First record of *Heterogaster cathariae* (Geoffroy, 1785) (Hemiptera, Heteroptera, Heterogastridae) in Luxembourg and first global DNA barcodes

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Abstract. The first record of the rare true bug species *Heterogaster cathariae* (Geoffroy, 1785) (Heteroptera, Heterogastridae) in Luxembourg was made in October 2022 using the community science application iNaturalist. The subsequent collection of four specimens – deposited at the National Museum of Natural History, Luxembourg – led to the first global production of genetic reference material (i.e. DNA barcodes) for the species.

Key words. First record, *Heterogaster* Schilling, 1829, Luxembourg, DNA barcode, community science.

1. Introduction

Scattered records of true bugs (Heteroptera) in Luxembourg were first comprehensively compiled by Reichling & Gerend (1994), who catalogued 525 species for the country (490 terrestrial and 35 aquatic). Later, these data and additional findings resulted in the first atlas of terrestrial bugs of Luxembourg (Reichling 2001), including distribution and ecological information. Between 2002 and 2006, a countrywide survey focusing on cicadas (Auchenorrhyncha) was performed (Niedringhaus et al. 2010a, b), with many true bugs collected as by-catch. In their provisional analysis of by-catch data and older datasets, Niedringhaus & Stöckmann (unpublished) identified a total of 535 species for Luxembourg, thereby revising some older records but likewise adding new discoveries. Since then, several other first records of true bug species were reported for Luxembourg (e.g. Schneider 2010, Schneider & Christian 2013, Vitali 2019, Schneider 2020, Siebenaler et al. 2021).

While visiting a vegetable garden plot in the Cents quarter of Luxembourg City on the 22nd of October 2022, the first author documented a number of Heteroptera species on catmint *Nepeta cataria* L. (Lamiaceae) by using his mobile phone camera and the application of the community science platform iNaturalist (iNaturalist.lu 2022a). Among these observations, adults and nymphs of a species unfamiliar to the first author, they were later identified by the iNaturalist community as *Heterogaster cathariae* (Geoffroy, 1785).

The genus *Heterogaster* Schilling, 1829 belongs to the family Heterogastridae, which was raised to family rank by Henry (1997). It comprises two New World and nine Old World species of seed bugs (Slater 1964, Slater & O'Donnell 1997). Until now, it is only represented by a single species in Luxembourg, namely the nettle ground bug *Heterogaster urticae* (Fabricius, 1775), mentioned as being present in the south of Luxembourg by Cungs (1991) and supported by data available at the Global Biodiversity Information Facility (GBIF.org, 2022a). It is a relatively common species in Europe and known to utilise the stinging nettle, *Urtica dioica* L. (Urticaceae), as its host plant (Péricart 1998, Ødegaard & Anders 2007, Wheeler 2013). In Luxembourg *H. urticae* is widespread in the Gutland region and rare in the Oesling region according to Reichling (2001). Another species of the genus, *Heterogaster artemisiae* Schilling, 1829, was reported from the neighboring countries of Belgium, France and Germany, but no observation has yet been made from Luxembourg. *H. artemisiae* is known to prefer *Thymus* L. (Lamiaceae) as a host plant. On further inspection, it became clear that records of *H. cathariae* from Luxembourg were not yet available in literature, although Péricart (1998) referred to a specimen collected in Torgny in May 1950, in « le Luxembourg belge » in Belgium, which might have led to some confusion in the past. In general *H. cathariae* is considered a rare species, primarily reported to occur on *N. cataria* and rarely on species of the genera *Mentha* L., *Melissa* L. and *Lycopus* L. (Malenovský et al. 2011, Dioli 1997). Only very few records of the species are currently publicly available (GBIF.org 2022b).

