JOURNAL of CONCHOLOGY

1 May 2024

Published by the Conchological Society of Great Britain and Ireland, established 1874

SHORT COMMUNICATION

Love story or fling? The first observation of *Vorticella* sp. (Ciliophora: Peritrichia) on the freshwater snail *Gyraulus rossmaessleri* (Auerswald, 1852)

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Abstract. Shells of *Gyraulus rossmaessleri* (Auerswald, 1852) infested with the bell-shaped peritrich ciliate *Vorticella* sp. were found in small, flooded soil depressions located in a floodplain meadow in the Upper Rhine Valley (north-eastern France). The observed prevalence was 100%. Analysis of the distribution of *Vorticella* aggregates on planispiral shells shows that the epibionts occur largely in the pseudo-cavities formed by the apex and umbilicus and mainly along the suture. This is the first case of ciliated epibionts on gastropods associated with temporary freshwater habitats inhabited by drought-adapted fauna. However, given the scarcity of observations of *G. rossmaessleri* in this region, it is difficult to affirm whether this epibiosis is relatively common.

Key words. Drought resistance, floodplain meadows, Planorbidae, refuge habitat, Upper Rhine Valley

ZooBank identifier. urn:lsid:zoobank.org;pub:3EA07371-5C27-4CA7-8DE5-8BC352A01346

DOI. https://doi.org/10.61733/jconch/4512

Mollusc shells can provide hard substrates for the colonisation and establishment of a wide range of organisms, from algae, bacteria, protists to macro-invertebrates, in both freshwater and marine habitats (Gutiérrez et al. 2003). Some of these biotic interactions include epibiosis, that is, non-parasitic facultative associations involving epibionts, organisms that remain attached to the surface of living substrates, the basibionts, during the sessile phase of their life cycle (Wahl 1989, 2009). While a large diversity of epibionts has been recorded on many marine gastropod and freshwater bivalve species (Abbott & Bergey 2007), only a few studies have reported gastropod-ciliated protist epibiosis in freshwater environments (López-Ochoterena 1964; Dias et al. 2006, 2008, 2010; Utz 2007; Sartini et al. 2018; Chartterjee et al. 2021) and none, to my knowledge, in temporary waterpool habitats, suggesting strong adaptations for both basibionts and epibionts (Strachan et al. 2015). In this short communication I report a case of epibiosis between the planorbid Gyraulus rossmaessleri (Auerswald, 1852) and a peritrich ciliate of the genus Vorticella Linnaeus, 1767. The basibiont, G. rossmaessleri is a widespread Euro-Siberian species found mainly in temporary water bodies (Vinarski

et al. 2006; Glöer 2019), which reaches its western limit in north-eastern France in the Upper Rhine Valley (Richling & Groh 2014; Umbrecht & Bichain 2018; Bichain et al. 2023). In this area, the species is strictly limited to the region known as the Ried, which comprises a mosaic of floodplain meadows and forests crossed by a dense network of rivers, springs, and phreatic streams (Sell et al. 1998). Typically, Ried ecosystems are permanently flooded during periods of rain and/or snowmelt from autumn (November) to spring (March–April), followed by a complete drying of the soil surface, also more or less continuous, from May to October. Snails can survive droughts in the wettest microhabitats, such as soil depressions, by withdrawing deep into their shell, which they close with an epiphragm. The epibiont, Vorticella species are suspension-feeding ciliates that live in two forms: the free-swimming telotroch and the sessile stalked trophont. The latter has contractile myonemes that allow Vorticella to pull the cell body, also called the zooid, against substrates. According to Warren (1986), in some species, such as Vorticella microstoma (Ehrenberg, 1830), a cyst can form around the body cell and detach from the stalk, allowing the encysted zooid to survive drought conditions for several weeks (Ranasinghe & Amarasinghe 2020). When favourable conditions return, the cyst breaks open, releasing the telotroch, which swims free before settling on a substrate where it develops a stalk and grows to full size.

