Türk. entomol. derg., 2026, 50 (3): 231-243
DOI: http://dx.doi.org/10.16970/entoted.1908135

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Microbial diversity in the Pale tussock moth, *Calliteara pudibunda* L., 1758 (Lepidoptera: Erebidae) larvae in the Eastern beech forest in Türkiye

Türkiye'deki Doğu kayın ormanlarında Kızıl kuyruklu kayın tırtılı, *Calliteara pudibunda* L., 1758 (Lepidoptera: Erebidae) larvalarındaki mikrobiyal çeşitlilik

Dönüş GENÇER[1*] Zeynep BAYRAMOĞLU[2] İsmail DEMIR[3]

Abstract

The Pale tussock moth, *Calliteara pudibunda* L., 1758 (Lepidoptera: Erebidae) is an important defoliator of deciduous forests, particularly *Fagus orientalis* Lipsky (Fagales: Fagaceae), with recurrent outbreaks in Türkiye. Despite its ecological and economic importance, its microbial communities remain poorly characterized. In this study, the microbiome of larvae collected during an outbreak in Bursa was analyzed by shotgun metatranscriptomic sequencing in Trabzon between 2023 and 2024. A total of 53.4 million paired-end reads were generated and taxonomically classified using Kraken2. The microbiome was dominated by Firmicutes and Proteobacteria, comprising more than 65% of bacterial reads, while Reoviridae and Baculoviridae were the most abundant viral families. Sequences related to Cypovirus 15 and Orgyia pseudotsugata multiple nucleopolyhedrovirus (OpMNPV) were detected. Electron microscopy further confirmed the presence of two baculoviruses, Calliteara pudibunda nucleopolyhedrovirus-Türkiye (CapuNPV-T1) and Calliteara pudibunda granulovirus-Türkiye (CapuGV-T). Archaeal sequences included extremophiles such as *Sulfolobus acidocaldarius* A (Sulfolobales: Sulfolobaceae) and methanogenic *Methanosarcina* spp. Kluyver & van Niel (Methanosarcinales: Methanosarcinaceae). These findings provide the first high-resolution insights into the microbiome and virome of *C. pudibunda* in Türkiye, highlighting microbial associations relevant to biological control strategies.

Keywords: Baculovirus, Cypovirus, *Calliteara pudibunda*, microbial diversity, shotgun metatranscriptomics

Öz

Kızıl kuyruklu kayın tırtılı, *Calliteara pudibunda* L., 1758 (Lepidoptera: Erebidae), özellikle Doğu kayını, *Fagus orientalis* Lipsky (Fagales: Fagaceae) üzerinde beslenen ve Türkiye'de dönemsel salgınlar oluşturan önemli bir yaprak zararlısıdır. Ekolojik ve ekonomik önemine rağmen, bu türün mikrobiyal toplulukları sınırlı düzeyde bilinmektedir. Bu çalışmada, Bursa'da bir salgın sırasında toplanan larvaların mikrobiyomu, 2023-2024 yıllarında Trabzon'da shotgun metatranskriptomik dizileme ile analiz edilmiştir. Toplam 53,4 milyon paired-end okuma elde edilmiş ve Kraken2 ile taksonomik sınıflandırma yapılmıştır. Mikrobiyomun, bakteriyel okumaların %65'inden fazlasını oluşturan Firmicutes ve Proteobacteria tarafından domine edildiği belirlenmiştir. Viral toplulukta Reoviridae ve Baculoviridae en baskın ailelerdir. Cypovirus 15 ve Orgyia pseudotsugata multiple nucleopolyhedrovirus (OpMNPV) dizileri tespit edilmiştir. Elektron mikroskobu analizleri, Calliteara pudibunda nucleopolyhedrovirus-Türkiye (CapuNPV-T1) ve Calliteara pudibunda granulovirus-Türkiye (CapuGV-T) varlığını doğrulamıştır. Arkea dizileri arasında *Sulfolobus acidocaldarius* A (Sulfolobales: Sulfolobaceae) ve metanojenik *Methanosarcina* spp. Kluyver & van Niel (Methanosarcinales: Methanosarcinaceae) yer almıştır. Bu bulgular, Türkiye'de *C. pudibunda* mikrobiyom ve viromuna ilişkin ilk yüksek çözünürlüklü verileri sunmakta ve biyolojik mücadele stratejileri açısından önem taşıyan mikrobiyal ilişkileri ortaya koymaktadır.

Anahtar sözcükler: Bakülovirüs, Cypovirus, *Calliteara pudibunda*, mikrobiyal çeşitlilik, shotgun metatranskriptomik

[1] Trabzon University, Şalpazarı Vocational School, Department of Property Protection and Security, 61670, Trabzon, Türkiye
[2] Recep Tayyip Erdoğan University, Pazar Vocational School, Department of Plant and Animal Production, 53300, Rize, Türkiye
[3] Karadeniz Technical University, Faculty of Science, Department of Biology, 61080, Trabzon, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: donus.gencer@trabzon.edu.tr
Received (Alınış): 12.03.2026 Accepted (Kabul ediliş): 28.07.2026 Published Online (Çevrimiçi Yayın Tarihi): 09.09.2026

Introduction

The pale tussock moth, *Calliteara pudibunda* L., 1758 (Lepidoptera: Erebidae) is a major defoliator of broadleaf forests, particularly beech, *Fagus orientalis* Lipsky (Fagales: Fagaceae), and has caused severe outbreaks across Europe (Novak et al., 1976; Mazzoglio et al., 2005; Kimoto & Duthie-Holt, 2006; Trofimova, 2012). The species is widely distributed throughout Europe and parts of Asia and is considered an important periodic forest pest in temperate deciduous ecosystems (Ipekdal & Avcı, 2023). Historically regarded as rare in Türkiye, the species reached outbreak levels in Bursa Province during 2018-2019, leading to complete defoliation of *F. orientalis* stands over several hundred hectares (Göktürk & Aksu, 2005; Ipekdal, 2022).

