

Short note

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***Anthonomus spilotus* (Coleoptera: Curculionidae):
new to the Belgian fauna**

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In Belgium there are fifteen species of the genus *Anthonomus* Germar 1817. Thirteen of these species are monophagous or oligophagous on Rosaceae [1] and four of them are considered economically important pests in Belgian fruit production: *Anthonomus pomorum* (Linnaeus, 1758) (apple), *Anthonomus pyri* (Gyllenhal, 1835) (apple, pear) [2], *Anthonomus rubi* (Herbst, 1795) (strawberry, raspberry, blackberry) and *Anthonomus rectirostris* (Linnaeus, 1758) (cherry). These species are omnipresent in Belgium and almost all of Europe. *Anthonomus spilotus* (Redtenbacher, 1847) (Fig. 1), a species that lives on *Pyrus* sp. [3,4,5,6,7,8,9,10], *Crataegus* sp. [4,7,8,10] and *Mespilus germanica* [4,7,8,10] seems to be as widespread in Europe as the earlier mentioned species of *Anthonomus*, however, its distribution and biology are poorly documented. *A. spilotus* has been observed in Belgium from 2014 onwards in nine locations with indications already of its presence in high numbers since 2012 (Table 1). The distribution and biology of *A. spilotus* in Europe will be clarified based on a literature overview and the recent findings of this species in Belgium.

Anthonomus spilotus was first described by REDTENBACHER (1847), who mentioned it to be not uncommon in fruit trees in Austria (Upper Austria). Most literature on *A. spilotus* appeared in the context of plant protection in pear growing, with the most reports from France. PUSSARD (1930) described the large populations in pear plantations in the Rhône valley and mentioned earlier reports from the south: Montpellier, Marseille, the Pyrenees and Corsica but also from more north: Allier, Nièvre, Seine-et-Marne, Seine-et-Oise (now Essonne, Yvelines and Val-d'Oise), Calvados, Oise, Aube and Somme. Later publications confirmed *A. spilotus* to be a pest of pear in almost all France [11,10] although rare in the north and Paris Basin [5,9]. INRA (French National Institute for Agricultural Research) has specimens in collection from Indre-et-Loire 2010 and barcoded the DNA of the species [12]. *Anthonomus spilotus*

is also considered present in Switzerland [11] and BOVEY & BAGGIOLINI (1967) reported it as a pest of pear trees in Romandy although only sporadically, and often overlooked. The weevil is also found in Germany [11]. RHEINHEIMER & HASSLER (2010) stated that *A. spilotus* is rare or even regionally disappeared in Germany, without recorded economic damage in pear orchards. Almost all records are from south and south-west Germany with all recent observations from Baden, Palatinate and Rhineland. The supposed presence of *A. spilotus* in The Netherlands is based on a species list from 1966 [11,13] but observations are rare or non-existent. *A. spilotus* is considered absent in Poland [14] but is present in Ukraine (Western Ukraine, Pruto-Dnistrov's'ke Mezhyrichchia) [15], Hungary [11] and Russia [11]. In the Czech Republic this weevil is considered present [11] but regionally endangered [16]. *A. spilotus* even occurs as far north as Southern Sweden [11,7], in particular Öland and Scania [14] with a recent specimen in the collection of the Naturhistoriska Riksmuseet [17]. *A. spilotus* is also reported in Southern Europe and Northern Africa with reports from Italy [11,5], Greece [11,5], Spain [11], Portugal [11], Morocco [3,4,10] and Algeria [3,4,10]. In the United Kingdom (Kent), *A. spilotus* was recorded for the first time in 2017 in pear orchards where it was already considered present for more than three years [8]. An overview of the countries with reports of *A. spilotus* is given in Figure 2. From the above it becomes clear that although widespread in Europe, *A. spilotus* is considered rare in most countries. This species was considered abundant and an occasional pest of pear only in France and even there recent records are rare. However, *A. spilotus* has recently appeared in high numbers in the UK and Belgium. While for the UK it is thought to be newly imported [8], it is possible that *A. spilotus* was a rare and overlooked species in Belgium, hence having a similar status as in Northern France, Northern Germany and The Netherlands. It is, however, remarkable how an overlooked species could become an economically important pest species in a few years. This could indicate that its habitat conditions have somehow become more favourable. These favourable conditions could possibly be explained partially by climate change and/or a shift in insecticide use in pear growing.