These two freshwater organisms, both adapted to dry conditions, are potentially able to occupy the same temporary water bodies. However, their interaction was never formally recorded until opportunistic sampling during a field study focussed on the distribution of *G. rossmaessleri* in north-eastern France (J.-M. Bichain unpublished data). The study area is in the Central Ried (Bernhard *et al.* 1992) about 30 km south of Strasbourg, France. Sampling was carried out on 3 November 2023 after the first autumn rains, which started on 18 October 2023. The sampled habitats are small, flooded soil depressions (Fig. 1A, B) at the edge between a wet oligotrophic grassland with *Molinia caerulea* (L.) Moench (EUNIS 2012 code: E3.511; EU Habitats

Directive code: 6410) and a small (c. 10,000 m²) Phragmites Adans. reed bed normally without free-standing water (EUNIS 2012 code: D5.11). Specimens were collected using a fine mesh net (500 μ m) and transported live to the laboratory. The epibionts on the shell of one specimen were scraped off (Fig. 1C-E) and photographed in vivo using a Tucsen 20 MP digital camera mounted on a Nikon YS100 microscope. The corresponding snail was removed from its shell and dissected to observe the diagnostic anatomical features of the reproductive system to confirm its specific identity (Meier-Brook 1983; Glöer 2019). The shells of all other snails were also examined to identify potential epibiont species diversity. After fixation in 70% ethanol, shells were photographed in two standard positions-upper side (upper shell surface of a crawling snail) and lower side (ventral shell surface of a crawling snail) (Fig. 2A, B)—using a digital camera mounted on a Motic SMZ171 stereomicroscope.



Figure 1. Sampling site of *Gyraulus rossmaessleri* in the Central Ried, north-eastern France. **A**, oligotrophic grassland with *Molinia caerulea* and a small *Phragmites* reed bed (geographical coordinates of the sampled site: 48.3311°N, 7.6415°E, altitude 158 m). **B**, flooded soil depressions where *G. rossmaessleri* was sampled at the edge of the reed bed (white arrows). **C**, epibionts on the upper side of the basibiont shell. **D**, details of the epibiont aggregate on the upper side of the basibiont shell. **E**, bell-shaped epibiont, *Vorticella* sp.

The maximum diameter and the flat surface area of each shell were measured using ToupView v. 3.7.7892-2016, as well as the total surface area occupied by epibionts. Finally, a grid was digitally superimposed on each photograph to estimate the epibiont distribution on the different surface areas of the shell, as shown in Figure 2.

Twenty snails, corresponding well to the species description of *G. rossmaessleri*, were collected. Shell diameters ranged from 2 to 4 mm (n = 20, mean = 2.64, standard deviation = 0.61), and only one epibiont species, belonging to the genus *Vorticella*, was found on them.

All shells (n = 20) were colonised by *Vorticella* on at least one side, including 13 on both sides. Epibiont frequency on the upper shell surface was 80%, with the area occupied ranging from 0.1% to 14.6% of the total shell surface (n =16, mean = 4.2%, standard deviation = 4.3%). The epibiont frequency on the lower side was 85%, with the occupied area ranging from 0.1% to 14.9% of the total shell area (n =17, mean = 5.6%, standard deviation = 5.3%). There is no significant correlation between the total shell surface area and the surface area occupied by epibionts (n = 20, Pearson correlation coefficient = 0.004). However, epibionts do not appear to be randomly distributed, but rather, they show a striking presence along the suture, from the first whorl on and near the protoconch (81.2%) and into the umbilicus (77.1%) to the edge of the aperture (Fig. 2A–C). Epibionts



Figure 2. Schematic representation of the distribution of *Vorticella* sp. on the shell of *Gyraulus rossmaessleri*. **A**, *Vorticella* distribution on the upper side (n = 16). **B**, *Vorticella* distribution on the bottom side (n = 17). **C**, upper and bottom distribution plotted on lateral view, the dotted lines indicate the pseudo-cavities in the upper and bottom sides. **D**, box plots showing the interquartile range (minimum, first quartile, median, third quartile, and maximum) of the surface area occupied by *Vorticella* sp. on the upper (**A**) and (**B**) lower sides.

tended, although not significantly, to occupy a larger area of the lower shell surface (Fig. 2D).

Few cases of epibiosis between freshwater gastropods and ciliated protists (peritrichids and/or suctorians) have been formally described. So far, six nominal species are listed as basiobionts: Physella acuta (Draparnaud, 1805) in a eutrophic stream from Brazil (Sartini et al. 2018); Lymnaea atenuata Say, 1829 (Ladislavella elodes (Say, 1821) according to MolluscaBase 2024), and Physella osculans (Haldeman, 1841) from a lake in Mexico (Lópes-Ochoterena 1964), ampullariid apple snails from southern Brazil (Dias et al. 2006, 2008, 2010; Utz 2007) with Pomacea lineata (Spix, 1827) (Pomacea linnaei (Philippi, 1852) according to MolluscaBase 2024), Pomacea figulina (Spix, 1827) (junior synonym of Pomacea canaliculata (Lamarck, 1822) according to MolluscaBase 2024), and Pomacea canaliculata (Lamarck, 1822), as well as Pomacea bridgesii (Reeve, 1856) from India (Chatterjee et al. 2021), but probably a misidentification, possibly introduced Pomacea diffusa Blume, 1957, or a species of the genus Pila Röding, 1798 (Robert Cowie personal communication).

