

New report of *Craspedacusta sowerbii* (Lankester, 1880) medusae in Belgium

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ABSTRACT. The jellyfish *Craspedacusta sowerbii* (Lankester, 1880) is known to be an invasive species in freshwater worldwide, except in Antarctica. In Belgium, *C. sowerbii* was first recorded in 1939 by Damas in the River Meuse. Since then, few further Belgian observations of this species have been made. Here, we report the presence and determine the gender of *C. sowerbii* medusae in an artificial pond in Forrières, in southern Belgium. This is the first record of this species in the province of Luxembourg. Our findings suggest that the population consists exclusively of females.

Keywords: *Craspedacusta sowerbii*, freshwater jellyfish, invasive species

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1. Introduction

Craspedacusta sowerbii (Lankester, 1880) (Cnidaria: Limnomedusae: Olindiidae) is a freshwater jellyfish considered to be an invasive species. Native to the Yangtze valley in China, *C. sowerbii* is now found on every continent except Antarctica (Dumont, 1994; Oualid et al., 2019). The life cycle of *C. sowerbii* includes both an attached polyp and swimming medusa (jellyfish) stage. However, most of the studies provided observations of the medusa form only. The polyp form is much more difficult to observe because it is only about a millimetre in size. In Belgium, *C. sowerbii* was first recorded in 1939 by Damas in the River Meuse. In his work, Damas reported finding numerous polyps of *C. sowerbii* attached to stones at the riverbed. He observed the jellyfish only in June and noted that the specimens were exclusively young, non-sexual specimens not exceeding 1.5 mm in size. Since then, there have been few scientific publications mentioning the presence of *C. sowerbii* in Belgium, though specific locations were not always provided (Parent, 1982; Dumont, 1994). Additionally, three sightings have been reported by naturalist groups and in local magazines targeted at the general public. The first concerns an artificial pond located in Anderlecht, a municipality in the city of Brussels (Symoens, 1953). The other two were reported in artificial environments: Lake Eau d'Heure in 2012 in

the province of Hainaut and, a year later in 2013, in the Kelchterhoef pond in the province of Limburg (Notre Nature, 2021).

In this work, a new record of *C. sowerbii* from an artificial pond in Southern Belgium is reported. Morphological methods were used to identify and sex the jellyfish.

2. Material and methods

2.1. Study area

During July of 2024, *C. sowerbii* medusae were observed in a small artificial pond constructed in 2006 in the Luxembourg Province. The pond is located in the village of Forrières at 202 m a.s.l. (50°07'58.81"N; 5°16'48.26"E), has a maximum depth of about 1 m and covers an area of around 3,000 square meters (Fig 1A, B). The water temperature, pH and conductivity were measured using a Flintronic multiparameter probe (EZ9908). NO²⁻, NO³⁻, GH, KH and Cl₂ values were roughly estimated using JBL test strips for water analysis.

2.2. Observation of live jellyfish

The high turbidity of the water limited visibility to just a few centimetres below the surface, allowing

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observation only of jellyfish near the water's surface (Fig. 1C). A visual inspection of the jellyfish distribution was undertaken throughout the pond. On July 11, four specimens were collected and placed in a 20-litre aquarium for 10 days to photograph and record videos using a Canon M50 digital camera. The aquarium was maintained at room temperature, with no artificial lighting or filtration. Daily, one litre of water was replaced with fresh pond water to provide the necessary plankton for feeding the jellyfish. On the tenth day, the jellyfish were fixed in a 4% formalin solution. A further 17 specimens were then captured and immediately fixed in the same solution.

2.3. Microscopic investigations

One specimen was dissected to isolate the tentacles and gonads. Tentacles were placed on a glass slide, stained for 5 minutes with haematoxylin and washed with fresh water. Gonads were also placed on a glass slide, stained for 5 minutes with haematoxylin, washed with fresh water, stained for 5 minutes with 2% eosin and washed again with fresh water. The tissues were covered with a coverslip and gently squashed so that they could be observed under the light microscope Swift SW380T equipped with a Canon M50 camera. The twenty other specimens were observed without staining under a Bresser Analyth STR stereomicroscope used with transmitted light and equipped with the same camera. The diameter of the jellyfish was measured on individuals fixed in formalin.

