

RESEARCH ARTICLE

Functional morphology of cirri in the barnacle *Amphibalanus improvisus* (crustacea: Balanidae)

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Funding information

Deutsche Forschungsgemeinschaft

Abstract

Barnacles rely heavily on their mobile cirri for food capture because of the sessile lifestyle. These filamentous food capturing devices are extended into the water current and perform undulating movements. Cuticular structures with corresponding musculature work together, to allow these highly repetitive movements. This paper studies the interplay between structure and function of the cirri using scanning electron microscopy (SEM), microcomputed tomography (μ CT) and high-speed video recordings (HSV) in *Amphibalanus improvisus*. Barnacles use the external cuticle structures (denticles and setae) for efficient grooming, food capturing, and providing support for the muscular system responsible for movement control. Entanglement of the cirri during extension is probably avoided through an interlocking of the serrate setae on cirri IV–VI, creating a “zipper-like” effect, which was recorded here using the HSV. We analyzed the muscular arrangement using μ CT and found a new flexor muscle in both the endo- and exopod of cirrus II. Supported by the intrinsic cirral muscles, the new flexor muscles may provide variable movements of the anterior cirri (cirri I–III), which is important for further food handling. Our results provide a foundation for further comparative studies of the feeding apparatus of barnacles and for possible implications in the area of bio-inspired robotics.

KEYWORDS

entanglement, feeding, prey capture, ultrastructure, zipper-like effect

1 | INTRODUCTION

Suspension feeders are organisms that feed by straining suspended particles from the water. This feeding strategy can be found throughout several animal groups in marine environments (Hamann & Blanke, 2022). They typically use a combination of detritus particles feeding and prey capturing, to maximize their energy uptake (Hamann & Blanke, 2022; Riisgård & Larsen, 2010). Within this ecological group of suspension feeders, sessility is a recurring phenomenon. Sessile organisms are firmly attached to the ground

and cannot self-locomote (Harris, 1990). As they cannot change their location, there is a need for a highly robust and efficient food capturing mechanism giving the animals an advantage of their sessile habit (Harris, 1990).

Within the balanomorphan barnacles, *Chelonibia testudinaria* has shown the ability to change its position on loggerhead turtles toward more preferable feeding positions over time (Chan, Wong, et al., 2021b), however most are sessile. Balanomorphan barnacles (Figure 1) rely mostly on active suspension feeding by undulated movement of their cirral fan (cirri IV–VI, posterior, Figure 1) in the

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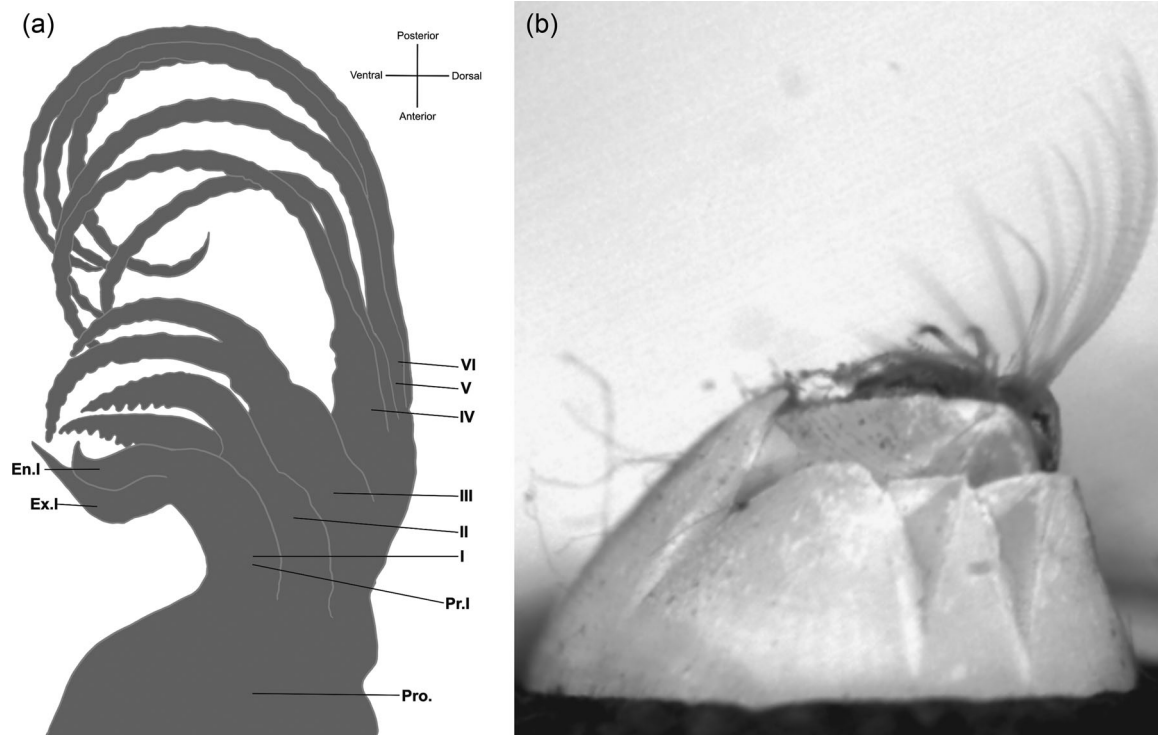


FIGURE 1 *Amphibalanus improvisus*. (a) Schematic drawing of the animal without shell showing prosoma (Pro) and thorax with the anterior cirri (I–III) and posterior cirri (IV–VI). Each cirrus is subdivided into the exopod (Ex.I), endopod (En.I) and protopod (Pr.I, highlighted on cirrus I). (b) *A. improvisus*, microphotograph overview showing the shell and posterior cirri extended into the water column.

water, where prey and other feeding particles are trapped in a mesh of setae (Høeg et al., 1994). When there is strong water movement, barnacles can alternatively extend the cirral fan into the water column, orienting it in the direction of the highest water flow (Anderson, 1981, 1994). The trapped food particles are then transported to the mouth appendages by the anterior cirri (cirri I–III, Figure 1; Anderson, 1981). The anterior cirri are often modified to maxillipeds, and the number of modified cirri varies between species (Anderson, 1994). For example, stalked barnacles like *Lepas anatifera* typically only have the first cirri pairs modified as maxillipeds whereas balanomorphan barnacles typically have the first two or three cirri pairs modified as maxillipeds (Anderson, 1994; Chan, Dreyer, et al., 2021a). Each anterior cirrus completes specific actions. For balanomorphan barnacles like *Balanus trigonus*, the endopods of cirri I encloses the tip of the oral cone and the exopods of cirri I are used to remove rejected food. Cirri II and III bring food down to cirri I and scrape particles off the posterior cirri (Anderson, 1994).

