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Structural differences between conic and predator-induced bent morphs of *Tetraclita rubescens* Nilsson-Cantell, 1931 (Cirripedia: Balanomorpha: Tetraclitidae) revealed by CT scanning

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ABSTRACT

Morphological variants within species can arise as defences against predation. Several species of barnacles are known to exhibit predator-induced variants in the form of bent morphs, in which the rim of the opercular opening is perpendicular to the base. Bent morphs had only been described until recently for species of *Chthamalus* Ranzani, 1817, but the Northeast Pacific barnacle *Tetraclita rubescens* (Nilsson-Cantell, 1931) is now also known to exhibit a bent morph along the Pacific coast of the Baja California Peninsula, Mexico in the equatorward part of the species' range. We show how the bent morph in *T. rubescens* is formed and how it compares with those of *Chthamalus* spp. We used X-ray computed microtomography (μCT) imaging technology to compare and quantify morphological differences between the bent and the typical, conic morphs of *T. rubescens*. All the bent specimens exhibited a bend in the same direction, towards the rostrum, and had a significantly shorter rostrum compared to the other shell wall plates. Conic specimens showed no significant disparity in length among their wall plates. The bent morph also has a relatively smaller opercular opening than the conic morph. We suggest that the bent morph has arisen as the result of recent range expansions of warm-water gastropod predators, an indirect result of climate warming (tropicalisation). A comparison with published information about the bent morphs of *Chthamalus* species revealed interspecific similarities and differences in shell shape, the direction of bend, and the predators that purportedly induce the bent phenotype. Further studies may clarify whether there are any life-history trade-offs and population impacts associated with bent morphs.

KEYWORDS: Baja California, barnacle shell morphology, Crustacea, dimorphism, Muricidae, predator-induced defence, rocky intertidal, tropicalisation

INTRODUCTION

Morphological variation within species can be explained by a multitude of ecological and evolutionary factors, including genetic differences caused by barriers to gene flow and natural selection (Wright, 1943; Mokady *et al.*, 2000), differences in habitat or environmental gradients (e.g. the effects of wave exposure on shell morphology; Forrester *et al.*, 2016; Alcaraz *et al.*, 2024), or biological interactions, such as predator-prey interactions (DeWitt *et al.*, 1999; Willmer *et al.*, 2005). Intraspecific differences in morphology among individuals living in habitats with similar environmental conditions are typically subtle (Crothers, 1985). In some species, however, two or more distinct morphs can be found living side-by-side, with different adaptations to survive and reproduce under the prevalent environmental conditions, but also with different associated trade-offs (Lively, 1999). A distinct morph may be representative of a unique genotype (Kawecki & Ebert, 2004), but it can also be a result of phenotypic plasticity in response to specific cues in the local environment (Bradshaw, 1965; West-Eberhard, 2008). A predator-induced morphological defence is the result of a specific cue indicating the presence of a

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predator (Freudiger *et al.*, 2021; Murua *et al.*, 2014). The conditions that favour predator-induced defences are the presence of a reliable and non-lethal predator cue (e.g. a chemical or physical cue), unpredictable risk of predation in time and space, the existence of trade-offs associated with the development of the defence, and benefits that outweigh the costs of developing the defence (Clark & Harvell, 1992; Tollrian & Harvell, 1999).

Predator-induced defences have been well studied in freshwater crustaceans such as species of *Daphnia* Müller, 1785 (Tollrian & Harvell, 1999). For example, the development of neckteeth in *D. pulex* Leydig, 1860 induced by the phantom midge larvae *Chaoborus* Krough, 1911 (Krueger & Dodson, 1981; Tollrian, 1993) or the crest development in *D. longicephala* Hebert, 1977 induced by the backswimmer (*Notonecta* Linnaeus, 1758) (Grant & Bayly, 1981; Barry, 2000). Several species of rocky intertidal barnacles exhibit a 'bent' morph in habitats where muricid gastropod predators exert high predation pressure on them (Lively, 1986a, b; Jarrett, 2008, 2009; Murua *et al.*, 2014; Fenberg *et al.*, 2023). In bent individuals, the rim of the opercular opening is turned perpendicular to the base of the barnacle shell, different from the typical conic morphology with the opercular rim oriented parallel to the base (Lively, 1986a, b; Jarrett, 2008, 2009; Murua *et al.*, 2014; Fenberg *et al.*, 2023). Life history trade-offs observed in bent morphs of barnacles include a lower reproductive output and slower growth compared to the respective conic morphs (Lively, 1986a; Jarrett, 2009). Bent-morph barnacles had until recently only been documented in three species of *Chthamalus* Ranzani, 1817: *C. anisopoma* Pilsbry, 1916, *C. fissus* Darwin, 1854, and *C. montagui* Southward, 1976 (see respectively Lively, 1986a,b; Jarrett, 2008, 2009; Murua *et al.*, 2014). Individuals of *C. fissus* can also develop into a third morph with a narrow operculum (Jarrett, 2008, 2009). The narrow morph and the bent morph are two separate responses; Jarrett (2009) suggests that the capacity to develop the bent morph is not present in all individuals or that the predator exposure within the study was below the threshold level required to induce the bent morph for some individuals. The bent and narrow morphs are defences against predation by the muricid gastropod *Mexacanthina lugubris* Sowerby, 1822, which preys by ramming its labral spine into the opercular opening of barnacles (Jarrett 2008). Using a mixture of observational and experimental approaches, the bent morph of each of these *Chthamalus* species has been found to be more resistant to predation than the respective conic morph living in the same habitat (Lively, 1986a; Jarrett 2008, 2009, 2018; Murua *et al.*, 2014).

