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Abstract

Recently, we discovered complex horizontal surface currents above the polyps of stony coral colonies (class: Anthozoa, order: Scleractinia), which seem to play a crucial role in nutrition and epithelial cleaning. Following this initial discovery, however, the question arose whether other groups of cnidarians produce similar currents. This study investigates horizontal surface currents in 30 cnidarian species from 3 classes and 11 orders. Utilizing video microscopy and particle tracking of red fluorescent microbeads, the research reveals species-specific variations in the presence and dynamics of surface currents. Thirteen out of 30 species, including scyphozoans, showed observable currents, challenging the original assumption that these were exclusive to Anthozoa. Additionally, all studied Octocorallia (subclass) and Hydrozoa (class) species were found to lack horizontal surface currents. Statistical analyses and machine-learning algorithms identified significant differences in track current parameters among orders. This research contributes to bridging a century-long knowledge gap, offering evolutionary insights, and emphasizing the ecological significance of horizontal surface currents in cnidarians. We additionally discuss the potential biological functions of surface currents, such as food capture, nutrient transportation, and surface cleanliness. Limitations include species coverage and possible stress effects from microbeads. Future directions involve expanding taxonomic coverage and conducting field experiments for a holistic understanding of cnidarian behaviors. This study establishes a foundation for expanding the understanding of exploring cnidarian biodiversity and potential conservation efforts.

Zusammenfassung

Kürzlich haben wir komplexe Oberflächenströmungen auf den Polypen und Kolonien von Steinkorallen (Klasse: Anthozoa, Ordnung Scleractinia) entdeckt, die offensichtlich eine entscheidende Rolle bei der Nahrungsaufnahme und Reinigung spielen. Nach dieser anfänglichen Entdeckung kam die Frage auf, ob es auch andere Gruppen von Cnidarian gibt, die ähnliche Strömungen auf ihrer Oberfläche entwickeln. Diese Studie untersucht horizontale Oberflächenströmungen anhand von 30 Cnidaria Arten aus 11 Ordnungen und erweitert dabei den Fokus über die bereits intensiv erforschten Scleractinia hinaus. Durch den Einsatz von Video-Mikroskopie und der Nachverfolgung (tracking) roter fluoreszierender Mikroplastikperlen zeigen die Ergebnisse artenspezifische Variationen in der Ausprägung von Oberflächenströmungen auf. Bemerkenswert ist, dass bei 13 von 30 Arten, einschließlich der Schirmquallen (Scyphozoa), beobachtbare Strömungen festgestellt wurden, was die ursprüngliche Annahme herausfordert, dass diese ausschließlich für Anthozoa charakteristisch sind. Des Weiteren wurde festgestellt, dass sämtliche untersuchten Arten der Achtstrahlertiere (Octocorallia) und Hydrozoa keine horizontalen Oberflächenströmungen aufweisen. Mithilfe statistischer Analysen und von Machine-Learning-Algorithmen wurden signifikante Unterschiede in den Strömungsparametern zwischen den Ordnungen identifiziert. Diese Forschung trägt dazu bei, eine seit Jahrhunderten bestehende Wissenslücke zu schließen. Sie liefert Einblicke in die Evolution des Phylums und betont die ökologische Bedeutung horizontaler Oberflächenströmungen von Cnidarian. Zudem werden potenzielle biologische Funktionen von Oberflächenströmungen, wie beispielsweise Nahrungsaufnahme, Nährstofftransport und Reinigung, diskutiert. Limitationen dieser Studie umfassen unter anderem die repräsentative Auswahl der Arten sowie mögliche Stressauswirkungen durch die Applizierung von Mikroplastikperlen. Zukünftige Forschungsrichtungen beinhalten eine Ausweitung der taxonomischen Abdeckung, und die Durchführung von Feldexperimenten, um ein ganzheitliches Verständnis des Verhaltens von Cnidaria zu erlangen. Diese Studie legt somit den Grundstein für das Verständnis der Erforschung der Biodiversität von Cnidaria und möglicher Schutzmaßnahmen.

Introduction

Life on Earth has been categorized into three overarching domains: Bacteria, Archaea, and Eukaria (Woese et al., 1990; Hug et al., 2016). In the domain of Eukaryotes and within the animal kingdom, we find the phylum Cnidaria, which comprises organisms like corals, jellyfish, and sea anemones. It is a highly diverse phylum of exclusively aquatic species predominantly inhabiting marine environments (Figure 1). The number of species within this phylum is estimated to range from 7,200 to 13,000 species (Daly et al., 2007).

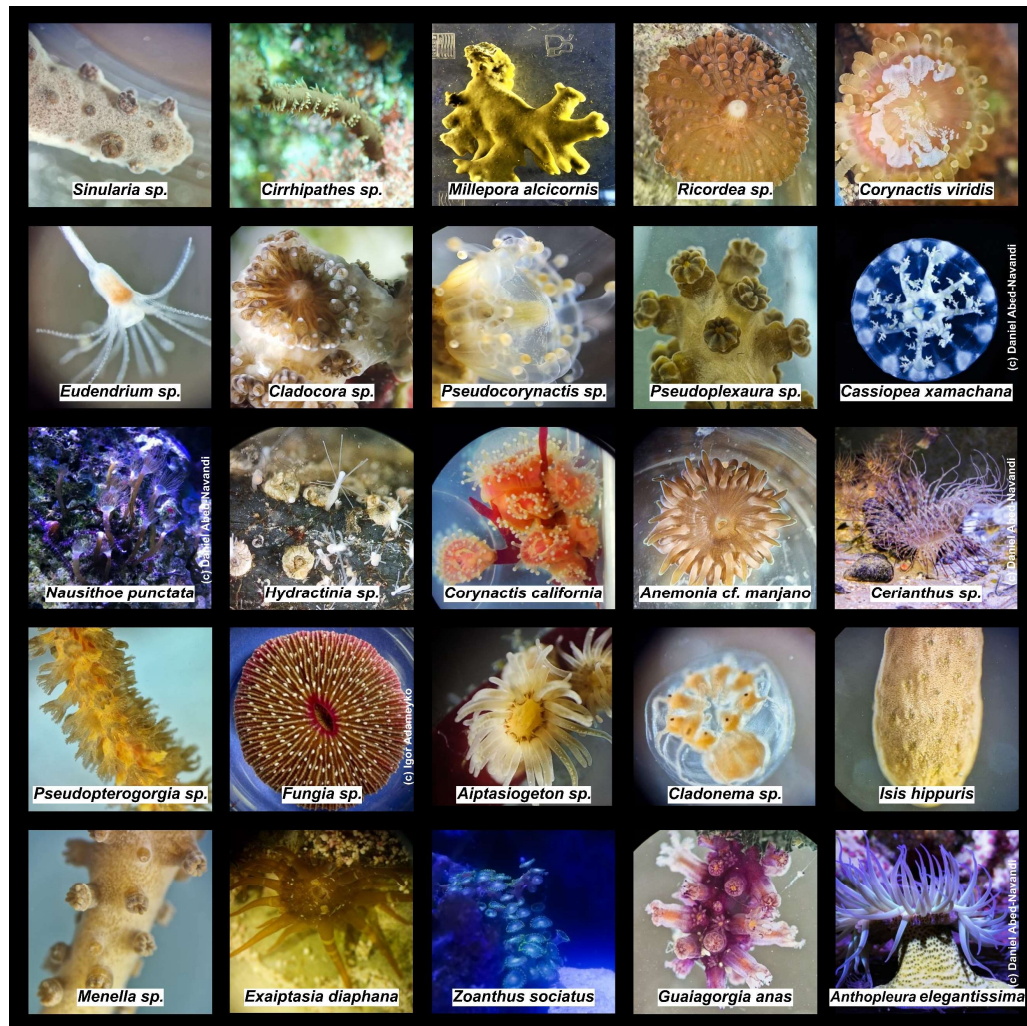


Figure 1 | Diversity of Cnidaria. Selection of species investigated within this study. *Cassiopea xamachana*, *Nausithoe punctata*, *Anthopleura elegantissima* and *Cerianthus* species: © Daniel Abed-Navandi. *Fungia* species: © Igor Adameyko.

The cnidarian phylum

The morphological diversity of cnidarians can be, as part of a broader context, attributed to the presence of two body forms within the phylum: the polyp and the medusae (Figure 2 A and B) (Tucker and Adams, 2014). Polyps are sessile structures and are firmly anchored to the substrate. They exhibit a cylindrical, vase-shaped body with an aboral end attached to the substrate, such as rocks, coral reefs, or the sea floor (Figure 2 C). Medusae are pelagic, free-swimming organisms. Their body shape is often likened to that of an open umbrella (Figure 2 D). Integral to their anatomy is one opening that serves as both a mouth and an anus and is encircled by tentacles (Figure 2 A and B) (Daly et al., 2007; Tucker and Adams, 2014). This opening is the entrance of the gastrovascular cavity in both polyps and medusae. All cnidarians display a relatively simple body plan characterized by two cell layers: the outer layer, the ectoderm, and the inner layer, the endoderm (or gastroderm). Between the two layers lies a non-cellular, gel-like matrix called the mesoglea, which provides structural support to the cnidarian body and acts as an elastic, connective tissue that allows for shape changes and movement (Turk and Kem, 2009; Tucker and Adams, 2014).

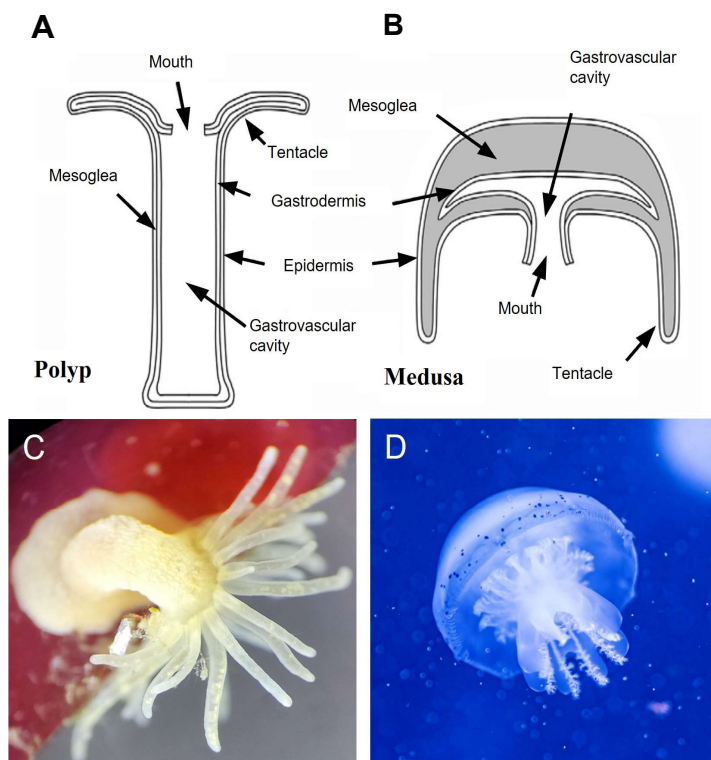


Figure 2 | Cnidarian morphologies. A displays a Polyp and B a Medusae. Some species have different body forms at different stages of their life cycle. C a typical cnidarian polyp illustrated by *Aiptasiogeton species*; D a typical cnidarian medusa illustrated by *Stomolophus meleagris* (© D. Abed-Navandi). (Adapted from Tucker and Adams, 2014)

Although cnidarians exhibit a wide range of shapes and sizes, they all share a distinctive and defining feature: nematocysts (Technau and Steele, 2011). These are specialized cells that are primarily located in the tentacles (Beckmann and Özbek, 2012). Nematocysts are small, capsule-like structures that contain an intricately coiled, harpoon-like thread loaded with venom. When stimulated by contact with prey or potential threats, nematocysts rapidly eject their venomous threads. This function serves multiple purposes for cnidarians, including predation, adhesion to surfaces, and defense (Turk and Kem, 2009).

Clades of the phylum Cnidaria

The phylum can be divided into three clades: Anthozoa, Medusozoa, and Myxozoa. The key differentiation among these cnidarian clades is related to their life stages and physical attributes. Anthozoa are characterized by stationary polyps, while Medusozoa include polyps and mobile medusae. Myxozoa are parasitic organisms with complex life cycles that involve hosts, including vertebrates and annelids (Koch and Grimmelikhuijzen, 2019).

Anthozoa

The most species-rich clade within Cnidaria is Anthozoa. One subclass of this clade is Hexacorallia, which encompasses the orders Actiniaria (sea anemones), Antipatharia (black corals), Ceriantharia (tube worms), Corallimorpharia, Scleractinia (hard corals) and Zoantharia (Daly et al., 2003 & 2007; Kayal et al., 2013). All Hexacorallia are individual polyps that can be solitary without forming true colonies, such as Actiniaria, Corallimorpharia, Zoantharia, and Ceriantharia, or colonial like Antipatharia and Scleractinia. Hexacorallia are present in marine environments across the globe. A significant number of species in tropical and subtropical regions have the capacity to establish a mutualistic association with photoautotrophic dinoflagellates (unicellular algae) of the family *Symbiodiniaceae* known as Zooxanthellae (Quattrini et al., 2020; McFadden et al., 2022). Certain species, such as scleractinians, exhibit the capacity to precipitate a calcium carbonate skeleton that allows them to construct three-dimensional coral reefs which are recognized as biodiversity hotspots (Corriero et al., 2019; McFadden et al., 2021).

Another subclass of Anthozoa is Octocorallia. Octocorallia, also called “soft corals” or “octocorals”, are predominantly colonial species. As of October 2022, this subclass has been classified into two orders: Malacalcyonacea and Scleralcyonacea (McFadden et al., 2022). Although octocorals occur in all marine regions, their highest diversity is in the deep sea (Cairns, 2007). In shallow waters, zooxanthellate octocorals are an important structural component of coral reefs, particularly in locations like the Caribbean, where they can be as abundant as, if not more abundant than, hard corals (Rodriguez et al., 2020).

Unlike hexacorallian polyps, which display a sixfold symmetry, octocorallian polyps exhibit an eightfold symmetry or multiples of eight (Conci et al., 2021). Additionally, the tentacles of soft coral polyps have finger-like extensions, also called pinnules (Fabricius and Alderslade, 2001).

The third subclass of Anthozoa is Ceriantharia, commonly referred to as “tube anemones”. These solitary anthozoans can be found across a wide range of oceanic environments, from the intertidal zones to the deepest depths of all oceans, including the Arctic Ocean (Molodtsova et al., 2023). Tube anemones typically inhabit an elongated tube formed by filaments of their special cnidae, known as ptychosysts, which are encrusted with sand or rock (Stampar et al., 2020).

Medusozoa

Medusozoa comprises species of the classes Cubozoa (box jellyfish), Staurozoa (benthic stalked jellyfish), Scyphozoa (true, motile jellyfish), and Hydrozoa (Hydroids, Siphonophores, and Hydromedusae). Medusozoa is set apart from Anthozoa through its biphasic life cycle, which involves a transition between a sessile polyp stage and a typically free-swimming medusoid stage (McFadden et al., 2022).

Cubozoa, commonly known as “box jellyfish”, is the smallest class within Cnidaria, with only 50 described species (Bentlage et al., 2010). It comprises two orders: Carybdeida and Chirodropida. Box jellyfish typically exhibit a wide pantropical distribution and are known for their extreme toxicity (Brinkman and Burnell, 2009; Kingsford and Mooney, 2014).

Scyphozoa includes approximately 220 species which are split up into two subclasses: Coronamedusae and Discomedusae. Members of both subclasses inhabit all oceans, from tropical to Arctic and Antarctic regions, and can be found in both shallow coastal waters and the open ocean (Hale, 1999). Certain species typically found in shallow waters possess the ability to form a mutual symbiotic relationship with Zooxanthellae (Helm et al., 2018).

The class of Staurozoa is represented by more than 50 species from 11 genera. They are bottom-dwelling (benthic) and are typically found in shallow, temperate waters (Miranda et al., 2018). This group of jellyfish displays a particularly unique morphology. The aboral part of the animal is attached to a substratum and organized like an Anthozoa polyp. Whereas the oral part is more similar to the body structure of a scyphozoan or cubozoan medusa (Maria and Valeriia, 2023).

The fourth class of Medusozoans is Hydrozoa. It is a heterogeneous group of more than 3000 species (Bouillon et al., 2006). Among these, there is a high degree of diversity in morphologies ranging from free-swimming Hydromedusae to reef-building, colonial species (Bouillon, 2006; Cartwright et al., 2008). Hydrozoans are found throughout all marine regions, inhabiting a wide range of environments, including the deep ocean and shallow tropical reefs. Like many other cnidarians, some hydrozoans can engage in photosymbiosis with Zooxanthellae (Olguín-López et al., 2019).

Myxozoa

Myxozoa are primarily endoparasitic cnidarians. This clade includes approximately 2200 species within 64 genera (Collins, 2009; Kayal et al., 2018). These microscopically small animals are common parasites of freshwater and marine fish (Dezfuli et al., 2021). Most Myxozoans need both a vertebrate host and an aquatic annelid host at different stages of their life cycle to develop and reproduce (Kent and Sanders, 2020).

Autogenously generated surface currents in cnidarians

The first documented record of surface currents in Cnidaria can be traced to the early 20th century. Indeed, in 1930, Charles Maurice Yonge, an English marine biologist, documented the existence of horizontal currents among 40 scleractinian species inhabiting the Great Barrier Reef (Yonge, 1930). He observed that these currents are primarily associated with cleansing functions and occasionally assist in transporting food to the mouth. However, since this founding publication, only a handful of studies explored this phenomenon (Lewis, 1975; Lewis, 1976; Fautin and Mariscal, 1991; Shapiro et al., 2014; Tambutté et al., 2021; Boudier et al., 2022; Pacherres et al., 2022).

Surface currents can be categorized into two distinct types: vertical and horizontal. These flows are likely generated by structures known as cilia. Cilia are fine, hair-thin, membrane-covered appendages that heterogeneously adorn the surface of various types of cells (Mitchell, 2007). Measuring approximately 10 – 20 µm in length, cilia are able to generate coordinated and rhythmic motions (Gilpin et al., 2020; Pacherres, 2021). Given their high degree of conservation among eukaryotic organisms, it is reasonable to infer that all Cnidaria species possess these organelles (Mitchell, 2007).

Biological functions of surface currents

Epidermal surface currents in cnidarians fulfill a diverse array of biological functions. They can contribute to the maintenance of a clean cnidarian surface by removing mucus or fine sediments and foreign particles (Bouderlique et al., 2022; Pacherres et al., 2022). Currents directed toward the cnidarian mouth can play a crucial role in facilitating food uptake. Moreover, a vertically oriented flow has the capacity to enhance and modify the effective diffusion of a multitude of substances, such as oxygen, at the surface of the cnidarians by modifying the dynamics of the diffusive boundary layer surrounding the tissue (Shapiro et al., 2014; Pacherres et al., 2020; Tambutté et al., 2021). Indeed, vertical vortices on the epidermal surface of scleractinians substantially enhance the transport of nutrients and oxygen by up to 400 % (Shapiro et al., 2014). These abilities demonstrate that cnidarians can shape and influence their microenvironments through the creation of a water flow on their surface.