3. Results

3.1. Observation of live jellyfish

The jellyfish were observed *in situ* for a period of three weeks. They were visible only on the surface of the water. Nevertheless, we were able to collect them on the bottom of the pond using a net in places where they were not visible on the surface. Their location varied from day to day, but they were observed in all parts of the pond during this period. The high turbidity of the water made it impossible to assess the density of the populations, but we never saw more than three jellyfish per square metre. The average temperature of the pond was 23.5°C, pH 8.2, conductivity was 451 μ S. Nitrite, nitrate and chloride ions were undetectable, hardness (GH) was between 125 and 250 ppm and alkalinity (KH) was around 250 ppm. In the aquarium, the jellyfish were active day and night, swimming actively towards the surface and dropping slowly to the bottom.

3.2. Morphology and sex determination

The average diameter of fixed specimens was 15 \pm 3 mm, ranging from 10 to 20 mm. Boothroyd et al. (2002) report that the average shrinkage due to 4% formalin is more or less 10%.

The taxonomic status of the jellyfish was determined according to the descriptions of Jankowski (2001) and Luskow et al. (2021). The manubrium extends onto the subumbrella (Fig. 2A), four gonads

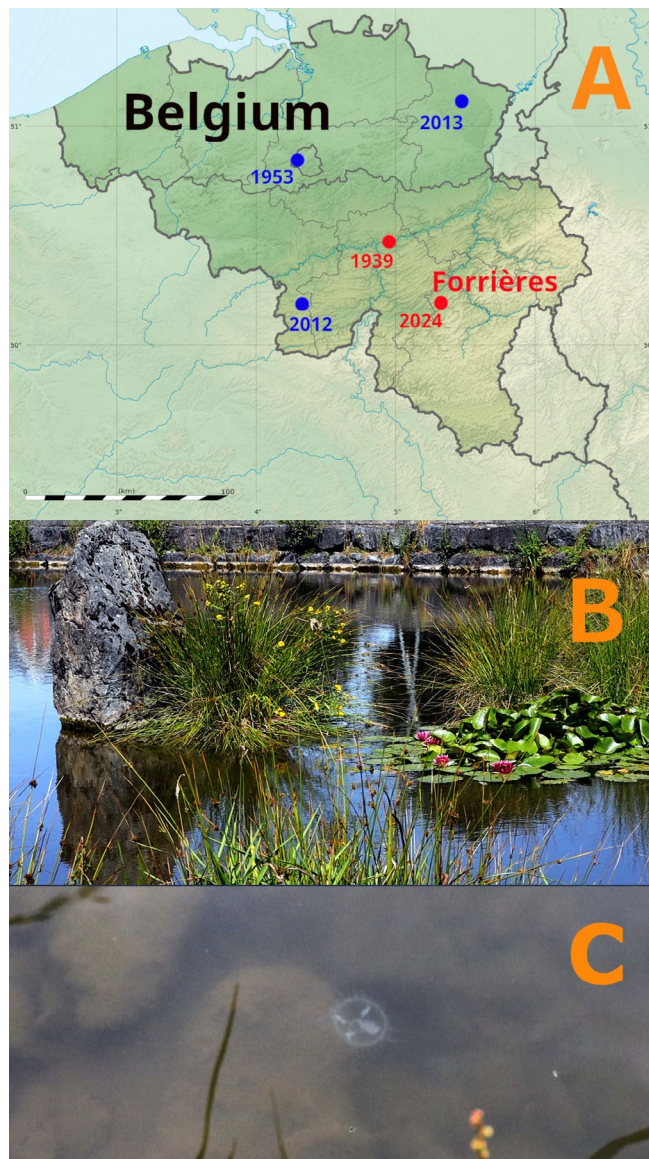


Fig.1. a) Location of Forrières on the map of Belgium. Blue dots : naturalist publications, red dots : scientific sightings. b) General aspect of the pond. c) Mature medusa videotaped during an observation on 25 July 2024.

are suspended along the radial canals (Fig. 2A, B), and four long perradial tentacles are visible in the extension of the radial canals (Fig. 2B). On the surface of the tentacles, the nematocysts are grouped together in plates (papillae), arranged in more or less complete parallel rings (Fig 2C, D).

