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First record in Belgium of *Trissolcus basalis* (Hymenoptera, Scelionidae), an egg parasitoid of economically important stink bugs (Hemiptera, Pentatomidae)

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Abstract. The scelionid parasitoid *Trissolcus basalis* (Wollaston,1858) has been detected in Belgium for the first time based on specimens reared from a parasitized egg mass of *Nezara viridula* (Linnaeus,1758) collected in an urban garden at Sint-Amandsberg, Ghent. Identification was based on adult morphology and DNA barcoding. This is presently believed to be the northernmost record in Europe of *T. basalis* and could be the consequence of a northward expansion of this species due to climate change. This first record may be of economic importance for the biological control of stink bug pests in Belgian vegetable and fruit production.

Keywords. Stink bug, egg parasitoid, Scelionidae, *Trissolcus basalis*, DNA barcoding.

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Introduction

Parasitoid wasps in the family Scelionidae (Hymenoptera: Platygastroidea) are gaining increasing attention for biological control due to their parasitism of eggs of key insect pests in agriculture. *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Scelionidae) belongs to the *basalis*-species group of the genus *Trissolcus* (*sensu* Johnson 1987; Talamas *et al*. 2017) and is a solitary egg parasitoid of stink bugs with an assumed worldwide distribution (Colazza & Bin 1995). The taxonomy of the superfamily Platygastroidea was recently revised, reaffirming the Scelionidae as a valid family (Chen *et al*. 2021).

Trissolcus basalis is a generalist and is known to parasitize the eggs of a wide range of pentatomid hosts (Jones 1988). However, the most common host is the southern green stinkbug *Nezara viridula*

(Linnaeus, 1758) (Hemiptera: Pentatomidae) (Colazza *et al*. 1995; McPherson & McPherson 2000). Due to increased global transportation and climate change, non-native species can establish populations beyond their natural distribution (Ziska *et al*. 2011). When non-native herbivores such as *N. viridula* invade cropping systems, they can quickly become serious pests. *Nezara viridula* is a highly polyphagous herbivore and due to its habitat expansion, it has become one of the most economically important species of Hemiptera causing damage to crops in the open field and greenhouses (Panizzi 2000; McPherson & McPherson 2000). Its geographic origin is uncertain but is assumed to be eastern Africa or the Mediterranean (Hokkanen 1986). Within the last decade, established breeding populations were documented throughout eastern and western Europe, including Belgium (Schmitz 1986; Panizzi 2000; Rédei & Torma 2003; Barclay *et al*. 2004; Werner 2005; Musolin 2012; Dethier & Chérot 2014; Aukema 2016; Grozea *et al*. 2016; Rabitsch 2016: Hemala & Kment 2017).

Trissolcus basalis has recently also been recorded parasitizing eggs of the brown marmorated stinkbug, *Halyomorpha halys* (Stål, 1855), in the United States (Balusu *et al*. 2019; Tillman *et al*. 2020). *Trissolcus basalis* has also been reported to have emerged from *H. halys* eggs in Italy (Rondoni *et al*. 2017). *Halyomorpha halys* is native to eastern Asia and has gained pest status in orchards and vegetable crops in most of the U.S.A., as well as in southern and western Europe (Hoebeke & Carter 2003; Wermelinger *et al*. 2008; Fogain & Graff 2011; Leskey *et al*. 2012; Lee *et al*. 2013; Rice *et al*. 2014; Haye *et al*. 2015; Bariselli *et al*. 2016). In Belgium, *H. halys* has been detected since 2017 and is assumed to have established overwintering breeding populations (Claerebaut *et al*. 2019). A first report from Haspengouw in July 2021 indicates that *H. halys* is already present in commercial pipfruit orchards in Belgium, but currently no damage has been reported (G. Peusens, pers. comm. 27 July 2021).

Established or augmented populations of egg parasitoids like *T. basalis* may assist in the biological control of stink bug pests, *N. viridula* in particular, and help relieve the imminent threat posed by *H. halys* on crop production in Belgium and other European countries (Cantón-Ramos & Callejon-Ferre 2010; Koch *et al*. 2017).

Material and methods

A single mass of 73 *N. viridula* eggs was collected from a *Stevia rebaudiana* leaf (Asteraceae) on 28 August 2020 in an urban garden at Sint-Amandsberg (Ghent), Belgium (51°3′20.666″ N, 3°44′53.297″ E). The egg mass was placed in a climatic chamber in the laboratories of the Department of Plants and Crops, Ghent University, set at 24°C, 18:6 h L:D and 70% RH (PHCBI MLR-352H-PE, Japan) and held for emergence of stink bug nymphs or adult parasitoids. Over 50 parasitoid adult wasps emerged from this egg mass with a sex ratio of 1:9 male to female.

A culture was started from a single isolated mated female wasp from the field-collected egg mass and kept at the above-mentioned climatic conditions by offering fresh (< 24 h old) *N. viridula* egg masses, from a culture maintained at the Department of Plants and Crops, Ghent University. The culture of the parasitoid was kept in polystyrene insect breeding dishes (100 × 40 mm; SPL Life Sciences Co., Korea). The adults of the F1 generation and all subsequent generations were fed with a drop of honey placed directly on the breeding dish. Water was provided via moistened synthetic cotton (Roltasoft Hartmann, Germany).

The parental specimen and four F1 specimens were morphologically identified using the key of Talamas *et al*. (2017). Specimens were deposited at the Ghent University Museum, Zoology Collections with collection numbers: UGMD_104422 and UGMD_104423.