2. Material and methods

2.1. Sampling site

The initial occurrence was recorded on the 22nd of October 2022 (commune of Luxembourg; with an accuracy of ca. 5m at the WGS84 coordinates 49.6186N, 6.1587E) via an observation of adult (iNaturalist.lu 2022b) and nymph (iNaturalist.lu 2022c) individuals using the iNaturalist app. The multiple individuals were spotted on planted catnip (*N. cataria*) (Fig. 1). The site was revisited on the 24th of October 2022, to collect specimens for further examination and deposition in the collections of the National Museum of Natural History, Luxembourg (MNHNL). Four specimens were collected at the same location as the

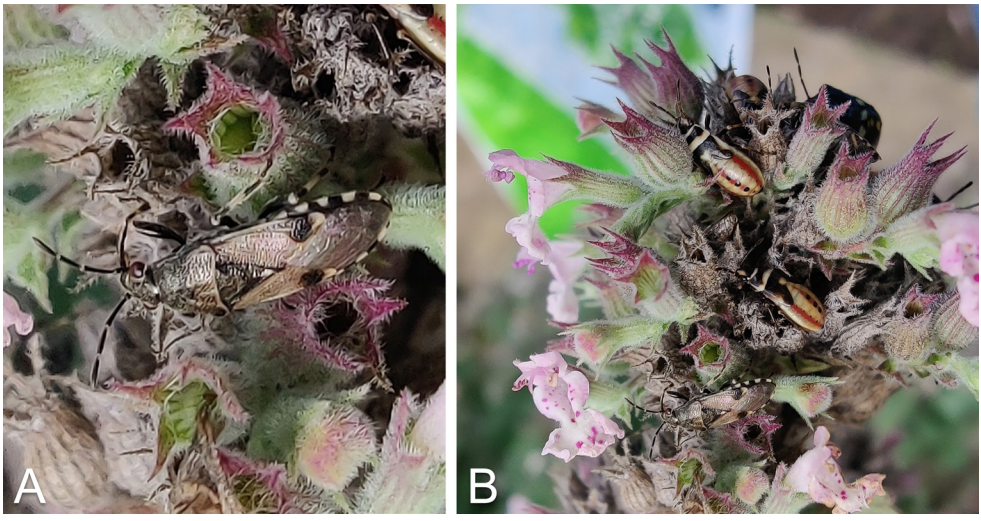


Fig. 1. Initial images uploaded to the community science platform iNaturalist on October 22nd 2022 by the first author showing an adult (A) as well as nymph (B) individuals on a catnip plant (*Nepeta cataria*). Based on these images, the initial identification of *Heterogaster cathariae* was made (images Paul Braun CC0).

