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Urban areas as overlooked reservoirs of insect diversity: first records of three *Sciophila* species (Diptera: Mycetophilidae) from Poland with DNA sequence data

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Abstract. Here, we present the first records of three *Sciophila* Meigen, 1818 species (*S. limbatella* Zetterstedt, 1852, *S. pomacea* Chandler, 2006, and *S. rufa* Meigen, 1830) from Poland, expanding the known distribution range of this poorly studied genus of fungus gnats (Mycetophilidae) in Europe. All specimens were found in urban environments in major Polish cities (Łódź and Kraków) during investigations of insect fauna inhabiting parasitic polypores on ornamental trees. Species were identified through morphological examination and confirmed using DNA barcoding. Molecular diversity and species assignment based on the COI mtDNA marker were assessed based on available public data of examined species. Occurrence data and available DNA barcodes were presented and compared with newly presented records. Members of *Sciophila* are associated with specific microhabitats and fungal hosts. Therefore, our findings underscore the complexity of urban ecosystems and the potential for these environments to support diverse and specialized insect fauna. Additionally, *Phellinus pomaceus* is identified as a host for *S. limbatella* for the first time. These results highlight the ecological value of overlooked urban microhabitats and contribute new insights into the biology of fungus gnats.

Keywords: fungus gnats, polypores, urban fauna, cities, DNA barcoding.

Introduction

Urbanization is one of the greatest threats to global biodiversity, constituting a pressing challenge for environmental conservation. Numerous studies highlighted its detrimental effects on ecosystems, including habitat fragmentation, elevated pollution levels, increased annual temperatures ("urban heat island" effect), and a range of other anthropogenic disturbances (Nielsen et al. 2014, Fenoglio et al. 2020). However, in contrast to the common perception of cities as ecological wastelands, growing evidence suggests that, despite intense anthropogenic pressures, urban areas can support surprisingly high levels of biodiversity. Cities not only accumulate species from surrounding ecosystems but can also harbor unique and even rare insect fauna (Sattler et al. 2011, Bauer et al. 2024).

The genus *Sciophila* is represented by about 60 species in the Palaearctic region, seven of which are known from Poland: *S. cliftoni* Edwards, 1925, *S. fuliginosa* Holmgren, 1883, *S. hirta* Meigen, 1818, *S. lutea* Macquart, 1826, *S. nigronitida* Landrock, 1925, *S. thoracica* Staeger, 1840, and *S. varia* (Winnertz 1864) (Bechev & Koç 2006, Mikołajczyk 2007). The biology of these species is poorly documented.

Larvae typically develop on the surface of lignicolous polypores, feeding on fungal spores. Still, they may also be found within the fruiting bodies of basidiomycete fungi, where they consume fungal tissue. Some species inhabit decaying wood, feeding on fungal hyphae that overgrow the substrate, or are possibly saprophagous (Hutson et al. 1980, Falk & Crossley 2005, Bechev & Koc 2006, Jakovlev 2012). This genus is also known for producing characteristic structures such as silky webs, mucilaginous tubes, and cocoons in which pupation occurs. The observation of such structures on parasitic polypores (*Phellinus pomaceus* and *Fomes fomentarius*) growing on *Prunus cerasifera* and *Fagus sylvatica* prompted us to detect these insects. These resulted not only in the identification of new species records but also in the determination of their fungal hosts.

Mycetophilidae are relatively understudied, partly due to their high diversity and the challenges of identification. Molecular techniques, particularly DNA barcoding of the mitochondrial COI gene (Hebert et al. 2003), have emerged as effective tools for confirming identifications and revealing cryptic species, especially in non-model arthropods. This approach is particularly valuable for uncovering so-called 'dark taxa'—understudied groups representing hidden biodiversity often missed by traditional taxonomy—highlighting the importance of integrative methods in species discovery and classification (Chimeno et al. 2022, Meier et al. 2025). Public data on BOLDSYSTEMS, currently with some 125 Holarctic BINs, of which only 50 are named, indicates that a major proportion of *Sciophila*'s species diversity remains unnamed.

The main aim of this paper is to present new records of *Sciophila* from Poland using integrative identification methods.

Material and methods

Material was collected in two major Polish cities: Łódź (51º46′N, 19º27′E; 655,000 inhabitants; area: 293 km²) and Kraków (50º03′N, 19º56′E; 804,000 inhabitants; area: 326 km²) (Fig. 1C) (Statistics Poland 2024).