Larvae are highly destructive, skeletonizing young leaves and buds before consuming entire laminae at later stages. Mass infestations severely reduce forest productivity and may cause complete defoliation during outbreak periods (Nilsson, 1978; Ipekdal & Avcı, 2023). Severe defoliation may reduce tree vigor and increase susceptibility to secondary biotic and abiotic stress factors (Ipekdal & Avcı, 2023). As in many forest Lepidoptera, the outbreak dynamics of *C. pudibunda* are influenced by natural epizootics, especially those caused by insect viruses (Myers & Cory, 2013; Gencer et al., 2018, 2019; Bayramoglu et al., 2018; Sevim et al., 2010). Baculoviruses are among the most important naturally occurring viral pathogens of Lepidoptera and frequently contribute to the collapse of outbreak populations in forest ecosystems (Cory & Myers, 2003; Jehle et al., 2006). Although baculoviruses infecting *C. pudibunda* have been reported (Krieg & Langenbuch, 1956; Krejmer et al., 2015; Gencer et al., 2024, 2025), no study has comprehensively investigated the wider microbial community of this species. Previous studies mainly focused on the characterization of viral pathogens, particularly nucleopolyhedroviruses associated with outbreak populations (Krejmer et al., 2015; Gencer et al., 2025).

Insects harbor diverse microbiomes that can shape host nutrition, immunity, development, and pathogen susceptibility (Engel & Moran, 2013; Chen et al., 2016; Mason et al., 2020). Recent studies have demonstrated that insect-associated microbial communities may vary according to developmental stage, host phylogeny, diet, and environmental conditions (Yun et al., 2014; Phalnikar et al., 2018). Yet, the microbiome and virome of *C. pudibunda* remain virtually unexplored, with only one recent Turkish report describing a nucleopolyhedrovirus infection (Gencer et al., 2025).

Here, we address this knowledge gap using shotgun metatranscriptomic sequencing of larvae collected during a major outbreak in Türkiye. Recent advances in next-generation sequencing technologies, particularly metatranscriptomic approaches, have enabled comprehensive characterization of insect-associated microbial communities without cultivation-based limitations (Shi et al., 2016; Wu et al., 2020). We hypothesize that *C. pudibunda* harbors a taxonomically rich and heterogeneous microbial community, including entomopathogens with biocontrol potential. This study provides the first high-resolution overview of the microbiome and virome of *C. pudibunda* in Türkiye, offering insights into host-microbe interactions and contributing to future studies on host-associated microbial diversity and insect-pathogen interactions.

Materials and Methods

Field investigations and sample collection

In 2019, surveys were conducted in the beech forests of Bursa Province, Türkiye, to assess outbreak intensity, population density, and host tree damage. Tree trunks, root collars, understory shrubs, and forest debris were examined. Larvae and pupae exhibiting symptoms of infection were collected together with apparently healthy individuals. Insects were transported in ventilated plastic boxes containing fresh foliage to the Microbiology Laboratory at Karadeniz Technical University in Trabzon for further analysis between 2023 and 2024.

For metatranscriptomic analyses, pooled whole-body samples consisting of 20 larvae were used for total RNA extraction in order to maximize the recovery of microbial and viral RNA and to better represent the outbreak population, as is commonly done in insect metatranscriptomic studies (Shi et al., 2016; Wu et al., 2020).

Macroscopic and microscopic examinations

The external morphology of dead and symptomatic larvae and pupae was inspected under stereomicroscopy. Internal tissues including fat body, trachea, hemocoel, and midgut were examined under phase-contrast microscopy (Nikon Eclipse E600). Liquefied tissues were used to prepare slides for dark-field and phase-contrast observation. Samples showing viral symptoms were preserved at −20°C until further analyses.

Microscopic dissections were performed aseptically using sterile forceps and needles under laboratory conditions. These dissections were conducted for pathological and microscopic observations only and were not used for organ-specific RNA isolation.

Microscopic observation of occlusion bodies (OBs)

Occlusion bodies were visualized by phase-contrast and dark-field microscopy at 20×-40× magnification. For scanning electron microscopy (SEM), 20 µl aliquots of suspensions were air-dried on stubs, gold-coated, and examined with a JEOL JSM-6610 microscope. Transmission electron microscopy (TEM) was performed on purified OBs fixed in glutaraldehyde and OsO4, embedded in resin, sectioned, and stained with uranyl acetate before examination with a JEOL JEM-1220 microscope at 80 kV.

