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Here I am! Evidence of a Hymenopteran larval parasitoid attacking the Box tree moth, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Crambidae) in Germany confirmed

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Abstract

The box tree pyralid moth (*Cydalima perspectalis*), which originates from Asia and has been introduced to Europe in 2006, has now infested several countries and continents outside its natural distribution. This herbivore is specialized on *Buxus* spp. and has destroyed not only many ornamental plantings, which are often of historical value, but also boxwood in its natural range, where it is often part of certain forest types. To date, no effective natural enemies are known, and plantings can only be saved by frequent insecticide applications, while natural boxwoods are threatened or have already gone extinct after massive outbreaks of this pest. During a monitoring in 2025, we discovered a parasitoid population in one of the first infested natural *Buxus* spp. stands in Europe, the boxwood forest near Grenzach-Wyhlen, Germany. According to our data, this Campoplegine larval parasitoid, *Eriborus* sp., is not native, but may have been accidentally introduced in the years after the introduction of its host nearby.

Key Words

Adventive biological control, Box tree moth, *Buxus* spp., parasitoid

Introduction

The Box tree moth (BTM), *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Crambidae) is an invasive species from Asia that was first observed in Europe in southwestern Germany in 2006 (Krüger 2008). Since then, the BTM has spread throughout Europe and has also infested natural forest stands of *Buxus* spp. in mountainous regions in southwestern Europe, the Balkans, Middle East and North Africa. Today, it is also found in Great Britain and Ireland, and since 2018, it has invaded North America (Coyle et al. 2022; Budziszewska and Bereś 2024). The species is monophagous on *Buxus* spp. Larvae feed on the foliage of the plants and, after complete defoliation, also on the bark of branches and trunks. The entire development takes place on the plant, with six to seven larval instars and pupation in a cocoon, which is usually spun between leaves (Nacambo et al. 2014). The moth lives for several weeks, visits flowers for nectar, and is capable of flying long distances (up to 5 km according to John and Schumacher (2013)) to find new host plants. The females oviposit in several clusters of about 10 to 20 eggs (Göttig and Herz 2016), mainly on the underside of leaves (Wan et al. 2014).

Since its arrival, BTM has caused severe damage to ornamental plants of the genus *Buxus* (especially *B. sempervirens* L.) throughout Europe and other infested regions (Kenis et al. 2013; Mitchell et al. 2018). Boxwood plays an important role as a plant of cultural heritage, especially as a structural element of historic sites such as castles, churches, and cemeteries, but also in private gardens. Nowadays, many box trees have been replaced by other species with a similar shape – or, where the plantings are still preserved, permanent monitoring for BTM is necessary, combined with careful pruning and frequent insecticide applications throughout the year. Even more critical is its spread to natural boxwood stands in mountainous regions or as part of certain forest types (Kenis et al. 2013). For instance, BTM has been reported damaging the natural *Buxus sempervirens* formations on rocky slopes, which are widespread in southern France and northwestern Italy and protected by the EU 92/43 Directive (Ferracini et al. 2022).

There are two relict sites of boxwood forests in Germany: one is located on the slopes of the Moselle near the cities of Bonn and Koblenz, the other on the slopes of the Rhine around the small town of Grenzach-Wyhlen near the Swiss border (Fuchs 1979; John and Schumacher 2013; Anonymous 2025). Both forest areas are under strict nature conservation and are known as sites with high biodiversity (Fuchs 1979). However, the boxwood forest in Grenzach-Wyhlen (the "Grenzach Boxwood Forest") in particular was already suffering from BTM infestation in early 2010 (John and Schumacher 2013; Kenis et al. 2013). It was one of the first natural habitats to be affected, probably due to its proximity to the first location where the moth was found (the town of Weil am Rhein, about 8 km away straight line; John and Schumacher (2013)).

During the invasion history to date, no effective natural enemies of BTM have been identified in the infested areas (Kenis et al. 2013; Göttig and Herz 2016; Martini et al. 2019; López et al. 2022; Brinquin et al. 2024; Budziszewska and Bereś 2024). Frequent insecticide applications are therefore necessary in ornamental plantings, particularly due to the multivoltine development of the moth and the requirement that the foliage of boxwood as an ornamental plant must look as unaffected as possible. However, the natural boxwood forests are considered more or less lost, at least in Germany, as their protected status in nature reserves also prohibits any insecticide treatment (John and Schumacher 2013; Anonymous 2025). On the other hand, the undisturbed and highly diverse environment of these habitats makes potential adaptation of antagonistic organisms more likely. Therefore, in the year 2025 – 19 years after the first detection of BTM – we initiated a survey of BTM populations, potential natural enemies, and the condition of the boxwood in these German relict forests.

