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NOTES AND COMMENTS

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Deformed wings in introduced solitary bees, *Megachile spp.*, independent of virus infections

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ABSTRACT

Insect wing deformities can be caused by various factors, including the Deformed wing virus (DWV). Symptomatic and asymptomatic specimens of the introduced solitary wild bees *Megachile sculpturalis* and *Megachile disjunctiformis* collected at eleven Central European locations were screened for DWV infections. Even though virus spillover is common, and DWV was detected in other bee species collected in the same habitat as *Megachile spp.,* neither DWV-A nor DWV-B were found in any of the samples ($N = 54$) including two symptomatic males with deformed wings. This indicates that other stressors were responsible for the observed clinical symptoms, thereby highlighting the necessity of differential diagnostics.

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Pathogens; *Megachile sculpturalis*; *Megachile disjunctiformis*; deformed wing virus; invasive species

Shared viruses between adjacent managed Western honey bees, *Apis mellifera*, and wild bees are common (Piot et al., 2022; Yañez et al., 2020). However, at present, there are hardly any virus data available for the two wild solitary bees, *Megachile sculpturalis* and *Megachile disjunctiformis,* which have been introduced into Europe (Bortolotti et al., 2018; Vereecken & Barbier, 2009). Unlike all other introduced wild bees to Europe, *M. sculpturalis* is currently in rapid range expansion extending its colonized area from East Spain to the Balkans (Lanner et al., 2022). Besides the displayed dispersal ability, the species was observed to compete for nesting resources with native bees (Lanner et al., 2020). For these reasons, *M. sculpturalis* was categorized as the first (potentially) invasive alien bee in Europe (IUCN Managing Invasive Alien Species to Protect Wild Pollinators, 2020).

Within a citizen science initiative (www.beeradar.info, Lanner et al., 2020), two *M. sculpturalis* males with deformed wings were reported (Figure 1). The most prevalent virus detected in managed as well as wild bees, the deformed wing virus (DWV), might have caused clinical symptoms of wing deformations and reduced life span (de Miranda & Genersch, 2010; Kevill et al., 2017). DWV comprises three master variants DWV-A/B/C, whereby variants A and B are known to be widespread in Europe and DWV-B is considered to be the most virulent strain (McMahon et al., 2016; Mordecai et al., 2016). Here, we, therefore, conducted a screening of these two and further sampled asymptomatic *M. sculpturalis* and *M. disjunctiformis* individuals to investigate the possible impact and occurrence of DWV-A and/or DWV-B.

Megachile sculpturalis ($N = 54$ including two symptomatic males; Table 1 pooled sample nr. 1–9) were sampled from eleven European locations and stored in RNAlater. Similarly, *M. disjunctiformis* ($N = 5$; Table 1 pooled sample nr. 10) were collected in Bologna, Italy stored in Zymo DNA/RNA shield. Bees were homogenized individually in PBS solution (Phosphate Buffered Saline; pH 7.4) using one glass bead (⌀ = 5 mm) and an electronic crushing shaker machine (Retsch Mixer Mill MM 300, Haan, Germany). Aliquots of the homogenates were pooled in 10 samples according to the sampling site including a group consisting only of the two bees with deformed wings (Table 1, sample nr. 7). Then, RNA was extracted using the NucleoSpin® RNA II kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. For reverse transcription, the extracted RNA was measured using a

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spectrophotometer (QuickDrop™, Molecular Devices, San Jose, CA, USA). Then, 0.5 µg of RNA and 0.75 µL of 100 mM of random hexamer primers were heated at 70 °C for 5 min and then cooled down to 4 °C. To obtain a final volume of 25 µL, a master mix, consisting of 5 µL M-MLV 5X Reaction Buffer (Promega, Fitchburg, Wisconsin, USA), 1.25 µL 10 mM dNTP Mix (2.5 mM each; Bioline, London, UK) and 1 µL (200 units) M-MLV reverse transcriptase (Promega, Fitchburg, Wisconsin, USA) was added and incubated at 37 °C for 60 min. For the screening of DWV-A and DWV-B, qPCR reactions were prepared using 6 µl of 2X reaction buffer (SensiFAST™ SYBR® No-ROX Kit, Meridian Bioscience, London, UK), 0.24 µl forward and reverse primer, 2.52 µl water and 3 µl of cDNA. Primer information is stored in the supplementary material (Table S1). Finally, qPCR was performed in a CFX96™ Real-Time PCR Detection Systems (BioRad, CA, USA) with the following settings: 3 min for 95 °C, 40 cycles of 3 sec at 95 °C and 30 sec at 57 °C. The amplification was followed by a melting curve analysis of the strand dissociation to verify the PCR product specificity. The analysis was performed by reading the fluorescence at 0.5 °C increments from 55 to 95 °C.

