

# Low-temperature tolerance of early juveniles of six terrestrial hermit crab species

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## Abstract

Because temperature strongly influences biological processes of ectotherms, it also plays a fundamental role in determining their geographical distribution. We evaluated the low-temperature tolerance of early juveniles of six terrestrial hermit crab species in the family Coenobitidae (genera *Birgus* and *Coenobita*), *B. latro*, *C. brevimanus*, *C. cavipes*, *C. purpureus*, *C. rugosus*, and *C. violascens* that occur in the northwestern Pacific region, Japan. A total of 30 laboratory-raised juveniles (about 1 mm in shield length) carrying gastropod shells were individually stocked in small plastic cups with sandy bottoms in temperature-controlled incubatory chambers at ~27°C. The temperature was reduced by 1°C every 48 h, and the juveniles were observed until all the crabs had died; the median lethal temperature (MLT) was estimated as the temperature at which 50% of the test juveniles had died. The MLT estimates varied significantly among the species, and the most northward distributed species, *C. purpureus*, had the lowest MLT values. The phylogeny, paleoceanography, paleogeography, and paleoclimatology suggest that cooler thermal regimes might have acted as an evolutionary force for the divergence of *C. purpureus* in the Pliocene. A negative correlation was found between the northern latitudinal limit of distribution and the MLT values, even after controlling for the phylogenetic relationships in the six

coenobitids. A temperature-dependent biogeography was thus recognized in terrestrial hermit crab species in the northwestern Pacific region, and global warming is expected to affect their geographical distributions.

**Keywords**

Biogeography; coconut crab; evolutionary force; land hermit crab; lethal temperature; thermal adaptation

