

A cryptic moth species drives major outbreak dynamics on oak without escaping its natural enemies

Markus Franzén^{a,b,*}, Romana Salis^b, Marcus Hall^b, Alvaro Gaytan^c, Anders Forsman^b, Tomas Roslin^d, Ayco J.M. Tack^e

^a Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping SE-581 83, Sweden

^b Center for Ecology and Evolution in Microbial Model Systems (EEMIS), Department of Biology and Environmental Science, Linnaeus University, Kalmar SE-391 82, Sweden

^c Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), Spanish National Research Council (CSIC), Seville 41012, Spain

^d Department of Ecology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

^e Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm SE-106 91, Sweden

ARTICLE INFO

Keywords:

Acrocercops brongniardella
Acrocercops andreneli
 Insect outbreaks
 Oak
 Parasitoids
 Species distribution
 COI barcoding
 Spatial autocorrelation

ABSTRACT

Global forest ecosystems are experiencing increasingly frequent and severe insect outbreaks, driven by complex interactions among climate change, land-use alterations, and shifting species distributions. Species that are morphologically indistinguishable — often referred to as cryptic species — can differ significantly in distribution, host use, and susceptibility to natural enemies and might thereby differ in outbreak dynamics. Yet, the contribution of cryptic species to temporal changes in the frequency and severity of insect outbreak dynamics remains poorly understood. Motivated by recent defoliation events in northern European oak forests, we investigated an emerging leaf-miner outbreak in Sweden. Through targeted surveys, rearing from 22 sites and Malaise trapping at 34 sites (56 sites total), we identified a pronounced spatial clustering of outbreaks at higher latitudes. The newly recognised cryptic species *Acrocercops andreneli* was strongly associated with these outbreaks, whereas sites with only *Acrocercops brongniardella* never showed outbreaks. Host-parasitoid networks related to the two cryptic moth species were strikingly similar. Our findings demonstrate the importance of cryptic species for outbreak dynamics and their consequences for host plant health in ways that are easily overlooked by traditional taxonomy. Moreover, such outbreak dynamics cannot always be linked to a lack of top-down control by natural enemies.

1. Introduction

Recent shifts in climate and land use are altering ecological communities, potentially triggering an increase in the frequency and severity of species outbreaks (Dale et al., 2001; Nyman et al., 2022). These changes pose significant challenges for forest ecosystem management, particularly as outbreak dynamics become more complex and less predictable (Kuuluvainen, 2009; Mina et al., 2022). At the same time, the application of molecular tools has revolutionised our understanding of species diversity, revealing species that are morphologically (nearly) indistinguishable — often referred to as cryptic species. These cryptic species may play crucial roles in triggering and maintaining outbreak dynamics (Fišer et al., 2018). While molecular surveys have shed light on biodiversity and geographic variation in species distributions

(Lavergne et al., 2010; Wang, 2024), it remains unclear whether and how cryptic species contribute to outbreak dynamics, leaving a vital gap in our ability to anticipate and manage emerging forest pests.

The prevalence of cryptic species poses a fundamental challenge for our understanding of outbreak dynamics in forest ecosystems. Cryptic species often exhibit distinct ecological characteristics, and recent evidence suggests that species within cryptic species complexes can differ in their host preferences, competitive abilities, and susceptibility to natural enemies (Gorayeb et al., 2021; Xu et al., 2021). Importantly, even subtle differences in life-history strategies and resource use between cryptic species are known to have a major influence on population and outbreak dynamics (Turchin, 2013; Wolz et al., 2024).

Parasitoid communities play a crucial role in regulating herbivore populations, but their interactions with cryptic species can be

* Corresponding author at: Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping SE-581 83, Sweden.

E-mail address: markus.franzen@liu.se (M. Franzén).

<https://doi.org/10.1016/j.foreco.2025.123181>

Received 19 May 2025; Accepted 13 September 2025

Available online 23 September 2025

0378-1127/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

remarkably complex, as DNA barcoding studies have revealed. For instance, (Smith et al., 2007) found that 15 apparently generalist parasitoid species actually comprised at least 73 distinct mtDNA lineages, many specialised on specific host caterpillars. Recent research has revealed that morphologically similar but genetically different host species often support distinct parasitoid assemblages, leading to species-specific patterns of top-down control (Grégoire and Gould, 2023). These differential interactions between parasitoids and cryptic hosts can significantly influence local population dynamics and ultimately affect outbreak trajectories. For example, if one of the cryptic species is invasive, it might have left its parasitoids behind (i.e. natural enemy release; Liu and Stiling, 2006; Roy et al., 2011). As another example, one of the cryptic species might have parasitoid interactions that result in higher amplitude oscillations (Turchin, 1999). Moreover, as environmental conditions shift, the strength and specificity of these parasitoid-host relationships may be altered, with important implications for biological control (Jervis et al., 2023).

Recent severe defoliation events in oak forests across the Western Palearctic have provided a compelling natural experiment to examine how cryptic species influence outbreak dynamics and interact with their parasitoid communities (Lyubenova et al., 2024). For decades, these defoliations were attributed to a single blotch leaf miner, *Acrocercops brongniardella* (Ekholm et al., 2019). However, the recent discovery of a cryptic species, *A. andreneli*, initially described outside oak forests (Nel et al., 2023), has challenged this assumption and raised new questions about the actual drivers of these outbreaks. Here, we focus on the distributional range of oak in Sweden to address four key questions: (1) What are the spatial patterns of the outbreaks and the two cryptic leaf miner species? (2) What is the relationship between their relative

abundances and infestation levels? (3) What is the diversity and structure of the parasitoid community of the two cryptic species? And (4) how parasitoid diversity and parasitism rate relate to infestation levels. By investigating these aspects, we reveal how cryptic diversity shapes outbreak dynamics, offering insights for understanding and managing forest pest outbreaks under changing environmental conditions.