RNA isolation and sequencing

Pools of larvae were subjected to total RNA extraction. Whole-body homogenates of pooled larvae were used for RNA isolation without organ-specific separation to capture microorganisms and viral sequences associated with different host tissues. RNA quality was assessed by TapeStation and Qubit fluorometry, with acceptance criteria of ≥10 ng/µl and RIN ≥7. Libraries were prepared using the Illumina Total RNA Prep Kit, involving rRNA depletion, fragmentation, cDNA synthesis, and indexing. Sequencing was carried out on the Illumina NovaSeq 6000 platform, generating 150 bp paired-end reads.

Bioinformatic analysis of shotgun metatranscriptomic data and taxonomic profiling

Raw reads were subjected to quality control with FastQC and trimmed with Trimmomatic (Bolger et al., 2014) to remove adapters and low-quality bases. Taxonomic classification of high-quality reads was performed with Kraken2 against the PlusPF database. To refine microbial profiles, reads were compared with curated databases (NCBI RefSeq, SILVA, Virus-Host DB) using Kraken2 and DIAMOND. Host reads were filtered by Bowtie2 mapping. Contigs were assembled with MEGAHIT or Trinity and annotated for functional and taxonomic features. Relative abundance of viral, bacterial, archaeal, and eukaryotic microorganisms was calculated and visualized using bar plots and Krona charts.

Results

Inspection on dead caterpillars

Macroscopic observations revealed that larval and pupal cadavers were extremely fragile, rapidly liquefying and disintegrating. This caused leakage of dark body fluids and contamination of surrounding vegetation. Many larvae were found hanging upside down on tree trunks and branches, resembling “baseball bats” (Figure 1). Similar symptoms were observed in both field- and laboratory-collected specimens, strongly suggesting a natural viral infection.



Figure 1. Larvae that suspended upside down on tree trunks and body structures liquefied.

Microscopy

Light microscopy of cadaver fluids showed dense, bright occlusion bodies (OBs) under both dark-field and phase-contrast microscopy (Figure 2). SEM revealed two OB phenotypes: smooth polyhedral crystals typical of nucleopolyhedroviruses, and irregular, pitted forms consistent with cypoviruses (Figure 3). TEM confirmed the presence of multiple baculoviruses, including a multinucleocapsid NPV and a granulovirus, designated as Calliteara pudibunda nucleopolyhedrovirus-Türkiye (CapuNPV-T1) and Calliteara pudibunda granulovirus-Türkiye (CapuGV-T) (Figure 4). While CapuNPV-T1 had been previously characterized (Gencer et al., 2024), our study also detected and focused on CapuGV-T.

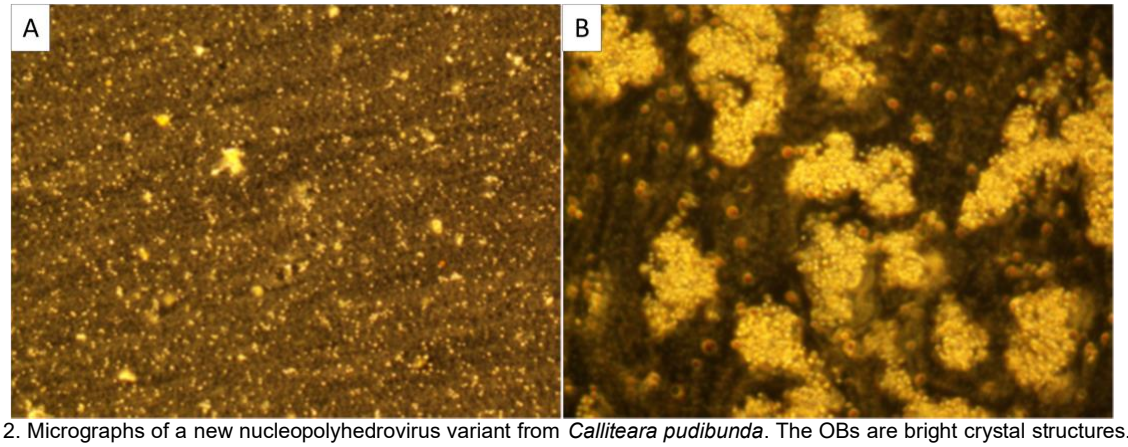


Figure 2. Micrographs of a new nucleopolyhedrovirus variant from *Calliteara pudibunda*. The OBs are bright crystal structures. Light microscopy (a) 20X; (b) 40X.