Aim of this study

This study builds upon the research laid out in the 2022 published article by Bouderlique et al. Their research focused primarily on the assessment and quantification of horizontal surface currents among cnidarians of the scleractinian order. This research on 14 species showed that each colony produced species-specific surface-associated currents spanning the entire colony. Furthermore, they also conducted further investigations to comprehend the factors that influence these currents and explore their potential function. Preliminary findings (unpublished pers. obs. Bouderlique) suggested that Hydrozoan, such as *Millepora alcicornis* (Linnaeus, 1758), did not exhibit surface currents. Despite their morphological similarities, Scleractinia (order) and Milleporidae (family) exhibit differences regarding the presence of horizontal surface currents. This finding suggested that surface currents might be exclusive to Scleractinia.

The main research purpose of this project is to assess the presence or absence of horizontal surface currents in Cnidaria and, if present, to determine how they might differ between multiple cnidarian orders. The central research questions are, therefore:

- (i) Which cnidarian orders and species display surface currents, and which do not?
- (ii) What insights about the evolutionary history of cnidarians can be derived from the presence or absence of surface currents?
- (iii) Are there distinct surface current patterns and do they differ among cnidarian species and orders?

Material and Methods

The foundation of the analysis are video recordings of fluorescent microbead movements, which served as a medium to display horizontal surface currents. 30 cnidarian species spanning 11 different cnidarian orders (Table 1) were analyzed. Twenty-seven were obtained from the Haus des Meeres (HdM), a public aquarium in Vienna, Austria, between January and August 2023. The remaining three species, *Millepora alcicornis*, *Stylaster roseus* (Pallas, 1766), and *Stichopathes* sp. (Brook, 1889), were analyzed during a two-week excursion at the CARMABI research station situated in Curaçao, Southern Caribbean Sea in September 2022.

Table 1: List of analyzed species

Species	Class	Subclass	Order	Origin
<i>Parazoanthus axinellae</i> (Schmidt, 1862)	Anthozoa	Hexacorallia	Zoantharia	HdM
<i>Zoanthus sociatus</i> (Ellis, 1768)				HdM
<i>Anemonia cf. manjano</i> (Carlgren, 1900)			Actiniaria	HdM
<i>Anemonia viridis</i> (Forsskål, 1775)				HdM
<i>Aiptasiogeton species</i> (Schmidt, 1972)				HdM
<i>Anthopleura elegantissima</i> (Brandt, 1835)				HdM
<i>Exaiptasia diaphana</i> (Rapp, 1829)				HdM
<i>Cirrhipathes species</i> (de Blainville, 1830)			Antipatharia	HdM
<i>Stichopathes species</i> (Brook, 1889)				Curaçao
<i>Corynactis californica</i> (Carlgren, 1936)			Corallimorpharia	HdM
<i>Corynactis viridis</i> (Allman, 1846)				HdM
<i>Pseudocorynactis species</i> (den Hartog, 1980)				HdM
<i>Ricordea species</i> (Duchassaing & Michelotti, 1860)				HdM
<i>Cladocora species</i> (Ehrenberg, 1834)			Scleractinia	HdM
<i>Fungia species</i> (Lamarck, 1801)				HdM
<i>Cerianthus species</i> (Delle Chiaje, 1841)		Ceriantharia	Spirularia	HdM
<i>Guaiaigorgia anas</i> (Grasshoff & Alderslade, 1997)		Octocorallia	Malacalcyonacea	HdM
<i>Isis hippuris</i> (Linnaeus, 1758)				HdM
<i>Menella species</i> (Gray, 1870)				HdM
<i>Pseudoplexaura species</i> (Wright & Studer, 1889)				HdM
<i>Pseudopterogorgia species</i> (Kükenthal, 1919)				HdM
<i>Sinularia species</i> (May, 1898)				HdM
<i>Cladonema species</i> (Dujardin, 1843)	Hydrozoa	Hydroidolina	Anthoathecata	HdM
<i>Eudendrium species</i> (Ehrenberg, 1834)				HdM
<i>Hydractinia species</i> (van Beneden, 1844)				HdM
<i>Millepora alcicornis</i> (Linnaeus, 1758)				Curaçao
<i>Stylaster roseus</i> (Pallas, 1766)				Curaçao
<i>Cassiopea andromeda</i> (Forsskål, 1775)	Scyphozoa	Discomedusae	Rhizostomeae	HdM
<i>Sanderia malayensis</i> (Goette, 1886)			Semaeostomeae	HdM
<i>Nausithoe punctata</i> (Kölliker, 1853)			Coronamedusae	Coronatae
HdM (Haus des Meeres)				

HdM (Haus des Meeres)

Sample preparation

After collection, specimens were transported to the laboratory and placed in individual glass containers (diameter: 20 cm, height: 10 cm) filled with water from their original tank at the HdM. Each specimen was fully covered with approximately 1 – 2 cm of seawater. To reduce potential stressors, all cnidarians were provided with a minimum of two to four hours rest in an artificial light-free environment. Only natural light (through the window) was allowed during this period to help the animals to acclimate to their new surroundings before recording.

Fluorescent microbeads preparation

The red fluorescent microbeads (Cospheric) in sizes 1 – 5 μm and 63 – 75 μm with a density of 1.3 g/cm^3 and 1.090 g/cm^3 , respectively, were first coated with the liquid surfactant Tween 80 (Sigma Aldrich), in a falcon tube for 12 hours. This was necessary due to the hydrophobic nature of the beads, which would otherwise cause them to float on the water surface. Afterward, the mixture of tween 80 and microbeads was diluted with Phosphate-Buffered Saline (PBS, Sigma Aldrich) and subsequently centrifuged for 10 minutes at 1000 g. Following this, the beads underwent additional five washes with PBS to remove excess of tween 80, as surfactants are known to be harmful to marine organisms (Jackson et al., 2015; Boudier et al., 2022).

For each species, 2 – 3 mL of the microbead solution was added to 7 – 8 mL unfiltered seawater from the original tank of the targeted species. This mixture was centrifuged at 2000 g for 10 minutes at room temperature three times. After each round of centrifugation, the beads that had floated on the water surfaces were removed, and fresh seawater from the original tank of the species was added to the falcon tube. According to the manufacturer, the microbeads emit light between 575 nm and 750 nm, which corresponds to red light, when excited with a light of the wavelength ranging from 500 nm to 700 nm. To minimize the signal-to-background ratio, it is necessary to reduce the ambient light. Excessive ambient light can diminish the contrast between the beads and the background, potentially causing the particle tracking software to be unable to detect and track the beads (Figure 3).

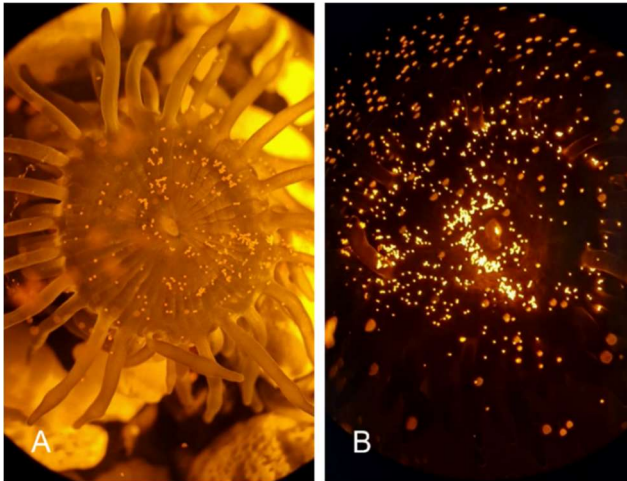


Figure 3 | Excessive light impairs video quality. Images show two *Anemonia cf. manjano* polyps, one with room lights on **A** and one with lights off **B**.

Experimental setup

Video recordings were generated for each species using three different specimens (polyps or colonies). *Millepora alcicornis* and *Cerianthus* sp. (Delle Chiaje, 1841) were each recorded only twice and once, respectively. The recordings were created using a fluorescent stereomicroscope (Leica, M165 FC) equipped with a camera holder. The videos were captured using a Samsung X23 camera. Additionally, a fluorescence microscopy illumination system (CoolLED) was affixed to the microscope (Figure 4).



Figure 4 | Experimental setup. Sample A was placed under the stereomicroscope, exposed to green light from the CoolLED system C and captured by the camera B.

Bead tracking experiments

After the beaker containing the sample was positioned under the microscope, approximately 100 – 200 μL of the prepared, diluted microbeads solution was gently applied near the cnidarian surface using a micropipette. Beads were released 1 – 5 mm above the specimen/colony. Due to their neutral buoyancy, slightly greater density than the surrounding seawater, and the previous preparation steps, most beads landed directly on the cnidarian surfaces. The recording of bead movements began once the water disturbances caused by the application of beads had subsided. Movements of the beads were continuously recorded until either the surface was entirely devoid of beads, or it was observed that there was no movement. The recording duration ranged between three to five minutes.

Data analysis

Pre-processing of the videos

The analysis required the initial extraction of the frames from the cnidarians' recordings as TIFF images using ffmpeg. The generated images can then be analyzed using Imaris or ImageJ. To optimize computational efficiency, every second frame was extracted, which was taken into account when assessing the speed of the tracks in the subsequent analyses. Following this, the extracted frames were reassembled as an Imaris (Bitplane, Zurich, Switzerland) file using the Imaris File Converter tool (Bouderlique et al., 2022). A list of the most important computational resources can be found in Table 2.

Table 2: Key computational resources

R version 4.1.3 (2022-03-10)	https://cran.r-project.org/bin/windows/base/ https://rdocumentation.org/packages/ggplot2/versions/3.4.2 (Valero-Mora, 2010) https://rdocumentation.org/packages/rstatix/versions/0.7.2 (Kassambra, 2023) https://rdocumentation.org/packages/svglite/versions/2.1.1 (Wickham, 2023)
Python version 3.9.0 (2020-10-05)	https://www.python.org/downloads/ https://github.com/LouisFaure/coral_paper/ https://pypi.org/project/matplotlib/ (Hunter, 2007) https://pypi.org/project/umap-learn/ (McInnes et al., 2018)
ffmpeg	https://www.ffmpeg.org/ (Tomar, 2006)
Imaris 9.6.0	https://imaris.oxinst.com/
ImageJ	https://imagej.net/ij/download.html

Processing of Imaris – microbeads tracking

The generated Imaris files (.ims files) were analyzed using the semi-automated tracking module of the Imaris Microscopy Image Analysis Software (Bouderlique et al., 2022). This module involves the automatic tracking of beads by the software with manual adjustments of parameters such as estimated spot diameter, tracking algorithm, maximum distances, and maximum gap size (Table 3). For each .ims file, it is crucial to select and configure these parameters anew to maximize track capture. This approach ensures the accurate identification and characterization of individual objects (referred to as spots), facilitating their interconnection across consecutive frames. This process enables the measurement of track trajectories by assessing the beads' positions in each frame. The final step in the tracking process involved manually filtering out generated tracks from floating beads not located directly on the surface of the animal.

Table 3: Adjustable parameters for object tracking in Imaris

Estimated spot diameter^(a)
The diameter of the spots. Spots with a smaller diameter will not be detected.
Tracking Algorithm^(a)
Autoregressive Motion: To predict the motion of objects.
Autoregressive Motion Expert: To predict the motion of multiple objects with adjacent or crossing paths.
Brownian Motion: To model the motion of each object as a random movement without a clear pattern.
Connected Components: To connect objects at different time points if their spheres overlap.
Lineage: To connect objects that undergo splitting.
Maximum Distance^(a)
The maximum distances at which two spots are still recognized as the same spot between 2 time points.
Maximum Gap Size^(a)
The maximum consecutive time points a track can have missing to continue.

^(a) Adapted from the IMARIS 9.2 Reference Manual

Key parameters

Raw data describing the tracks and the position of the spots in each frame was exported from Imaris as Excel files. To comprehensively characterize the tracks, eight key parameters were computed from the raw data:

- 1) The number of turns per track which represents the counts of how often the track changes its direction.
- 2) Intersections indicate the number of times the track intersects with itself.
- 3) Track length (μm)
- 4) Track displacement length (μm) measures the overall distance covered by the track, which quantifies its spatial movement.
- 5) Average track speed ($\mu\text{m}/\text{sec}$)
- 6) Maximum track speed ($\mu\text{m}/\text{sec}$)
- 7) Speed variance ($\mu\text{m}/\text{sec}$)
- 8) Sinuosity is a dimensionless measure of the straightness or curvature of a path (Cummins and Wilzbach, 2019). Here it was calculated by the track's total length divided by the track's straight-line length.

Statistical analysis

Given that the primary objective was to uncover whether other cnidarian orders exhibited horizontal surface currents, I opted to group the species into their respective orders for the statistical testing. One Kruskal-Wallis test was conducted for each parameter to determine if there were statistically significant differences in the tracks of cnidarian orders that displayed horizontal surface currents. Additional post hoc tests (Dunn's tests) were applied to assess which pairwise comparisons were statistically significantly different. A value of $\alpha = 0.5$ was set.

Uniform Manifold Approximation and Projection (UMAP)

UMAP, is a machine-learning algorithm, used to reduce dimensionality, and facilitate the visualization and comprehension of large, high-dimensional datasets (McInnes et al., 2018). Initially created for visualizing single-cell data, the algorithm has become the most widely used method in single-cell data analysis since its publication in 2018 (Tjoonk, 2023). It constructs a weighted graph by determining a specific number of nearest neighbors for each data point, reflecting the distances between them. The goal is to discover a low-dimensional representation that accurately preserves the relationships between data points in their respective groups, so-called "neighborhoods" (McInnes et al., 2018; Diaz-Papkovich et al., 2021). Following a similar approach as in Boudier et al. (2022), this study utilized UMAP to group the tracks of each species displaying horizontal surface currents. Here the grouping was based on their similarity, considering the aforementioned eight key parameters. In the resulting UMAP groups, more similar tracks are positioned closer together. Subsequently, I applied the Leiden algorithm to refine the classification of the tracks (Traag et al., 2019). After this, the proportion of each Leiden group (LG) was calculated for every species, thus determining the composition of LG's for each species. Next, pairwise Euclidean distances of LG composition between species were computed using the Scipy Python package to visually represent the similarities or differences in track type composition among species (Virtanen et al., 2020).

Results

Thirty cnidarian species were analyzed, comprising 22 anthozoans, 5 hydrozoans, and 3 scyphozoans. This covers 11 orders (Zoantharia, Actiniaria, Antipatharia, Corallimorpharia, Scleractinia, Spirularia, Malacalcyonacea, Anthoathecata, Rhizostomeae, Semaestomeae, and Coronatae).

In the upcoming section, there will be a summary of the analyzed species to give a broad overview of which orders and species exhibit or do not exhibit horizontal surface currents. This will be followed by a detailed analysis of the track data.

Characterization of the presence and absence of horizontal surface currents

Order Zoantharia

Two Zoantharia species were studied (Table 1): *Parazoanthus axinellae* (Schmidt, 1862) of the family Parazoanthidae (Figure 5 A), and *Zoanthus sociatus* (Ellis, 1768) from the family of Zoanthidae (Figure 5 E).

Parazoanthus axinellae (n = 3) immediately underwent contraction after the beads were applied, followed by a swift closure of the tentacle ring (Figure 5 B-D). There were no visible surface currents seen in this species. *Zoanthus sociatus* (n = 3) displayed horizontal surface currents on the oral side of the polyp, both within the tentacle ring and on the tentacles (Figure 5 F-H). After an initial contraction of the tentacle ring following the application of beads, the polyp reopened, and all beads were transported away from the mouth. Beads were cleared from the central disc within 28 seconds (Figure 5 F), 78 seconds (Figure 5 G), and 34 seconds (Figure 5 H).

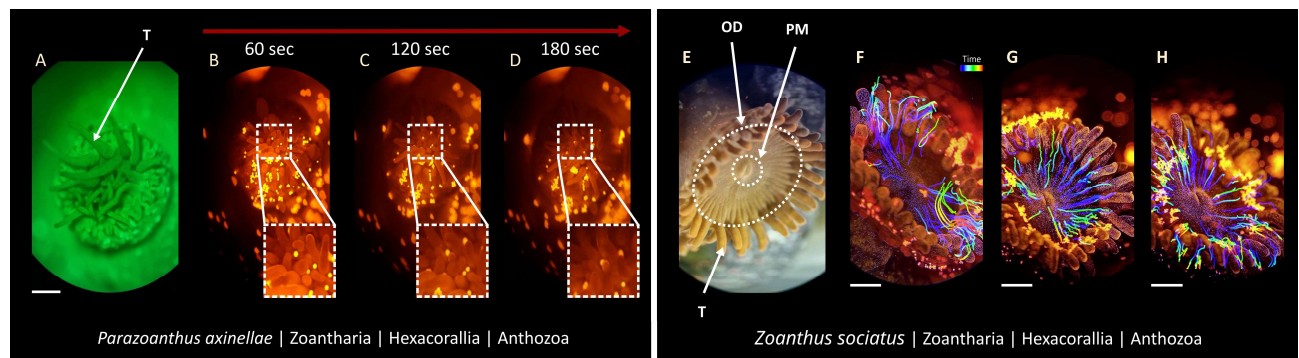


Figure 5 | Horizontal surface currents in the order Zoantharia (Class: Anthozoa, Subclass: Hexacorallia). Overhead perspective of **A** *Parazoanthus axinellae* under green, fluorescent light and **E** *Zoanthus sociatus* under white light. The positions of beads at three different time points in a single polyp of *Parazoanthus axinellae* (**B-D**) and time-coded tracks on three polyps of *Zoanthus sociatus* (**F-H**) under red fluorescent light. PM: Polyp mouth, OD: Oral disc, T: Tentacle. White bars equal 1 mm.

Order Actiniaria

In total, five actiniarian species were analyzed. The species *Anemonia* cf. *manjano* (Calgren, 1900) (Figure 6 A), *Anemonia viridis* (Forsskål, 1775) (Figure 6 I) and *Anthopleura elegantissima* (Brandt, 1835) (Figure 6 Q) belong to the Actiniidae family. *Aiptasiogeton* sp. (Schmidt, 1972) (Figure 6 E) and *Exaiptasia diaphana* (Rapp, 1829) (Figure 6 M) are classified in the Aiptasiidae family (Table 1).

Anemonia cf. *manjano* (Figure 6 B-D), *Anemonia viridis* (Figure 6 J-L), and *Anthopleura elegantissima* (Figure 6 R-T) (all $n = 3$) exhibited no horizontal surface currents (Figure 6 B-D, J-L and R-T). Despite active polyp movement, microbeads on different surfaces remained stationary.

However, both Aiptasiidae species showed horizontal surface currents. In *Aiptasiogeton* sp. ($n = 3$), the beads displayed a direction away and towards the polyp's mouth and were removed within 27 seconds (Figure 6 F), 45 seconds (Figure 6 G), and 25 seconds (Figure 6 H) following application. In *Exaiptasia diaphana* ($n = 3$), the majority of beads displayed a direction away from the polyp's mouth. The beads were cleared from the oral disc within 50 seconds (Figure 6 N), 26 seconds (Figure 6 O), and 36 seconds (Figure 6 P) post-application.

