Recent invasion and eradication of two members of the *Euwallacea fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae) from tropical greenhouses in Europe

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DNA extraction and Sequencing

After taxonomic determination, all individuals were stored in absolute ethanol at -20°C. DNA of three individuals from Poland, nine individuals from Italy, three individuals from Erfurt and 17 from Berlin. All individuals were extracted using the GenElute Mammalian Genomic DNA miniprep kit (Sigma-Aldrich, Steinheim, Germany). DNA was eluted in 100 µl elution solution (10 mM Tris, 1 mM EDTA) and stored at -20°C.

A fragment of the mitochondrial COI gene was PCR amplified using the primers /Lep-R1 (Hebert et al. 2004). Each reaction was set up in a total of 25µl including 7.5µl H_2O, 12.5µl DreamTaq PCR Master Mix (ThermoFisher Scientific, Waltham, Massachusetts), 1.25 µM of each primer and 2.5µl template DNA. All reactions were performed in an Eppendorf Mastercycler Gradient (Eppendorf; Hamburg, Germany) with the following conditions: 95°C 3min followed by 35 cycles 95°C 30 s, 46°C 30 s, 72°C 1 min followed by a final extension at 72°C for 10 min. PCR products were run on a 1% agarose gel stained with SYBRsafe (Thermo Fisher Scientific) and Sanger sequenced by a commercial provider (Eurofins MWG Operon, Ebersberg, Germany). Sequences were aligned with CodonCode Aligner v8.0 (CodonCode Dedham, MA, USA), manually edited and compared to sequences available in Genbank using the BLAST algorithm in NCBI.

Individual beetle specimens from the two Dutch outbreak locations (NL1 = 18 specimens, NL2 = 14 specimens) were morphological identified to genus level and used for DNA extraction with the High Pure PCR template preparation kit (Roche, Basel, Switzerland) according to the manufacturers' instructions. Insect specimens were crushed in lysis buffer with a micro-pestle prior to DNA extraction. The DNA extracts from each specimen were used for library preparation with the NEBNext® Ultra II DNA Library Prep kit for Illumina. Library prep quality and yield was measured with the 2100 Bioanalyzer (Agilent, CA, USA), and samples which resulted in products of expected size (500-700 bp) were sequenced using an Illumina MiSeq, a NextSeq2000, or a NovaSeq6000 with at least 2 gigabases (Gb) output.

Assembly of mitogenomic sequences

Paired-end Illumina sequence reads were uploaded to CLC genomics workbench v20 (Qiagen, CA, USA) and subjected to a pipeline that combined *de novo* assembly and blast based identification to detect putative mitogenomic contigs. The pipeline contained the following analysis steps: trim reads (Quality trim: 0.05; Ambiguous limit: 2), *de novo* assembly (mapping mode: map reads back to contigs; update contigs: ON; length fraction: 0.8; similarity fraction: 0.9; minimum length: 1000), extract consensus (threshold: 0; low coverage handling: insert N), and megablast with a local database containing complete Coleoptera mitogenomes as reference (maximum alignments: 25; maximum E-value: 1e-20; minimum percentage identity: 80%). Contigs producing blast hits with the mitogenomic reference set were regarded as putative mtDNA contigs and were structurally and functionally annotated using the online Mitos2 tool (http://mitos2.bioinf.uni-

leipzig.de/index.py, last accessed 14 June 2021). The annotations were manually updated where needed to correct for misidentification of stop codons of mitogenomic protein coding genes. The partial mitochondrial *cox1* gene was extracted from the 15,822 to 16,323 bp mitogenomic sequences with 260 to 18,124 x average read for further analysis.