In Belgium, *A. spilotus* was always considered present [11] based on its occurrence in neighbouring countries, without actual reported findings or specimens in collections. Recently the species was observed at nine locations (two natural reserves and seven pear orchards). An overview of the observations is given in Table 1; the sampling locations are depicted in Fig. 2B. In 2014, a single observation of *A. spilotus* was made on wild pear in a natural reserve in Han-sur-Lesse (Location b). In 2015, observations were not only made on wild pear in Auffe (Location c) but also a large population was found in a commercial pear orchard in Boëlhe (Location a), where it had already been observed for three years. In 2016, signs

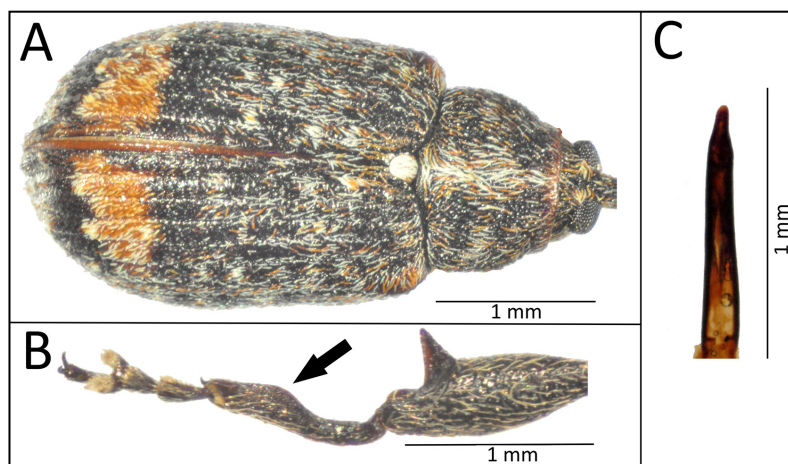


Fig. 1 – Morphological characteristics of *Anthonomus spilotus* Germar, 1817. **A.** Elytra, pronotum and head: forehead hairs directed towards the eyes and reaching over them, elytra elongated and not widening near the end and pale elytral fascia. **B.** Foreleg: inside of protibia strongly curved at the apical half (arrow). **C.** Median lobe of aedeagus: slightly asymmetrical and narrowly pointed.

TABLE 1

List of observations of *Anthonomus spilotus* in Belgium.

