

Supplementary material

Exoskeletons of mud crabs, *Scylla serrata*, of different sizes: body weight, surface morphology, internal tissue structure, and mechanical resistance

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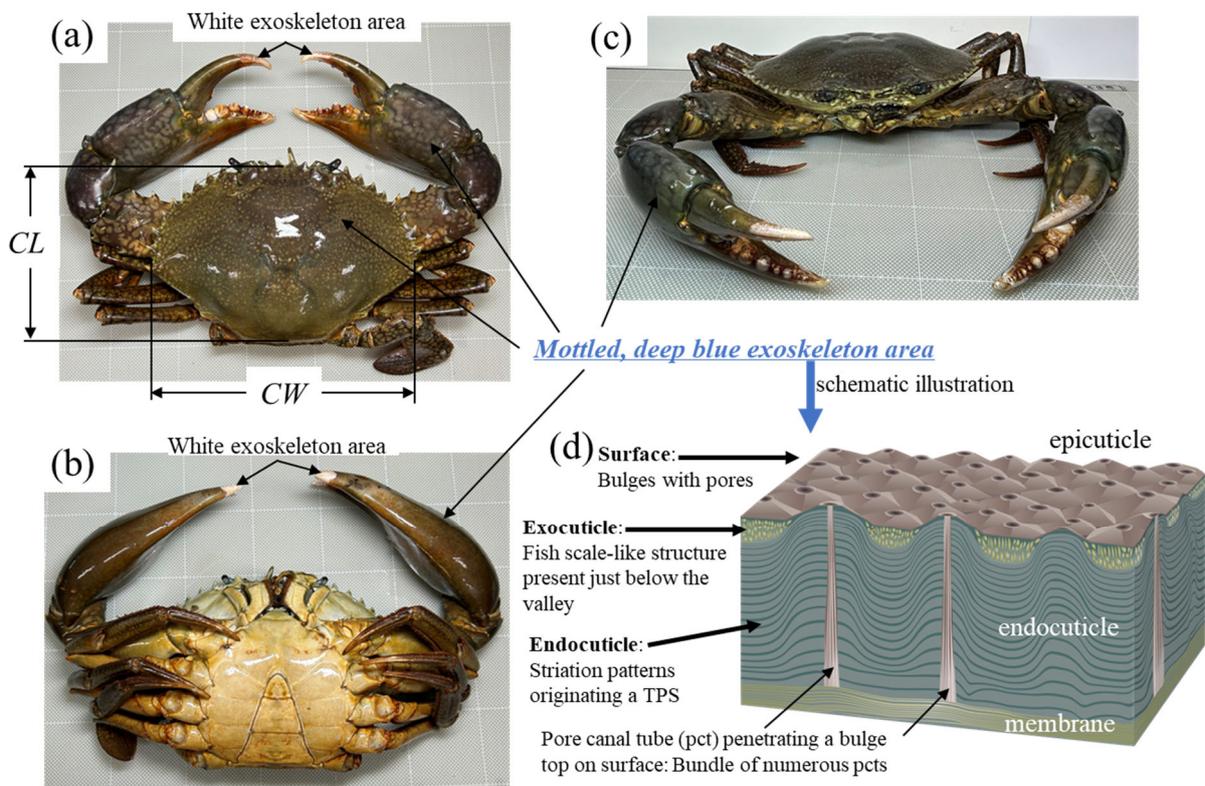


Figure S1. The male mud crab with body weight (BW)=1442 g, carapace width (CW)=169.6 mm, and carapace length (CL)=115.1 mm: (a) top, (b) bottom, and (c) front. (d) Schematic illustration of the surface and longitudinal section of a mottled, deep-blue exoskeleton. The exoskeleton is composed of epicuticle (thinnest wax layer of the outermost surface), exocuticle, endocuticle (thickest layer), and membrane (adjacent layer to cells).

(a) Male



(b) Female

Figure S2. Sex of mud crabs: (a) male (body weight: 1270 g) and (b) female (body weight: 1100 g). The shape of the abdomen is a thin triangular apron for males, while the female's is wider.

