



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Aquatic
Sciences and Assessment
Division of Microbial Ecology

Investigative collaboration to find cause and effect of mysterious mass die-off of the invasive alien species Fringed Water Lily (*Nymphoides peltata*)

*Undersökande samarbete för att finna orsak och verkan av mystisk massdöd hos den
invasiva främmande arten sjögull
(*Nymphoides peltata*)*

Version 1.0



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CONTENTS

CONTENTS	3
ACKNOWLEDGEMENTS	4
SAMMANFATTNING (SVENSKA)	5
SUMMARY (ENGLISH)	6
1. BACKGROUND	7
2. OVERVIEW OF KNOWN <i>N. PELTATA</i> PATHOGENS	8
2.1 BIOLOGY OF <i>SEPTORIA</i>	9
3. RECONSTRUCTED TIMELINE OF 2023	11
4. METHODS	12
4.1 COORDINATED SAMPLING AND OBSERVATIONS 2024	12
<i>Observations</i>	12
<i>Sampling</i>	12
4.2 ISOLATION OF FUNGI AND FUNGUS-LIKE ORGANISMS	12
4.3 IDENTIFICATION OF FUNGI AND FUNGUS-LIKE ORGANISMS	13
5. RESULTS	14
5.1 KOLL PÅ SJÖGULL	14
5.1.1 <i>Outreach and communications</i>	14
5.1.2 <i>Survey observations</i>	14
5.2 FUNGI AND FUNGUS-LIKE ORGANISMS ASSOCIATED WITH <i>N. PELTATA</i> IN SWEDEN	21
5.2.1 <i>First report of Septoria villarsiae from Sweden</i>	22
6. KEY FINDINGS AND IMPLICATIONS	23
REFERENCES	24
APPENDICES	25
APPENDIX 1: KOLL PÅ SJÖGULL SURVEY IN 3 LANGUAGES	25
APPENDIX 2: DETAILS OF METHODS FOR DNA EXTRACTION, SEQUENCING, AND DNA-BASED IDENTIFICATION	28
APPENDIX 3: EXAMPLES OF OUTREACH AND COMMUNICATION	30
APPENDIX 4: EXPANDED DETAILS OF SAMPLES AND ORGANISMS IDENTIFIED USING ITS DNA SEQUENCES	31

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SAMMANFATTNING (SVENSKA)

Under 2023 observerades i delar av Sverige ett utbrett bladfall eller död hos *Nymphoides peltata* (sjögull), en invasiv art som är skadlig för ekosystem i sötvatten. Initiala undersökningar tydde på att infektion av svamp eller svampliknande organismer var en möjlig anledning, men vid den tidpunkten kunde länken inte fastställas på grund av brist på övergripande kunskap om de olika patogener som angriper *N. peltata* i Sverige, liksom information om växternas tillstånd eller förändringar i deras tillstånd före den förmodade växt döden. Föreliggande projekt utformades för att fylla dessa kunskapsluckor genom: i) insamling av observationer från 2023 för att upprätta en tidslinje för händelserna; ii) samordning av observationer och prover för 2024; iii) genomgång av kunskap om patogener som angriper *N. peltata*; iv) kartläggning av svampar i Sverige som länkats till *N. peltata*. Arbetet genomfördes med en kombination av engagemang från allmänheten, samarbete med myndigheter och intressegrupper samt vetenskaplig forskning vid Sveriges Lantbruksuniversitet. Vi har sett att liknande växt död eller bladfall tidigare rapporterats från Asien men inte observerats i Västeuropa. Rapportering från allmänheten och myndigheter tyder på att patogen som angriper *N. peltata* är vitt utbredda i Sverige, och att majoriteten rapporter innehåller observationer av skador på angripna växter. Det är emellertid fortsatt oklart huruvida växterna dör, reagerar på en infektion med att tappa bladen, och/eller försvagas av en infektion. Genom att identifiera svampar och svampliknande organismer (t.ex. protister) från infekterade växtprover kunde femton potentiella patogener identifieras. Att notera är att *Septoria villarsiae*, en patogen som länkats till allvarlig förstörelse av *N. peltata* i andra länder (t.ex. Sydkorea), för första gången dokumenterades i Sverige. Ett orsakssammanhang mellan infektion, bladfall, försämrad kondition hos växten eller växt död har ännu inte rapporterats i den vetenskapliga litteraturen och kräver ytterligare undersökningar innan man kan förstå infektionens inverkan. Det är ännu okänt om den variant av *S. villarsiae* som återfinns i Sverige är särskilt virulent, eller om alla *N. peltata*-kloner i regionen är mottagliga för den. Emellertid tyder exempelvis observationer under 2024 i Mälaren på att vissa *N. peltata*-kloner kan vara mindre påverkade. Detta väcker oro beträffande potentialen för vidare spridning av en resistent växtgenotyp. Dessutom finns det fortfarande signifikanta oklarheter i vår kunskap om *Septoria*-svampar, inklusive huruvida de kan infektera arter som är av betydelse i svenska sötvattens- eller terrestra ekosystem. Dessa frågor, bland andra, behöver utredas för att införlivas i system för effektiv kontroll av denna invasiva växt innan någon svamp kan övervägas som en potentiell biologiskt kontrollagent för *N. peltata* i Sverige. I alla händelser kan närvaron av naturligt förekommande patogener som kan försämra växtens tillstånd synergistiskt tjäna till att gynna utrotningsinsatserna.

SUMMARY (ENGLISH)

In 2023, the large-scale leaf drop or death of *Nymphaoides peltata* (sjögull, fringed water lily), an invasive species detrimental to freshwater ecosystems, was observed in parts of Sweden. While initial investigations suggested fungal or fungus-like infections as a possible cause, the link could not be made at that time due to a lack of comprehensive knowledge regarding the diversity of *N. peltata* pathogens in Sweden and information about the status or changes in status of the plants before the putative plant deaths. This project was designed to fill these knowledge gaps through the: i) collection of observations from 2023 to produce a timeline of events; ii) coordination of observations and samples for 2024; iii) review of knowledge on pathogens of *N. peltata*; iv) survey of *N. peltata*-associated fungi in Sweden. The work was undertaken through a combination of public engagement, collaboration with public authorities and interest groups, and scientific investigation at the Swedish University of Agricultural Sciences. We find that similar plant death or leaf drop events have been previously reported from Asia but not observed in western Europe. Reports submitted by the public and public authorities suggest the presence of *N. peltata* pathogens are widespread in Sweden, with lesions observed on affected plants in most reports. However, it remains unclear whether the plants are being killed, dropping their leaves in response to infection, and/or weakened by infection. Through the identification of fungi and fungus like-organisms (e.g., protists) from infected plant samples, fifteen potential plant pathogens were identified. Notably, *Septoria villarsiae*, a pathogen linked to severe *N. peltata* devastation elsewhere (e.g., South Korea), was documented for the first time in Sweden. Causation between infection, leaf loss, reduced plant condition, or plant death for any pathogen of *N. peltata* has not yet been reported in the scientific literature and requires additional investigation before the impacts of infection can be understood. It remains unknown if the *S. villarsiae* strain present in Sweden is particularly virulent, or if all clones of *N. peltata* in the region are susceptible to it. However, observations from 2024 in Lake Mälaren, for example, suggest that some *N. peltata* clones may be less affected. This raises concerns about the potential for a resistant plant genotype to spread more widely. Further, important uncertainties in our knowledge of *Septoria* fungi exist, including whether they can infect species important in Swedish freshwater or terrestrial ecosystems. These question, among others, must addressed to inform effective control of this invasive plant and before any fungus can be considered as a potential biocontrol agent for *N. peltata* in Sweden. Regardless, the presence of naturally occurring pathogens that may diminish the condition of the plant can synergistically benefit efforts towards eradication.

1. BACKGROUND

Nymphoides peltata (Sjögull, Fringed Water Lily, Yellow Floating Heart), is an aggressive invasive plant that negatively impacts freshwater ecosystems and organisms. Actions to control its spread also impede outdoor activities (e.g. boating and fishing). This plant is found in streams and lakes across mid and southern Sweden. Costly, labor-intensive efforts to control *N. peltata* are ongoing, primarily using large floating frames with light-blocking materials. This is a slow and expensive process, and recolonization of previously treated areas remains possible.

In summer to early autumn 2023, observers of *N. peltata* in Lake Mälaren reported unexpected mass “deaths” of these plants, at the scale of “flera fotbollsplaner” (several football fields)¹. Because this phenomenon was newly observed in 2023, no framework existed to systematically collect the observations needed to understand its progression, timing, and other key aspects—such as whether the plants were dying entirely, including their rhizomes, or only experiencing leaf detachment (leaf drop). Also, there was no systematically collected information about changes to impacted water bodies that could provide explanations for the deaths.

One possible explanation for this phenomenon is that the *N. peltata* were killed by a fungal or fungus-like pathogen (e.g. Oomycetes, which are protists rather than fungi). To preliminarily assess the potential for the deaths being pathogen-related, leaves were collected (Joel Segersten and Stina Drakare, SLU) and brought to SLU. Lesions on the blades were visually inspected and were found to be similar to examples from the literature for known pathogens of *N. peltata*² (Figure 2); motivating further work.

The existence of a naturally occurring fungal pathogen for *N. peltata* in Sweden could synergistically increase the effectiveness of ongoing work to kill and remove the plant. However, appropriate and careful pre-study must be done before any pathogen could be intentionally employed as a biocontrol agent without risk to Sweden’s flora or crops.

NEEDS:

The following needs were identified in cooperation with stakeholders representing public agencies and local associations, including Havs- och Vattenmyndigheten (HAV; Swedish Agency for Marine and Water Management), Mälarens Vattenvårdsförbund (Lake Mälaren Water Conservation Association), and the Sjögull Nätverk (Water lily network, including city, county, and regional governments).

- Collection of observations from 2023.
- Coordinated observations for the 2024 season
- Review of state of knowledge of *N. peltata* fungal infections
- Survey of *N. peltata*-associated fungi in Sweden

SCOPE OF PROJECT:

To address the identified needs, a project was proposed and subsequently funded via HAV with the following scope:

1. Inform on *N. peltata* fungal infections.
2. Recreate timeline of events 2023.
3. Coordinate sampling and observations from the field.
4. Isolate and identify *N. peltata*-associated fungi and fungus-like organisms.