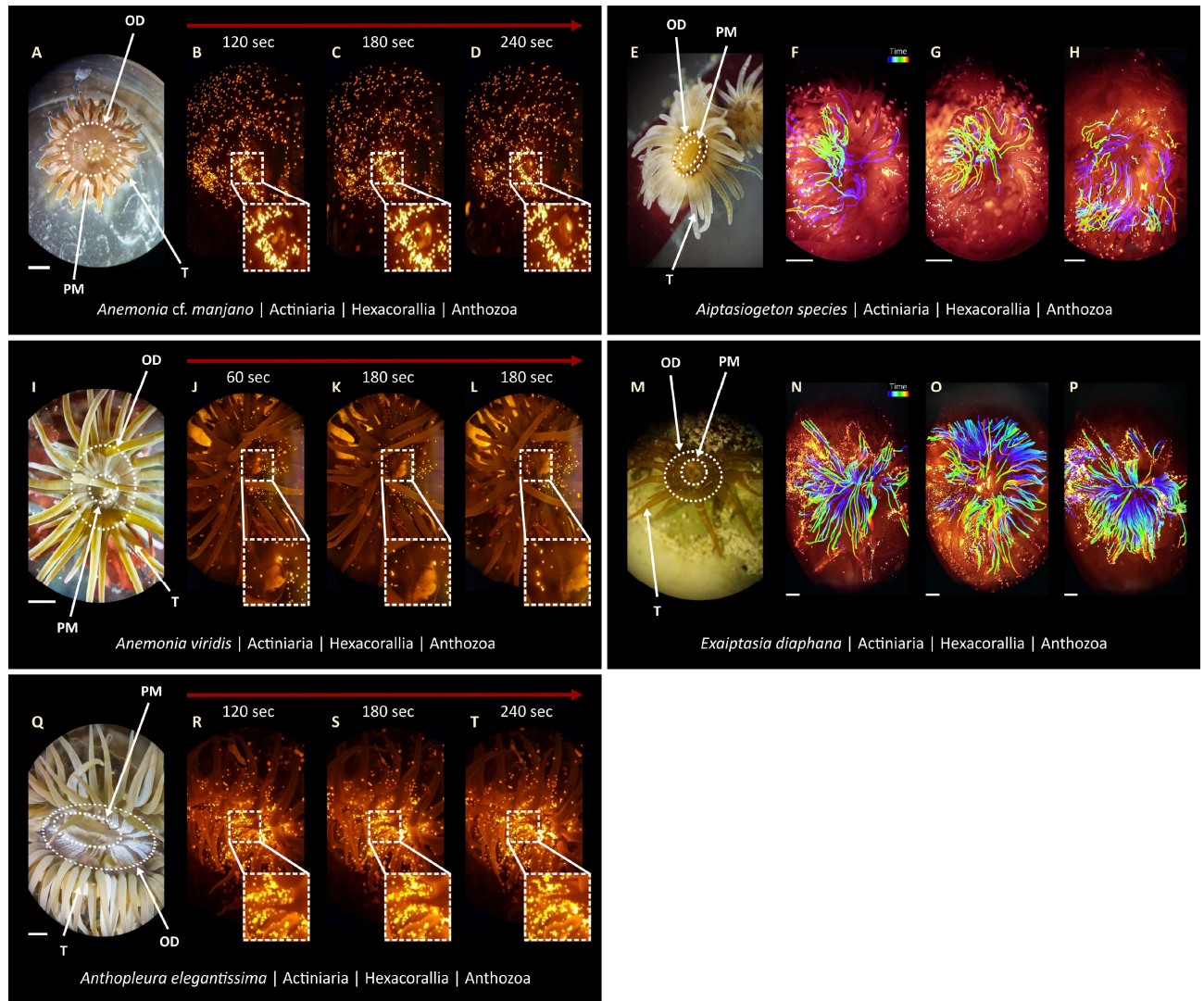


Figure 6 | Horizontal surface currents in the order Actiniaria (Class: Anthozoa, Subclass: Hexacorallia). Overhead perspective of **A** *Anemonia cf. manjano*, **E** *Aiptasiogeton* sp., **I**, *Anemonia viridis*, **M**, *Exaiptasia diaphana* and **Q** *Anthopleura elegantissima* under white light. The position of beads at three different time points in a single polyp of *Anemonia cf. manjano* (**B-D**), *Anemonia viridis* (**J-L**), *Anthopleura elegantissima* (**R-T**), and time-coded tracks on three polyps of *Aiptasiogeton* sp. (**F-H**) and *Exaiptasia diaphana* (**N-P**) under red fluorescent light. PM: Polyp mouth, OD: Oral disc, T: Tentacle. White bars equal 1 mm.

Order Antipatharia

Two species were analyzed (Table 1): *Cirrhipathes* sp. (Blainville, 1830) (Figure 7 A) and *Stichopathes* sp. (Figure 7 E), both of the family Antipathidae. Both species exhibited horizontal surface currents.

Beads that landed on the stem and the base of the polyps of *Cirrhipathes* sp. ($n = 3$) are moved toward the outside of the tentacle tips and then to the center of the polyps (Figure 7 B-D). Only a few beads remained within the tentacle ring around the mouth of the polyps. The majority of beads transported to the tentacle tips by horizontal surface currents were subsequently swept away by the movement of the surrounding water. The beads were transported from the aboral end of the polyps to the tentacle tips within 35 seconds (Figure 7 B), 23 seconds (Figure 7 C), and 22 seconds (Figure 7 D) after their application. In *Stichopathes* sp. ($n = 3$), the horizontal surface currents displayed a lower degree of complexity (Figure 7 F-H). The applied beads moved along the axis of the stem of the colony and were removed from the colony within 39 seconds (Figure 7 F), 43 seconds (Figure 7 G), and 27 seconds (Figure 7 H).

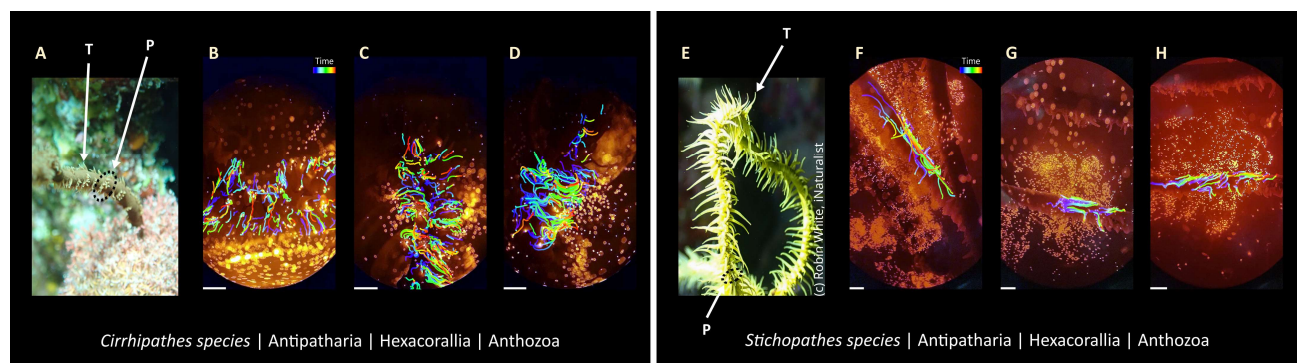


Figure 7 | Horizontal surface currents in the order Antipatharia (Class: Anthozoa, Subclass: Hexacorallia). Overhead perspective of **A** *Cirrhipathes* sp. and **E** *Stichopathes* sp. under white light. Time-coded tracks on three polyps of *Cirrhipathes* sp. (**B-D**), and *Stichopathes* sp. (**F-H**) under red fluorescent light. P: Polyp, T: Tentacle. White bars equal 1 mm. *Stichopathes luetkeni*: © Robin White.

Order Corallimorpharia

Four corallimorpharian species were studied (Table 1), namely *Corynactis californica* (Calgren, 1936) (Figure 8 A), *Corynactis viridis* (Allman, 1846) (Figure 8 E), *Pseudocorynactis* sp. (den Hartog, 1980) (Figure 8 I) and *Ricordea* sp. (Duchassaing & Michelotti, 1860) (Figure 8 M).

Corynactis californica (Figure 8 A-D), *Corynactis viridis* (Figure 8 E-H), and *Pseudocorynactis* sp. (Figure 8 I-L) (all $n = 3$) are of the family Corallimorphidae. The overall trend indicated less noticeable movement on the tentacles compared to the region within the tentacle ring and around the polyp mouth. The majority of beads that landed on the oral side of the polyp were oriented toward the mouth, consequently entering the mouth. Notably, in *Pseudocorynactis* sp., the mouth extended more upward than in the other two species, resulting in fewer beads directly entering the polyp's mouth.

In *Corynactis californica*, the oral disc underwent bead clearance within 108 seconds (Figure 8 B), 36 seconds (Figure 8 C) and 116 seconds (Figure 8 D) after the beads were applied. Similarly, in *Corynactis viridis*, the beads were taken up into the polyp's mouth in the span of 129 seconds (Figure 8 F), 138 seconds (Figure 8 G), and 62 seconds (Figure F H). Meanwhile, *Pseudocorynactis* sp. exhibited bead transport toward the upward-facing polyp mouth within 2 seconds (Figure 8 J), 4 seconds (Figure 8 K), and 2 seconds (Figure 8 L) after application.

Ricordea sp. ($n = 3$) is a member of the Ricordeidae family. This species exclusively exhibited surface currents on the polyp's mouth, which features folding that resembles an accordion. The applied beads are drawn into the folds and underwent a continuous back-and-forth or up-and-down motion (Figure 8 N-P). Some beads became trapped in this current within the folds, neither entering nor exiting it. Beads not trapped in the currents were swiftly directed into the respective polyp's mouth within 3 seconds (Figure 8 N), 5 seconds (Figure 8 O), and 4 seconds (Figure 8 P) from the outer edge of the polyp's mouth.

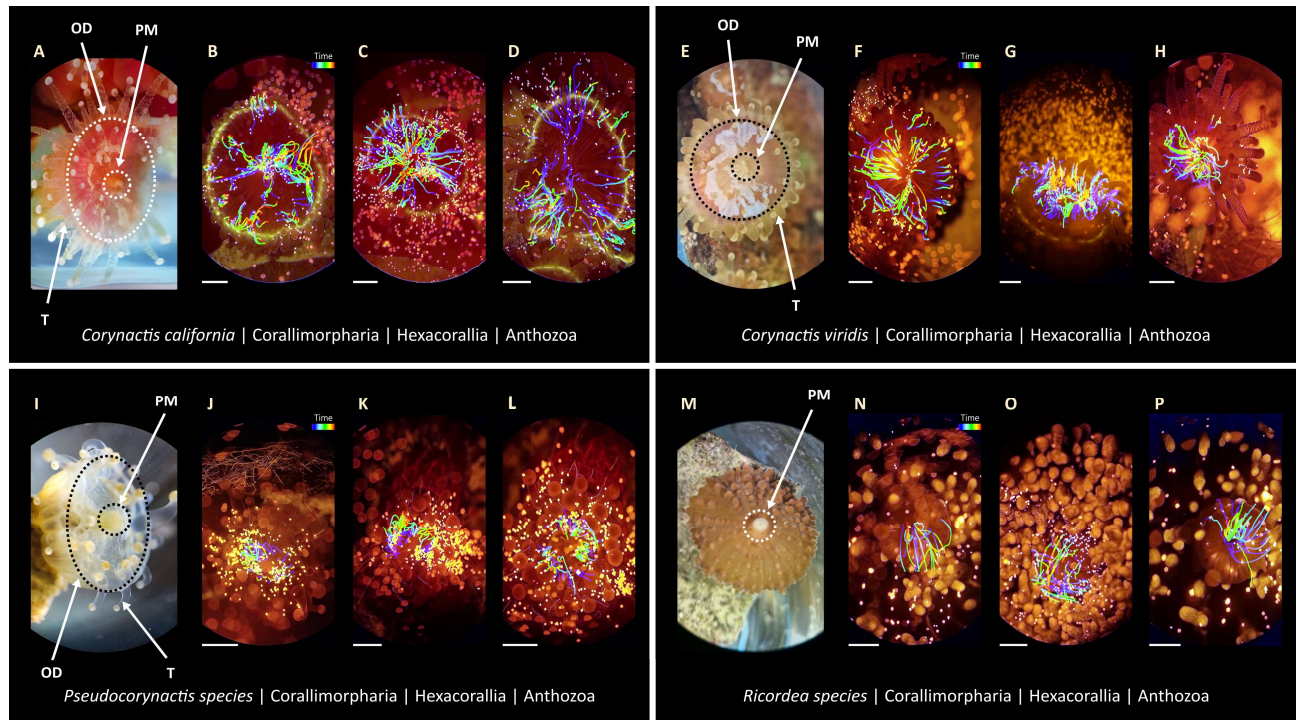


Figure 8 | Horizontal surface currents in the order Corallimorpharia (Class: Anthozoa, Subclass: Hexacorallia). Overhead perspective of **A** *Corynactis californica*, **E** *Corynactis viridis*, **I** *Pseudocorynactis* sp. and **M** *Ricordea* sp. under white light. Time-coded tracks on three polyps of *Corynactis californica* (**B-D**), *Corynactis viridis* (**F-H**), *Pseudocorynactis* sp. (**J-L**), and *Ricordea* sp. (**N-P**) under red fluorescent light. PM: Polyp mouth, OD: Oral disc, T: Tentacle. White bars equal 1 mm.

Order Scleractinia

Two scleractinian species *Cladocora* sp. (Ehrenberg, 1834) (Figure 9 A) of the Cladocoridae family and *Fungia* sp. (Lamarck, 1801) (Figure 9 E) of the family Fungiidae were analyzed (Table 1).

In *Cladocora* sp. ($n = 3$), the beads moved in both directions, away and toward the mouth (Figure 9 B-D). Beads that are transported away from the mouth are conveyed over the tentacle ring and proceed along the side of the polyp and toward neighboring polyps. In contrast, beads directed toward the mouth become entangled in a surface-associated mucus. None of the beads were transported into the mouth of the polyp. Here the polyps oral disc were cleared of beads in 22 seconds (Figure 9 B), 33 seconds (Figure 9 C) and 43 seconds (Figure 9 D) following the beads application.

In the solitary and free-living *Fungia* sp. ($n = 3$), the horizontal surface currents were primarily directed away from the polyp's mouth (Figure 9 F-H). The microbeads moved through the ridges on the coral surface. The beads that settle on the costae are transported away into the ridges. The applied beads were transported from the center of the polyp to the outer edge of the polyp within 59 seconds (Figure 9 F), 77 seconds (Figure 9 G) and 102 seconds (Figure 9 H).

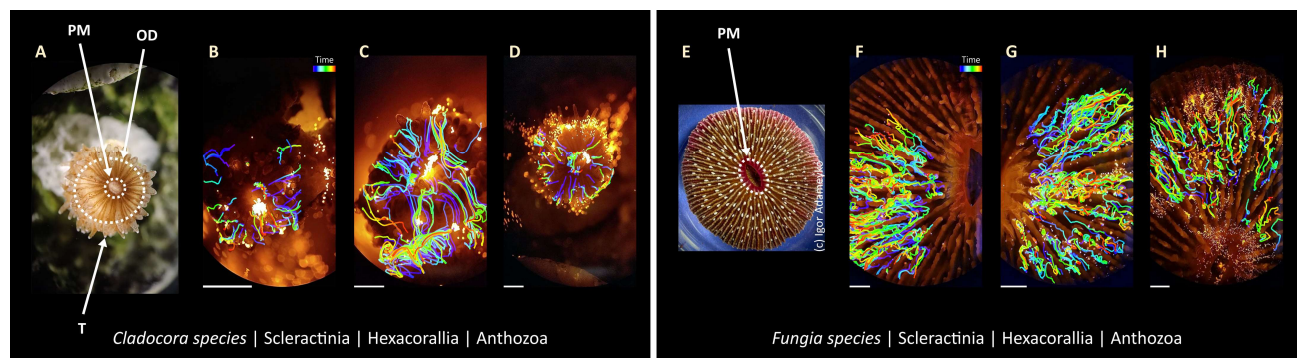


Figure 9 | Horizontal surface currents in the order Scleractinia (Class: Anthozoa, Subclass: Hexacorallia). Overhead perspective of A *Cladocora* sp. and E *Fungia* sp. under white light. Time-coded tracks on three polyps of *Cladocora* sp. (B-D) and *Fungia* sp. (F-H) under red fluorescent light. PM: Polyp mouth, OD: Oral disc, T: Tentacle. White bars equal 1 mm. *Fungia* species: © Igor Adameyko.

Order Spirularia

One single specimen belonging to the genus *Cerianthus* (Table 1) of the family Cerianthidae was evaluated (Figure 10 A). Upon the application of beads, the tube-dwelling anemone contracted into a ball-like shape. The beads congregated at the center of the animal, driven by the pronounced movement of the tentacles (Figure 10 B-D). Following this, the individual withdrew into its tube, carrying the microbeads along. The beads were actively moved by the organism themselves rather than being moved by surface currents.

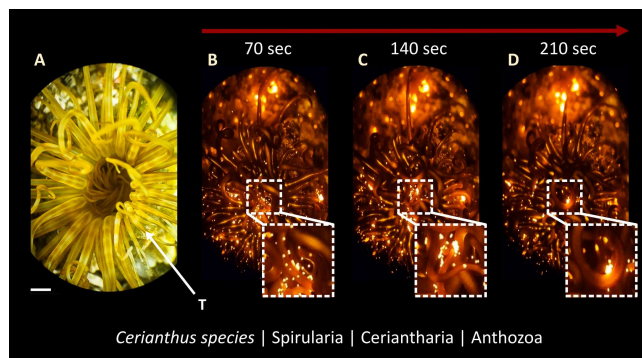


Figure 10 | Horizontal surface currents in the order Spirularia (Class: Anthozoa, Subclass: Ceriantharia). Overhead perspective of **A** *Cerianthus* sp. under white light. The position of beads at three different time points in a single polyp of *Cerianthus* sp. (**B-D**) under red fluorescent light. T: Tentacle. White bar equals 1 mm.

Order Malacalcyonacea

Six Octocorallia species of the order Malacalcyonacea were analyzed (Table 1): *Guaiaigorgia anas* (Grasshoff & Alderslade, 1997) (Figure 11 A), *Menella* sp. (Gray, 1870) (Figure 11 E), *Isis hippuris* (Linnaeus, 1758) (Figure 11 I), *Pseudoplexaura* sp. (Wright & Studer, 1889) (Figure 11 M), *Pseudopterogorgia* sp. (Kükenthal, 1919) (Figure 11 Q), and *Sinularia* sp. (May, 1898) (Figure 11 U) belonging to the families Paramuriceidae, Isididae, Plexauridae, Pterogorgiidae and Sinulariidae respectively.

Neither *Guaiaigorgia anas* (Figure 11 B-D), nor *Menella* sp. (Figure 11 F-H), *Isis hippuris*, (Figure 11 J-L) *Pseudoplexaura* sp. (Figure 11 N-P), *Pseudopterogorgia* sp. (Figure 11 R-T), and *Sinularia* sp. (Figure 11 V-X) (all n = 3) displayed horizontal surface currents.