Despite the relative abundance of ecological, experimental, and biogeographic studies on the patterns and processes associated with predator-induced defences in barnacles, there has been surprisingly little research on the similarities and differences in shell structure of the bent and conic morphs, either within or between species or among genera. For example, it is unknown whether the same wall plates contribute to the bent form of the shell in all the relevant taxa. Previous studies on *C. anispoma* and *C. montagui* have demonstrated different, species-specific directions of bending due to different patterns of growth of the wall plates, (i.e. favoured growth of the left or right lateral plate) in *C. anisopoma* (Lively, 1984, 1986a, b), but favoured growth of the rostrum and the rostro-lateral wall plates in *C. montagui* (Murua *et al.*, 2014). Nor is it known if these distinct morphs are representative of a unique genotype or a result of phenotypic plasticity in response to the specific cues in their local environment. Fenberg *et al.* (2023) recently reported on the presence of a bent morph in *Tetraclita rubescens* (Nilsson-Cantell, 1931), demonstrating that bent morphs are not limited to *Chthamalus* spp., nor even to Chthamaloidea, because *Tetraclita* Schumacher, 1817 is a member of a different superfamily of sessile barnacles, the Coronuloidea (Newman & Ross, 1976; Chan *et al.*, 2017, 2021).

There are fundamental differences in shell structure in the genera that produce bent morphs. Namely, species of *Tetraclita* tend to be much larger than *Chthamalus* spp. as adults (> 40 mm in shell diameter), and they have external ribs with a thatched appearance (Sanford & Swezey, 2008). Species of Tetraclitidae have four or six plates, while those of Chthamalidae have four, six, or eight wall plates (Kim *et al.*, 2019; Chan *et al.*, 2021). The species of interest, *T. rubescens* has only four wall plates, which are thicker than the six wall plates of the *Chthamalus* spp. that also exhibit the bent morph (Fig. 1; Darwin, 1854). In *Tetraclita*, the two plates that lie at the ends of the longitudinal axis of the shell are the carina and rostrum (Fig. 1). The carina is the plate closest to where the cirri extend out through the aperture of the operculum, and the rostrum lies directly across from the carina. The plates on either side are the lateral plates.

We aimed to build an understanding of the differences in shell structure between the bent and conic morphs of *T. rubescens* by using a combination of CT scanning and microscope observations of specimens. We also compare the bent morph of *T. rubescens* with descriptions in the literature on the species of *Chthamalus* that produce bent morphs to identity similarities and differences in how bent morphs are formed in each species (Fig. 2). Finally, we review the predators (muricid gastropods) that purportedly induce the development of bent morphs in each species and their feeding traits.

Study system

Tetraclita rubescens is typically found individually or in dense aggregations within the middle portion of the rocky intertidal zone. Its geographic range extends from Cape Mendocino, California, USA (~40.3°N) to Magdalena Bay, Baja California Sur state, Mexico (~24.5°N) (Fenberg *et al.*, 2023). This extensive north-south range means that it encounters a variety of muricid gastropod predators, which can broadly be categorised as cool- or

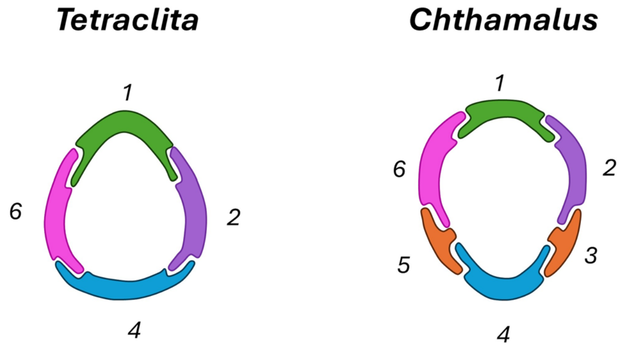


Figure 1. The shells of *Tetraclita* sp. (four plates) and *Chthamalus* sp. (six plates). Plate labels: 1, carina; 2, lateral A; 3, rostro-lateral; 4, rostrum; 5, rostro-lateral; 6, lateral B. Adapted from Darwin (1854).

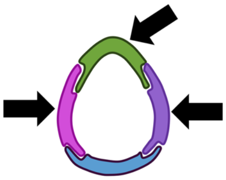
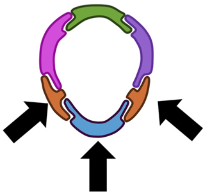
	Tetraclita rubescens	***Chthamalus anisopoma***	***Chthamalus montagui***	***Chthamalus fissus***
Location	Northern California to Baja California Sur	Gulf of California	British Isles	Southern California to Baja California Sur
Direction of bend	Bent towards the rostrum, overgrowth of the carina and both lateral plates	Bent morph is a result of differential growth of the lateral plates (not specified which plate)	Bent towards the carina, a result of overgrowth of the rostrum and rostro-lateral plates	No data on the direction of bend
Shape				
Predators	*Mexacanthina lugubris, Stramonita biserialis*	*Mexacanthina angelica*	*Nucella lapillus*	*Mexacanthina lugubris*
Predation style	*M. lugubris:* uses labral spine *S. biserialis:* selective drilling into thinner shell structures, toxins and group foraging	Labral spine is forced through the opercular plates	The opercular plates are pried apart using the proboscis OR by drilling using the radula and chemical dissolution	The labral spine is rammed into the opercular opening to force or break apart opercular plates to gain access to the soft tissue
References	Fenberg *et al*., 2023; observations herein	Lively, 1984, 1986a, b; Mokady *et al*., 2000	Murua *et al*., 2014; Crothers 1985	Jarrett 2008, 2009; Fenberg *et al*., 2023

Figure 2. Summary of literature data on barnacle species that exhibit a bent morph, including information on their geographic range, direction of shell bend, shape, predators that purportedly induce the bent morph, and their style of predation. These barnacles may have other predators, but only those thought to be associated with, or to be sympatric with bent-morph barnacles are listed. The diagrams of the shell-wall plates of *Chthamalus* and *Tetraclita* species are colour-coded as shown in Figure 1; the arrows indicate which plates outgrow the others, when known.