2. OVERVIEW OF KNOWN *N. PELTATA* PATHOGENS

Three fungal pathogens have been reported from *N. peltata*, *Septoria villarsiae* (Figure 1), *Puccinia scirpi*, and *Uromyces nymphoidis* (Table 1). Prior to this study, only *P. scirpi* had been reported from Sweden, although all three species had previously been reported from Europe.

According to the USDA Fungal Databases³, *P. scirpi* is a generalist pathogen, capable of causing disease in diverse plants (Table 1). *Septoria villarsiae* and *U. nymphoidis* reportedly infect and cause disease only on *N. peltata* (host-specific pathogens or specialists). However, the practice of naming species within *Septoria* has relied on an “assumption” that these fungi are host-specific. **This means that the same fungus on two different hosts could have two different names⁴**. Thus, based only on the lack of reports of this fungus on other hosts, these authors cannot confidently determine that *S. villarsiae* only causes disease in *N. peltata* (see also *Biology of Setporia*).

Of these pathogens, only *S. villarsiae* has been implicated in infections causing plant death. In South Korea, leaf spots caused by this fungus had been observed to form even before the leaves surfaced under some conditions, “devastating whole host populations”². In response to questions sent via email, Dr. H.D. Shin, Professor Emeritus, Division of Environmental Science and Ecological Engineering, Korea University:

“In Korea, this plant is one of the popular pond plants, but frequently devastated by Septoria infection.”
Personal communication to J. Anderson 15 January 2024.

Nathan Harms, Senior Research Biologist, Aquatic Ecology and Invasive Species Branch, US Army Engineer Research and Development Center, USA answered:

*“Regarding pathogens- we’ve identified a few from *N. peltata*, including *Septoria villarsiae* (in the USA and South Korea), and a rust, *Puccinia scirpi* (South Korea). I believe *S. villarsiae* could cause die-offs, because I have seen dead and dying plants here in the US, but never something so dramatic as what you describe.”* Personal communication to J. Anderson 11 January 2025.

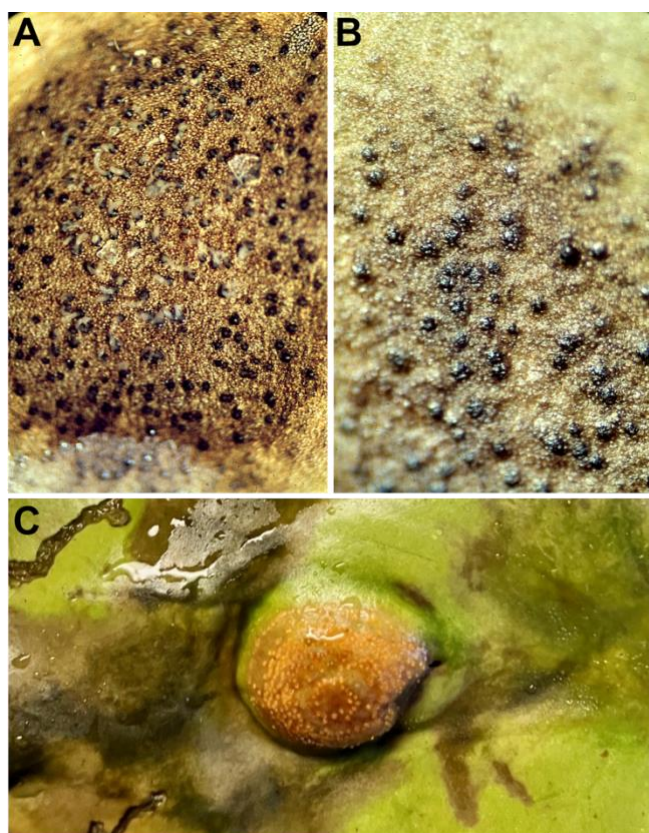


Figure 1. A,B) Close up images of *S. villarsiae* infection on *N. peltata* leaf by Klok and van der Velde 2023. The black dots are a type of reproductive structure/fruiting body (pycnidia) where conidia (asexually produced spores) are produced. DOI: 10.7717/peerj.16689/fig-6 CC BY 4.0. C) Possible *Puccinia scirpi* infection on *N. peltata*. Photo by J. Anderson.

Septoria villarsiae is a verified causal agent of leaf spots/lesions on *N. peltata*² (Figure 1) according to work that that fulfills Koch’s postulates for establishing the causal link between a microorganism and disease (the gold standard in pathogen testing). However, we find no published experimental evidence that *S. villarsiae* infection causes plant death. Rather, the ability to kill the plants is anecdotal or assumed. Further, although *S. villarsiae* has been found

in other parts of western Europe and the United States (invasive ranges of *N. peltata*), scientists studying *N. peltata* predators and pathogens in those areas report that they are not aware of any large-scale phenomena similar to events in Sweden 2023 (e.g. personal communication to J. Anderson from Sonja Stutz, Weed Biological Control, Centre for Agriculture and Bioscience International (CABI) Switzerland 15 January 2024; personal communication to J. Anderson from N. Harms 11 January 2025). Thus, the ability of *S. villarsiae* to directly kill its hosts is not confirmed

2.1 Biology of *Septoria*:

There is very little information available on *S. villarsiae* in existing literature. Therefore, we can only generalize from other species within the same genus to build a foundational understanding of this fungus.

- **The genus:** The genus *Septoria* currently includes more than 500 species.
- **Taxonomic problems:**
 - The genus is polyphyletic, meaning that it likely contains species that are not actually close relatives to “true” *Septoria* fungi. Rather, these organisms may share morphological features/ecologies but are in fact from different fungal genera or families⁴⁻⁶.
 - The genus includes cases where a fungus has been given more than one name based on the different hosts it was found on. Likewise, a *Septoria*-like fungus on *N. peltata* would be reported as *S. villarsiae*, even if it was a different fungus with spores that do not conform with the species description (e.g. De Souza et al 2021⁷). Thus, more robust host information is needed before action towards biocontrol could begin.
- **Reproduction:** *Septoria* refers to the asexual states of the fungi, where spores (conidia) are produced by mitotic cell divisions within a black, ball-like, structures (pycnidium). No sexual state for *S. villarsiae* has been reported, so we do not have more details about its full life cycle. When reproducing sexually (forming spores through meiosis), the fungi will make pseudothecia with sack-like structures called asci.
- **Dispersal:** The asexually produced spores may be spread to new plants through the air or via splashes of water from rain etc. The sexual spores would look different from the asexually produced spores and may disperse differently.
- **Overwintering:** These fungi may overwinter as mycelium within plant litter or as spores.
- **Impacts on hosts:** *Septoria* fungi vary widely in impacts on their host plants. They may cause mild leaf spotting, weaken plants through leaf damage that reduces plant production or causes leaf drop, and potentially cause plant death.
- **Host specificity:** Some *Septoria* species are known to infect multiple host plant species^{4,8} and host jumping to new plant families (evolving to infect new hosts) is part of the evolutionary history of these fungi⁴. However, according to Verkley et al. in 2013, their work indicated “that most species of *Septoria* have narrow host ranges, being limited to a single genus or a few genera of the same plant family.”⁴
 - *Nymphoides peltata* is in the family Menyanthaceae.
 - ***Menyanthes trifoliata* (Vattenklöver), Menyanthaceae, is also found in Sweden and may be important for diverse butterflies and moths⁹**

Table 1: Known fungal pathogens of *N. peltata* as per USDA fungal databases³

Species	Reported from:		Other reported host plants
	Europe	Sweden	
<i>Puccinia scirpi</i>	Bulgaria Lithuania Norway Poland United Kingdom	7 reports (6 specify <i>N. peltata</i> host).	<i>Bolboschoenus maritimus</i> <i>Ficinia levysiae</i> <i>Ficinia</i> sp. <i>Ficinia trichodes</i> <i>Holoschoenus vulgaris</i> <i>Limnanthemum grayanum</i> <i>Nymphoides forbesii</i> <i>Nymphoides grayana</i> <i>Nymphoides indica</i> subsp. <i>occidentalis</i> <i>Nymphoides</i> sp. <i>Schoenoplectus lacustris</i> <i>Schoenoplectus litoralis</i> <i>Schoenoplectus tabernaemontani</i> <i>Schoenoplectus validus</i> <i>Scirpus articulatus</i> <i>Scirpus hotarui</i> <i>Scirpus lacustris</i> <i>Scirpus litoralis</i> <i>Scirpus maritimus</i> <i>Scirpus mucronatus</i> <i>Scirpus</i> sp. <i>Scirpus sylvaticus</i> <i>Scirpus tabernaemontani</i> <i>Scirpus triqueter</i> <i>Scirpus tuberosus</i> <i>Scirpus validus</i>
<i>Septoria villarsiae</i>	Netherlands Poland Romania	No reports in Artportalen	None reported
<i>Uromyces nymphoidis</i>	Romania	No reports in Artportalen	None reported
Reports from Sweden are based on observations in Artportalen ¹⁰ . USDA Fungal Databases ³ and Artportalen accessed on 16 June 2025. Note: there are synonymous names for <i>Nymphoides peltata</i> that may be reported in the USDA database, including <i>Limnanthemum peltatum</i> .			

3. RECONSTRUCTED TIMELINE OF 2023

Through discussions and communications with stakeholders (see Needs above), observations from the field in 2023 were collected and are summarized in Figure 2. It appears that the plants' first leaves emerged as normal in May. However, already by 21 June, unhealthy plants with browning or spotted leaves were observed in the Arboga River, and this was considered atypical based on previous observations. These symptoms were observed before the start of what was reported as a "rainy" July, which could have impacted water levels. By 15 July, large numbers of detached leaves or dead plants were seen to be drifting out into open water, beyond the depth where the plants normally grow. At Skillingeudd, photos taken by drone show initial patches of *N. peltata* on 21 June (which emerge before other aquatic plants), that were notably absent by 17 August (surrounding aquatic plants of other species were unaffected). By the end of summer, *N. peltata* leaves were not abundant or were absent from the Arboga River (one source of *N. peltata* in Lake Mälaren). Note, however, that ***N. peltata* was observed in that river again in 2024**, suggesting that at least some plants survived the 2023 season.

