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# Epibiotic Relationship Between Peritrich Ciliate *Vorticella* spp. Dujardin, 1841 and Aquatic Duckweed *Lemna minor* L. Roots in Freshwater Pond

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ABSTRACT

An epibiosis between ciliate *Vorticella* spp. and the roots of aquatic plant *Lemna minor* in a freshwater pond proved that ciliates generally employing them as an anchor substrate while at the same time filtering suspended particles from the passing water. The duckweed is floating aquatic plants that offer lush microbial fauna to the ciliates. This association shows a commensal relationship, in which *Vorticella* spp. adhere to duckweed roots as a substrate for colonization and thereby gain access to a nutrient-rich microhabitat of pond water and strain food particles without any damage to aquatic weeds, but duckweed does not seem to derive any direct advantage from the association with *Vorticella* spp. Number and size of duckweed roots influence the colonization of ciliates, both in cluster and solitary. The present observational study confirms that *Vorticella* spp. has a permanent epibiotic association with roots of Lemna minor in freshwater ponds. Colonies and single individuals are present in different root lengths (11–27 mm), with longer roots having more individuals based on larger attachment and aggregation surface area. Root length does not limit colonization; short and long roots are both effective substrates for attachment. The widely and uneven distribution of clustered colonies suggests cooperative advantages such as efficient feeding and defence. In general, Vorticella exists as an opportunistic epibiont on the duckweed root, which is ecologically significant for a renewable habitat for protozoan ciliates.

## 1. Introduction

An epibiotic association between aquatic plant and protozoa plays a significant function in keeping the biotic community intact and facilitating nutrient cycling in aquatic communities. A common example is an epibiotic association between the peritrich ciliate *Vorticella* and aquatic duckweed roots (*Lemna minor* L.). Peritrich ciliate genus *Vorticella* Dujardin [1] is a cosmopolitan and abundant protozoan, ecologically significant as members with their sessile lifestyle attaching by contractile stalks to any substrate within freshwater environments. The variety of ciliates, like *Vorticella* spp. harboured on the various living and moving surfaces, such as aquatic plants, insects, and molluscs, by which they get a new habitat and filter suspended particles, which enhances nutrient recycling in the microbial food webs [2]. Duckweed is a free-floating aquatic plant, commonly found in the freshwater ponds. The roots of duckweed provide a large surface area for a variety of microorganisms, especially protozoans for attachment which explore fresh nutrient rich aquatic habitat always. The epibiotic association of *Vorticella* with the roots of duckweed has a significant impact on nutrient cycles and microbial biology of aquatic environments [3]. The roots of duckweed offer a solid surface that is enriched by root exudates and organic particles, providing for microbial communities such as diverse protozoans. The occurrence of *Vorticella* on *Lemna* roots is an epibiotic association where *Vorticella* gains from having access to a nutrient-rich microhabitat and effective feeding currents provided by ciliary action, which permits suspension feeding from bacteria and other small particles in the water [4, 5].

This epibiotic relationship is predominantly viewed as commensal because *Vorticella* acquires a habitat without exerting evident injury to the host plant, with some research indicating possible indirect advantages like regulation of microbial communities in the vicinity of the roots, influencing the health of the plant. Freshwater ponds offer a vibrant condition where epibiotic interactions befit ecosystem function by unifying microbial and macrophyte habitats. The epibiotic association of *Vorticella* and duckweed roots is an ecologically important as it has significant influence on nutrient cycling and cleaning and purifying the aquatic ecosystem [6]. *Vorticella* shows stalked morphology to attach itself tightly on substrates like *Lemna* roots, where it finds favour of being able to access suspended organic particles via ciliary feeding currents, and duckweed roots being a sheltered place enriched with nutrients from root exudates and the surrounding water [4,7].

While the association is mostly considered to be commensal, with *Vorticella* gaining habitat and food without injuring the duckweed, the association has impacts on local microbial community structure and nutrient cycling at the plant-water surface. *Vorticella* epibiosis is research that contributes to the understanding of the ecological functions of peritrich ciliates as microhabitat colonizers and their possible impact on water and aquatic plant health. Additionally, the study of this interaction contributes to an understanding of the diversity and distribution of protozoan epibionts in freshwater communities and their adaptive functions to being attached to dynamic biological surfaces like duckweed roots, floating at the surface of freshwater areas, allowing for oxygen exchange and nutrient uptake [6,7]. Freshwater ecosystems rely on such biotic association for maintaining microbial diversity as well as ecosystem functioning. Duckweeds are known for their fast growth, efficient nutrient uptake, and refuge to epiphytic organisms, such as ciliates [8-10].