| Date | | Observations | | | Host | | Location | | | | |
|------|----|--------------|----|-----------------------|---|-----------|---|----------------|------|--------------|----------|
| YYYY | MM | DD | N° | Life stage(s) | Signs of presence on the host plant | Certainty | Species | Habitat type | Code | Municipality | Province |
| 2012 | - | - | 1 | - | Curled/deformed leaves with necrotic edges (pupal chambers) | Likely | <i>Pyrus communis</i> L. cv. Conference | Orchard | a | Geer | Liège |
| 2013 | - | - | 2 | - | Curled/deformed leaves with necrotic edges (pupal chambers) | Likely | <i>Pyrus communis</i> L. cv. Conference | Orchard | a | Geer | Liège |
| 2014 | - | - | 3 | - | Curled/deformed leaves with necrotic edges (pupal chambers) | Likely | <i>Pyrus communis</i> L. cv. Conference | Orchard | a | Geer | Liège |
| 2014 | 06 | - | 4 | Adult | - | Certain | <i>Pyrus pyraster</i> | Nature reserve | b | Rochefort | Namur |
| 2015 | 04 | - | 5 | Adult | - | Certain | <i>Pyrus pyraster</i> | Nature reserve | c | Rochefort | Namur |
| 2015 | 05 | 07 | 6 | Larvae | Strong reduction in foliage and fruit set | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | a | Geer | Liège |
| 2015 | 05 | - | 7 | Adult | - | Certain | <i>Pyrus pyraster</i> | Nature reserve | c | Rochefort | Namur |
| 2015 | 06 | 12 | 8 | Adults | - | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | a | Geer | Liège |
| 2016 | - | - | 9 | - | Curled/deformed leaves with necrotic edges (pupal chambers) | Likely | <i>Pyrus communis</i> L. cv. Conference | Orchard | d | Gingelom | Limburg |
| 2017 | 03 | 31 | 10 | - | Punctures in leaf buds | Likely | <i>Pyrus communis</i> L. cv. Conference | Orchard | e | Faimes | Liège |
| 2017 | 04 | 10 | 11 | Larvae/ Adult | Punctures in leaf buds + curled leaves with necrotic edges | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | d | Gingelom | Limburg |
| 2017 | 04 | 12 | 12 | Larvae/ Adult | Punctures in leaf buds + curled leaves with necrotic edges | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | d | Gingelom | Limburg |
| 2017 | 04 | 26 | 13 | Larvae/ Pupae | Pupal chambers on leaves + delayed/inhibited sprouting of leaf buds | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | d | Gingelom | Limburg |
| 2017 | 04 | 26 | 14 | Larvae/ Pupae | Pupal chambers on leaves + delayed/inhibited sprouting of leaf buds | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | f | Gingelom | Limburg |
| 2017 | 05 | 26 | 15 | Adults | - | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | d | Gingelom | Limburg |
| 2017 | 05 | 26 | 16 | Adults | - | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | f | Gingelom | Limburg |
| 2017 | 05 | 31 | 17 | Adults | Small holes in leaves made by feeding adults | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | d | Gingelom | Limburg |
| 2017 | 05 | 31 | 18 | Adults | Small holes in leaves made by feeding adults | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | f | Gingelom | Limburg |
| 2018 | 03 | 16 | 19 | Adult | - | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | f | Gingelom | Limburg |
| 2018 | 03 | 30 | 20 | Adults/ Eggs/ Larvae | Punctures in leaf buds + curled leaves | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | f | Gingelom | Limburg |
| 2018 | 04 | 06 | 21 | Adults | Punctures in leaf buds + curled leaves + reduction in foliage | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | f | Gingelom | Limburg |
| 2018 | 04 | 09 | 22 | Adults | Punctures in leaf buds | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | a | Geer | Liège |
| 2018 | 04 | 09 | 23 | Adults/ Larvae | Punctures in leaf buds + curled leaves | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | g | Kortessem | Limburg |
| 2018 | 04 | 20 | 24 | Adults/ Larvae | Curled leaves | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | g | Kortessem | Limburg |
| 2018 | 05 | 02 | 25 | Adults/ Larvae/ Pupae | Pupal chambers on leaves | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | g | Kortessem | Limburg |
| 2018 | 05 | 07 | 26 | Adults/ Pupae | Pupal chambers on leaves | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | g | Kortessem | Limburg |
| 2018 | 05 | 16 | 27 | Adult | - | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | a | Geer | Liège |
| 2018 | 05 | 16 | 28 | Adults | - | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | h | Hoeselt | Limburg |
| 2018 | 05 | 16 | 29 | Adults | - | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | f | Gingelom | Limburg |
| 2018 | 05 | 16 | 30 | Adults/ Larvae/ Pupae | Pupal chambers on leaves + reduction in foliage | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | i | Riemst | Limburg |
| 2018 | 05 | 29 | 31 | Adults/ Larvae/ Pupae | Pupal chambers on leaves | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | i | Riemst | Limburg |
| 2018 | 06 | 04 | 32 | Adults/ Pupae | Pupal chambers on leaves + small holes in leaves made by feeding adults | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | i | Riemst | Limburg |
| 2018 | 06 | 18 | 33 | Adults | Small holes in leaves made by feeding adults | Certain | <i>Pyrus communis</i> L. cv. Conference | Orchard | i | Riemst | Limburg |

of its presence were noticed in a second pear orchard in Jeuk (Location d) and in 2017, the population in this orchard was confirmed. At about 1 km from the latter, another pear orchard (Location f) was found to host a population of *A. spilotus* in 2017. Also in 2017, a pear orchard with signs of *A. spilotus* was discovered in Les Waleffes (Location e). In 2018, *A. spilotus* was detected in the other three pear orchards: in Kortesseem (Location g), Hoeselt (Location h) and Membruggen (Location i). The latter location contained a large population.