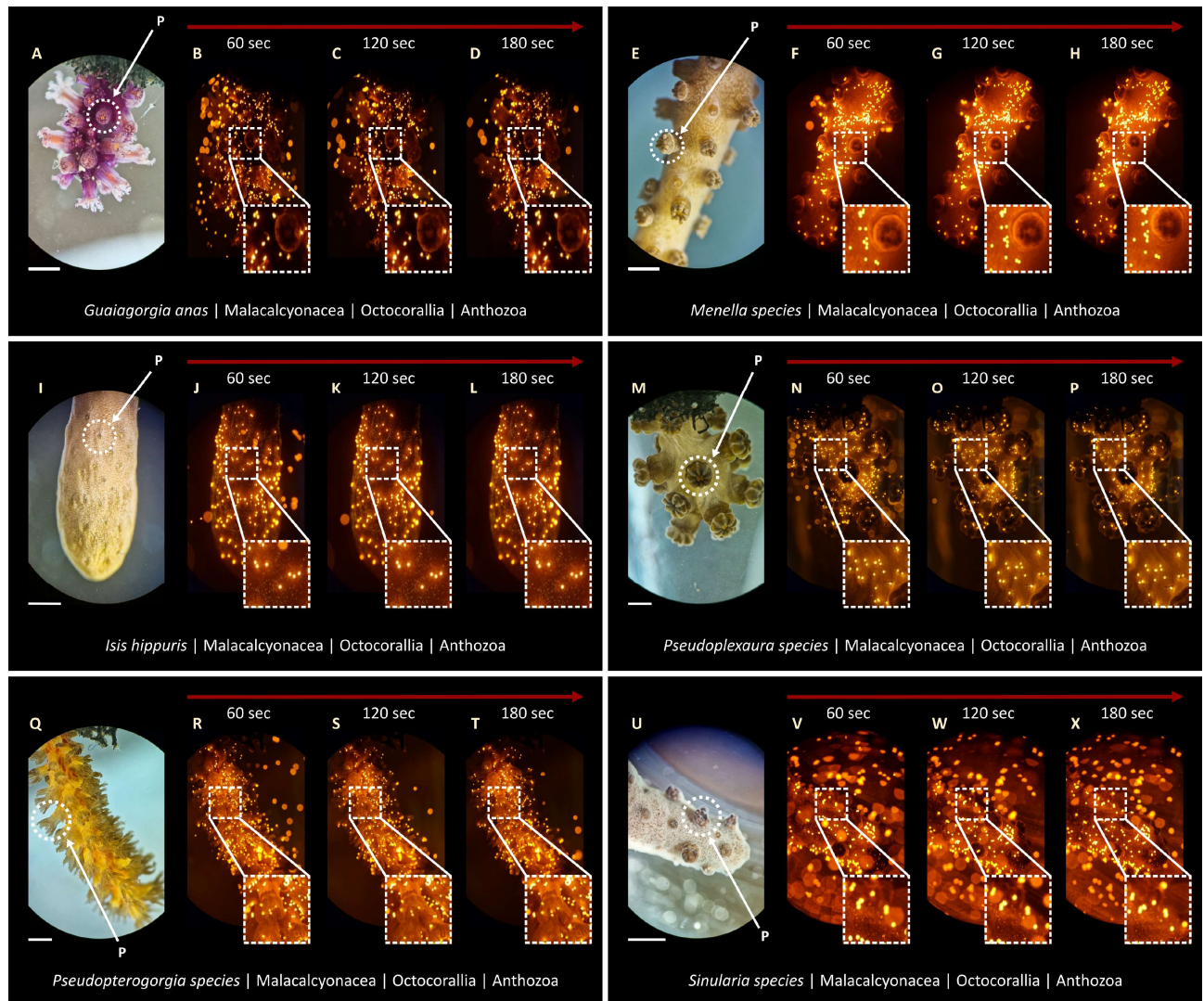


Figure 11 | Horizontal surface currents in the order Malacalcyonacea (Class: Anthozoa, Subclass: Octocorallia). Overhead perspective of **A** *Guaiagorgia anas*, **E** *Menella* sp., **I**, *Isis hippuris*., **M**, *Pseudoplexaura* sp., **Q** *Pseudopterogorgia* sp. and **U** *Sinularia* sp. under white light. The position of the beads at three different time points in a single polyp of *Guaiagorgia anas* (**B-D**), *Menella* sp. (**F-H**), *Isis hippuris* (**J-L**), *Pseudoplexaura* sp. (**N-P**), *Pseudopterogorgia* sp. (**R-T**) and *Sinularia* sp. (**V-X**) under red fluorescent light. P: Polyp. White bars equal 1 mm.

Order Anthoathecata

The species *Cladonema* sp. (Dujardin, 1843) (Figure 12 A), *Eudendrium* sp. (Ehrenberg, 1834) (Figure 12 E), *Hydractinia* sp. (van Beneden, 1844) (Figure 12 I), *Millepora alcicornis*, (Figure 12 M) and *Stylaster roseus* (Figure 12 Q) of the families Cladonematidae, Eudendriidae, Hydractiniidae, Milleporidae and, Stylasteridae, respectively were analyzed (Table 1).

Cladonema sp. (Figure 12 B-D), *Eudendrium* sp. (Figure 12 F-H), *Hydractinia* sp. (Figure 12 J-L), and *Stylaster roseus* (Figure 12 R-T) (all $n = 3$) and *Millepora alcicornis* (Figure 12 N-P) ($n = 2$) did not show any horizontal surface currents.

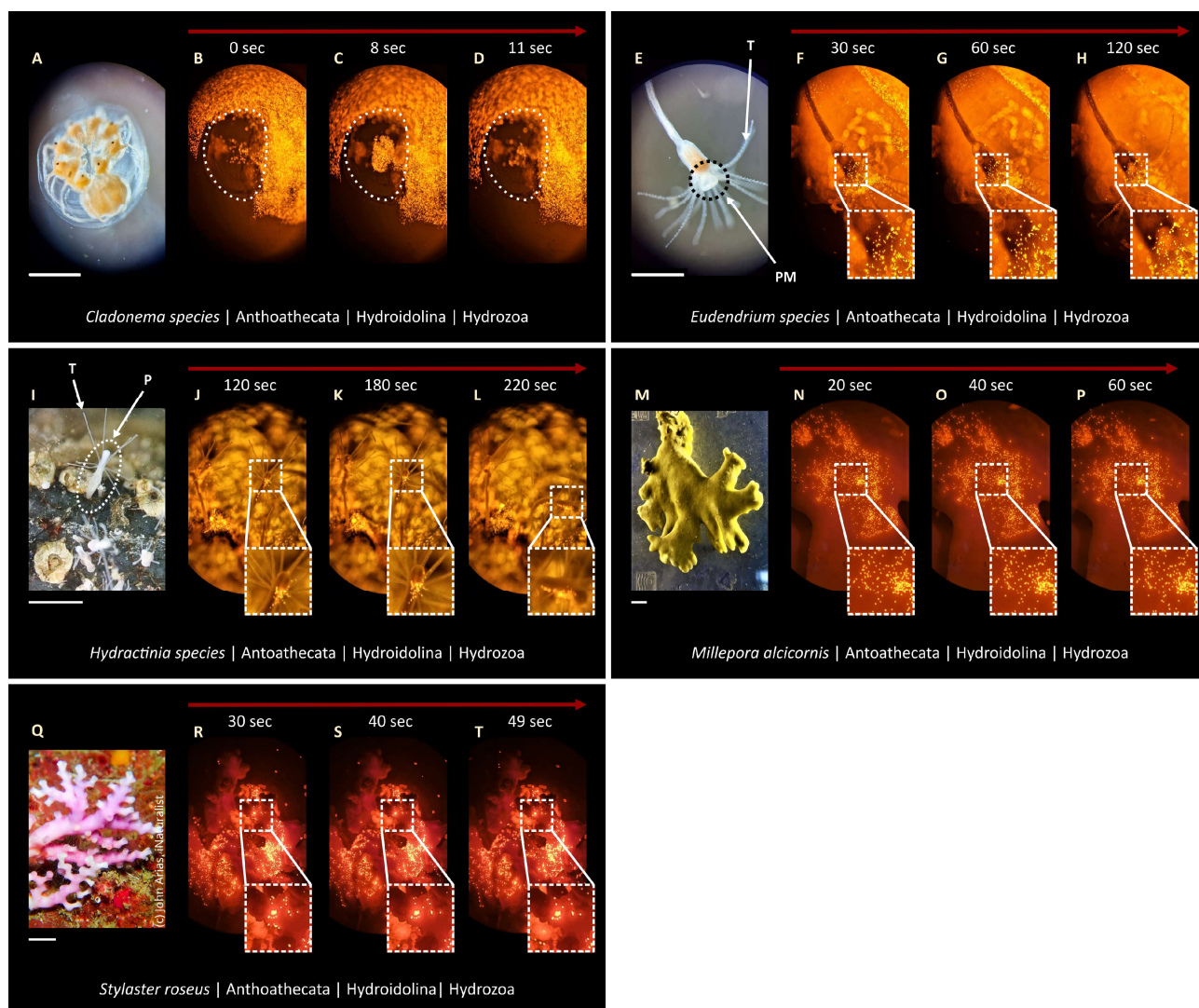


Figure 12 | Horizontal surface currents in the order Anthoathecata (Class: Hydrozoa, Subclass Hydroidolina). Overhead perspective of **A** *Cladonema* sp., **E** *Eudendrium* sp., **I**, *Hydractinia* sp., **M**, *Millepora alcicornis*, and **Q** *Stylaster roseus* under white light. The position of beads at three different time points in a single polyp or medusae of *Cladonema* sp. (**B-D**), *Eudendrium* sp. (**F-H**), *Hydractinia* sp. (**J-L**), *Millepora alcicornis* (**N-P**) and *Stylaster roseus* (**R-T**) under red fluorescent light. P: Polyp, T: Tentacle. White bars equal 1 mm. *Stylaster roseus*: © Jonh Arias.

Order Rhizostomeae

Three polyps of *Cassiopea andromeda* (Forsskål, 1775) (Figure 13 A) of the family Cassiopeidae were recorded (Table 1). Because the individual polyps were 1 – 2 mm in size, it was necessary to use smaller beads (1 – 5 μm). The majority of beads showed movement both along the sides of the polyp and across the oral disc but not on the tentacles (Figure 13 B-D). There was a transfer of the beads from the lateral sides toward the edge of the oral disc and the mouth of the polyp. On the oral disc, most of the beads moved toward the mouth of the polyp in the course of 14 seconds (Figure 13 B), 6 seconds (Figure 13 C) and 6 seconds (Figure 13 D) after application. Only a few beads were moving away from the mouth of the polyp. No beads were observed entering the polyp's mouth.

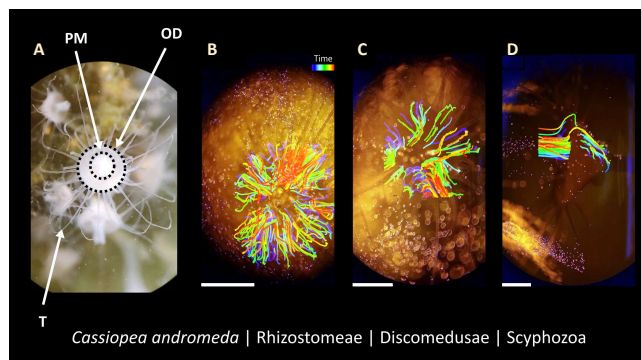


Figure 13 | Horizontal surface currents in the order Rhizostomeae (Class: Scyphozoa, Subclass: Discomedusae). Overhead perspective of **A** *Cassiopea andromeda* under white light. Time-coded tracks on three polyps of *Cassiopea andromeda* (**B-D**) under red fluorescent light. PM: Polyp Mouth, OD: Oral disc, T: Tentacle. White bars equal 1 mm.

Order Semaestomeae

Three polyps of *Sanderia malayensis* (Goette, 1886) (Figure 14 A) of the Pelagiidae family showed horizontal surface currents (Table 1). To visualize these, microbeads measuring between 1 – 5 μm in size were utilized. The flow patterns observed in *Sanderia malayensis* polyps are similar to those found in the *Rhizostomeae*. Along the oral disc of the polyp, the microbeads exhibit bidirectional movement (Figure 14 B-D). Most of the beads moved toward the polyp mouth within 7 seconds (Figure 14 B), 6 seconds (Figure 14 C), and 6 seconds (Figure 14 D) after application. Only a few beads were transported away from the polyp's mouth. Meanwhile, on the side of the polyp, the flow is more pronounced near the outer tentacle rim compared to the regions of the polyp stem that are situated further down.

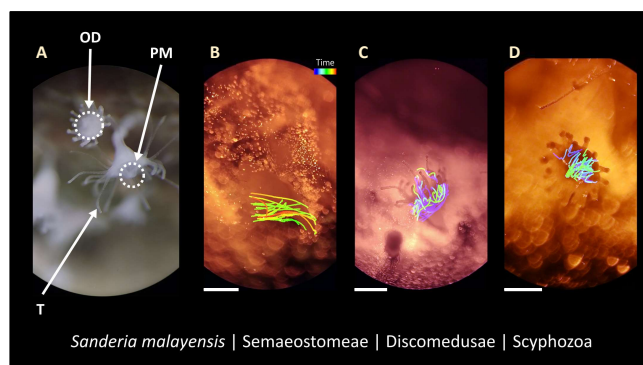


Figure 14 | Horizontal surface currents in the order Semaestomeae (Class: Scyphozoa, Subclass Discomedusae). Overhead perspective of **A** *Sanderia malayensis* under white light. Time-coded tracks on three polyps of *Sanderia malayensis* (**B-D**) under red fluorescent light. PM: Polyp Mouth, OD: Oral disc, T: Tentacle. White bars equal 1 mm.

Order Coronatae

Nausithoe punctata (Kölliker, 1853) (Figure 15 A) of the family Nausithoidae was analyzed (Table 1). The analysis centered on *Nausithoe punctata* polyps (n = 3), with all recordings captured from a lateral perspective. Most beads settled on the peridermal tube. Due to the polyp's rapid withdrawal into its tube in response to even slight disturbances, only a limited number of beads landed on the lateral section of the polyp's top. The beads displayed no movement induced by surface currents (Figure 15 B-D).

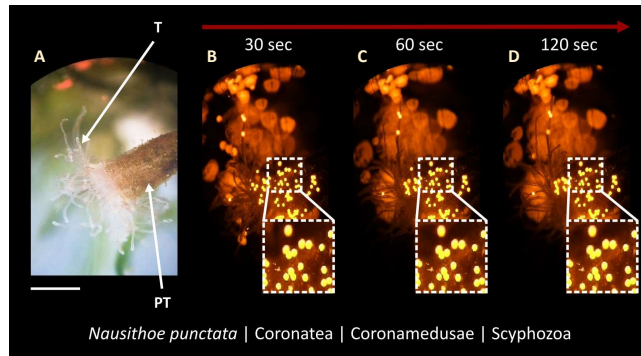


Figure 15 | Horizontal surface currents in the order Coronatae (Class: Scyphozoa, Subclass: Coronamedusae). Overhead perspective of **A** *Nausithoe punctata* under white light. The position of beads at three different time points in a single polyp of *Nausithoe punctata* (**B-D**) under red fluorescent light. PT: Peridermal Tube, T: Tentacle. White bar equals 1 mm.

Surface current divergence in the cnidarian phylum

Amongst the 30 studied cnidarian species, 17 had no surface currents, while 13 displayed clear horizontal flows. I was able to reveal that horizontal surface currents were exclusive to certain classes, particularly Anthozoa and Scyphozoa (Figure 16). The orders Zoantharia and Actiniaria stood out because they hosted both species that exhibited horizontal surface currents and those that did not. In contrast, all other examined orders comprised species that either exhibited or did not exhibit these currents. Noteworthy to mention is that the analysis excluded species from the Cubozoa and Staurozoa classes due to their unavailability at the HdM.

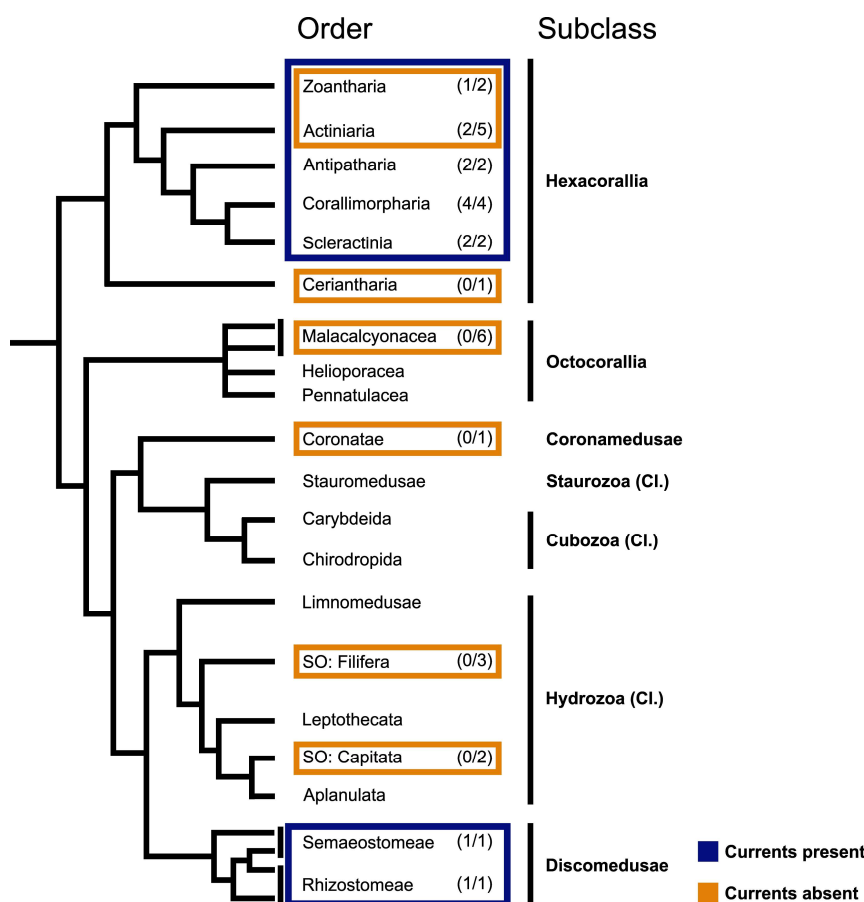


Figure 16 | Phylogenetic Tree of Cnidaria. Absence (orange) and Presence (blue) of horizontal surface currents across all analyzed orders (orders without color were not subject to this study). The numbers in parentheses indicate how many species, out of all the investigated species of the corresponding order, displayed horizontal surface currents. The suborders (SO) Filifera and Capitata belong to the order Anthoathecata. Cl.: Class. (Adapted from Marques and Collins 2004 and Kayal et al., 2013)

Track analysis

For track analysis, the characteristics were extracted from the raw track data generated in Imaris. Subsequently, for all further analytical steps, the 13 species displaying horizontal surface currents were grouped into their corresponding orders, resulting in seven orders in total. This grouping involved the aggregation of tracks from all specimens belonging to the same species (three recordings per species) (Table 4). For the statistical analysis, one Kruskal-Wallis test was performed for each of the eight explanatory key parameters (i.e., track turns, sinuosity, track length, displacement length, average speed, maximum speed, speed variance, and intersections), followed by a post hoc test (Dunn's test). The comparison of the seven orders with each other led to 21 pairwise comparisons per parameter, resulting in a total of 168 comparisons.