## 2. Experimental Methods

The research was performed in a freshwater pond within the Isabella Thoburn College (26.871°N, 80.942°E), Lucknow, Uttar Pradesh, India, with typical lentic conditions and high development of the aquatic plant *Lemna minor* L. Sampling was done in the monsoon season (July to August 2025), when *Lemna minor* was at maximum abundance. Sampling of duckweed was done using a sterile glass beaker (wide-mouth) in order to cause minimum disturbance to the roots of the plants to maintain epibiotic communities. Single *Lemna minor* plants with healthy roots were gently isolated from the colony, and roots were rinsed gently with fresh water to eliminate contaminants. *Lemna minor* roots were viewed using a compound light microscope at 40x to 100x magnifications. *Vorticella* spp. was characterized morphologically by stalked peritrich features typical of the genus, such as contractile stalks, bell-shaped zooids, and ciliary

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structures, according to taxonomic keys of Warren [7], Foissner et al. [2], and Kovalchuk [3]. Photographs were obtained with a camera.

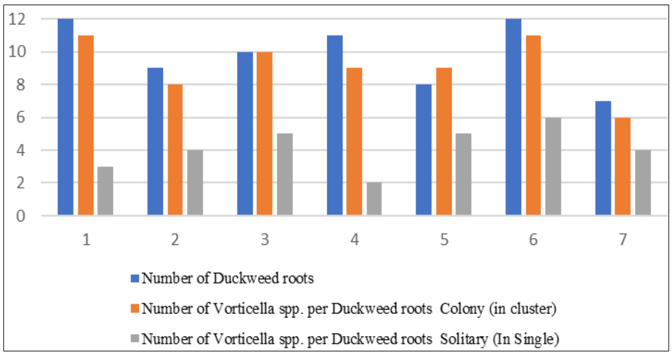
The colonization density of *Vorticella* colonies on *Lemna minor* roots was measured by counting the number of colonies per root or area from several randomly chosen root pieces. The length of the roots was taken with a microscale using a binocular microscope at 40x magnification [11]. Colonization types like cluster formation, size of the colony, and the sites of attachment on the roots were observed.

## 3. Results and Discussion

The *Vorticella* spp., peritrich ciliates, are a frequently occurring group that commonly colonize *Lemna minor* roots in aquatic pond ecosystems. Root samples of different lengths (approximately 11 to 38 mm) showed consistent colonization by *Vorticella*, with colony (clumped) and solitary forms occurring. There were varying numbers of *Vorticella* per root, ranging from more than ten individuals, showing a strong epibiotic community. Roots with higher mean length had more colonizing ciliates, which indicates that root surface area is a significant factor affecting colonization density [12].

**Table 1** Variation in *Vorticella* spp. colony and solitary on duckweed roots

| S. No. | Duckweed roots length (mm) | Number of Duckweed roots | Number of *Vorticella* spp. per Duckweed roots | |
|---|---|---|---|---|
| | | | Colony (in cluster) | Solitary (In Single) |
| 1 | 21.4± | 12 | 11 | 3 |
| 2 | 18.0± | 9 | 8 | 4 |
| 3 | 19.2± | 10 | 10 | 5 |
| 4 | 26.8± | 11 | 9 | 2 |
| 5 | 15.0± | 8 | 9 | 5 |
| 6 | 27.0± | 12 | 11 | 6 |
| 7 | 11.0± | 7 | 6 | 4 |