2. Materials and methods

2.1. Study system and area

Our study was conducted across the range of oak (*Quercus* spp.) in Sweden (latitude 55° to 61°) (Fig. 1). The study focused on two cryptic species within the leaf-mining moth genus *Acrocercops*: *A. brongniardella* and *A. andreneli* (Lepidoptera: Gracillariidae). These species represent a cryptic species complex, defined here as morphologically indistinguishable species that can only be reliably separated through molecular analysis or detailed examination of genitalia structures (Hending, 2024). Based on external morphology, adults of both species exhibit identical characteristics, including a wingspan of 8–10 mm and distinctive brownish, white, and black patterned forewings (Fig. 1). This morphological similarity led to their treatment as a single species until a detailed examination revealed two distinct species (Nel et al., 2023). Following the description of *A. andreneli* as a new species, the species was quickly reported from Sweden (Bengtsson, 2024) where it is both common and widespread in major parts of the country (Iwaszkiewicz-Eggebrecht, 2024; Miraldo, 2024).

Acrocercops brongniardella exhibits a univoltine life cycle tightly synchronised with oak phenology. Adults emerge from overwintering

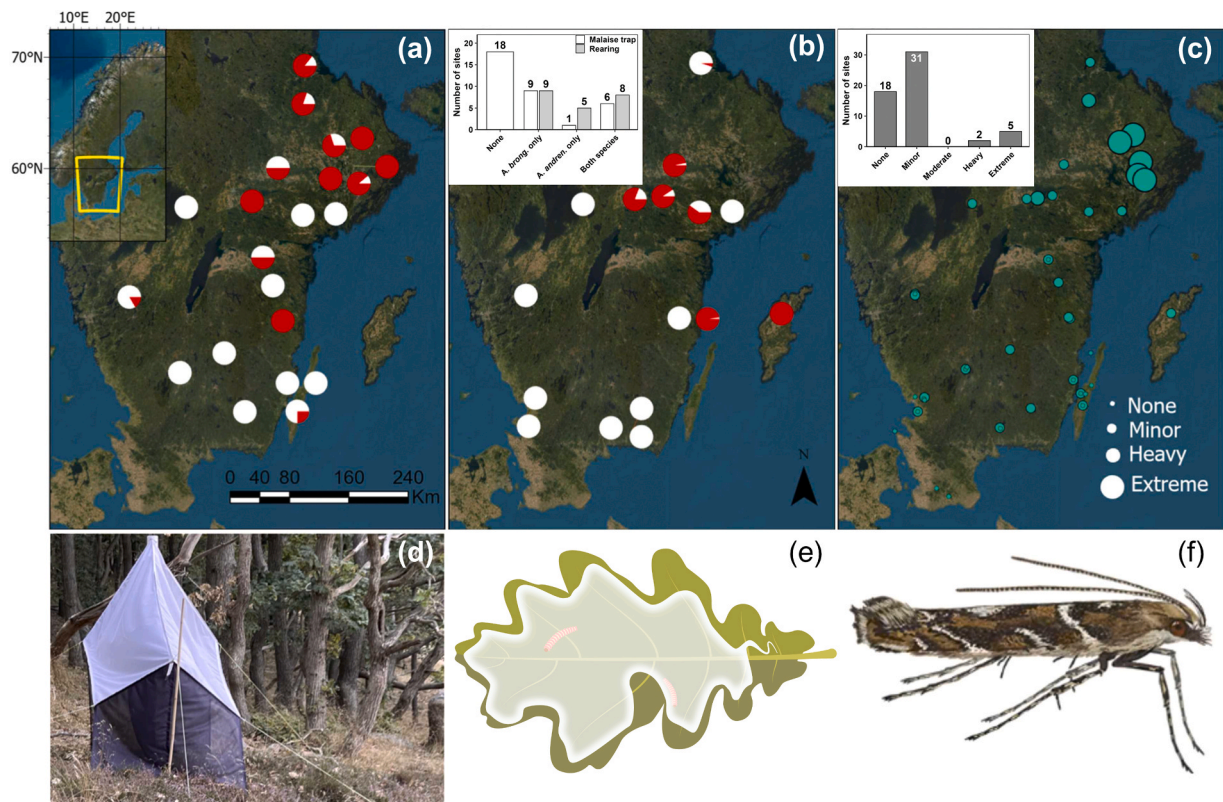


Fig. 1. Overview of the study system, infestation levels, and distribution of two cryptic *Acrocercops* species across the range of oak (*Quercus*) in Sweden. (a, b) Relative abundances of *A. brongniardella* (white) and *A. andreneli* (red) across sampling sites based on reared leaf mines. The inset figure in (b) shows the number of sites occupied by each species and method. (c) Infestation severity at each site, with circle size proportional to the infestation score (none, minor, moderate, heavy, or extreme). The inset in (c) shows the frequency distribution of infestation severity scores. (d) A Malaise trap used for capturing adult moths, (e) a leaf mine of *Acrocercops* on *Quercus robur* (illustration by Marc Hauber), and (f) an adult *Acrocercops* moth. The yellow rectangle in the map inset (top-left) indicates the study region within Sweden.

diapause coinciding with oak leaf expansion and remain active until May. Females oviposit single eggs along leaf veins on the upper leaf surface, with typically 5–6 eggs per leaf, though up to 25 eggs per leaf have been recorded. Larval development spans approximately one month, during which first instars create characteristic serpentine mines that often merge into larger communal blotch mines as larvae develop. Upon completing development, larvae exit their mines and descend via silk threads to pupate in flat cocoons on substrates beneath host trees, with adult emergence occurring after approximately 20 days (Bengtsson et al., 2011). These moths serve as important indicators of forest health and contribute to oak ecosystem dynamics through the effects of herbivory on carbon dynamics and as prey for natural enemies (Ekholm et al., 2019). In contrast, the life history of the recently described *A. andreneli* (Nel et al., 2023) remains largely unknown, highlighting a significant knowledge gap in understanding the ecological differences between these cryptic species. The primary natural enemies of *Acrocercops* species are parasitoid wasps from the families Braconidae and Eulophidae (Ermolaev, 2024; Shaw and Askew, 1976), which are key regulators of leaf-miner populations (Muraleedharan et al., 1988).