410 g mud crab

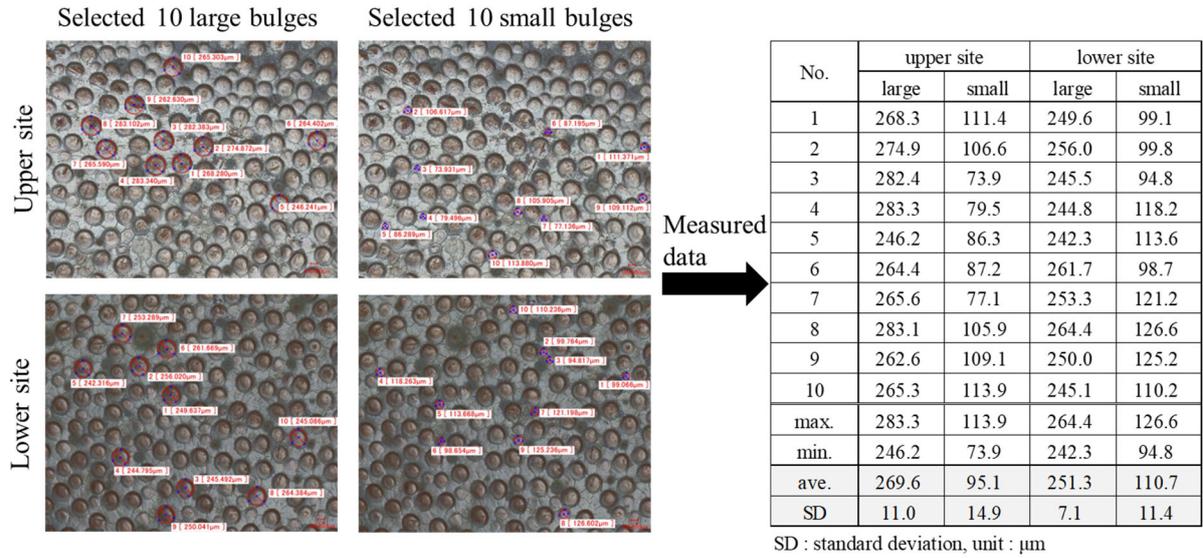


Figure S3. The surface morphology of the upper site and lower site on the carapace of a 410 g mud crab. The bulge diameter was measured using software installed on a 3D laser scanning microscope (VK-X200/210, Keyence Corporation, Osaka, Japan).

985 g mud crab

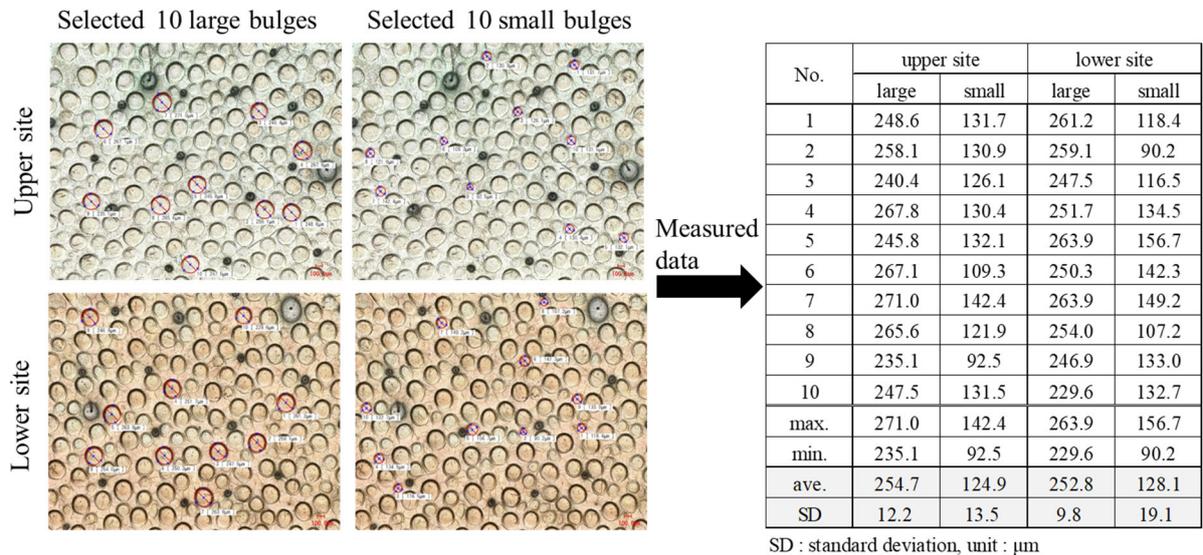


Figure S4. The surface morphology of the upper site and lower site on the carapace of a 985 g mud crab. The bulge diameter was measured using software installed on a 3D laser scanning microscope (VK-X200/210, Keyence Corporation, Osaka, Japan).