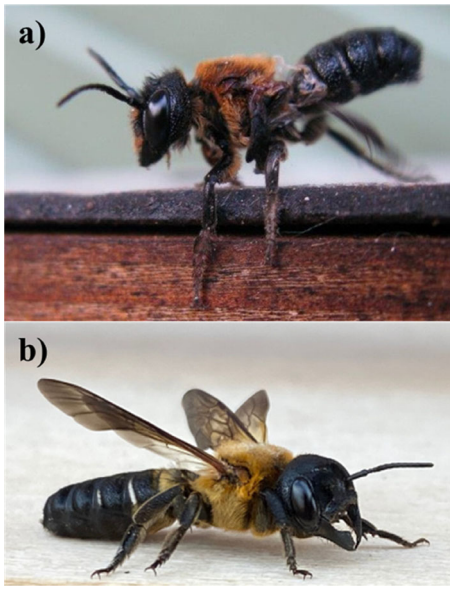

Figure 1. Adult *Megachile sculpturalis*: (a) male displaying deformed wings (CC by 4.0 Merz); (b) asymptomatic female.

Standard curves dilutions (10^{-2} to 10^{-5} ng/reaction) for DWV-A and DWV-B were prepared from purified PCR products and combined with positive controls containing the target viruses. As negative controls, the following reactions were added: 1: RNA-extraction and RT control without bee sample; 2: PCR negative control, using water instead of cDNA template).

Even though positive PCR controls and the 28S rRNA target gene performed as expected, neither DWV-A nor DWV-B were detected in any of the samples including the symptomatic males. The third variant, DWV-C, is considered to have a minor prevalence compared to DWV-A/B and so far, unknown transmission routes (Beaurepaire et al., 2020; Mordecai et al., 2016). Therefore, this third variant was not considered. Despite sampling efforts at eleven areas with documented DWV infections in bees (Berthoud et al., 2010; Bordin et al., 2022) and large numbers of managed honey bee colonies, we found no evidence of any DWV-A and DWV-B in our samples. Similarly, other cases of deformed wings showed that environmental stressors (e.g., increased humidity) might have caused these symptoms (Huwiler et al., 2020). However, these negative findings do not imply that *M. sculpturalis* may not carry other pathogens known to be widespread in its non-native habitat. Indeed, 65% of a local population was tested positive for Sacbrood virus (SBV, Cilia et al., 2022). Combined with the present data suggesting rather low chances for DWV at least for the tested solitary bees, this indicates that there might be differences between viruses and bee host species in terms of actual spillover rates.

Further screenings of possible co-introduced pathogens originating in its natural environment seem to be required for these introduced bee species. Potential spillovers of co-introduced pathogens are among the main threats associated with invasive alien species (Russo, 2016). The example of the co-

Table 1. Sampling information for the screened wild bees *Megachile sculpturalis* and *Megachile disjunctiformis*.

Sample	Coordinates N	Coordinates E	Locations	Sex / Life stage	Date
1	47,263978	11,365871	Innsbruck (Austria)	2 F	11.07.2021
2	44,512436	11,332794	Bologna (Italy)	6 F	25.07.2021
	45,48331	10,726754	Lazise (Italy)	1 M / 1 F	31.07.2021
3	47,280321	11,41104	Innsbruck (Austria)	3 F	07.08.2021
4	46,673701	11,152571	Meran (Italy)	2 M	12.06.2021
	46,49418	11,346366	Bozen (Italy)	8 M, 3 F	16.07.2021
	46,666662	11,19592	Labers (Italy)	3 M	19.07.2021
5	NA	NA	Switzerland	1 M / 1 F	31.07.2020
6	46.730836	7.673244	Oberhofen (Switzerland)	3 L	15.08.2020
	46.181846	46.181846	Carouge (Switzerland)	2 NA, 1 M	19.08.2020
7*	46.730836	7.673244	Oberhofen (Switzerland)	2 M	05.07.2019
8	48.153767	16.396103	Vienna (Austria)	4 M, 4 F	30.07.2020
9	46,2935871	7,88438226	Visp (Switzerland)	6 F	27.07.2021
10	44,524029	11,349484	Bologna (Italy)	5 M	07.07.2021

Coordinates, Location, Sex and Years are given. (1–9: *M. sculpturalis*; 10: *M. disjunctiformis*; * = symptomatic males, F = female, M = male, L = larvae).

introduced pathogen spillover from managed *Bombus terrestris* demonstrates the tremendous negative effects on Argentinian endemic *Bombus sp.* (Arbetman et al., 2013). Therefore, further screenings of associated pathogens are advised for those introduced bees.

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