2.2. Field sampling and targeted rearing

To examine the distributions and relative abundances of the two cryptic leaf miners, *A. brongniardella* and *A. andreneli* (Fig. 1), we employed two complementary sampling approaches: (1) leaf collection of infested leaves for rearing (Figs. 1 and 2) Malaise trap monitoring (Fig. 1). Leaf collection for rearing occurred at 22 sites from May 20 to June 10 2023, where oak leaves were collected and stored in muslin bags under refrigeration until laboratory processing. At each site, a standardised search protocol was implemented, with a minimum survey

time of 45 min per location. The number of leaves collected was naturally dependent on infestation levels and ranged from 3 to 414 leaves per site. In the laboratory, leaves were placed into clear cylindrical plastic containers (118 mm diameter × 150 mm height) with tight-fitting lids. To standardise rearing conditions, we put three leaves per container. The containers were maintained in an unheated facility and monitored weekly for adult emergence. Successful emergence occurred in 441 out of 480 rearing containers, with considerable variation across sites (range: 1–138 containers per site; mean: 19; median: 7), and a total of 632 moths emerged. To identify the *Acrocercops* species present at a given location, we barcoded individual moths from each site (mean: 6.41, median: 5, range 1–16). Barcoding of the 118 individuals identified 63 *A. andreneli* and 55 *A. brongniardella* (Fig. S1). For each site, we calculated the proportion of *Acrocercops* species based on single-specimen barcoding data by dividing the number of individuals of each *Acrocercops* species by the total specimens sequenced. Malaise traps were established at 34 locations as part of a broader biodiversity monitoring programme investigating oak-associated insects in southern Sweden (Forsman et al., 2024). These traps operated continuously from May 2022 to November 2023, with collection intervals of 5–30 days. All samples were preserved in 96 % ethanol at 4°C until they were processed for DNA metabarcoding. For each site, we calculated the proportion of *Acrocercops* species based on metabarcoding by dividing the number of sequence read counts of each *Acrocercops* species by the total number of sequence read counts of both specimens combined. To link the relative abundance of each species to infestation levels, we surveyed all 56 oak stands between August 14–18, 2023. At each site, ten trees were examined for approximately two minutes each using binoculars from each cardinal direction to assess the entire canopy. Across the 56 study sites, infestation levels were classified using an ordinal scoring

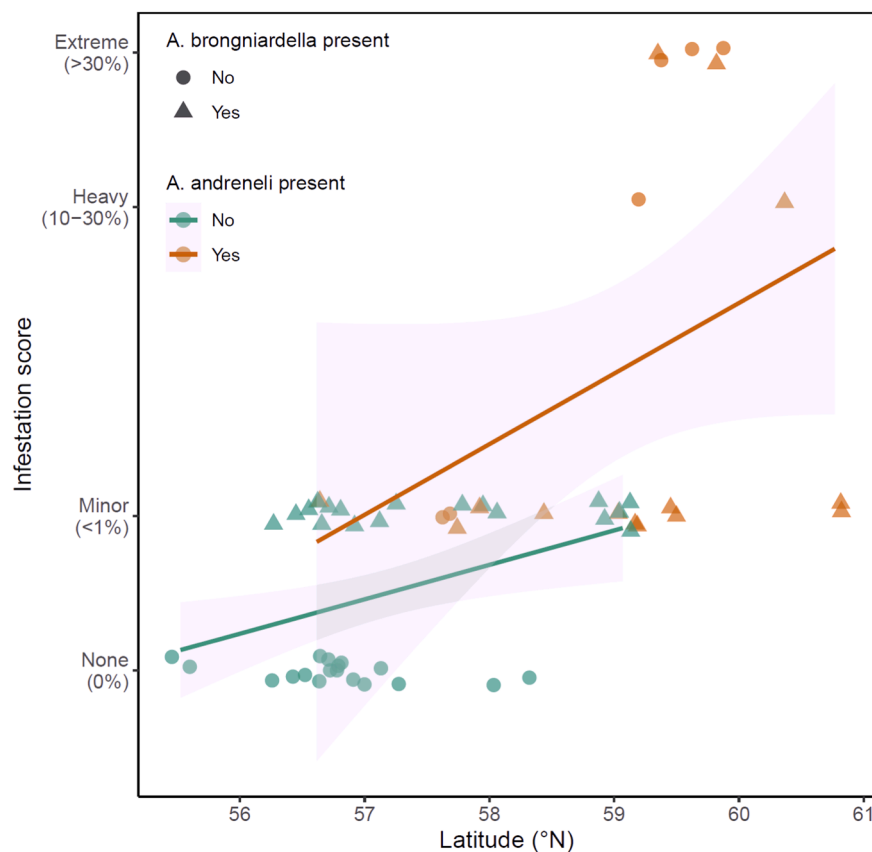


Fig. 2. Latitudinal patterns of *Acrocercops* infestation severity in relation to species presence across the distribution of oak (*Quercus*) in Sweden. Each point represents a site, with colour indicating *A. andreneli* presence (green = absent, orange = present) and shape indicating *A. brongniardella* presence (circle = absent, triangle = present). The two lines (green and orange) show the predicted infestation severity, with 95 % confidence intervals shaded in grey.

system from no infestation ($n = 18$ sites, 32.1 % of all sites), minor (<1 % leaves infested, $n = 31$, 81.6 %), moderate (1–10 %, $n = 0$), heavy (10–30 %, $n = 2$, 5.3 %), and extreme (>30 %, $n = 5$, 13.2 %).

To assess parasitoid community structure, we conducted systematic leaf rearings across study sites (see above). All emerging parasitoids were collected and subjected to DNA sequencing to ensure accurate species delineation. Based on single-specimen barcoding, we constructed host-parasitoid networks, estimated site-specific parasitoid species richness, and calculated parasitism rates as the ratio of emerged parasitoids to total moth emergence (combined counts of *A. andreneli* and *A. brongniardella*, which were always > 0). Pearson's correlation between parasitoid species richness and moth count revealed a strong positive association ($r = 0.86$, 95 % CI: 0.66–0.95, $t(16) = 6.72$, $p < 0.001$), indicating confounding with sample size. Consequently, our data do not permit a meaningful exploration of parasitoid species richness patterns related to our fourth research question.

2.3. Molecular analysis

To establish the identity of both leaf miners and parasitoids, we employed two complementary molecular approaches: metabarcoding of Malaise trap bulk samples and single-specimen barcoding of hatched adults from the rearing programme individuals. DNA extraction, amplification and sequencing of the bulk samples and single specimens were performed at the Canadian Centre for DNA Barcoding using their standard protocols. For metabarcoding, we amplified the cytochrome c oxidase I (COI) gene using the BF3 + BR2 primer pair (Elbrecht et al., 2019), with three PCR replicates per sample as well as positive and negative controls. Libraries were sequenced on an Illumina NovaSeq (2×250 bp).