**Graph 1** Colonization of Vorticella spp. duckweed roots

Rooted duckweed samples range from 11.0 to 27.0 mm (Fig. 1b). There were 7 to 12 roots per duckweeds (Fig. 1b). It was observed that more *Vorticella* in clusters than in solitary conditions, suggesting a preference for *Vorticella* to aggregate upon colonizing duckweed roots. Both solitary (single) and colony (cluster) forms of *Vorticella* were observed (Table 1 and Graph 1). On the duckweed roots (21.4 mm, 19.2 mm, 27.00 mm), the colony/individuals per root is greater than the solitary, whereas on other roots (18.0 mm, 15.0 mm, 11.0 mm), solitary forms were moderate (Table 1; Fig. 1b). Longest roots, 27.0 mm (Table 1 and Fig. 1b), contained the greatest number of roots observed, twelve (12) in quantity (Fig. 1), and also the greatest numbers of *Vorticella* colonies, 11, while 9 were solitary. Moderate root lengths (21.4 mm, 26.8 mm, 19.2 mm) had intermediate *Vorticella* counts, reflecting that clustering is prevalent over a number of length categories. Shortest roots (11.0 mm) still accommodate quite a good number of *Vorticella* (6 in colony in clusters and 4 in solitary), indicating even duckweed roots of short lengths are successful substrates for colonization (Fig. 1b). Both shorter and longer roots can accommodate high numbers of *Vorticella* colonies, but absolute numbers will be higher on roots of bigger sample sizes. Duckweed roots of different lengths are good substrates for *Vorticella*, accommodating solitary and colonial ciliate stages. Dominance of clusters colonies showed aggregated colonization of duckweed roots, which provide ecological advantages to the protozoa viz feeding efficiency. Colonization by *Vorticella* (both solitary and clusters) was present along the full range of root length, from very short (11.0 mm) roots to longer roots (27.0 mm), this represent that root length does not limit colonization, though the greatest colony numbers are on longer roots (Fig. 1c), whereas solitary forms are always fewer than colonies, their occurrence on the root, shows some *Vorticella* remain single attachments, perhaps because of competition, spatial restraint and colony dispersal processes (Fig. 2b).

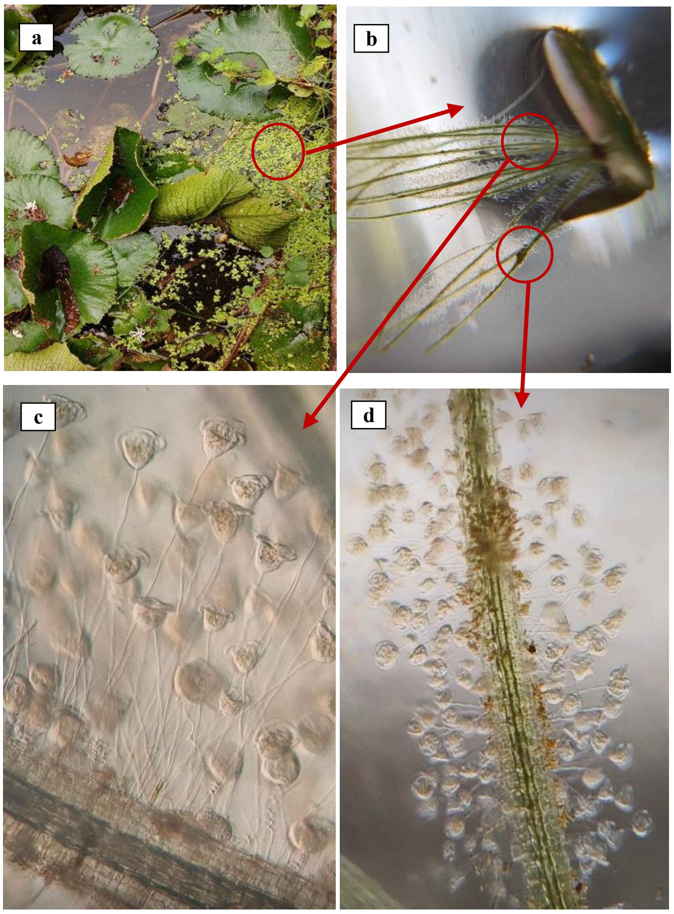



**Fig. 1** a) Fresh water pond with duckweeds; b) enlarge view to duckweed, *Lemna minor* with roots; c) & d) Colony of *Vorticella* spp. harboured on duckweed roots