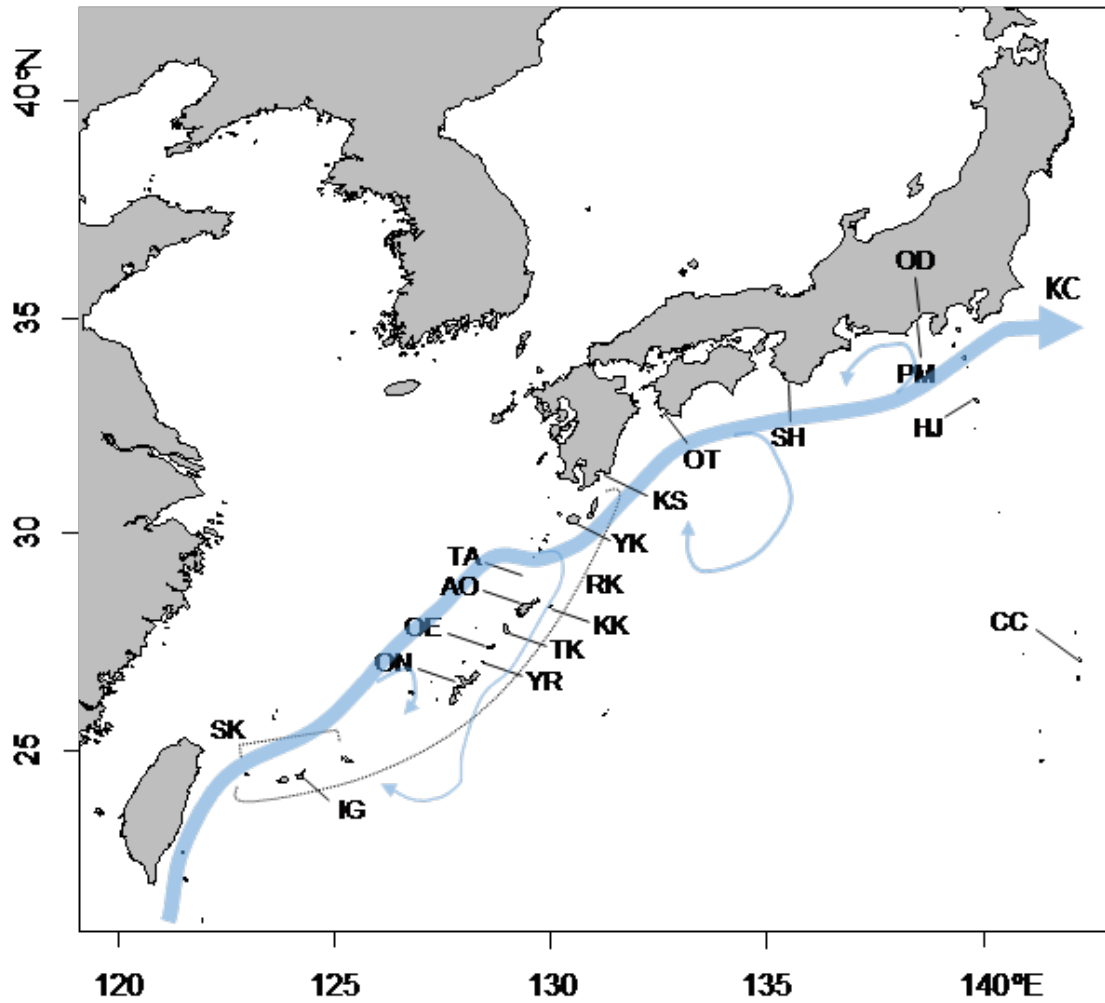
**Supplementary material**

**Table S1.**

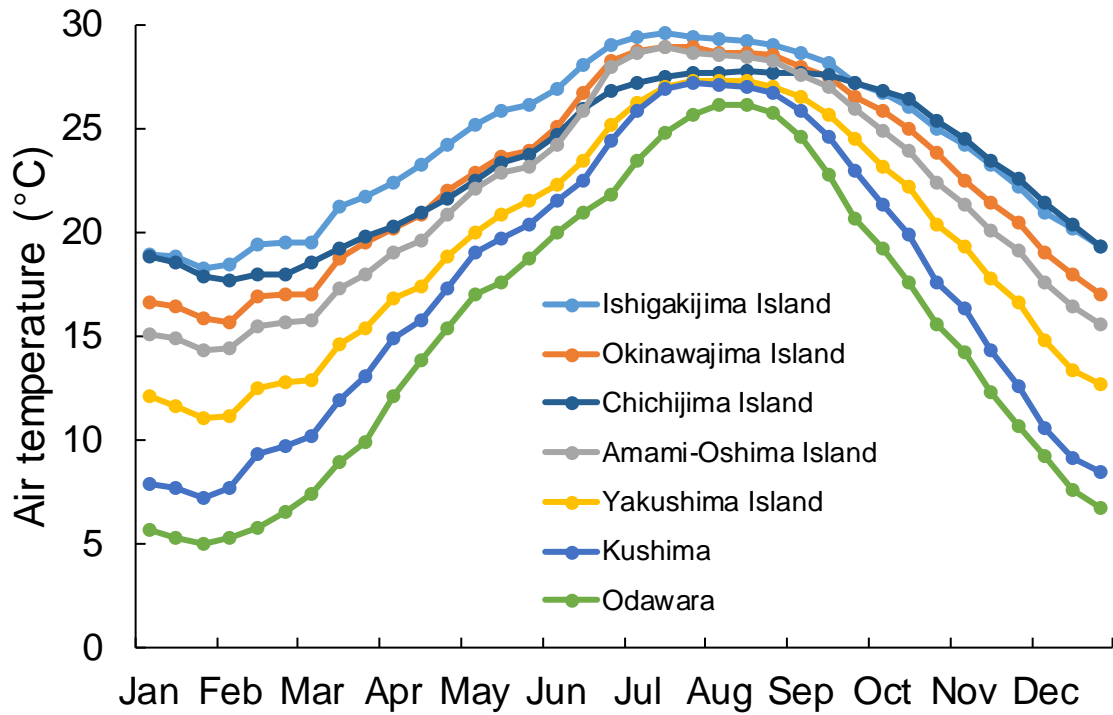
Median lethal temperature (MLT) estimates for evaluating the low-temperature tolerance of early juveniles of six terrestrial hermit crab species.

Species	Brood no.	Lot no.	Number of test juveniles	Number of moulted juveniles	MLT (°C)	
					Estimate	Standard error
<i>Birgus latro</i> (Linnaeus, 1767)	A	1	15	0	11.153	0.016
		2	15	0	11.607	0.025
	B	1	15	0	12.294	0.046
		2	15	0	12.313	0.023
<i>Coenobita brevipennis</i> Dana, 1852	A	1	10	0	12.809	0.295
		2	10	0	11.509	0.066
		3	10	0	12.398	0.192
	B	1	10	2	11.606	0.098
		2	10	2	12.600	0.119
		3	10	0	12.369	0.077
<i>Coenobita cavipes</i> Stimpson, 1858	A	1	15	1	9.544	0.207
		2	15	0	9.757	0.302
	B	1	15	1	9.652	0.083
		2	15	0	10.121	0.065
<i>Coenobita purpureus</i> Stimpson, 1858	A	1	10	2	9.179	0.146
		2	10	0	7.627	0.041
		3	10	1*	8.674	0.149
	B	1	15	2	5.533	0.015
		2	15	1	8.545	0.148
<i>Coenobita rugosus</i> Milne-Edwards, 1837	A	1	10	5*	10.259	0.092
		2	10	6	10.836	0.167
		3	10	1	10.131	0.107
	B	1	10	3	10.180	0.053
		2	10	3	9.897	0.229
		3	10	1	9.835	0.281
<i>Coenobita violascens</i> Heller, 1862	A	1	10	0	12.188	0.099
		2	10	0	12.777	0.054
		3	10	0	15.723	0.170
	B	1	15	2	12.420	0.171
		2	15	2	13.688	0.103

