



Annual Report 2025

Biological control of European frogbit, *Hydrocharis morsus-ranae*

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Cover photo: Robin Champion, a student from the Lycée of Porrentruy, collecting *Hydrocharis morsus-ranae* in Gals, Switzerland.

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Summary

1. European frogbit, *Hydrocharis morsus-ranae* L. (Hydrocharitaceae) is a free-floating aquatic macrophyte native to Europe which has become invasive in North America (NA). *Hydrocharis morsus-ranae* was introduced into NA as an ornamental in the early 1930s. It is most abundant in eastern NA where it is currently not listed as a federal noxious weed in the US, but is well established in northern New York and southeastern Michigan. It is considered a Class B state noxious weed in Vermont and an invasive aquatic plant in Maine. In Canada it is regulated as a prohibited species in Ontario where it is most prolific. This report summarizes the work conducted at CABI developing a biological control programme for North America in 2025.
2. In 2025, we were able to visit 40 sites in seven different countries. We found *Hydrellia* flies at ten sites relatively widespread between Switzerland, United Kingdom, and Sweden, the weevil *Bagous puncticollis* at five sites in Slovenia, Hungary, Slovakia and Sweden, and the smut *Tracya hydrocharidis* at four sites in United Kingdom and Italy. We collected genetic material from *H. morsus-ranae* at 33 sites and demography data and plant chemistry samples at four sites.
3. In 2025, host-range testing started with larval development of *H. albifrons* under no-choice conditions. We were able to test eight species with some development recorded on several *Potamogeton* species, but none on *Elodea densa*, *A. plantago-aquatica* and *S. latifolia*.
4. Larval development of the weevil *Bagous puncticollis* has never been observed before. In 2025, we were able to obtain full development from egg to adult by offering turions of *Hydrocharis morsus-ranae* to newly emerged larvae. This is the first time *H. morsus-ranae* has been confirmed as host for *B. puncticollis*.
5. In 2026, we plan to test additional non-target plants under no-choice conditions and to conduct dual-choice tests for *H. albifrons*, with a particular focus on species where development was recorded in 2025. Investigations into the biology and life cycle of *B. puncticollis* are ongoing. We aim to establish a stable rearing colony of *B. puncticollis* and begin preliminary host range testing. We plan to conduct field surveys to locate more wild populations of *B. puncticollis*, with the aim of establishing a larger and more robust rearing colony using field-collected individuals.

1. Introduction

Hydrocharis morsus-ranae (European frogbit) is a free-floating aquatic macrophyte with heart-shaped leaves and small white flowers with three petals (Plate 1a) (Catling *et al.*, 2003). It can be found in slow moving waters, typically in bays, ponds, open marshes and ditches, and along protected edges of lakes and rivers (Jacono, 2009). It is native through much of Europe and parts of temperate Asia as well as North Africa (Cook and Löönd, 1982). In its native range *H. morsus-ranae* has declined or has been extirpated throughout parts of its European range and is of conservation concern in several areas (Preston and Croft, 1997). *Hydrocharis morsus-ranae* was introduced into North America as an ornamental in the early 1930s. The initial introduction into Ottawa is thought to originate from the Zurich Botanical Gardens, Switzerland (Dore, 1968; White *et al.*, 1993). From late summer through autumn, winter buds (turions) are formed on the stolons and it is estimated that a single plant can produce approximately 100 to 150 turions which favour its spread (Plate 1b) (Nault and Mikulyuk, 2009; Scribailo and Posluszny, 1984; Dore, 1968). Now it is most abundant in eastern North America, with mats completely covering waterbodies (Plate 2). It is currently not listed as a federal noxious weed, but it is well established in northern New York and southeastern Michigan, where it is considered a Class B state noxious weed in Vermont and an invasive aquatic plant in Maine (NRCS 2023). In Canada it is present in southeastern Ontario and western Quebec where it is one of the five invasive plants that cause major impacts on the natural ecosystem (Catling *et al.*, 2003). It is not regulated as a noxious weed in any Canadian province. It has also been reported on the west coast of North America, where it is established in Washington, and is an A list noxious weed in California (NRCS 2023).

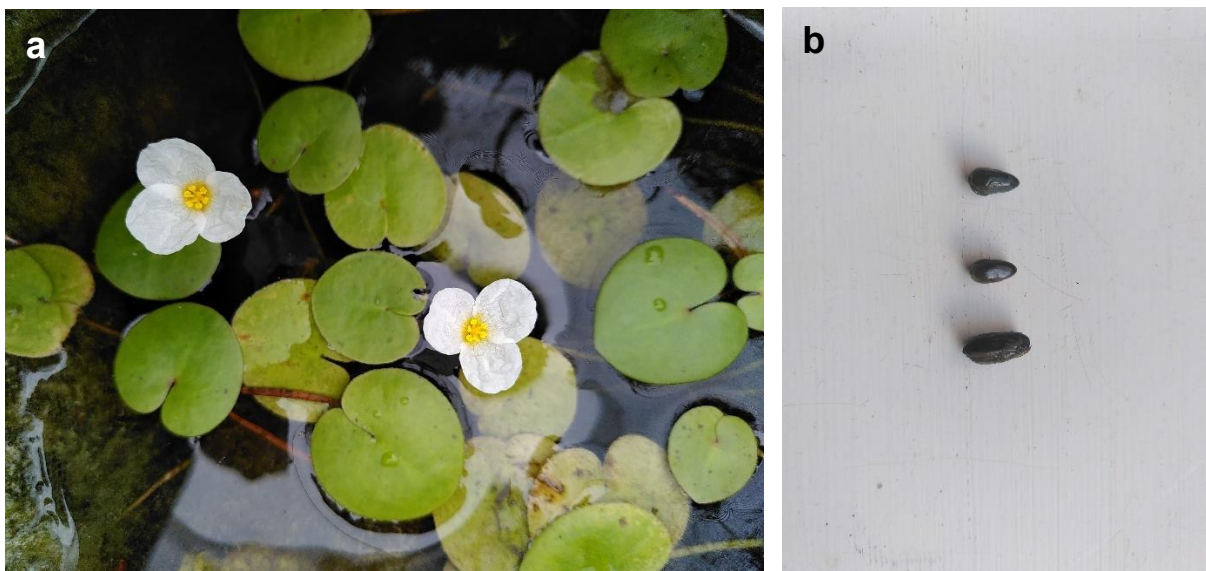


Plate 1. Flowering *Hydrocharis morsus-ranae* (a) and overwintering turions (b).