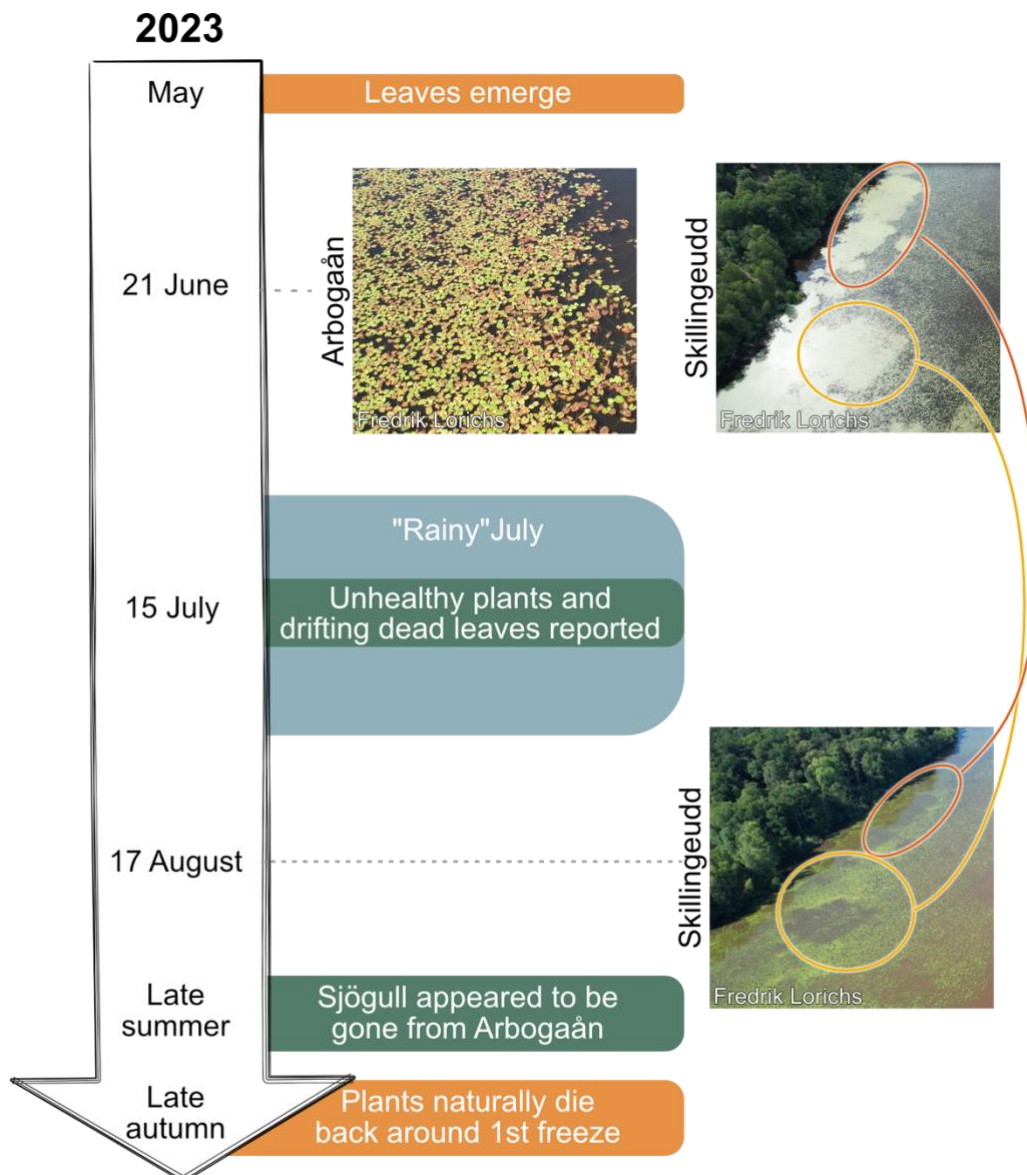


Figure 2. Summary of observations from 2023 (green and blue) within the context of the plant's annual cycle (orange). Photos illustrate the events. Black circle indicates a drifting patch of detached leaves, Connected orange circles indicate the same location in different photos. Note that observers thought there were no hydrological changes that would have impacted all sites where the plant phenomenon was observed.

4. METHODS

4.1 Coordinated sampling and observations 2024

Observations:

To collect standardized reports on the state of *N. peltata* across the 2024 growing season, the Koll på Sjögul (Watch on Fringed Water Lily) online survey was developed to be used by representatives of public authorities, members of the public, and other interested parties (Appendix 1). The survey was made available in Swedish, English, and German, as per stakeholder requests to support inclusive participation by tourists at some sites and was implemented through the European Union KoboToolbox Server (<https://www.kobotoolbox.org/>).

The reporting survey was designed to enable collection of information on the health status of the plants, signs of infection, and locations of populations and infected plants across the 2024 growing season with the goal of producing a comprehensive time-line of events for 2024. It was also designed to support the selection of samples for sending to SLU for further work.

To engage survey participants social media, a video, and a web page were developed by SLU Communicator Ulrika Jansson Klintberg. and additional communications were coordinated by C. Tennfors, Länsstyrelsen Västmanlands län.

Sampling:

Based on evidence of infection in photos submitted to the survey, representatives of public authorities were then directly contacted and asked to submit samples for the isolation and identification of fungi and fungus-like organisms. Sampling was performed according to a protocol co-developed by Anderson, Tennfors, and participating stakeholders. Briefly, it was requested that three individual plants/samples were collected per site, with care not to create fragments that could increase the spread of the plant. Each plant was to be individually placed into a sealable plastic bag labeled with the date and place of collection and kept cool, but not frozen, before prompt shipment to SLU (with packaging keep the sample cool in transit). Metadata about the collection site (coordinates, photos, status of the plants and populations, date, time, reporter name, agency, and contact information; Appendix 1) were collected through the survey. It is noted, however, that some samples were sent directly, bypassing the observation reporting process. As such, the survey did not produce a complete and standardized observational record.

4.2 Isolation of fungi and fungus-like organisms

The goal of the isolation and identification process was to identify potential fungi and fungus-like pathogens associated with *N. peltata*. This “survey” approach was necessary because there is insufficient information available on the pathogens of this plant, including their virulence and distributions, to proceed directly to a “diagnostic” approach.

To isolate fungi from *N. peltata* leaf blades, petioles, rhizomes, and root samples, the samples were rinsed in deionized water to remove surface debris and areas of interest around lesions or discoloration were dissected and retained (subsamples) for further processing. For samples in good condition (little or limited decomposition), the subsamples were processed using a two-step surface sterilization approach to kill microbes that were on, but not growing within, the plant tissue (30 seconds in 70% ethanol, then 60 seconds in 0.5% bleach, finishing with two washes in sterile deionized water). The subsamples were then cut into smaller pieces and placed in Petri dishes on ¼ Potato Dextrose Agar (PDA; with added agar to give 1.5% agar)

with 500 mg/L each of Streptomycin Sulfate and Penicillin G to reduce bacterial growth (Figure 3). This work was carried out using sterile techniques to decrease chances for contamination by non-relevant microorganisms.

In the case that the material was in poor condition, but was still somewhat intact, the subsamples were instead washed three times in sterile deionized water before placement on the PDA. Note, however, that this process can allow growth of fungi that were on the surface of the plant material (either coincidentally or in interaction with the plant). Also, these subsamples may host decomposer fungi rather than pathogens. Materials that arrived in highly decomposed states or after freezing could not be processed.

Growth of microbes from the subsamples was monitored for a minimum of one month. When growth was observed, it was isolated to a fresh Petri dish with the goal of growing each organism alone, separate from other species (pure culture). This process was repeated up to six times to try to achieve pure cultures of the different organisms. However, in some cases this was not possible, preventing further characterization of the fungi or fungus-like organisms from the subsample using approaches within the scope of this project. In a small number of cases, the isolates died in culture before growth for sequencing was possible. This is expected given that the lab conditions may be suitable for most fungi, but some may have special requirements for growth.



Figure 3. Example of pieces of petiole on 1/4 PDA with fungal growth visible from the piece on the middle-right. Photo by Aprajita Singh.

4.3 Identification of fungi and fungus-like organisms

The isolation process (above) can produce multiple pure cultures of the same fungus from within a plant and from multiple plants. To reduce redundancy in the next steps, efforts were made to select representatives from groups of highly similar looking cultures. It must be noted, therefore, that the results are **not quantitative** indicators of how abundant an organism was or its true distribution among the locations sampled.

The selected fungi and fungus-like organisms that were successfully isolated into pure culture were grown in a malt extract peptone broth (17 g/L malt extract, 2.5 g/L peptone) to produce tissue suitable for DNA extraction. DNA was extracted using a commercially available kit (Qiagen PowerSoil Pro). The region of the fungal DNA that is the current standard for fungal barcoding is ITS (Internal transcribed Spacer region of the ribosomal DNA operon, Appendix 2). DNA sequence of this part of fungal genomes was sent to Eurofins Scientific for sequencing (Appendix 2). The sequences were then compared to a database (US National Database for Biotechnology Information - NCBI¹¹) using Megablast to identify organisms by match to the most similar DNA sequences in the database (Appendix 2). In some cases, this led to species level identifications. For isolates where this was not possible, the lowest level of taxonomy (most specific) shared among the top 10 BLAST hits is reported. Ecological predictions for the identified taxa were made based on review of the ecologies of top 10 BLAST hits per isolate within each identified taxon or group and require testing for verification.

5. RESULTS

5.1 Koll på Sjögull

5.1.1 Outreach and communications

Between April and October 2024, observations from nature were submitted by members of the public and representatives of public authorities or interest groups via digital reporting to a survey called “Koll på Sjögull” (Appendix 1).

Information about the project and survey was shared directly with the Sjögull Network (authorities and groups working to eradicate the plant) and with the public through diverse mechanisms including posters (C. Tennfors), social media and a web page (supported by SLU Communicator Ulrika Jansson Klintberg), presentations by Anderson and additional outreach was performed by County Boards (Länsstyrelserna).

Examples of outreach materials produced (Appendix 3):

YouTube:	https://www.youtube.com/shorts/vIPA20SVbhw
Social media:	https://www.linkedin.com/posts/slu_sjaemgull-medborgarforskning-invasivaarter-activity-7196037843872366593-l8W1
Website:	https://old.slu.se/institutioner/vatten-miljo/forskning/forskningsprojekt/alla-forskningsprojekt/bio/fume/sjogull/

5.1.2 Survey observations

The observation and sampling campaign generated 26 reports, 15 from authorities and 11 from the public. Most reporters correctly identified *N. peltata*, with only 5 reports featuring misidentified plants. Of the misidentified plants, four were water lilies (näckrosor), possibly including *Nymphaea alba* and *Nuphar lutea*. These reports highlight the difficulty for the public to distinguish *N. peltata* from other water plants, which can be managed proactively in future “citizen science” efforts.