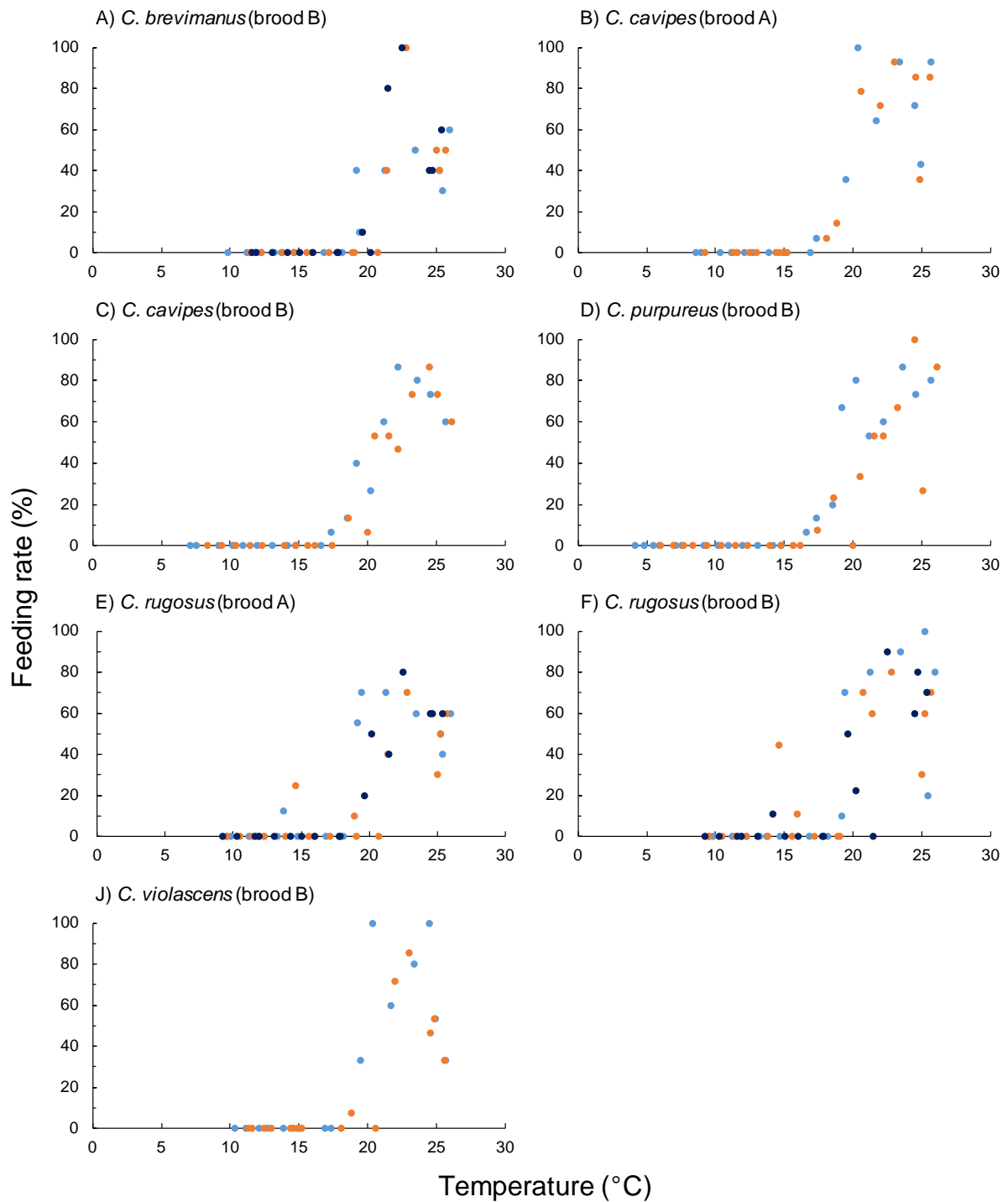
\* One individual died during moulting and was not included in the analysis.