Raw reads were received as paired-end merged demultiplexed fastq files. Primers were trimmed using cutadapt v4.4 (Martin, 2011). Sequences were then processed using APSCALE v1.6.3 (Buchner et al., 2022) using default parameters. The bioinformatics workflow included quality filtering (418 ± 10 bp, maximum expected error = 1), OTU clustering at 97 % similarity, and taxonomic assignment using the MIDORI 2 database v GB257 (Hleap et al., 2021). We applied hierarchical identity thresholds (species ≥ 98 %, genus ≥ 95 %, family ≥ 90 %, order ≥ 85 %) and conservative abundance filtering (0.005 % minimum read abundance) with control-sample subtraction (Keck et al., 2023).

For single-specimen analysis, we processed up to 15 moths per site, and all emerged parasitoids. The COI gene was amplified using the LCO1490 and HCO2198 primers (Folmer et al., 1994), and bi-directional Sanger sequencing was performed on an ABI 3730 sequencer (Applied Biosystems), yielding 118 high-quality, barcode-compliant sequences. Multiple sequence alignment was conducted with MUSCLE v.5.1 under automatic algorithm selection, producing a final alignment of 559 bp, including 46 variable sites (8.2 %) and a GC content of 29 %. Model selection in Modeltest-NG 0.1.6 identified TrN+I as the best-fit evolutionary model according to AICc and BIC.

Genetic distance estimates in MEGA v.11.0.16 using the K2P model indicated a clear separation between the two *Acrocercops* species. Intraspecific distances ranged from 0 % to 2.4 % for *A. brongniardella* and 0–0.7 % for *A. andreneli*, whereas interspecific distances spanned 4.6–7.0 %. Analysis in Fabox v. 1.61 identified 28 distinct haplotypes: 8 in *A. andreneli* (63 specimens) and 20 in *A. brongniardella* (55 specimens). These relationships were visualised as a TCS haplotype network in popART (Fig. S1).

Species assignments from metabarcoding and single-specimen data were cross-validated against the BOLD database and GenBank, paying particular attention to distinguishing between the two *Acrocercops* species and identifying parasitoid taxa. The observed genetic divergence (>4.6 %) provided a robust framework for assigning metabarcoding OTUs to either species, provided that sequence similarity surpassed the 98 % threshold.

2.4. Statistical analyses

All statistical analyses were performed in R version 4.4.2 (R Core Team, 2024). Spatial maps were created to visualise species occurrences using ArcGIS Pro 3.3.0 (ESRI).

2.4.1. Spatial patterns of the outbreaks and the two cryptic leaf miner species

To explore spatial patterns in outbreak severity, we modelled the infestation score (scale 0–4) of each of the 56 oak sites as a function of latitude (continuous) using glmmTMB (version 1.1.10) (Brooks et al., 2017) as it can handle spatial structuring with negative binomial distribution to account for overdispersion in the infestation score. Spatial autocorrelation in model residuals was evaluated using Moran's I test with a k-nearest neighbour approach ($k = 4$) implemented in the spdep package version 1.3–8 (Bivand et al., 2017).

2.4.2. Relationship between relative abundances of the two cryptic species and infestation levels

To examine how the two *Acrocercops* species relate to infestation severity, we modelled the infestation score (ordinal scale 0–4) at each site as a function of the presence of *A. brongniardella* (binary: present/absent), *A. andreneli* (binary: present/absent) and latitude at the 56 sites. Ordinal logistic regression was implemented using the polr function from the MASS package (version 7.3–64) (Ripley et al., 2013) to account for the ordered nature of the infestation scores, although the spatial structure can't be included in ordinal regressions. In a complementary analysis, to further examine how the two *Acrocercops* species relate to infestation severity, we investigated the proportion of *A. andreneli* in the local *Acrocercops* population and latitude using multinomial logistic regression (as the ordinal regression did not converge). For this purpose, infestation severity was reclassified into three categories based on the percentage of affected oak leaves: none (0 %), minor (<1 %), and severe (>10 %), with the original heavy (10–30 %) and extreme (>30 %) categories grouped. These models were implemented using the nnet package version 7.3–20 (Ripley et al., 2016).

2.4.3. The structure of the parasitoid community of the two cryptic species

To describe the parasitoid community, we focused on parasitoid species richness and host-parasitoid network metrics. For the subset of sites with rearing data ($n = 22$), parasitoid species richness was quantified. In our study system—with only two host species—the use of detailed network metrics (e.g. connectance) risks obscuring the central pattern of parasitoid sharing. Instead, we present an aggregated bipartite food web using the bipartite package version 2.20 (Dormann, 2008) from data pooled across all sites, which clearly visualises host-parasitoid interactions.

3. Results

3.1. Spatial patterns of the outbreaks and the two cryptic leaf miner species

Acrocercops infestation was detected at 67.9 % (38 of 56) of surveyed sites (Fig. 1a,b). Among these sites, the proportion of *A. andreneli* ranged from 0 to 1 (median = 0.109, mean = 0.394). Across all 56 sites, 18 had neither *A. brongniardella* nor *A. andreneli*, 18 had only *A. brongniardella*, 6 had only *A. andreneli*, and 14 had both species (Fig. 1a,b). Infestation severity was highly structured in space, shown by Moran's $I = 0.347$, $p < 0.0001$, and infestation severity increased significantly with latitude—with the most severe cases occurring above 59°N (Fig. 1a, Table S1).

3.2. Relationship between relative abundances of the two cryptic species and infestation levels

Latitude and both species showed significant positive associations with infestation severity (Fig. 2, Table 1). Sites where *A. andreneli* was present had dramatically higher infestation levels, and outbreaks were exclusively observed at these sites with an extreme infestation (Fig. 2, Table 1). In contrast, while *A. brongniardella* presence was also a significant predictor, its effect was considerably less pronounced (Fig. 2, Table 1). Additionally, each one-degree increase in latitude was associated with a 2.12-fold increase in the odds of higher infestation severity (Fig. 2, Table 1). The multinomial regression confirmed that increasing latitude and a higher proportion of *A. andreneli* were significantly associated with elevated odds of severe infestation (Table S2).