warm-water species depending on their biogeographic ranges. The cool-water species are *Nucella emarginata* (Deshayes, 1839), *N. ostrina* (Gould, 1852), *N. canaliculata* (Duclos, 1832), and *Acanthinucella spirata* (Blainville, 1832); the latter three rarely prey on *T. rubescens* whereas *N. emarginata* commonly preys on this barnacle in California (West, 1986; Sanford & Swezey, 2008; Fenberg *et al*., 2023). The warm-water species that *T. rubescens* may encounter in the more southerly part of its range are *Mexacanthina lugubris, Stramonita biserialis* (Blainville, 1832) and *Plicopurpura columellaris* (Lamarck, 1816) (see Fenberg *et al*., 2023). These warm-water predators have historically been restricted to the subtropics and tropics, but their ranges are currently expanding polewards as a part of tropicalisation (Fenberg *et al*., 2014, 2023; Wallingford & Sorte, 2022), which has resulted in an increase in predation pressure on *T. rubescens* in the equatorward part of its range (Fenberg *et al*., 2023; Zarzyczny *et al*., 2024).

Tropicalisation is the result of climate-induced range shifts, namely the poleward expansion of tropical species and the accompanying retreat of temperate species, causing cascading effects on species interactions and marine biogeographic structure (Zarzyczny *et al*., 2024). Fenberg *et al*. (2023) observed that bent morphs of *T. rubescens* are found only in the equatorward part of its range, where it overlaps with the warm-water predatory gastropods. By contrast, the bent morph is absent or very uncommon where the barnacle overlaps with the ranges of the cool-water predators. Fenberg *et al*. (2023) therefore suggested that one or more of the warm-water predators triggers the development bent morphs in *T. rubescens*.

MATERIAL AND METHODS

Locations and data collection

Approximately 200 individuals of *Tetraclita rubescens* were collected from three sites where the bent morph was present along the west coast of Baja California Sur, Mexico: Santa Rosalillita (28.65°N, 114.28°W), Punta Morro Santo Domingo (28.24°N, 114.09°W), and Bahía Asunción (27.12°N, 114.39°W). Barnacles were carefully removed using a thin-bladed oyster knife to prevent damage to the shells, and immediately stored in 95% ethanol. Conic individuals are cone-shaped and defined by the rim of the opercular opening oriented parallel to the base of the barnacle. By contrast, the rim of the opercular opening is perpendicular to the base in bent morphs (Fig. 3). While the conic morph is more common in the medium to larger size classes, i.e. barnacles of 15–40 mm in shell diameter (Fenberg *et al.*, 2023), the bent morph is typically smaller, usually just 5–20 mm in shell diameter.

Microscopic examination

Fifty bent specimens of *T. rubescens* were dissected under a stereoscopic dissecting microscope (Zeiss Stemi 2000-C, Oberkochen, Germany) in order to determine the direction of bend by examination of the shell wall plates. The soft bodies were removed along with the opercular plates (terga and scuta), and the four plates of the shell wall were identified using Darwin's (1854) illustrations as a guide (Fig. 1). Barnacles were turned over and positioned so their outline matched the teardrop shape in Darwin's (1854) diagram, with the pointed carina at the top and the flatter rostrum at the bottom. The plates and direction of bend were then identified, and photos were taken in dorsal and ventral view of specimens representing the conic and bent morphs. For ease of reference, the lateral plates were labelled as 'lateral A' situated clockwise from the carina in the dorsal view, and 'lateral B' situated anticlockwise from the carina. Both bent and conic individuals were examined, the direction of bend (defined by the plate the opercular opening was facing) was noted for each bent individual. A notch was also noticed only on the bent barnacles and so the presence or absence was noted for each bent individual. The notch is an indentation/slit that is observable in some bent individuals, it is an extension of the opercular opening that involves damage to the carina (Fig. 3C).

Figure 3. Photographs taken in the field showing conic and bent morphs of *Tetraclita rubescens* living side by side (**A**). A large conic individual exhibiting the diamond/kite shape of the opercular opening that is typical of the conic morph, with two attached juvenile conic individuals (**B**). A bent individual with a notch in its carina, alongside a specimen of the muricid gastropod *Mexacanthina lugubris*, a predator of *T. rubescens* that is thought to induce the bent morphology (**C**).

CT imaging

X-ray imaging is a non-destructive technique that measures the extent to which X-rays are attenuated (i.e. absorbed or scattered) as they pass through a specimen (Withers *et al.*, 2021). The resulting image, known as a radiograph, reflects the structural and material density of specimens and can be interpreted as a two-dimensional (2D) attenuation map (Fig. 4A, C). Computed tomography (CT) builds on this by collecting many such images from different angles around a rotating specimen (Withers *et al.*, 2021). These are reconstructed using computational algorithms into a three-dimensional (3D) dataset that reveals the internal structure of the specimen (Fig. 4B). Computed microtomography (μCT) is a variation of conventional CT that uses specialised hardware to achieve resolution up to several orders of magnitude higher than that achieved through conventional systems (Withers *et al.*, 2021).

Twenty preserved specimens in total were scanned, 10 conic and 10 bent individuals. The overall diameter of the scanned specimens was 4.8–17.2 mm for bent specimens and 13.9–23.0 mm for conic specimens. Each barnacle was placed in a test tube, surrounded by bits of polystyrene foam and other packing material to keep it stationary during scanning, thereby avoiding motion artifacts (blurring) or other distortions of the images (Vila-Comamala *et al.*, 2011).