The gonads of the 20 jellyfish were examined under the transmitted light stereomicroscope to detect oocytes. The gonads are elongated and flattened laterally. They are larger near the edge of the umbrella and smaller near the stomach (Fig. 2E). All gonads of the 20 jellyfish contain oocytes between 50 and 150 μ m in size (Fig. 2F). They appear dark and are clearly visible inside the gonad (Fig. 2E). All the jellyfish sampled were therefore female. The ovaries of the smallest jellyfish (10 mm in diameter) contain far fewer oocytes (Fig. 2G). In larger individuals, the number of oocytes appears to be highly variable (from 10 to more than 300 oocytes per gonad, Fig. 2H).

4. Discussion and conclusion

There is very few scientific literature documenting sightings of *C. sowerbii* in Belgium. This is the first time this jellyfish has been found in Forrières and even in the province of Luxembourg. The origin of this population is unclear, but it is likely related to the introduction of polyps or resistant forms known as podocysts. Under adverse conditions, *C. sowerbii* polyps can encyst, forming a chitinous shell. This podocyst stage can endure complete desiccation for up to 40 years (Bouillon and Boero, 2000). Podocysts can be carried by plants or animals, such as birds or fish (El Mousaoui, 2015). This may explain the presence of *C. sowerbii* in Forrières, where ornamental water lilies are present and where ducks, geese and grey cranes (*Grus grus*) are frequently observed. Additionally, local fishermen regularly bring in fish.

In temperate zones, the jellyfish occurrence generally coincides with the warmest temperatures (DeVries, 1992; Purcell, 2005). The Forrières pond is very shallow and its temperature can easily rise in summer, even in depth. This could explain the appearance of jellyfish. Some researchers therefore suggest that the increase in the number of sightings of the jellyfish form is due to recent climate change (Lundberg and Svensson, 2003; Marchessaux et al., 2022). However, there is currently no evidence of this.

The jellyfish collected in Belgium measured 10–20 mm in diameter (fixed specimens). Considering an estimated 10% shrinkage in 4% formalin, their in vivo size would range from approximately 11 to 22 mm. These dimensions are fully consistent with the typical size range reported for *Craspedacusta sowerbii*, whose medusae generally reach 5–25 mm and occasionally up to 25–30 mm under warm conditions (25–30 °C) (Lüskow et al., 2021). Therefore, the Belgian specimens fall within the expected range for this species and correspond to the medium-sized, sexually mature individuals frequently recorded in lentic environments of temperate regions.

Determining the sex of *Craspedacusta sowerbii* fixed in formalin requires microscope examination of the gonads in sexually mature individuals. All specimens examined from the pond in Forrières were available for sex determination and were of the same sex: female. Our observations are consistent with other studies (Boothroyd et al., 2002). Outside of China, very few reports have documented populations with medusae of both sexes, e.g.: a pond in Southampton Quarry at Richmond, USA (Rice, 1958), Lake Mead, Nevada, USA (Deacon and Haskell, 1967), Haruta-ike, an artificial pond in Chikuma City, Nagano, Japan (Peterson et al., 2022), Lake Santo of Monte Terlagio, Italy (Morpurgo et al., 2024).

