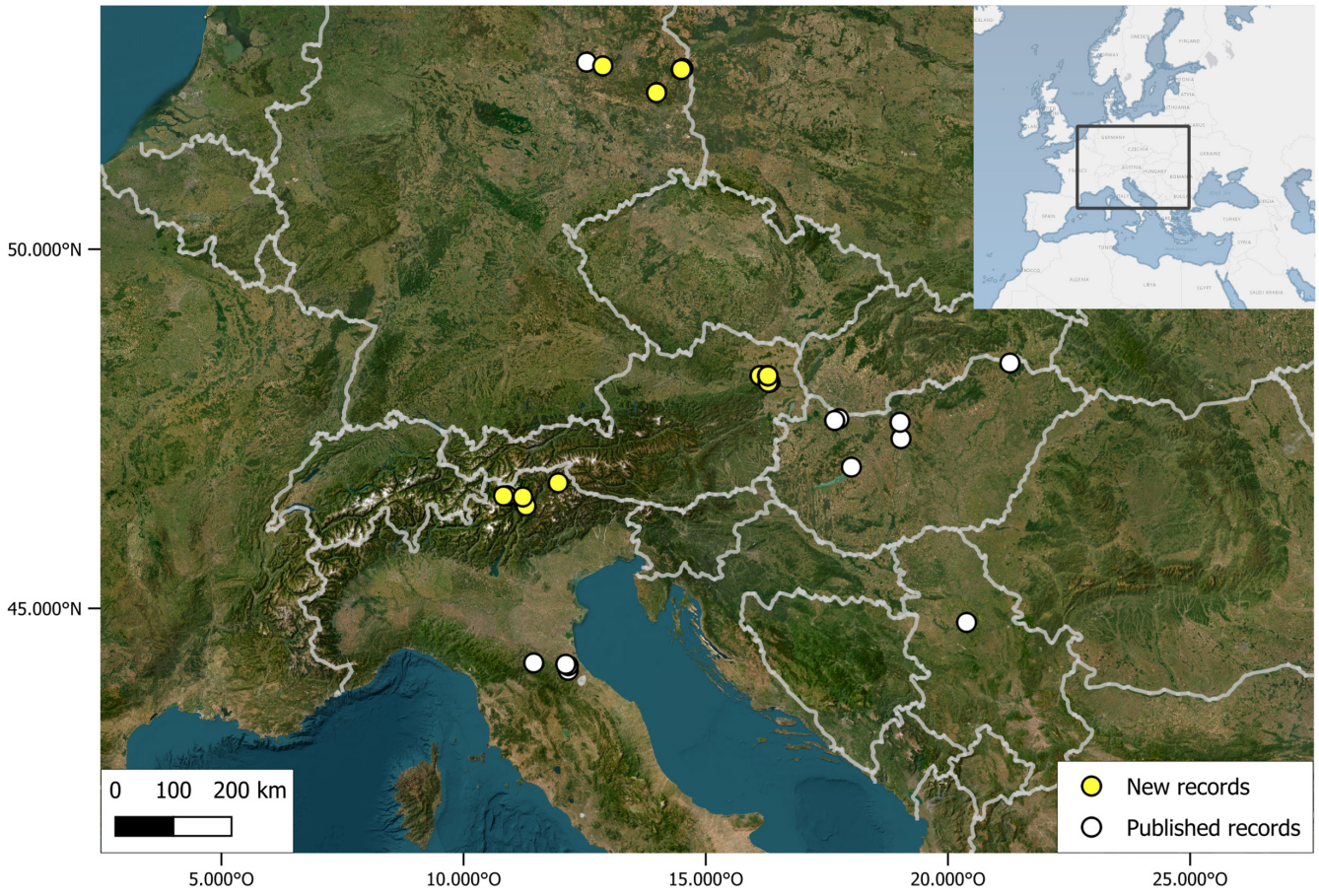



FIGURE 1 Current reports of *M. mumecola* in Europe. Yellow points indicate new records for Italy in 2022 as well as for Austria and Germany in 2023. White points indicate published records from earlier years (Borbély et al., 2021; EPPO, 2022; Panini et al., 2017; Petrović-Obradović, 2021). [Colour figure can be viewed at wileyonlinelibrary.com]