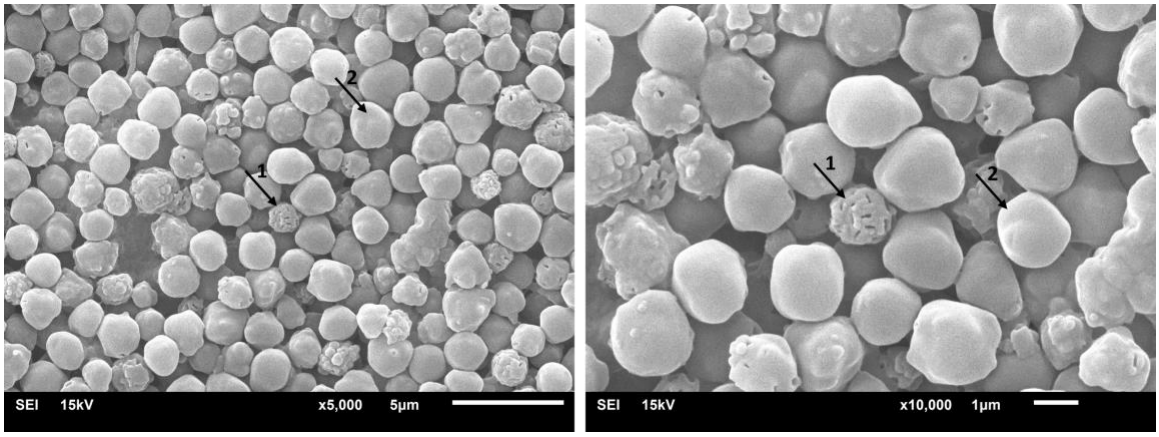


Figure 3. Scanning electron micrograph of polyhedral OBs (a) 5000X and (b)10000X. Arrow 1 indicates an example of a Cypovirus, while an arrow 2 points to a representative of a Nucleopolyhedrovirus.

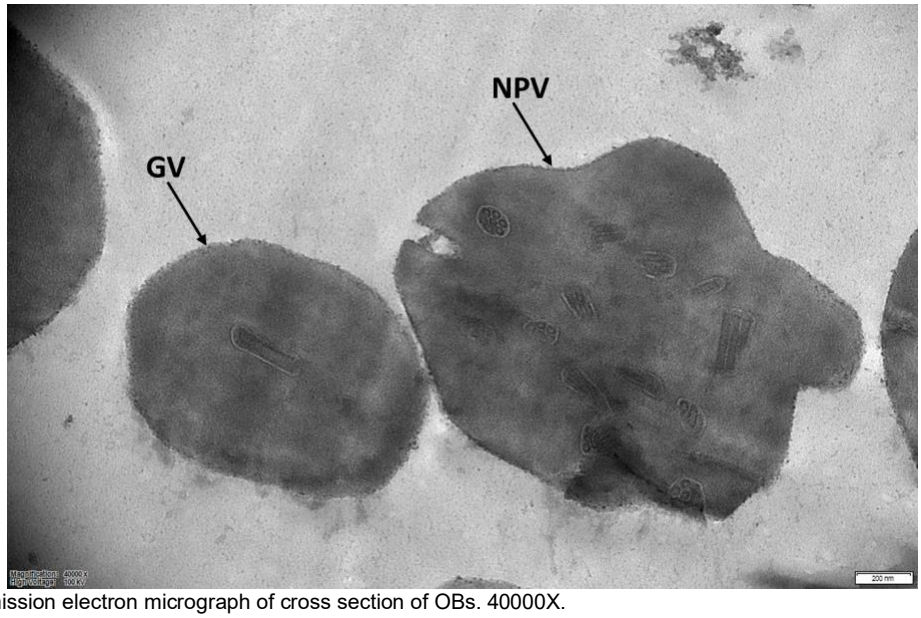


Figure 4. Transmission electron micrograph of cross section of OBs. 40000X.

Microbial community analysis

Metatranscriptomic profiling of the microbial community associated with *C. pudibunda* revealed that Bacteria constituted the most dominant domain, followed by Eukaryota, Viruses, and Archaea. Among bacterial taxa, the phyla Firmicutes and Proteobacteria were the most prevalent, with representative genera including *Staphylococcus*, *Klebsiella*, and *Haemophilus*, suggesting a diverse assemblage of both commensal and potentially opportunistic microbial species. Within the viral community, members of the families Reoviridae and Baculoviridae were particularly prominent. Notably, sequences corresponding to Cypovirus 15 and Orgyia pseudotsugata multiple nucleopolyhedrovirus (OpMNPV) were detected, indicating active or residual viral infections and supporting the hypothesis of host-virus interactions within the larval virome. Although Archaea were less abundant, their presence was marked by thermophilic species such as *Sulfolobus acidocaldarius* A (Sulfolobales: Sulfolobaceae) and methanogenic taxa including *Methanosarcina* spp. Kluyver & van Niel (Methanosarcinales: Methanosarcinaceae) and *Methanobacterium* spp. Kluyver & van Niel (Methanobacteriales: Methanobacteriaceae), reflecting potential metabolic diversity in the host-associated archaeal community. Eukaryotic reads were predominantly of human origin-likely due to environmental contamination or handling-but also included fungal taxa such as *Botrytis cinerea* Pers. Ex Nocca & Balb.

(Helotiales: Sclerotiniaceae) and *Saccharomyces* spp. Meyen ex E.C.Hansen (Saccharomycetales: Saccharomycetaceae), as well as unidentified protist-related sequences, potentially representing parasitic or environmental eukaryotes. These findings highlight the complex and multi-domain nature of the *C. pudibunda* microbiome, encompassing both symbiotic and environmental organisms.

The abundance distribution of the selected microbial domains in the analyzed samples is illustrated in Figure 5. The X-axis represents the microbial domains, while the Y-axis shows their total abundance measured in read counts. Among these domains, Bacteria exhibited the highest abundance, exceeding 3.5 million reads. Eukaryota and Viruses followed with notably lower abundances, approximately 150,000 and 70,000 reads, respectively. Archaea contributed the least to the microbial community, with a minimal abundance relative to the other domains.