Each cirrus consists of a proximal protopod (homologous to the coxa basis of other crustacea; Chan et al., 2008) and two distal rami: exopod and endopod, which are divided into several segments, connected by a posterior hinge (Cannon, 1947). Small hair-like structures called setae are found on the segments of all cirri. They serve a vital part of the food capture and further food handling processes inside the mantle cavity by the maxillipeds (Chan et al., 2008). The setae show great variation in morphology, with each type

corresponding to a certain function and can serve mechanical and sensory functions (chemo- and mechanoreceptors; Garm, 2004a). The setal morphology has previously been analyzed using scanning electron microscopy (SEM) and setal function was discussed based on the morphology data (Chan et al., 2008; Garm, 2004b; Hunt & Alexander, 1991; Walley, 2011).

Barnacle cirri show great developmental plasticity depending on the environmental conditions they are exposed to and throughout the seasons (Kaji & Palmer, 2016; Neufeld, 2012). Marchinko and Palmer (2003) found that the cirri of barnacles in wave exposed environments are shorter and stouter than those in more protected areas and Kaji & Palmer (2016) showed that fusion of the leg segments can also be seen in more exposed areas. These changes allow the cirri to withstand higher velocities and reduce the risk of damage (Marchinko & Palmer, 2003). The longer and thinner cirri in more protected environments can sieve larger volumes of water, however, they are more prone to breakage (Marchinko & Palmer, 2003). Kim et al. (2020) investigated an extreme example of this in the whale barnacles *Coronula diadema* and *Conchoderma auritum*. The height of the cirral segments is much greater than the width when compared to other species. These much thicker segments can withstand the strong currents and allow them to feed without the cirri breaking off.

Darwin (1854) initially studied the morphology and anatomy of the cirri, finding a singular flexor muscle extending over the entire length of each cirrus (used solely for the contraction of the cirri) and

concluded that hydraulic pressure is used to extend them. This was further investigated and confirmed in several later papers (Anderson, 1981, 1994; Anderson & Southward, 1987; Cannon, 1947; Pfeiffer & Lowe, 1989). Gutman (1960) showed that hydraulic pressure is generated by compression of the prosoma, leading to a hemolymph influx into the thorax and cirri, causing them to unfurl due to the increased pressure. For the furling action of the cirri toward the oral cone, the flexor muscle in each cirrus contracts and the fluid is pushed back into the prosoma, bringing the barnacle's mantle back into the resting position. The movements of cirri I–III are difficult to study because of the calcareous plates of the barnacle (Hunt & Alexander, 1991). As the result, only few attempts have been made to study these movements comprehensively in a living animal. Anderson (1981) successfully dissected the live body of a sessile barnacle out of the shell but did not make any claims on the movement of the cirri. Hunt and Alexander (1991) inserted a scope between the opercular plates to film inside the shell but mostly focused on the movement of the mouthparts. Only by studying an in vivo dissected animal, is it possible to analyze the movement and functional morphology of the anterior cirri properly.

This study aims to understand the functional morphology of the barnacle cirri, which has not been studied to date. New study approaches, such as a microcomputed tomography (μ CT) based 3D-reconstruction of the muscular system of *Amphibalanus improvisus* (crustacea: balanidae) and high-speed videography (HSV) of the cirri motion were used. Furthermore, we used SEM focusing on a comparison of the cuticular surface microstructure of functionally different cirri. In this study, we aim to answer the following questions. (1) Does the part of the feeding apparatus which is primarily responsible for prey capture (cirri IV–VI) contain specialized folding mechanisms with particular structure and alignment that allow for a continuous, consistent movement of the cirri? (2) Is the part of the feeding apparatus, which is responsible for grooming and the transportation of food to the oral cone (cirri I–III), actively steered by the organism using muscle movement? (3) Are the size, shape, and distribution of specialized cuticular microstructures important during the capture and transportation of food particles? The combination of selected techniques should provide a basis for future comparison of the obtained data to those from other barnacle groups. Additionally, our results may be used in the bioinspired soft robotics, to increase longevity of structures during high-repetition movements, such as in soft-rigid hybrid manipulators (Culha et al., 2016).

2 | MATERIALS AND METHODS

2.1 | Animals

The sessile barnacles examined in this study are *A. improvisus* (Darwin, 1854), or bay barnacle, a cross-fertilizing, hermaphroditic crustacean species, which produces around 1000 eggs per season (Furman & Yule, 1990). In recent years, there has been some debate on the classification of the species. *Balanus improvisus* belongs to the

Balanus amphitrite complex, which have all been transferred to the genus *Amphibalanus* in the subfamily Amphibalanidae (Chan, Wong, et al., 2021; Clare & Høeg, 2008). This is the classification that will be used in our study. The specimens were collected in the Baltic Sea (Strande, near Kiel, Germany). The barnacles were kept in an aquarium with filtered Baltic seawater at Kiel University, Germany and fed with *Artemia salina* (Linnaeus 1758, Branchiopoda: Artemiidae) every 3 days.

2.2 | Preparation

To prepare the *A. improvisus* samples for the μ CT and SEM, the barnacles ($n = 5$) were placed in 20 mmol magnesium chloride solution for 24 h to relax the muscles (Butt et al., 2008). Subsequently, they were dissected using tweezers, extracting the animal out of its shell through the operculum, taking care not to damage the tissues.

2.3 | μ CT

Prior scanning, the samples were stained with Lugol's iodine solution for 1 week and washed thoroughly with water. The specimens ($n = 3$) were subsequently dehydrated in ascending ethanol series, critical point dried (Balzers CPD030; Baltic Praeparation, Niesgrau, Germany, or Leica EM CPD300; Leica Biosystems) after being dehydrated in an ascending ethanol sequence from 70% to 100%. A SkyScan 1172 desktop μ CT scanner (Bruker micro-CT) was used to scan the specimens. The following scanning specifications were used: two segments, 360° scan, 0.25° rotation steps, X-ray source: 40 kV, 250 μ A. The scans were then reconstructed in the NRecon software and segmented in the Amira software (Thermo Fisher Scientific). The open-source 3D creation suite Blender 2.82a (Blender Foundation) was used for the final visualization.

2.4 | HSV

The HSV were taken using two different methods. First, the barnacles ($n = 5$) in aquarium were filmed in frontal view with a tripod mounted Photron FASTCAM Color (Photron) HSV camera with a 60 mm Nikon lens (Nikon Corporation) at a frame rate of either 250 or 500 frames per second (fps) and a resolution of 1024 \times 1024 pixels.

Additionally, for closeups of the cirri, the same HSV camera was connected to an Olympus SZX12 stereomicroscope (Olympus Cooperation). The barnacles were placed in a small dish, completely submerging them in Baltic seawater. Occasionally, some water was replaced with cold water, to ensure that the water had a suitable temperature. *A. salina* were added to the dish, to trigger and to record the feeding process.

The HSV footage were later edited in the open-source 3D creation suite of the Blender video editor (V2.8 Blender Foundation, www.blender.org).

2.5 | SEM

For SEM, cirri I–VI were dissected and critical point dried (see above). These samples were then sputter-coated with 10 nm thick gold-palladium layer using a Leica Bal-TEC SCD500 (Leica Biosystems). A Hitachi S-4800 (Hitachi High Technologies Corp.) SEM was used for higher-magnification images and a tabletop Hitachi TM3000 (Hitachi High-Technologies Corp.) SEM for overview images. The images were processed in the software Affinity Photo (Serif Ltd.).