Specimens from Location d and f of 2017 were identified using the key of DIECKMANN (1968) and were added to the collections of the Royal Belgian Institute of Natural Sciences. Morphological characteristics for *A. spilotus* are depicted in Fig. 1: forehead hairs directed towards the eyes and reaching over them, elytra elongated and not widening near the end, pale elytral fascia, inside of protibia strongly curved at the apical half and median lobe slightly asymmetrical and narrowly pointed. Specimens from Location i of 2018 were also molecularly identified as follows: genomic DNA was isolated from individual adults using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc, Germany) following the manufacturer's protocol. The *COI* (Cytochrome c oxidase subunit 1) region was amplified using primer cocktails designed by CRUAUD *et al.* 2010 [18] and GERMAIN *et al.* 2013 [19] (Table 2). PCR was carried out in a 50 µl reaction volume including 2 µl of genomic DNA, 22 µl of Milli-Q and 25 µl of BIO-X-ACT™ Short Mix and 0.2 µl of each 10 µM primer cocktail. PCR conditions for *COI* were: 94°C for three minutes, five cycles of 94°C for 30 seconds, 45°C for 30 seconds and 72°C for 60 seconds, followed by 35 cycles of 94°C for 30 seconds, 51°C for 60 seconds and 72°C for 60 seconds, with a final extension at 72°C for 10 minutes. PCR products were purified and sent for sequencing using M13F (5'-TGTAACGACGGCCAGT-3') and M13R (5'-CAGGAAACAGCTATGAC-3') primers [20]. The