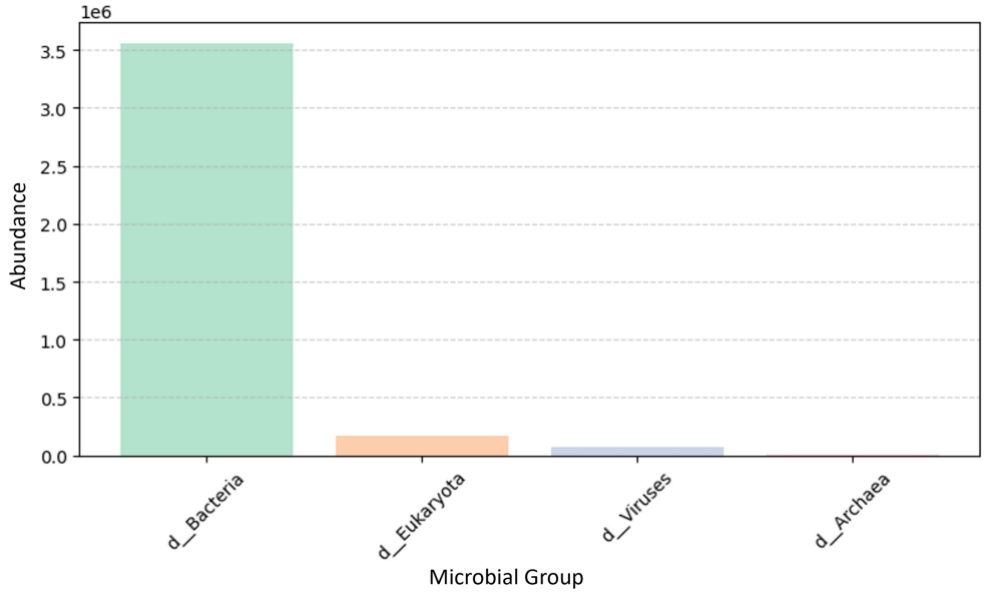


Figure 5. Abundance analysis of microbial communities - top 4 most abundant microbial taxa.

The bar chart illustrates the top 20 most abundant bacterial taxa identified in the samples analyzed, based on the number of assigned reads. The most dominant taxon corresponded to the domain Bacteria, with an abundance exceeding 3.5 million reads. This was followed by members of the phylum Firmicutes and the class Bacilli, both of which exhibited high relative abundance, highlighting the prominent representation of Firmicutes, particularly Bacilli, in the microbial community.

Further taxonomic breakdowns reveal a high abundance of *Staphylococcus* species, notably *Staphylococcus aureus* Rosenbach (Bacillales: Staphylococcaceae), highlighting their substantial contribution to the overall bacterial composition. Similarly, members of the Proteobacteria phylum, especially the classes Gammaproteobacteria and orders such as Enterobacterales and Pasteurellales, also appear prominently among the top taxa. Genera such as *Klebsiella*, *Haemophilus*, and *Salmonella* are present in lower but still notable quantities.

Overall, the data demonstrate the dominance of Firmicutes and Proteobacteria within the microbial community, with specific genera contributing significantly to the bacterial landscape. This distribution pattern may reflect environmental, host-associated, or clinical factors influencing microbial composition (Figure 6).

The bar chart illustrates the top 20 most abundant viral taxa in the dataset. Viruses showed the highest overall abundance, followed by members of the kingdom Orthornavirae, suggesting a predominance of RNA viruses within the sampled community.



Figure 6. Relative abundance of the top 20 bacterial taxa detected in *Calliteara pudibunda* larvae, dominated by Firmicutes and Proteobacteria.

A more detailed taxonomic breakdown shows a significant presence of the phylum Duplornaviricota and its affiliated class Resentoviricetes, order Reovirales, and family Reoviridae, with genera such as Cypovirus and Cypovirus 15 appearing among the top taxa. This indicates a strong representation of double-stranded RNA viruses within the Reoviridae family.

Additionally, taxa belonging to the phylum Naldaviricetes, particularly the family Baculoviridae, are prominently represented. Notable members include Alphabaculovirus and specific species like Orgyia pseudotsugata multiple nucleopolyhedrovirus, which are known insect-infecting viruses.

Other notable viral groups include Pisuviricota (especially the class Duplopiviricetes and the order Durnavirales), as well as less abundant but still detectable members of the Betabaculovirus genus and Partitiviridae family.

Overall, the data suggest a diverse virome dominated by RNA viruses from the Orthornavirae kingdom, with substantial contributions from families known to infect both invertebrates and plants. This composition may reflect specific host associations or environmental conditions present in the sampled ecosystem (Figure 7). The bar chart displays the top 20 most abundant archaeal taxa identified in the samples, based on abundance count. The most dominant taxon corresponded to the domain Archaea, with an abundance exceeding 500 reads. Among the most prevalent archaeal phyla, Crenarchaeota clearly dominates, particularly the class Thermoprotei and its subsequent orders and families, including Sulfolobales and Sulfolobaceae. Notably, *Sulfolobus* and *S. acidocaldarius* A are among the top-ranked genera and species, suggesting a strong representation of thermophilic archaea in the microbial community.

The phyla Euryarchaeota and Thaumarchaeota also appear within the top 20 taxa, although with significantly lower abundance. Within Thaumarchaeota, the order Nitrosopumilales and the genus *Nitrosopumilus*, including *Nitrosopumilus maritimus* Könneke, Bernhard, de la Torre, Walker, Waterbury & Stahl (Nitrososphaerales: Nitrosopumilaceae), are present, indicating the existence of ammonia-oxidizing archaea, which may play key roles in nitrogen cycling.