2.6 | Nomenclature

The description and terminology of the structures found on the cuticle using SEM follows Garm (2004b) for crustacean cuticular structures and the muscle terminology follows the nomenclature of Anderson (1981). The nonfunctional (nonmoving) point of a muscle will be termed “origin point” and the functional point of the muscles will be called the “termination point.”

3 | RESULTS

3.1 | SEM

The cuticular body of *A. improvisus* sits inside a calcareous shell. For this study, the shell has been removed to describe the anatomy more clearly. Figure 2 shows the body, which is divided into two morphologically simplified parts, the prosoma and the thorax, the latter carrying the six feeding arms. The body has three pairs of large uniform posterior cirri (IV–VI) and three pairs of shorter, unequal anterior cirri (I–III). Each cirrus consists of a protopod, endopod, and exopod. They are split into small segments covered in hair-like setae.

3.1.1 | Cirri I–III

Cirrus I (Figure 3a) has a long exopod and a short endopod. The median surface of both endo- and exopod carry serrate (Figure 3a4) and multicuspidate (Figure 3a3) setae. The lateral surface of both carries nearly no setae. The protopod carries pappose setae (Figure 3a1) along its posterior margin. Further cuticular structures are comb-like rows of denticles (Figure 3a2) on the median surface of the protopod, approximately 10 μ m apart from one another.

Cirrus II (Figure 3b) has two short rami, similar in size. The median surface of both rami carries pappose (Figure 3b2) and serrulate (Figure 3b4) setae and the lateral surface of both carry nearly no setae, except near to the tip of each ramus. Further cuticular structures are fan-like denticles on the median surface of the protopod, along with pore-like structures (Figure 3b1). The denticles (Figure 3b3) can be found on the lateral and median edges of each segment.

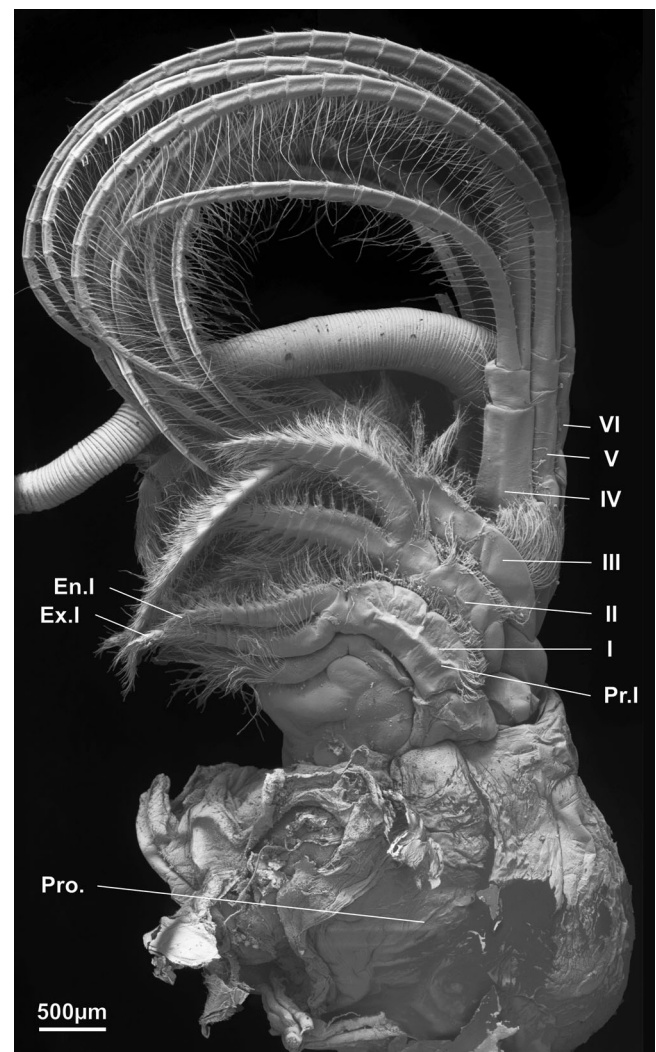


FIGURE 2 *Amphibalanus improvisus*, scanning electron micrograph of the body, lateral view with the shell removed, showing prosoma (Pro) and thorax with the anterior cirri (I–III) and posterior cirri (IV–VI). Each cirrus is subdivided into the exopod (Ex.I), endopod (En.I) and protopod (Pr.I) (highlighted on cirrus I).

Cirrus III has two long, similar-sized rami (Figure 3c). The median surface of both rami carries serrate (Figure 3c1) and serrulate (Figure 3c2) setae. Comb-like denticles often contaminated with algae (Figure 3c4) are found on the median surface of the protopod and some following segments. The protopod carries pappose setae (Figure 3c3).

3.1.2 | Cirri IV–VI

In contrast to cirri I–III, cirri IV–VI are long and slender, making up the cirral fan. All these cirri are similar, so only the cirrus IV is presented here in detail. The cirrus IV has two long, nearly identical cirri (Figure 3d). Each segment carries seven pairs of setae anterolaterally, the longest being the distal pair, getting progressively shorter. The

