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First Record of *Nemorilla floralis* (Fallén 1810) (Diptera, Tachinidae) Parasitism on Box Tree Moth – *Cydalima perspectalis* (Walker 1859) (Lepidoptera, Crambidae) Larvae

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Abstract: Box tree moth, *Cydalima perspectalis* is native to East Asia where it occurs on *Buxus* trees. Its natural distribution includes China, Japan, Korea and India, and it was accidentally introduced into Europe, Middle East, North America and north Africa through the trade in *Buxus* spp., where it can cause considerable damage. In the native range of *C. perspectalis*, three tachinids (Diptera, Tachinidae) are known to parasitize larvae of *C. perspectalis*, including *Exorista* sp., *Pseudoperichaeta nigrolineata* (Walker 1853) and *Compsilura concinnata* (Meigen 1824). Data on tachinid parasitoids parasitizing *C. perspectalis* on the European continent include only *P. nigrolineata*. Field collection of mature larvae and pupae of *C. perspectalis* during July 2020 in four locations in Split – Dalmatia County in Croatia resulted in collection of live tachinid larvae that were reared to adults. The tachinid specimens were identified at the Natural History Museum, London, UK, as *Nemorilla floralis* (Fallén, 1810) (Diptera, Tachinidae). Conservatively, 1.2 – 2.4 % parasitism rate was achieved. This is the first world record of *N. floralis* parasitism on *C. perspectalis* larvae, which can be added as a potential tool for *C. perspectalis* biological control.

Keywords: *Buxus*; invasive pest; parasitism rate; Croatia

1. Introduction

A non-native species with negative ecological, economic, or health consequences is called an invasive alien species [1]. Biological invasions are considered a major threat to biodiversity and the functioning of natural ecosystems [2]. Box tree moth, *Cydalima perspectalis* (Walker 1859) (Lepidoptera, Crambidae), is one such species native to eastern and southern Asia where it is a native pest of *Buxus* trees [3,4]. Its natural distribution includes China, Japan, Korea and India [4]. This pest was accidentally introduced to Europe through the trade in box tree, *Buxus* spp. where it can cause considerable damage [5]. The invasive characteristic of *C. perspectalis* has been clearly demonstrated by its rapid spread and establishment along the European Union countries after its first detection in southwestern Germany and in the Netherlands in 2007 [6,7]. *Cydalima perspectalis* has subsequently continued to spread, and has been identified in Switzerland, France, Austria, Belgium, Czech Republic, England, Hungary, Italy Liechtenstein, Slovakia, Slovenia, Croatia, Romania and Turkey [3,8,9]. This pest has been a serious problem in Croatia since its arrival in 2013, causing significant damage on *B. sempervirens* plants [10].

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In the native range of *C. perspectalis*, which is South and East Asia [11], there are already data on the occurrence of natural parasitoids of *C. perspectalis*, three of which belong to the order Diptera (larval parasitoids) and seven to the order Hymenoptera (egg, larval and pupal parasitoids) [4]. Within the order Diptera, three tachinids (Tachinidae) are known to parasitize larvae of *C. perspectalis*, including *Exorista* sp. a parasitoid that can cause up to 47.4% of larval and pupal mortality, and two highly polyphagous parasitoids, *Pseudoperichaeta nigrolineata* (Walker 1853) and *Compsilura concinnata* (Meigen 1824) [4].

Data on opportunistic parasitoids of *C. perspectalis* on the European continent include *P. nigrolineata*, a tachinid fly reported from Switzerland [4] that parasitizes larvae of *C. perspectalis*, *Stenomalina* cf. *communis* (Ness 1834) (Hymenoptera, Pteromalidae), a parasitoid wasp in Britain [5], and *Apechthis compunctator* (L.) (Hymenoptera, Ichneumonidae), a pupal parasitoid reported from Switzerland [4]. Studies in natural boxwood forests in France and Spain report parasitization by the tachinid fly *C. concinnata* [12,13].