Scanning was performed at the μ-VIS X-ray Imaging Centre, University Hospital Southampton (https://muvis.org), using a custom-designed X-ray μCT based on the Nikon XTH 225 ST system (Nikon, Tokyo, Japan) but optimised for histology applications. Each specimen was imaged using a tungsten target operating at 75 μA and 110 kVp (8W), with a 0.08 mm aluminium filter to shape the

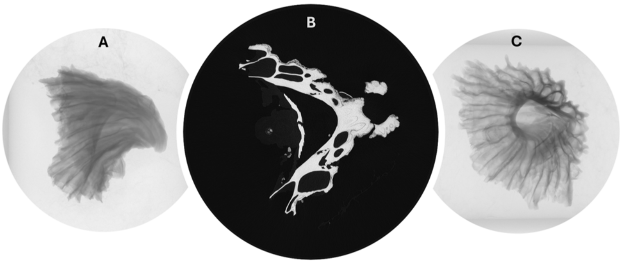


Figure 4. Representative X-ray imaging outputs from micro-computed tomography (μCT) of an individual barnacle (Specimen MVIS06586). Radiograph acquired at 0° projection angle (**A**); corresponding central slice from the reconstructed 3D volume (**B**); radiograph at 90° (**C**). The radiographs illustrate X-ray attenuation contrast (specimen structure and material density), whereas the reconstructed slice reveals internal structural detail.

X-ray spectrum. In total, 3001 projections were acquired over a 360° rotation. Each projection was averaged across four frames, with an exposure time of 125 ms per frame to enhance signal-to-noise ratio. The source-to-detector distance was fixed at 1,137 mm, while the source-to-object distance was adjusted to between 30 and 90 mm depending on specimen size, to maximise resolution. This resulted in an effective isotropic voxel size ranging between 4 and 13 µm, with an average size of 9.5 µm for the cohort. Further information on the scan parameters for each specimen can be found in Supplementary material Table S1. Reconstruction was performed by using Nikon's filtered back-projection software to generate 32-bit volume datasets, which were subsequently saved as 1.296 × 10^8-bit volume files.

Data analysis

We used ORS Dragonfly software, vers. 2022.2 (https://www.theobjects.com/dragonfly) for visualisation of µCT datasets and to measure and analyse the specimen. The wall plates of the shells of both bent- and conic-morph specimens were manually segmented using the segmentation tool, 'regions of interest' (ROIs); this was done by colouring each plate in all slices of each specimen. Seven ROIs were generated for each specimen: carina, lateral A, lateral B, rostrum, opercular plates, and two other regions including sand/grit and epibionts.

The measurement tool was used to measure the length of each shell wall plate for all 20 specimens. Each plate was measured along its midline, from the base to the opercular margin, by adding together multiple short lines to ensure the most accurate measurement (Fig. 5C). The circumferences of the base and opercular rim were also measured by summing small, straight-line measurements made around these parts of the barnacle (Fig. 5A, B).

Straight-line measurements were used to find the absolute-value disparities in length (mm) between all possible pairs of plates: carina-rostrum (CR), carina-lateral A (CA), carina-lateral B (CB), rostrum-lateral A (RA), rostrum-lateral B (RB), and lateral A-lateral B (AB). Length measurements of each plate were made from the base to the opercular margin. The mean absolute-value difference for all six pairwise differences in shell-wall plate lengths was calculated for each individual. The six sets of pairwise differences were then compared between all 10 individuals of each morph, as well as between the two sets of 10 individuals each representing the bent and conic morphs, in both cases by using the Wilcoxon rank-sum test.

The base:operculum circumference ratio was compared between morphs to confirm whether the operculum is relatively smaller in the bent morph. To standardise the ratio, the basal circumference of each specimen was expressed as a multiple of the opercular circumference. This allowed for direct comparison among individuals regardless of their absolute size differences. The multipliers were then compared between the bent and conic morphs using the Wilcoxon rank-sum test. All statistical testing was done using R studio.

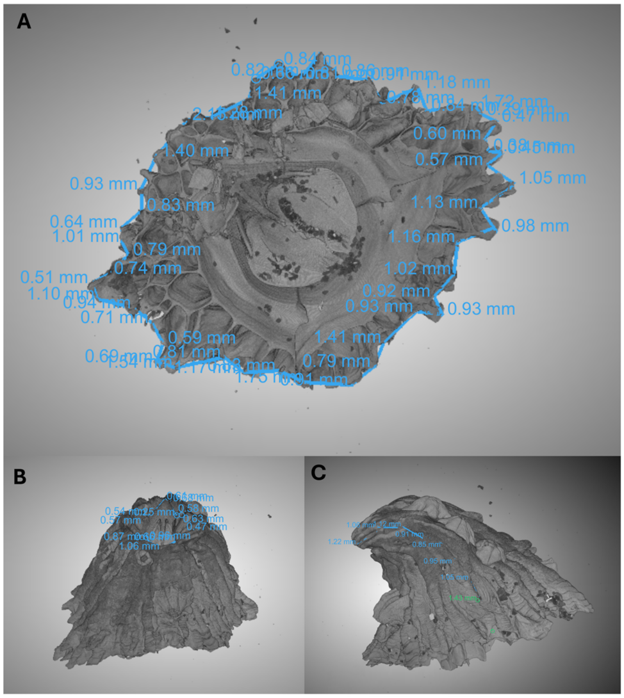


Figure 5. Images from Dragonfly (https://www.theobjects.com/dragonfly) showing how measurements were made. The circumference of the base (**A**) and the opercular opening (**B**) was measured by using several small straight lines to trace the base/opercular, the lengths were then added together. Plates were measured using the same straight-line tool to measure the middle of the plate, demonstrated here on the bent specimen MVISO6586 (**C**).