In Łódź, fruiting bodies of *Phellinus pomaceus* (Fig. 1A) colonized by unidentified larvae were collected from cherry plum trees (*Prunus* *cerasifera*) growing in a residential green area between October and December 2022. Imagoes were later reared, as most species of fungus gnats can only be reliably identified in their adult form (Ševčík 2010, Dubiel & Mikołajczyk 2019). Collected fruiting bodies were placed in glass containers (Fig. 1B) lined with a 2 cm layer of moist, previously heat-sterilized coconut fibre and regularly sprayed with non-chlorinated water to maintain humidity. Rearing was conducted under controlled room-temperature conditions. Individuals of *Collembola* sp. were introduced to reduce mould growth on the decaying fungal material, as discussed later.

In Kraków, papery cocoons were collected in July 2021 from the underside of *Fomes fomentarius* fruiting bodies growing on a European beech (*Fagus sylvatica*) tree in Park Lotników Polskich (Aviator's Park) (Fig. 1D, E). Adult specimens later emerged. Another individual was also observed at this site, emerging from the underside of a sporocarp growing on Norway maple (*Acer platanoides*). When they emerged, they immediately flew away from the cocoon without touching the surface.

Figure 1. Localization and materials: (A) *Phellinus pomaceus* collected; (B) rearing container with collected *Phellinus pomaceus*; (C) location of Łódź and Kraków in Poland, (based on a map by Poznaniak, available at Wikimedia Commons, modified from the original, licensed under CC BY-SA 3.0.); (D) *Fomes fomentarius* with *Sciophila* cocoons found; (E) collected cocoons.

Collected adult specimens were preserved in 97% ethanol. Male genitalia were dissected and macerated in 10% potassium hydroxide (KOH) for 24 hours. Photographic documentation was carried out using a Leica M205 C stereomicroscope equipped with a Leica DMC5400 camera. Image processing, including focus stacking, was performed using Leica Application Suite X (LAS X) software.

Morphological identification to the species level was based on male genital characters, following the identification keys of Bej-Bienko (1964) and Hutson et al. (1980), and illustrations provided by Zaitzev (1982).

DNA was extracted from a single leg using the Chelex method (Casquet et al. 2012). A 650 bp fragment of the cytochrome c oxidase subunit I (COI) gene was amplified using primers LCO1490-JJ and HCO2198-JJ (Astrin & Stüben 2008). Details of the DNA amplification and sequencing protocols are provided in Dettner et al. (2024).

The resulting sequences were edited, primer-trimmed, and aligned using Geneious 11.1.5 (Kearse et al. 2012). Sequence identity was verified by BLAST (Altschul et al. 1990) comparisons with published sequences. All newly obtained COI sequences were deposited in GenBank (accession numbers PV634331–PV634336; Table S1). Additionally, the sequences were submitted to the Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert 2007) to obtain Barcode Index Numbers (BINs). BINs cluster genetically similar DNA sequences and serve as tentative species equivalents (Ratnasingham & Hebert 2013). New sequences were compared with 36 publicly available COI sequences of *Sciophila limbatella*, *S. pomacea*, and *S. rufa*, retrieved from the BOLD via studies by Kjærandsen (2022a), Roslin et al. (2022), Mantič et al. (2020), and other public data (Table S1). All sequences, including associated metadata and trace files, are available in the DS-SCILIM dataset (http://dx.doi.org/10.5883/DS-SCILIM).

Intra- and interspecific genetic distances were calculated using the Kimura 2-parameter model (K2P; Kimura 1980) via BOLD's analytical tools ("Distance Summary" and "Cluster Sequences"). A phenogram was constructed in MEGA X (Kumar et al. 2018) using the neighbor-joining method (Saitou & Nei 1987), based on K2P distances, with 1,000 bootstrap replicates.

Occurrence data for *S. limbatella*, *S. pomacea*, and *S. rufa* were retrieved from the Global Biodiversity Information Facility (GBIF.org (8 May 2025) GBIF Occurrence Download https://doi.org/10.15468/dl.2juubu, doi.org/10.15468/dl.8kts5j, doi.org/10.15468/dl.ge7kzz). The distribution map was created in QGIS 3.20.2 using base layers from Natural Earth (Free vector and raster map data @ naturalearthdata.com).