It is continuing to expand its range, becoming more abundant and considered invasive with socio-economic and environmental impacts (Catling *et al.*, 2003). The negative impacts are typical for floating macrophytes and include displacing native plant

species, reducing biodiversity, decreasing water quality and flow, clogging irrigation pumps, impeding recreational activities, and diminishing aesthetic value. As with many macrophytes, once established, it can be extremely difficult to control. Any mechanical method that fragments plants such as harvesting or hand pulling are likely to favour the growth and spread of this species. There are no biological control agents already developed for *H. morsus-ranae*, but several herbivores have been recorded in the native range with two insects, *Bagous puncticollis* Boheman (Coleoptera, Curculionidae) and *Hydrellia albifrons* Fallén (Diptera, Ephydriidae), the pathogen *Tracya hydrocharidis* Lagerh (Basidiomycota, Doassansiaceae) are the most promising (Table S1). Thus, the biological control programme was initiated in 2024 with support from the US Army Corps of Engineers to investigate the potential for developing biological control agents against *H. morsus-ranae*. During surveys in the native range, we also sampled *Stratiotes aloides*, another Hydrocharitaceae species that is starting to get invasive in North America and grows in the same habitat. Although these two plant species seem to share partly the same herbivores according to the literature (Table S1), our first results do not confirm this and we expect to find on each of the plants a specific ephydrid fly and a specific weevil (Häfliger et al., 2026).



Plate 2. Invasion of *Hydrocharis morsus-ranae* in Michigan, USA. Photo credit: Dr. Ian A. Knight.

2. Work Programme for Period under Report

The work plan for 2025 is outlined below. Results are reported in subsequent sections.

Surveys

- Extend field surveys to Sweden, UK, Hungary, and Serbia or Czech Republic.

***Bagous puncticollis* (Coleoptera, Curculionidae)**

- Survey specific sites known for this weevil in Czech Republic, Hungary, Sweden, UK;
- If successful with collections, study biology and establish a rearing;
- Initiate preliminary host-specificity tests on hosts recorded in the literature, *Stratiotes aloides* and *Elodea canadensis*.

***Hydrellia albifrons* (Diptera, Ephydriidae)**

- Maintain a rearing colony at CABI;
- Continue studying the biology to try and understand the overwintering of this fly;
- Initiate preliminary host-specificity tests on hosts recorded in the literature, *Potamogeton* species, *Alisma plantago-aquatica*;
- Set up an experiment to explore the potential impact this fly may have.

***Tracya hydrocharidis* (Basidiomycota, Doassansiceae)**

- Search for the smut *Tracya hydrocharidis* in UK.

3. Surveys

3.1 Field surveys

Hydrocharis morsus-ranae is present throughout Europe (Plate 3) although local populations have declined or have been extirpated in recent years linked to pollution and habitat destruction.

METHODS Based on GBIF and iNaturalist databases (GBIF, 2024; iNaturalist, 2025), we drew maps using QGIS 3.30 Hertogenbosch and selected sites according to the different criteria such as: record date of *H. morsus-ranae* not older than 2021, not in a protected site or reserve, type of water body, accessibility and herbivores of interest records. Between May and August 2025, we conducted four field trips. At each site, plants were checked visually for insects and sampled for dissection in the lab.

RESULTS We were able to visit 40 sites, including ponds, channels and rivers in eight different countries: Switzerland, France, Sweden, United Kingdom (UK), Hungary, Slovakia, Czech Republic, Italy and Slovenia (SI) (Plate 3). We found the ephydrid fly *Hydrellia albifrons* at ten sites relatively widespread between Switzerland, United Kingdom, and Sweden, the weevil *Bagous puncticollis* at four sites in Slovenia, Hungary, Slovakia and Sweden, and the smut *Tracya hydrocharidis* at four sites in the United Kingdom and Italy.