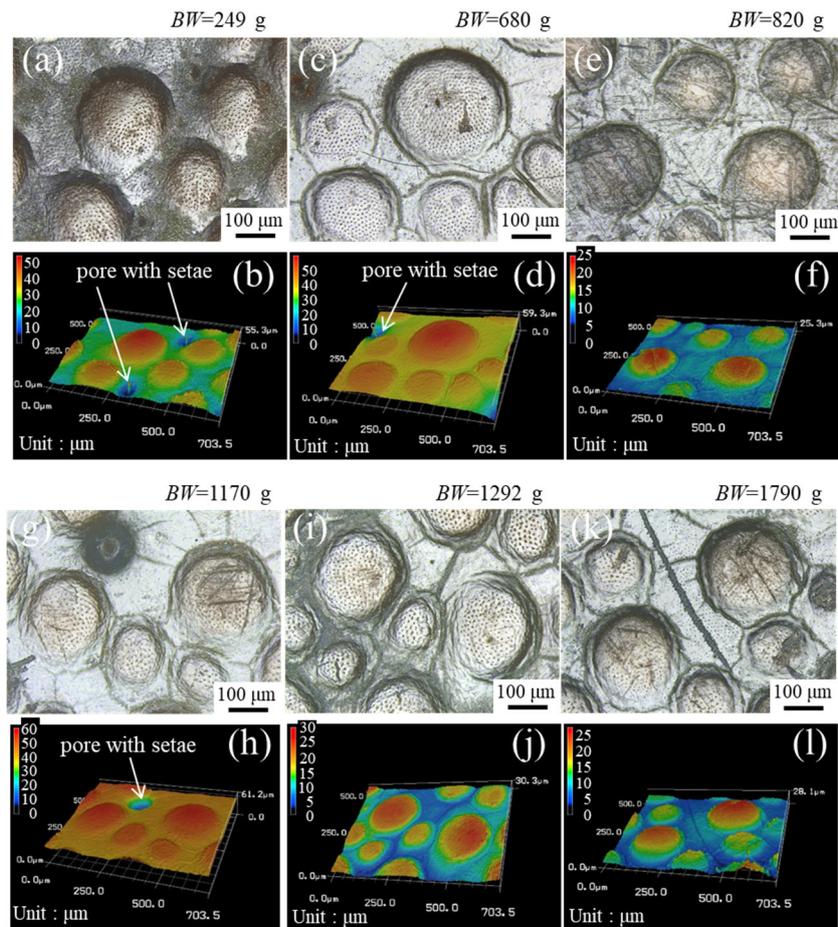
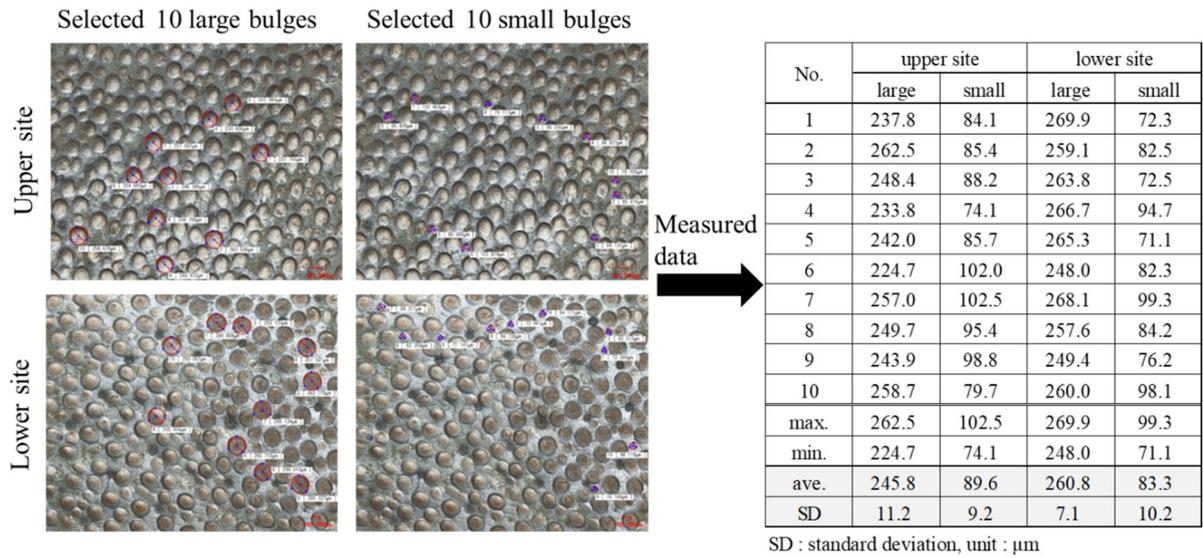
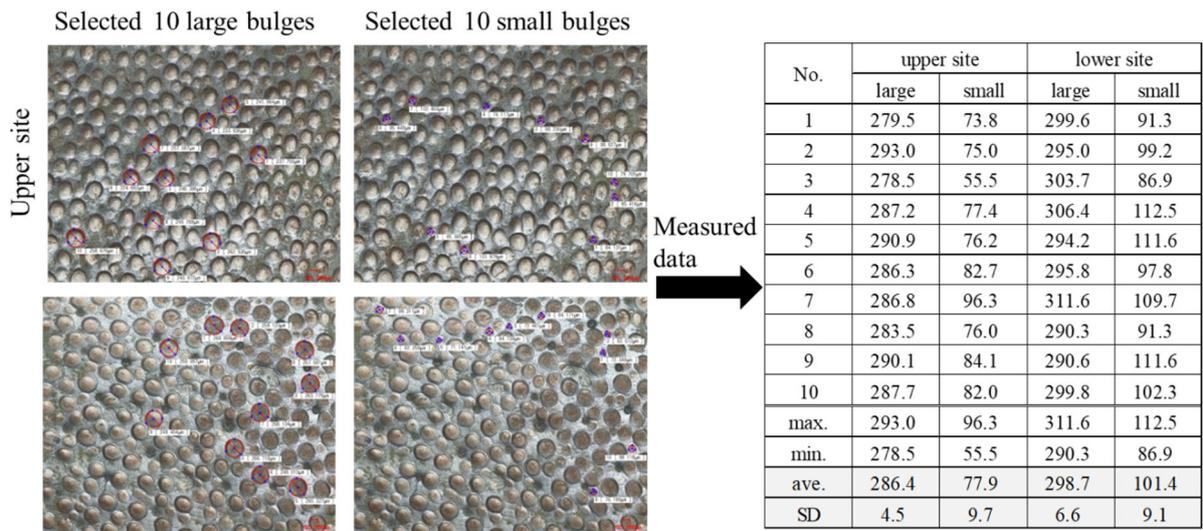