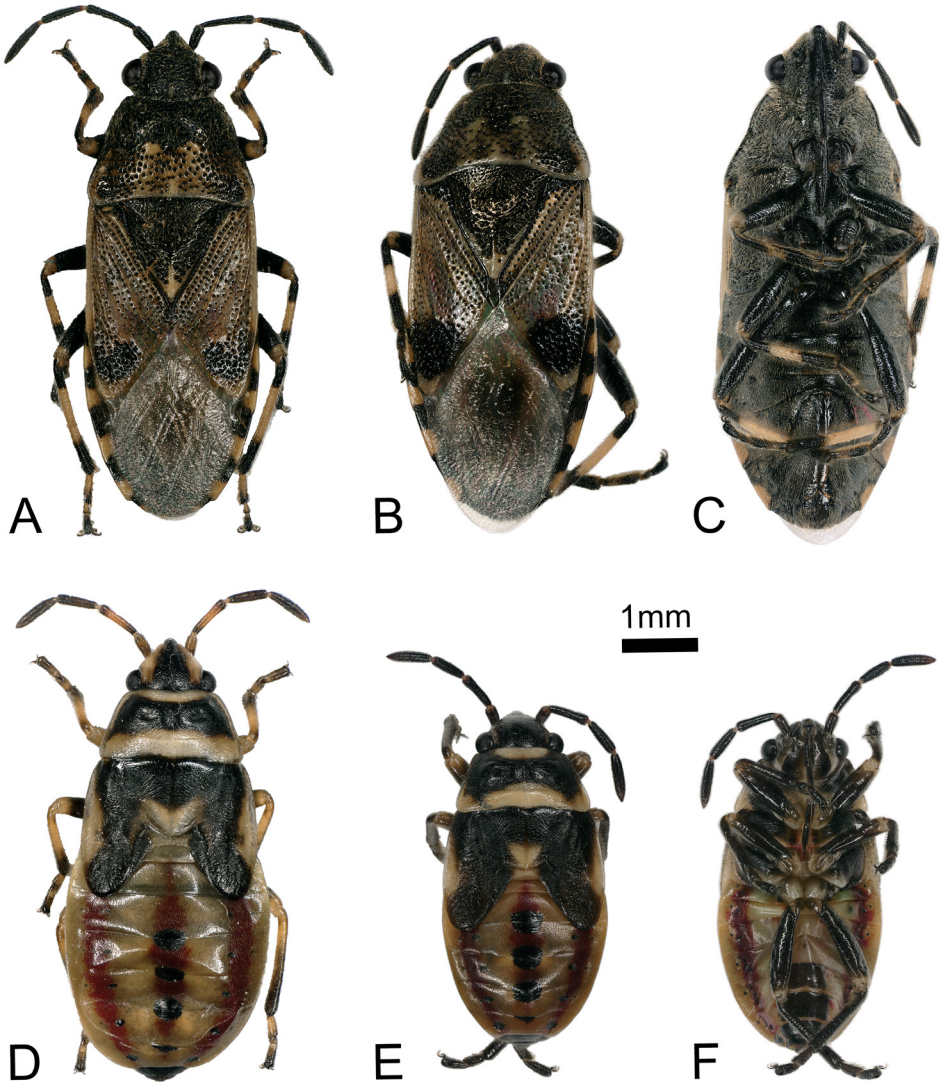


Fig. 2. *Heterogaster cathariae* specimens collected on 22nd of October 2022 in Luxembourg deposited at the MNHNL invertebrate collection: (A) dried adult: MNHNL138189, (B&C) adult female conserved in ethanol: MNHNL138822, (D) dried nymph: MNHNL138190, (E&F) nymph in ethanol: MNHNL139839 (image MNHNL CC-BY).

initial occurrence. The collected specimens comprised one adult and one nymph preserved in ethanol as well as one adult and one nymph temporarily stored in a household freezer. The specimens were subsequently integrated into the collections of the MNHNL with the following collection numbers: MNHNL139839 for the nymph and MNHNL138822 for the female (both preserved in ethanol) and MNHNL138189 for the adult and MNHNL138190 for the nymph (both preserved as dried material). The latter two were prepared by Francesco Vitali (MNHNL). Images were produced using a VHX-6000 (Keyence) digital microscope using focus stacking and image stitching (Fig. 2). On the 8th of July 2023 the site was visited anew to confirm the presence of the species (iNaturalist.lu 2023).

2.2. Specimen identification

Roland Lupoli, who initially provided the taxonomic annotation of *H. cathariae* on iNaturalist, was sent the digital microscope images of the collected specimens for confirmation. Among the Heteroptera, the Lygaeoidea have five-veined membranes, and species in the family Heterogastridae possess an alternating black and white bicolor dorsal connexivum and a slightly longitudinally keeled scutellum at the top. The genus *Heterogaster* is characterized by the presence of a small distal spine on the profemurs (absent in the genus *Platyplax* Fieber, 1860) and sinuated lateral edges of the pronotum (straight in *Platyplax*). The tibiae of *H. cathariae* have two black rings whereas three are observed in *H. urticae* and *Heterogaster affinis* Herrich-Schaeffer, 1835. The size of *H. cathariae* is between 6.4 and 7.5 mm while it is less than 5.5 mm in *H. artemisiae*.