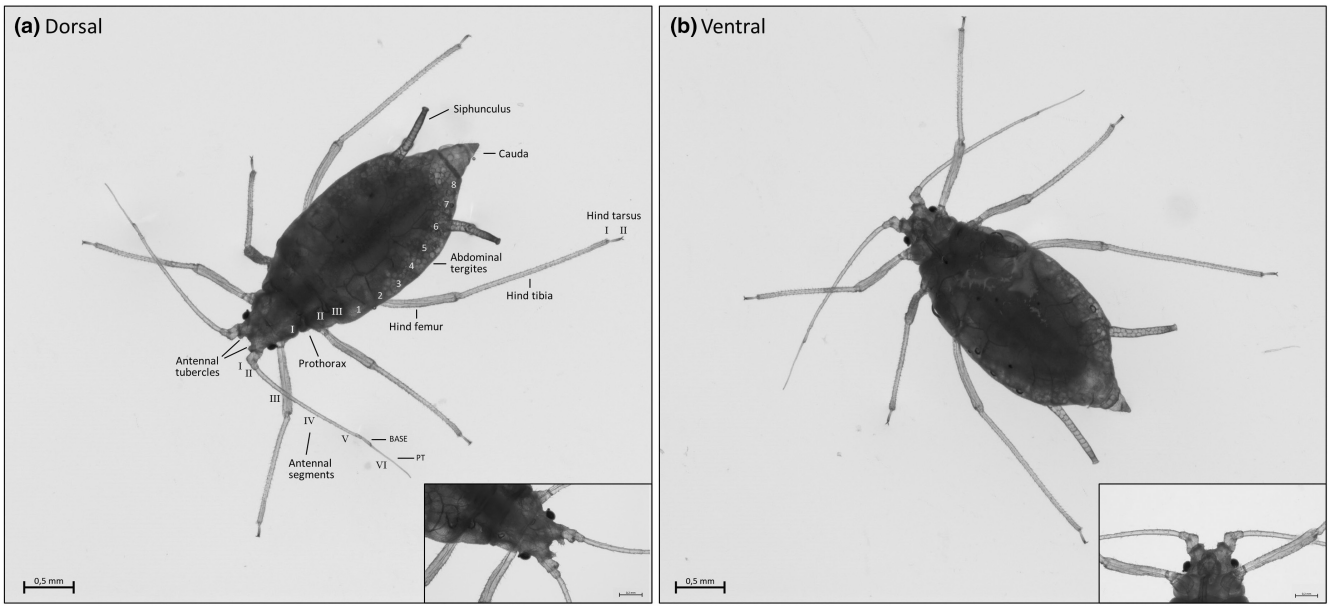



FIGURE 2 Morphological characteristics of wingless *M. mumecola*: dorsal view (a) and ventral view (b). The antennal tubercles are detailed in the bottom right corner.

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TN93+I for COI and T92+G for 12S as the best fitting nucleotide substitution mode. Subsequently, Maximum Likelihood (ML) phylogeny was reconstructed with bootstrap analysis to estimate node support including 1000 replicates.

## 3 | RESULTS AND DISCUSSION

In our study, the invasive apricot aphid, *M. mumecola* was recorded at all investigated apricot orchards (Figure 1). The morphological identification was based on the key by Blackman and Eastop (1994), where *M. mumecola* is characterized overall by its pale green colour, well-developed antennal tubercles, a cauda slightly longer than its width, a hairless tubular siphunculus, and hairs on antennal segment III (Figure 2). The aphid species was detected in Austria for the first time in several places, underpinning the progressing spread of this invasive aphid throughout Europe. In all organically maintained orchards included in the current study, heavy infestations led to the previously described symptoms (Panini et al., 2017). The levels of infestation led to major difficulties in biological aphid management at the investigated sites in Austria.

Preventive measures to control aphids in fruit cultivation include removing infested shoots, implementing targeted irrigation and fertilization, using agronomical practices that conserve and promote beneficial insects through ecological practices (Furmanczyk et al., 2022). Additionally, both integrated and organic productions have access to insecticides for direct control. In organic farming, this includes applications of mineral and plant oils, neem, pyrethrins or soaps (Penvern et al., 2010). A screening showed that insecticides allowed for organic production have a minor efficiency in the successful control of *M. mumecola* compared to those used in integrated pest management (Tabet et al., 2023). Therefore, especially in organic farming, where fewer control options are available, the production of large colonies caused considerable damage.

