

Phylogenetic analysis of several *Otiorhynchus* spp. (Coleoptera: Curculionidae) present in The Netherlands

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Introduction

Otiorhynchus forms a large genus of weevils in the family Curculionidae. Many species belong to *Otiorhynchus* and are important pests of soft fruit and ornamental plants. Within this genus the vine weevil *Otiorhynchus sulcatus* (Fabricius, 1775) and the clay-coloured weevil *O. singularis* (Linnaeus, 1767) have been well known in The Netherlands since 1848. However, in the last 10 years the established presences of several other species from Southern Europe like *O. crataegi* (Germar, 1825), *O. aurifer* (Boheman, 1843), *O. armadillo* (Rossi, 1792) and *O. salicicola* / *apenninus* (Heyden, 1908) has also been reported in The Netherlands (Figure 1).

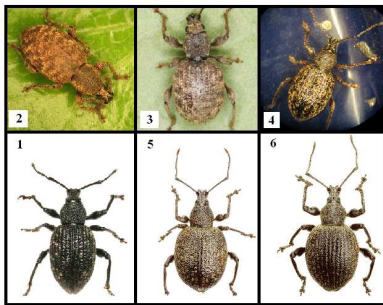


Figure 1. Several *Otiorhynchus* species, 1: *Otiorhynchus sulcatus*, 2: *O. singularis*, 3: *O. crataegi*, 4: *O. aurifer*, 5: *O. armadillo* and 6: *O. salicicola* (source 5,6: T.Heijerman).

Phylogenetic analysis

To help with species discrimination, a molecular study was undertaken. The phylogenetic relationship between these species was analysed based on the DNA nucleotide sequences of the mitochondrial cytochrome oxidase subunit II (COII) and cytochrome oxidase subunit III (COIII) genes. The phylogenetic trees have been generated from alignments of obtained sequences (Figure 2 and Figure 3).

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CLUSTAL 2.0.12 multiple sequence alignment
O.salicicola1      ACTCCAGAGAGAGCATCCCCACTTATAGAACACTTATATCTTCCATG 50
O.salicicola2      ACTCCAGATAGAGCATCCCCACTTATAGAACACTTATATCTTCCATG 50
O.aurifer1        ACTTCAGGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.aurifer2        ACTTCAGGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.armadillo1      ACTTCAGGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.armadillo2      ACTTCAGGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.singularis1     ACTTCAGGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.singularis2     ACTTCAGGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.cribicollis_EF583370_
O.vaucheri_EF583371_
O.sulcatus1       TCTCCATGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.salicicola2     ACTTCAGGATAGAGCATCCCCCTTATAGAACACTTATATCTTCCATG 50
O.crataegi1       ACTTCCA-GACAGGGCCCTCTCCACTATAGAGCACTTTATCTTCCATG 49
O.crataegi2       ACTTCCA-GACAGGGCCCTCTCCACTATAGAGCACTTTATCTTCCATG 49
  
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Figure 2. Alignment of nucleotide sequences of (partial) COII gene for several *Otiorhynchus* species. Presence of *O. cribicollis* and *O. vaucheri* in The Netherlands is not yet reported. Underlined nucleotides indicate *O. salicicola* specific sequence.

Phylogram

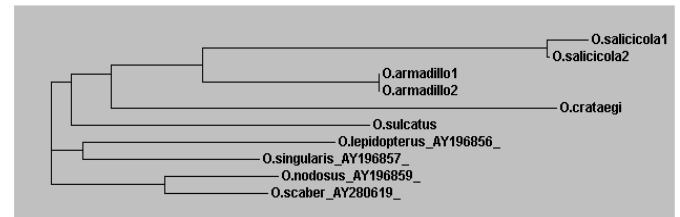


Figure 3. A consensus phylogenetic tree generated with CLUSTALW2 program from the alignment of nucleotide sequences of (partial) COIII gene for several *Otiorhynchus* species. Presence of *O. lepidopterus*, *O. nodosus* and *O. scaber* in The Netherlands is not yet reported.

Specific detection

Our study showed that both COII and COIII gene sequences can discriminate between *Otiorhynchus* species; especially *O. armadillo* and *O. salicicola*, which are morphologically very close and difficult to determine by classical methods. The variations in COII and COIII sequences are useful for development of species-specific PCR assays (Figure 4) which can be used in early identification and detection (eggs and larvae).



Figure 4. PCR products amplified with specific primers for *O. salicicola*. Lane 1-2: *O. salicicola*; Lane 3-4: *O. aurifer*; Lane 5-6: *O. armadillo*; Lane 7: *O. sulcatus*; Lane 8: *O. singularis*; M: 100 bp Ladder (Promega).

The other *Otiorhynchus* spp. present in The Netherlands like *O. ovatus* (Linnaeus, 1758), *O. raucus* (Fabricius, 1777), *O. rugosostriatus* (Goeze, 1777) *O. dieckmanni* (Magnano, 1979), and *O. meridionalis* (Gyllenhal, 1834) may be analyzed in the future.