3.3. The structure of the parasitoid community of the two cryptic species

A total of 72 parasitoid specimens representing 20 taxa from six families were reared from 18 sites. The community comprised Eulophidae (38 individuals, nine species), Braconidae (19 individuals, five species), Pteromalidae (eight individuals, three species), Ichneumonidae (three individuals, one species), Tachinidae (three individuals, one species) and Cynipidae (one individual, one species). Site-specific parasitoid richness ranged from 0 to 6 taxa (mean $\pm$ SE: 2.36 ± 0.42 , $n = 22$). Both moth species supported diverse parasitoid assemblages, with *A. brongniardella* hosting 17 taxa and *A. andreneli* 18 taxa and parasitoids found in low numbers exhibited host specificity, as expected by chance (Fig. 3).

4. Discussion

Species overlooked by traditional taxonomy may alter community dynamics and outbreak trajectories. New molecular methods now allow the resolution of such taxa and, thus, a dissection of their ecological roles. Focusing on a newly discovered cryptic species, *A. andreneli*, and its traditionally recognised sibling species, *A. brongniardella*, we found that the cryptic taxon actually caused the most severe outbreaks on oaks. The strong spatial autocorrelation in infestation patterns indicates that outbreak severity is geographically structured, with neighbouring sites showing more similar infestation levels than would be expected by chance. This spatial clustering, combined with the pronounced latitudinal gradient in infestation severity, suggests that *A. andreneli* may be particularly well-adapted to conditions in eastern Sweden (Table S1). This spatial pattern could reflect underlying environmental gradients or resource availability in northern oak forests (Hagen et al., 2007).

4.1. Cryptic species and invasion dynamics

One of the most striking results of this study is that *A. andreneli* was present at every site experiencing heavy or extreme infestation (Fig. 2). This precise correspondence with severe (>10 % leaf area) oak damage calls for a re-evaluation of the previous framing of *A. brongniardella* as

the primary driver of outbreaks (Kirichenko, 2023; Musolin, 2022). That a species causes outbreaks almost everywhere where present is rare in forest pest systems (Shorohova et al., 2009) – and this pattern would have remained hidden unless the presence of *A. andreneli* had been resolved by molecular tools. Thus, our results underscore how cryptic congeners can dramatically reshape our understanding of outbreak dynamics (Ghelardini et al., 2016; Nahrung et al., 2023). The disproportionate impact of *A. andreneli* aligns with growing evidence that morphologically similar species can exhibit markedly different ecological effects (Bickford et al., 2007). The pattern suggests underlying physiological or behavioural adaptations that enhance the competitive ability of *A. andreneli*. These patterns emphasise the critical importance of molecular diagnostic tools, such as COI barcoding, for accurate pest surveillance and species delimitation (Ekholm et al., 2019; Hebert et al., 2003).

What has been documented as *Acrocercops brongniardella* - which may, in some cases, represent *A. andreneli* due to potential historical misidentification - is currently undergoing well-documented range expansion in Russia, with recent records from both northern European Russia and Western Siberia (Ermolaev, 2024). First records from Omsk (2013), Yekaterinburg (2016), and St Petersburg (2018) demonstrate its spread into novel territories, while the species complex has historically caused outbreaks in other regions such as Kyiv (Ermolaev, 2024). In European Russia, the expansion has progressed through the Leningrad Oblast, with subsequent records from Gatchina and Sestroretsk (Musolin et al., 2022), while in Western Siberia, established populations now occur in both Omsk and Tyumen Oblasts, where the species is considered non-native and has produced notable outbreaks. While the spatial distribution pattern of this *Acrocercops* species complex in Russia is well-documented, its historical presence, current status, and precise species identity in other regions, such as Sweden, requires further investigation. Based on patterns observed in documented range expansions, we can only speculate that distribution likely results from a combination of natural dispersal and human-mediated movement. Although adult moths can disperse naturally through flight (Chapman et al., 2002), the discontinuous nature of new populations suggests anthropogenic dispersal mechanisms may be important, potentially facilitated by oak nursery trade and plant transport (Roques et al., 2014; Stenlid and Oliva, 2016). This multi-modal dispersal strategy would parallel patterns observed in other significant forest pests, including emerald ash borer and horse chestnut leaf miner (Grabenweger et al., 2010; Herms and McCullough, 2014). The successful establishment and subsequent high infestation rates of this *Acrocercops* species in newly documented areas emphasise the importance of comprehensive monitoring programs. Examination of historical specimens in regional collections across its potential range, including Sweden, could help elucidate not only distribution patterns and colonisation history but also clarify the historical and current species composition (Elith, 2017). Field observations by amateur lepidopterists in Stockholm suggest increasing severity of infestations as well as increased dominance of *A. andreneli* over *A. brongniardella* since the year 2000, though comprehensive quantitative surveys are needed to verify this (Hellberg pers. comm.).

4.2. Parasitoid community structure and biological control potential

The parasitoid communities reared from *A. andreneli* and *A. brongniardella* were largely shared between the two species, offering limited evidence of host specialisation. Although some parasitoid lineages appeared only in one host species (potential specialists), these cases were typically singletons and thus likely represent random occurrences rather than true host specialisation. This limited specialisation presents an interesting contrast to established host-parasitoid coevolutionary theory (Grégoire and Gould, 2023). Overlapping parasitoid assemblages between these cryptic moths suggest limited host discrimination by their natural enemies at present. However, parasitoid-host interactions can shift over time following species establishments, as demonstrated in

Table 1
Factors influencing oak leaf infestation severity across 56 sites across the distributional range of oak (*Quercus*) in Sweden. Results from ordinal logistic regression examining the effects of species presence and latitude on infestation severity. Model fit: AIC = 74.75, Residual deviance = 62.75. Shown are coefficient estimates, odds ratios (exp(Estimate)), standard errors (SE), χ^2 values (df = 1) and P values.