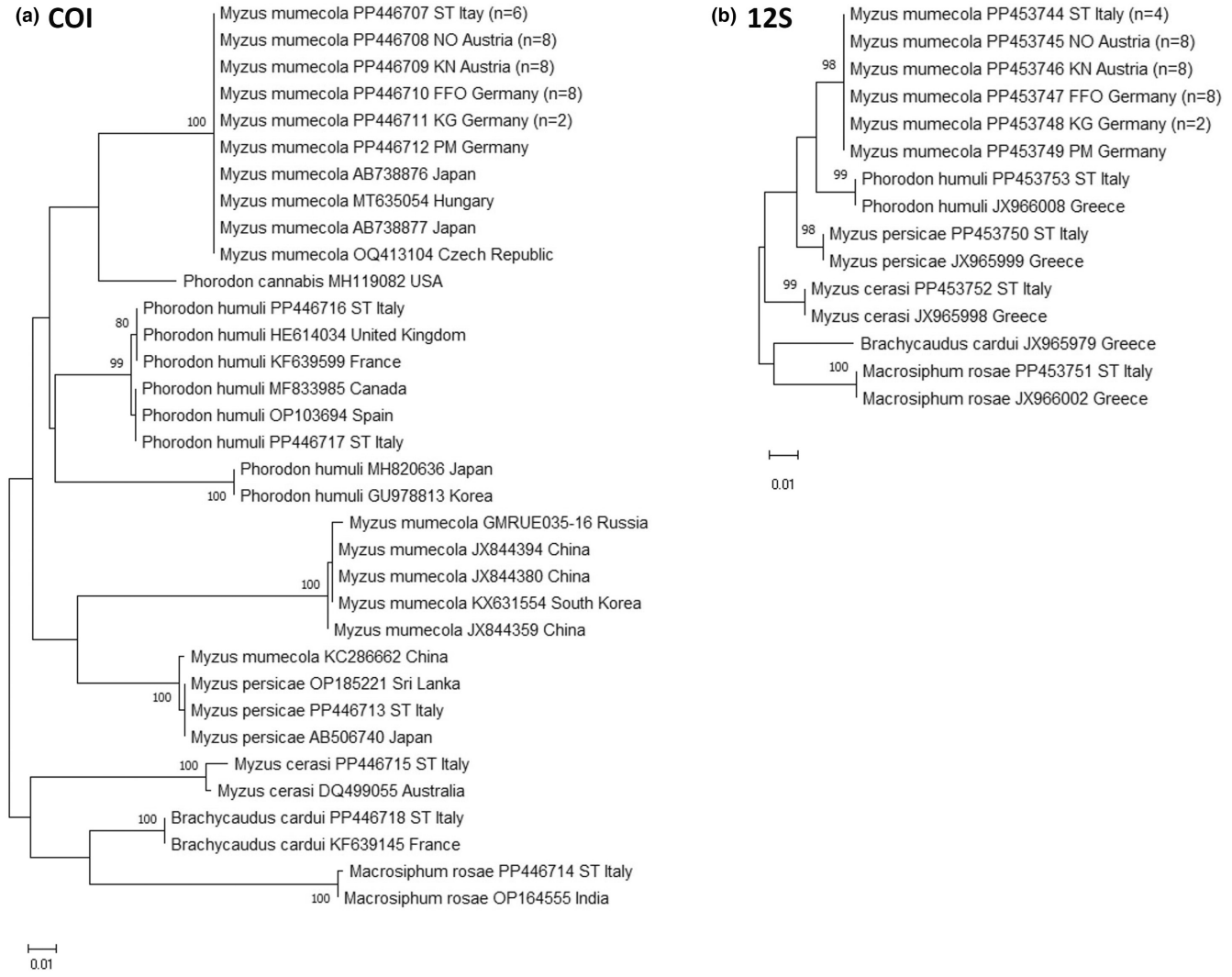



FIGURE 3 Maximum likelihood (ML) tree based on COI (a) and 12S (b) sequences from aphids collected in different locations in South Tyrol, Italy (ST); Niederösterreich, Austria (NO); Klosterneuburg, Austria (KN); Frankfurt (Oder), Germany (FFO); Krugau, Germany (KG); Potsdam-Mittelmark, Germany (PM). Sequences from NCBI GenBank and one sequence from BOLD Systems (GMRUE035-16) were used as references.