2.3. DNA barcode generation

Until this study, no DNA barcode of *H. cathariae* was available. Hence, the aim was to produce the global first DNA barcodes for the species. DNA was isolated by mechanically breaking a front and a middle leg of the specimens MNHNL138822 and MNHNL139839, and placing them into a TissueLyser II (Qiagen, Hilden, Germany). DNA extraction was performed using the Sherlock AX kit (A&A Biotechnology, Gdańsk, Poland), following the manufacturer's instructions. The only two changes to the protocol included an adaptation of the overnight incubation temperature to 50 °C and DNA elution with 20 µL of low TE buffer. DNA extracts were quantified using a Drop-Sense 16 spectrophotometer (Trinean, Gentbrugge, Belgium). The primer pair LCO1490-JJ (5'-CHAC WAAYCATAAAGATATYGG-3') and HCO2 198-JJ (5'-AWACTTCVGGRTGVCCA AARAA-TCA-3') of Astrin & Stüben (2008) was used to amplify the Folmer-fragment of the cytochrome *c* oxidase subunit I (COI) gene by polymerase chain reaction (PCR). Each PCR contained 12.5 µL Promega Gotaq, 0.5 µM of each primer, 20-35 ng DNA and nuclease free water up to a final volume of 25 µL. PCRs started with 2 min initial denaturation at 92 °C, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 47.5 °C for 15 s and extension at 72 °C for 30 s. The final incubation time was set at 72 °C for 1 min. PCRs were run in a Mastercycler nexus (Eppendorf, Hamburg, Germany). PCR products were purified following the protocol of the Agencourt AMPure XP PCR Purification kit (Beckman Coulter, U.S.A.). DNA sequencing was performed using the Promega ProDye™ Terminator sequencing system. Each PCR contained 4 µL purified DNA, 0.5 µL ProDye Master Mix, 2 µL sequencing buffer, 0.25 µL sequencing primer (10µM) and 3.25 µL nuclease free water. PCRs started with 1 min initial denaturation at 96 °C, followed by 25 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s and extension at 60 °C for 4 min. PCRs were run in a Mastercycler nexus (Eppendorf, Hamburg, Germany). Sequencing PCR products were purified following the Agencourt CleanSEQ Dye-Terminator Removal protocol (Beckman Coulter, U.S.A.). PCR products were sequenced using an ABI 3730XL capillary sequencer (Applied Biosystems). Sequences were quality-checked, edited and trimmed in Geneious R6 version 6.1.8 (Kearse et al. 2012).

2.4. Digitisation of *Heterogaster* specimens in the MNHNL collection

To gain further information on the occurrence of the genus *Heterogaster* in Luxembourg, thorough examination of the pinned Heteroptera specimens in the collection of the MNHNL was conducted. All of the identified specimens were carefully imaged, including their labels, using a Canon R7 digital camera (Fig. 3). The metadata associated with these specimens were either added to the existing records in the collection management system of the MNHNL (Recorder-Lux database) or new entries were created as necessary. In addition, a subset of exemplary specimens was subjected to detailed imaging using a Canon R7 camera (Fig. 4).

