

Supplementary data

Healing and regeneration in horseshoe crab, with possible application to trilobite injuries

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1. Supplementary figures

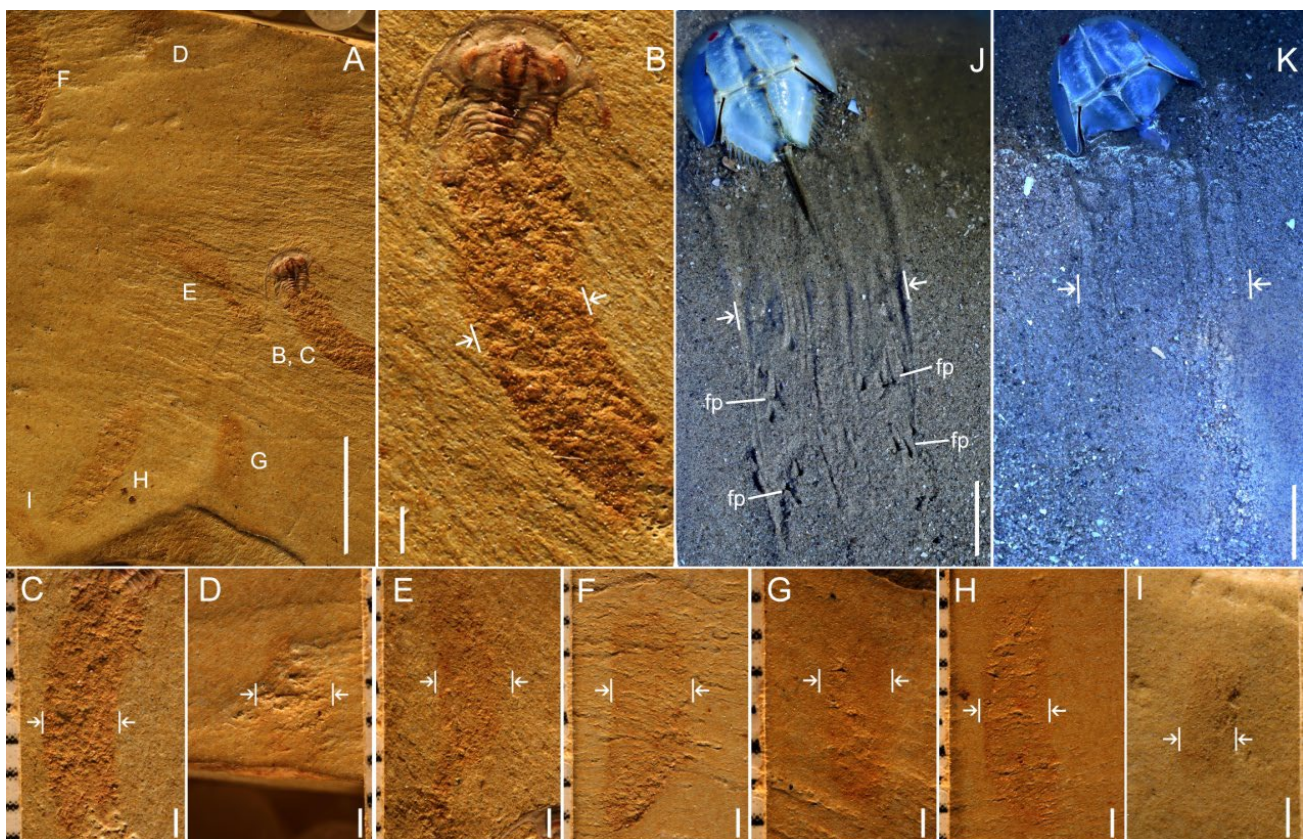


Figure S1. Comparison of crawling traces of a trilobite and a horseshoe crab. **(A–I)** An injured juvenile trilobite *E. intermedia* (SKLELI-EOM001) from the Cambrian Chengjiang Lagerstätte, magnified in **(B)** followed by one ploughing **(C)** and six surrounded **(D–I)** aequilate trails; **(J)** A crawling trail of an uninjured horseshoe crab (XMU499) with two rows of footprints, the trail has the same width with the maximal width of tergite; **(K)** A crawling trail of the same horseshoe crab losing the whole telson and half of the opisthosoma after an artificial injury, showing the absence of footprints. Abbreviation: fp, footprints. Scale bars represent: 5 mm **(A)**; 1 mm **(B–I)**; 5 cm **(J, K)**.