Parameter	Estimate	Odds ratio	SE	χ^2	P-value
<i>A. brongniardella</i> presence	2.37	10.70	0.777	11.57	< 0.001
<i>A. andreneli</i> presence	12.62	302000	0.005	11.18	< 0.001
Latitude	0.75	2.12	0.400	4.25	0.039

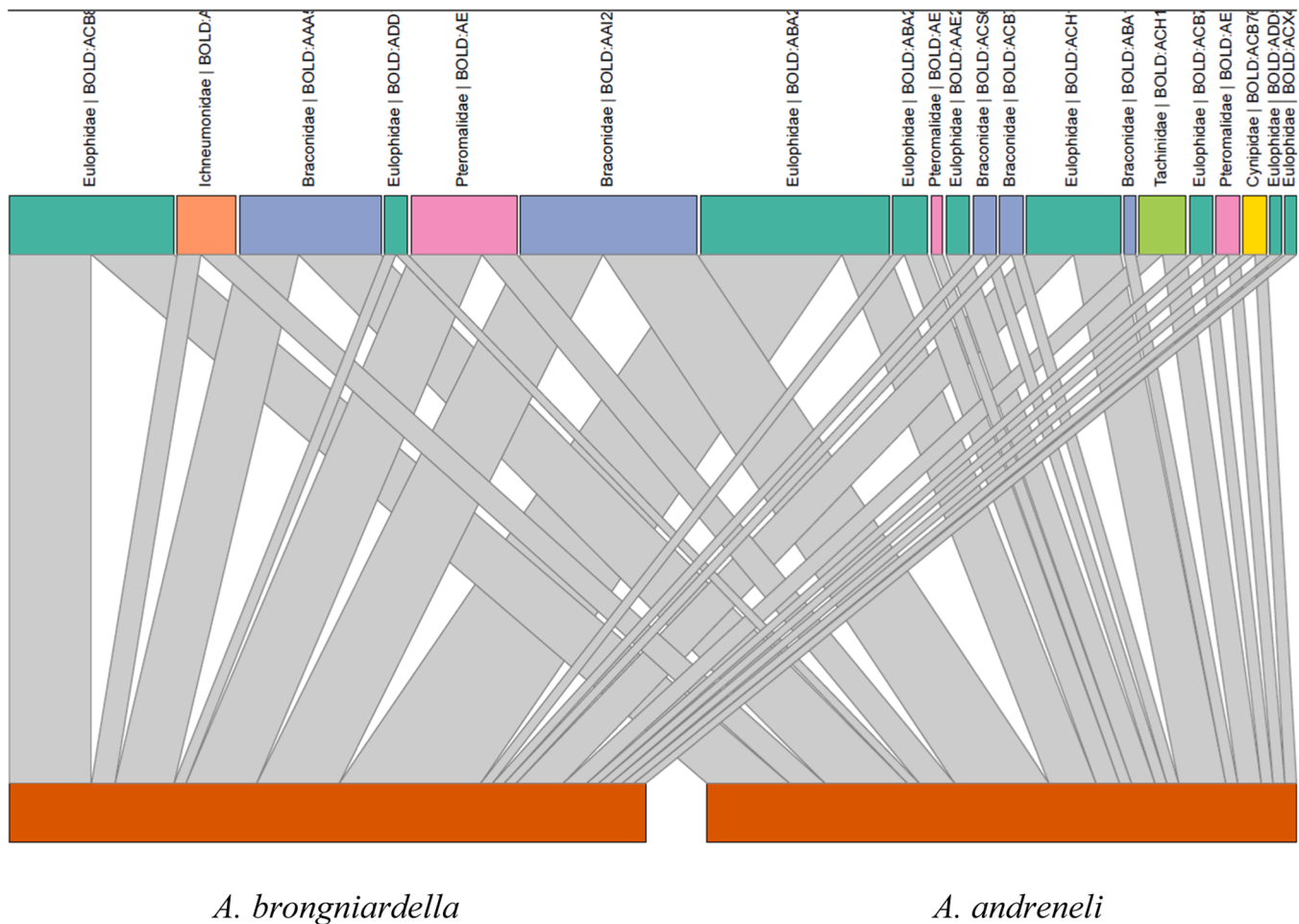


Fig. 3. Host–parasitoid network structure for two cryptic *Acrocercops* leaf-mining moths (*A. brongniardella* and *A. andreneli*) across eighteen Swedish oak forests. The two host species are shown in orange at the bottom of the network, while the parasitoid taxa at the top are colour-coded by family. Specifically, Eulophidae are represented in dark green, Pteromalidae in purple, Braconidae in blue, Cynipidae in yellow, Tachinidae in light green, and Ichneumonidae in orange. Grey bars indicate attack frequencies, with bar width proportional to interaction strength.

nettle-feeding butterflies (Audusseau et al., 2021). This temporal dynamic suggests that current parasitoid community composition may represent an early stage of interaction, with potential for divergence as natural enemies adapt to newly established or detected host populations. Nonetheless, widespread sharing of parasitoid species among leaf-miner species in even different genera and families has been previously demonstrated by (Blanchet et al., 2018; Kaartinen et al., 2010; Tack et al., 2011). Hence, it may come as no surprise that two hosts, almost indistinguishable to the eye, share a large part of their parasitoid complements. These findings have implications for biological control approaches targeting cryptic pest complexes, as they suggest that cryptic species may be part of more generalist parasitoid communities than previously anticipated (Panzavolta et al., 2021). *A. andreneli* seems to be attacked by parasitoid species emerging from *A. brongniardella*, which should – in theory – cause both apparent competition between the species and bring them both under parasitoid control (Godfray, 1994; Tack et al., 2009). Only longitudinal studies of host-parasitoid dynamics will resolve this conundrum.

4.3. Future research directions and management implications

Although we aimed to explore the relationships among parasitoid species richness, parasitism rate, and infestation levels, our sampling approach did not permit adequate control for confounding variables inherent in the simultaneous sampling of hosts and parasitoids.

Consequently, it was not possible to disentangle the effects of sampling effort from biological variation, precluding robust conclusions regarding the regulatory role of parasitoid communities. Further research employing sampling strategies that explicitly account for these confounders is required.