Figure S5. (a,c,e,g,i,k) Laser scanning microscope images of the upper site of the exoskeleton surface of mud crabs of different body weights and (b,d,f,h,j,l) their 3D color maps: (a,b) 249 g, (c,d) 680 g, (e,f) 820 g, (g,h) 1170 g, (i,j) 1292 g, and (k,l) 1790 g.

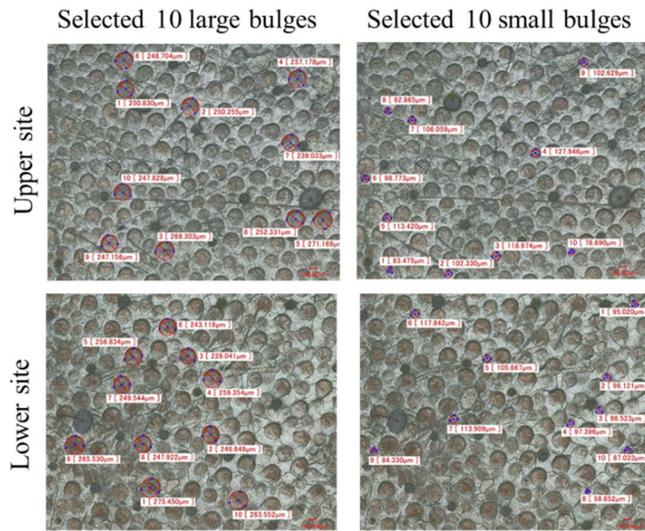
(a) 249 g mud crab



(b) 680 g mud crab



(c) 820 g mud crab

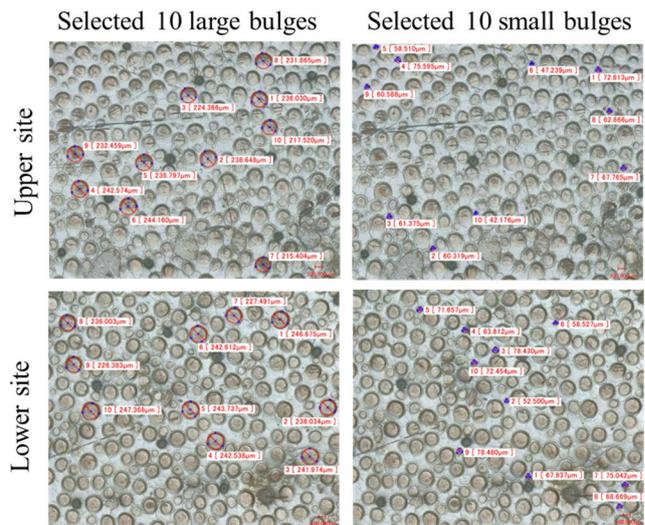


Measured data →

No.	upper site		lower site	
	large	small	large	small
1	250.8	83.5	275.5	95.0
2	250.2	102.3	246.6	98.1
3	268.3	118.9	228.0	96.5
4	257.1	127.9	259.4	97.4
5	271.1	113.4	256.8	105.7
6	248.7	98.7	243.1	117.8
7	239.0	106.1	249.5	113.9
8	252.3	92.8	247.9	58.6
9	247.1	102.6	265.5	84.3
10	247.8	76.7	263.6	87.0
max.	271.1	127.9	275.5	117.8
min.	239.0	76.7	228.0	58.6
ave.	253.2	102.3	253.6	95.4
SD	9.3	14.8	12.8	15.8