Furthermore, twenty frozen adult individuals of both sexes from the F2 generation, originating from a single mated female were pooled for DNA extraction and mitochondrial cytochrome c oxidase I (COI) fragment sequencing. DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen). The DNA samples were quantified using a NanoDrop2000 spectrophotometer (Thermo Scientific). At least 20 ng of genomic DNA was used per PCR. The 5'-COI region was PCR-amplified using the primers [LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO-2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (FOLMER *et al*. 1994). The PCR was performed in a 50 µl reaction volume: 1 µl DNA, 29.5 µl molecular grade water, 10 µl 5X Green GoTaq Flexi PCR buffer, 2.5 µl dNTPs (25 mM each), 5 µl $MgCl_2$, 1.5 µl of each primer (1 µM each), 0.2 µl GoTaq G2 Flexi DNA polymerase (5 u/µl) (Promega Corp.,Wisconsin, USA). Thermocycling conditions were optimized to shorten reaction times and included initial denaturation at 94 °C for 300 s, followed by 35 cycles of 94 °C for 30 s, annealing at 41 °C for 45 s and extension at 72° C for 60 s; then further 600 s at 72°C for

Figure 1 – Lateral habitus of *Trissolcus basalis* (female) collected near Ghent, Belgium.

Figure 2 – Dorsal habitus of *Trissolcus basalis* (female).

Figure 3 – Ventrolateral habitus of *Trissolcus basalis* (female).

final extension. All PCR products were purified using the E.Z.N.A Cycle Pure Kit (Omega Bio-tek Inc, Georgia, USA) following the manufacturer's instructions and sent for sequencing to an external service (LGC Genomics GmbH, Berlin, Germany). The obtained forward and reverse sequences were inspected and a consensus sequence was made using the BioEdit Software ver. 7.2.0. The consensus sequence was compared with sequences present in the GenBank database by similarity search using the Basic Local Alignment Search Tool (http://ncbi.nlm.nih.gov/BLASTn), to confirm the taxonomic identity. The COI sequence generated was deposited in GenBank (MZ087751).

We checked collections at the Royal Belgian Institute of Natural Sciences, The Entomological Conservatory at Gembloux and online databases including fauna-eu.org (DE JONG *et al*. 2014) (accessed 20 October 2021) and gbif.org (GBIF, accessed 20 October 2021) to confirm that our record is in fact the first record in Belgium.

Figure 4 – Anterior head of *Trissolcus basalis* (female).

Results

Both the morphological and molecular identifications with barcoding converged to the same species: *Trissolcus basalis*. The BLASTn query returned over 10 sequences of *T. basalis* with a percent identity of 100% and E-values of 0.0, therefore showing high similarity with other *T. basalis* sequences. According to Talamas *et al*. (2017), *T. basalis* can be identified by the combination of the following characters: vertex without hyperoccipital carina, netrion sulcus incomplete, mesopleuron with episternal foveae shallowly impressed, metapleuron without setation and without well-defined paracoxal sulcus, mesoscutal humeral sulcus present as a smooth furrow and second metasomal tergite with longitudinal striation (Figs 1–4).

Discussion

The parasitized egg mass collected at Sint-Amandsberg (Ghent) represents the first record of *T. basalis* in Belgium and indicates the presence of an established breeding population. There have been no commercial releases of this species for biological control nor any known laboratory cultures in the area, excluding introduction from these sources.

Although *T. basalis* is considered to be globally distributed (Jones 1988; Colazza 1995; Talamas 2017), its distribution within Europe has been rather sparsely documented, with records from Cyprus, Montenegro, Portugal, Spain, Italy, Hungary, France and Germany (Awan *et al*. 1990; Colazza 1995; Tortorici *et al*. 2019; Awad *et al*. 2021). These European specimens were collected in more southern countries or regions. Records from France were from the Aquitaine and Provence-Alpes-Côte-d'Azur, two southern regions (USMENT00896070-00896071, 00896037-00896040, 00896055-0089604060, and 0089629, examined by Talamas *et al*. (2017)). The specimens from Germany (SMNS_Hym_Sce_000805-000806, examined in Awad *et al*. (2021)) were collected in the most southern state, Baden-Württemberg. Our record from Belgium would therefore constitute the northernmost record of *T. basalis* at present in Europe. It is possible that only in recent decades, *T. basalis* has colonized Belgium and other parts of northwestern Europe or has become ubiquitous enough to be detected in this area due to the northward habitat expansion of its prime host *N. viridula*. Additionally, the warming of the climate in northwestern Europe could have played a role in the gradual northward habitat expansion of *T. basalis* following its main host *N. viridula*.

In order to aid further research on the distribution of this parasitoid wasp in Europe, we have mapped all European records (Fig. 5).

More extensive studies on egg parasitoids of Pentatomidae in Belgium and Western Europe will greatly increase our knowledge of biological control of the various stink bug species currently causing economic damage in the area.

Given the economic importance of some of their pentatomid hosts, it will likely be beneficial to support or attract these parasitic wasps to agricultural fields and commercial fruit orchards in a conservation biological control approach (Rahat *et al*. 2005). In this context, it may be warranted to further investigate the physical and chemical cues by which these parasitoid wasps locate and recognize their hosts (Bin *et al*. 1993; Mattiacci *et al*. 1993) or plant resources (Martorana *et al*. 2017). Moreover, the interactions between populations of *T. basalis* and other species of egg parasitoids deserve attention. This knowledge will be instrumental in designing lntegrated Pest Management (IPM) strategies against stink bug pests in Belgium and its neighbouring countries.

Figure 5 – Map of Europe with highlighted countries where *T. basalis* was recorded (according to TALAMAS *et al*. 2017; TORTORICI *et al*. 2019 and AWAD *et al*. 2021).

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