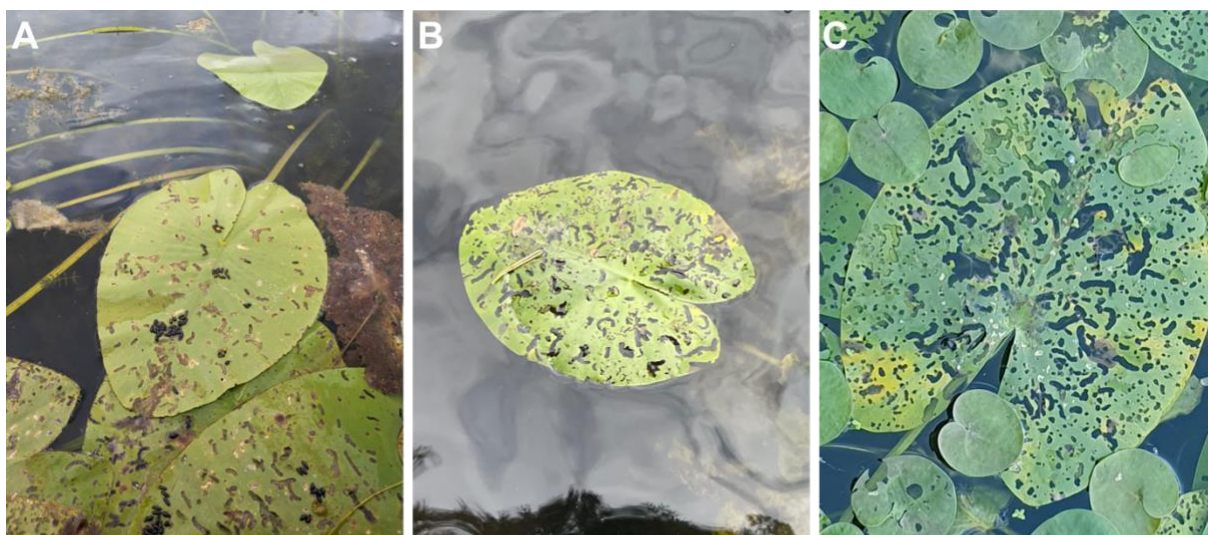


Figure 4. Waterlilies (näckrosor) are common in Swedish waterbodies, including *Nymphaea alba* and *Nuphar lutea*. The blades of these leaves show different damage (apparently from insects) than is seen on *N. peltata*, as also visible in figure C. A) Lillsjön, N6601593.93, E657864.227, 19 June 2024. B) Spånen, N6307482.009, E475363.654, 24 August 2024. C) Torshällaån N6588522.534, E583543.729, 21 July 2024. Images submitted to Koll på Sjögull survey. Coordinates = Sweref 99 TM

Natural enemies – herbivory: The water lily reports are useful for comparison of damage patterns with *N. peltata*. The insects that feed on the water lilies do not appear to also feed on *N. peltata*, or not to the same extent (Figure 4). There was little evidence of insect damage/herbivory to *N. peltata* in the photos submitted. This differs from reports from the native plant range and the United States (invaded range)¹², suggesting a potential enemy-release for the plant in Sweden, which could support invasion.

Defoliated or weakened vs “die-off”: *Nymphoides peltata* was observed in 2024 in locations that were impacted by putative “mass die-offs” in 2023, including the Arboga River and several locations in Lake Mälaren. This supports the occurrence of leaf drop or die-back in 2023 rather than a “die-off”. However, the density of the colonies was possibly reduced in early summer 2024.

Unfortunately, the level of participation achieved in the Survey was not sufficient to build a good overview of plant and colony status across the season. As such, critical questions remain, such as: i) whether plants are being killed by infection or rather are weakened due to leaf spots and leaf drop; ii) if some plants or colonies are resistant to infection and may replace the susceptible plants.

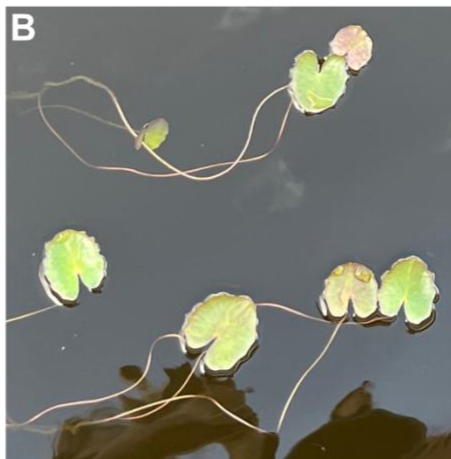
A sample of photos submitted to the survey are presented here (Figures 5-7) by waterbody, when known. Followed by a summary map (Figure 8) and figures illustrating the types of lesions reported (Figures 9 and 10).

Väringen 2024



Figure 5. Images illustrating the condition of *N. peltata* on 30 August, 2024 at one site in Väringen, near Frövi. The leaves are exhibiting diverse states of yellowing or senescence. The enlarged portions of the photo show *S. villarsiae*-like lesions. N6591530.143 E520577.746. Coordinates = Sweref 99 TM.

Lake Mälaren 2024



14 June
Västerås kommun
Västmanlands län



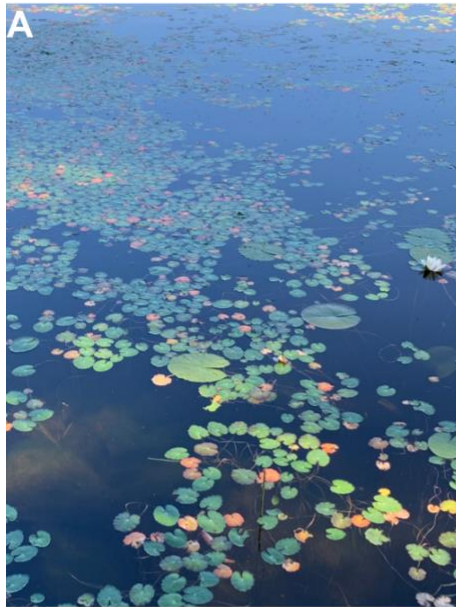
2 July
Kungsör kommun
Västmanlands län



16 September
Ekerö kommun
Stockholms län

Figure 6. Images illustrating the condition of *N. peltata* June, July, and September 2024 at three sites in Lake Mälaren. A-D) These leaves do not show *S. villarsiae*-like lesions, however, the leaves do not appear to be healthy, even at the June observation. E&F) This observation (Tureholm) is both later in the season and to the east of the others. Abundant *S. villarsiae*-like lesions are visible. A,B) N6594612.907 E580954.994. C,D) N6588507.638 E562227.471. E,F) N6578057.888 E654917.111. Coordinates = Sweref 99 TM.

Sommen 2024



26 June

Boxholms kommun

Östergötlands län



26 June

Boxholms kommun

Östergötlands län



9 August

Tranås kommun

Jönköpings län

Figure 7. Images illustrating the condition of *N. peltata* on 26 June and 4 September, 2024 at three sites in Sommen, near Tranås. The leaves at the three sites are exhibiting diverse states of senescence. Some potential insect damage is visible, but less than the other waterlilies (figure 4). B&F) The leaf blades have *S. villarsiae*-like lesions. F) The petioles are brownest at the blade, and increasingly green towards the rhizome. The rhizome and roots appear intact. A,B) N6437290.026 E501728.767. C,D) N6437141.871 E501797.548. E,F) N6432583.944 E501988.276. Coordinates = Sweref 99 TM.

A review of all submitted photos was undertaken to qualitatively assign a status to the pictured plants as having “Possible infection” by a fungal agent or “No evidence of infection”, depending on the visibility of lesions on the blades. When the report did not include photos or the photos did give a clear view of the status, they were classified as “Undetermined”. The photos indicating evidence for infection are collected in Figures 9 and 10. In almost all cases, the lesions appear *Septoria*-like, with the possible exclusion of the image labeled “Västmanland”, which is difficult to determine given the quality of the photo.