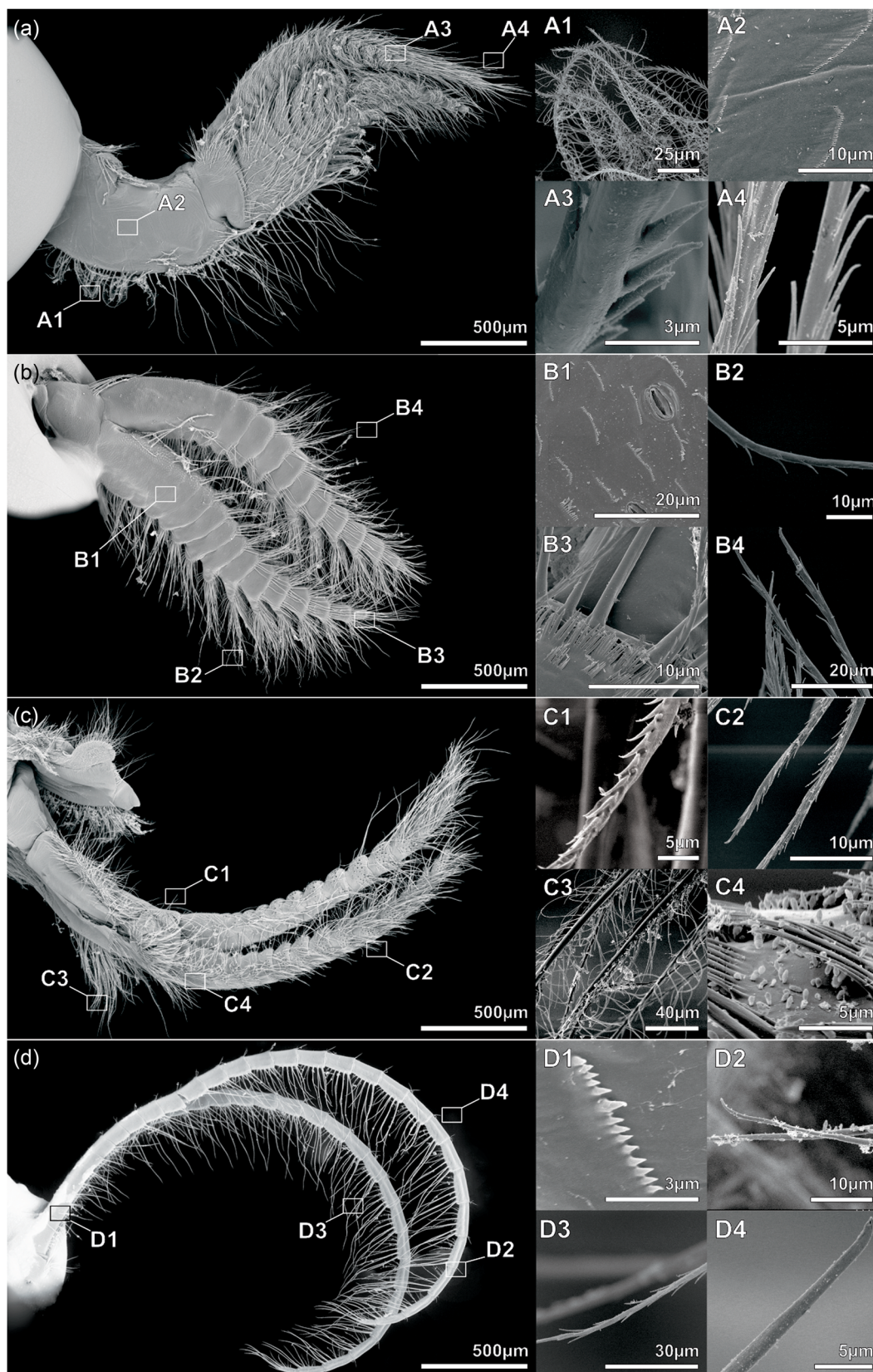


FIGURE 3 *Amphibalanus improvisus*, scanning electron micrograph of surface microstructures of cirri I–IV of the feeding limb. (a) Median view of the cirrus I, (a1) pappose setae, (a2) fan-like denticles on the median side of protopod I, (a3) multicuspitate setae, (a4) serrate setae. (b) Lateral view of the cirrus II, (b1) denticles and pore-like structures, (b2) pappose setae, (b3) denticles, (b4) serrulate setae. (c) Lateral view of the cirrus III, (c1) serrate setae, (c2) serrulate setae, (c3) pappose setae, (c4) comb-like denticles with trapped algae. (d) Lateral view of the cirrus IV, (d1) comb-like denticles, (d2) simple setae, (d3) serrate setae, (d4) simple setae with the terminal pore.

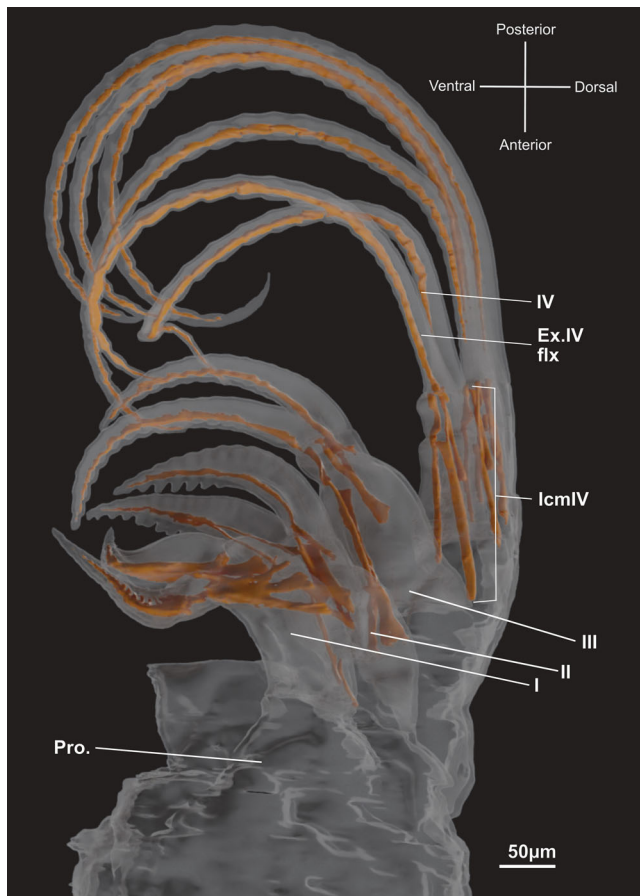


FIGURE 4 *Amphibalanus improvisus*, medial view of the 3D visualization of the cirri based on computerized tomography data. The cuticle is shown transparent, and the muscles are highlighted in orange. Ex.IV flx, exopod cirrus IV flexor muscle; IV Icm, cirrus IV intrinsic cirral muscles.

five longest setal pairs are serrate (Figure 3d3) and the two smaller ones are simple setae (Figure 3d2). Comb-like denticles (Figure 3d1) are found on the median surface of the protopod, in fewer numbers than on cirri I–III. Shorter setae with a terminal pore at the distal end are found on the posterior edge of the cirri, one on each segment (Figure 3d4).

3.2 | μ CT

This study is focused on the flexor muscles and intrinsic cirral musculature of *A. improvisus*. Figures 4 and 5 show the 3D visualizations of the barnacle thorax and the corresponding musculature. Extrinsic cirral musculature and body musculature are not shown there. An overview of the musculature is given in the Supporting Information: Table S1

Figure 4 shows the lateral view (right side of specimen) of the flexor muscles and intrinsic cirral muscles (Icm) in cirri I–VI. Details of cirri I–IV are given in Figure 5 and those of cirri V–VI are only shown in the overview image. The detailed view of cirrus IV is used

to describe the muscles of cirri V–VI, as they are similar in cirri V–VI.

3.2.1 | Cirri I–IV

Figure 5a shows the musculature of the cirrus I. Both exopod and endopod feature one muscle each (Ex.I flx in the exopod and En.I flx in the endopod). Exopod flexor I originates from the anterior joint of the protopod connecting it to the exopod and terminates at the second-to-last cirral segments of the exopod. En.I flx originates from the posterior part of the joint between the protopod and the endopod and terminates at the second-to-last endopod cirral segments. In each segment, the muscle fibers of En.I flx and Ex.I flx are attached to the antero-distal joint. There are three intrinsic cirral muscles (Icm.I–III) found in the protopod of the cirrus I. The muscle Icm.I originates from the medial wall of the protopod and terminates at the medial wall of the second endopod cirral segment. The muscle Icm.II originates from the medial wall of the protopod and terminates at the left lateral wall of the second endopod cirral segment. The muscle Icm.III originates on three different points, spread distally on the posterior wall of the protopod and terminates on the joint connecting protopod and exopod. The muscle Icm.IV is found in cirrus III, it terminates in the upper part of the protopod and runs toward the body region.