Here we report on the occurrence of a hymenopteran larval parasitoid that parasitizes BTM larvae in the boxwood forest of Grenzach-Wyhlen. As described in a recent publication by Kenis et al. (2025) and unknown to us at the start of our investigation, the species has already been detected in the region in June 2024 and in April 2025. We tracked the presence of this parasitoid in the Grenzach Boxwood Forest throughout the season 2025. Accordingly, it can be assumed that the species has established itself here. This allows us to predict the prospects for boxwood recovery due to the unexpected appearance of this antagonist.

Methods

Three surveys were conducted in the nature reserves of the Grenzach Boxwood Forest (about 150 ha, 47.555°N, 7.673°E) on May 08/09, June 19/20, and August 25/26, 2025. After consulting with the responsible nature conservation authority and receiving the approval for collection, we limited our sampling to the arthropod fauna found directly on boxwood trees. We took visual and suction samples as well as beating samples. Here we report only on the results of direct sampling of BTM larvae and their leaf cocoons. To collect these, we walked slowly along marked hiking trails in the forest (Bauckner et al. 2005) and carefully examined box trees near the trails for feeding damage. During each survey, BTM larvae and several pupae were collected. In addition, oval cocoons were found inside some of the leaf cocoons formed by BTM (Fig. 1). All collected insects and cocoons were taken to the laboratory and incubated in a climate chamber at 23 ± 2 °C, 70 ± 10% relative humidity, and long day conditions (16 h light/8 h darkness) until hatching. It was not possible to rear the BTM larvae collected on the first date because no *Buxus* spp. foliage was available. We thus froze the larvae immediately after collection and dissected them to determine whether they were infested with any parasitoids.

From the collected parasitoid cocoons inside BTM leaf cocoons, at least three different hyperparasitoid species as well as one primary parasitoid species emerged. Furthermore, parasitoid larvae were found in dissected BTM larvae. Single specimens were subjected to molecular analysis. DNA extraction was done following the protocol of Cruaud et al. (2019) without destruction in the case of adult wasps to maintain them for further morphological identification. Standard PCR conditions were used for amplification of the target sequence COI region (cytochrome c oxidase subunit I) using primers LCOI490 (5-GGTCAACAAATCATAAAGATATTGG-3) and HCO2198 (5-TAAACTTCAGGGTGACCAAAAAATCA-3) (Folmer et al. 1994). Furthermore, also primer Hym28S-F (5´- GTAAACCTGAGAAACCCAAAAGAT-3´) and Hym28S-R (5´- CCTGAAAGTACCCAAAGCAGTAG-3´) targeting 28S ribosomal gene (Pook et al. 2017) were used. After Sanger sequencing (Microsynth Seqlab GmbH, Germany), obtained nucleotide sequences were compared with sequences of similar species in GenBank using a Nucleotide BLAST search on the National Center for Biotechnology Information (NCBI) platform.

Several specimens were determined morphologically by one of the authors (M. Riedel) and compared also with available reference material.

Results

All detected parasitoid cocoons (Fig. 1) were found inside BTM leaf cocoons. Beside the parasitoid cocoon, the head capsule and the larval skin of the host were visible (Fig. 2). At the first sampling event, five parasitoid

Figure 1. Parasitoid cocoon found inside the leaf cocoon of BTM, collected in the Grenzach Boxwood Forest at 19.06.25. Scale bar: 2000 µm. Picture: A. Herz, JKI.

Figure 2. Parasitoid cocoon beside beside the remains of the host larva, including the head capsule of the host. Scale bar: 1000 µm. Picture: A. Herz, JKI.

cocoons were collected, from which one was already empty and four were hyperparasitized (Table 1). Three hyperparasitoids emerged from the cocoons in the laboratory. Two were ichneumonids (one male, one female) and belong to a *Mesochorus* species (det. M. Riedel). From the third one, a chalcidoid wasp emerged. The last cocoon was opened and the dead parasitoid inside was successfully barcoded as *Mesochorus* sp. by Hym28S primer.

At the second sampling event, six parasitoid cocoons were collected. Two of them were empty but showed the emergence holes of the chalcidoid and the primary parasitoid. The other four cocoons did not develop and after dissection showed deceased larval or pupal remains. At the third sampling event, we collected 19 parasitoid cocoons. We obtained in total three primary parasitoids (all females) belonging to the ichneumonid subfamily Campopleginae, after incubation in the laboratory (Fig. 3). Twelve cocoons were already left, three other cocoons remained intact and one was hyperparasitized by a chalcidoid wasp.

At the first survey (beginning of May), we collected 22 BTM larvae from different places within the forest. After freezing, all were dissected under the stereomicroscope and 72% of them contained a parasitoid larva (1st *caudata*-stage (Fig. 4) or 2nd to 3rd instar parasitoid larva (Fig. 5)). Molecular analysis was conducted for some of them. According to the barcoding using COI primers, the obtained sequences matched the published one with accession number PP234971.1 (*Eriborus* sp. from Korea; Kim et al. (2024)) to 100%. When using Hym-28S primers, in some larvae a possible hyperparasitization by a *Mesochorus* sp. was also detected.