Table 4: Number of tracks per species and per order

Species	Number of tracks (per species) ^{(a),(b)}	Order	Number of tracks (per order) ^(c)
<i>Zoanthus sociatus</i>	375	Zoantharia	375
<i>Aiptasiogeton species</i>	481	Actiniaria	1487
<i>Exaiptasia diaphana</i>	1006		
<i>Cirrhipathes species</i>	609	Antipatharia	790
<i>Stichopathes species</i>	181		
<i>Corynactis californica</i>	492	Corallimorpharia	1101
<i>Corynactis viridis</i>	294		
<i>Pseudocorynactis species</i>	203		
<i>Ricordea species</i>	112		
<i>Cladocora species</i>	435	Scleractinia	1214
<i>Fungia species</i>	779		
<i>Cassiopea andromeda</i>	888	Rhizostomeae	888
<i>Sanderia malayensis</i>	159	Semaeostomeae	159

^(a) Number of tracks depends on applied microbead quantity

^(b) The sum of all tracks from specimen of the same species

^(c) The sum of all tracks from species of the same order

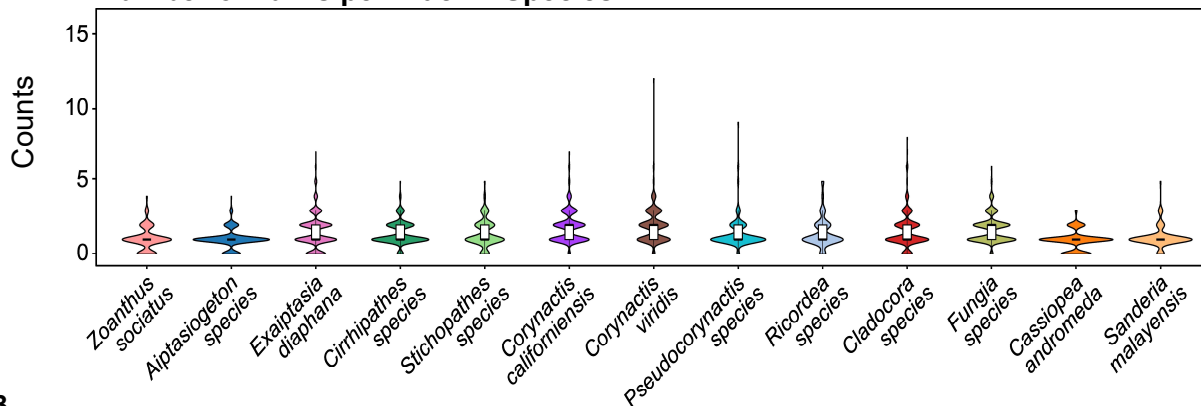
Number of track turns

Statistically significant differences among the seven orders were observed regarding the number of track turns ($\chi^2(6) = 547.08$, $p < 0.0001$) (Figure 17 B). Fifteen comparisons were statistically significant (Appendix A). The comparisons Zoantharia – Rhizostomeae, Zoantharia – Semaestomeae, Actiniaria – Antipatharia, Actiniaria – Semaestomeae, Corallimorpharia – Scleractinia, as well as Rhizostomeae – Semaestomeae, did not show statistically significant differences.

When looking at the medians of the species, *Fungia* sp., *Corynactis californica*, and *Corynactis viridis* exhibited a median of 2, while the remaining 10 species had a median of 1 (Figure 17 A) (Appendix I).

A

Number of Turns per Track – Species



B

Number of Turns per Track - Orders

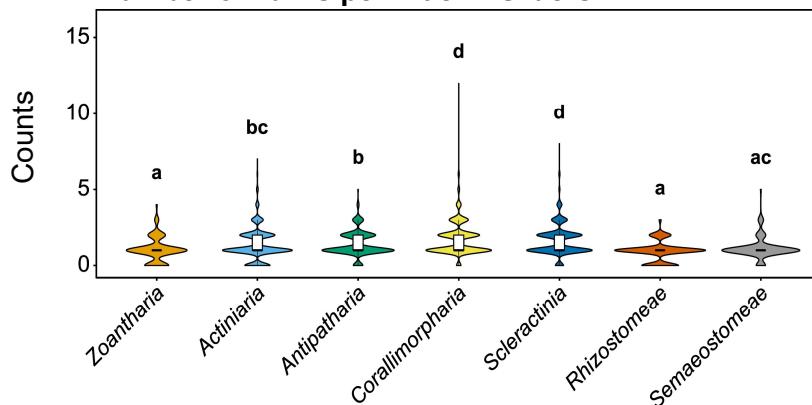


Figure 17 | Track turns – distribution. Number of turns per track for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn's test). Orders with the same letters show no statistically significant difference.

Sinuosity

Statistical testing revealed a significant difference among all seven orders ($\chi^2(6) = 949.29$, $p < 0.0001$) (Figure 18 B). Seven pairwise comparisons did not show statistically significant differences (Appendix B). These comparisons were Zoantharia – Actiniaria, Zoantharia – Rhizostomeae, Actiniaria – Rhizostomeae, Antipatharia – Scleractinia, Antipatharia – Semaestomeae, Corallimorpharia, – Semaestomeae, as well as Scleractinia – Semaestomeae.

Exaiptasia diaphana had the lowest median of 1.15, whereas *Pseudocorynactis* sp. showed the highest median of 2.71 (Figure 18 A) (Appendix I).

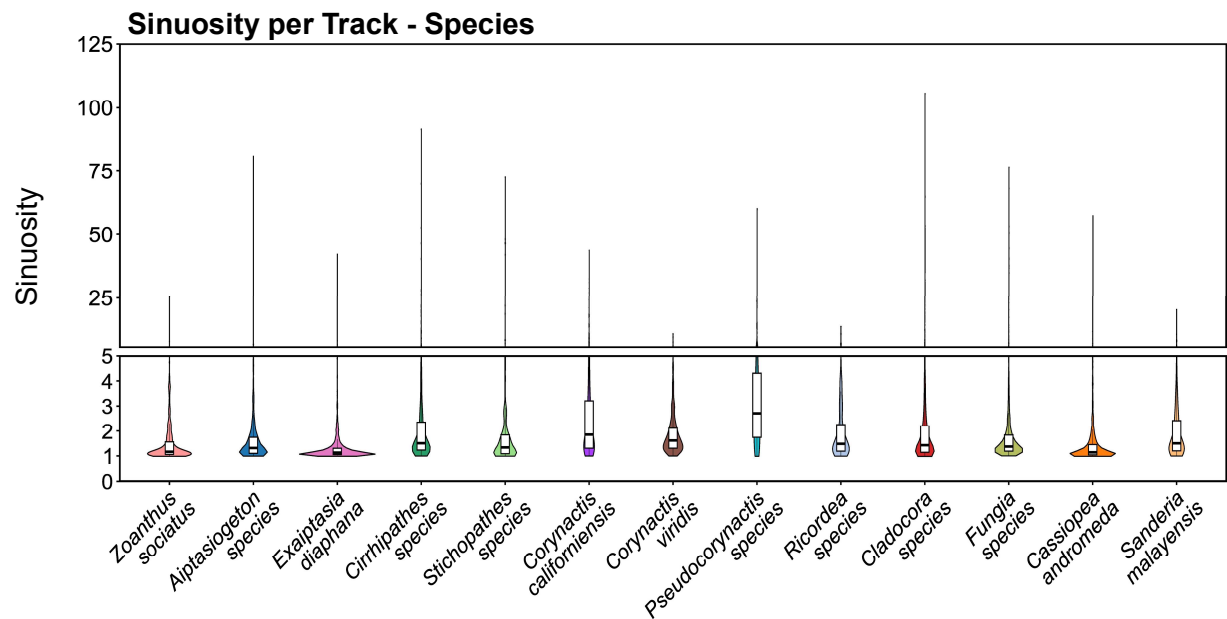
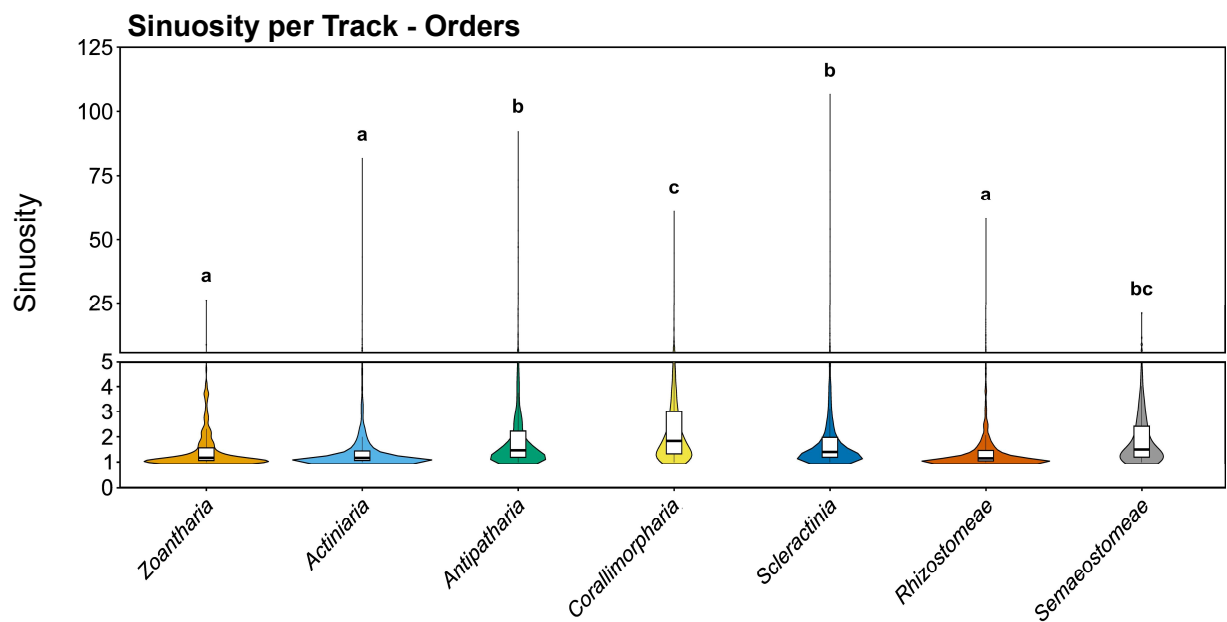
A**B**

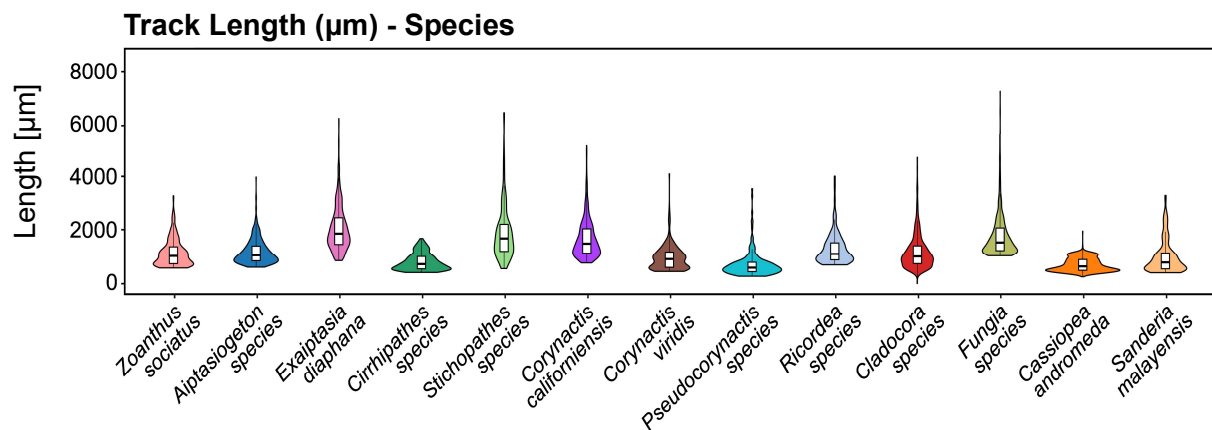
Figure 18 | Sinuosity – distribution. Sinuosity per track for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn's test). Orders with the same letters show no statistically significant difference.

Track length

A statistically significant difference among all seven orders was identified ($\chi^2(6) = 1853.4, p < 0.0001$) (Figure 19 B). Only Zoantharia and Corallimorpharia, as well as Antipatharia and Semaestomeae, were not statistically significantly different (Appendix C).

On the species level, *Pseudocorynactis* sp. had the lowest median of track length with 578.68 μm , and *Exaiptasia diaphana* had the highest value of 1794.91 μm (Figure 19 A) (Appendix I).

A



B

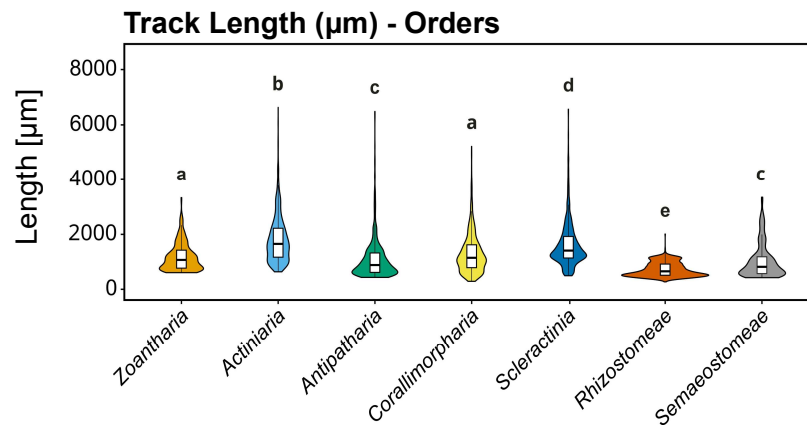


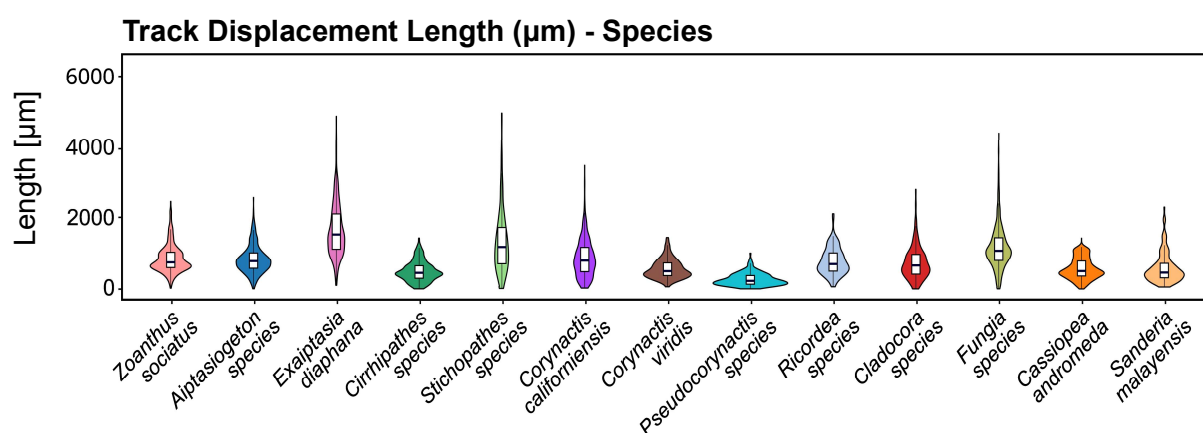
Figure 19 | Track length – distribution. Track length for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn's test). Orders with the same letters show no statistically significant difference.

Track displacement length

Track displacement length of the studied orders displayed statistically significant differences ($\chi^2(6) = 1474.9$, $p < 0.0001$) (Figure 20 B). The five comparisons Antipatharia – Corallimorpharia, Antipatharia – Rhizostomeae, Antipatharia – Semaestomeae, Corallimorpharia – Semaestomeae, and Rhizostomeae – Semaestomeae were not statistically significantly different (Appendix D).

Similar to the track length, *Pseudocorynactis* sp. exhibited the lowest median with 221.69 μm , whereas *Exaiptasia diaphana* showed the highest median with 1462.19 μm (Figure 20 A) (Appendix I).

A



B

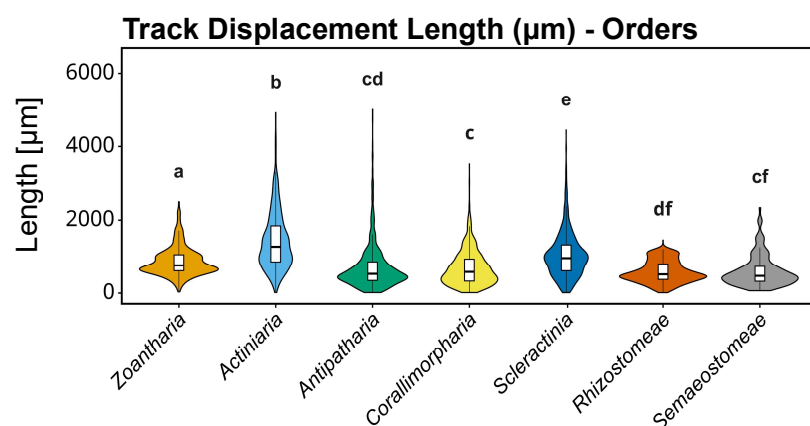


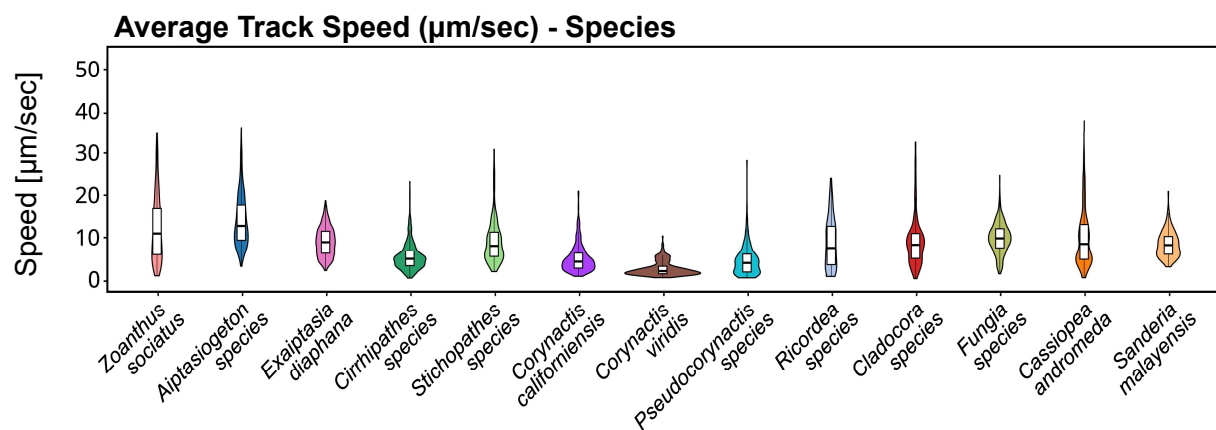
Figure 20 | Track displacement length – distribution. Track displacement length for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn's test). Orders with the same letters show no statistically significant difference.

Average track speed

All seven orders exhibited statistically significant differences in their average track speed ($\chi^2(6) = 1442.1$, $p < 0.001$) (Figure 21 B). Four comparisons displayed no statistically significant differences: Zoantharia – Actiniaria, Scleractinia – Rhizostomeae, Scleractinia – Semaestomeae, and Rhizostomeae – Semaestomeae (Appendix E).