Figure 5b shows the musculature of the cirrus II. Both exopod and endopod feature two muscles each. These muscles were named flexor muscle 1 (flx.I) and flexor muscle 2 (flx.II). The muscle exopod flexor I (Ex.I flx.I) originates from the left lateral wall of the protopod and terminates at the second-to-last cirral segments of the exopod. The muscle endopod flexor I (En.I flx.I) originates from the left lateral wall of the protopod and terminates at the second-to-last cirral segments. The muscle endopod flexor II (En.II flx.II) originates from the medial inside wall of the protopod and terminates at the sixth last segment. The muscle exopod flexor II (Ex.II flx.II) originates from the anterior part of the joint between the protopod and exopod and terminates at the eighth-to-last segments. In each segment, muscles En.II flx.I and Ex.II flx.I attach to the anterior walls of the cuticle. There are four intrinsic cirral muscles (Icm.II–IV) found in the protopod of the cirrus II. The muscle Icm.II.I originates from the medial wall of the protopod and terminates at the anterior joint between the protopod and endopod. Muscle Icm.II.II also originates from the medial wall of the protopod and terminates at the posterior point of the joint between protopod and endopod. Muscle Icm.II.III has three different origin points, all three are situated on the left lateral wall of the protopod, however, staggered distally. The termination point is at the most anterior point of the joint between the protopod and exopod. The muscle Icm.II.IV originates from the anterior wall of the thoracic cuticle and terminates at the anterior wall of the protopod, below the joint between the exopod and protopod.

Figure 5c shows the musculature of the cirrus III. Both exopod and endopod feature one muscle each (Ex.III flx in the exopod and

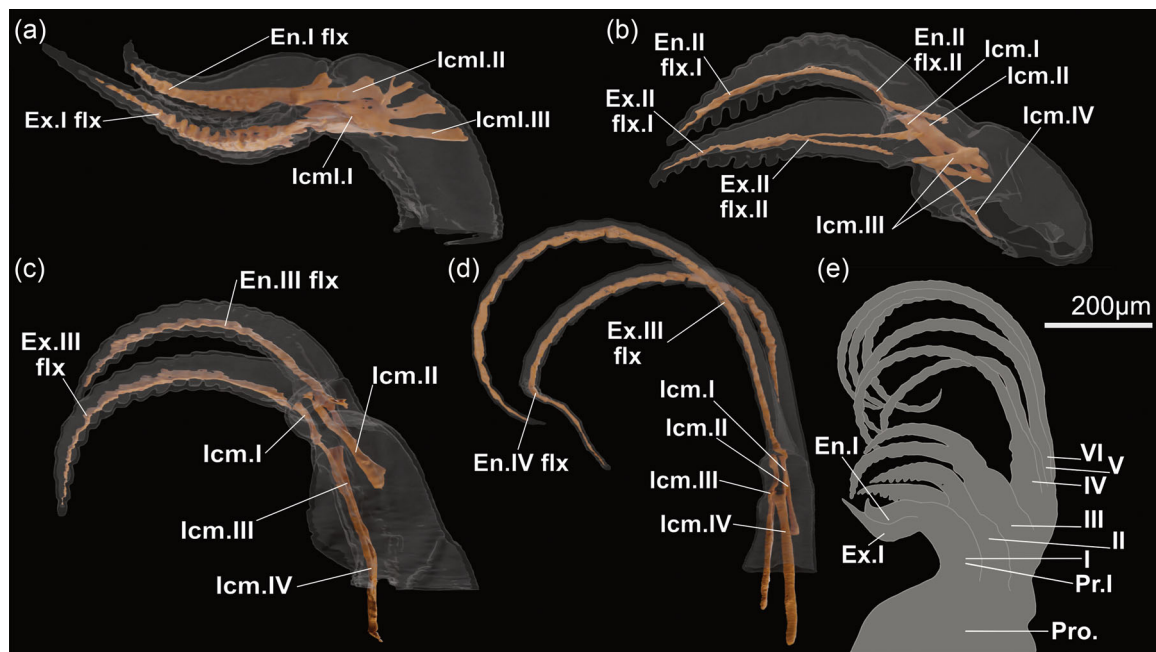


FIGURE 5 *Amphibalanus improvisus*, lateral view of the 3D visualization of cirri I–IV based on microcomputed tomography data. The cuticle is shown transparent, and the muscles are highlighted in orange. (a) Right lateral view of the cirrus I, (b) right lateral view of the cirrus II, (c) right lateral view of the cirrus III, (d) right lateral view of the cirrus IV. (e) Schematic drawing. En.I flx, endopods flexor muscle I; En.II flx, endopod flexor muscle II; Ex.I flx, exopod flexor muscle I; Ex.II flx, exopod flexor muscle II; Icm.I–IV, intrinsic cirral muscles I–IV.

En.III flx in the endopod). The muscle Ex.III flx originates from the antero-distal joint of the protopod connecting it to the exopod and terminates at the second-to-last cirral segments of the exopod. The muscle En.III flx originates from the left lateral wall of the protopod and terminates in the second-to-last endopod cirral segments. In each segment, the muscular strands of En.III flx and Ex.III flx attach to the antero-distal joint. There are four intrinsic cirral muscles (Icm.III.I–IV) found in the protopod of the cirrus III. The muscle Icm.III.I is a short muscle originating from the anterior wall of the protopod and terminates at the anterior joint between the protopod and exopod. The muscle Icm.III.II originates from the left lateral wall of the protopod and terminates at the posterior point of the joint between the protopod and endopod. The muscle Icm.III.III originates from the right lateral wall of the thoracic cuticle and terminates at the right lateral wall of the protopod. The muscle Icm.III.IV originates from the left lateral wall of the thoracic cuticle and terminates at the left lateral wall of the protopod.

Figure 5d shows the musculature of cirrus IV. The exopod and endopod each contain one muscle, which is the flexor muscle (Ex.IV flx). The muscle Ex.IV flx originates from the left lateral wall of the protopod and terminates at the second-to-last cirral segments of the exopod. The muscle En.IV flx originates from the fifth endopod cirral segment and terminates at the second-to-last endopod cirral segments. In each segment, the muscular strands of En.IV flx and Ex.IV flx are attached to the antero-distal joint of each segment. There are four intrinsic cirral muscles (Icm.IV.I–IV) found in the protopod of cirrus IV. The muscle Icm.IV.I originates from the right lateral wall of the protopod and terminates at the

anterior joint between the protopod and endopod. The muscle Icm.IV.II originates from the right lateral wall of the protopod and terminates at the right lateral wall of the first exopod segment. The muscle Icm.IV.III originates from the left lateral wall of the thoracic cuticle and terminates at the anterior joint between the exopod and protopod. The muscle Icm.IV.IV originates from the posterior wall of the thoracic cuticle (not pictured) and terminates at the right lateral wall of the protopod.

3.3 | Movements of the cirral fan

We used HSV to record the movement of the cirral fan in and out of the operculum. Barnacle individuals were observed from both lateral and frontal views, to obtain qualitative data on the motion sequence. For clarification, see the schematic drawing prepared for each video sequence (Figure 6). Original HSV footage is provided in Supporting Information: Videos S1 and S2).