In this study, a high (100%) single-species epibiont prevalence was found on the planorbid, G. rossmaessleri. Dias et al. (2006, 2008) also observed a high incidence of ciliate protist infestation on the Pomacea spp. (82.6% to 100%), while Sartini et al. (2018) recorded 60.7% on the invasive Physella acuta. However, all these studies reported infestation of basibionts by several epibiont species from different trophic levels. According to Dias et al. (2006), gastropod shells may therefore provide microenvironments for communities of ciliated protists where complex trophic interactions occur. For example, some epibionts can feed on bacteria and/or algae on the shell surface (e.g. peritrichids), while others are predators (e.g. suctorians) of other ciliates and/or are basibionts (hyperepibiosis) of other organisms, such as rotifers (Chatterjee et al. 2021). Two species of peritrichid ciliates (Vorticella sp. and Epistylis sp.) were observed on shells of Gyraulus sp. collected in Lake Virpazar, Montenegro. Some shells were occupied by a single species, others by both (Michal Horsák personal communication).

In my sample, only one epibiont species seems to be present. The first factor that could explain this single-species epibiont community is probably related to this flood-todrought prone habitat, which is highly selective and severely limits the number of freshwater species that can live there. Indeed, for several months these small soil depressions dried up, as did the other habitats around them. The collected planorbid snails probably survived the dry period before regaining their full biological function with the first rains 15 days earlier. Apart from micro-crustaceans such as daphnids and copepods, no other invertebrates were found during sampling. This suggests that this environment is only colonised by fauna typical of ephemeral aquatic habitats (Strachan *et al.* 2015) and that this particular *Vorticella* species is probably drought resistant.

A second factor is probably the small size of *G. rossmaessleri* shells, which have a total surface area of about 5–20 mm², whereas *Physella* or *Pomacea* shells must have at least 10–500 times more surface area. Large surfaces provide more available microhabitats and therefore, fewer competitive interactions between potential epibiont species (Utz & Coats 2005).

In this study, I report that both the lower and upper sides of the G. rossmaessleri planispiral shells constitute pseudocavities (Fig. 2C) that are predominantly occupied by Vorticella along the suture. These pseudo-cavities probably provide protection for the epibiont from dislocation due to mechanical forces associated with the snail's movements. In addition, the suture also provides more favourable attachment sites while the angle formed between successive whorls makes the area near the suture more protected for cyst storage and/or telotroch attachment. This is consistent with the observations of Dias et al. (2006, 2008) and Sartini et al. (2018), indicating that epibiont distributions are not the result of simple random processes but are linked to complex structural features of shells (e.g. size, shape, ornamentation, and texture). Furthermore, experimental evidence suggests that peritrich ciliates actively select living substrates and attachment sites on basibionts (e.g. Fernandez-Leborans et al. 2006). The benefits for an epibiont to colonize living substrates are notably (i) to remain in favourable habitats when environmental changes occur (Dias et al. 2008) and (ii) to disperse with the basibiont, thus contributing to gene flow between populations (Wahl 1989).

Based on these observations, I construct the following scenario. With the first autumn rains, the soil of the swamps becomes saturated with water such that the soil depressions quickly flood and remain inundated after a few days. The quiescent snails become active again and the *Vorticella* cysts release the swimming zooids. Shells are rapidly colonised in these relatively confined habitats. Zooids attached to the edges of the last whorl become detached via general snail activity, while zooids protected along the suture zones can develop to maturity. Snails and their epibionts can be dispersed by successive floods during the rainy season, potentially colonising new habitats. The deepest soil depressions also provide frost-free water in winter. When the water recedes in early summer, the snails and their epibionts withdraw into these depressions, which are the first places to flood and the last places to dry. These depressions remain moist when the surrounding environment is completely dry.

Little is currently known about the biology and ecology of *G. rossmaessleri*, and the distribution of populations in France is still poorly understood. Further research is needed to determine whether this epibiosis is common in these floodplain meadow ecosystems or whether it is purely coincidental.

ACKNOWLEDGEMENTS

I would like to thank Robert Cowie, Adrienne Jochum, and Julien Ryelandt, as well as Michal Horsák and an anonymous reviewer for their corrections and comments on the manuscript. This study is part of the "Grand Est" Regional Action Plan for freshwater molluscs (Action A03 of LIFE Biodiv'Est, France, Grand Est).

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