Figure S2. An artistic reconstruction of the imaginary Cambrian predator-prey ‘arms race’ scene *in situ* for the injured trilobites.

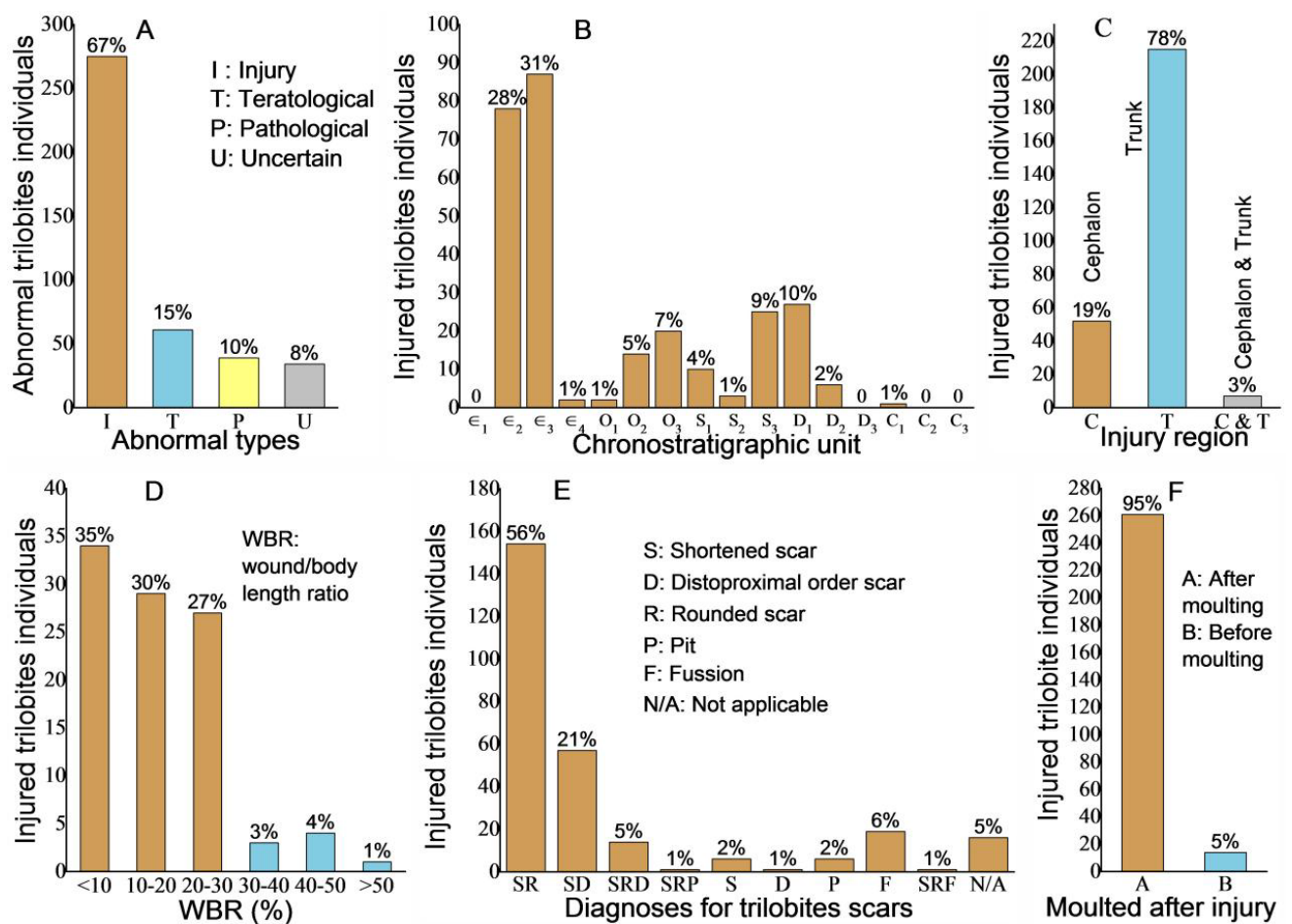


Figure S3. Statistics on 409 selected abnormal trilobite fossils ranging from Cambrian to Carboniferous. **(A)** Statistics of three abnormal types; **(B–F)** The numbers of injured trilobite individuals analysed according to chronostratigraphic unit **(B)**, injury region **(C)**, the wound/body length ratio **(D)**, injury scar types **(E)** and potential subsequent moulting taken place after the injury inferred according to the present integrative analyses **(F)**. See details for each fossil record listed in Table S3.

2. Supplementary tables

Table S1. Amputation experiments using late juveniles and first larvae of the horseshoe crab *T. tridentatus*.

Experimental batches	Injured regions	Duration	Morphological descriptions	Figures/Tables
I. Healing* (Experimental time: 2019/3/12–2019/11/08)	Cephalothorax & opisthosoma (WSR: 20.5%–64.0%)	Day 0	Seven juveniles (≥ 14 -instar, body width 9.5–19.5 cm) manually injured; one uninjured juvenile as control (XMU483, > 14-instar, width 20.4 cm)	Table S2
		Day 1	The wound surface becoming light blue in colour; white-floc (blood) observed in seawater. See the detailed supplementary observation on the haemostasis process in Batch III	Figure 1C (1 day)
		Day 5	White-floc (blood) disappeared	
		Day 10	The wound surface becoming light yellow in colour	
		Day 24	The first injured individual (XMU485, > 14-instar, width 19.0 cm) died	
		Day 27	The wound surface becoming golden beige	Figure 1C (27 day)