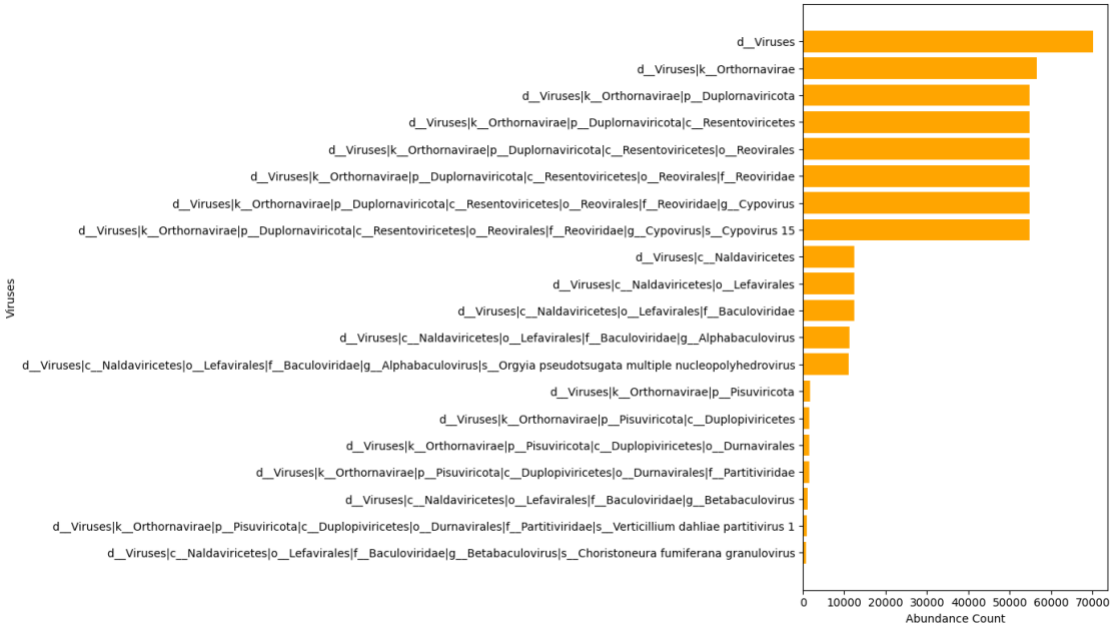


Figure 7. Relative abundance of the top 20 viral taxa in *Calliteara pudibunda* larvae, dominated by Reoviridae and Baculoviridae.

Members of the Euryarchaeota phylum, including classes such as Methanobacteria, Methanomicrobia, and Methanococci, are also detected, though at lower abundance levels. Genera such as *Methanobacterium* and *Methanosarcina* point to the presence of methanogenic archaea, suggesting potential involvement in anaerobic carbon cycling processes.

In summary, the archaeal community is predominantly composed of thermophilic taxa within the Crenarchaeota phylum, with secondary contributions from nitrifying and methanogenic archaea. This composition may reflect extreme or specialized environmental conditions present in the sampled habitat (Figure 8). The bar chart illustrates the top 20 most abundant eukaryotic taxa detected in the analyzed samples, based on abundance count. The domain Eukaryota showed the highest overall abundance, followed by a substantial representation of the kingdom Animalia, particularly members of the phylum Chordata. Within this phylum, the class Mammalia and its subordinate clades show high abundance, with the order Primates, family Hominidae, genus *Homo*, and species *Homo sapiens* L. (Primates: Hominidae) represented at progressively lower taxonomic levels. This indicates a substantial presence of human-associated DNA, likely derived from host or environmental contamination. Besides metazoans, fungal taxa from the kingdom Fungi, particularly within the phylum Ascomycota, also shows significant abundance. Noteworthy genera and species include *B. cinerea*, a known plant pathogen, and members of the orders Helotiales and Saccharomycetales, such as *Saccharomyces* spp. species, which are often associated with fermentation and environmental niches.

Additionally, protist taxa such as Aconoidasida from the phylum Sporozoa are present, albeit with lower abundance. These organisms may be parasitic or free-living eukaryotes commonly found in environmental or host-associated microbiomes.