SD : standard deviation, unit : μm

(d) 1170 g mud crab

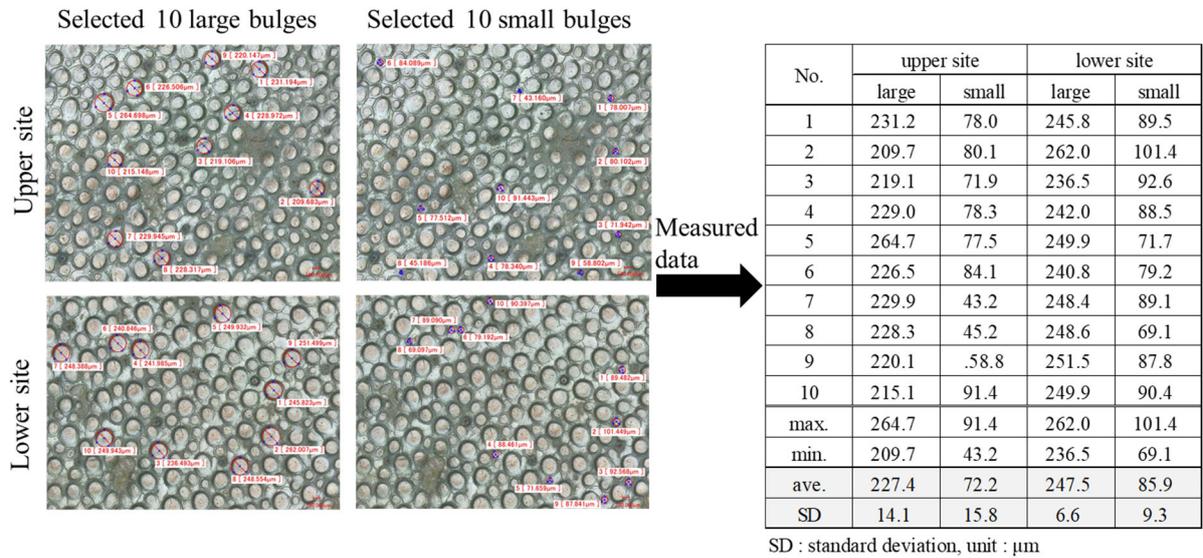


Measured data →

No.	upper site		lower site	
	large	small	large	small
1	236.0	72.8	246.7	67.9
2	238.6	60.3	238.0	52.5
3	224.4	61.4	241.9	78.4
4	242.6	75.6	242.5	63.8
5	236.8	58.5	243.7	71.7
6	244.1	47.2	242.6	58.5
7	215.4	67.7	227.5	75.0
8	231.9	62.9	236.0	68.7
9	232.5	60.6	226.4	78.5
10	217.5	42.1	247.4	72.5
max.	244.1	75.6	247.4	78.5
min.	215.4	42.1	226.4	52.5
ave.	232.0	60.9	239.3	68.8
SD	9.4	9.8	7.0	8.0

SD : standard deviation, unit : μm

(e) 1292 g mud crab



(f) 1790 g mud crab

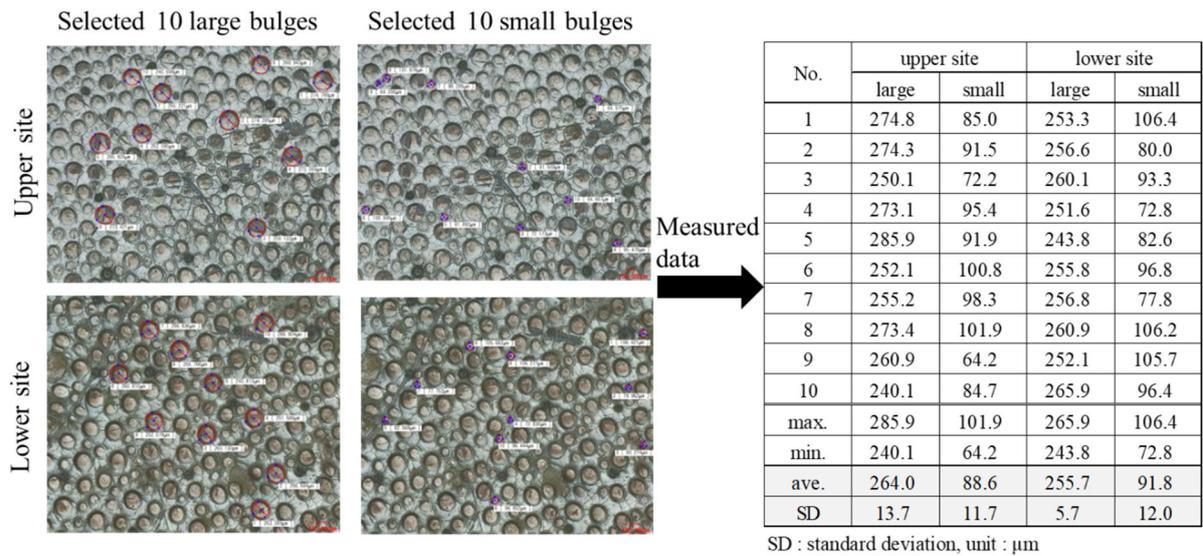


Figure S6. The surface morphology of the upper site and lower site on the carapace of male mud crabs with body weights of (a) 249 g, (b) 680 g, (c) 820 g, (d) 1170 g, (e) 1292 g, and (f) 1790 g. The bulge diameter was measured using software installed on a 3D laser scanning microscope (VK-X200/210, Keyence Corporation, Osaka, Japan).