Results

Sciophila limbatella Zetterstedt, 1852
(Figs. 2A–C)

Sciophila limbatella Zetterstedt, 1852:4130
Sciophila limbatella – Hutson et al. 1980:51, fig. 204; Zaitzev 1982a:45, fig. 10.3

Material: Two males and three females of *S. limbatella* were reared from *Phellinus pomaceus* fruiting bodies collected at 15. X, 14.XI and 4.XII.2022 from roadside *Prunus cerasifera* trees at two locations in the city of Łódź (1. GPS: N 51.798064°, E 19.473730° and 2. GPS: N 51.792558°, E 19.469325°) (Fig. 2A, sites 1 and 2).

Comments: *S. limbatella* has a transpalaearctic distribution. This distinctly coloured species is characterized by a striking contrast between its black body and significantly lighter, yellowish legs. The wings are approximately 5 mm long, lack microtrichia, and the Sc2 vein terminates before the base of Rs (Hutson et al. 1980).

The larvae of this poorly known species are presumed to be sporophagous. They develop on the surface of tough, woody polypores under the protective cover of a silky web produced by the larvae themselves (Falk & Crossley 2005). We document for the first time *Phellinus pomaceus* as a host fungus for *S. limbatella*. Moreover, contrary to our observation of roadside trees, this fungus species is generally reported to prefer moist, shady, and damp habitats (Hutson et al. 1980, Falk & Crossley 2005, Bechev & Koç 2006).

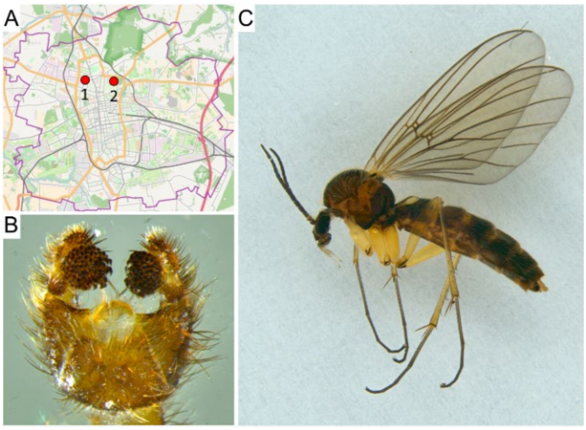

Figure 2. *Sciophila limbatella*: (A) map of Łódź city with location of the sites marked (map retrieved from © OpenStreetMap, licensed under the Open Database License [ODbL]); (B) male genitalia, dorsal view; (C) female habitus.

Sciophila pomacea Chandler, 2006
(Figs. 3A-C)

Sciophila pomacea Chandler, 2006:86 nom. nov.
= *Sciophila ochracea* Stephens in Walker 1856:41, junior primary homonym
Sciophila ochracea – Hutson et al. 1980:52, fig. 204; Zaitzev 1982a:54, fig. 12.7

Material: One male specimen of *S. pomacea* was reared from a *Phellinus pomaceus* fruiting body collected at 15.XI.2022 from a roadside *Prunus cerasifera* tree in Łódź (GPS: N 51.792558°, E 19.469325°) (Fig. 2A, site 1).

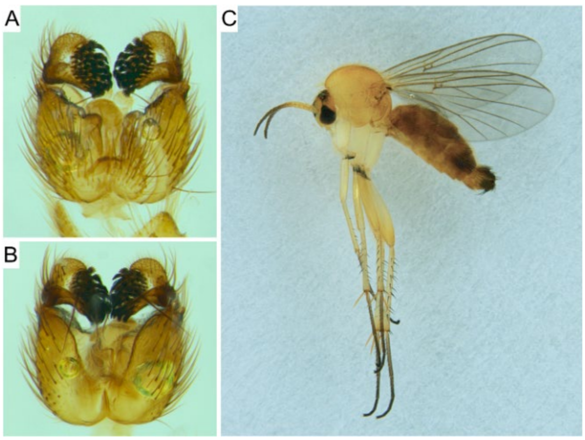

Figure 3. *Sciophila pomacea*: (A) male genitalia, dorsal view; (B) male genitalia, ventral view; (C) male habitus.

Comments: This small fungus gnat species has wings not exceeding 3.5-4.5 mm in length. The body and legs are almost uniformly ochraceous. Microtrichia are absent from the wing membrane (except for the extreme base), and the Sc2 vein terminates after the base of Rs. The anterior tibia lacks an antero-dorsal row of bristles (Hutson et al. 1980).