Figure 6 shows the extension and contraction of the cirri during a single beat. Cirri IV–VI emerge still curled from the operculum (Figure 6a,b). They begin to uncurl distally until they reach an unfurled, extended shape (Figure 6d). Cirri I–III also begin to extend outwards. The begin of withdrawal is shown in Figure 6e. During the withdrawal, the cirral fan (cirri IV–VI) withdraws and curls uniformly back into the shell. In Figure 6f, cirri II–III begin to wrap around the cirral fan and continue to do so until all cirri are back in the shell (Figure 6h). Figure 7 shows the frontal view of the cirral fan extension out of the shell. The focus is made here on the

endopod of cirrus VI and exopod of cirrus V. It shows how the two rami part from one another in a "zipper-like" fashion. The segments unfurl distally, with the setae of each segment holding the rami together until enough pressure pulls the hooks apart. This happens for each segment until the endopod and exopod are no longer in contact, but the setae remain in a "web-like" network. Thus, the cirri do not only unfurl out, but also separate from one another.

4 | DISCUSSION

4.1 | Setal microstructure

There are five main types of setae found on the cirri of *A. Improvisus*: pappose, multicuspitate, serrate, serrulate, and simple (Figure 3). Furthermore, denticles and pore-like structures can also be found.

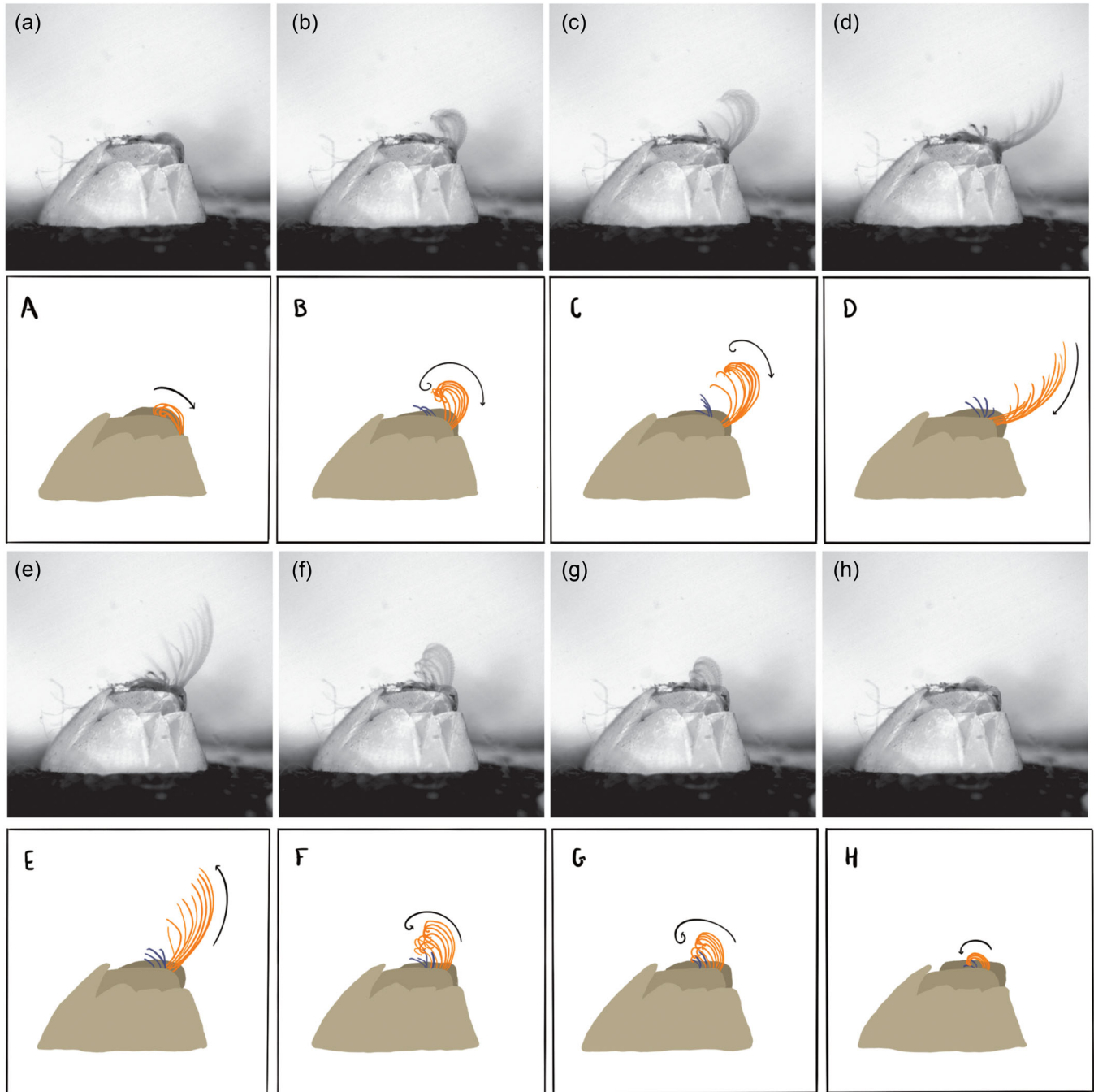


FIGURE 6 *Amphibalanus improvisus*, sequence of cirral fan movements (HSV) showing both the extension and contraction of cirri during a single beat in a side view. (a) Opening of the operculum. (b) Cirri IV–VI extend out of shell. (c) Cirri I–III also extend out of shell. (d) The furthest point of extension. (e) Beginning of the contraction of cirri IV–VI. (f) Curling of cirri IV–VI. (g) Cirri IV–VI are nearly fully curled up. (h) All cirri are back in the shell. Frame rate: 500 fps. fps, frames per second; HSV, high-speed video.