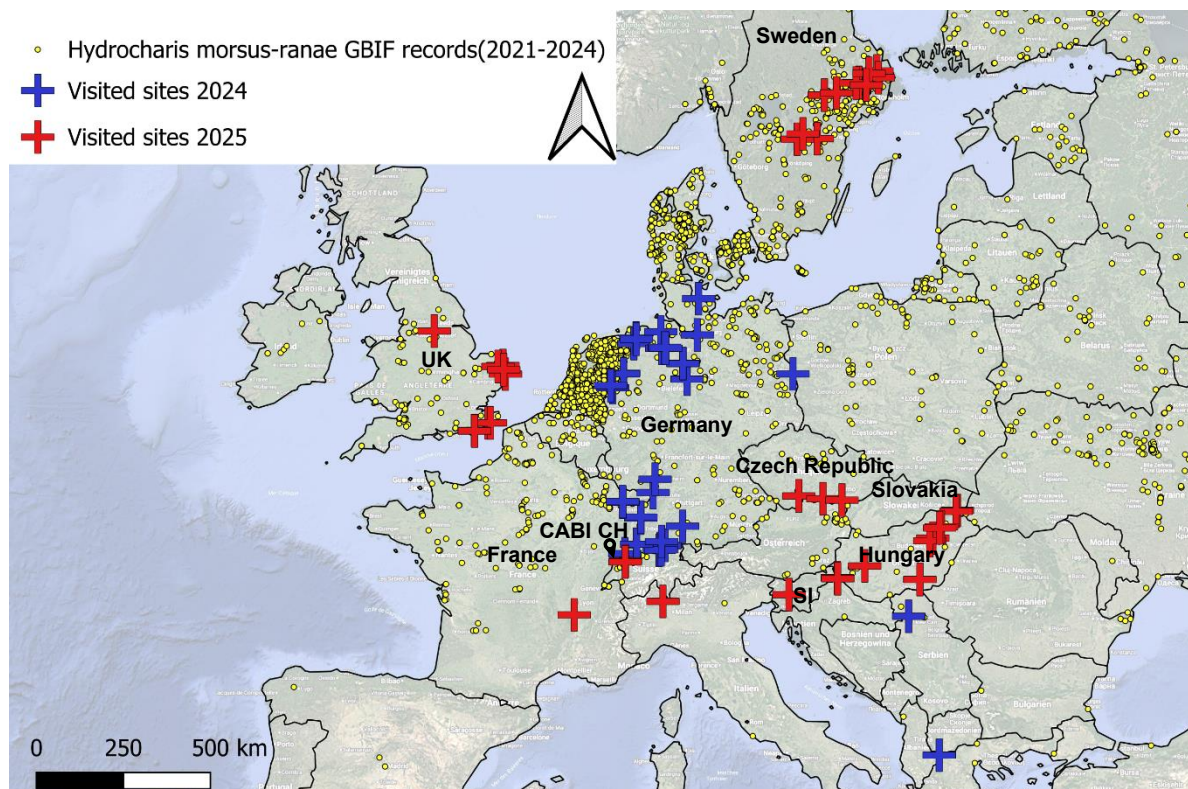


Plate 3. *Hydrocharis morsus-ranae* distribution throughout Europe. Yellow dots are records from 2021 to 2024. Blue crosses represent sites visited in 2024 and red crosses in 2025.

3.2 Genetic study

The geographic source of introduced plant populations can be extremely useful to understand the history of invasion to guide future surveys in search for appropriate natural enemies. This is done through molecular matching of introduced with native populations, in which plant tissues from introduced populations are sampled, their DNA extracted, analysed and then compared. It is especially important when the species has a large geographic range because potential biological control agents may be locally adapted to a particular plant genotype.

METHODS At each site, between one and ten plants were sampled, where up to three leaves were taken per plant, cleaned and dried with paper towelling and placed individually in a plastic bag with silica gel labelled with the site name and the date. These samples were then sent to Prof. Ryan Thum, Montana State University, Department of Plant Sciences and Plant Pathology for analysis.

RESULTS We were able to collect genetic material at 33 different sites from Sweden, Hungary, United Kingdom, Czech Republic, Italy, Slovenia and all samples were sent in August 2025. These are yet to be analysed, and results will be available soon.

3.3 Demography study

METHODS In order to understand the demography of the plants at sites visited, the patch size of the site was estimated as well as the total *H. morsus-ranae* coverage. We recorded visually if any of the *H. morsus-ranae* were flowering and what other plant species were present at the site. Five quadrats were placed along a 25 meter transect where possible. The first quadrat contained *H. morsus-ranae* and each quadrat was taken from at least five meters apart. The quadrat size was 0.25m² subdivided into four equal quadrants (Plate 4a). We estimated the distance of the quadrat centre to shore, water depth, percent cover of target and non-target plants. We took a picture of each quadrat and counted the number of flowers and fruits. We took three plants per quadrat to record whether turions were present. Once quadrats were completed, we collected six leaves from six different plants randomly collected in the site for leaf chemistry (Plate 4b). A picture of the leaves was taken and then placed separately in bags of silica gel and sealed in an envelope or plastic bag.

RESULTS We took demography and chemistry data at four different sites in Switzerland and United Kingdom. The raw data as well as samples for plant chemistry were sent in August 2025 to Dr. Ian Knight, from US Army Corps of Engineer for analysis.

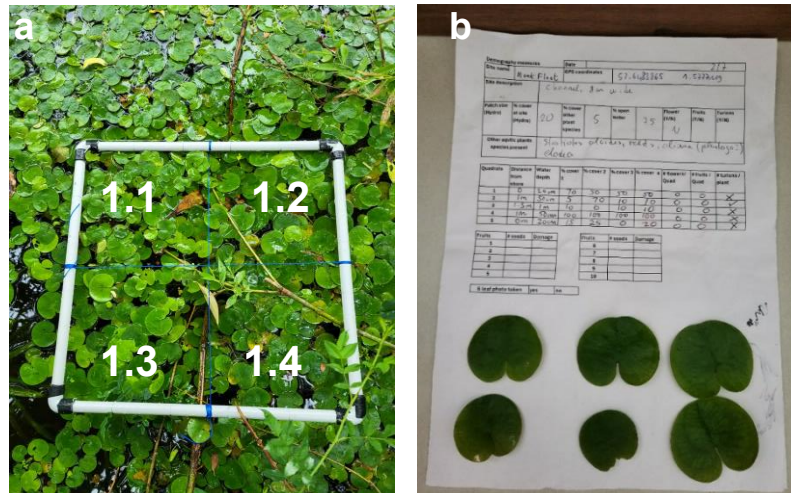


Plate 4. An example quadrat of the site “Muckfleet” (52.648379° N, 1.577679° W), in the United Kingdom. The quadrat is subdivided by four (1.1, 1.2, 1.3, 1.4) and *Hydrocharis morsus-ranae* cover is estimated (100% - 100% - 100% - 100%) (a); Leaf chemistry collection, leaves were saved in silica gel for future analysis (b).

4. Plant cultures

Plant populations of *H. morsus-ranae* from different origins were maintained at CABI. In spring 2025, cage-enclosed pools of ~450 litres were prepared with 20 litres potting soil, fertilizer and rainwater. Turions from four different populations, two from Switzerland and two from Germany, were added, one population each per pool in order to protect them from *H. albifrons* infestation. Different setups were tried to favour their growth such as shade vs no shade (Plate 5a), square vs round pools, addition of standing dead reeds (Plate 5b) vs *Butomus umbellatus* rhizomes (Plate 5c). A mixed population from turions or plants collected from multiple sites were released in three outdoor pools of ~3000 litres intermixed with potted *Butomus umbellatus* (Plate 5d).

Non-target species were either collected in the field in Switzerland or purchased online and kept in open pools or tanks (Table 1).