Sciophila pomacea has a transpalaearctic distribution and is closely associated with Polyporaceae fungi growing on Rosaceae and mainly recorded from gardens, old orchards, as well as natural woodlands and hedgerows. Larvae are most often found on fruiting bodies of fungi from the genera *Fomes* and *Phellinus*, but have also been observed on *Trametes versicolor*. They develop under a silky web (Falk & Crossley 2005).

Sciophila rufa Meigen, 1830
(Figs. 1D-E; 4A-C)

Sciophila rufa Meigen, 1830:295
Sciophila rufa – Hutson et al. 1980:51, fig. 206; Zaitzev 1982:60, fig. 14.2

Material: One male and one female of *S. rufa* were obtained from cocoons (1E) collected on 27.VII.2022 from the underside of *Fomes fomentarius* fruiting bodies (1C) growing on a *Fagus sylvatica* tree in Park Lotników Polskich (Aviator's Park), Kraków (GPS: N 50.0411°, E 19.5936°) (Fig. 4D). An additional specimen was collected from the same site on 19.IX.2023.

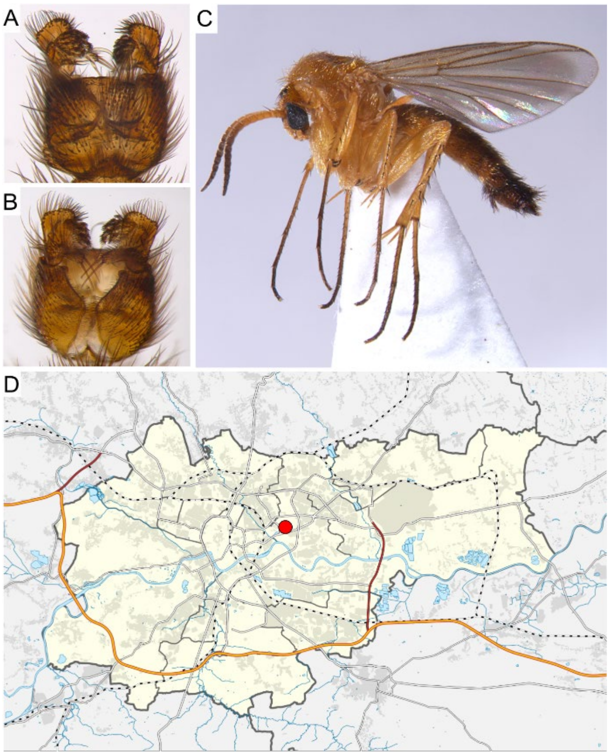

Figure 4. *Sciophila rufa*: (A) male genitalia from dorsal side; (B) male genitalia from ventral side: (C) male habitus; (D) map of Kraków with location of the site marked (based on a map by NordNordWest, available at Wikimedia Commons and modified from the original, licensed under CC BY-SA 3.0.).

Comments: *S. rufa* is widely distributed across the transpalaearctic region. It is a large species, with wings measuring 5-6.5 mm in length. The body is intensely ochraceous. Microtrichia are absent on the wing membrane, except at the extreme base, and the Sc2 vein terminates after the base of Rs. Additionally, macrotrichia are present at the base of the antennae, and an antero-dorsal row of bristles is present on the anterior tibia (Hutson et al. 1980).

Larvae develop primarily on *Fomes fomentarius* fruiting bodies but have also been observed on *Phellinus igniarius*. They live beneath a web and pupate in paper-like cocoons, which are attached either directly to the fungus or to nearby tree bark (Hutson et al. 1980, Falk & Crossley 2005). In Kraków, larvae develop on the same tree with another rare fungus gnat, *Keroplatus tipuloides* (Keroplatidae) (Mielczarek & Szwałko 2021).

We successfully sequenced five specimens of *S. limbatella* and one specimen of *S. pomacea*, while the sequencing of *S. rufa* (a single specimen) failed. The *S. limbatella* sequences were assigned to BIN: ACW1790 (mean p-distance: 0.41%), and the *S. pomacea* sequence to BIN: ACU9134 (mean p-distance: 0.57%). In the BOLD database, these species are assigned to the same BINs, and no additional BINs are currently known for them. In contrast, *S. rufa* shows higher genetic diversity, with two BINs reported: a single individual from the Czech Republic assigned to BIN: AEF9859, and several specimens from Norway and Finland assigned to BIN: ACM2865 (mean p-distance: 0.31%). The genetic distance between these two BINs is 3.07%.