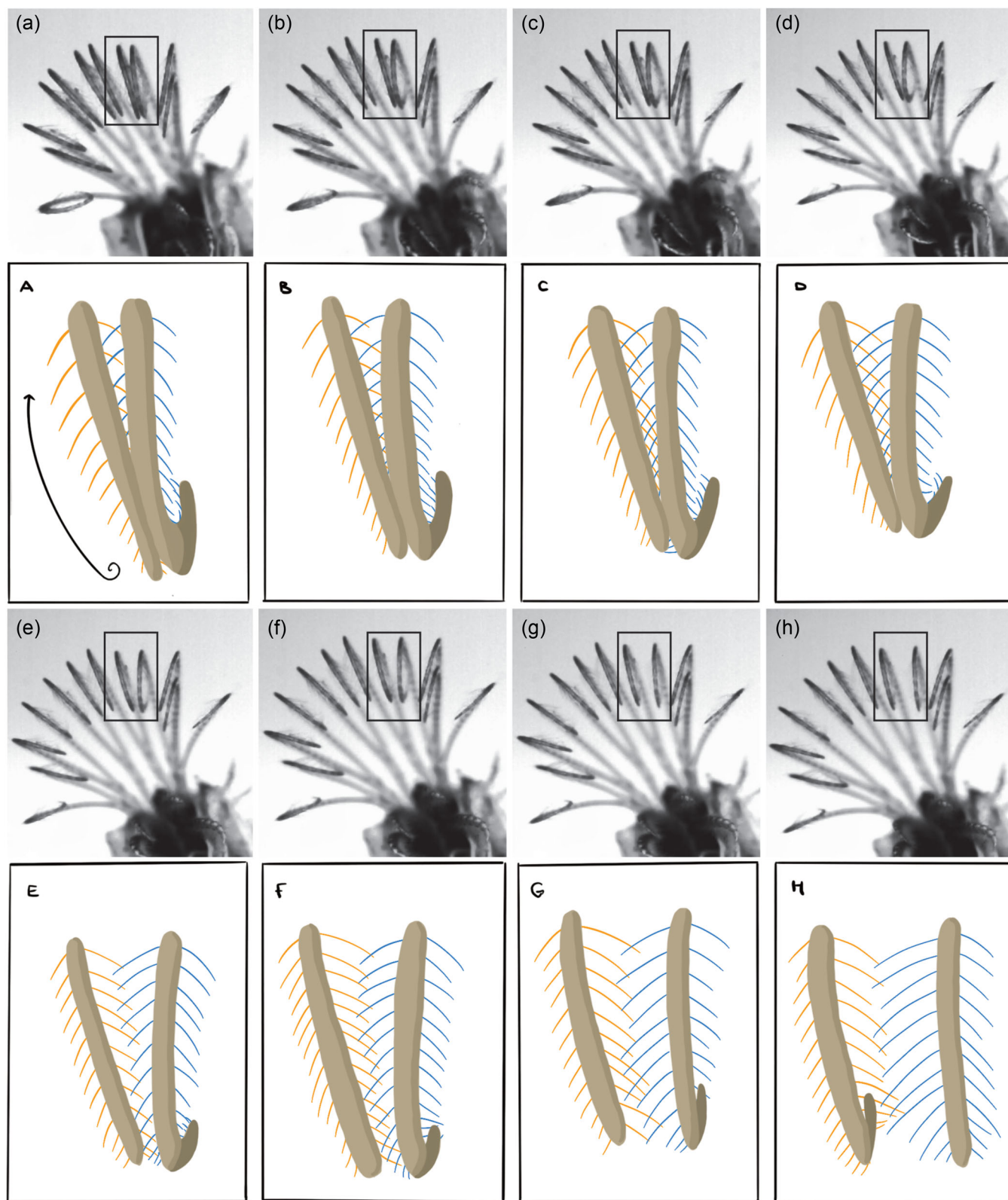


FIGURE 7 *Amphibalanus improvisus*, close-up sequence of cirral fan movements (HSV) in the frontal view. Focus is on the endopod of cirrus VI and on the exopod of cirrus V. (a) Cirri begin to unfurl distally. (b) Beginning of the distal separation of setae. (c–e) Separation of setae. (f) Cirri are no longer touching each other; they are only connected by setae. (g–h) Cirri are fully separated, setae continue to separate more during unfurling, however, stay together as a “mesh.” Frame rate: 250 fps. fps, frames per second; HSV, high-speed video.

Pappose setae are present on the protopod of cirri I–III (Figure 3a1,3b2,3c3). According to Garm (2004b), pappose setae are often found in areas that have little food interaction, which corresponds to the positioning of these setae on the cirri of *A. improvisus*. The function of these setae, as described by Garm (2004b) and further investigated in other barnacle species by Chan et al. (2008), is to prevent food particles from escaping by forming a capsule-like structure around the particles while the food is being captured and handled. When looking at Figure 2, this function becomes apparent, as the pappose setae close the gaps between the protopods of cirri I–IV. This supports previous statements by Chan et al. (2008) and Garm (2004b). Other possible functions of these setae are to generate a water current inside the shell for filter feeding, as also seen in some decapod crustaceans, such as *Thalassinidea* (Garm, 2004a).

Multicuspidate setae are found on both the endopods and exopods of cirri I (Figure 3a3). Garm (2004b) stated that their function is the rough handling of food particles. Anderson (1994) noted that cirri I have two different functions: the endopods transfer food to the oral cone and the exopods make up the rejection mechanism and enclose the oral cone. Both functions are rough handling movements, which correspond to the setal type found on these structures and support the findings of Garm (2004b). Multicuspidate setae on cirrus II might also be responsible for combing/cleaning the setae of cirrus III.

Furthermore, serrate setae are also responsible for the rough handling of food particles, such as collection, holding, and shredding (Garm, 2004a). The robust, stronger denticles can withstand the rough handling of food particles and are not prone to breakage (Garm, 2004a). Serrate setae can be found on the exopod of cirrus I (Figure 3a4), which are responsible for the rough handling and rejection of food, whereas cirri II transfer the food particles from cirrus III to cirrus I. Grooming is another important function of the serrate setae (Garm, 2004b), which would explain the wide distribution of this setal type on cirri of *A. improvisus* (Figure 3a4,c1,d3).

The serrate setae also form a strong connection between the setae in the capture net of cirri IV–VI. The setules of the serrate setae could act as “hooks” holding the setae in contact. Figures 6 and 7 show HSV sequences of the “zipper-like effect” that has not been described previously. It can be hypothesized that further function of the serrate setae is to avoid entanglement of the fine, long and flexible cirri IV–VI through this “hook-like” system, by allowing a distally-directed extension and contraction and making it more efficient. It would also make the grooming and “combing” of food particles more efficient, as cirrus III must only brush along the structures instead of also having to detangle them. This use of the serrate setae would answer our initial question of whether the prey capture structures contain specialized folding mechanisms that allow for a continuous, consistent movement due to the structure and alignment of the cirri. We call this mechanism the “zipper-like effect,” which has not been reported before for the food capture mechanism in barnacles. Additionally, the “hook-like” function of the serrate setae in the cirral net could provide extra support for feeding

in rougher environments and higher velocities. It could help to prevent breakage and to firmly grip the food particles (Garm, 2004a).

The core function of serrulate setae is in the relocation and probing of food particles, as described by Garm (2004a), which corresponds to the location of these setae on cirrus II (Figure 3b4) and III (Figure 3c2). Cirrus III rub, by setae covered endopods and exopods, along the undersides of the cirral net, which removes the food particles from the net for further processing (Anderson, 1994). Here, little pressure is applied to the food particles, which likely indicates that serrulate setae are responsible for soft food handling. Anderson (1994) and Garm (2004a) claims are supported by our data on serrulate setae in this study.

The simple setae presumably have more of a sensory function than a mechanical one in *A. improvisus*. Small simple setae are found on the posterior edge of cirri IV–VI (Figure 3d4), one at each joint. These setae have a terminal pore, indicating their possible function as chemoreceptors (Chan et al., 2008). These could be responsible for probing the surrounding water to gain chemical information (Garm, 2004b). Five pairs of setae are found on the anterior edge of cirrus IV, the smallest, most proximal pair of them are simple setae (Figure 3d2). These could be responsible for sensing possible food particles in the cirral net.