RESULTS

Microscopic inspection

Dissection and inspection of 50 bent specimens of *T. rubescens* showed that all were bent in the same direction, with the opercular opening always facing towards the rostrum. The rostrum is the smallest plate, and the lateral plates and the carina are, therefore, the plates that undergo positive differential growth. Notches were only observable in bent morphs; a notch was present in 35 of the individuals examined (70%), it is always an extension of the opercular opening appearing as damage to the centre of the carina (Fig. 3C).

Micro-CT analyses

The wall plates in shells of *T. rubescens* showed structural differences both within and between the bent and conic morphs. In dorsal and ventral view, both morphs exhibit a teardrop-shaped outline, which is typical for *Tetraclita* (Darwin, 1854). The opercular opening is clearly visible in the conic morph (Fig. 6A, B), as are all the wall plates of the shell when viewed from the ventral side (Fig. 6B). In stark contrast, however, neither the operculum nor the rostrum is visible in ventral view in the bent morph (Fig. 7A). This is because the carina and both lateral plates outgrow the rostrum to such an extent that the opercular opening comes to lie perpendicular to the base (Fig. 7C, E, F).

All four of the shell wall plates were similar in length in the conic morph and rather uniform in shape (Fig. 6C–F); in the bent morph individuals, however, the rostrum was shorter than the other three plates (Fig. 7D–F). This is the main reason for the significant disparity between the two morphs mean absolute value of pairwise plate-length differences (W = 89, $P = .004$; Fig. 7A). Confirmation of this assertion is provided by the pairwise comparisons of plate-length combinations in bent specimens (blue boxplots in Fig. 8B, Table 1), where significant differences in absolute-value disparity only occur in pairwise comparisons involving the rostrum (viz. the CR, RA, and RB pairings; Table 1). By contrast, the corresponding

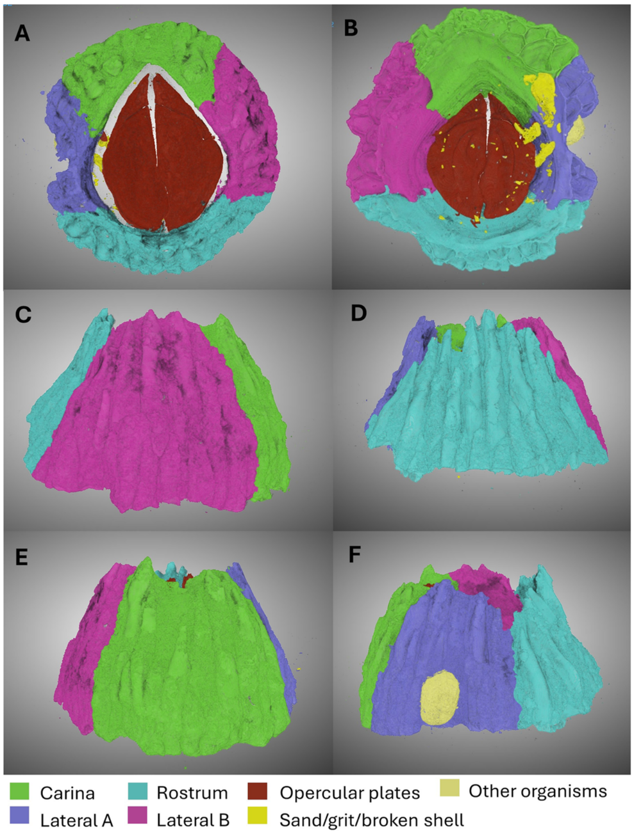


Figure 6. Images from Dragonfly (https://www.theobjects.com/dragonfly) showing an example of a conic-morph barnacle (*Tetraclita rubescens* specimen MBIO08862) with segmented plates and other objects coloured as follows: blue, rostrum; green, carina; pink, lateral A; purple, lateral B; red, operculum; pale yellow, another animal, viz., a limpet; and dark yellow, sand/grit or broken shell. Dorsal view (**A**); ventral view (**B**); lateral view mainly of lateral B plate (**C**); anterior view mainly of rostrum (**D**); posterior view mainly of carina (**E**); lateral view mainly of lateral A plate (**F**).

pairwise combinations of plate-length measurements in conic specimens showed no significant differences in absolute-value disparity at all among themselves (red boxplots in Fig. 8B; Table 1). There were, however, significant differences between bent and conic barnacles for the same three pairwise comparisons as above that involve the rostrum (CR, RA, RB), but not for any other plate pairings (*cf.* blue vs red boxplots in Fig. 8B; Table 1).

A significant difference in the base:operculum circumference ratio of the shell was found between the bent and conic morphs of *T. rubescens* (W = 98, *P* < .001). The bent barnacles typically had a circumference ratio between 3.3:1 and 5.8:1, which was generally larger than, and barely overlapped, the 2.2:1 to 3.9:1 range of this circumference ratio in conic barnacles (Fig. 9). The bent morph thus had a relatively smaller operculum than the conic morph when compared to the size of the shell base.

DISCUSSION

Comparison of the bent and conic morphs

All bent-morph individuals of *T. rubescens* have their opercular opening facing the shortest wall plate, the rostrum. This is because the carina and both lateral plates continue to grow while the rostrum slows or stops its growth. Differential growth of the rostrum in relation to the other wall plates is the key for understanding how the bent morph arises in *T. rubescens*. There is a significant difference within-individual plate-length variation between the bent and conic morphs, which is mostly attributable to the shorter rostrum of the bent morph (Fig. 8). Individuals of the conic morph, despite some variation due to erosion, broken bits of shell, and unevenness of outline due to position on the rock, show no significant within-individual differences in shell-plate length.