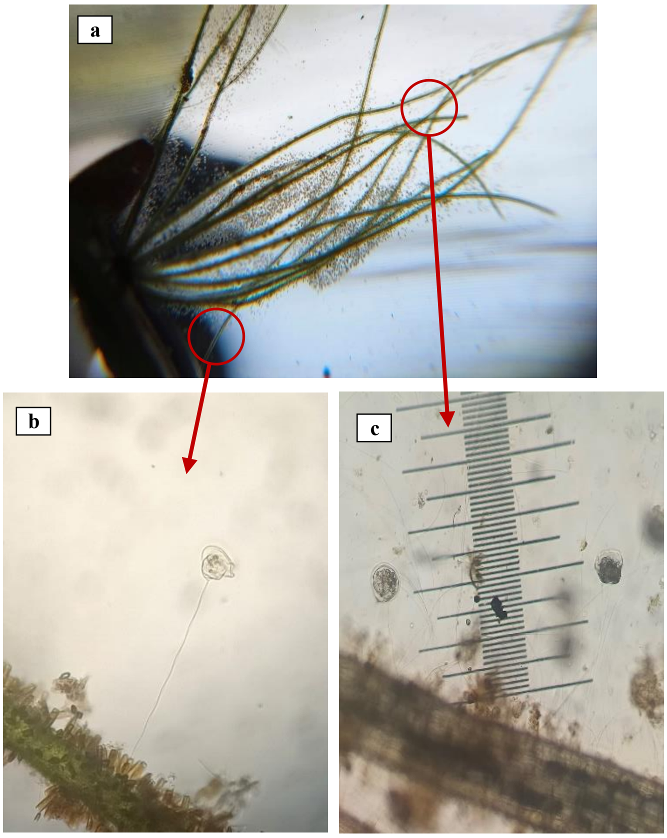



**Fig. 2** a) Enlarge view to duckweed, *Lemna minor* with roots; b) & c) solitary *Vorticella* spp. harboured on duckweed roots

*Vorticella* is a family of peritrich ciliates with an inverted bell-shaped body connected to a substrate by a long, thin, contractile stalk [1]. The bell-shaped body (zooid) is not connected anteriorly and is encircled by a ring of cilia massed in the peristomial zone surrounding the mouth. The stalk

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is fixed on surfaces like aquatic vegetation or rocks and has a contractile specialized organelle known as the spasmoneme that helps in the quick contraction of the stalk and movement. Cilia are dense around the mouth opening (peristome), arranged in a number of concentric rows in a whirl-like pattern (peristomial groove) (Fig. 3a). The cilia beat to create currents of water that help to drive food particles towards the cytostome (cell mouth), aiding in filter-feeding. The stalk and the main body beyond the peristome are devoid of cilia but possess kinetosomes for mechanical support (Figs. 3a and b).

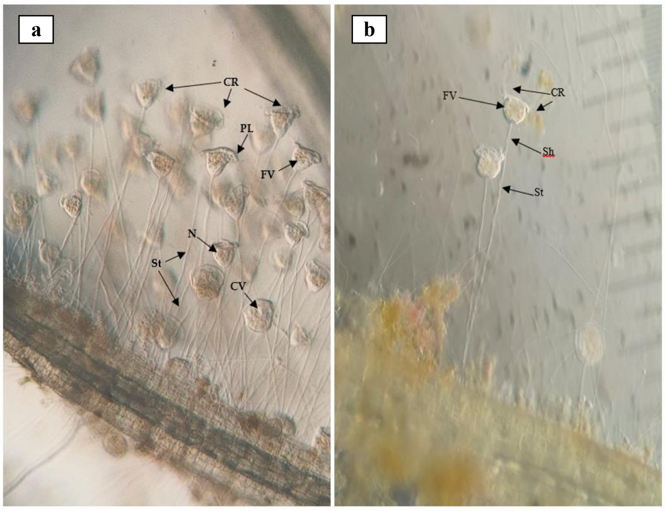


**Fig. 3** a) Colony of *Vorticella* spp. on duckweed root; and b) Solitary *Vorticella* spp. on duckweed root CR-ciliary row; PL-peristomial lip; CV-contractile vacuole; FV-food vacuole; N-nucleus; Sh-sheath; St-Stalk

The stalk is distinguished by its high contractility as a result of the spasmoneme, a helical bundle of contractile fibrils. When contracting, the stalk spirals as a spring, bringing the cell body toward the substrate; this is a defense or stimulus response (Figs. 3a and b). It has a big semicircular macronucleus responsible for metabolic activities, and a smaller micronucleus responsible for reproduction. Cytoplasm is separated into ectoplasm and endoplasm [7].