As anthropogenic pressures intensify through factors such as nursery trade and climate change, detecting and monitoring cryptic diversity becomes crucial for effective forest management (Mina et al. 2022). Our results suggest that integrating molecular diagnostics with traditional surveillance methods could significantly improve early detection of emerging pest threats (Hebert et al., 2003). Understanding the temporal dynamics of this system presents another critical research direction. Historical collections could provide valuable insights into whether *Acrocercops* has maintained a long-term presence in the region or represents a recent introduction. Longitudinal studies of both host and parasitoid community dynamics will also be needed to understand the overall scope of parasitoids in controlling host abundances. Such temporal analyses would help distinguish between invasion-driven outbreak dynamics and responses to environmental change (Wang, 2024). This historical perspective will enhance our ability to predict outbreaks and develop targeted management strategies. As the first step, we need to adequately identify the taxa causing the outbreaks, the enemies attacking them and the patterns in these communities. Our study makes a key contribution to this.

CRediT authorship contribution statement

Romana Salis: Data curation, Formal analysis, Visualization, Writing – review & editing. **Marcus Hall:** Conceptualization, Methodology, Validation, Writing – review & editing. **Tomas Roslin:** Conceptualization, Investigation, Project administration, Supervision, Visualization, Formal analysis, Methodology, Resources, Validation, Writing – review & editing. **Ayco Tack:** Conceptualization, Investigation, Project administration, Supervision, Visualization, Writing – review & editing. **Funding acquisition, Methodology, Resources, Validation, Writing – original draft.** **Markus Franzén:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Anders Forsman:** Project administration, Supervision, Visualization, Resources, Validation, Writing – review & editing. **Alvaro Gaytán:** Conceptualization, Methodology, Validation, Investigation, Supervision, Writing – review & editing.

Ethics approval

Fieldwork was conducted under permits issued by the Swedish Environmental Protection Agency and complied with all local regulations for protected areas. No protected species were sampled.

Declaration of Generative AI and AI-assisted technologies in the writing process

No generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Funding

Stiftelsen Seydlitz MP bolagen, KVA, Magnus Bervalls foundation and Formas supported this research via the Swedish National Research Programme on Climate (grant 2021-02142).

Declaration of Competing Interest

The authors declare that they have no known competing financial or non-financial interests that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Amadeus Nilsson, Bafraw Karimi, Carina Romero Ugarph, Dave Karlsson, Genoveva Elisabeth Zimmermann, Jonas Lundqvist, Lena Carlsson, and Sara Forsman for their assistance with field sampling, insect rearing, barcode preparation, and data management. Station Linné generously provided laboratory space and facilities. We are also grateful to Marc Hauber for preparing the illustration of the *Acrocercops* leaf mine.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.foreco.2025.123181](https://doi.org/10.1016/j.foreco.2025.123181).

Data Availability

Data will be made available on request.

References

Audusseau, H., et al., 2021. Rewiring of interactions in a changing environment: nettle-feeding butterflies and their parasitoids. *Oikos* 130, 624–636.

- Bengtsson, B., et al. 2011. Nationalnyckeln till Sveriges flora och fauna. - Fjärilar: Bronsmalar-rullvingemalar. Lepidoptera: Roeslerstammiidae-Lyonetiidae. ArtDatabanken, SLU, Uppsala.
- Bengtsson, B.Å., 2024. Anmärkningsvärda fynd av småfjärilar (Microlepidoptera) i Sverige 2023. *Entomol. Tidskr.* 145, 1–21.
- Bickford, D., et al., 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155.
- Bivand, R., et al., 2017. Package 'spdep'. - spatial dependence: weighting schemes. *Stat. R. Package Version* 1, 1–5.
- Blanchet, F.G., et al., 2018. Related herbivore species show similar temporal dynamics. *J. Anim. Ecol.* 87, 801–812.
- Brooks, M.E., et al., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R. J.* 9, 378–400.
- Buchner, D., et al., 2022. APSCALE: advanced pipeline for simple yet comprehensive analyses of DNA metabarcoding data. *Bioinformatics* 38, 4817–4819.
- Chapman, J.W., et al., 2002. High-altitude migration of the diamondback moth *Plutella xylostella* to the UK: a study using radar, aerial netting, and ground trapping. *Ecol. Entomol.* 27, 641–650.
- Dale, V.H., et al., 2001. Climate change and forest disturbances: climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms, or landslides. *BioScience* 51, 723–734.
- Dormann, C.F., et al., 2008. Introducing the bipartite package: analysing ecological networks. *R News* 8, 8–11.
- Ekholm, A., et al., 2019. Host plant phenology, insect outbreaks and herbivore communities – the importance of timing. *J. Anim. Ecol.* 89, 829–841.
- Elbrecht, V., et al., 2019. Validation of COI metabarcoding primers for terrestrial arthropods. *PeerJ* 7, e7745.
- Elith, J., 2017. *Invasive Species: Risk Assessment and Management*. Cambridge University Press, pp. 93–129.
- Ermolaev, I., et al., 2024. Parasitoids (Hymenoptera: Pteromalidae, Eulophidae, Braconidae) of the leaf blotch miner moth *Acrocercops brongniardella* (Lepidoptera: Gracillariidae) in the Ufa City outskirts. *Russian Entomol. J.* 33, 374–382.
- Fiser, C., et al., 2018. Cryptic species as a window into the paradigm shift of the species concept. *Mol. Ecol.* 27, 613–635.
- Folmer, O., et al., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- Forsman, A., et al., 2024. Latitudinal gradients of biodiversity and ecosystem services in protected and non-protected oak forest areas can inform climate smart conservation. *Geogr. Sustain.* 5, 647–659.
- Ghelardini, L., et al., 2016. Drivers of emerging fungal diseases of forest trees. *For. Ecol. Manag.* 381, 235–246.
- Godfray, H.C.J., 1994. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press.
- Gorayeb, E.S., et al., 2021. Performance and preference of *Bemisia tabaci* on cucumbers: understanding the recent outbreaks of Mediterranean cryptic species in Brazil. *Crop Prot.* 143, 105468.
- Grabenweger, G., et al., 2010. Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe. *Biol. Invasions* 12, 2797–2813.
- Grégoire, J.-C., Gould, J.R., 2023. Forest insect–natural enemy interactions. In: *Forest Entomology and Pathology*, 1. Entomology. Springer International Publishing Cham, pp. 141–167.
- Hagen, S.B., et al., 2007. Shifting altitudinal distribution of outbreak zones of winter moth operophtera *brumata* in sub-Arctic birch forest: a response to recent climate warming? *Ecography* 30, 299–307.
- Hebert, P.D., et al., 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 270, 313–321.
- Hending, D., 2024. Cryptic species conservation: a review. *Biol. Rev.* 100, 258–274.
- Hermes, D.A., McCullough, D.G., 2014. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. *Annu. Rev. Entomol.* 59, 13–30.
- Hleap, J.S., et al., 2021. Assessment of current taxonomic assignment strategies for metabarcoding eukaryotes. *Mol. Ecol. Resour.* 21, 2190–2203.
- Iwaszkiewicz-Eggebrecht, E., et al., 2024. High-throughput biodiversity surveying sheds new light on the brightest of insect taxa. *Proc. R. Soc. B* 292, 20242974.
- Jervis, M.A., et al., 2023. Population Dynamics. - In: *Jervis's Insects as Natural Enemies: Practical Perspectives*. Springer, pp. 591–667.
- Kaartinen, R., et al., 2010. Revealing secret liaisons: DNA barcoding changes our understanding of food webs. *Ecol. Entomol.* 35, 623–638.
- Keck, F., et al., 2023. Navigating the seven challenges of taxonomic reference databases in metabarcoding analyses. *Mol. Ecol. Resour.* 23, 742–755.
- Kirichenko, N., et al., 2023. Trophic associations and harmfulness of native and alien leaf mining moth species in Siberia. *Sib. Lesn. Zh.* 1, 85–97.
- Kuuluvainen, T., 2009. Forest management and biodiversity conservation based on natural ecosystem dynamics in Northern Europe: the complexity challenge. *AMBIO A J. Hum. Environ.* 38, 309–315.
- Lavergne, S., et al., 2010. Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. - annual review of ecology. *Evol. Syst.* 41, 321–350.
- Liu, H., Stiling, P., 2006. Testing the enemy release hypothesis: a review and meta-analysis. *Biol. Invasions* 8, 1535–1545.
- Lyubenova, A., et al., 2024. Prospects for oak cultivation in Europe under changing environmental conditions and increasing pressure from harmful organisms. *Forests* 15, 2164.

- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.* 17, 10–12.
- Mina, M., et al., 2022. Managing for the unexpected: building resilient forest landscapes to cope with global change. *Glob. Change Biol.* 28, 4323–4341.
- Miraldo, A., et al., 2024. Data of the Insect Biome Atlas: a metabarcoding survey of the terrestrial arthropods of Sweden and Madagascar. *Scientific Data* 12, 835.
- Muraleedharan, N., et al., 1988. Natural enemies of certain tea pests occurring in Southern India. *Int. J. Trop. Insect Sci.* 9, 647–654.
- Musolin, D.L., et al., 2022. Invasive insect pests of forests and urban trees in Russia: origin, pathways, damage, and management. *Forests* 13, 521.
- Nahrung, H.F., et al., 2023. Forest insect biosecurity: processes, patterns, predictions, pitfalls. *Annu. Rev. Entomol.* 68, 211–229.
- Nel, J., et al., 2023. Description d'une quatrième espèce européenne du genre *acrocerops wallengren*, 1881, A. *Andreneli* sp. n. (Lepidoptera, Gracillariidae, Gracillariinae). *Revue de l'Association Roussillonnaise d'Entomologie* 32, 108–112.
- Nyman, T., et al., 2022. A curated DNA barcode reference library for parasitoids of Northern European cyclically outbreaking geometrid moths. *Ecol. Evol.* 12, e9525.
- Panzavolta, T., et al., 2021. Alien invasive pathogens and pests harming trees, forests, and plantations: pathways, global consequences and management. *Forests* 12, 1364.
- R Core Team** 2024. **R: A Language and Environment for Statistical.** R version 4.4.2.
- Ripley, B., et al., 2013. Package 'mass'. *Cran R.* 538, 113–120.
- Ripley, B., et al., 2016. Package 'nnet'. R. Package Version 7, 700.
- Roques, A., et al., 2014. Climate warming and past and present distribution of the processionary moths (*Thaumetopoea* spp.) in Europe, Asia minor and north Africa. *Processionary moths and climate change: an update.* Springer, pp. 81–161.
- Roy, H., et al., 2011. Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids? *BioControl* 56, 451–468.
- Shaw, M., Askew, R., 1976. Ichneumonoidea (Hymenoptera) parasitic upon leaf-mining insects of the orders Lepidoptera, Hymenoptera and Coleoptera. *Ecological Entomology* 1, 127–133.
- Shorohova, E., et al., 2009. Natural stand structures, disturbance regimes and successional dynamics in the Eurasian boreal forests: a review with special reference to Russian studies. *Ann. For. Sci.* 66, 1–20.
- Smith, M.A., et al., 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proc. Natl. Acad. Sci.* 104, 4967–4972.
- Stenlid, J., Oliva, J., 2016. Phenotypic interactions between tree hosts and invasive forest pathogens in the light of globalization and climate change. *Philos. Trans. R. Soc. B Biol. Sci.* 371, 20150455.
- Tack, A.J., et al., 2009. Competition as a structuring force in leaf miner communities. *Oikos* 118, 809–818.
- Tack, A.J., et al., 2011. Can we predict indirect interactions from quantitative food webs?—an experimental approach. *J. Anim. Ecol.* 80, 108–118.
- Turchin, P., 1999. Population regulation: a synthetic view. *Oikos* 84, 153–159.
- Turchin, P., 2013. *Complex Population Dynamics: A Theoretical/empirical Synthesis* (MPB-35). Princeton University Press.
- Wang, S., et al., 2024. Towards mechanistic integration of the causes and consequences of biodiversity. *Trends Ecol. Evol.* 39, 689–700.
- Wolz, M., et al., 2024. Response of parasitoid communities to insecticide application during a *lymantria dispar* outbreak in mixed oak forests. *J. Appl. Ecol.* 61, 2774–2785.
- Xu, X., et al., 2021. Molecular identification of leafmining flies from Australia including new *liriomyza* outbreaks. *J. Econ. Entomol.* 114, 1983–1990.