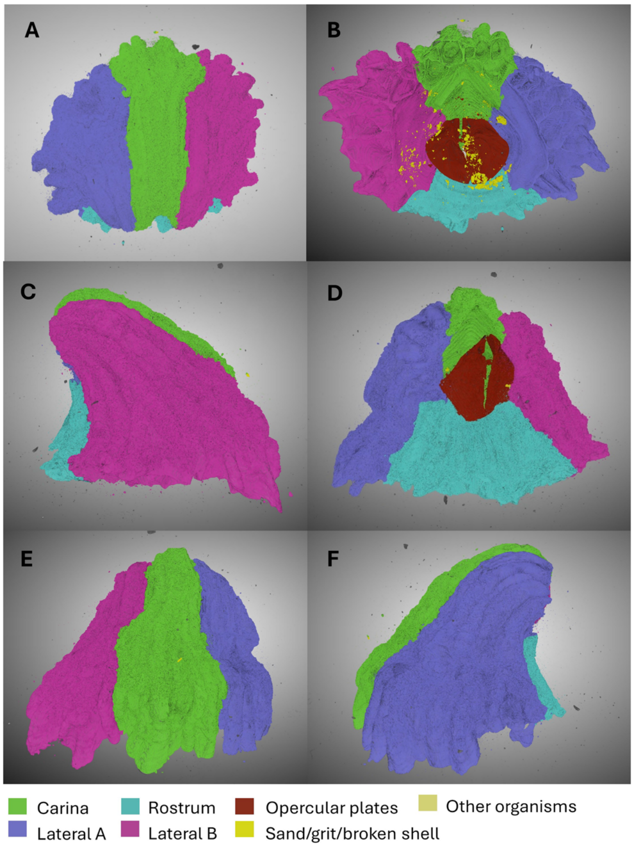


Figure 7. Images from Dragonfly (https://www.theobjects.com/dragonfly) showing an example of a bent-morph barnacle (*Tetraclita rubescens* specimen MBIO08843) with segmented plates and other objects coloured as in Figure 6. Dorsal view (**A**); ventral view (**B**); lateral view mainly of lateral B plate (**C**); anterior view mainly of rostrum (**D**); posterior view mainly of carina (**E**); lateral view mainly of lateral A plate (**F**).

Bent specimens of *T. rubescens* have a larger base:operculum circumference ratio than conic ones (Fig. 9), because the relative size of the opercular opening is smaller in bent-morph barnacles. A smaller *absolute* size of the opercular opening might, on average, be expected in the bent morph because such barnacles are typically smaller overall than conic ones (Fenberg *et al.*, 2023), but it is not immediately clear why the *relative* size of the opening should also be smaller in the bent morph. Part of the explanation may be that barnacles grow in such a way that the oldest parts of the wall plates surround the opercular opening and the basal parts represent the newest growth (Crisp & Bourget, 1985). The opercular opening can thus become larger through erosion/abrasion

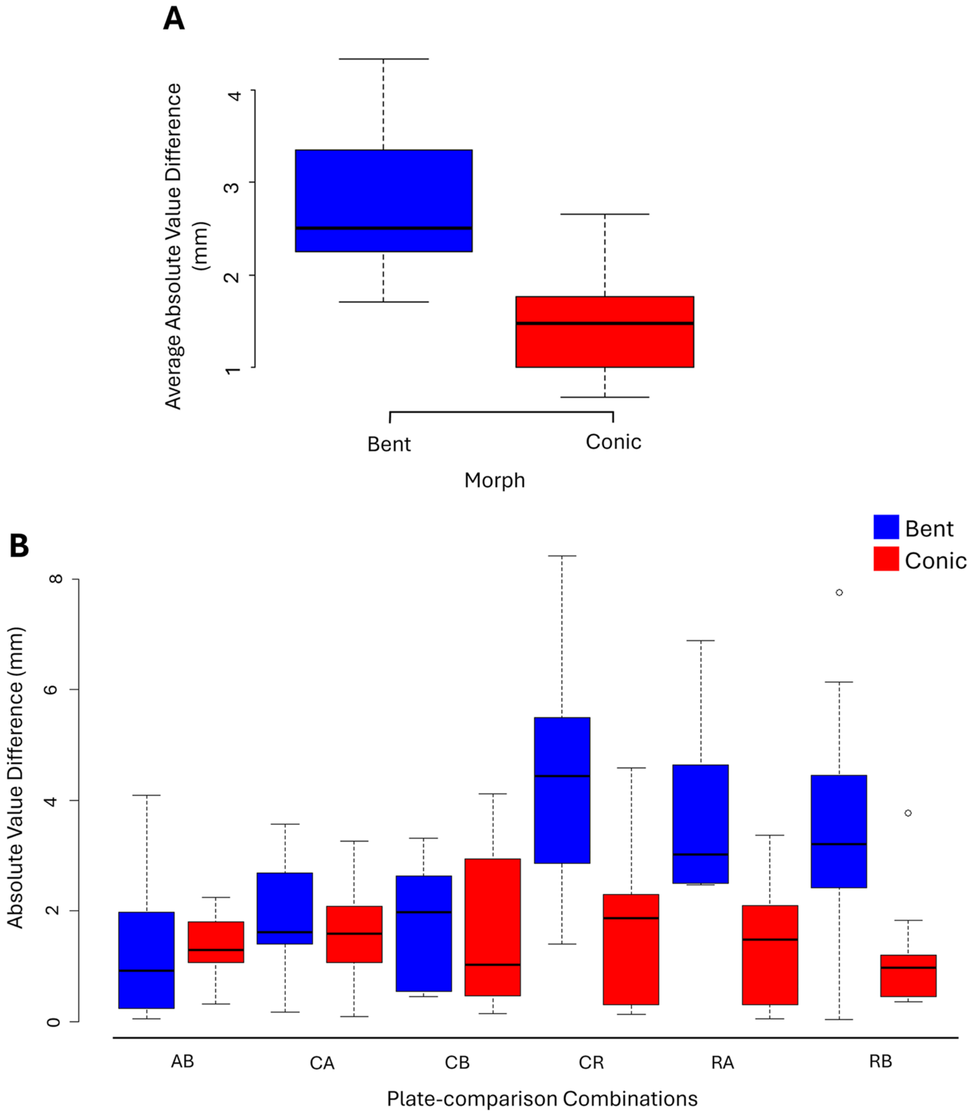


Figure 8. Boxplots showing absolute-value length differences between shell-wall plates for the bent (blue) and conic (red) morphs of *Tetraclita rubescens* (W = 89, $P = .004$) (**A**). Boxplots showing the absolute-value length differences in the following pairwise plate combinations: AB, lateral A and lateral B; CA, carina and lateral A; CB, carina and lateral B; CR, carina and rostrum; RA, rostrum and lateral A; RB, rostrum and lateral B. The elevated blue boxes signify a greater difference in the absolute-value lengths when comparing the rostrum to the carina and both lateral plates (**B**).