The Neighbor-Joining tree (Supplementary Fig. S1) clearly demonstrates that the newly sequenced individuals are not genetically distinct from one another or from existing sequences in the BOLD database. The geographic distribution of available sequences is notably biased, with a strong overrepresentation from Scandinavia (see Kjærandsen 2022a). Of the 42 sequences analyzed, only nine originate from outside this region—specifically, six from Poland, and one each from the Czech Republic, Russia, and Bulgaria.

Discussion

Urban context of the study

Despite growing research on urban fauna, many insect groups remain poorly studied in urban environments, including the Mycetophilidae. This dipteran family includes at least 924 species recorded in Europe, making it the third largest family of nematoceran flies (Søli & Kjærandsen 2008). Despite their abundance and ecological importance, the biology of many species remains poorly understood. In fact, the fungal hosts of more than half of all fungus gnat species are still unknown (Jakovlev 2012). Thus, our study, reporting new records of Mycetophilidae species from Poland and their association with specific polypore hosts, contributes not only to a better understanding of the distribution of these flies but also to insight into their biology.

The *Sciophila* species reported here are associated with specific microhabitats and fungal hosts (Ševčík 2010, Falk & Crossley 2005). Consequently, their presence in large cities highlights the complexity of urban ecosystems and their potential to support diverse and specialized insect fauna. This is likely due to the heterogeneity of habitats and microhabitats in urban landscapes, including remnant green spaces, unmanaged plots, and small habitat patches with preserved large and old trees. Beyond the insect-fungus interaction, this study also highlights the ecological duality of parasitic polypores. Although mainly known for their negative impact on urban trees, posing a serious problem for the maintenance of ornamental trees and shrubs in city green spaces (Pegler & Waterston 1968, Gáper et al. 2014), these fungi can also contribute positively to local biodiversity. Their fruiting bodies provide a unique and well-defined microhabitat that can be colonised by numerous invertebrate species (Gdula et al. 2021). As underscored by Kjærandsen (2022b), in the absence of natural habitats with similar qualities, creating fauna depots with large logs of dead wood in park and semi-park landscapes would further help the survival of particular fungus gnats dependent on lignicolous fungi or decaying wood.

The finding of *S. limbatella* in a highly urbanised environment is particularly noteworthy. This species is typically associated with pine and mixed forests (Hutson et al. 1980, Falk & Crossley 2005, Bechev & Koç 2006), so its presence in a remnant urban area may suggest greater ecological adaptability than previously assumed.

In the case of *S. pomacea*, its presence in urban environments may be linked to the frequent planting of ornamental fruit trees of the genus *Prunus*, including cherry plums (*P. cerasifera*), examined in our study. Due to their aesthetic value, such trees are often planted along roadsides, in parks, and in city squares, and generally do not receive the same level of intensive care as those in cultivated orchards, where numerous plant protection treatments, including the elimination of parasitic polypores, are routinely undertaken. Consequently, it can be hypothesized that the urban environment may paradoxically support higher densities of this species than in agricultural or natural forest areas.

In contrast, *S. rufa* is one of the most widespread representatives of the genus and appears to exhibit broad ecological tolerance (Falk & Crossley 2005). This species is frequently encountered in a variety of habitat types, including both natural and anthropogenic environments.

Molecular data

Molecular identification using COI DNA barcodes confirmed the identification of both successfully sequenced species. Low intraspecific distances and assignment to single BINs for *S. limbatella* and *S. pomacea*, along with comparison to public sequences in BOLD, support accurate species identification (Supplementary Fig. S1). Therefore, it is assumed that the morphological identification of *S. rufa* is also precise, despite the absence of molecular barcode data for this species. However, the situation appears more complex: BOLD currently lists two BINs assigned to *S. rufa*, with a p-distance of 3.07%. One BIN (BOLD: AEF9859) is represented by a single specimen from the Czech Republic, while the other (BOLD: ACM2865) includes nine specimens from the Nordic countries. This may reflect the presence of two cryptic species or a species complex at a geographical level. Further sequencing data is needed to test this hypothesis.