In the second survey (mid of June), only one BTM larva was found in the field. It was reared further in the laboratory and a parasitoid cocoon developed one week later. On 6th of July, a Campopleginae female emerged from this cocoon. Provided with BTM host larvae, honey and water, this female was kept in a rearing cage and survived for

Figure 3. Adult Campoplegine female: *Eriborus* sp. reared from *C. perspectalis* that had been collected in the Grenzach Boxwood Forest. Picture: M. Pink, JKI.

Figure 4. Young parasitoid larva dissected from a BTM-larva that had been collected in the Grenzach Boxwood Forest on 09.05.25. Scale bar 200 µm. Picture: A. Herz, JKI.

Figure 5. Parasitoid larva from a dissected BTM-larva that had been collected in the Grenzach Boxwood Forest on 09.05.25. This parasitoid larva was positive for *Eriborus* sp. (COI primer) and *Mesochorus* sp. (HYM28S primer), thus hyperparasitized. Scale bar 1000 µm. Picture: A. Herz, JKI.

more than nine weeks. The provision of host larvae was limited, nevertheless the female produced two progeny parasitoid cocoons from which one F1 female emerged. Thus, thelytoky of this parasitoid was confirmed.

In the third survey (end of August), again few BTM larvae were observed in the field and we collected 15 BTM larvae in total for further rearing in the laboratory. From these, we obtained nine parasitoid cocoons (parasitism rate 60%), from which six females hatched after about one week.

In total, we obtained ten living females belonging to Campopleginae from our field collection of parasitized BTM in the Grenzach Boxwood Forest. Several were barcoded by using primers mentioned above. The molecular analysis using COI primers revealed again 100% identity to the published nucleotid sequence with accession number PP234971.1. (*Eriborus* sp. from Korea; Kim et al. (2024)). Barcodes obtained from one parasitoid larva (JKI-Code: 250509-2.1.-L1-P01) and two females (JKI-Code: 250826-11, 250826-12) were deposited in GenBank (accession numbers PX734134, PX734135, PX734136). One of these females (250826-11) was also examined for the morphological characters after DNA-extraction.

The comparison of the morphological characters (females 250620-04, 250826-11; det. Riedel) suggests that this parasitoid belongs to the genus *Eriborus*, however it differs from all known European *Eriborus* species (see Horstmann 1987; Haraldseide 2023). The morphology is indeed identical with the one described by Kim et al. (2024) as *Eriborus* sp. This parasitoid species will be formally described in a separate paper in the near future together with the observed hyperparasitoid *Mesochorus* sp. mentioned above. The specimens are actually deposited in the private collection of one of the authors (M. Riedel) but will be permanently deposited in the Zoologische Staatssammlung Munich (ZSM, Bavarian State Collection of Zoology).

Table 1. Number of parasitoids (*Eriborus* sp.) and hyperparasitoids, dissected from collected larvae of the box tree moth *Cydalima perspectalis* (BTM) or collected as parasitoid cocoons inside leaf cocoons of the host at the Grenzach Boxwood Forest in 2025.

	1st Survey 08./09.05.25	2nd Survey 19./20.06.25	3rd Survey 25./26.08.25
Collected BTM larvae	22	1	15
Larvae parasitized by *Eriborus* sp.	16	1	9
Hyperparasitized larvae	≥ 2	0	0
Collected *Eriborus* sp. cocoons	5	6	19
Hyperparasitized by *Mesochorus*	3	n.d.	n.d.
Hyperparasitized by *Theronia*[1]	0	1	n.d.
Hyperparasitized by Chalcidoidea	1	1	1

[1]: A known pseudohyperparasitoid, e.g. a parasitoid species that parasitises other parasitoids but only once they have left the primary host (Broad et al. 2018). Here, the parasitoid was found as a cadaver in a Campoplegine cocoon and barcoded with Hym28S as *Theronia* sp. (EU378804.1).
n.d.: not determined because some cocoons were already empty.

Discussion

The main objective of our study was to investigate whether, almost 20 years after the arrival of BTM, new host-parasitoid associations between native parasitoids and the introduced host could have developed. In Germany, this has been confirmed for some tachinid fly species (*Nemorilla floralis* (Fallén), *Pseudoperichaeta nigrolineata* (Walker) according to Tschorsnig (2017); *Compsilura concinnata* (Meigen), Herz and Lüdke, unpublished). However, the Campoplegine parasitoids reared from our BTM material showed morphological and molecular characters of the species that had been reared from BTM in South Korea in the years 2022 and 2023 for the first time (Kim et al. 2024). Independently of our research and just one month before our official faunistic survey started in May 2025, a Swiss team collected BTM larvae in Switzerland and also in Grenzach-Wyhlen from which parasitoids emerged. They were identified as the same *Eriborus* sp. (Kenis et al. 2025). Thus, the species is already present in Switzerland too.