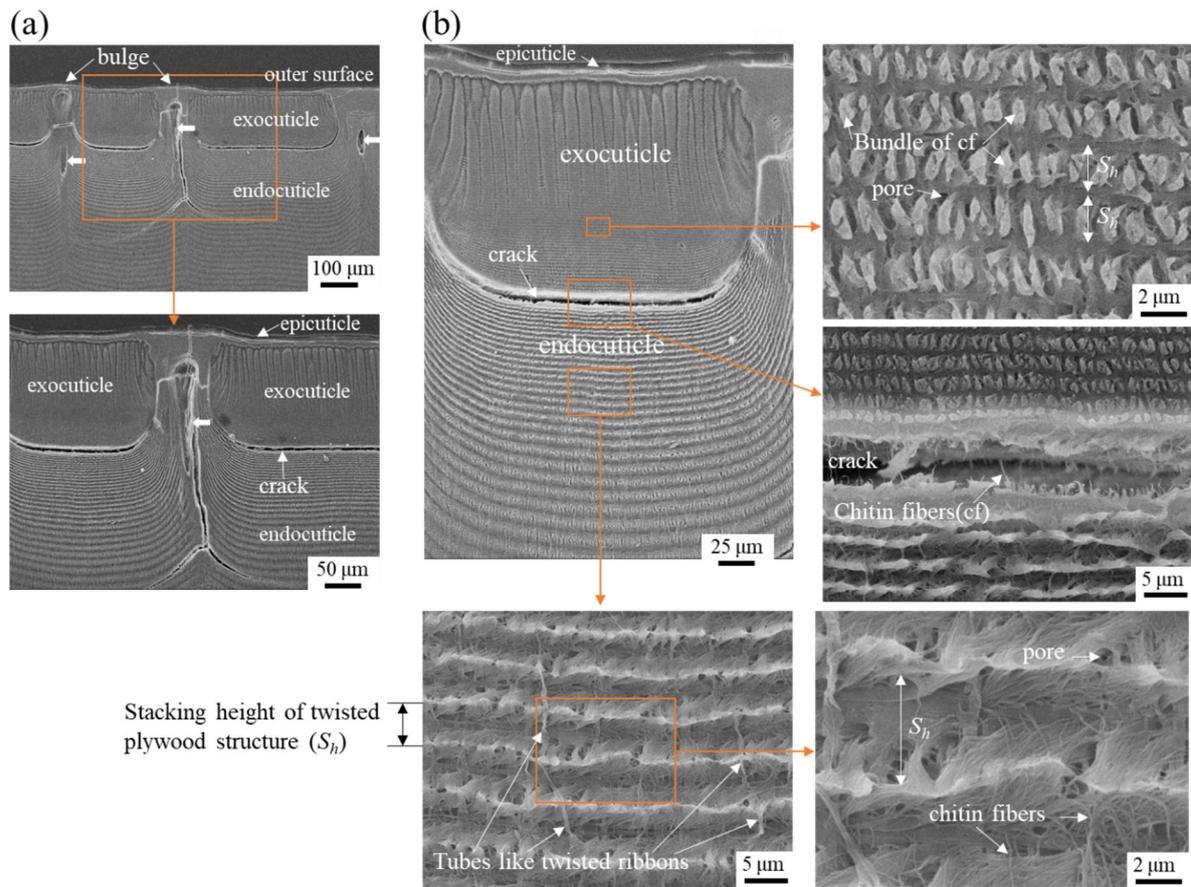


Figure S7. Scanning electron microscope (JCM-7000, JEOL, Tokyo, Japan) images of the polished surface: (a) near the outer surface with some bulges, (b) near the outer surface at a different location. Here, as a sample, a carapace of a 1292 g mud crab was prepared by polishing and gold coating. Cracks occur at the boundary (intermediate layer) between the exocuticle and endocuticle layers. The white arrows denote the traces of pore canal tubes observed on the polished surface in (a). When the polished sample is left in the SEM chamber for several hours, many cracks appear on the sample surface. These cracks are preferentially observed in the pore canal tubes and intermediate layer, which are dominated by the organic phase due to rapid drying under vacuum.