Rows of denticles were found on all cirri (Figures 3a2,3b1,3c4,3d1). Garm (2004a) and Garm and Watling (2013) suggested that their function is to scrape debris off other structures: this can be confirmed by the algal cells found behind the structures. Garm and Watling (2013) also mentioned that these structures could help in increasing the robustness of the cirri. Chan et al. (2008) took note of these denticles but did not comment on them further. Herein, more algae were found within the dense areas of denticle combs, such as along the edges of each cirral segment (Figure 3c4). Another possible function of these denticle combs could be to reduce drag on the surface of cirri by creating small turbulences, like in the case of the sharkskin microstructure (Dean & Bhushan, 2010). Furthermore, pore-like structures were found on the cuticle of cirrus II. The only information about such structures can be found in the study on *Lithoglyptes bicornis*, burrowing barnacle, where similar structures have been found near comb-like denticles and assumed that they may secrete substances to soften the substrate of their host (Kolbasov, 2000). As *A. improvisus* is not a parasitic barnacle, these pores probably do not have the same function, even if the structures are comparable. Another similar structure in the funnel-canal sense organs of *Carcinus maenas* (crustacea: decapoda) has also been found as a mechanoreceptor (Schmidt & Gnatzy, 1984). It may be worthwhile investigating these structures of *A. improvisus* in this context.

A. improvisus has rich microstructure on its cuticle surface. Some previous claims on the function of these structures were supported in the present study, but also some new insights were gained, such as the existence of the pore-like structures in the proximity of the comb-like denticles and the “zipper-like effect” of cirri IV–VI. Our initial question of whether the size, shape, orientation, and distribution of specialized microstructures are important during the capture

and transportation of food particles can be positively answered by the findings on the setal microstructure.

4.2 | Musculature

Noninvasive μ CT-based 3D-visualizations of the skeletal-muscle organization of the flexor and lcm revealed some novel details of the muscular equipment of *A. improvisus* cirri (Figures 4 and 5). The muscles of *A. improvisus* cirri have not been extensively studied to date: however, comparisons can be drawn to other species of the *Amphibalanus* genus (Anderson, 1994).

The singular flexor muscles in cirrus I (Figure 5a), cirrus III (Figure 5c), and cirrus IV (Figure 5d), used for the contraction of the cirri segments, are arranged similarly on the anterior edge, with a connection point to the cuticle at each antero-distal joint of the segments. The connection point in each cirral segment would explain the subsequent furling from the tip to the base of each cirrus, so that the cirri are packed and unpacked properly with the help of the “zipper-like” effect described above. The simple remoter and promotor furling (Anderson, 1994) of cirri IV–VI can be seen in Figure 7. In cirrus IV, the origin of muscle Ex.IV flx appears to be in the fifth segment. This artifact is probably caused by some impact on the muscles by the sample preparation for μ CT scanning (Figure 5d).

In the μ CT images of cirrus II (Figure 5b), another muscle with typical muscle appearance and a similar density to the other muscles was found in both the endopod and exopod, indicating a new formerly undescribed muscle. Here, it has been named muscle flx.II. This muscle may be responsible for a more controlled movement either in the retraction or extension. The presence of this muscle would contradict the statements made in previous papers on the cirral musculature of barnacles (Anderson, 1994; Gutman, 1960); however, further investigation on this topic is necessary in the future. For example, a cross-section on cirrus II could be prepared and examined under a light microscope to determine whether flx.II is indeed a muscle or something else.

Cirri I–IV appear to have four lcm. lcmIV could, however, not be found in cirri I (Figure 5a). This may be an artifact in the data set, as according to Anderson (1994), four lcm muscles can be found in the first cirri of *Semibalanus balanoides*. The true purpose of the lcm has not been previously investigated, however, they likely are responsible for the orientation of the cirri (direction of their extension). This could be explained by the slight differences in their origin and termination points. Anderson (1994) also hypothesized that they are responsible for the more varied maxilliped movements. The lcm III and IV extend from the protopod to inside the cuticle on the right lateral wall (Figure 5c,d). Their origin and termination points are similar, which could explain their quite similar orientation and movements. This is different from cirri I and II, where the lcm muscles all originate within the protopod and where the origin and termination points are vastly different from one another, possibly causing the more varied movements (Figure 5a,b). Future studies should be conducted to determine whether the lcm could also be responsible for the changing orientation of the cirral fan toward the strongest water flow (Anderson, 1981, 1994). Many statements on the

musculature of barnacles by Anderson (1994) and Gutman (1960) are supported by our study, however, some are contradicted due to the presence of a second flexor muscle in both the endopod and exopod of cirrus II (Figure 5b). It must be stated that our initial question that the part of the feeding apparatus important for grooming and the transportation of food to the oral cone is actively steered by the organism using muscle movement was attempted but could not be entirely verified in this study. In future studies, the extrinsic cirral muscles and body musculature responsible for the fluid transfer should be reconstructed and analyzed to provide more information on the more varied movements of cirri I–III.

5 | CONCLUSIONS

This study provides insights into the functional morphology of the food capture and transfer of *A. improvisus*. It seems that the cuticle surface microstructures of the barnacle cirri provide support for the muscular movements by an interlocking of the serrate setae on cirri IV–VI and creating a “zipper-like” effect, to avoid entanglement of the cirri during extension and contraction. The orientation of the lcm of cirri I–III appears to aid in the more varied movements of these cirri, as well as through the second flexor muscle (newly described here) in the endopod and exopod of cirrus II. Results of some previous studies on the cirral structures of barnacles were confirmed here, new structures were described and links between function and morphology were attempted. Additional structures, such as the extrinsic cirral muscles and lcm of cirri V and VI could be reconstructed, to provide more information on the movement of the anterior and posterior cirri. The present results on the “zipping mechanism” and underwater capturing device in general might be interesting for possible implications in the area of bio-inspired robotic grippers.

AUTHOR CONTRIBUTIONS

Sebastian Büsse and Stanislav N. Gorb designed the project and developed the concept of the study. Lena M. Hindenberg and Sebastian Büsse did the μ CT analysis and postprocessing. Lena M. Hindenberg and Sebastian Büsse did the HS-Video recordings. Lena M. Hindenberg analyzed the data. Sebastian Büsse and Stanislav N. Gorb did the supervision and project administration. Lena M. Hindenberg and Sebastian Büsse wrote the original manuscript and all authors edited, read, and approved the final version.

ACKNOWLEDGMENTS

We thank the entire Functional Morphology and Biomechanics group for their help and advice, especially Fabian Bäuml, Alexander Köhnsen, Thies Büscher, and Benedikt Josten. Special thanks go to Dennis Petersen for help with collecting and rearing the investigated barnacles and for many elucidating discussions. S. B. was directly financed by the DFG grant BU3169/1–2.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data supporting our findings are presented in the paper and the Supporting Information, respectively. The raw data of the video footage is available on Zendo: [10.5281/zenodo.6513713](https://zenodo.org/record/6513713).

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How to cite this article: Hindenberg, L. M., Gorb, S. N., & Büsse, S. (2022). Functional morphology of cirri in the barnacle *Amphibalanus improvisus* (Crustacea: Balanidae). *Journal of Morphology*, 283, 1439–1450. <https://doi.org/10.1002/jmor.21517>