of the opercular margins of these plates as an individual grows (Darwin, 1854). Because conic individuals tend to be larger and older than bent ones, this trend could explain why their opercular openings are also larger. A small opercular opening, however, serves for defence in other barnacles. In *Chthamalus fissus*, for example, a separate narrow-operculum morph exists alongside the bent and conic morphs (Jarrett, 2008). A narrow operculum may make it difficult for the predatory snail *Mexacanthina lugubris* to feed by making it hard for the snail to insert its labral spine into the operculum. The same snail is also a common predator of *T. rubescens*, especially in the southern portion of its geographic range along the Pacific coast of the Baja California Peninsula where the bent morph is common (Fenberg *et al.*, 2023). In this region, the relatively small operculum of bent morphs may give it an advantage against predation by *M. lugubris* over the conic morph.

One other notable difference between the conic and bent morphs in *T. rubescens* is the notch, which impacts the opercular opening and carina. The notch was observed in 70% of the bent barnacles examined microscopically (Fig. 3C), but none of the conic barnacles. The notch may be the result of failed predation attempts (caused by the labral spine of *M. lugubris*, for example), or it may be a structural adaptation that provides more space for extension of the feeding cirri in bent individuals with relatively small opercular openings. There is no other record of such a notch or any equivalent structure in the species of *Chthamalus* that have a bent morph. Field and laboratory observations of gastropod feeding behaviour with respect to both the bent and conic morphs, and the feeding behaviour of both barnacle morphs, will be needed to gain a better understanding of the notch.

It is unknown how feeding or copulation or other such functions might be affected in bent barnacles of any species. A reduced

Table 1. Comparison of mean absolute-value length differences in pairwise plate combinations between conic and bent morphs of *Tetraclita rubescens* and within the individual morphs (see Fig. 8B), showing W and *P* values from the Wilcoxon rank-sum test.

	Plate combination	**W value**	***P* value**	**Significance**
Comparison of absolute-value length difference for each plate combination between the conic and bent morph.	AB	36	0.3071	
	CA	60	0.4725	
	CB	56.5	0.6500	
	CR	84	0.0113	*
	RA	91	0.0022	**
	RB	84	0.0113	*
Plate combination absolute-value length comparisons for the bent morph barnacles	CA-CB	48	0.9097	
	CA-CR	13.5	0.0065	**
	CB-CR	14	0.0073	**
	RA-CR	41.5	0.5452	–
	RA-CA	88	0.0046	**
	RA-CB	87	0.0058	**
	RB-CR	39	0.4274	–
	RB-CA	79	0.0312	*
	RB-RA	46	0.7913	–
	AB-CR	8	0.0017	**
	AB-CA	29	0.1211	
	AB-CB	29	0.1212	
	AB-RA	6	0.0010	***
	AB-RB	16	0.0113	*
Plate combination absolute-value length comparisons for the conic morph barnacles	CA-CB	52	0.9097	
	CA-CR	40	0.4727	
	CB-CR	46.5	0.8205	
	RA-CR	42	0.5705	
	RA-CA	46.5	0.8205	
	RA-CB	47.5	0.8798	
	RB-CR	38	0.3845	
	RB-CA	30	0.1403	
	RB-RA	44	0.6775	
	AB-CR	39.5	0.4495	
	AB-CA	45.4	0.7623	
	AB-CB	52	0.7623	
	AB-RA	52	0.9097	
	AB-RB	65	0.2729	

Level of significance:

$^{*}P < 0.05$,
$^{**}P < 0.01$,
$^{***}P < 0.001$;
– indicates where both combinations include the rostrum.

feeding rate in comparison to conic barnacles could negatively affect the energy acquisition, physiology, and growth rate of bent-morph barnacles (Lively 1986a; Jarrett 2009, 2018). Similar trade-offs might also affect bent morphs of *T. rubescens*, supported by their generally smaller size compared to conic morphs (Fenberg *et al.*, 2023); however, further study into morph-associated differences in reproductive output, physiology, growth rate, and mortality is required to fully understand the life history trade-offs. Additionally, more detailed examination of the soft-tissue anatomy of both morphs might reveal whether the presence and direction of bending are associated with any protective internal realignment of features, such as the reproductive organs.

Comparison of *Tetraclita* to other species with bent morphs

Among barnacles, bent morphs have only been observed among species of *Chthamalus* until recently. Like the bent morph of *T. rubescens*, the bent morphs of *C. anisopoma* (see Lively, 1986a, b) and *C. fissus* (see Jarrett, 2008) occur along temperate and tropical/sub-tropical rocky shorelines of the Eastern Pacific (Fenberg *et al*, 2023), whereas the bent morph of *C. montagui* occurs in the British Isles (Murua *et al.*, 2014). In all these species, the bent morphs appear to be a response to predation pressure by certain muricid gastropods (Lively, 1986a, b; Jarrett, 2008, 2009; Murua *et al.*, 2014; Fenberg *et al.*, 2023).

