



## EMERGING THREAT IN ITALIAN WATERS: FIRST DETECTION OF THE ROSETTE AGENT *SPHAEROTHECUM DESTRUENS* AND ITS EXPANDING FISH HOST RANGE

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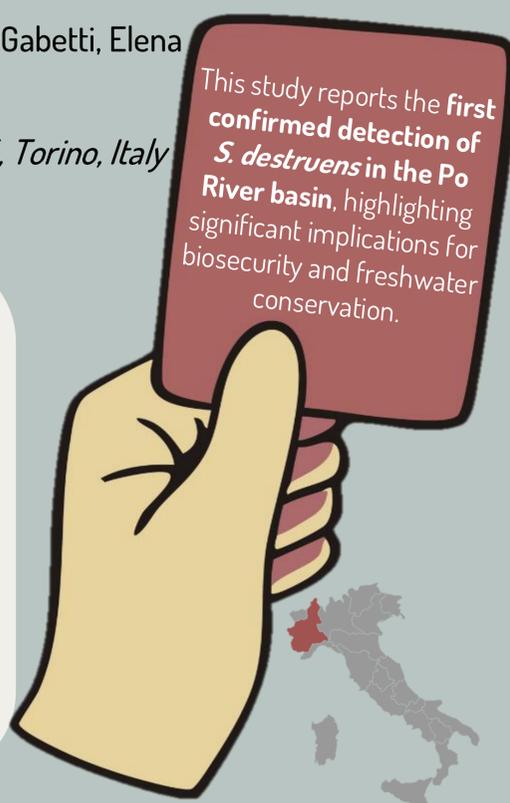
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### Introduction

Emerging aquatic pathogens represent a growing threat to freshwater biodiversity, aquaculture, and ecosystem stability. *Sphaerothecum destruens* (the "Rosette Agent") is an **obligate intracellular parasite** with a broad host range, high pathogenicity, and a capacity for cryptic spread via asymptomatic carriers, most notably the invasive topmouth gudgeon (*Pseudorasbora parva*).

*S. destruens* has been implicated in **severe population declines across Europe**, with mortality rates exceeding 90% in some aquaculture systems. Its wide environmental tolerance, prolonged spore survival, and frequent absence of early clinical signs render detection and control particularly challenging.

The Po River basin, Italy's largest and most ecologically and economically significant freshwater system, is home to diverse native and non-native fish communities, providing a critical context for assessing the emergence and spread of the pathogen under investigation.



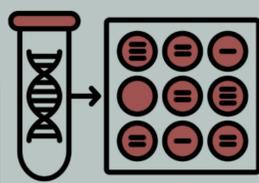
### Material and methods



Fish were sampled on 20 August 2024 at three sites along the metropolitan stretch of the **Po River** near Turin (Moncalieri, Murazzi, and San Mauro),



Sampling was performed using standardized **electrofishing** (CEN EN 14011:2003). **Kidney, spleen, and liver tissues were isolated for molecular analyses.**



DNA was extracted using Nucleospin Tissue kit (Macherey- Nagel). The primers **SdACH F** and **SdACH R**, and probe **SdACH P**, targeting the **18S rRNA gene** of *S. destruens*, designed by Combe et al. (2022), were used in **real-time qPCR**.



The validated assay had a limit of detection of 25 gene copies and a limit of quantification of 100 gene copies. Each sample was analysed in triplicate. Samples were classified as **potentially positive (Cycle threshold (Ct) > 38)** or **positive (Ct ≤ 38)** when at least one replicate showed amplification.

### Results and discussion

- All sites were dominated by non-native species (e.g., *Barbus barbus*, *Rhodeus sericeus*, and *Pseudorasbora parva*)
- S. destruens* was detected in both native (*Padogobius bonelli*) and non-native (*P. parva* and *B. barbus*) fish species.

**Table 1.** Fish species identified as positive or potentially positive based on cycle threshold (Ct) values; rep1, rep2, and rep3 indicate the corresponding replicates.

Station	Species	Ct_rep 1	Ct_rep 2	Ct_rep 3	Detection
Moncalieri	<i>Barbus barbus</i>	NA	NA	37.80	<b>Positive</b>
Murazzi	<i>Barbus barbus</i>	37.17	37.07	38.62	<b>Positive</b>
	<i>Barbus barbus</i>	NA	NA	41.08	<b>Uncertain</b>
	<i>Barbus barbus</i>	NA	40.13	NA	<b>Uncertain</b>
	<i>Pseudorasbora parva</i>	NA	NA	40.18	<b>Uncertain</b>
	<i>Padogobius bonelli</i>	39.33	38.84	40.17	<b>Uncertain</b>
San Mauro	<i>Barbus barbus</i>	37.56	38.21	36.5	<b>Positive</b>
	<i>Barbus barbus</i>	34.72	34.75	35.17	<b>Positive</b>



The detection of *P. parva* (a known carrier) underscores its key role in the introduction and spread of *S. destruens* in Italy, marking the **first documented occurrence in the region**.



*S. destruens* prevalence varied across sites, ranging from 3.45 % at Moncalieri to 19.23 % at Murazzi and 5.26 % at San Mauro. Species-specific prevalence was high, with 42.86 % for *Barbus barbus* at Murazzi and 50 % for *P. parva*, highlighting **strong site and host dependent variability in infection patterns**.

This study documented the **first detection of *S. destruens* in *B. barbus* and *P. bonelli***, thereby expanding the known host range of the pathogen.



The absence of visible clinical signs in infected fish supports evidence that infections often remain subclinical, enabling silent persistence and spread.



Asymptomatic invasive species facilitate the silent spread of *Sphaerothecum destruens*, posing a serious but often undetected threat to native fish biodiversity. **Proactive molecular surveillance and integrated biosecurity strategies are essential to prevent long-term ecological damage.**



**MORE INFO**

### References

Combe, M., et al., 2022. Towards unravelling the rosette agent enigma: spread and emergence of the co-invasive host-pathogen complex, *Pseudorasbora parva*-*Sphaerothecum destruens*. *Sci. Total Environ.* 806, 150427.

### Contacts

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