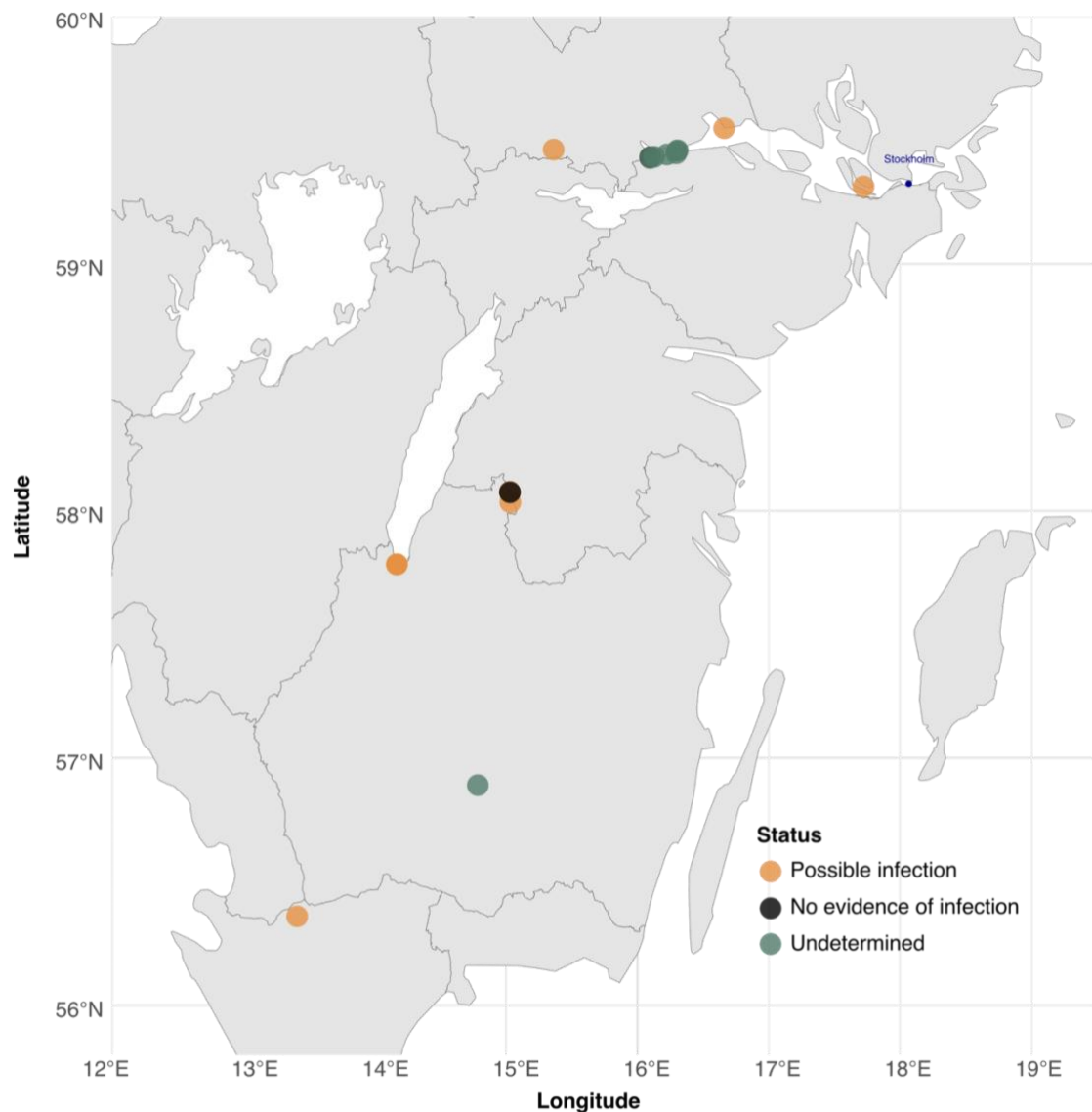


Figure 8. Summary of observations from photos submitted during 2024. Gold circles indicate that signs of probable infection were evident in the photos. Black circles indicate that the photos did not show infection-like lesions. Green circles indicate cases where samples were submitted without photos or where the status could not be determined from the photos. Note that some reports did not include coordinates but were identifiable to, and are plotted here in, Jönköping and Växjö. Map made using <https://github.com/borstell/swemapdata/>.

Jönköpings län 2024



Figure 9. Collection of photos reported from Jönköpings län that show evidence of possible infection by fungi or fungus-like organisms. Most lesions appear *Septoria*-like. It is noted that the rhizomes and roots (when visible) appear healthy.

Reports from 2024



Figure 10. Collection of photos from reports from different areas of central and southern Sweden that show evidence of possible infection by fungi or fungus-like organisms. In almost all cases, the lesions appear *Septoria*-like, with the possible exclusion of the image labeled "Västmanland", which is difficult to determine given the quality of the photo,

5.2 Fungi and fungus-like organisms associated with *N. peltata* in Sweden

All reports to the digital survey from public authorities were followed up with a request to send the samples for further processing. The subset of samples that were received in suitable condition were processed for the isolation of fungi and fungus-like organisms. Samples were considered suitable if they had not been frozen and were not in an advanced state of decomposition. Problems with shipping and the postal system rendered several shipments unsuitable.











































		Sample Processing							
		 Surface Sterilized  Sterile water rinsed  Both of above							
				Brändeborgsfjorden Sommen Köpingsviken Kungsör Kungsörstorp Brobyvikens utlopp Munkhammar Gösholmen Kvicksund					
Plant pathogens	ID	Phylum							
	<i>Alternaria</i> sp.	Ascomycota							
	<i>Botrytis</i> sp.	Ascomycota							
	<i>Epicoccum</i> sp.	Ascomycota							
	<i>Fusarium</i> sp.	Ascomycota							
	<i>Ilyonectria</i> sp.	Ascomycota							
	<i>Pseudopithomyces chartarum</i>	Ascomycota							
	<i>Sarcocladium</i> sp. (<i>S. Strictum</i>)	Ascomycota							
	<i>Septoria</i> sp.	Ascomycota							
	<i>Stemphylium vesicarium</i>	Ascomycota							
Possible plant pathogens	<i>Pythium</i> sp.	Oomycota*							
	<i>Penicillium</i> sp.	Ascomycota							
	<i>Plectosphaerella</i> sp.	Ascomycota							
	Pleosporales	Ascomycota							
	<i>Ceratobasidium</i> sp.	Basidiomycota							
Other	<i>Mucor hiemalis</i>	Mucoromycota							
	<i>Filosporella</i> sp.	Ascomycota							
	Helotiales	Ascomycota							
	<i>Leptodontidium</i> sp.	Ascomycota							
	<i>Penicillium corylophilum</i>	Ascomycota							
	Pezizomycotina	Ascomycota							
	<i>Tolypocladium</i> sp.	Ascomycota							
	<i>Trichoderma</i> sp.	Ascomycota							

Figure 11. Summary of the fungi and fungus-like organisms recovered from *N. peltata* samples from 2023 and 2024 organized by their predicted ecology. The origin of the organisms is indicated to the right with symbols coding for how the samples producing the isolates were processed. ID indicates the lowest shared taxonomic level among the top 10 BLAST hits per isolated strain (sequences with high similarity to the strain). The ecological predictions are based on review of the top 10 BLAST hits per isolate within these groupings and require testing for verification. Surface sterilization should yield isolates of organisms growing within the plant tissue, whereas water rinsed samples may also yield organisms on the plant surface. *Oomycota is a fungus-like group within Chromista, not a fungal phylum.

The results for the 64 isolates that were successfully isolated to single organisms, sequenced and identified to the most specific possible taxonomic level are summarized in Figure 11. See Appendix 4 for expanded details about locations, plant samples, and isolated organisms.

The isolated organisms could be grouped into 22 identifications based on the DNA sequence results (Figure 11). These are organized by the predicted ecology for the organism, which was based on an overview of existing literature for each of the top 10 BLAST hits reported. It must be emphasized that **these are subjective categorizations** and may differ from the ecology of the isolated strain upon experimentation, more refined taxonomic identification, or the genetic variation within a species or taxon.

Ten of the identified species/groups are probable plant pathogens. The probable fungal pathogens notably **include *Septoria*** (more below) in addition to fungi from the genera *Alternaria*, *Epicoccum*, *Ilyonectria*, *Pseudopithomyces* and *Stemphylium*. A fungus-like protist, *Pythium* (Oomycota), which is also a probable pathogen, was also recovered. These organisms were isolated from samples where surface sterilization was possible, increasing the likelihood that the fungi were growing within the plants. Three other probable pathogens, *Botrytis*, *Fusarium*, and *Sarcocladium*, were isolated from surface-sterilized samples, which can allow for the isolation of fungi that are coincidentally on the plant, as well as those growing within the plant, meaning the nature of their association with *N. peltata* remains unclear.

Five taxa are here classified as possible plant pathogens, indicating that the evidence of their pathogenicity was unclear or that there was variation in the predicted ecologies for the top 10 BLAST hits for the species in that group. These fungi include, *Penicillium* sp., *Plectosphaerella* sp., *Ceratobasidium* sp., *Mucor hiemalis*, and an isolate identifiable only to the fungal order Pleosporales. The remaining seven taxa are unlikely to be plant pathogens.

5.2.1 First report of *Septoria villarsiae* from Sweden

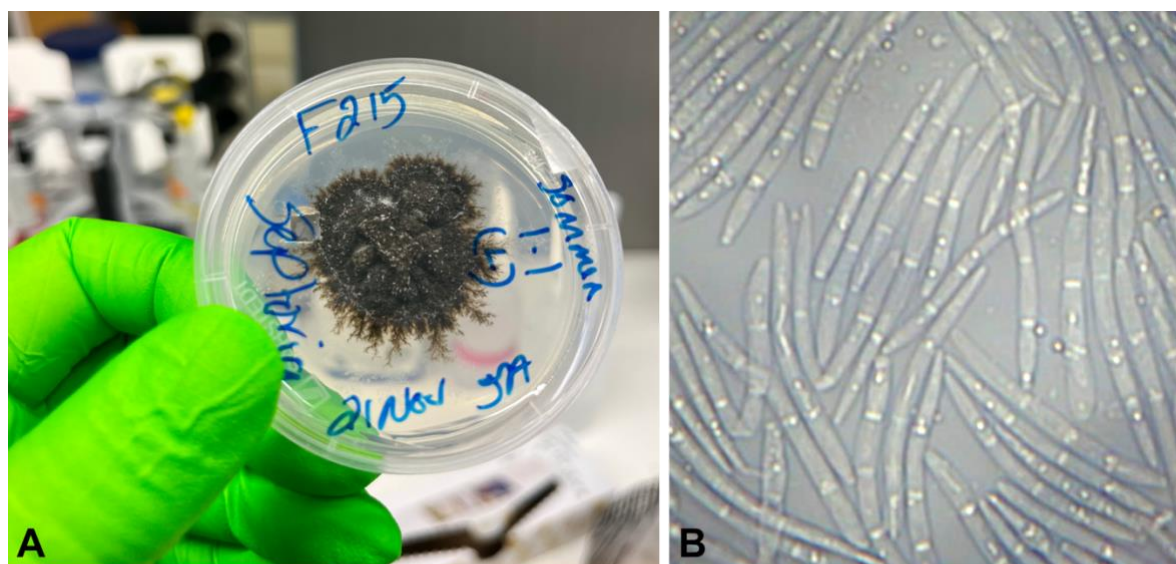


Figure 12. *Septoria villarsiae*, isolate F215, originated from a blade of *N. peltata* collected in Sommen, Östergötland, Sweden in July 2024. A) F215 growing on Potato Dextrose Agar produces a distinctive dark brown-black colony with abundant pycnidia and "dendritic" edge growth. Photo by J. Anderson. B) Spores (conidia) of F215. Spores are 27.8µm to 45.1µm long and 2µm to 4.8µm wide. Photo by Ziming Wang.

A species from the genus *Septoria* was isolated from Sommen from a plant sample that was surface sterilized. This implies that the fungus was indeed growing within the plant and is unlikely to be the coincidental growth of a spore from a different host. Examination of the isolate, F215, under the microscope was then performed. The isolate produces hyaline (clear), multiseptate (typically 2 to 3), asexual spores (conidia) that measured 27.8µm to 45.1µm x 2µm to 4.8µm. The spores are consistent with the original description of the species from 1864 which specifies that the spores are “1/20 to 1/30 of a millimeter long” (33.33µm – 50µm) and multiseptate¹³. This isolate is thus identified as *Septoria villarsiae* and constitutes the first documented report from Sweden to the best knowledge of these authors.

6. KEY FINDINGS AND IMPLICATIONS

In 2023, large numbers of *N. peltata* seemed to die, leading to reports of “mass die-offs”. Given that plants were again observed in affected locations in 2024 (e.g. Arboga River), it remains unclear whether, or to what extent, the plants died, had leaf loss, or may have experienced reduced fitness due to leaf spots and leaf loss. Thus, the impacts of 2023 and ongoing infections remains an open question. However, pathogens that diminish the condition of the plant may synergistically benefit efforts towards plant removal.