Several hypotheses may explain the unisexual character of the population observed at Forrières. It may result from a founder effect, where genetic factors determine sex, and a single polyp or podocyst gives rise to medusae of only one sex. In 1924, Payne proposed parthenogenetic development of the eggs of *Craspedacusta ryderi*, but true parthenogenesis, i.e. the development of unfertilized eggs, has never been

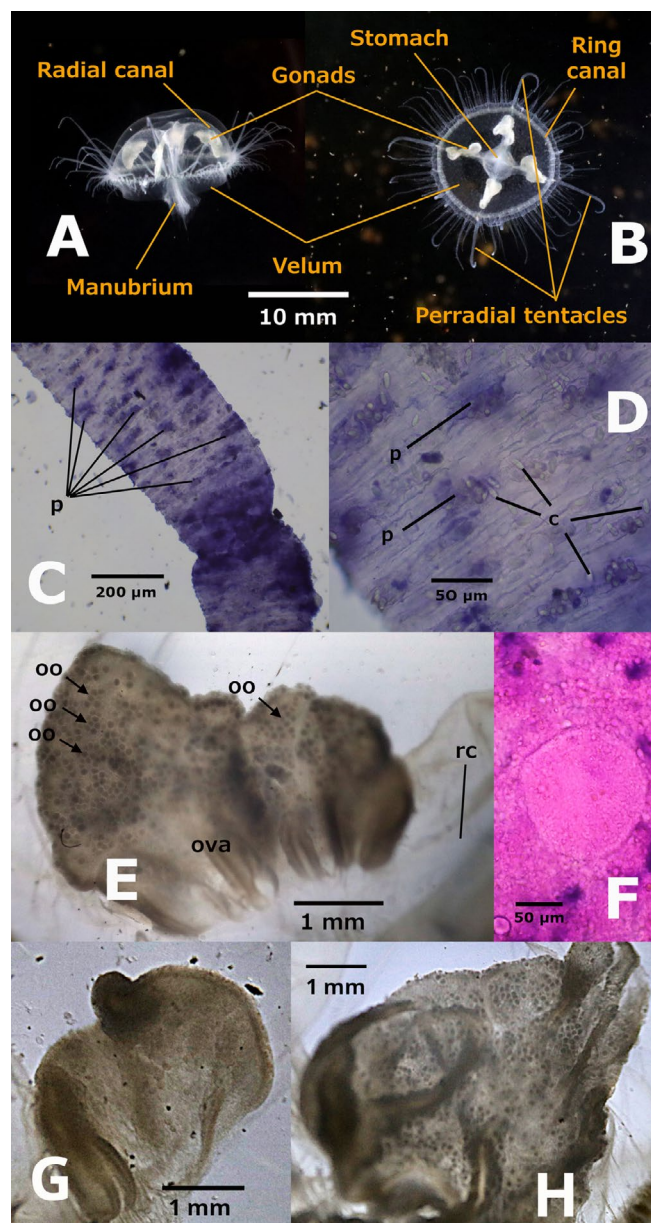


Fig.2. A-B. Side view (A) and top view (B) of the *C. sowerbii* medusae observed in aquarium. C. Tentacle showing the circular arrangement of the nematocyst plates or papillae (p). D. High magnification of papillae (p) showing cnidocytes (c). E. General view of the gonads: ovary (ova) showing oocytes (oo). rc = radial canal. F. High magnification of a large oocyte stained with hematoxylin/eosin. G. Ovary of small individual (10 mm in diameter) with few, sparsely distributed oocytes. H. Ovary of large individual (20 mm in diameter) with numerous oocytes at various stages of maturation.

demonstrated in *C. sowerbii*. In 2000, Carré and Carré reported that the sex of medusae in the genus *Clytia* is temperature-dependent, suggesting that environmental factors can influence sex determination in some hydrozoans. Recent molecular analyses revealed the existence of distinct genetic lineages within the *C. sowerbii* species complex, some of which appear to correlate with the sex of medusae (Schachtl, 2019; Wang, 2022). While environmental factors may modulate the timing and maturation of gonads, they do not satisfactorily explain the widespread unisexual swarms, which are best interpreted as the result of an underlying genetic mechanism; genome-based sex markers are still needed to confirm the exact pathway.

Our report indicates the presence of *C. sowerbii* in a Belgian region where it has not yet been described. It is the first report in Belgium that specify the sex of the *C. sowerbii* jellyfish population observed. It would be useful to survey other ponds in the region to assess the extent of the invaded areas. To do this, polyp forms should be detected.

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Conflict of Interest

The authors declare no conflicts of interest.

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