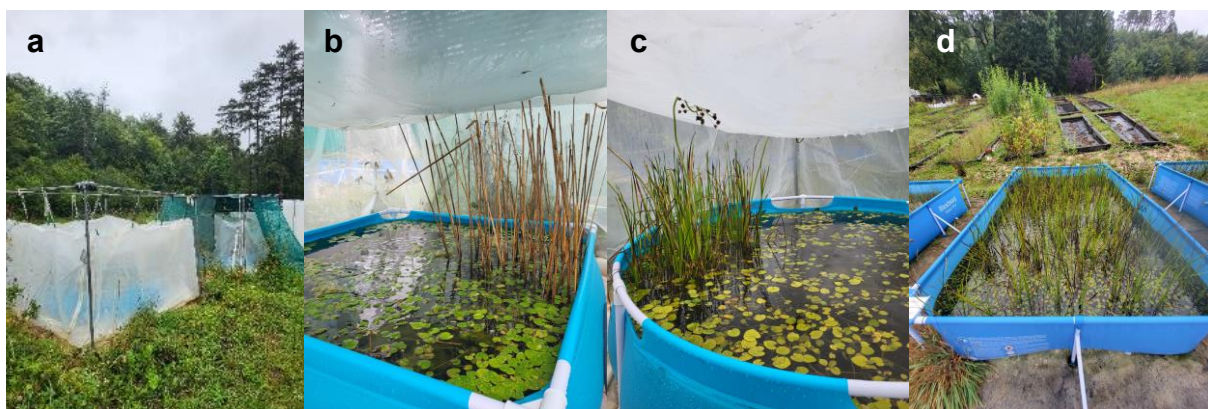


Plate 5. Cage-enclosed pools with shade or no shade (a) *Hydrocharis morsus-ranae* with standing dead reeds (b), or *B. umbellatus* plants (c) and outdoor pool together with *B. umbellatus* (d).

Table 1. Plant species tested to assess the host specificity of *H. albifrons*, ordered by phylogenetic distance from *Hydrocharis morsus-ranae* (Onezoom, 2024; Soltis *et al.*, 2011). The native distribution was based on the GBIF database and Plant of the World Online of the Royal Botanic Gardens, Kew. Abbreviations, CH: Switzerland, USA: United States of America, SA: South America, Eur: Europe, NA: North America.

Plant species	Growth form	Origin	Native distribution
HYDROCHARITACEAE			
<i>Hydrocharis morsus-ranae</i> L.	Emerged	Field collected, Europe	Eur, Asia and Africa
<i>Hydrocharis laevigata</i> (syn. <i>Limnobium laevigatum</i>) (Humb. & Bonpl. Ex Willd.) Byng & Christenh.	Emerged	Purchased online	SA
<i>Elodea densa</i> (Planch.) Casp.	Submerged	Purchased from local nursery, CH	SA
ALISMATACEAE			
<i>Alisma plantago-aquatica</i> L.	Emerged	Purchased from local nursery, CH	Eur and Asia
<i>Sagittaria latifolia</i> Willd.	Submerged and emerged	Purchased from local nursery, USA	NA
POTAMOGETONACEAE			
<i>Potamogeton lucens</i> L.	Submerged	Purchased	Eur and Asia
<i>Potamogeton natans</i> L.	Submerged and emerged	Field collected, Switzerland	Eur, Asia, Africa and NA
<i>Potamogeton crispus</i> L.	Submerged	Field collected, Switzerland	Eur, Asia and Africa
<i>Potamogeton perfoliatus</i> L.	Submerged	Field collected, Switzerland	Eur, Asia, NA

5. *Hydrellia albifrons* (FALLÉN) (Diptera, Ephydriidae)

5.1 Introduction

Four shore fly species are recorded as herbivores on European frogbit (Table S1). However, since identification of *Hydrellia* species is difficult, many of the old records may be misidentified and *H. albifrons* is most likely the only *Hydrellia* species developing on *H. morsus-ranae*, with *H. morsus-ranae* as the only host (Stuke pers. comm., ephydrid specialist, University Bremen). *Hydrellia albifrons* is widespread in Europe and also known from North Africa and Pakistan (Kahanpää and Zatwarnicki, 2015).

Females typically lay eggs on the leaf surface (Plate 6b). After about five days, larvae hatch and immediately start mining the leaf. Under laboratory conditions, development through three instars to the puparium is completed within two weeks. Pupation occurs in leaf petioles and on the leaf underside. Adults emerge approximately one week after pupation. We observed up to three generations per year. A few adults emerged in spring 2025 from puparia overwintered in a wooden shed in yoghurt cups filled with water, confirming the puparia as overwintering stage for the species (Häfliger *et al.*, 2025). We have a population of *H. albifrons* established in the garden at CABI (47.373084 °N, 7.325497 °W).

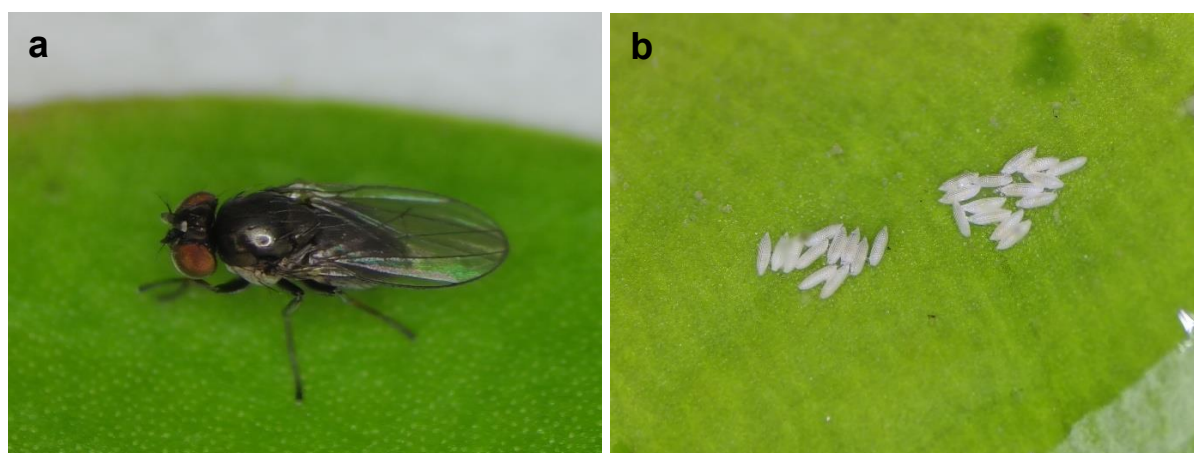


Plate 6. Adult *Hydrellia albifrons* (a) and eggs on leaf surface (b).

Most of the *Hydrellia* species are associated with aquatic habitats and several species have been considered in other weed biological control programs (Wheeler and Center, 2001; Grodowitz *et al.*, 1997; Martin *et al.*, 2013; Moffat *et al.*, 2024), and some species are also known as pests on cultivated rice *Oryza sativa* (Viajanthe and Heinrichs, 1986; Pathak and Khan, 1994; El-Habashy, 2011). More than 200 *Hydrellia* species are known on aquatic plant species in the palearctic region (Rodrigues *et al.*, 2014).