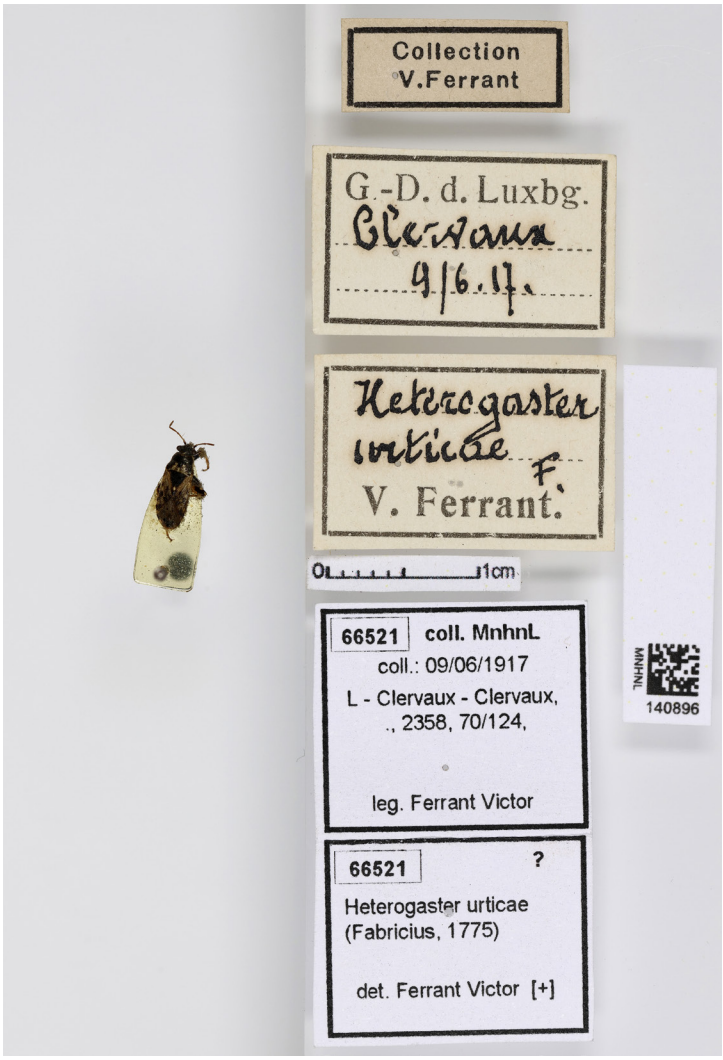


Fig. 3. *Heterogaster urticae* specimen: MNHNL140896 collected and determined by Victor Ferrant on the 9th of June 1917 in Clervaux, deposited in the MNHNL collection (image MNHNL CC-BY).

3. Results

The *H. cathariae* specimens were found on a catnip plant together with four other true bug species, i.e. *Dolycoris baccarum* (Linnaeus, 1758), *Nezara viridula* (Linnaeus, 1758), *Pyrrhocoris apterus* (Linnaeus, 1758) and *Nezara viridula* (Linnaeus, 1758). For two of the collected specimens of *H. cathariae*, an adult female (MNHNL138822) and a nymph (MNHNL13983), the first global barcodes for the species were generated and uploaded to the Barcode of Life Data System (BOLD) repository under the BOLD-Process ID: MNHNL173-22 and MNHNL174-22. The COI barcodes of both specimens were found to be identical. When compared to all currently available DNA barcode entries in the BOLD database, the genetic similarity values were below 90% for several species. These include an unidentified Hemiptera from Gabon (87.41%, GMGGB1009-17), an unidentified Hemiptera from Australia (87.31%, HEQT938-11), *H. urticae* from Germany (86.51%, GMGMM1363-14), and *Heterogaster behrensii* (Uhler, 1876) from the United States (86.09%, SICOD1017-19).

A total of 12 *H. urticae* specimens were identified within the MNHNL collections.