All bent-morph individuals of *T. rubescens* are bent in the same direction, with the carina and lateral plates outgrowing the rostrum, resulting in a bend towards the rostrum (Fig. 7A, D). A comparison to the literature on the species of *Chthamalus* shows that this is not the only way to produce bent morphs (Fig. 2). In dorsal view, *Chthamalus* spp. have the same teardrop shape as *T. rubescens* but with the rostrum, not the carina, at the pointed end. It also has two additional rostro-lateral plates (making a total of six plates in the shell wall), one on each side between the rostrum and the lateral plate (Fig. 1) (Darwin, 1854). The bend in *C. anisopoma* is the

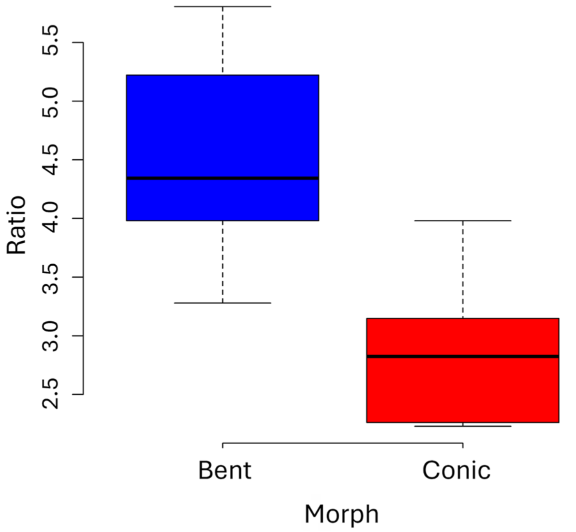


Figure 9. Boxplots showing the base:operculum circumference ratio of bent and conic morphs of *Tetraclita rubescens* (W = 98, $P < .001$). The larger ratios for the bent morph shows that bent individuals generally have a relatively smaller opercular opening than conic individuals.

result of differential growth that favours the lateral plates (Lively, 1986a, b, 1999), resulting in a bend, though it is not specified which lateral plate or the specific direction of bend. In *C. montagui*, it is the result of differential growth favouring the rostrum, such that the shell is bent towards the carina (Murua *et al.*, 2014) and the rostrum becomes similar in shape to the carina of the bent morphs of *T. rubescens*. *Chthamalus montagui* is preyed on by *Nucella lapillus* (Linnaeus, 1758), which pries the opercular plates open either with its proboscis or by boring a hole with the radula while also employing chemical dissolution (Crothers, 1985).

While there is no information in the literature on the direction of the bend in *Chthamalus fissus*, the same predatory snail, *Mexacanthina lugubris*, likely induces the bent morph in both *C. fissus* and *T. rubescens*. This snail attacks by ramming its labral spine into the opercular opening (Jarrett, 2008, 2009; Fenberg *et al.*, 2023), a simple and efficient strategy that does not rely on drilling and chemical dissolution. *Tetraclita rubescens* and *M. lugubris* are both commonly found along the Pacific coast of the Baja California Peninsula (Fenberg *et al.*, 2014), being particularly abundant where the bent morph occurs and where many active predation events have been recorded (Fenberg *et al.*, 2023). There are, however, regions where these species overlap but the bent morph of *T. rubescens* is rare or absent (along the Pacific northern Baja California Peninsula and southern California). This may be due to insufficient predation pressure or to the lack of a genetic capacity to produce the bent morph in these barnacle populations (Fenberg *et al.*, 2023). Furthermore, *M. lugubris* appears to preferentially prey on *C. fissus* in regions where *T. rubescens* is uncommon. In these areas (e.g. northern Baja California), the bent and narrow-operculum morphs of *C. fissus* are common but the bent morph of *T. rubescens* is rare (Jarrett, 2008; Fenberg *et al.*, 2014, 2023). Site-dependent differences in relative abundance between these two barnacle species likely result in prey switching, with more pressure on *C. fissus* and selection in favour of its bent morph there (Fenberg *et al.*, 2023). Further comparison of the bent morphs of *C. fissus* and *T. rubescens* could provide valuable insight into the mechanics of how bent morphs are formed across these two different species and genera that share a predator.

The sister species of *C. fissus* is *C. anispoma* (see Wares, 2009), which have bent morphs that are induced by *Mexacanthina angelica* (Oldroyd, 1918), which is the sister species to *M. lugubris* (Lively, 1986a, b; Marko & Vermeij, 1999; Jarrett, 2008, 2009). These predator/prey pairings occur on opposite sides of the Baja California Peninsula. We know that the bent morph in *C. anispoma* is caused by differential growth favouring the lateral plates of the shell wall (Mokady *et al.*, 2000). Therefore, *C. fissus* likely becomes bent in a similar way, both on account of relatedness and because *M. lugubris* preys in the same way as *M. angelica* (Lively, 1986b, 1999; Jarrett, 2008, 2009) by forcing its labral spine through the opercular plates. It is still unknown whether bent morphs only form in individuals with a unique genotype or due to phenotypic plasticity triggered by specific cues in the local environment. Field and laboratory experiments are essential for obtaining a better understanding of how and why bent morphs are formed in *T. rubescens*, its life history trade-offs, and whether bent morphs will become increasingly common as warm water predators continue to expand poleward with climate warming.

Further study and analysis of differences in life history, physiology, reproductive output and soft-tissue anatomy are needed to fully understand the differences between the two morphs of *T. rubescens*. *Tetraclita rubescens* bends differently from other barnacle species with bent morphs (viz. several species of *Chthamalus*), each of which appears to have a unique pattern of differential growth of wall plates resulting in a bend, but more research on *C. fissus* is needed. Furthermore, genomic and gene-expression analysis will be beneficial for understanding the mechanisms of barnacle bending and also whether the bent morphs of different kinds of barnacle is a phenotypically plastic or an evolutionary response to predation.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. CT scan parameters.

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