- **First reported *N. peltata* leaf drop/die-off events in Europe:** From consultation with experts, we find that leaf drop/die-off events of this type have not been reported from this region before.
- **Evidence of pathogen involvement:** Lesions on affected plants and the isolation of fifteen probable or possible plant pathogens suggest that one or more pathogens may contribute to diminished condition of *N. peltata* in Sweden. Notably, plants with signs of infection were found in most of the waterbodies sampled. Suggesting that the pathogens may be distributed beyond Lake Mälaren and the Arboga River (where reports from 2023 originated).
- **First documented report of *Septoria villarsiae* in Sweden:** Finding *S. villarsiae* in Sweden is noteworthy because it is the only pathogen that has been linked to severe *N. peltata* devastation in other regions.
- **Key knowledge gaps:**
 - The survey in 2024, did not provide a complete picture of:
 - The progression or distribution of infection.
 - The impact of infection in 2023 in the following year.
 - The direct causal link between *S. villarsiae* infection and plant death still relies largely on anecdotal evidence rather than published experimental proof.
 - It is unknown if the *S. villarsiae* strain present in Sweden is particularly virulent, or if all clones of *N. peltata* in the region are susceptible to it. Observations from 2024 in Lake Mälaren (Anderson personal observation, Stora Aspholmen) suggest that some *N. peltata* clones may be less affected.
 - It is important to experimentally confirm the pathogenicity and virulence of the isolated fungi, understand the environmental triggers contributing to the widespread leaf drop/die-offs, and assess the host range of *Septoria villarsiae* in Sweden.
- **Potential for Biocontrol (with caution):** Fungal pathogens offer a tantalizing possibility for a "synergistic" approach to managing *N. peltata*. However, any consideration of using such a fungus as an active biocontrol agent would require extensive and careful pre-study to assess host specificity and mitigate risks to Sweden's native flora and fauna. The known taxonomic complexities and potential for host-jumping within the *Septoria* genus underscore this critical need for thorough research before any deployment.

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APPENDICES

APPENDIX 1: Koll på Sjögull survey in 3 languages

Swedish - Translation by Karl Lundén, SLU.

Koll på Sjögull

Detta projekt är ett samarbete mellan Mikrobiell ekologi, Institutionen för vatten och miljö, SLU, och Havs- och vattenmyndigheten. För mer information om detta projekt besök <https://www.slu.se/sjogull>.

Om dig

***Om dig**
Information om hur vi behandlar personuppgifter finns nedan.

☐ Att uppdatera om sjögulls hälsa är INTE en del av mitt jobb. Jag är en "medborgarforskare".

☐ Jag har ett ansvar relaterat till mitt jobb att uppdatera om sjögull.

Här kan du berätta hur sjögull ser ut där du är. Det är redan till hjälp om du svarar en gång, men det är också bra för oss att höra om, eller hur, situationen förändras under sommaren och hösten. Du är välkommen att svara på enkäten igen!


Berätta för oss var växterna är. På många enheter kan du placera en punkt på kartan eller klicka på symbolen nedan. På iPhone kan du använda Google Chrome och Firefox, se till att godkänna platstjänster. Om detta inte fungerar, se andra alternativ nedan.

latitud (x,y *)


longitud (x,y *)

altitud (m)

noggrannhet (m)



☐ Gula fläckar som bildar knölar.



☐ Det är inte som bilderna, eller så är du inte säker.

***Avgör omfattningen på angreppet på ett enskilt blad. Hur mycket av bladet ser påverkat ("sjukt") ut enligt dig?**

☐ Bara en del av bladet ser påverkat ut.

☐ Ungefär halva bladet ser påverkat ut.

☐ Nästan hela bladet ser påverkat ut.

***Avgör omfattningen av angrepp på en hel grupp av sjögull. Är bara ett fåtal växter påverkade ("sjuka") eller är de flesta växter angripna enligt dig?**

☐ Bara några växter ser påverkade ut.

☐ Ungefär hälften av växterna ser påverkade ut.

☐ De flesta/alla ser påverkade ut.

Foton

Bilder du tillhandahåller kan inkluderas i rapporteringen av projektets resultat, inkludera inte någon person eller personer i bilderna du skickar in. Eftersom vi inte samlar in information om er som är medborgarforskare (såvida du inte anger din e-postadress ovan) kan vi inte erkänna dig som fotograf.

Ta ett foto av sjögullpopulationen (grupp sjögullväxter nära dig).
Vi vill se hur växtpopulationen ser ut.

Ta ett foto på 1 påverkat "sjukt" blad om du ser något.
Vi vill se hur sjukdomen ser ut på ett typiskt blad.

Om alternativet ovan inte fungerade för dig kan du antingen ange en [Plus Code](#) (t.ex. från Google Maps) eller beskriva din plats så tydligt som möjligt.


***När ser du växterna**


Berätta vad du ser

***Titta på gruppen av sjögull. Tycker du att de verkar**

☐ Friska


☒ Sjuka (helt eller delvis)





***Är tecken på sjukdom som en av dessa bilder eller något annat?**

☐ Bruna/svarta fläckar eller fält.



Personuppgifter

De uppgifter du registrerar här kommer att behandlas av SLU för att administrera din anmälan eller ditt besök. Läs mer om behandling av personuppgifter på www.slu.se/personuppgifter.

***Ja, jag förstår att mina uppgifter lagras och behandlas i samband med att jag skickar in detta formulär.**


☐ OK


***Ja, jag förstår och accepterar att SLU kommer använda insända uppgifter för forskning. SLU kan även använda mina uppgifter till utbildning, miljöanalys och information till allmänheten, utan ersättning.**

☐ OK

Har du frågor om detta arbete? Du kan kontakta oss på sjogull@slu.se.
<https://www.slu.se/sjogull>.

Tack för att du tar dig tid att hjälpa oss förstå vad som händer i sjögull i år. Du får gärna anmäla igen! Det är bra för oss att veta hur växterna ser ut under olika tider på sommaren och hösten.





Koll på Sjögull

Augen auf für die europäische Seekanne (Nymphoides peltata): Dies ist ein Gemeinschaftsprojekt der Schwedischen Universität für Agrarwissenschaften (SLU) und HaV dem Schwedischen Amt für Meer und Wasser. <https://www.slu.se/sjogull>.

▼ **Wer bist du?**

*** Wer bist du?**
Information über die Behandlung deiner persönlichen Daten findest du weiter unten.

☐ Ich bin ein Citizen Science-Forscher und arbeite nicht beruflich mit der europäischen Seekanne und deren Krankheiten.

☐ Ich arbeite beruflich mit der europäischen Seekanne und deren Krankheiten.

Hier kannst du beschreiben wie die Seekannen an deinem Standort aussehen. Für uns ist es wertvoll wenn du den Momentanzustand beschreibst aber noch besser ist es, wenn du beschreiben kannst, ob oder wie sich der Zustand über den Sommer und Herbst verändert. Du kannst dieses Formular mehrmals ausfüllen!


Beschreiben Sie uns, wo die Pflanzenstandorte sind. Auf vielen Geräten können Sie einen Punkt auf der Karte einzeichnen oder auf das Symbol unten klicken. Auf dem iPhone können Sie Google Chrome oder Firefox verwenden. Stellen Sie dabei sicher, dass Sie Ortungsdienste akzeptieren. Wenn dies nicht funktioniert, können Sie unten weitere Alternativen einsehen.

Breite (x,y °)


Länge (x,y °)

Höhe (m)

Präzision (m)



☐ Gelbe Flecken die Geschwülste bilden



☐ Das sieht nicht wie auf den Bildern aus - ich bin unsicher.

*** Bewerte das Ausmaß des Krankheitsangriffes auf einem Blatt. Wie groß ist die angegriffene ("kranke") Oberfläche des Blattes?**

☐ Nur ein Teil des Blattes sieht angegriffen aus.

☐ Ungefähr die Hälfte des Blattes sieht angegriffen aus.

☐ Fast das ganze Blatt sieht angegriffen aus.

*** Bewerte das Ausmaß des Krankheitsangriffes auf einen Bestand. Sind nur einzelne Pflanzen angegriffen ("krank") oder ein Großteil der Pflanzen?**

☐ Nur einzelne Pflanzen sehen angegriffen aus.

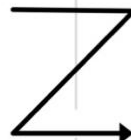
☐ Ungefähr die Hälfte des Bestandes sieht angegriffen aus.

☐ Die meisten/alle Pflanzen sehen angegriffen aus.

▼ **Fotos**

Fotos die du schickst können Teil eines Schlussberichtes werden und deshalb dürfen die Fotos keine Person oder Personen beinhalten. Da wir keine Information über Citizen Science-ForschereInnen sammeln (mit Ausnahme der freiwilligen Angabe der Email-Adresse) können wir dich nicht als Fotograf angeben.

Mache ein Foto des Seekannenbestandes oder Teilen des Bestandes.
Wir möchten gerne sehen wie der Bestand aussieht.




Wenn die obige Alternative bei Ihnen nicht funktioniert hat, können Sie entweder einen [Plus Code](#) (z.B. von Google Maps) eingeben oder Ihren Standort so deutlich wie möglich beschreiben.

*** Wann beobachtest du die Seekannen**


▼ **Beschreibe was du siehst.**

*** Beobachte den Seekannenbestand**
Wirkt der Bestand?

☐ gesund




☒ krank (der ganze Bestand oder Teile des Bestandes)



*** Sehen die Krankheitszeichen aus wie auf einem dieser Bilder oder anders?**

☐ Braune/schwarze Flecken



Mache ein Foto des Seekannenbestandes oder Teilen des Bestandes.
Wir möchten gerne sehen wie der Bestand aussieht.

Mache ein Foto des angegriffenen Blattes.
Wir möchten sehen wie ein typisches angegriffenes Blatt aussieht.

▼ **Persönliche Daten**

Die von dir registrierten Daten werden von der Schwedischen Universität für Agrarwissenschaften (SLU) benutzt um dein Nachricht oder deinen Besuch zu verwalten. Mehr Information (auf Schwedisch) findest du hier www.slu.se/personuppgifter

*** Ich bin damit einverstanden, dass meine Daten gelagert und behandelt werden wenn ich dieses Formular einschalte.**



☐ OK

*** Ich bin damit einverstanden, dass die Schwedische Universität für Agrarwissenschaften (SLU) meine Daten für Forschung, Umweltanalysen und Information für die Allgemeinheit kostenlos benutzt.**

☐ OK

Wenn du Fragen hast kannst du uns via sjogull@slu.se mailen.
<https://www.slu.se/sjogull>.