		Day 33	The second injured individual (XMU499, 14-instar, width 9.5 cm) died	
		Day 77	The wound surface becoming beige brown	Figure 1C (77 day)
		Day 90	The wound surface becoming chestnut brown	Figure 1C (90 day)
		Day 118	Tissues obtained from wound regions for μ -CT 3D analyses, and fully regenerated exoskeletons have been detected after 3D construction	Figure 1D–O
		Day 153	The third injured individual (XMU484, > 14-instar, width 19.5 cm) died	
		Day 170	The fourth injured individual (XMU487, > 14-instar, width 17.7 cm) died	
		Day 179	The fifth (XMU481, > 14-instar, width 18.5 cm) and six injured individuals (XMU489, > 14-instar, width 17.5 cm) died	
		Day 211	The seventh individual (XMU488, > 14-instar, width 18.2 cm) died, (all injured individuals died)	
		Day 241	The uninjured individual for control still alive. Moulting not observed in all individuals.	
II. Crawling traces (2019/3/12)	Opisthosoma (WSR: 62.2%)	0 hour	One 14-instar individual (XMU499, width 9.5 cm) was manually injured. The picture shows the crawling trace before injury.	Figure S1J
		0–2 hours	The whole body immediately twisted and then kept unmovable	
		2 hours	Crawled on the sandy bottom for five minutes; its crawling trace was recorded by photography	Figure S1K
		2–5 hours	Crawled into the sands, kept unmovable	
III & IV. Haemostatic & Moulting* (2021/04/27–2021/12/04)	Cephalothorax (WSR: 22.2%–30.0%)	0 hour	Four juveniles (≥ 14 -instar), manually injured. Body width, 10.2 cm, 13.4 cm, 13.7 cm, 13.1 cm; WSR: 22.2%, 30.0%, 26.4%, 29.1%)	Figure 1A
		0.5 hour	The wound bleeding and surrounded by white-floc	

Table S1. Cont.