It is possible that this *Eriborus* species was accidentally introduced to the first locations of the BTM invasion near Weil am Rhein and Basel in the late 2000s. On the other hand, it could also be the case that it has only recently reached this region, as Kenis et al. (2025) report the discovery of this *Eriborus* sp. during an insect survey in Basel in June 2024. In any case, the thelytoky, long survival time and probably also the high host searching ability of this potentially specific parasitoid certainly favour the successful establishment of even few introduced specimens. But becoming easily a victim of European hyperparasitoid species, as well as intensive pesticide treatment of BTM hosts or the removal of boxwood plants from ornamental plantings, may have hampered the rapid population growth of this species and its spread to other regions outside the Grenzach Boxwood Forest or neighbouring sites. We have found at least three species of hyperparasitoids, parasitizing larvae or cocoons of the primary parasitoid during the whole season, albeit at low numbers. Their role has yet to be clarified as to whether they have a harmful or rather stabilizing effect on the trophic cascade of *Buxus* sp., BTM and parasitoids (Poelman et al. 2022).

When we began our visits in 2025, we expected to find only single patches of surviving box trees in the Grenzach Boxwood Forest according to previous reports (John and Schuhmacher 2013; Kenis et al. 2013). However, it appears that the boxwood is currently recovering (Fig. 6) and even older trees have several years old, almost undamaged foliage (Fig. 7). In addition, the overall presence of BTM was rather low throughout the season. Further observations will be necessary in the coming years to track the population dynamics of the herbivore, its antagonist, and its hyperparasitoids, as well as the status of the *Buxus* stands in Grenzach-Wyhlen. At present the qualitative impression is rather positive, in contrast, for example, to the situation in the boxwood forests further north at the river Moselle, where mass outbreaks of BTM occurred in 2025 (own observation).

Figure 6. Boxwood Forest Grenzach-Wyhlen in 2025: location where the first five parasitoid cocoons were found (09.05.2025). Picture: A. Herz, JKI.

Given the possible origin of the parasitoid in Asia and its high host specificity (still to be confirmed) for BTM, we can assume another case of "accidental" biological control (Weber et al. 2021; Fenn-Moltu et al. 2024; Kenis et al. 2025) in Germany, in addition to recent evidence for other host/parasitoids systems (Dieckhoff et al. 2021; Martin et al. 2023). We assume that the further spread and establishment of this species may help to limit mass outbreaks of BTM and to save box trees especially in nature reserves where the use of insecticides is prohibited. However, at least in Germany and across borders to other countries, regions with natural boxwood are quite disjunct. Larger ornamental plantings in parks or gardens have already been displaced or are treated with frequent cutting and insectide applications to remove all BTM larvae. This

Figure 7. Left: Understorey boxwood, showing several annual shoots undestroyed, along the hiking trail in the Boxwood Forest at Grenzach-Wyhlen (09.05.25). Right: BTM-leaf cocoon with parasitoid cocoon inside on boxwood (19.06.25). Pictures: A. Herz, JKI.

situation makes it difficult for the parasitoid to successfully establish itself in a particular location without a host reservoir. As consequence, wasps need to disperse over a wider spatial range without host and its host plant. Our monitoring in 2025 was limited to the natural boxwood sites at Rhine (Grenzach-Wyhlen) and Moselle (around Bad Bertrich, Treis-Kalden) as well as a few irregular checks in other parks in southwestern Germany. However, it was only in the Grenzach Boxwood Forest, where we were able to observe the cocoons of *Eriborus* sp.

Even so, Kenis et al. (2025) found parasitized BTM larvae in ornamental plantings in Switzerland.

Hence, introducing this parasitoid into gardens and parks with extensive boxwood plantations could be of interest, as it could reduce the overall prevalence of BTM in this situation. Historic gardens often contain older boxwood stands with larger trees alongside the strictly formal, typically Baroque hedges. Parasitoids and hosts could survive in latent densities in such "forest corners." It might therefore be advisable to actively release and establish the parasitoid in locations outside its currently known range. In this case, at least in Germany, approval from the nature conservation authority is likely to be required which also stipulates a risk assessment for non-target species. In addition to monitoring the spatial and temporal occurrence of *Eriborus* sp., its host range and possible non-target effects must now be clarified – and this requires further research.

Author contributions

Conceptualization, investigation, data curation and analysis: all authors. Original draft preparation: Annette Herz. Review and editing: Danilo Lüdke, Matthias Riedel. All authors have read and agreed to the published version of the manuscript.

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