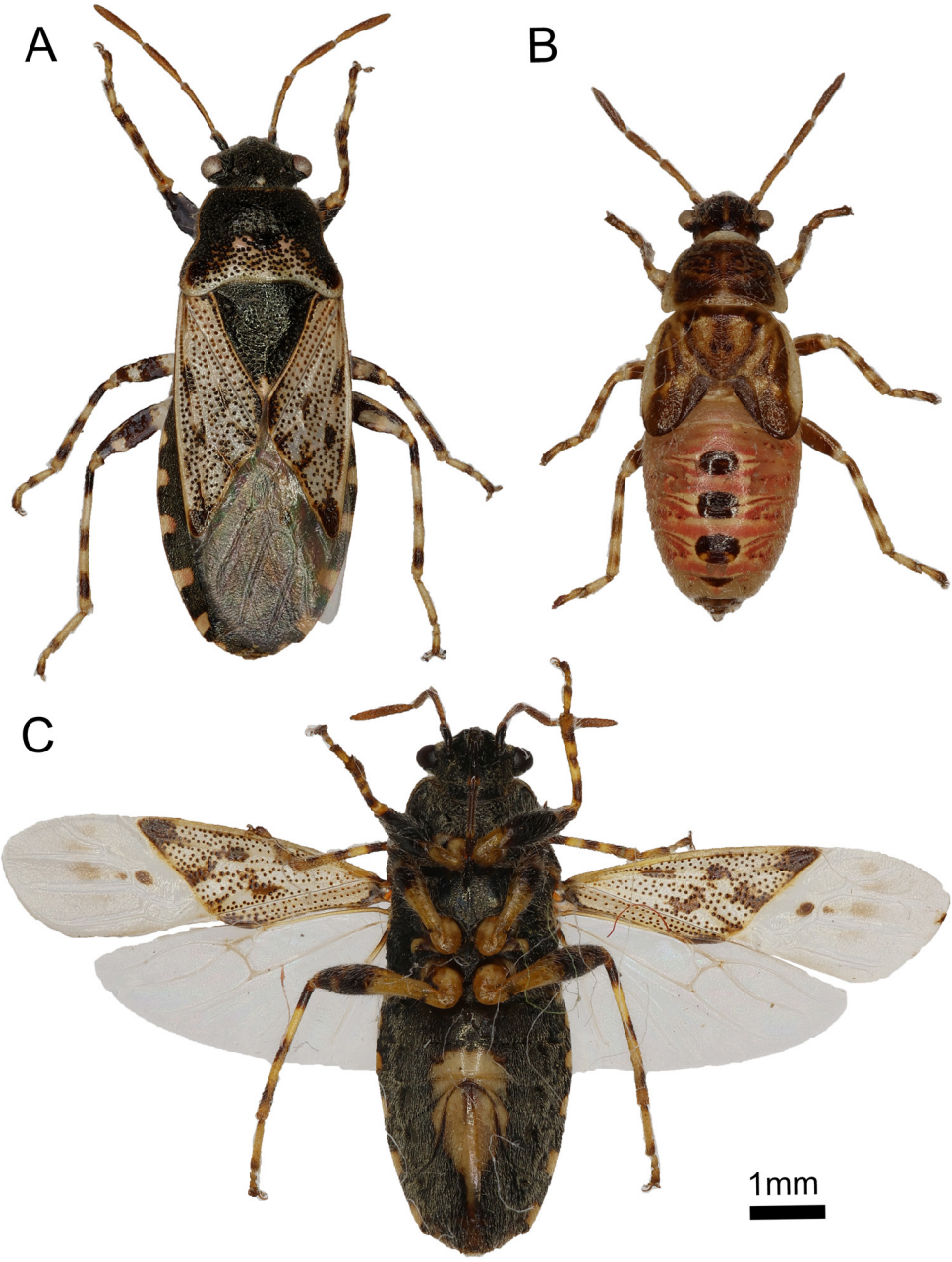


Fig. 4. *Heterogaster urticae* dried specimens from the MNHNL collection: (A) adult male: MNHNL140889, (B) nymph: MNHNL140897, (C) adult female: MNHNL140899 (image MNHNL CC-BY).

These specimens were given the MNHNL registration numbers MNHNL140888 to MNHNL140899 (Table 1). The oldest specimen in the collection was collected by Victor Ferrant on June 9th, 1917, in Clervaux (Fig.3), while the most recent specimen was collected by Léopold Reichling on October 11th, 1995, in Mondercange. All data and

images of the specimens are now accessible through the mdata.mnhn.lu and GBIF.org portals.