Experimental batches	Injured regions	Duration	Morphological descriptions	Figures/Tables
III & IV. Haemostatic & Moulting* (2021/04/27–2021/12/04)	Cephalothorax (WSR: 22.2%–30.0%)	1 hour	The original connective tissue is extruded from the wound; a thin pale-yellow film over the connective tissue is forming; the white-floc disappeared	Figure 1B (1 hour)
		2 hours	The extruded connective tissue is retracting into the exoskeleton of the wound; the new pale-yellow film unchanged	Figure 1B (2 hours)
		4 hours	The extruded connective tissue is still retracting; the new pale-yellow film unchanged	Figure 1B (4 hours)
		8 hours	The wound is completely haemostatic and the wound region is fully covered by the pale yellow film; the extruded connective tissue is still retracting	Figure 1B (8 hours)
		16 hours	The extruded connective tissue is mostly retracted into the exoskeleton of the wound	Figure 1B (16 hours)
		24 hours	No apparent morphological changes observed	
		48 hours	No apparent morphological changes observed	
		Day 3–68	The wound is healing. The healing process is recorded in detail during Batch I	
		Day 69	One 14-instar individual (width 10.2 cm) moulting on the 68th day	Figure 1P
		Day 112	One individual (width 13.4 cm) died; moulting not observed throughout	
		Day 115	Another individual (width 13.7 cm) died; moulting not observed throughout	
		Day 178	The third individual (width 13.1 cm) died; moulting not observed throughout	
		Day 221	The 14-instar injured individual (width 10.2 cm) still alive	
V. Moulting (2021/06/24–2021/10/26)	Cephalothorax (WSR: 20.0%–30.0%)	0 day	440 first larvae (width 6.0–7.0 mm) selected: 400 larvae manually injured and 40 control individuals uninjured	Figure 1Q (left)
		Day 1–42	Checking for moulting everyday	
		Day 43	Newly moulted larvae (number of injured larvae vs. number of uninjured larvae in the control group): 1 vs. 0	Figure 1Q (right)
		Day 44	The whole moulted larva is selected for μ -CT 3D analyses; the wound is healed and fully regenerated	Figure 1R
			exoskeleton has been detected after 3D construction	
		Day 50	Newly moulted larvae: 3 vs. 1; all these four larva (injured) are selected for μ -CT 3D construction	
		Day 57	Newly moulted larvae: 1 vs. 3	
		Day 69	Newly moulted larvae: 19 vs. 4; one newly moulted larva (injured) is selected for μ -CT 3D construction	
		Day 92	Newly moulted larvae: 39 vs. 4	
		Day 124	Newly moulted larvae: 0 vs. 0	
		Day 124	All moulted larvae: 63 (15.8%) vs. 12 (30.0%); all larvae of the control group dead and the experiment ends	

Notes: the experimental batches I and II were conducted in Xiamen University; the Batches III–V were conducted in Guangxi Institute of Oceanology.

* The injury in the first batch is much heavier than that in third batch: the WSR value (the wound/sagittal length ratio) is 20.5%–64.0% and 22.2%–29.1%, respectively.

Table S2 Statistics for eight horseshoe crab juveniles during the healing experiment (Batch I).

Specimens	Body length (cm)*	Body width (cm)	Injury location	Wound length (cm)	Wound/Body length	Survival days
XMU481	20.0	18.5	Cephalothorax	4.1	20.5%	179
XMU487	18.0	17.7	Opisthosoma	4.2	23.3%	170
XMU489	17.9	17.5	Cephalothorax	5.4	30.2%	179
XMU488	18.1	18.2	Opisthosoma	6.3	34.8%	204
XMU484	20.0	19.5	Cephalothorax + opisthosoma	4.1+5.4	47.5%	153
XMU499	9.8	9.5	Opisthosoma	6.1	62.2%	30
XMU485	20.0	19.0	Cephalothorax + opisthosoma	5.3+7.5	64.0%	24
XMU483	20.5	20.4	Control individual	Not injured	Not injured	> 241

* The horseshoe crab telsons were not included in measurement.