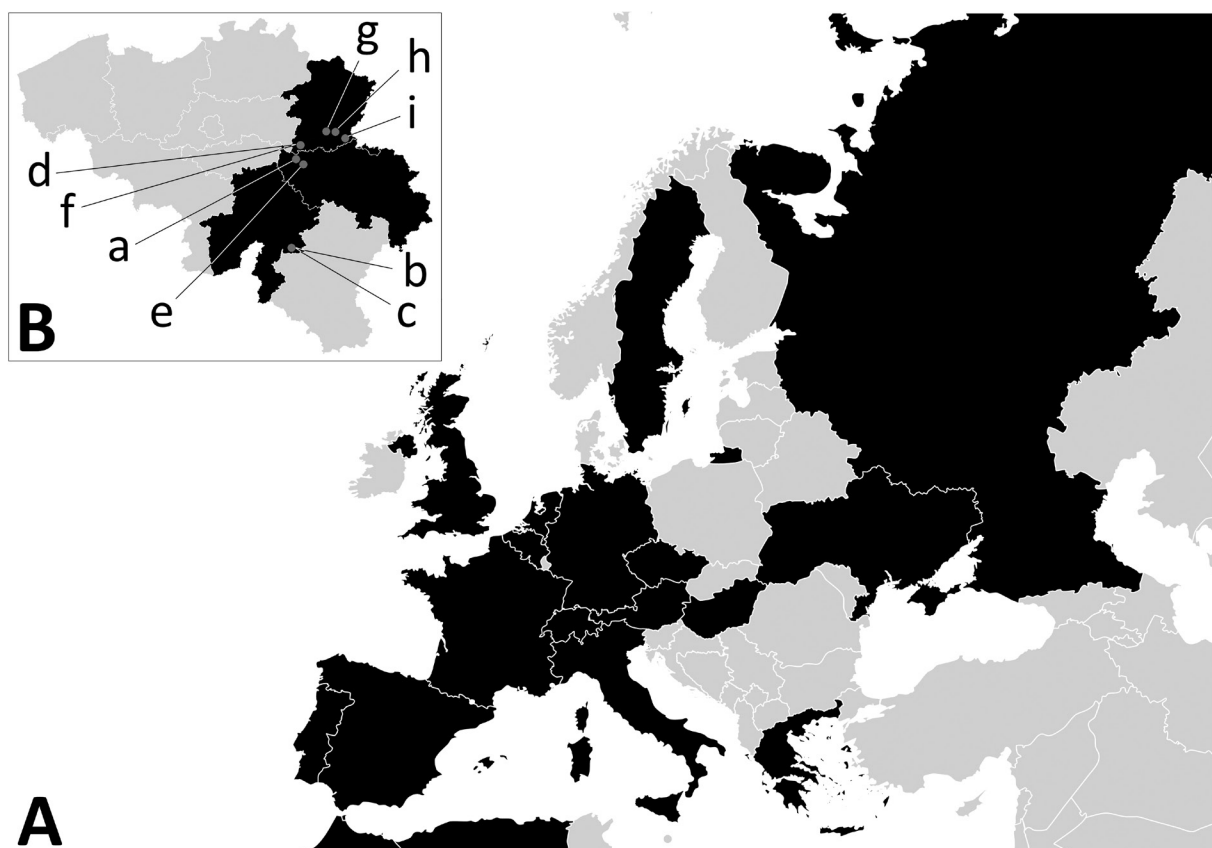


Fig. 2 – A. Overview of the geographical distribution of *Anthonomus spilotus* in Europe, the species is considered (at least regionally) present in the countries marked in black. B. Overview of the Belgian locations where *A. spilotus* was found, letters referring to the location codes in Table 1.

TABLE 2

PCR primer cocktails used to amplify *COI*.

| Primer name | Primer sequence (5'–3') | References |
|----------------|--|------------|
| Forward | | |
| LCO1490puc_t1 | TGTA AACACGACGGCCAGTTTTC AACWAATCATAAAGATATTGG | [18] |
| LCO1490Hem1_t1 | TGTA AACACGACGGCCAGTTTTC AACTAAYCATAARGATATYGG | [19] |
| Reverse | | |
| HCO2198puc_t1 | CAGGAAACAGCTATGACTAAACTTCWGGRTGWCCAAARAATCA | [18] |
| HCO2198Hem2_t1 | CAGGAAACAGCTATGACTAAACYTCAGGATGACCAAAAAAYCA | [19] |
| HCO2198Hem1_t1 | CAGGAAACAGCTATGACTAAACYTCDGGATGBCCAAARAATCA | [19] |

quality of the Sanger sequencing reads was first assessed in Sequence Scanner v2.0. The sequences were edited and analysed using software packages of Chromas 2.00 (Technelysium, Helensvale, QLD, Australia) and BioEdit 7.0.4.1 [21]. The INRA in-house reference sequence of *A. spilotus* [12] and the sequence of a closely related and most morphologically similar species, *Anthonomus ulmi* (DeGeer, 1775), available in the GenBank: KM450134.1 [22], were also imported in BioEdit. The alignment of