The observation data uncovers that *Vorticella* spp. is present on duckweed roots of different lengths, both in the form of colonies and solitary entities. Colony is predominant in all the length groups with values always comparable or higher than the solitary numbers, especially on roots of intermediate length like 19.2–27.0 mm. For very short roots (11.0 mm), solitary and colony counts are still similar, which is an indication that the substrate is not in any way limiting the colonization, but may impact aggregation dynamics. The roots measured between 21–27 mm, they support somewhat higher *Vorticella* densities (up to 11 colonies and 6 solitary), possibly because these root lengths would provide the best possible surface area or microhabitat for the attachment and feeding of ciliates. Least variation among clusters and single forms at low and high ends of root length emphasizes the colonization efficacy and versatility of *Vorticella* in exploiting the duckweed microhabitat.

A study shows that freshwater snail-inhabiting ciliated protozoans discovered that 85% of twelve live snails contained the peritrich ciliate *Epistylis niagarae*. The vacant snail shells did not contain any ciliates, as they were immobile. This abundance emphasizes that *Epistylis niagarae* establishes a certain biological association with living snails, in that they are mobile, offering a nutritious environment as opposed to merely colonizing any convenient hard substrate. Ciliated protozoa, *Epistylis*, are environmentally important, serving as pivotal elements of the aquatic food web and ecosystem, and they play a role in the general ecological processes of freshwater communities [3].

The epibiotic relationship of *Vorticella* spp. with *Lemna minor* roots is a common aquatic epibiotic association in which protozoa use plant surfaces for attachment, nutrition, and reproduction. The roots offer a firm substrate supplemented with microbial communities like bacteria and biofilms that serve as the food source of *Vorticella* [12-14]. The ciliate, *Vorticella*, has dual strategies for its survival in harsh ecological conditions. They harboured as solitary and colonial forms on the aquatic duckweed. The development of *Vorticella* colony in the clusters provides benefits such as increased feeding efficiency and stress resistance in diverse environmental situations, whereas solitary forms could facilitate dispersal to new substrates.

Epibiotic interaction promotes the adaptability of *Vorticella* in its existence with fluctuating pond conditions. The earlier research on colorization in ciliates in agglomeration emphasizes the function of submerged aquatic vegetation as microhabitats for varied communities of epibionts, which are highly active in nutrient circulation and energy transmission in freshwater ecosystem food webs [1, 15]. The intimate association between *Vorticella* spp. and *Lemna minor* roots reveals a commensal relationship in a pond ecosystem, where epibionts play an important role in the aquatic ecosystem [16]. The previous finding confirms that peritrich ciliates, especially *Vorticella*, are opportunistic epibionts and flourish on available substrata in aquatic conditions and gain an advantage from a nutrient-rich ecosystem [1, 17]. Colony in clusters improves feeding rates and enables the *Vorticella* to be protected from physical stresses, with solitary animals indicating dispersal and minimal competition for space. The adaptability of *Vorticella* on the duckweed root lengths is beneficial in dynamic aquatic environments. Duckweed roots offer a continually renewable, ubiquitous substrate in ponds that sustains recurring ciliate populations that play significant roles in microbial food webs and water quality improvement [10, 15, 18].

## 4. Conclusion

The present study demonstrates that *Vorticella* spp., a group of peritrich ciliates, exhibit a strong and consistent epibiotic association with the roots of *Lemna minor* in freshwater pond habitats. Colonization occurs across a wide range of root lengths (11–27 mm), with both colony (cluster) and solitary forms were observed, though colonies predominate. Duckweed roots with greater lengths supported large numbers of *Vorticella* colonies due to increased surface area that facilitates aggregation and firm attachment. The presence of both clusters of colonies and solitary individuals across all root sizes of duckweeds suggests that root length size is not a limiting factor for colonization; both short and long roots serve as effective substrates. Ultimately, the comparatively greater numbers on longer roots suggest increased scope for cooperative or space-aggregated colonization, offering ecological advantage in the form of more efficient feeding and self-protection. The results are pointing to the *Vorticella* spp., being opportunistic epibionts successfully thrive in the pond ecosystems and take advantage of both the biological and the physical characteristics of duckweed roots. *Vorticella* colonization in cluster patterns highlights the importance of aquatic macrophytes as renewable habitats for microbial communities, consequently maintaining ecological balance and promoting functional ecosystem processes.

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