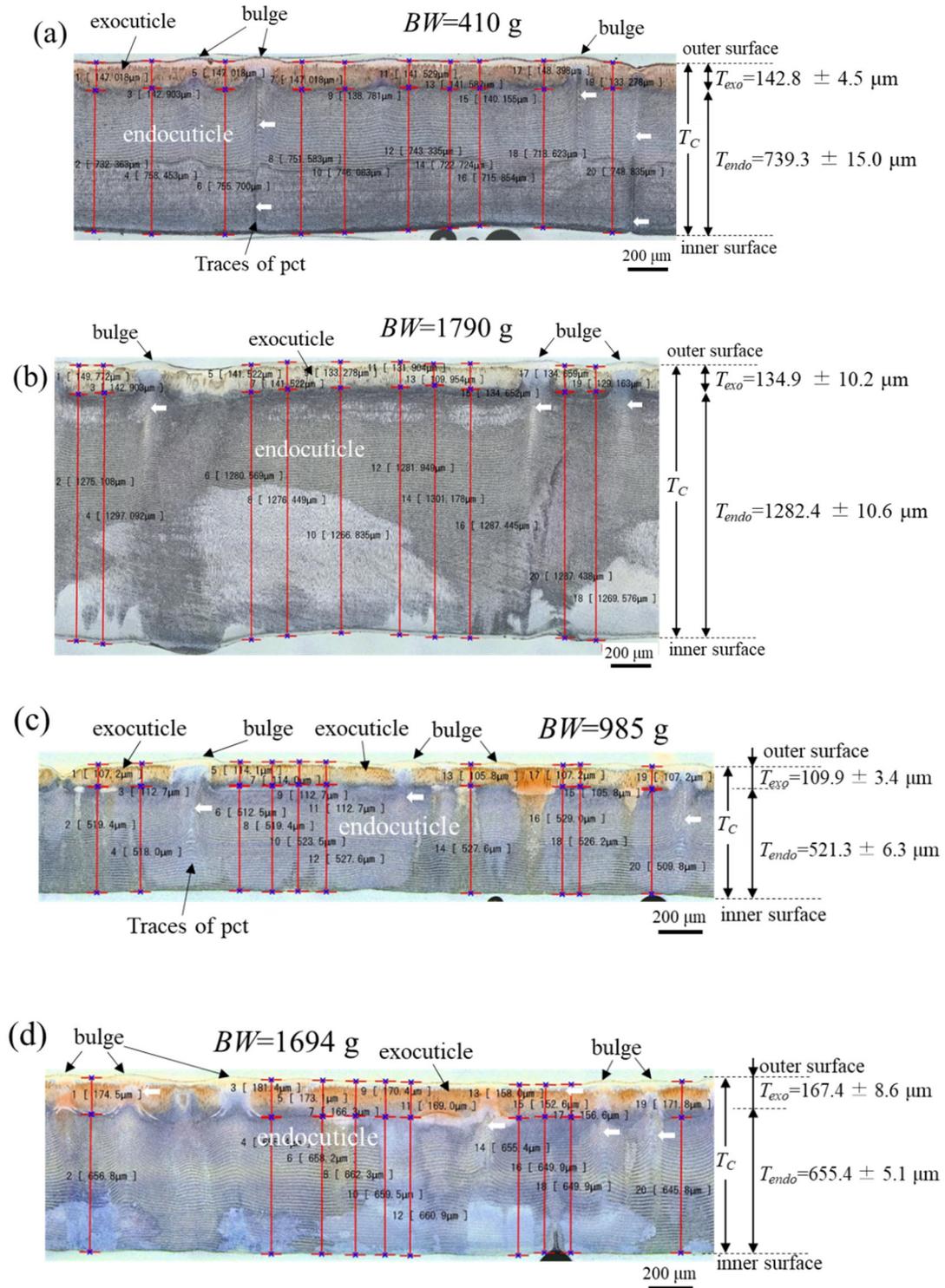


Figure S8. Optical micrographs of the polished surface in the x - y cross section of (a) 410 g and (b) 1790 g mud crabs, including measurement results of exocuticle thickness (T_{exo}) and endocuticle thickness (T_{endo}), and (c) 985 g and (d) 1694 g mud crabs with significantly thinner endocuticles. The T_C denotes the exoskeleton thickness ($= T_{exo} + T_{endo}$), and the white arrow denotes traces of pore canal tubes observed on the polished surface. The thickness of each layer was measured at 10 locations.