5.2 Host range testing

From June to September 2025, we established the first host-range trials, set up as no-choice larval development tests.

METHODS In June 2025, the first plants with mines were collected from the “garden” plants of mixed populations and *H. albifrons* puparia were moved to Petri-dishes with a damp filter paper. Once adults emerged, pairs (one male with one female) of *H. albifrons* were set up individually in cylinders with 2-3 plants of *H. morsus-ranae* and yeast mixed with sugar in a yellow bottle lid as a protein source to stimulate egg laying (Plate 7a). After a maximum of three days, before the first eggs hatch (Häfliger *et al.*, 2025), three eggs were delicately collected with a fine brush. One to two plants per control or non-target plant species were prepared in a cylinder and the three eggs were deposited, one egg per leaf (Plate 7b). From one adult cylinder, i.e. one female, a maximum of three replicates per test plant species were set up as well as two to three controls at the same time. Larval development was tracked until pupation after which puparia were collected and isolated in Petri dishes (5 x 0.5 cm) on moist filter paper until adult emergence. All adults were kept in alcohol for further morphological or molecular identification. With this method we were able to set up between three and 13 replicates of eight non-target species and 47 controls (Table 2). Control plants were taken from the caged pools when possible (Plate 5a) or from the Swiss population “Gals” from the outdoor pools (Plate 5d). Non-target plant species originated from field collected populations or cultured plants from outdoor pools (Plate 5d).

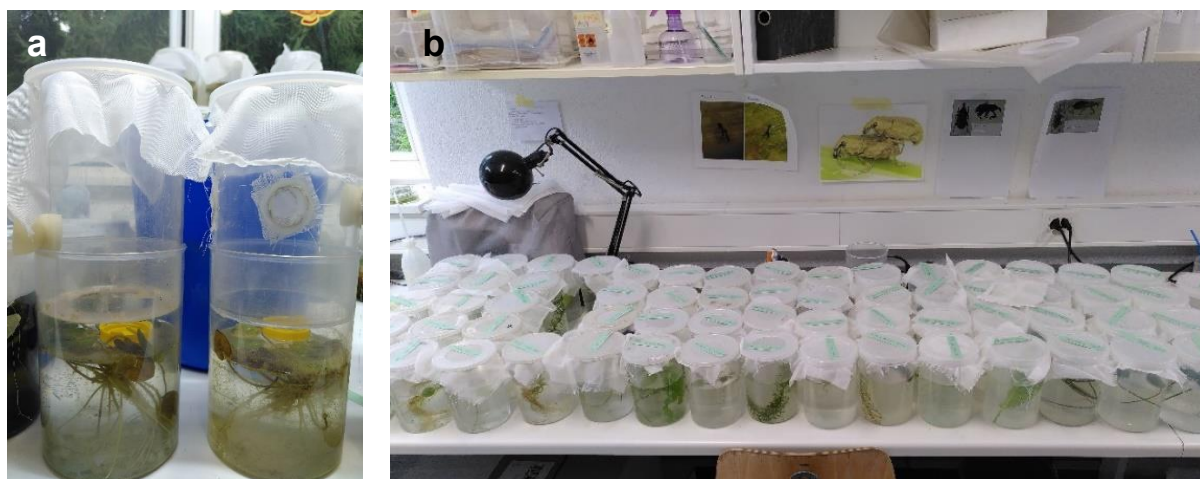


Plate 7. Cylinders (a) for egg production and (b) for no-choice tests with non-target plant species and *H. morsus-ranae* as a control.

RESULTS Of the eight species tested, *Elodea densa*, *Alisma plantago-aquatica* and *Sagittaria latifolia* did not support the development of *H. albifrons* (Table 2). In contrast, one adult from 30 eggs emerged from *H. laevigata* and all *Potamogeton* species tested supported variable development to adult of *H. albifrons* with between 26.7% - 40% success rate, while the control, *H. morsus-ranae*, supported a higher development rate to adult of 61.7% (Table 2). It appears that the rate of puparia failing to develop to adults varies between species, with highest failure rate on *P. natans* and lowest on *P. crispus*. However, since some of the puparia that did not emerge may overwinter, we cannot compare mortality rates of puparia here. *Hydrellia albifrons* needed slightly more time on average on the *Potamogeton* species than on the control to develop to puparia, 18.4 - 21.2 days vs. 16.2 days, and to adult 23.6 – 28.3 days vs. 22.7 days

(Table 2). Unfortunately, the field collected plants of *P. perfoliatus* were already infested by *Hydrellia fascitibia* von Roser, and eggs or first instar larvae were missed when setting up the tests. Out of the 33 *H. albifrons* eggs set up, 23 adults emerged: four were identified as *Hydrellia albifrons*, four as *H. fascitibia*, and 15 could not be identified. These tests were therefore excluded from the results due to contamination and will be repeated in 2026.

Table 2. The development success and time in days of *Hydrellia albifrons* from egg to puparia and adult under no-choice conditions.

Plant species	# reps set up	# valid reps	# eggs set up	% puparia $\pm$ SE	Days to puparia $\pm$ SE	% adults $\pm$ SE	Days to adult $\pm$ SE
<i>Hydrocharis morsus-ranae</i>	47	47	141	71.6 $\pm$ 3.8	16.2 $\pm$ 0.3	61.7 $\pm$ 4.1	22.7 $\pm$ 0.3
<i>Hydrocharis laevigata</i>	10	10	30	3.3 $\pm$ 3.3	NA	3.3 $\pm$ 3.3	25
<i>Elodea densa</i>	10	10	30	0		0	
<i>Alisma plantago-aquatica</i>	10	10	30	0		0	
<i>Sagittaria latifolia</i>	3	3	9	0		0	
<i>Potamogeton lucens</i>	10	10	30	33.3 $\pm$ 8.6	18.4 $\pm$ 1	26.7 $\pm$ 8.1	26.7 $\pm$ 0.9
<i>Potamogeton natans</i>	10	10	30	56.7 $\pm$ 9	21.2 $\pm$ 0.9	40.0 $\pm$ 8.9	28.3 $\pm$ 1.3
<i>Potamogeton crispus</i>	13	13	39	37.5 $\pm$ 7.7	20.4 $\pm$ 1*	32.5 $\pm$ 7.5	23.6 $\pm$ 0.9
<i>Potamogeton perfoliatus</i>	11	0	33	-	-	-	-

* *Potamogeton crispus* “days to puparia” data are likely unreliable, as the reported development time to adulthood appears unrealistically short. This may be due to plant quality, which could have made puparia harder to detect, leading to delayed recording of pupation date.