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**TABLE 1** Accession numbers of the obtained sequences for COI and 12S region deposited in NCBI GenBank.

| Aphid species | Country | Host plant | Sample | COI | 12S |
|---|---|---|---|---|---|
| *Myzus mumecola* | Italy | *Prunus armeniaca* | 22MM54M | PP446707 | PP453744 |
| *Myzus mumecola* | Austria | *Prunus armeniaca* | 23M04NO | PP446708 | PP453745 |
| *Myzus mumecola* | Austria | *Prunus armeniaca* | M2301KN1 | PP446709 | PP453746 |
| *Myzus mumecola* | Germany | *Prunus armeniaca* | 23MMDE11 | PP446710 | PP453747 |
| *Myzus mumecola* | Germany | *Prunus armeniaca* | 23MMDE3 | PP446711 | PP453748 |
| *Myzus mumecola* | Germany | *Prunus armeniaca* | 23MMDE6 | PP446712 | PP453749 |
| *Myzus persicae* | Italy | *Prunus persica* | 22MM1B | PP446713 | PP453750 |
| *Macrosiphum rosae* | Italy | *Rosa* sp. | 23MM2B | PP446714 | PP453751 |
| *Myzus cerasi* | Italy | *Prunus avium* | 23MM3B | PP446715 | PP453752 |
| *Phorodon humuli* | Italy | *Prunus serrulata* | 23MM4B | PP446716 | PP453753 |
| *Phorodon humuli* | Italy | *Prunus domestica* | 23MM5B | PP446717 | – |
| *Brachycaudus cardui* | Italy | *Prunus armeniaca* | 23MM7B | PP446718 | – |

Molecular characterization based on the two loci COI and 12S did not show any genetic differences in obtained sequences between the populations from Italy, Austria, and Germany. These findings implicate a progressive spread of this invasive species within Europe, probably caused by the introduction of a single population.

Molecular identification by COI sequence analysis for all 33 *M. mumecola* samples from Austria, Italy, and Germany revealed an identical consensus sequence of 594 bp with no ambiguities to available *M. mumecola* sequences in NCBI GenBank from Japan, Italy (Panini et al., 2017), the Czech Republic, Hungary (Borbély et al., 2021) and thus, support the morphological assignment to *M. mumecola* (Figure 3a). Surprisingly, our samples, together with all other COI sequences from Europe and sequences from Japan, clustered closer to the genus *Phorodon* than to the genus *Myzus*. Even more surprising was the clustering of *M. mumecola* sequences from China, which clustered closer to the COI sequences of the aphid *M. persicae*, resulting in two distinct COI sub-groups.

The identity of other aphid species could be confirmed by BLASTN analysis of the obtained COI sequences to sequences from NCBI GenBank (Figure 3) and available literature: *P. humuli* (accession number: HE614034, King et al., 2015; MF833985, DeWaard et al., 2019; MH820636, Li et al., 2020; GU978813, Lee et al., 2011), *M. persicae* (OP185221, Marasinghe et al., 2023; AB506740, Komazaki et al., 2010), and *Myzus cerasi* (DQ499055, Valenzuela et al., 2007).

Sequences of the 12S locus were obtained for 31 *M. mumecola* samples, with an identical sequence length of 320 bp (Figure 3b). No reference sequence for *M. mumecola* was available in databases. The highest sequence similarity of 98.8% for the obtained sequences was found to *M. persicae* (JX965999) as well as to *P. humuli* (JX966008), both from Greece (Papasotiropoulos et al., 2013). Alignment of the obtained 12S sequence of *M. mumecola* to the corresponding sequences of *M. persicae* or *P. humuli* revealed seven single nucleotide changes which differentiated *M. mumecola* from the two mentioned aphid species. Therefore, the 12S locus can be used in addition to the COI locus for the molecular identification of *M. mumecola*. One COI and 12S sequence per region was deposited in NCBI GenBank (Table 1).

Further research on the *M. mumecola* phylogeny and a morphological characterization of the two putative COI sub-groups is needed for verification of the correct assignment of the invasive aphids to one species and finally to the species originally described as *M. mumecola*.

## AUTHOR CONTRIBUTIONS

**Sabine Oberhofer:** Validation; writing – original draft; writing – review and editing; methodology; data curation. **Monika Riedle-Bauer:** Validation; investigation; writing – review and editing. **Mirjam Weissmann:** Validation; writing – review and editing. **Agnes Andrae:** Validation; writing – review and editing. **Ulrike Holz:** Validation; writing – review and editing. **Sabine Oettl:** Validation; writing – review and editing; methodology. **Urban Spitaler:** Conceptualization; validation; investigation; writing – original draft; writing – review and editing; methodology; data curation.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

DNA sequences were deposited in NCBI GenBank. Locations are available in the supplementary material of this article. https://www.ncbi.nlm.nih.gov/genbank/.

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