According to Fauna Europaea [14], all three tachinid species (*P. nigrolineata*, *S. communis* and *C. concinnata*) are widely distributed on the European continent and are therefore presumed to range in Croatia. However, so far there has been no evidence of natural enemies of *C. perspectalis* in Croatia [10]. In addition to the identified parasitoids, laboratory studies on eight *Trichogramma* species showed potential for the highly polyphagous *Trichogramma brassicae* Bezdenko 1968 and *Trichogramma dendrolimi* Matsumura 1926 (Hymenoptera, Trichogrammatidae) as biological control agents, based on their ability to locate and reproduce on eggs of *C. perspectalis* [15]. Beneficial predators such as *Chrysoperla carnea* (Stephens 1836) (Neuroptera, Chrysopidae) and *Orius majusculus* (Reuter 1879) (Heteroptera, Anthocoridae) have been observed preying on *C. perspectalis* eggs [16] and therefore, conserving natural enemies like *O. majusculus* and implementing the release of beneficial insects like *C. carnea* can be considered as supportive tools in the biological control of *C. perspectalis* [16].

Identifying potential biological control agents of *C. perspectalis* in both the native and introduced ranges of the pest is of considerable importance when a biological control program is being considered for an invasive pest. Here we report on a parasitic insect not previously known to attack *C. perspectalis*.

2. Materials and Methods

Field collection of mature larvae and pupae of *C. perspectalis* was conducted on 22nd –30th July 2020 in four locations in Split – Dalmatia County in Croatia (Figure 1). Larvae and pupae were collected on naturally infested *B. sempervirens* individual plants or hedgerows in private house gardens. These preimaginal stages were progeny of the second adult generation, and the purpose of field collection was to establish a laboratory colony.

Fresh pupae from location Poljica were sent by bonded air carrier and delivered to USDA APHIS PPQ S&T Forest Pest Methods Laboratory (FPML) in Massachusetts, USA on August 21, 2020, while larvae from other locations were transferred to the laboratory of University of Split for pupation. The shipped pupae remained in transit for 7 days before delivery to FPML. Upon reception of the insect material in FPML, it was observed that one *C. perspectalis* pupa had an exit hole and a live tachinid larva next to it (which pupariated immediately (< 1 hour), while another tachinid larva had emerged and pupariated during shipment (Figure 2). The puparia were held individually in glass vials plugged with cotton. On August 30, two adult tachinids emerged (Figure 3). They were pinned and delivered to the Natural History Museum in United Kingdom for identification.

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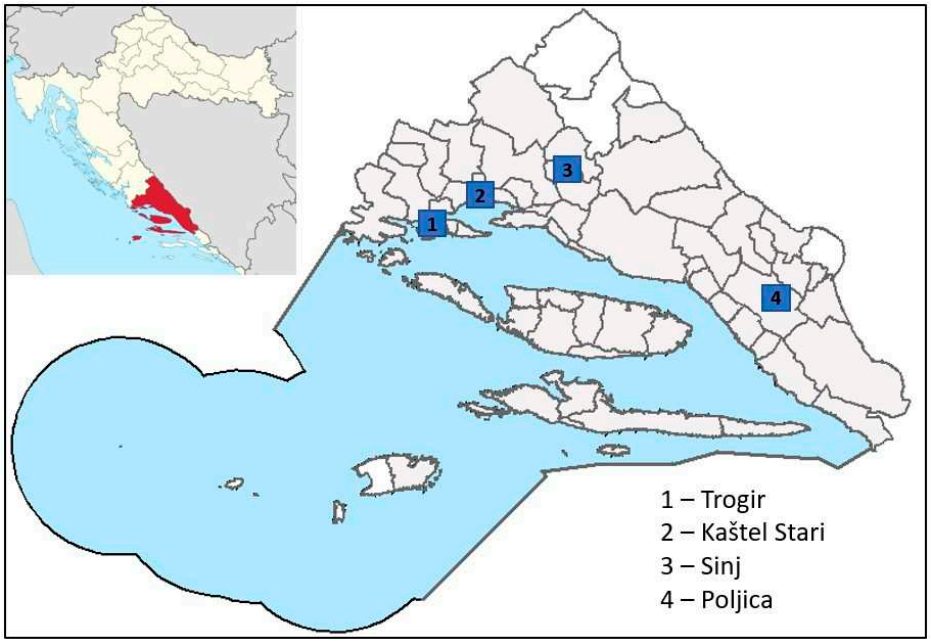

Figure 1. Map of the collection sites in Split – Dalmatia County. The inset shows the location (in red) of the collection area in Croatia.