Corynactis viridis showed the lowest median for average track speed of 2.23 $\mu\text{m}/\text{sec}$, while *Aiptasiogeton* sp. had the highest median of 12.33 $\mu\text{m}/\text{sec}$ (Figure 21 A) (Appendix I).

A



B

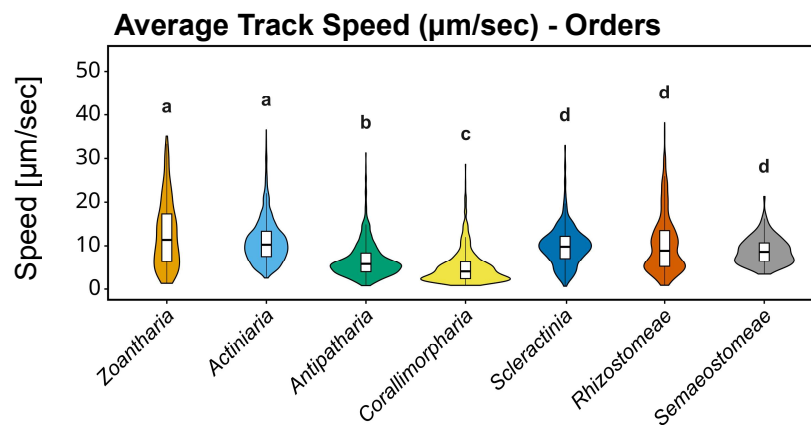


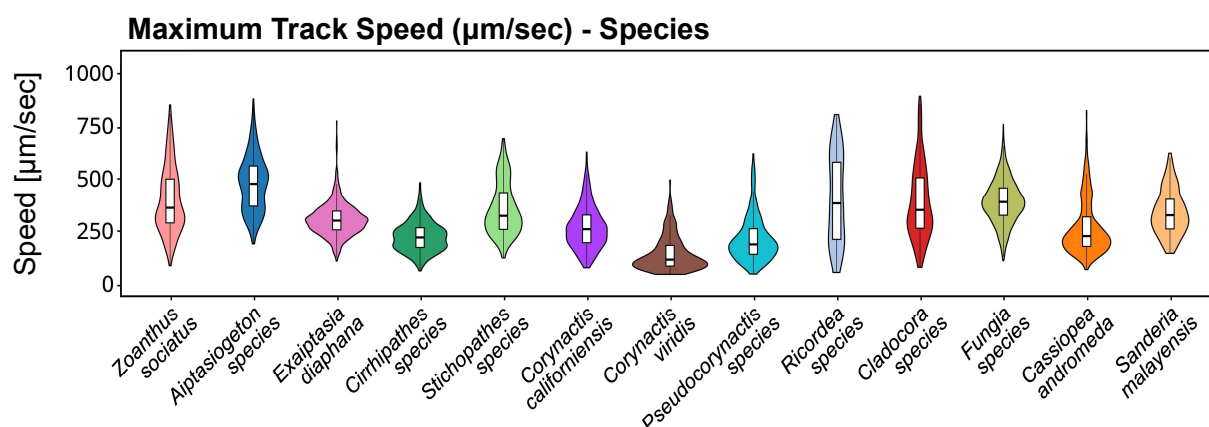
Figure 21 | Average track speed – distribution. Average track speed for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn’s test). Orders with the same letters show no statistically significant difference.

Maximum track speed

The maximum track speed across all seven orders exhibited statistically significant differences ($\chi^2(6) = 1424$, $p < 0.001$) (Figure 22 B). Here, the comparisons Zoantharia – Scleractinia, Zoantharia – Semaestomeae, Actiniaria – Semaestomeae, Antipatharia – Corallimorpharia and Antipatharia – Rhizostomeae exhibited no statistically significant differences (Appendix F).

Similar to the average track speed, *Corynactis viridis*. displayed the lowest median in maximum track speed with 115.79 $\mu\text{m}/\text{sec}$, while *Aiptasiogeton* sp. had the highest with 454.92 $\mu\text{m}/\text{sec}$ (Figure 22 A) (Appendix I).

A



B

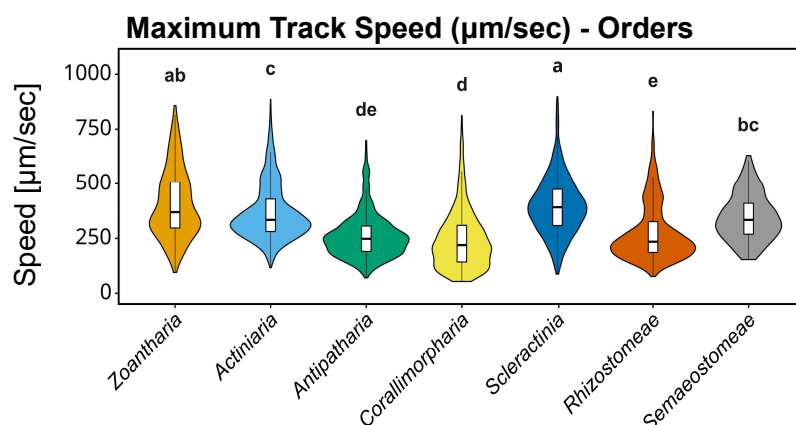


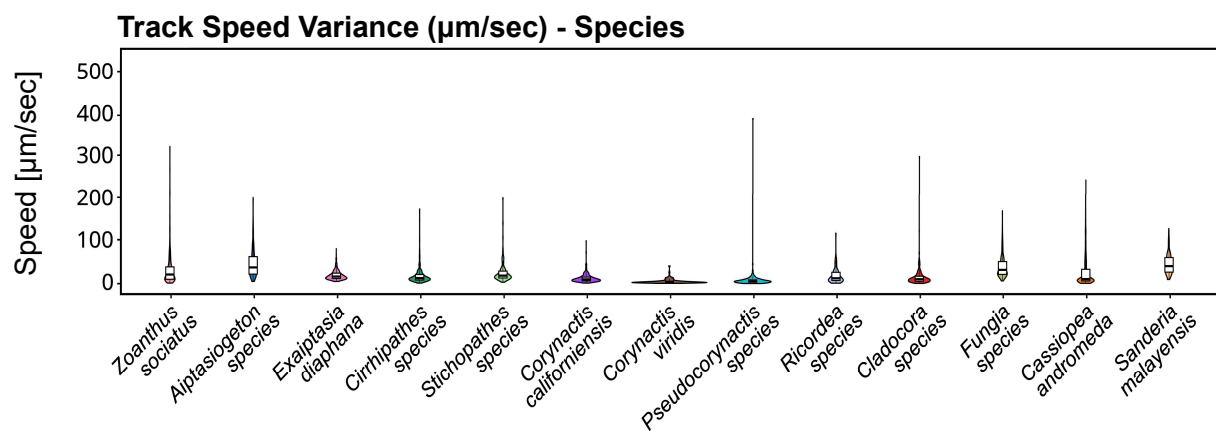
Figure 22 | Maximum track speed – distribution. Maximum track speed for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn's test). Orders with the same letters show no statistically significant difference.

Track speed variance

Additionally, the seven orders showed a statistically significant difference in speed variation ($\chi^2(6) = 1267.3$, $p < 0.0001$) (Figure 23 B). The comparisons Zoantharia – Actiniaria, Zoantharia – Scleractinia, Actiniaria – Scleractinia, and Antipatharia – Rhizostomeae did not show statistically significant differences (Appendix G).

The lowest median in track speed variances was displayed by *Corynactis viridis* with 3.9 $\mu\text{m}/\text{sec}$, while *Sanderia malayensis* showed the highest median with 312.75 $\mu\text{m}/\text{sec}$ (Figure 23 A) (Appendix G).

A



B

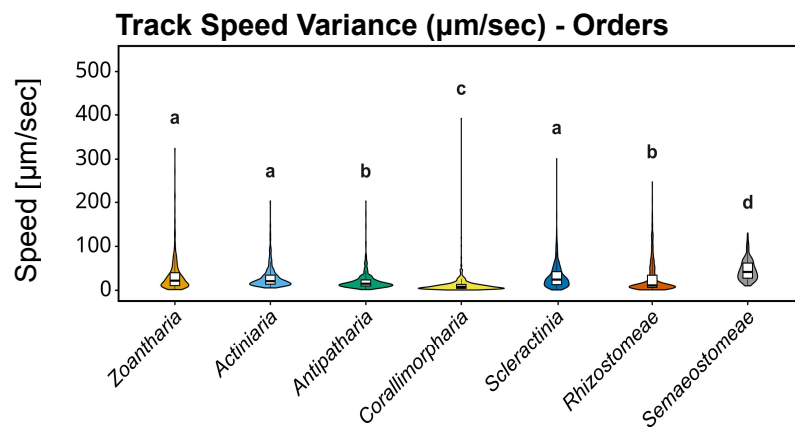


Figure 23 | Track speed variance– distribution. Track speed variance for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn’s test). Orders with the same letters show no statistically significant difference.

Intersections

Statistical analysis indicated significant differences in the number of intersections among the orders ($\chi^2(6) = 777.53, p < 0.001$) (Figure 24 B). Seven comparisons did not display statistically significant differences. These are Zoantharia – Actiniaria, Zoantharia – Scleractinia, Zoantharia – Rhizostomeae, Zoantharia – Semaestomeae, Actiniaria – Rhizostomeae, Antipatharia – Semaestomeae and Scleractinia – Semaestomeae (Appendix H).

When looking at the species level, *Zoanthus sociatus*, *Aiptasiogeton* sp., *Exaiptasia diaphana*, *Ricordea* sp., *Cladocora* sp., and *Cassiopea andromeda* had the lowest median with 0 intersections, while *Corynactis viridis* had the highest, with 8.5 intersections (Appendix I).

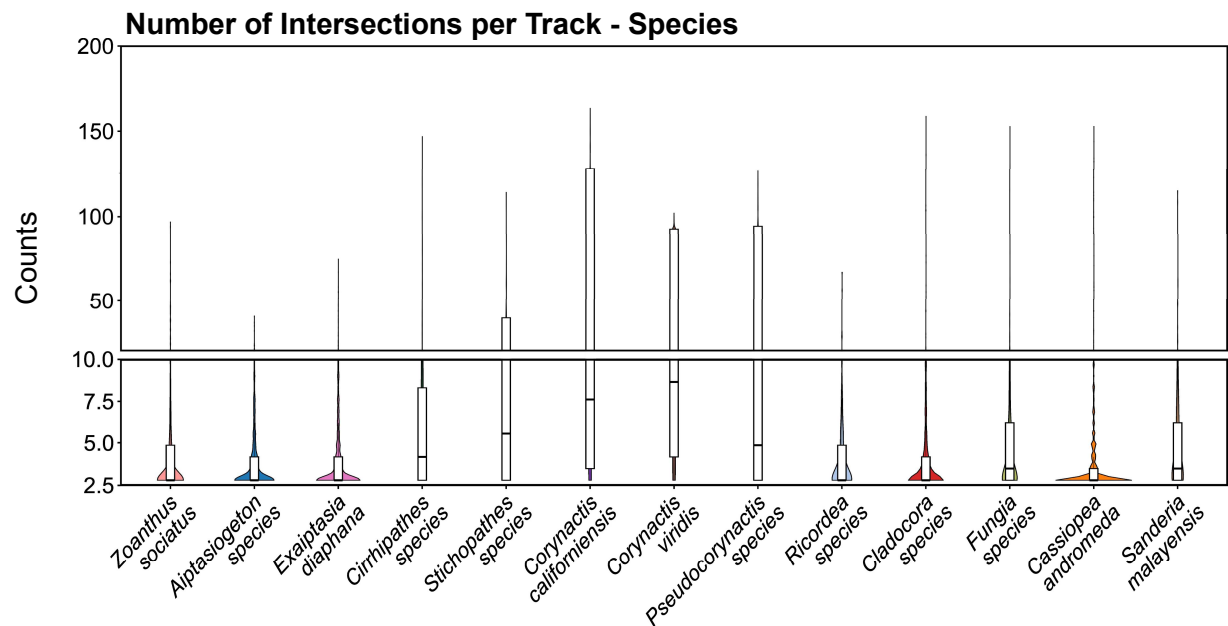
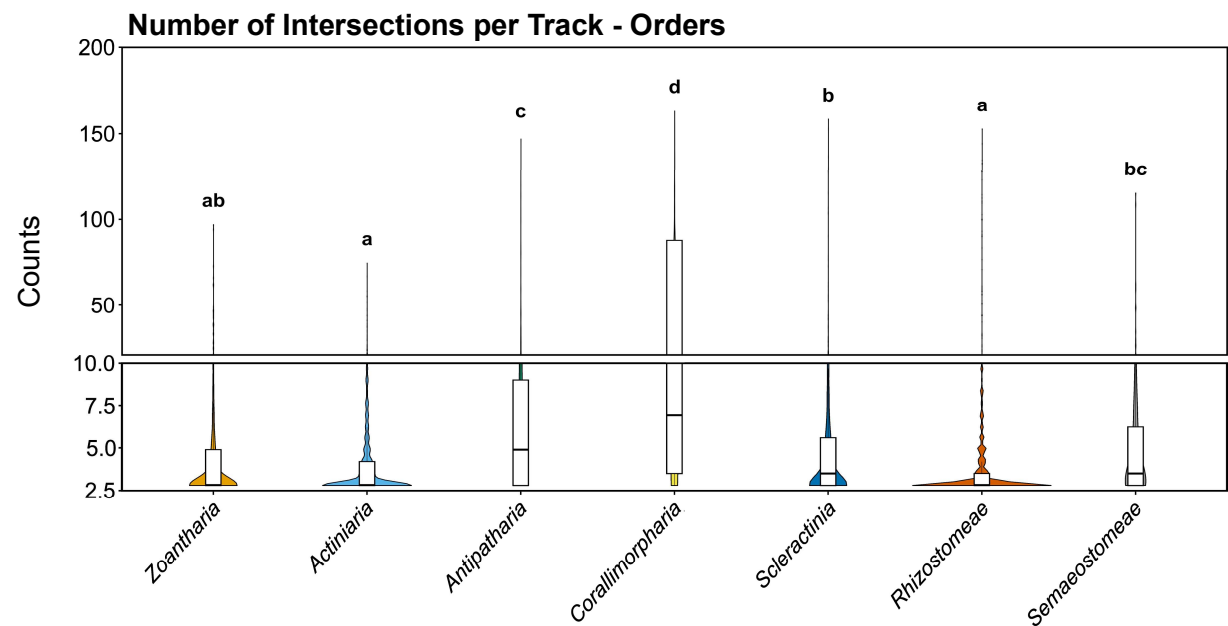
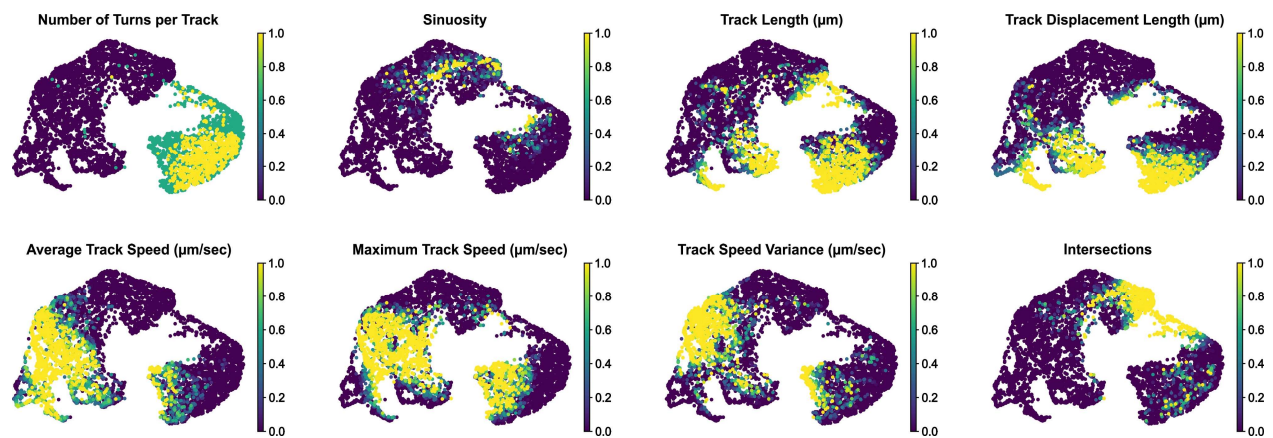
A**B**

Figure 24 | Intersections per track – distribution. Track intersections for **A** species and **B** orders. Boxplots display Median and Interquartile Range. Letters in **B** indicate significance after post hoc test (Dunn's test). Orders with the same letters show no statistically significant difference.

UMAP track analysis

The UMAP algorithm grouped 6014 tracks from 13 cnidarian species (Table 4) based on their similarity for each of the eight key parameters (see section Material and Methods – key parameters). Within each parameter, tracks that showed higher similarity were grouped into so-called “neighborhoods”; the color coding reflects the parameter value of each track. The result is a 2D projection of multidimensional track data for each parameter (Figure 25 A) and each species (Figure 25 B).

A



B

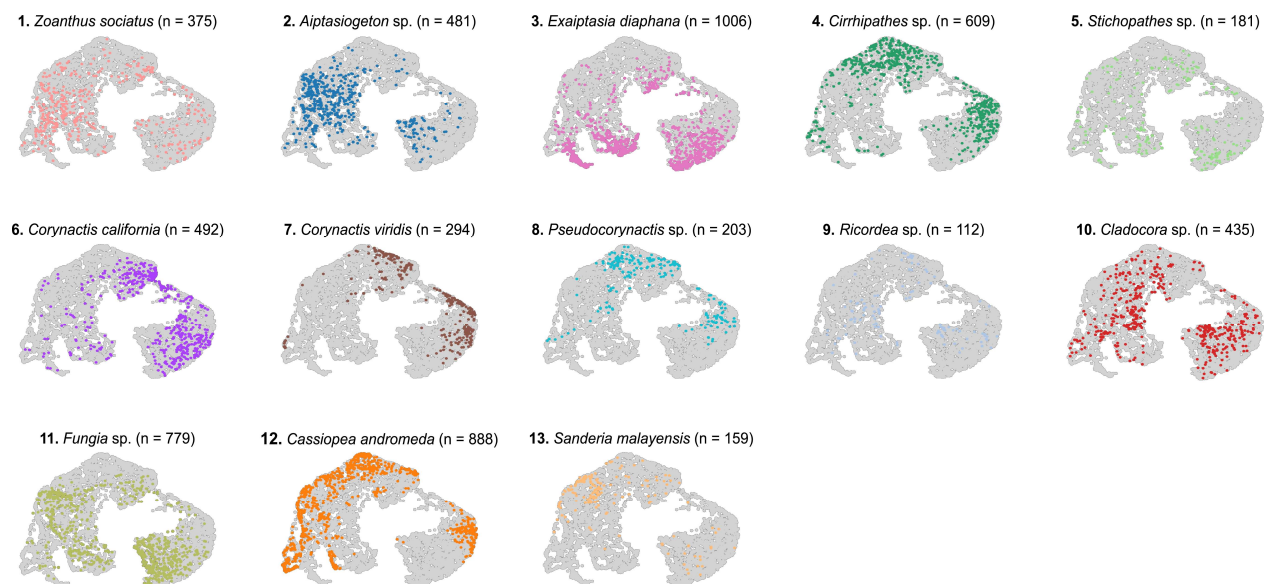


Figure 25 | Track classification. **A** UMAP 2D projection of the track distribution for each parameter and **B** for each species. Colors in **A** indicate that each track-value was standardized within the range of 0 to 1. Numbers in **B** the sum of all tracks for the respective species.