4. Discussion

H. cathariae was recorded for the first time in Luxembourg, representing distribution records at the northernmost extent of its known range. The occurrences were documented in a vegetable garden, specifically on a catnip plant that had been planted several years ago. This suggests that the species may have a synanthropic association, being found in close proximity to human-influenced habitat. While the host plant *N. catari* is considered regionally extinct in the wild (Colling 2005), it is commonly used as an ornamental garden plant, although multiple hybrids (e.g. *Nepeta* × *faassenii*) are sold (Stearn 1959). This highlights the potential of ornamental plants serving as hosts for rare insect species. The subsequent re-detection of *H. cathariae* in 2023 suggests

Table 1. Overview of *Heterogaster urticae* and *Heterogaster cathariae* specimens of the MNHNL collections.

Species	Catalogue nr.	Count	Preparation	Collection date	Collector	Collection location
H. urticae	MNHNL140896	1 adult	pinned	1917-06-09	V. Ferrant	Clervaux
H. urticae	MNHNL140898	1 adult	pinned	n/a	V. Ferrant	Walferdange
H. urticae	MNHNL140894	1 adult	pinned	1978-02-26	L. Reichling	Jardin Reichling, Luxembourg
H. urticae	MNHNL140892	2 adults ♀♂	pinned	1979-12-07	L. Reichling	Biisserweg, Luxembourg
H. urticae	MNHNL140890	1 adult	pinned	1982-10-09	N. Schneider	Bonnevoie, Luxembourg
H. urticae	MNHNL140899	1 adult ♀	pinned	1984-01-25	L. Reichling	Brill, Schifflange
H. urticae	MNHNL140897	1 nymph	pinned	1986-09-04	L. Reichling	SW Kayler Plateau, Esch-sur-Alzette
H. urticae	MNHNL140893	3 adults	pinned	1987-09-17	N. Schneider	Roeser, abri vitré
H. urticae	MNHNL140888	1 adult ♀	pinned	1991-08-17	N. Schneider	Altwies carrière, Mondorf-les-Bains
H. urticae	MNHNL140895	1 adult ♂	pinned	1992-06-15	L. Reichling	S. de Schifflange
H. urticae	MNHNL140891	1 nymph	pinned	1995-08-10	L. Reichling	NW Born
H. urticae	MNHNL140889	1 adult ♂	pinned	1995-08-11	L. Reichling	Jardin Melchior, Mondercange
H. cathariae	MNHNL138822	1 adult ♀	ethanol	2022-10-24	P. Braun	Cents, Luxembourg
H. cathariae	MNHNL139839	1 nymph	ethanol	2022-10-24	P. Braun	Cents, Luxembourg
H. cathariae	MNHNL138189	1 adult	pinned	2022-10-24	P. Braun	Cents, Luxembourg
H. cathariae	MNHNL138190	1 nymph	pinned	2022-10-24	P. Braun	Cents, Luxembourg

that the species has become established at the site in question.

The iNaturalist platform fostered international collaboration of citizen scientists and taxonomic experts, and even led to the first global production of genetic reference material (i.e. DNA barcodes) for a rare species, easing future DNA-based discoveries in semi-automated assessment programs additional to classic morphological reportings.

The related species *H. artemisiae* may also be found in Luxembourg in the near future. This species is known to visit *Artemisia* L. and *Thymus* L. which are common in gardens and the wild (Esenbekova et al. 2017). In fact, an occurrence of *H. artemisiae* has already been reported by Raoul Gerend from just 1 km south of the Luxembourgish-French border on the Stromberg mountain (France) adjacent to the Mosel River (GBIF.org 2023).

The finding presented in the present study highlights the potential of community science tools like iNaturalist in detecting new species occurrences. From the initial photographing of *H. cathariae* to the collection of specimens and subsequent taxonomic and DNA identification, only a short amount of time elapsed. This was made possible by the first author, who was both a naturalist and an employee of the MNHNL, recognizing the significance of the observation and initiating further analyses. The ability to revisit the site and collect specimens ensured scientific evidence of the occurrence. It raises the question of how many of these first records go unnoticed by the wider scientific community.

Overall, this discovery demonstrates the valuable contribution of community science platforms and the need for timely engagement by local or national naturalist and scientific communities in validating and documenting these first records.

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