5.3 Discussion

Preliminary host-range results suggest that *H. albifrons* has a relatively restricted host range within the family Hydrocharitaceae, however, under no-choice conditions it is capable of developing to adult on several species within the genus *Potamogeton*. On one hand this is not surprising given that in the literature *H. albifrons* has been recorded from *Potamogeton* species. However, the development time also appeared to vary among plant species with larvae of *H. albifrons* requiring more time to develop on *Potamogeton* species, suggesting sub-optimal hosts. According to the specialist, *H.*

albifrons is most likely monophagous and only developing on *H. morsus-ranae* (Stuke pers. comm., ephydrid specialist, University Bremen). Given this, additional experiments are needed to determine the realised host range of *Hydrellia albifrons*. In 2026, more detailed choice experiments will be conducted under both laboratory and outdoor conditions to refine our understanding of the host selection.

6. *Bagous puncticollis* BOHEMAN (Coleoptera, Curculionidae)

Bagous puncticollis is reported as rare and endangered in most European countries (Bogusch, 2017; Sprick, 2000). Most literature records consider *B. puncticollis* as oligophagous, with *H. morsus-ranae*, *Stratiotes aloides* and *Elodea canadensis* as host plants (Caldara and O'Brien, 1998; Dieckmann, 1983; Sprick, 2000). However, these records are only from adult collections, while larval development has never been observed (Sprick, 2000) and nearly nothing is known about the biology of this weevil.

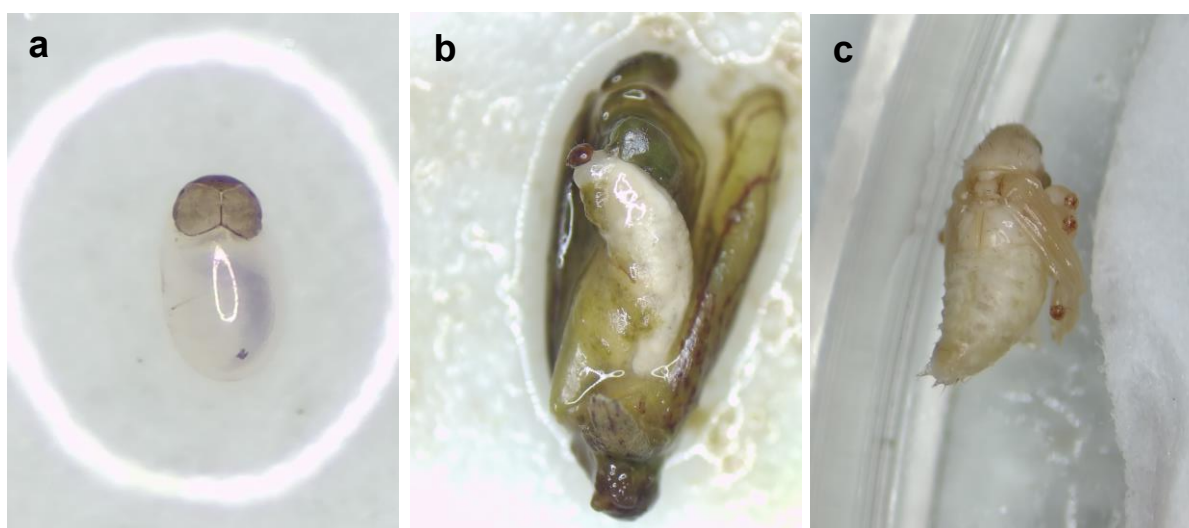


Plate 8. *Bagous puncticollis* egg (a), third instar larva in turion (b) and pupa in Petri dish (c).

6.1 Rearing trials

During a fieldtrip at the end of May 2025, a total of fourteen weevils were found on *Hydrocharis morsus-ranae* during a field trip through Slovenia (1), Hungary (11) and Slovakia (2). These weevils were set up for rearing in the laboratory in cylinders and cups on *H. morsus-ranae*. The weevils readily fed on the plants as seen by the typical holes in the leaves (Plate 9b) and survived well the whole summer. However, we never found eggs or larvae. We also offered a few turions, and *S. aloides* and *E. canadensis* plants to the weevils. This also did not trigger oviposition. At the end of July, we found two additional weevils at a field site in Sweden. On plants offered during the travel, we

found 15 eggs in the leaf petioles. However, no more eggs were obtained in the lab, and none of the hatching larvae established on plants offered. Finally, a single female collected in early August in Hungary laid 48 eggs in the lab setup. Since we had no rearing success so far transferring newly hatched larvae on *H. morsus-ranae* plants, we tried offering turions in Petri dishes on a moist filter paper. Larvae entered the turions within a few minutes and developed to mature third instar larva within about ten days. Pupation occurred mostly in the Petri dish (Plate 8c), either freely on the filter paper or under the filter paper, wherefrom the adults emerged a few days later. Currently we have 13 field collected and 12 lab reared adults overwintering in cylinders filled with water and plant debris as a swimming platform.

6.2 Discussion

We were able to show that *B. puncticollis* must develop on turions of *H. morsus-ranae*. This is a big step forward; however, we still need to learn about the biology of this weevil, especially oviposition triggers. There are also several open questions regarding pupation. In the lab setup, pupation was never successful under submerged conditions and only occurred on or under a nearly dry filter paper. No pupation was obtained on or in turions or in mud that was offered to the third instar larvae in the dishes. It is still unknown where pupation occurs in the field. Currently, we assume that the turions that developed in our rearing might not have been large enough (0.6-1.0 cm) and that mature third instar larvae may stay in the turions for pupation, if they are large enough, but this remains to be tested. We also want to check whether overwintering weevils may oviposit in turions in spring, before they develop to plants. In addition, we plan to offer turions of *S. aloides* to test whether larval development would be possible on this plant.