Results

When combining the UMAP projection of each parameter (Figure 25 A) with the individual species UMAP track projections (Figure 25 B1-B13), it becomes apparent that each species exhibits a unique combination and quantity of tracks that are located in these neighborhoods.

Zoanthus sociatus (Figure 25 B1) exhibited a grouping of tracks mainly defined by high average speed, high maximum speed, and high speed variance.

Aiptasiogeton sp. (Figure 25 B2) had many tracks with increased average track speed, high maximum track speed, and, to a lesser extent, a high speed variance. In contrast, *Exaiptasia diaphana* (Figure 25 B3) displayed a high number of tracks that were not only longer (compared to the tracks of the other species) but also had a longer displacement length and a high number of track turns.

The antipatharian species *Cirripathes* sp. (Figure 25 B4) and *Stichopathes* sp. (Figure 25 B5) portrayed different patterns. *Cirripathes* sp. had two track groupings outside any neighborhood that was indicative of particularly high values in all parameters. *Stichopathes* sp., however, did not show any track grouping with tracks spread across various neighborhoods.

Corynactis californica (Figure 25 B6) exhibited one track grouping in the area that is characterized by a high number of intersections, and another partly located in the region characterized by high number of track turns. *Corynactis viridis* (Figure 25 B7) and *Pseudocorynactis* sp. (Figure 25 B8) both displayed one grouping of tracks with a high number of track turns and one grouping that was located outside any neighborhood indicative of high values in all parameters. Similar to *Stichopathes* sp., *Ricordea* sp. (Figure 25 B9) also lacked a specific track grouping, with tracks distributed throughout the entire 2D projection.

Both *Cladocora* sp. (Figure 25 B10) and *Fungia* sp. (Figure 25 B11) featured tracks with a high number of track turns, high average speed, and maximum speed. Additionally, *Cladocora* sp. showed many tracks with high speed variance. In comparison, *Fungia* sp. had numerous tracks located where tracks with particularly high length or track displacement length were found.

Cassiopea andromeda (Figure 25 B12) had many tracks grouped in locations outside neighborhoods with particularly high values. Additionally, it had tracks marked by high average speed, maximum speed, speed variance, track length, and track displacement length.

Sanderia malayensis (Figure 25 B13) displayed a single cluster of tracks characterized by a high speed variance.

Leiden track analysis

In this analysis step, I utilized the Leiden algorithm to identify groups within the UMAP 2D projection of the multidimensional track data from Figure 25 A (Traag et al., 2019). This yielded five Leiden groups (LG) that are locally partitioned from each other (Figure 26).

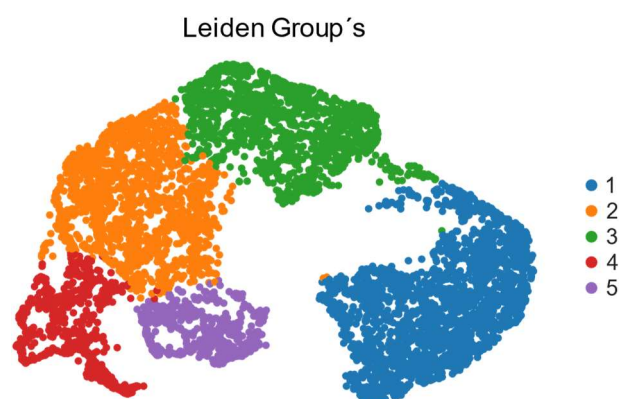


Figure 26 | Leiden group's. Grouping of the tracks into 5 distinct groups, according to the Leiden algorithm (1= blue; 2 = orange; 3 = green; 4 = red and 5 = purple).

LG one (blue) is situated in the UMAP projection, where tracks are primarily characterized by a multitude of turns. However, within this area, there were also tracks grouped together that stand out for their considerable length and displacement length, a high average speed, maximum speed, and speed variance. Particularly in the upper segment of LG one, adjacent to LG 3, there is an abundance of tracks that feature a high number of intersections.

LG two (yellow) is found within the UMAP projection, where tracks displayed elevated values for average speed, maximum speed, and speed variance.

The third LG (green) is positioned where the UMAP projection displays tracks featuring a substantial number of intersections, high sinuosity, and, to a lesser extent, tracks of considerable length.

In the UMAP projection, LG four (red) is located where tracks are distinguished by their substantial length, displacement length, and also by their elevated values in average speed, maximum speed, and speed variance.

Lastly, LG five (purple) is very similar to group four. However, this group encompasses the region in the UMAP projection where, in comparison to LG 4, there are more tracks that are particularly long and have a notably long displacement length, but fewer tracks characterized by high average speed, maximum speed, and speed variance.

Results

Next, the composition of the five LG for each species was determined (Figure 27 A). Following this step, the Euclidean distances between pairwise species compositions were calculated to assess the differences and similarities among each species' composition (Figure 27 B).

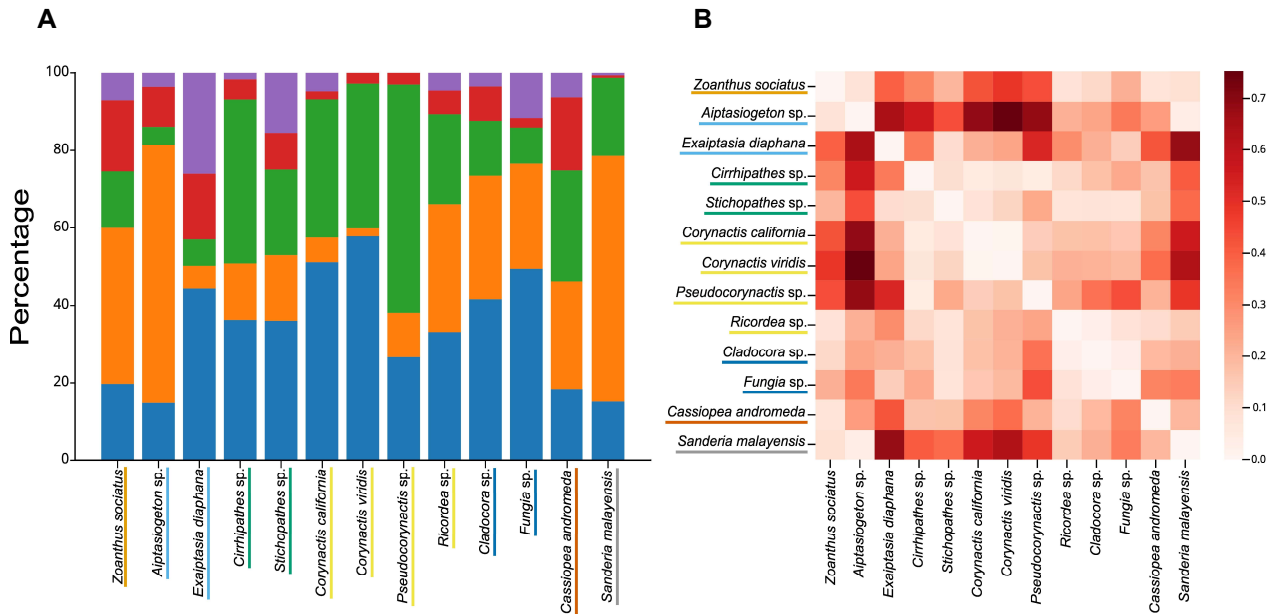


Figure 27 | Species comparison. **A** Composition of the five Leiden group from Figure 26 for each species in percentage. **B** Euclidean distances between each composition depicted in a heatmap. Colors indicate the corresponding orders (light orange: Zoantharia; light blue: Actiniaria; dark green: Antipatharia; yellow: Corallimorpharia; dark blue: Scleractinia; dark orange: Rhizostomeae and gray: Semaestomeae).

Zoanthus sociatus showed the highest similarity with *Aiptasiogeton* sp. and the second-highest similarity with *Cassiopea andromeda*, as well as *Sanderia malayensis* (Figure 27 A-B). In *Zoanthus sociatus*, *Aiptasiogeton* sp., and *Sanderia malayensis*, the majority of tracks were in LG 2, indicated by tracks with high average track speed, maximum speed, and speed variance. This fact could also be the reason why their compositions were the most distinct when compared to all the other species (Figure 27 B). Additionally, *Zoanthus sociatus* is similar to *Cassiopea andromeda* in that both had a comparable proportion of LG 1 (track turns) and LG 5 (track length and track displacement length).

Aiptasiogeton sp. was also notably similar to *Sanderia malayensis* (Figure 27 A-B). Both species, in comparison to all others, not only had the highest number of tracks grouped into LG 2 (average speed, maximum speed, speed variance) but also exhibited the fewest tracks that were located in LG 1 (track turns).

Exaiptasia diaphana exhibited the highest similarity with the antipatharian species *Stichopathes* sp. and the scleractinian species *Fungia* sp (Figure 27 A-B). Compared to all other species, these three species displayed the highest proportion of LG 5, indicating that they had the longest tracks on average with a high displacement length.

The compositions of *Cirripathes* sp and *Stichopathes* sp. are fairly similar because both species shared a similar proportion of tracks that are indicated by a comparably higher number of track turns (LG 1) (Figure 27 A-B). However, *Cirripathes* sp. was more similar to the corallimorpharian species *Pseudocorynactis* sp., *Corynactis californica*, and *Corynactis viridis*. Out of all species, all four had the biggest proportion of LG 3, which is mainly characterized by tracks that, in comparison to other tracks, feature a high number of intersections and a high sinuosity. Additionally, *Corynactis viridis* and *Pseudocorynactis* sp. did not exhibit any tracks that are grouped in LG 5 (track length and track displacement length) (Figure 27A).

Ricordea sp. is also very similar to *Cladocora* sp., in that both species have a similar proportion of tracks characterized by a higher average speed, maximum speed, and speed variance (LG 2), as well as a higher track length, and track displacement length (LG 5).

Discussion

This research project was driven by the realization that while Scleractinia exhibit horizontal surface currents, hydrozoans, like *Millepora alcicornis* do not. This formed the initial hypothesis that such currents were specific to anthozoans. This study, therefore, aimed to evaluate if this holds true. The primary purpose of the research was to assess the presence or absence of horizontal currents on the epidermal surfaces of cnidarians beyond scleractinians.

Through video microscopy and computer-assisted particle tracking, this study successfully provides visual proof of the existence of such surface currents in various cnidarian orders and species of the Anthozoa and Scyphozoa classes, with observed speeds reaching a maximum of nearly 1 mm per second (e.g., *Aiptasiogeton* sp. displayed tracks with a maximum speed of up to 962 $\mu\text{m}/\text{sec}$). I determined that on a species level, 13 out of the 30 studied species showed surface currents. These currents follow similar intricate trajectory in every individual of the same species. On the order level, 7 out of the 11 studied orders include at least one species that exhibits horizontal surface currents. These currents follow similar intricate trajectory in every individual of the same species.

While the overall flow direction is either toward or away from the polyp mouth, each species displays a unique arrangement of horizontal surface currents which is rooted in variations of the describing key parameters: track turns, sinuosity, track length, track displacement length, average track speed, maximum track speed, speed variations and intersections.

Furthermore, the analysis has shown that none of the analyzed six octocorallian species (subclass) and five hydrozoan species (class) display these currents.

Unveiling evolutionary patterns

The observed distinctions in surface current behaviors among anthozoans, hydrozoans, and scyphozoans offer insightful glimpses into the diverse evolutionary strategies adopted by these distinct groups. This divergence suggests that, throughout Cnidaria's evolution, the trait of having horizontal surface currents underwent multiple instances of acquisition or loss across various classes and subclasses (Figure 16). The dynamic nature of surface current adaptations among different cnidarian subclasses provides a nuanced perspective on their evolutionary history and underscores the complexity of ecological and physiological adaptations within this diverse phylum.

Biological functions of horizontal surface currents

Cnidaria are predominantly sessile organisms and are commonly perceived to be entirely dependent on their surrounding environments (Pacherres et al., 2020). As a result, these animals must find alternative ways to sustain and provide for themselves and/or neighboring polyps. One way to do this is through the means of surface currents. Vertical vortices can help the individual polyp or colony to manipulate their immediate water environment and increase the availability of oxygen or nitrogen (Shapiro et al., 2014; Pacherres 2021; Pacherres et al., 2022). Horizontal surface currents are equally significant, though they have predominantly been documented in Scleractinians until now (Bouderlique et al., 2022). This study introduces the novel observation of horizontal surface currents in other orders, suggesting they likely play a crucial role in processes such as food uptake or the cleansing of polyps or colony surfaces. This hypothesis is consistent with previous research findings, suggesting that horizontal surface currents, particularly those described in Scleractinia, may play a role in feeding and cleaning. This expands our understanding of how these currents contribute to the ecological dynamics of Cnidaria (Yonge, 1930; Shapiro et al., 2014; Bouderlique et al., 2022).

Food capture and uptake

Horizontal surface currents in certain species of this phylum may be linked to processes associated with food acquisition (Bouderlique et al., 2022). The analysis of fluorescent microbeads revealed that solitary anthozoans, such as corallimorpharians, or actiniarians gather the beads in the central region around the mouth before ingestion. This suggests that these horizontal currents are helpful for feeding. Additionally, the potential significance of horizontal surface currents for food uptake is further confirmed by the fact that these currents are particularly pronounced directly around the mouth of the polyps. The findings presented here are consistent with the observations made by Bouderlique et al. (2022), wherein a directional movement of free-moving particles into the mouths of polyps of the scleractinian species such as *Echinopora lamellosa* (Esper, 1791) was observed.

Transportation purposes

Consistent with preceding findings from Boudier et al. (2022), is the observation that cnidarian species, including those of the order scleractinians, are actively clearing their surfaces with horizontal surface currents. This suggests that these currents may have similar functions in different cnidarian orders.

These currents seem to play a role in cleansing the polyp surface, particularly in the region surrounding the mouth within the tentacle ring. Both solitary species, such as *Zoanthus sociatus* and *Exaiptasia diaphana*, and colonial species, exemplified by *Cladocora* sp. and *Cirrhipathes* sp., predominantly redirect the applied beads away from the polyp mouth. Given that cnidarian polyps and medusae possess a single exterior opening serving dual roles as both a mouth and anus, this specific area of the polyp is particularly important (Daly et al., 2007). Consequently, it becomes essential to develop strategies to maintain the cleanliness of this region, particularly in species known to inhabit benthic marine environments characterized by substantial sedimentation, such as intertidal zones like sea anemones such as *Exaiptasia diaphana* (Camillo et al., 2020).

Moreover, horizontal surface currents might influence chemical microenvironments by enhancing the transport and dispersion of substances such as oxygen, particularly in species hosting zooxanthellae and forming colonies. Previous observations in scleractinian species have indicated that vertical vortices contribute to oxygen distribution in colony areas with fewer symbionts (Pacherres et al., 2022). Consequently, it is reasonable to propose that horizontal surface currents could also play a similar role in redistributing oxygen across cnidarians and colony surfaces.

Surface current variability in Octocorallia and Scleractinia

Octocorallia (subclass) and Scleractinia (order) are both Anthozoa. However, based on the findings of this study, they differ in the display of horizontal surface currents.

Scleractinians of various growth forms, such as *Echinopora lamellosa* (foliaceous growth form), *Fungia* sp. (solitary and free-living), or *Stylophora pistillata* (Esper, 1792) (branching growth form), exhibit intricate networks of horizontal surface currents (Boudier et al., 2022). All six examined Octocorallia species lack such currents. This suggests that either octocorals may have developed alternative strategies to compensate for the absence of these currents, or conversely, scleractinians may have generated the currents to compensate for the absence or deficiency of something that Octocorallia possess.

Furthermore, Octocorallia, (e.g., *Menella* sp. or *Guaiaorgia anas*) demonstrate greater flexibility in extending and stretching their polyps than scleractinians. This flexibility could help minimize exposure to sedimentation. In sedimentation, octocoral polyps can rise above the sediment, mitigating potential sedimentation effects and facilitating the removal of particles from the entire colony surface. This adaptation may eliminate the necessity for horizontal surface currents to clean the surfaces.

Another aspect that could potentially explain why scleractinians exhibit horizontal surface currents while octocorals do not is that octocorals are known to release terpenoids into the surrounding water, which can act as ichthyotoxins during periods of stress, such as predation (Rodriguez et al., 2020). This could suggest that octocorals have developed alternative mechanisms for protection and, as a result, do not depend on horizontal surface currents for defense.

Additionally, these variations may offer insights into the level of independence exhibited by individual polyps in Scleractinia and Octocorallia. Scleractinian polyps, being less "individualistic" and residing in closer proximity have been compelled to adapt and evolve to this lifestyle. Consequently, it is logical to facilitate the distribution of resources, such as food, nutrients, or oxygen, throughout the entire colony via horizontal surface currents. While Octocorallia also live colonially, individual polyps are more flexible and exhibit greater plasticity, allowing them to supply themselves individually, more easily, and making them more independent.

Study limitations

While I employed a novel and robust method to display horizontal surface currents utilizing red fluorescent microbeads, it is necessary to acknowledge several limitations in interpreting the study findings. The number, and selection of species are constrained due to the sourcing of Cnidaria from the local public aquarium, Haus des Meeres thereby introducing biases in the representativity of cnidarian species.

Additionally, the application of beads as a tracer for surface currents may act as a stress factor for all animals, implying that the recordings were not conducted under entirely stress-free conditions. A commonly observed reaction to stress in all cnidarians is the production of mucus (Bakshani et al., 2018). While mucus production was evident across all species, certain orders, like Actiniaria or Scleractinia, displayed notably elevated levels of mucus production, which could potentially impact the observed behaviors.

External environmental parameters, including water temperature or salinity, were not consistently monitored during the experiments, limiting the ability to account for potential influences on the recorded surface currents.

Another limitation of this study is that a maximum of only three individuals from each species were recorded, compromising the results' robustness. Moreover, all individuals of the same species obtained from the HdM are clones formed through fragmentation or other forms of asexual reproduction.

Given that most species were recorded from above, with exceptions in scyphozoan polyps, Antipatharia, and *Cladocora sp.*, the identification of horizontal surface currents was limited to the oral side of the polyp. This limitation hinders a comprehensive evaluation of whether specific species, particularly solitary polyps, manifest surface currents on their lateral surfaces.

Outlook

The path forward in this research involves addressing several crucial aspects to enhance the depth and scope of our understanding. It is imperative to broaden the taxonomic coverage by incorporating more species, encompassing a greater range of orders, and including additional classes. Field experiments will provide valuable insights, allowing us to observe and document phenomena in a real-world context. To deepen our understanding of the biological functions of horizontal surface currents, such as their involvement in food uptake or surface cleaning, conducting additional, more detailed experiments is crucial. Even within a single order, like Scleractinia. Species such as *Echinopora lamellosa* or *Acropora lamarcki* (Veron, 2000) predominantly exhibit horizontal surface currents toward the mouth, while others, like *Fungia sp.*, show currents away from the mouth (Bouderlique et al., 2022). This suggests that these currents are adapted to specific needs within each species. Furthermore, the importance of already acquired data on species, encompassing factors like geographical distribution, skeletal formation, and symbiotic relationships with Zooxanthellae, must not be overlooked. Integrating this existing information into our analysis is essential for gaining a thorough understanding of the dynamics of surface currents in these ecosystems, whether present or absent. Given that the project is still underway, the intention is to incorporate the already collected data into the analysis, enriching our perspective and providing a more comprehensive view of these environmental dynamics.