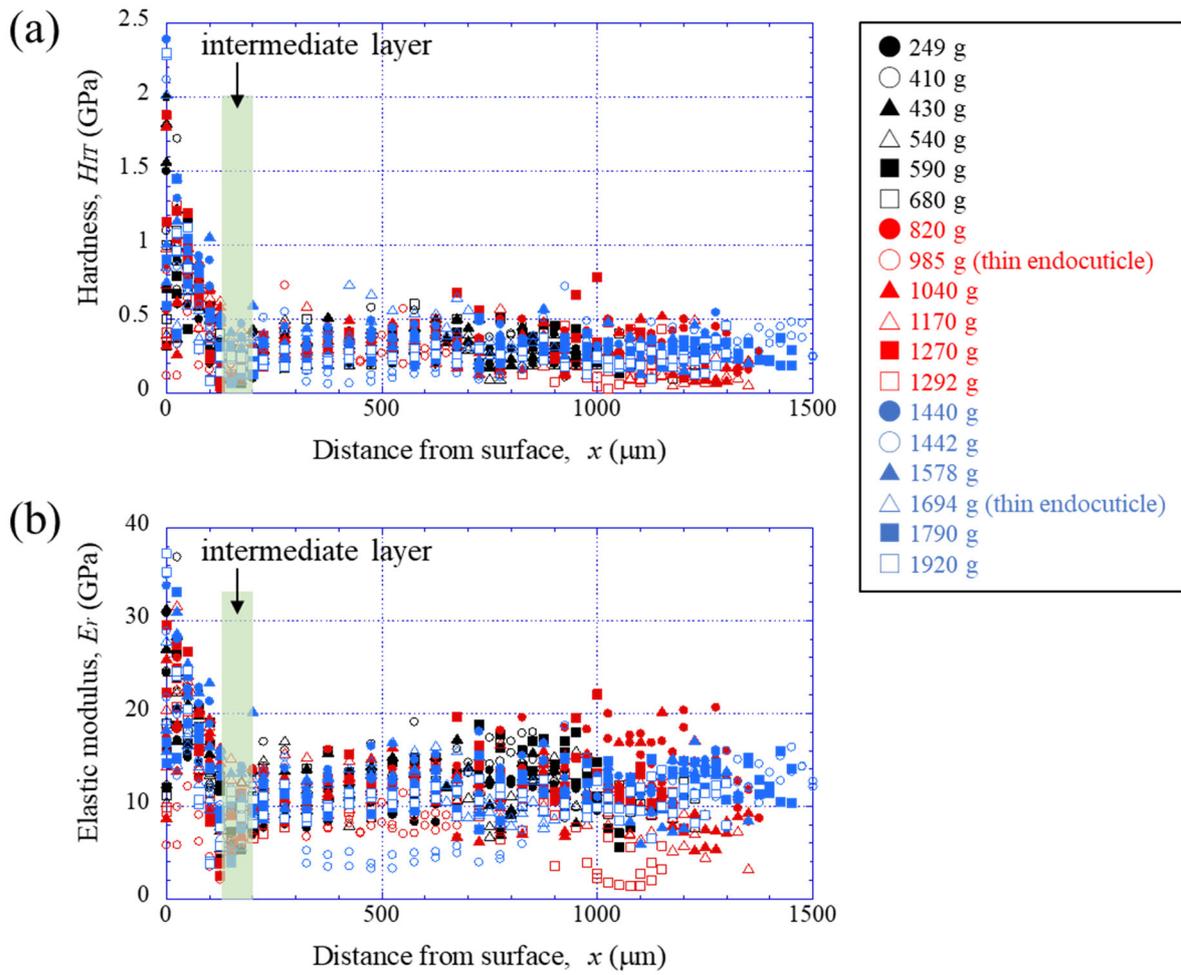


Figure S9. Distributions of (a) hardness (H_{IT}) and (b) elastic modulus (E_r) with distance from the outer surface, x , on two lines in a carapace cross section of all mud crabs weighing 249 g to 1700 g.

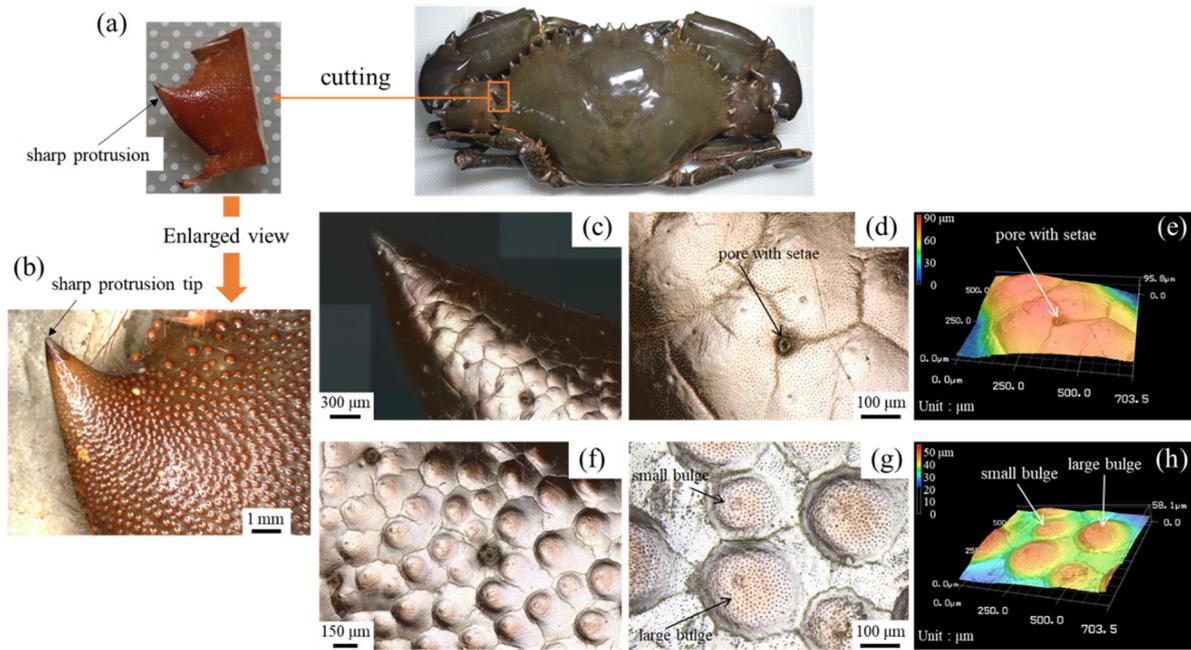


Figure S10. (a) The sharp protrusion on the carapace edge of a 680 g mud crab and (b) optical micrograph near the sharp protrusion tip. The surface morphology of the (c–e) tip and (f–h) base of the sharp protrusion: (c,f) merged laser scanning microscope images, (d,g) enlarged views, and (e,h) 3D color map.