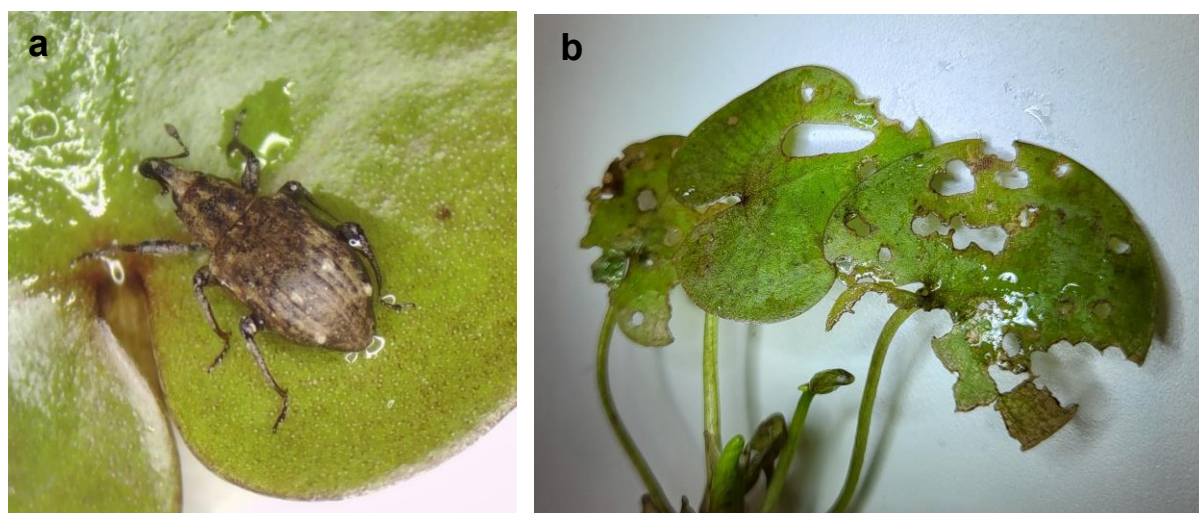


Plate 9. Adult *Bagous puncticollis* collected on *Hydrocharis morsus-ranae* in Hungary (a), and typical adult feeding marks on a plant exposed to weevils in the rearing setup (b).

7. *Tracya hydrocharidis* (Basidiomycota, Doassansiaceae)

The smut fungus, *Tracya hydrocharidis*, produces lesions starting with white leafspots on the underside of floating leaves which contain immature spore balls and later brown and finally mature black spore balls occur in the leaves and petioles (Ainsworth, 2023; Van Steenwinkel et al., 2022). *Tracya hydrocharidis* grows exclusively on plants of the genus *Hydrocharis*, suggesting good specificity to *H. morsus-ranae* (Van Steenwinkel et al., 2022). It was described as critically endangered in Britain (Ainsworth, 2023), however, even though it can be challenging to spot directly in the field, it seems that its status could be reconsidered as it seems more widespread and only unnoticed (Ainsworth, 2023). Identification directly in the field can be challenging, the smut causes yellowing of the leaves, but its presence could only be confirmed back in the lab under a binocular microscope. We were able to identify the smut at four different sites, three in UK (out of 10 sites) and one in Italy, supporting the idea that the smut is more widespread than initially thought. In the laboratory, the sporidia were isolated and placed in liquid nitrogen for long-term storage.

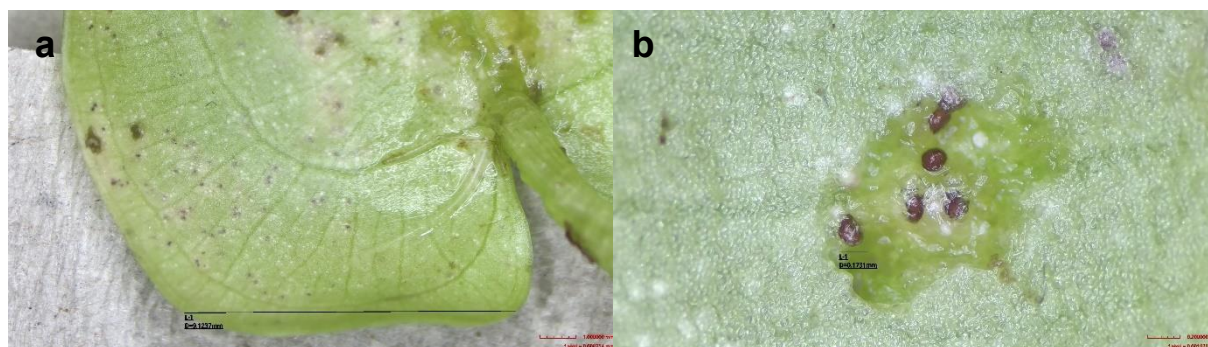


Plate 10. *Hydrocharis morsus-ranae* leaf infected with the smut, *Tracya hydrocharidis* (a). close up of *Tracya hydrocharidis* spore balls (b).

8. Work Programme Proposed for 2026

The following work programme is being proposed for 2026.

Test plant list

- Develop test plant list relevant to NA for the family Hydrocharitaceae.

Hydrellia albifrons (Diptera, Ephydriidae)

- Maintain a rearing colony at CABI;
- Redo the no-choice tests with *Potamogeton perfoliatus*;
- Conduct choice tests both in the lab and outdoors with plants in the genus *Potamogeton*.

Bagous puncticollis (Coleoptera, Curculionidae)

- Survey specific sites known for this weevil in Hungary in an attempt to collect more weevils and study the biology in the field and in the lab;
- Establish a rearing at CABI;
- Initiate preliminary host-specificity tests on hosts recorded in the literature, *Stratiotes aloides* and *Elodea canadensis*.

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11. Supplementary material

Table S1. Phytophagous arthropods and pathogens associated with *Hydrocharis morsus-ranae* in the literature. M = monophagous, O = oligophagous, P = polyphagous.