Conclusion

This research builds on knowledge from previous research efforts dating back almost 100 years (Yonge et al., 1930). It significantly contributes to our understanding of horizontal surface currents in cnidarians, addressing a pivotal knowledge gap prompted by preliminary observations indicating the absence of such currents in hydrozoans, specifically *Millepora alcicornis*. The study successfully invalidated the initial hypothesis that horizontal surface currents are exclusive to anthozoans. In fact, they extend across various cnidarian orders within the Anthozoa and Scyphozoa classes.

Utilizing advanced techniques such as video microscopy and computer-assisted particle tracking, the study visually demonstrated the intricate trajectories of horizontal surface currents, highlighting species-specific behaviors across the observed 30 species and 11 orders. Notably, the absence of these currents in Octocorallia and Hydrozoa underscores the potential influence of external factors such as resource availability or habitat conditions.

The divergence in surface current behaviors between anthozoans, hydrozoans, and scyphozoans sheds light on distinct evolutionary strategies. We can infer that during the evolution of Cnidaria, the trait of having horizontal surface currents was either acquired or lost multiple times in different subclasses. This newfound knowledge changes the perception we have today of cnidarian phylogeny.

This study explored the potential biological functions of these currents, emphasizing their crucial role in nutrient transportation, food uptake, and the cleansing of polyp surfaces. The findings align with the broader ecological context, highlighting the significance of horizontal surface currents for the survival and maintenance of sessile cnidarians, particularly in a nutrient-poor environment, such as found in tropical coral reefs.

Cnidaria stand as integral components of marine biodiversity, warranting heightened care and attention to ensure their protection and preservation. Given their pivotal role in sustaining diverse marine ecosystems, particularly within reef environments, a more comprehensive and systematic understanding of Cnidaria becomes imperative. This imperative understanding is essential not only for the well-being of Cnidaria but also for the broader ecological balance and the survival of numerous other species intricately linked to these vital marine organisms.

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Appendices

Appendix A: P-values of the post hoc test (Dunn's test) – **track turns**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	2.88×10^{-8}	6.04×10^{-7}	****
2		Antipatharia	5.73×10^{-13}	1.20×10^{-11}	****
3		Corallimorpharia	9.36×10^{-36}	1.97×10^{-34}	****
4		Scleractinia	5.27×10^{-30}	1.11×10^{-28}	****
5		Rhizostomeae	5.20×10^{-2}	1	ns
6		Semaeostomeae	3.52×10^{-1}	1	ns
7	Actiniaria	Antipatharia	2.84×10^{-3}	5.96×10^{-2}	ns
8		Corallimorpharia	9.78×10^{-27}	2.05×10^{-25}	****
9		Scleractinia	9.76×10^{-20}	2.05×10^{-18}	****
10		Rhizostomeae	2.98×10^{-25}	6.27×10^{-24}	****
11		Semaeostomeae	5.31×10^{-3}	1.11×10^{-1}	ns
12	Antipatharia	Corallimorpharia	2.92×10^{-10}	6.13×10^{-9}	****
13		Scleractinia	1.51×10^{-6}	3.18×10^{-5}	****
14		Rhizostomeae	1.59×10^{-31}	3.33×10^{-30}	****
15		Semaeostomeae	2.81×10^{-5}	5.90×10^{-4}	***
16	Corallimorpharia	Scleractinia	4.35×10^{-82}	1	ns
17		Rhizostomeae	7.43×10^{-2}	9.14×10^{-81}	****
18		Semaeostomeae	8.40×10^{-15}	1.77×10^{-13}	****
19	Scleractinia	Rhizostomeae	4.28×10^{-12}	8.99×10^{-11}	****
20		Semaeostomeae	6.21×10^{-72}	1.30×10^{-70}	****
21	Rhizostomeae	Semaeostomeae	1.59×10^{-2}	3.33×10^{-1}	ns

Appendix B: P-values of the post hoc test (Dunn's test) – **sinuosity**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	4.65×10^{-1}	1	ns
2		Antipatharia	9.32×10^{-23}	1.96×10^{-21}	****
3		Corallimorpharia	3.41×10^{-55}	7.16×10^{-54}	****
4		Scleractinia	1.79×10^{-19}	3.75×10^{-18}	****
5		Rhizostomeae	1.10×10^{-1}	1	ns
6		Semaeostomeae	6.85×10^{-13}	1.44×10^{-11}	****
7	Actiniaria	Antipatharia	1.95×10^{-50}	4.10×10^{-49}	****
8		Corallimorpharia	2.09×10^{-133}	4.38×10^{-132}	****
9		Scleractinia	4.66×10^{-50}	9.79×10^{-49}	****
10		Rhizostomeae	1.86×10^{-1}	1	ns
11		Semaeostomeae	5.04×10^{-18}	1.06×10^{-16}	****
12	Antipatharia	Corallimorpharia	7.46×10^{-12}	1.57×10^{-10}	****
13		Scleractinia	7.01×10^{-2}	1	ns
14		Rhizostomeae	3.08×10^{-48}	6.46×10^{-47}	****
15		Semaeostomeae	4.65×10^{-1}	1	ns
16	Corallimorpharia	Scleractinia	4.04×10^{-22}	8.48×10^{-21}	****
17		Rhizostomeae	3.31×10^{-116}	6.95×10^{-115}	****
18		Semaeostomeae	2.52×10^{-3}	5.29×10^{-2}	ns
19	Scleractinia	Rhizostomeae	2.11×10^{-46}	4.42×10^{-45}	****
20		Semaeostomeae	8.24×10^{-2}	1	ns
21	Rhizostomeae	Semaeostomeae	1.64×10^{-19}	3.44×10^{-18}	****

Appendix C: P-values of the post hoc test (Dunn's test) – **track length**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	1.22×10^{-41}	2.56×10^{-40}	****
2		Antipatharia	2.13×10^{-5}	4.47×10^{-4}	***
3		Corallimorpharia	7.56×10^{-2}	1	ns
4		Scleractinia	7.30×10^{-22}	1.53×10^{-20}	****
5		Rhizostomeae	8.69×10^{-42}	1.83×10^{-40}	****
6		Semaeostomeae	7.88×10^{-5}	1.66×10^{-3}	**
7	Actiniaria	Antipatharia	6.53×10^{-125}	1.37×10^{-123}	****
8		Corallimorpharia	1.72×10^{-64}	3.62×10^{-63}	****
9		Scleractinia	3.43×10^{-8}	7.20×10^{-7}	****
10		Rhizostomeae	0.01×10^{-10}	0.01×10^{-9}	****
11		Semaeostomeae	1.46×10^{-43}	3.07×10^{-42}	****
12	Antipatharia	Corallimorpharia	1.41×10^{-15}	2.95×10^{-14}	****
13		Scleractinia	2.80×10^{-74}	5.87×10^{-73}	****
14		Rhizostomeae	4.72×10^{-31}	9.90×10^{-30}	****
15		Semaeostomeae	2.19×10^{-1}	1	ns
16	Corallimorpharia	Scleractinia	1.62×10^{-28}	3.41×10^{-27}	****
17		Rhizostomeae	2.25×10^{-96}	4.72×10^{-95}	****
18		Semaeostomeae	1.56×10^{-8}	3.27×10^{-7}	****
19	Scleractinia	Rhizostomeae	3.69×10^{-221}	7.75×10^{-220}	****
20		Semaeostomeae	6.31×10^{-29}	1.33×10^{-27}	****
21	Rhizostomeae	Semaeostomeae	8.94×10^{-8}	1.88×10^{-6}	****

Appendix D: P-values of the post hoc test (Dunn's test) – **track displacement length**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	7.68×10^{-30}	1.61×10^{-28}	****
2		Antipatharia	2.84×10^{-15}	5.96×10^{-14}	****
3		Corallimorpharia	5.79×10^{-14}	1.22×10^{-12}	****
4		Scleractinia	9.63×10^{-4}	2.02×10^{-2}	*
5		Rhizostomeae	8.58×10^{-23}	1.80×10^{-21}	****
6		Semaeostomeae	4.56×10^{-11}	9.57×10^{-10}	****
7	Actiniaria	Antipatharia	2.35×10^{-150}	4.94×10^{-149}	****
8		Corallimorpharia	1.12×10^{-169}	2.35×10^{-168}	****
9		Scleractinia	1.06×10^{-32}	2.22×10^{-31}	****
10		Rhizostomeae	3.02×10^{-194}	6.33×10^{-193}	****
11		Semaeostomeae	5.05×10^{-53}	1.06×10^{-51}	****
12	Antipatharia	Corallimorpharia	3.23×10^{-1}	1	ns
13		Scleractinia	1.88×10^{-51}	3.95×10^{-50}	****
14		Rhizostomeae	2.51×10^{-2}	5.27×10^{-1}	ns
15		Semaeostomeae	1.42×10^{-1}	1	ns
16	Corallimorpharia	Scleractinia	5.54×10^{-54}	1.16×10^{-52}	****
17		Rhizostomeae	5.57×10^{-4}	1.17×10^{-2}	*
18		Semaeostomeae	4.06×10^{-2}	8.53×10^{-1}	ns
19	Scleractinia	Rhizostomeae	2.11×10^{-73}	4.42×10^{-72}	****
20		Semaeostomeae	2.98×10^{-22}	6.27×10^{-21}	****
21	Rhizostomeae	Semaeostomeae	8.35×10^{-1}	1	ns

Appendix E: P-values of the post hoc test (Dunn's test) – **average track speed**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	7.87×10^{-1}	1	ns
2		Antipatharia	8.18×10^{-49}	1.72×10^{-47}	****
3		Corallimorpharia	9.78×10^{-106}	2.05×10^{-104}	****
4		Scleractinia	1.25×10^{-3}	2.63×10^{-2}	*
5		Rhizostomeae	2.95×10^{-7}	6.20×10^{-6}	****
6		Semaeostomeae	1.33×10^{-4}	2.79×10^{-3}	**
7	Actiniaria	Antipatharia	7.96×10^{-94}	1.67×10^{-92}	****
8		Corallimorpharia	9.62×10^{-231}	2.02×10^{-229}	****
9		Scleractinia	6.04×10^{-6}	1.27×10^{-4}	***
10		Rhizostomeae	1.48×10^{-12}	3.10×10^{-11}	****
11		Semaeostomeae	3.36×10^{-5}	7.06×10^{-4}	***
12	Antipatharia	Corallimorpharia	1.69×10^{-16}	3.55×10^{-15}	****
13		Scleractinia	2.13×10^{-57}	4.48×10^{-56}	****
14		Rhizostomeae	3.73×10^{-35}	7.83×10^{-34}	****
15		Semaeostomeae	1.21×10^{-10}	2.55×10^{-9}	****
16	Corallimorpharia	Scleractinia	4.80×10^{-158}	1.01×10^{-156}	****
17		Rhizostomeae	9.31×10^{-107}	1.96×10^{-105}	****
18		Semaeostomeae	8.62×10^{-29}	1.81×10^{-27}	****
19	Scleractinia	Rhizostomeae	4.61×10^{-3}	9.68×10^{-2}	ns
20		Semaeostomeae	4.26×10^{-2}	8.94×10^{-1}	ns
21	Rhizostomeae	Semaeostomeae	5.94×10^{-1}	1	ns

Appendix F: P-values of the post hoc test (Dunn's test) – **maximum track speed**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	1.59×10^{-3}	3.34×10^{-2}	*
2		Antipatharia	1.73×10^{-58}	3.62×10^{-57}	****
3		Corallimorpharia	3.83×10^{-80}	8.03×10^{-79}	****
4		Scleractinia	6.16×10^{-1}	1	ns
5		Rhizostomeae	2.39×10^{-54}	5.03×10^{-53}	****
6		Semaeostomeae	2.88×10^{-3}	6.05×10^{-2}	ns
7	Actiniaria	Antipatharia	5.88×10^{-79}	1.23×10^{-77}	****
8		Corallimorpharia	2.51×10^{-126}	5.26×10^{-125}	****
9		Scleractinia	4.17×10^{-8}	8.75×10^{-7}	****
10		Rhizostomeae	2.45×10^{-74}	5.14×10^{-73}	****
11		Semaeostomeae	2.33×10^{-1}	1	ns
12	Antipatharia	Corallimorpharia	8.87×10^{-3}	1.86×10^{-1}	ns
13		Scleractinia	1.24×10^{-114}	2.61×10^{-113}	****
14		Rhizostomeae	2.55×10^{-1}	1	ns
15		Semaeostomeae	4.75×10^{-17}	9.98×10^{-16}	****
16	Corallimorpharia	Scleractinia	1.01×10^{-171}	2.12×10^{-170}	****
17		Rhizostomeae	8.06×10^{-5}	1.69×10^{-3}	**
18		Semaeostomeae	1.02×10^{-23}	2.14×10^{-22}	****
19	Scleractinia	Rhizostomeae	2.22×10^{-110}	4.66×10^{-109}	****
20		Semaeostomeae	2.19×10^{-4}	4.60×10^{-3}	**
21	Rhizostomeae	Semaeostomeae	5.00×10^{-15}	1.05×10^{-13}	****

Appendix G: P-values of the post hoc test (Dunn's test) – **track speed variance**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	1.10×10^{-1}	1	ns
2		Antipatharia	1.04×10^{-8}	2.18×10^{-7}	****
3		Corallimorpharia	2.10×10^{-68}	4.40×10^{-67}	****
4		Scleractinia	8.95×10^{-2}	1	ns
5		Rhizostomeae	2.05×10^{-14}	4.31×10^{-13}	****
6		Semaeostomeae	2.27×10^{-16}	4.76×10^{-15}	****
7	Actiniaria	Antipatharia	1.27×10^{-24}	2.67×10^{-23}	****
8		Corallimorpharia	9.13×10^{-180}	1.92×10^{-178}	****
9		Scleractinia	8.37×10^{-1}	1	ns
10		Rhizostomeae	2.94×10^{-40}	6.17×10^{-39}	****
11		Semaeostomeae	2.37×10^{-16}	4.97×10^{-15}	****
12	Antipatharia	Corallimorpharia	7.22×10^{-49}	1.52×10^{-47}	****
13		Scleractinia	1.01×10^{-23}	2.12×10^{-22}	****
14		Rhizostomeae	2.24×10^{-2}	4.71×10^{-1}	ns
15		Semaeostomeae	5.31×10^{-39}	1.11×10^{-37}	****
16	Corallimorpharia	Scleractinia	1.46×10^{-166}	3.07×10^{-165}	****
17		Rhizostomeae	4.12×10^{-37}	8.65×10^{-36}	****
18		Semaeostomeae	2.75×10^{-102}	5.78×10^{-101}	****
19	Scleractinia	Rhizostomeae	2.77×10^{-38}	5.81×10^{-37}	****
20		Semaeostomeae	1.06×10^{-15}	2.23×10^{-14}	****
21	Rhizostomeae	Semaeostomeae	1.43×10^{-47}	3.01×10^{-46}	****

Appendix H: P-values of the post hoc test (Dunn's test) – **intersections**. The table provides the p-value, adjusted p-value (Bonferroni correction), and significance level for all 21 pairwise comparisons of the 7 orders that display horizontal surface currents.

Nr.	Order 1	Order 2	p-value	p-value (adjusted)	significance
1	Zoantharia	Actiniaria	2.54×10^{-1}	1	ns
2		Antipatharia	1.99×10^{-16}	4.17×10^{-15}	****
3		Corallimorpharia	1.17×10^{-43}	2.46×10^{-42}	****
4		Scleractinia	2.25×10^{-2}	4.73×10^{-1}	ns
5		Rhizostomeae	2.51×10^{-3}	5.26×10^{-2}	ns
6		Semaeostomeae	3.13×10^{-3}	6.57×10^{-2}	ns
7	Actiniaria	Antipatharia	8.92×10^{-40}	1.87×10^{-38}	****
8		Corallimorpharia	6.64×10^{-112}	1.39×10^{-110}	****
9		Scleractinia	2.14×10^{-7}	4.49×10^{-6}	****
10		Rhizostomeae	4.56×10^{-3}	9.58×10^{-2}	ns
11		Semaeostomeae	3.46×10^{-5}	7.27×10^{-4}	***
12	Antipatharia	Corallimorpharia	2.12×10^{-11}	4.46×10^{-10}	****
13		Scleractinia	8.14×10^{-17}	1.71×10^{-15}	****
14		Rhizostomeae	1.21×10^{-46}	2.53×10^{-45}	****
15		Semaeostomeae	6.57×10^{-3}	1.38×10^{-1}	ns
16	Corallimorpharia	Scleractinia	2.52×10^{-62}	5.29×10^{-61}	****
17		Rhizostomeae	5.88×10^{-112}	1.23×10^{-110}	****
18		Semaeostomeae	9.68×10^{-11}	2.03×10^{-9}	****
19	Scleractinia	Rhizostomeae	3.65×10^{-13}	7.67×10^{-12}	****
20		Semaeostomeae	8.59×10^{-2}	1	ns
21	Rhizostomeae	Semaeostomeae	6.34×10^{-8}	1.33×10^{-6}	****

Appendix I: Median of the eight key parameters - per species.

Order	TT	S	TL	DL	AS	MX	SV	I
<i>Zoanthus sociatus</i>	1	1.18	1001.50	712.44	10.64	345.78	20.55	0
<i>Aiptasiogeton species</i>	1	1.33	1024.51	745.98	12.33	454.92	36.24	0
<i>Exaiptasia diaphana</i>	1	1.15	1794.91	1462.19	8.70	288.57	16.10	0
<i>Cirrhipathes species</i>	1	1.52	706.53	433.65	4.95	213.76	12.64	2
<i>Stichopathes species</i>	1	1.35	1625.08	1128.59	7.86	309.94	18.08	4
<i>Corynactis californica</i>	2	1.85	1441.01	759.89	4.32	250.46	9.84	7
<i>Corynactis viridis</i>	2	1.63	886.62	478.99	2.23	115.79	3.90	8.5
<i>Pseudocorynactis species</i>	1	2.71	578.68	221.69	4.04	183.02	5.73	3
<i>Ricordea species</i>	1	1.49	1073.04	667.07	7.39	365.90	12.21	0
<i>Cladocora species</i>	1	1.44	983.07	630.19	8.10	336.16	10.23	0
<i>Fungia species</i>	2	1.39	1482.72	1026.78	9.56	371.85	30.81	1
<i>Cassiopea andromeda</i>	1	1.16	624.45	480.95	8.29	219.72	10.41	0
<i>Sanderia malayensis</i>	1	1.52	765.59	441.78	8.05	312.75	39.09	1

Abbreviations: TT (Track Turns), S (Sinuosity), TL (Track length), and DL (Displacement Length) in μm ; AS (Average Speed), MS (Maximum Speed), and SV (Speed Variance) in $\mu\text{m}/\text{sec}$; I (Intersections)