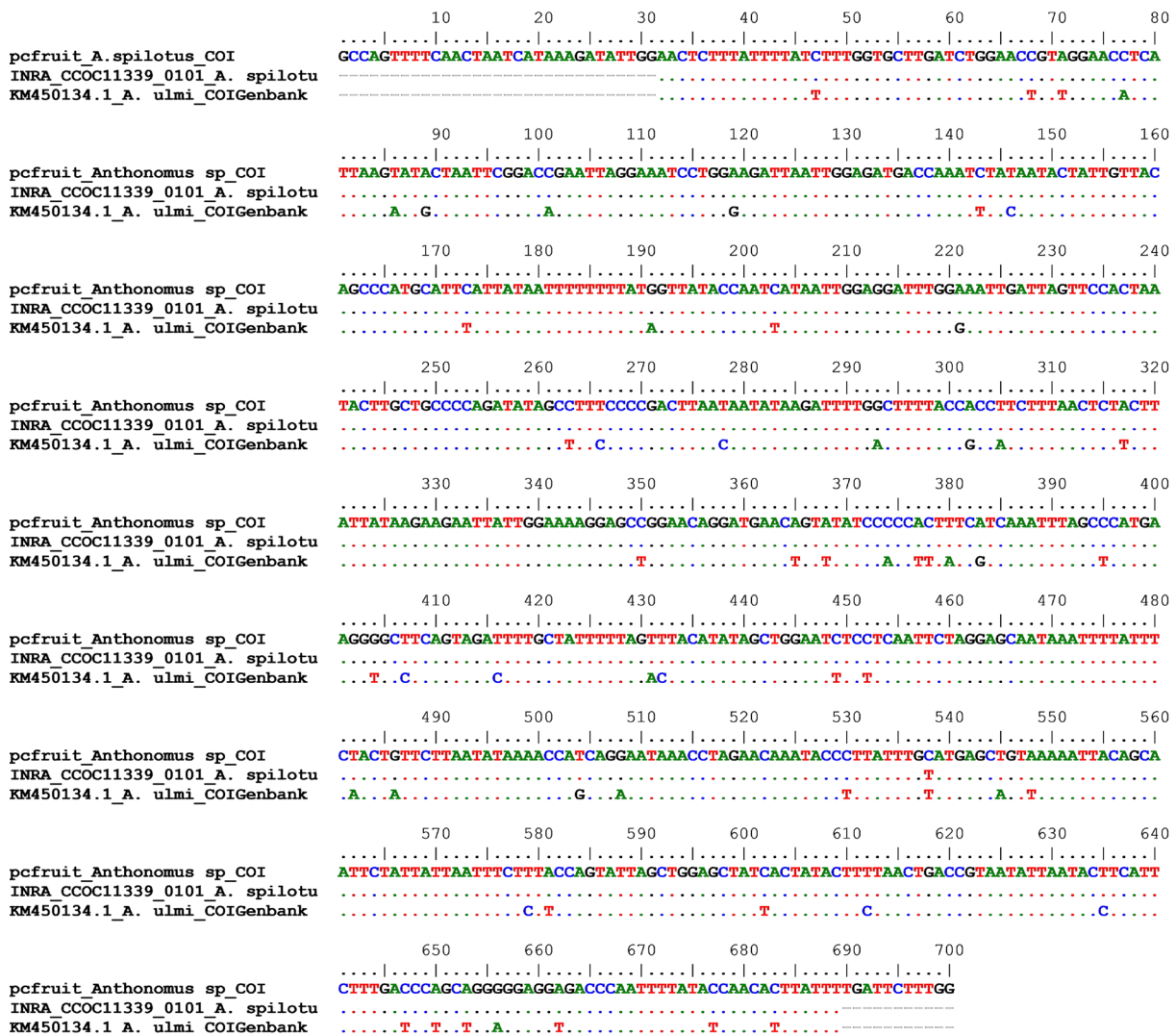


Fig. 3 – Alignment of *COI* sequences of the Belgian population of *Anthonomus spilotus* (Location i, 2018), The French population of *A. spilotus* (Indre-et Loire, 2010) and *Anthonomus ulmi*.

the sequences showed 99% and 91% similarity between the Belgian specimens and the French (Indre-et-Loire) population of *A. spilotus* and *A. ulmi*, respectively (Fig. 3). It is therefore both morphologically and molecularly confirmed that *A. spilotus* is present in Belgian pear orchards.

When rearing adults from collected pupae (Locations d and f in 2017, Location i in 2018), parasitoid wasps (Hymenoptera: Pteromalidae and Hymenoptera: Ichneumonidae) emerged, indicating the presence of antagonists of this species in Belgium.

A tentative phenology of *A. spilotus* in Belgium can be drawn up from the observations in Table 1. Overall adult activity was observed from end March till June, which is in line with the biology described from neighbouring countries: the end of hibernation/start of oviposition occurred at the end of March [5,3,7] and emergence of the new generation adults took place in June [7,3]. The characteristic feeding and oviposition punctures with droplets of plant sap made by the hibernated adults in leaf buds, leaf petioles and spurs and the resulting inhibited sprouting, leaf drop and deformations [3,9,7] were clearly observed. The leaf feeding of the new adults before aestivation [7] could also be seen. The larvae that were observed were mostly feeding on the leaf while sheltered in a leaf that remained rolled up, characterized by necrotic leaf edges. Pupae were found in hard blackened pupal chambers formed on the leaf edges or petioles. All this is analogous to earlier descriptions of this weevil's biology [7,3,9]. In 2017 (Locations d, f) and in 2018 (Location g), pupation was noted at the end of April and the beginning of May, respectively, while for both France and Germany it is described as generally occurring at the end of May.

The authors will further investigate the distribution, host plant specificity and phenology of *A. spilotus*, and to this end, would greatly appreciate receiving information regarding further records of this species in Belgium.

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