Taxa	Host range	Plant part attacked	Feeding mode	Host plants	Source
COLEOPTERA					
Curculionidae					
<i>Bagous chinensis</i>	O	Stem?	Borer?	<i>Hydrilla verticillata</i> , <i>Elodea nuttallii</i> , <i>Egeria densa</i> , <i>Vallisneria natans</i> , <i>Hydrocharis morsus-ranae</i>	Zhang <i>et al.</i> 2012
<i>Bagous limosus</i>	P	Stem	Borer	<i>Potamogeton</i> spp., <i>Hydrocharis morsus-ranae</i> , <i>Myriophyllum</i>	Labat 2024; Ellis 2001-2025
<i>Bagous puncticollis</i>	O?	Unknown (leaf/ stem)	Unknown (borer)	<i>Hydrocharis morsus-ranae</i> , <i>Stratiotes aloides</i> and <i>Elodea canadensis</i>	Caldara & O'Brien 1998; Sprick 2000; Pešić 2007
Chrysomelidae					
<i>Galerucella grisea</i>	P	Leaf	External	<i>Lysimachia</i> , <i>Hydrocharis morsus-ranae</i> , <i>Hypericum</i> , <i>Juncus</i>	https://dbif.brc.ac.uk
DIPTERA					
Ephydriidae					
<i>Hydrellia albifrons</i>	P?	Leaf	Larvae miner	<i>Potamogeton</i> , <i>Hydrocharis morsus-ranae</i> , <i>Alisma plantago-aquatica</i>	Kahanpää 2015
<i>Hydrellia cochleariae</i>	P?	Leaf	Miner	<i>Stratiotes</i> , <i>Potamogeton</i> , <i>Callitriche</i> , <i>Hydrocharis morsus-ranae</i>	Kahanpää 2015; https://dbif.brc.ac.uk
<i>Hydrellia griseola</i>	P?	Leaf	Miner	Alismataceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cyperaceae, Gramineae, Hydrocharitaceae (<i>Hydrocharis morsus-ranae</i> & <i>Stratiotes aloides</i>), Labiatae, Leguminosae, Lemnaceae, Liliaceae, Polygonaceae, Typhaceae	Hesler 1995; https://dbif.brc.ac.uk
<i>Hydrellia mutata</i>	P?	Leaf	Miner	<i>Alisma</i> , <i>Stratiotes</i> , <i>Lemna</i> , <i>Hydrocharis morsus-ranae</i>	Kahanpää 2015; https://dbif.brc.ac.uk
HEMIPTERA					
Aphidinae					
<i>Rhopalosiphum nymphaeae</i>	P	Leaf	External	<i>Prunus</i> spp., <i>Acorus</i> , <i>Alisma plantago-aquatica</i> , <i>Azolla filiculoides</i> , <i>Baldellia</i> , <i>Butomus umbellatus</i> , <i>Calla</i> , <i>Callitriche</i> , <i>Ceratophyllum submersum</i> , <i>Echinodorus</i> ,	Halder 2020; Milenković 2022; Ellis 2001-2025

Taxa	Host range	Plant part attacked	Feeding mode	Host plants	Source
<i>Elodea</i> , <i>Glyceria</i> , <i>Hippuris vulgaris</i> , <i>Holcus lanatus</i> , <i>Hydrocharis</i> , <i>Juncus</i> , <i>Lemna</i> spp., <i>Marsilea</i> spp., <i>Menyanthes</i> , <i>Myriophyllum spicatum</i> , <i>Nasturtium officinale</i> , <i>Nelumbo</i> , <i>Nuphar lutea</i> , <i>Nymphaea alba</i> , <i>Nymphoides</i> , <i>Pistia</i> , <i>Polygonum</i> , <i>Potamogeton natans</i> , <i>Ranunculus aquatilis</i> , <i>Sagittaria sagittifolia</i> , <i>Salvinia</i> spp., <i>Saxifraga aquatica</i> , <i>Schoenoplectus lacustris</i> , <i>Scirpus</i> , <i>Secale cereale</i> , <i>Sparganium</i> , <i>Stratiotes aloides</i> , <i>Trapa</i> , <i>Triglochin</i> , <i>Typha</i> spp.					
LEPIDOPTERA					
Crambidae					
<i>Cataclysta lemnata</i>	O	Leaf	External	<i>Azolla</i> , <i>Lemna</i> , occasionally <i>Stratiotes aloides</i> , <i>Hydrocharis morsus-ranae</i>	Van der Velde 1988; Ellis 2001-2025
<i>Elophila nymphaeata</i>	P	Leaf	Larvae miner	<i>Alisma plantago-aquatica</i> , <i>Callitriche palustris</i> , <i>Catabrosa aquatica</i> , <i>Hydrocharis morsus-ranae</i> , <i>Leersia</i> , <i>Myosotis laxa</i> , <i>Nuphar lutea</i> , <i>Nymphaea alba</i> , <i>Nymphoides peltata</i> , <i>Persicaria amphibia</i> , <i>Potamogeton</i> spp., <i>Sparganium emersum</i> , <i>Stratiotes aloides</i> , <i>Stuckenia pectinata</i>	Vallenduuk & Cuppen 2004; Ellis 2001-2025
<i>Munroessa icciusalis</i>	P	Leaf	External	<i>Eichhornia crassipes</i> , <i>Limnobium spongia</i> , <i>Orontium aquaticum</i> , <i>Potamogeton diversifolius</i> , <i>Sparganium americanum</i> , <i>Hydrocharis morsus-ranae</i>	Catling <i>et al.</i> 2003; Stoops 1998; Ellis 2001-2025
<i>Parapoynx stratiotata</i>	P	Leaf	External	<i>Alisma plantago-aquatica</i> , <i>Callitriche palustris</i> , <i>Ceratophyllum demersum</i> , <i>Elodea</i> , <i>Hydrocharis morsus-ranae</i> , <i>Myriophyllum</i> spp., <i>Nymphaea alba</i> , <i>Potamogeton crispus</i> , <i>Stratiotes aloides</i> , <i>Trapa natans</i>	Ellis 2001-2025
<i>Synclita occidentalis</i>	O	Leaf	External	<i>Lemna</i> , <i>Hydrocharis morsus-ranae</i>	Catling 2003 <i>et al.</i> ; Bashar <i>et al.</i> 2007
FUNGI					
BASIDIOMYCOTA					
Doassansiaceae					
<i>Tracya hydrocharidis</i>	M	Leaf	Leaf spot pathogen	<i>Hydrocharis morsus-ranae</i>	Van Steenwinkel 2022

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