Vielen Dank dass du dir Zeit genommen hast um uns zu helfen. Schicke gerne weitere Berichte damit wir besser verstehen, wie die Seekannen über den Sommer und Herbst aussehen.

English

Koll på Sjögull

Status of Fringed Water Lily: This project is a cooperation between the Division of Microbial Ecology, in the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences (SLU), and the Swedish Agency for Marine and Water Management (Hav). For information about this project visit <https://www.slu.se/sjogull>.

About you

***About you**

☐ Reporting on this plant is NOT part of my job responsibilities. I am a "Citizen Scientist".

☐ I have a job-related responsibility to report on this plant.

Here you can tell us about how the Fringed Water Lilies look where you are. It is already helpful to us if you answer once, but is also good for us to hear if or how the situation changes throughout the summer and fall. You are welcome to respond to this survey again!


Tell us where the plants are that you are reporting. On many devices, you can place a point on a map or click on the symbol below. On iPhone, use Google Chrome or Firefox (Safari does not always work well, depending on your settings). Make sure you allow the browser to access your location. If these options do not work, see other alternatives below.

latitude (x,y *)

longitude (x,y *)

altitude (m)

accuracy (m)



***What is the extent of damage on a single leaf. How much of the leaf looks affected ("sick", infected) to you?**

☐ Only part of the leaf looks infected/"sick".

☐ About half of the leaf looks infected/"sick".

☐ Almost the whole leaf looks infected/"sick".

***What is the extent of the infection/"sickness" in a group of plants? Are only a few plants affected ("sick") or do you think most plants are affected?**

☐ Only a few plants look infected/"sick".

☐ Around half of the plants look infected/"sick".

☐ Most/all of the plants look infected/"sick".

Photos

The photos you provide may be included in reports of the project results. Do not include any person or people in the photos you submit. Because we do not collect personal information about you who are "Citizen Scientists" (unless you provide your email address above) we cannot credit you as the photographer.

Take a photo of the plant population (the group of plants near you).

Take a photo of 1 affected leaf if you see any.

Personal information

The information you provide here will be used at SLU to administer this report and project. Read more about the handling of personal information at <https://www.slu.se/en/about-slu/contact-slu/personal-data/>.

***Yes, I understand that my information will be stored and processed in connection with the submission of this survey.**

☐ OK

***Yes, I understand and accept that SLU will use the submitted information for research. SLU can also use my information for teaching, environmental analysis and to report results to the public, without credit or compensation.**



Tell us about what you see.

***Look at a group of plants. Do you think they look healthy?**

☐ Healthy

☒ Infected/sick (some or all plants)

***Are the signs of infection/"sickness" similar to one of these photos or do they look different?**

☐ Brown/black spots or patches

☐ Gold/yellow spots that form bumps

☐ They don't look like the photos, or I am not sure.

Personal information

The information you provide here will be used at SLU to administer this report and project. Read more about the handling of personal information at <https://www.slu.se/en/about-slu/contact-slu/personal-data/>.

***Yes, I understand that my information will be stored and processed in connection with the submission of this survey.**



☐ OK

***Yes, I understand and accept that SLU will use the submitted information for research. SLU can also use my information for teaching, environmental analysis and to report results to the public, without credit or compensation.**

☐ OK

Do you have questions about this project? You can contact us at sjogull@slu.se or visit us at <https://www.slu.se/sjogull>.

Thank you for taking a moment to help us understand what is happening with the Fringed Water Lily this year. Please report again! It is important for us to hear how the plants look at different times in the summer and fall.

APPENDIX 2: Details of methods for DNA extraction, sequencing, and DNA-based identification

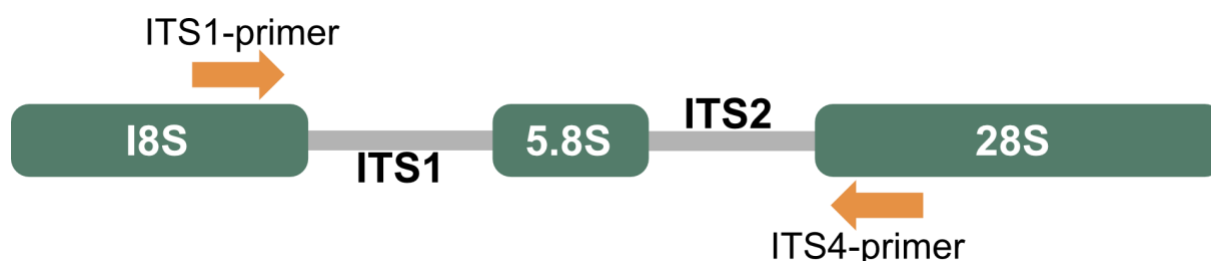


Figure A2.1: Schematic of the Internal Transcribed Spacer (ITS) Region. Amplification using ITS1 and ITS4 primers produces DNA sequences with ITS1, 5.8S rDNA, and ITS2.

The ITS region (above) was amplified using ITS1 + ITS4 primer combination except when it was suspected the organism was an Oomycete, then combination ITS1Oo + ITS4 was used instead^{14,15} (primer sequences and cycle details follow). Amplification was performed using Thermo Scientific DreamTaq DNA Polymerase (EP0702). The results were checked using gel electrophoresis. Successful reactions were cleaned using Invitrogen PureLink PCR Purification Kit (K3100-01 and K3100-02), quantified using Invitrogen Qubit dsDNA Broad Range Assay Kit (Q33265) and sent for sequencing at Eurofins Scientific using both forward and reverse primers. Resulting sequences were quality trimmed and assembled into contigs in Geneious Prime 2025.1.2 using the Geneious assembler. The assembled sequences were then compared to the NCBI Core nucleotide database using Megablast (through Geneious Prime), with the top 10 best matching hits returned. The taxa identified as best hits for each sequence were reviewed manually in MycoBank¹⁶ to retrieve their current taxonomic information and through online searches to produce a subjective prediction of the likely ecology of the organism.

Primers:

ITS1 (forward)	5' TCCGTAGGTGAACCTGCGG
ITS1Oo (forward)	5' GGAAGGATCATTACCACA
ITS4 (reverse)	5' TCCTCCGCTTATTGATATGC

PCR Amplification cycle			
Step	Temperature	Time	Cycles
Initial denaturation	95°C	3 min	1
Touchdown denaturation	95°C	30 sec	Start 54°C -1°C/cycle End 49°C 6 cycles
Touchdown annealing	54°C to 48°C	30 sec	
Touchdown extension	72°C	1 min	
Denaturation	95°C	30 sec	25
Annealing	48°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	15 min	1

APPENDIX 3: Examples of outreach and communication

Website:

<https://old.slu.se/institutioner/vatten-miljo/forskning/forskningsprojekt/alla-forskningsprojekt/bio/fume/sjogull/>

Koll på sjögull

SENAST ÄNDRAD: 10 JULI 2024

Den invasiva vattenväxten sjögull har precis som många andra invasiva arter lätt för att sprida sig, växer snabbt och är tålig. Därför kom det som en överraskning när sjögullen plötsligt drabbades av en okänd sjukdom och till och med försvann från vissa platser under sommaren 2023. Nu arbetar forskarna för att kartlägga denna oväntade vändning. Kan du hjälpa till att lösa gåtan?

Enkät koll på sjögull

Kortfilm om projektet (Youtube)

Sjögull beskrivs ofta som vacker men förrädisk. Den liknar den gula näckroosen, men tar över där den växer. Växten sprider sig över stora ytor och påverkar då ekosystemet och hur vi människor kan nyttja vattnet. Sjögull är en främmande invasiv art men inte giftig.

Bakgrund till den nya sjögullsforskningen

Eftersom sjögull ofta innebär stora problem vill vi få bort den och undvika vidare spridning. Invasiva arter kan vara svåra att bekämpa och i fallet sjögull har bekämpningsmetoderna hittills bara varit lokala, fungerat tillfälligt och varit dyra.

Förväningen blev stor när det under sommaren 2023 visade sig att sjögullen på några platser fick en sjukdom som gjorde att de dog ut. En teori till varför många sjögullsväxter dog är att sjögullen angreps av svampar. Därför har forskningsprojektet startat för att se om detta möjligtvis kan stämma.

Den nya forskningen är ett samarbete med allmänheten

Forskarna är intresserade av att veta om växten verkar frisk, eller om bladen exempelvis är fläckiga och verkar angripna av sjukdom på minsta sätt. Den här informationen kommer forskarna få från de personer från allmänheten som vill rapportera sina fynd genom en webbaserad enkät.

Så här kommer forskarna använda observationerna

Genom enkätsvaren hoppas forskarna få dokumentation av angrepp på sjögull och kunna förstå när och var angreppen sker. Den inrapporterade informationen hjälper forskarna att avgöra hur provtagning på angripna vatten och växter kan ske. Proverna kan därefter analyseras på laboratorium och förhoppningsvis kunna visa vad det är som angriper sjögullen, om det är en akvatisk svamp eller inte.



Den främmande och invasiva vattenväxten Sjögull är en vacker blomma som liknar vår inhemska gula näckros. Foto: Peter Olsson-Scholz.

Fakta:

Korta fakta om sjögull

- latinskt namn: *Nymphoides peltata*
- Svenskt namn: sjögull
- Organism: växter
- Livsmiljö: Sötvatten
- Ursprung: Asien, och Centraleuropa
- Första fynd i Sverige: 1850-1899
- Risk för att vara invasiv i Sverige (riskklass): 5 - mycket hög risk

Om sjögull

Sjögullen blommar med sina gula, fransiga kronblad under juli till augusti. Blomman sticker upp några centimeter över vattenytan, där de mörkgröna bladen flyter. Den trivs i lugna näringsrika vatten som vikar och hamnar.

Sjögull bildar massiva bestånd och sprider sig snabbt genom både frö och vegetativa delar. De kan växa snabbt och rotar sig i botten ned till tre meters djup. Risken för spridning börjar i mitten av april och till första frosten då växten går i vila.

Med sina täta bestånd täcker sjögullen vattenytan och begränsar ljusinflödet och därmed syretillgången i vattnet. Arten utgör ett hinder vid bad, fiske och båt fart.