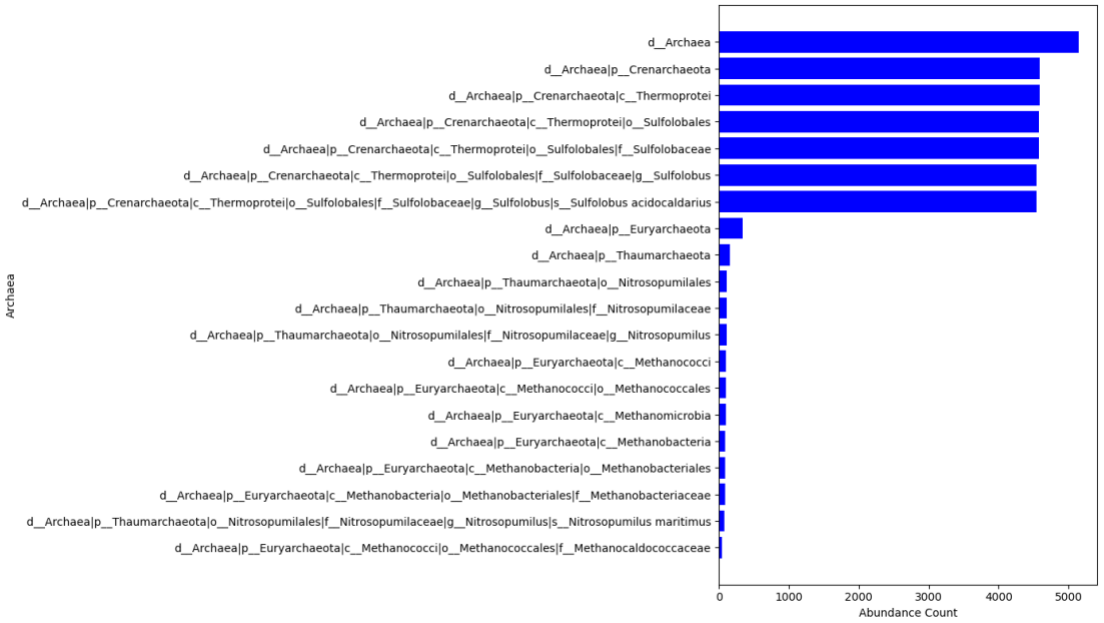


Figure 8. Relative abundance of the top 20 archaeal taxa in *Calliteara pudibunda* larvae.

In summary, the eukaryotic community profile is dominated by Metazoan sequences, especially those of human origin, followed by a diverse range of fungal taxa and a minor representation of protists. This distribution suggests a mixture of host-related and environmental eukaryotic DNA within the sample composition (Figure 9).

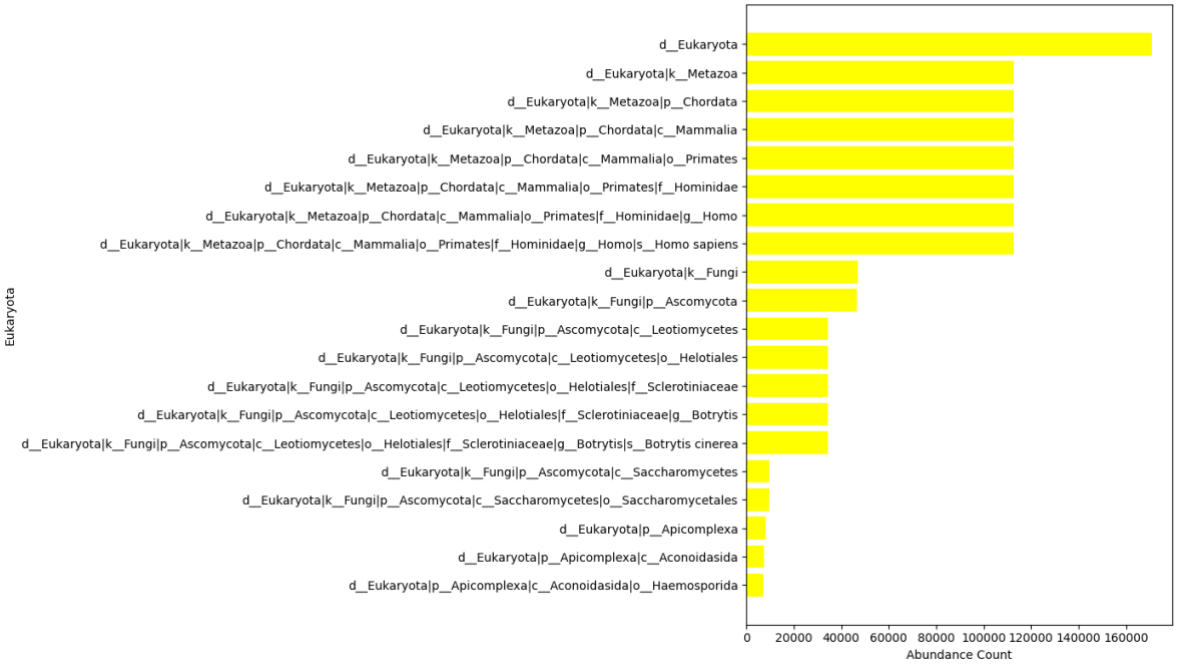


Figure 9. Relative abundance of the top 20 eukaryotic taxa in *Calliteara pudibunda* larvae, including metazoan and fungal sequences.

Discussion

This study provides the first comprehensive metatranscriptomic characterization of the microbial community associated with *C. pudibunda* larvae in the eastern beech forests of Türkiye. Our findings reveal a highly diverse and taxonomically rich microbiome, comprising bacteria, viruses, archaea, and eukaryotic microorganisms, each contributing uniquely to the host-microbe ecological dynamics.

Among the microbial domains detected in *C. pudibunda*, Bacteria represented the most abundant group, a finding consistent with previous studies on lepidopteran-associated microbiota (Chen et al., 2016; Mason et al., 2020). Taxonomic profiling revealed that the bacterial community was largely dominated by members of the phyla Firmicutes and Proteobacteria. Genera such as *Staphylococcus, Klebsiella* and *Haemophilus* were particularly prevalent, mirroring observations in other lepidopteran hosts including *Manduca sexta* L., 1763 (Lepidoptera: Sphingidae) and *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) (Chen et al., 2016).