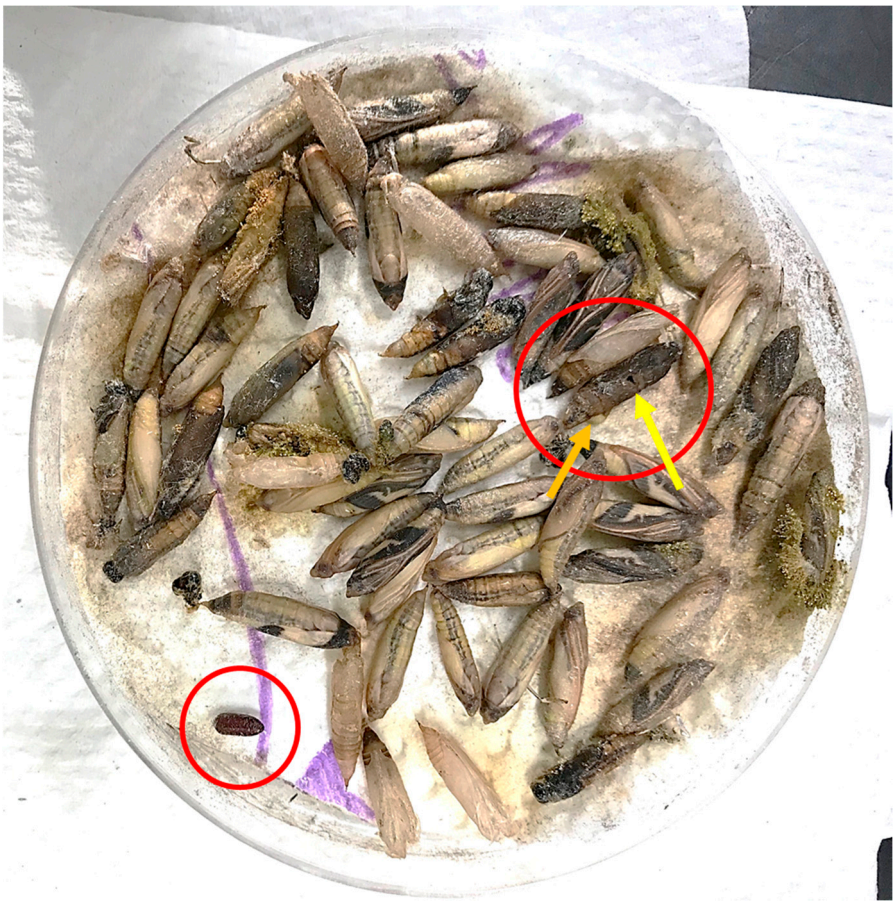

Figure 2. *Cydalima perspectalis* pupae that arrived from Croatia on 21 August 2020. One tachinid puparium was present (small red circle). A tachinid larva (orange arrow) was discovered below a black moth pupa with an exit hole (yellow arrow).

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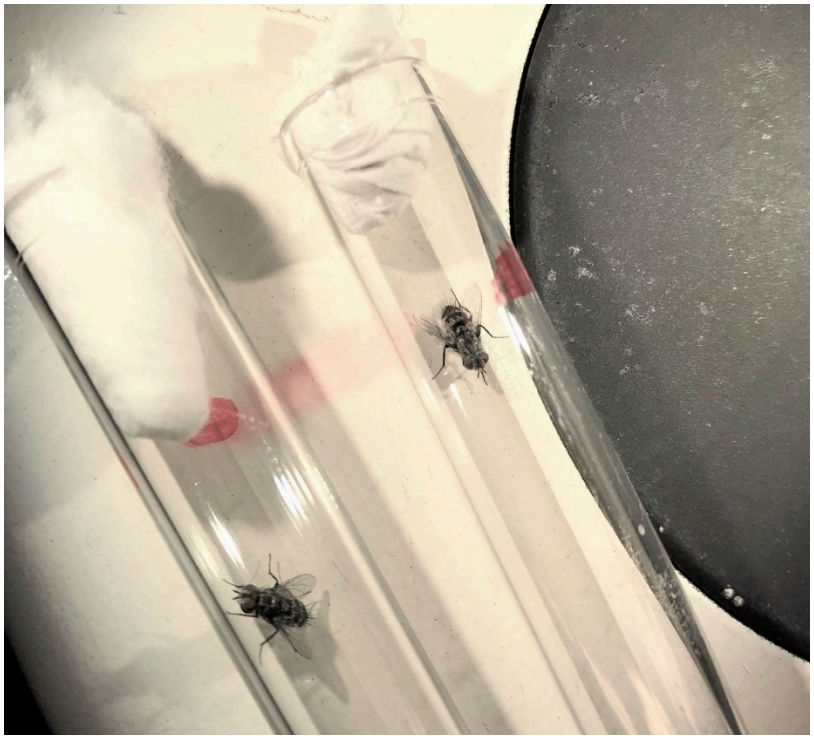

Figure 3. Tachinid fly adults emerged after maturation.