Om projektet

Namn: "Undersökande samarbete för att finna orsak och verkan av mystisk massdöd hos den invasiva främmande arten sjögull (*Nymphoides peltata*)"

Finansierat av Havs- och vattenmyndigheten, enl. Dnr 2023-001094

Kontakta projektet via mejladressen: sjogull@slu.se

Kontaktinformation

Jennifer Anderson, Forskare
Institutionen för vatten och miljö, Avdelningen för mikrobiell ekologi
jennifer.anderson@slu.se

Länkar

[Rapporten tar emot information om var det finns sjögull](#)

[Sjögull i Artfakta.se](#)

[Om främmande invasiva arter på Naturvårdsverkets hemsida](#)

[Föreläsning på engelska om akvatiska svampar av forskaren Jennifer Anderson på Biotopias youtubekanal](#)

Dela:



SIDANSVARIG: VATTENMILJÖ WEBB@SLU.SE

Social media:

https://www.linkedin.com/posts/slu_sjaemgull-medborgarforskning-invasivaarter-activity-7196037843872366593-l8W1

SLU - Swedish University of Agricultural Sciences
54,180 followers
1 yr · 🌱

Forskarna har startat ett spännande forskningsprojekt för att undersöka vad som orsakade massdöden av sjögullen sommaren 2023. Sjögull är en invasiv vattenväxt som finns i Mälardalen och söderut. På sommaren täcker den stora delar av sjöar och vattendrag och försvårar för andra organismer att leva där. Massdöden av sjögullen är därför mycket intressant och forskningen ska undersöka vad som orsakade denna. Kanske var det ett svampangrepp? Forskaren Jennifer Anderson säger:

- Jag behöver din hjälp för att lösa detta mysterium. Vi vill se om växtdöden beror på en svamp och i så fall förstå om det kan hjälpa till att bekämpa sjögull.

Du kan delta i forskningsprojektet genom att rapportera in dina observationer av sjögull – angripen eller ej. Dina uppgifter kommer att bidra till att identifiera var och när angreppen sker och med förhoppningen att kunna hitta en lösning för att bekämpa denna invasiva art. Du hittar mer information om forskningsprojektet och hur du kan delta på www.slu.se/sjogull

#sjögull #medborgarforskning #invasivaarter #forskningssamarbete

Show translation



**Sjögull beskrivs ofta
som vacker men
förrädisk.**

SLU

Peter Olsson Skol

APPENDIX 4: Expanded details of samples and organisms identified using ITS DNA sequences

- **Organism ID:** The lowest (most specific) shared taxonomic level among the top 10 BLAST hits per isolated strain (sequences with high similarity to the strain).
- **Predicted Ecology:** The ecological predictions are based on review of the ecologies of top 10 BLAST hits per isolate within these groupings and require testing for verification. Pathogen = likely plant pathogen. Possible = possible plant pathogen. Other = not likely plant pathogen.
- **Isolate ID:** The lab ID of the isolated fungus or fungus-like organism from the study.
- **Plant Part:** The part of the plant that the organism was isolated from. *Denotes sample processed by sterile water rinses rather than surface sterilization. Surface sterilization should yield isolates of organisms growing within the plant tissue, whereas water rinsed samples may also yield organisms on the plant surface.
- **Sample Origin:** The name of the water body sampled or a nearby landmark or place for reference.
- **North and East:** Coordinates of the sample origin in Sweref 99 TM.
- **Sample label:** The label on the original sample as provided by the collector or na (not available).

Brändeborgsfjorden June 2024

Organism ID	Predicted Ecology	Isolate ID	Plant Part	North	East	Sample label
<i>Alternaria sp.</i>	Pathogen	F37P	petiole*	6260651.32	482572.354	Svamp B (9)
<i>Botrytis sp.</i>	Pathogen	F36L	blade*	6261251.53	482890.919	Svamp A (7)
<i>Ilyonectria sp.</i>	Pathogen	F38P	petiole*	6260876.57	482234.687	Svamp C (20)
<i>Pythium sp.</i>	Pathogen	F37L	blade*	6260651.32	482572.354	Svamp B (9)
<i>Pythium sp.</i>	Pathogen	F38L	blade*	6260876.57	482234.687	Svamp C (20)

Sommen - Östergötland July 2024

Organism ID	Predicted Ecology	Isolate ID	Plant Part	North	East	Sample label
<i>Ceratobasidium sp.</i>	Possible	F39R	rhizome	6437290.03	501728.767	Sommen 1.1
<i>Leptodontidium sp.</i>	Other	F40R	rhizome	6437141.87	501797.548	Sommen 1.2
<i>Penicillium corylophilum</i>	Other	F39P	petiole	6437141.87	501797.548	Sommen 1.2
<i>Penicillium sp.</i>	Possible	F6	blade	6437290.03	501728.767	Sommen 1.1
<i>Plectosphaerella sp.</i>	Possible	F40L	blade	6437141.87	501797.548	Sommen 1.2
<i>Pseudopithomyces chartarum</i>	Pathogen	F214	blade	6437141.87	501797.548	Sommen 1.2
<i>Pythium sp.</i>	Pathogen	F39L	blade	6437290.03	501728.767	Sommen 1.1
<i>Septoria sp.</i>	Pathogen	F215	blade	6437290.03	501728.767	Sommen 1.1

Lake Mälaren – organized by sample origin

Organism ID	Predicted Ecology	Isolate ID	Plant Part	Sample Origin	North	East	Sample label	Date m-y
<i>Filosporella</i> sp.	other	F4	blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Fusarium</i> sp.	Pathogen	F211	petiole*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Ilyonectria</i> sp.	Pathogen	F10	blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Ilyonectria</i> sp.	Pathogen	F11	blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Ilyonectria</i> sp.	Pathogen	F204	blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Ilyonectria</i> sp.	Pathogen	F207	petiole, blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Ilyonectria</i> sp.	Pathogen	F208	petiole, blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Pythium</i> sp.	Pathogen	F9	na	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Pythium</i> sp.	Pathogen	F200	petiole*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Sarcocladium</i> sp. (S. Strictum)**	Pathogen	F210	blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Stemphylium vesicarium</i>	Pathogen	F16L	blade	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Stemphylium vesicarium</i>	Pathogen	F17	blade	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Stemphylium vesicarium</i>	Pathogen	F16L	blade	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Stemphylium vesicarium</i>	Pathogen	F216	blade	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Trichoderma</i> sp.	other	F209	petiole, blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Trichoderma</i> sp.	other	F30	Petiole, blade*	Broby.	6590277.3	572906.079	UB	Aug-23
<i>Epicoccum</i> sp.	Pathogen	F100-4	blade	Gös.	6591810.59	574016.85	U-län6	Oct-24
<i>Epicoccum</i> sp.	Pathogen	F100-3	blade	Gös.	6591810.59	574016.85	U-län6	Oct-24
<i>Pythium</i> sp.	Pathogen	F100-5	blade	Gös.	6591810.59	574016.85	U-län6	Oct-24
<i>Alternaria</i> sp.	Pathogen	F18	blade	Koping.	6593063.64	560544.795	KO	Aug-23
<i>Mucor hiemalis</i>	Possible	F19	blade*	Koping.	6593063.64	560544.795	KO	Aug-25
<i>Mucor hiemalis</i>	Possible	F19B	blade	Koping.	6593063.64	560544.795	KO	Aug-23
<i>Mucor hiemalis</i>	Possible	F3	blade*	Koping.	6593063.64	560544.795	KO	Aug-25
<i>Penicillium</i> sp.	Possible	F214	na	Koping.	6593063.64	560544.795	KO	Aug-23
Pleosporales	Possible	F203	petiole*	Koping.	6593063.64	560544.795	KO	Aug-23
Pleosporales	Possible	F32P	petiole*	Koping.	6593063.64	560544.795	KO	Aug-23
Pleosporales	Possible	F12	petiole*	Koping.	6593063.64	560544.795	KO	Aug-23
<i>Tolypocladium</i> sp.	other	F8	petiole*	Koping.	6593063.64	560544.795	KO	Aug-23

Lake Mälaren Continued

Organism ID	Predicted Ecology	Isolate ID	Plant Part	Sample Origin	North	East	Sample label	Date m-y
<i>Alternaria sp.</i>	Pathogen	F104-2	blade	Kungsör	6588223.61	562239.27	U-län11	Oct-24
<i>Pythium sp.</i>	Pathogen	F104-1	blade	Kungsör	6588223.61	562239.27	U-län11	Oct-24
<i>Filosporella sp.</i>	other	F41R-3	rhizome	K-torp.	6588533.42	562267.112	na	Jun-24
<i>Filosporella sp.</i>	other	F41R4	root	K-torp.	6588533.42	562267.112	na	Jun-24
Helotiales	other	F212	root	K-torp.	6588533.42	562267.112	na	Jun-24
<i>Ilyonectria sp.</i>	Pathogen	F41L	blade	K-torp.	6588533.42	562267.112	na	Jun-24
Pezizomycotina	other	F41R	rhizome	K-torp.	6588533.42	562267.112	na	Jun-24
<i>Alternaria sp.</i>	Pathogen	F20B	blade*	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Alternaria sp.</i>	Pathogen	F14	blade	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Alternaria sp.</i>	Pathogen	F217-1	petiole	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Fusarium sp.</i>	Pathogen	F21-1	petiole*	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Fusarium sp.</i>	Pathogen	F22	petiole*	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Fusarium sp.</i>	Pathogen	F22P	petiole*	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Fusarium sp.</i>	Pathogen	F21P	petiole*	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Ilyonectria sp.</i>	Pathogen	F15	petiole	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Ilyonectria sp.</i>	Pathogen	F1	na	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Mucor hiemalis</i>	Possible	F217	petiole	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Mucor hiemalis</i>	Possible	F217-2	petiole	Kvik.	6592020.52	574613.474	KV	Aug-23
Pleosporales	Possible	F205	petiole*	Kvik.	6592020.52	574613.474	KV	Aug-23
<i>Filosporella sp.</i>	other	F103-2	petiole	Munk.	6590522.66	573526.21	U-län8	Oct-24
<i>Filosporella sp.</i>	other	F103-4	petiole	Munk.	6590522.66	573526.21	U-län8	Oct-24
<i>Pythium sp.</i>	Pathogen	F102	petiole	Munk.	6590522.66	573526.21	U-län8	Oct-24
<i>Pythium sp.</i>	Pathogen	F103-6	petiole	Munk.	6590522.66	573526.21	U-län8	Oct-24