Broderick et al. (2004) demonstrated that *Bacillus* spp. Berthold (Bacillales: Bacillaceae) and *Enterococcus* spp. Thiercelin & Jouhaud (Lactobacillales: Enterococcaceae) are frequently found in the midgut of *M. sexta*, playing mutualistic roles in digestion and nutrient assimilation. Similarly, Engel and Moran (2013) highlighted that insect-associated bacteria often perform crucial functions such as vitamin synthesis, immune modulation, and xenobiotic detoxification. However, some of the detected taxa in this study, such as *K. pneumoniae* and *Haemophilus influenzae* D (Enterobacteriales: Pasteurellaceae), have also been implicated in opportunistic infections, suggesting potential roles in secondary pathogenesis, particularly in immunocompromised or virally infected hosts.

The enrichment of Bacilli and members of the family Staphylococcaceae may also reflect environmental exposure of larvae or contamination during feeding on plant surfaces, as reported in surface microbiota analyses of lepidopteran larvae (Mason et al., 2020). Furthermore, post-viral dysbiosis -characterized by the overrepresentation of facultative pathogens- has been observed in other insect-virus interaction studies, suggesting that viral infections may modulate host microbial homeostasis (Chen et al., 2016).

The metatranscriptomic analysis of *C. pudibunda* unveiled a complex and diverse virome, predominantly comprising RNA viruses from the kingdom Orthornavirae, with a significant presence of the family Reoviridae. Notably, sequences corresponding to Cypovirus species were identified, aligning with previous reports of cytoplasmic polyhedrosis virus (CPV) infections in various lepidopteran pests, where CPVs have been associated with sublethal infections and developmental delays (Zhou et al., 2014; Martemyanov et al., 2023). These findings underscore the widespread occurrence and ecological relevance of CPVs in natural insect populations. In addition to RNA viruses, DNA viruses belonging to the family Baculoviridae, particularly Orgyia pseudotsugata multiple nucleopolyhedrovirus (OpMNPV), were also detected. Baculoviruses have long been recognized for their potential as environmentally safe biocontrol agents in forestry and agriculture, owing to their high host specificity and efficacy under field conditions (Szewczyk et al., 2006; Gencer et al., 2024). Their use has been especially successful in managing populations of major defoliating pests such as *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and *H. armigera* (Erlandson et al., 2007; Baillie & Bouwer, 2012). Comparative studies in other insect hosts also support the high viral diversity observed in this study. Qi et al. (2023) reported that more than 800 RNA viruses from 39 families have been identified in over 300 agricultural insect species, including members of the Lepidoptera, Hemiptera, and Diptera. This suggests that the virome of *C. pudibunda* is consistent with broader patterns of viral richness and complexity in phytophagous insect taxa (Medd et al., 2018). Furthermore, electron microscopy analyses (SEM and TEM) revealed the presence of multiple occlusion body morphologies, supporting the coexistence of at least two distinct baculovirus genotypes: CapuNPV-T1 and CapuGV-T. Such co-infections are not uncommon among Lepidoptera and may result in genetic exchange, synergistic effects, or altered pathogenicity, influencing viral evolution and biocontrol potential

(Wennmann & Jehle, 2014; Ferrelli & Salvador, 2023). This finding aligns with observations in *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), where multiple nucleopolyhedrovirus (SeMNPV) genotypes co-occurred within the same host population (Georgievska et al., 2010).

Archaea, although less abundant, presented a distinct profile predominantly composed of thermophilic taxa such as *S. acidocaldarius* A, suggesting the possible resilience and adaptability of these extremophiles in insect hosts or microhabitats (Quehenberger et al., 2017). The presence of methanogenic archaea, including *Methanosarcina* and *Methanobacterium*, hints at potential interactions in the gut environment, possibly contributing to host digestion and carbon cycling (Chellapandi et al., 2018). This reflects a broader ecological significance beyond infection and pathogenicity, pointing toward symbiotic relationships.

The eukaryotic community was largely composed of host-associated metazoan sequences, especially those from *H. sapiens*, likely representing environmental or laboratory contamination. Nonetheless, the detection of fungal taxa such as *B. cinerea* and *Saccharomyces* spp. suggests diverse environmental interactions. These fungi, known for their roles in plant pathology and fermentation, may have originated from surrounding vegetation or substrate (Peay et al., 2016). Additionally, protist-related sequences affiliated with the phylum Apicomplexa may indicate potential parasitic interactions yet to be characterized in *C. pudibunda*.

These findings underscore the complexity of host-microbe interactions and highlight the importance of using high-throughput sequencing to unravel microbial ecology in forest pest populations.

Conclusion

Our study provides novel insights into the microbial ecology of *C. pudibunda* and establishes a foundational framework for future investigations of host-associated microbial communities in forest Lepidoptera. Metatranscriptomic analyses revealed a diverse microbiome and virome composed of bacterial, viral, archaeal, and eukaryotic microorganisms associated with outbreak populations of *C. pudibunda* in Türkiye. The detection of baculovirus- and cypovirus-related sequences, together with electron microscopy observations, supports the coexistence of multiple viral agents within the host population. These findings contribute to the understanding of microbial diversity and host–microbe interactions in *C. pudibunda* and provide baseline data for future ecological and biological control studies.

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