The distribution of occurrence and sequence data reveals a strong bias toward records from Scandinavia (Fig. 5). This pattern likely results from targeted sampling and barcoding campaigns conducted by national initiatives, such as NorBOL (Norway, www.norbol.org), FINBOL (Finland, https://en.finbol.org/), and the Swedish Insect Inventory Program (SIIP, https://www.stationlinne.se). These initiatives provide invaluable molecular and occurrence data across numerous taxonomic groups, including so-called "Dark Taxa." Large-scale Malaise trap sampling efforts are significantly advancing our understanding of poorly studied taxa (Roslin et al. 2022, Buchner et al. 2025). For example, Mycetophilidae have been proposed as a flagship group among Dark Taxa, as demonstrated in a case study from Singapore (Meier et al. 2025), where barcoding efforts revealed 120 species, 115 of which appear to be new to science.

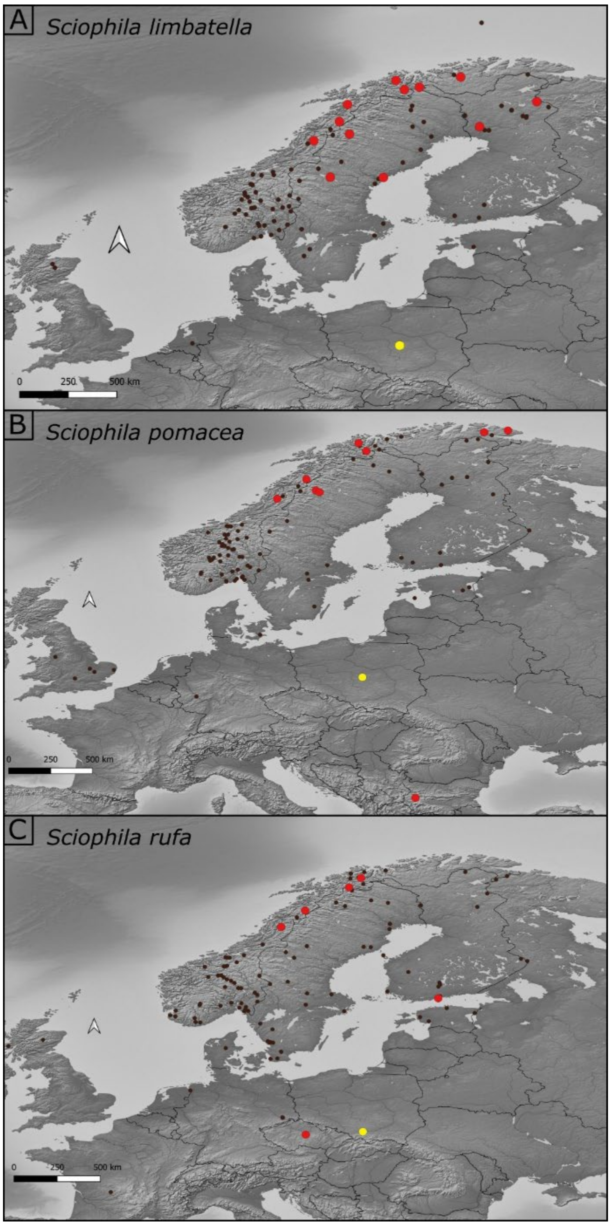

Figure 5. Distribution of a) *Sciophila limbatella*, b) *Sciophila pomacea*, and c) *Sciophila rufa* based on GBIF occurrence records and public molecular data. Red circles indicate public molecular data from BOLD (see details in Table S1), yellow circles represent new data generated in this study, and black dots show previously known species records from GBIF (GBIF.org).

Rearing method

In addition, we propose a simple improvement to the larval rearing method: introducing springtails (*Collembola* spp.) to reduce fungal overgrowth in rearing containers. Although adequate ventilation was provided by the low height of the containers and the use of mesh covers, mold growth remained a recurring problem, resulting in significant larval mortality. However, this issue was greatly mitigated by the addition of springtails, which preferentially feed on mold rather than the body of a ligneous polypore, slowing mold development and increasing the larva's chances of survival until adulthood.

Conclusions

In light of our observations, it seems relevant to ask about the actual state of knowledge of the Mycetophilidae fauna in Poland. As three new species were found during the time- and space-limited observations, it can be assumed that many species remain unrecorded. Every addition to the molecular reference library at the country scale is valuable for further identification or wider biogeographic context studies. Our study also shows that the urban environment, despite high anthropogenic pressure, can be an essential refugium even for highly specialized insect groups, and that the preservation of even small enclaves of greenery and retaining old trees infected with parasitic fungi can be of real importance for the protection of less obvious elements of the fauna. Therefore, we suggest that urban areas should not be considered solely as threats to biodiversity.

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