3. Results

From four collection sites, two specimens of Diptera emerged from pupae of *C. perspectalis* collected at Poljica (Table 1). Of the 83 moth pupae sent to FPML from Poljica, 9 adults emerged in transit, 53 emerged in the lab, 20 died in transit of unknown causes, and the remaining pupa was determined to have been parasitized. We cannot be certain whether both tachinids emerged from one or two hosts because mold may have concealed an exit hole in another dead pupa; an examination of the dead pupae under a dissecting microscope did not reveal an additional exit hole. Dead pupae were not dissected to determine if they were parasitized. Conservatively, therefore, 1.2 – 2.4 % parasitism rate was achieved.

The tachinid specimens were identified at the Natural History Museum (NHM), London, UK, as *Nemorilla floralis* (Fallén, 1810) (Diptera, Tachinidae). Specimens were photographed and deposited at NHM.

Table 1. Overview of the origin of *C. perspectalis* populations collected: Location, GPS coordinate, elevation, date of collection, number of individuals collected and number of *N. floralis* emerged.

Location	GPS coordinates	Elevation (meters)	Date of larva and pupa collection	Number of larvae and pupae collected	Number of *N. floralis* emerged
Trogir	43°31'17.5"N 16°15'32.8"E	5	22.07.2020.	52	0
Kaštel Stari	43°33'08.2"N 16°21'14.9"E	18	22.07.2020.	35	0
Poljica	43°24'56.4"N 17°07'43.2"E	414	30.07.2020.	83	2
Sinj	43°42'13.8"N 16°38'23.2"E	319	28.07.2020.	43	0

The following Figures 4 and 5 represent one of the *N. floralis* specimens.

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Figure 4. Lateral view of *Nemorilla floralis* adult.

Figure 5. Dorsal view of *Nemorilla floralis* adult.

4. Discussion

Data on the occurrence of natural enemies and their impact on the population reduction of *C. perspectalis* are predominantly reported in its native range of South and East Asia [4,11]. In Asia, *C. perspectalis* has several natural enemies, including two egg parasitoids, five larval parasitoids, and two pupal parasitoids belonging to the Tachinidae, Braconidae, Chalcididae, Encyrtidae, and Ichneumonidae [4]. Natural enemies in Europe include the tachinid *P. nigrolineata,* the pteromalid *S. communis*, and the ichneumonid *A. compunctator* [5,8]. Studies in natural boxwood forests in France have reported 1.83% parasitization by the tachinid fly *C. concinnata* [12]. *Compsilura concinnata* was the only parasitoid to emerge from 800 5th and 6th instar larvae collected over 5 years in Catalonia,

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Spain; parasitism rates at six locations ranged from 0 to 20% [13]. In that study, 0.5% of 42 larvae that died before pupation were found to be infected by a strain of the fungus *Beauveria bassiana* (Bals. - Criv.) Vuill., 1912 (Ascomycota: Hypocreales), another potential control agent [13]. In this research, we confirm that at least 1.2 – 2.4 % parasitism rate was achieved by *N. floralis*, depending on whether one or two hosts had been parasitized and whether other tachinids in the collection had died in transit along with their hosts.

Nemorilla floralis is native to the Palearctic region, where it is reported to be widely distributed in central and western Europe and Scandinavia. *Nemorilla floralis* presence has been confirmed in Croatia [17] and neighboring countries too [18]. *It is* an endoparasitoid of larvae of almost twenty moth species but so far there is no record of its parasitism on *C. perspectalis* [19]. *C. perspectalis* was only recently found in Africa [20] and North America [21,22]. Due to the short recent history of *C. perspectalis* invasion in over 40 countries of Europe, Middle East, Africa and North America [23], and the significant number of other hosts parasitized by *N. floralis*, further discovery of parasitism of *C. perspectalis* larvae by *N. floralis* is likely to occur in the fly's native range in other parts of Europe.

5. Conclusions

This is the first record of *N. floralis* parasitism on *C. perspectalis* larvae. This common and generalist parasitoid may potentially augment biological control of *C. perspectalis* in Europe.

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Conflicts of Interest: The authors declare no conflict of interest.

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