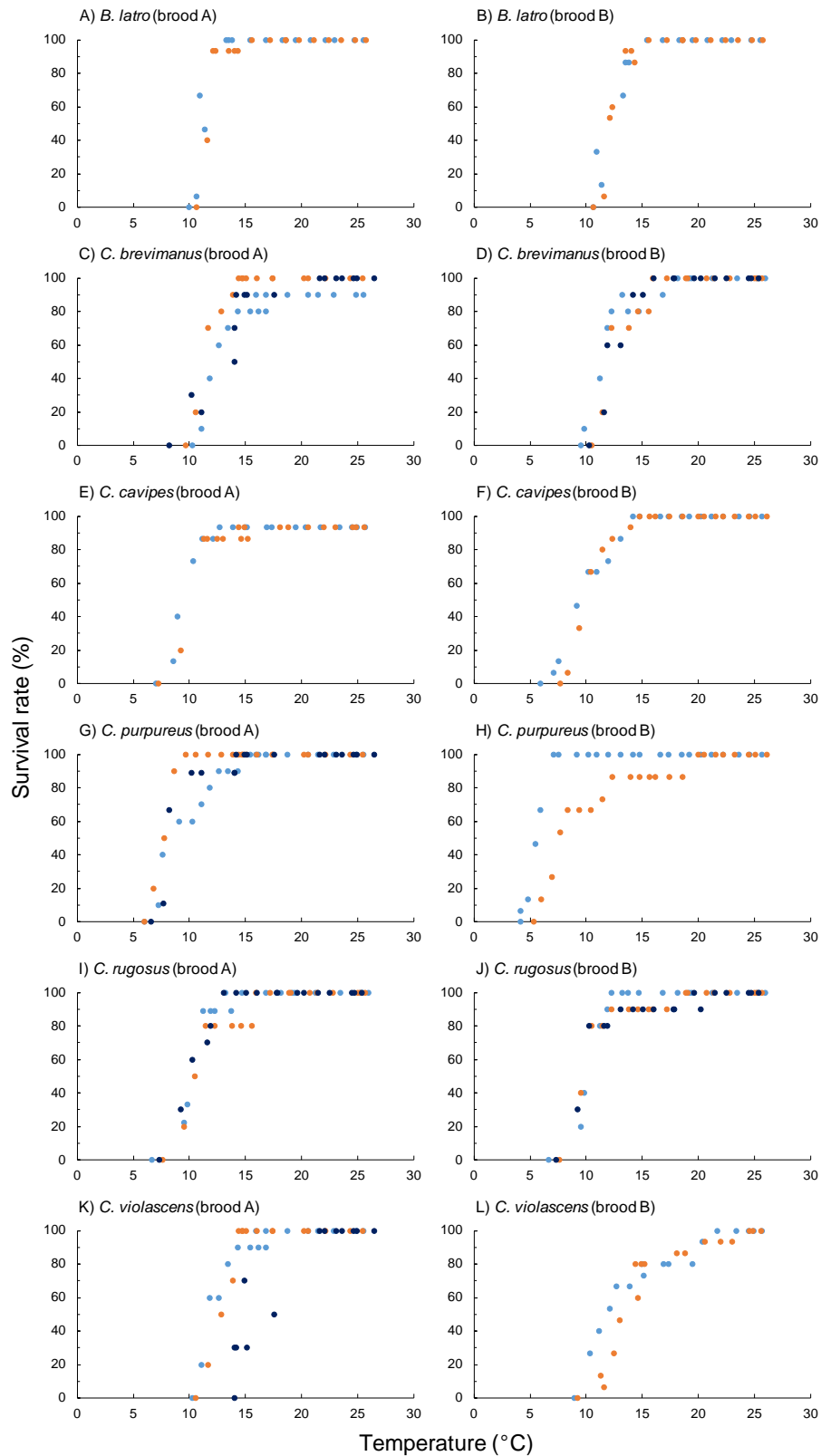
**Figure S1.** Map showing the Ryukyu Archipelago and the Pacific coast of Japan with information on the Kuroshio Current (KC) in the northwestern Pacific region. Blue lines show the KC and its countercurrents. Dotted lines indicate the Ryukyu Archipelago (RK) and Sakishima Islands (SK), respectively. Other locations referred to in the main document and the supplementary fig. S2 are as follows: IG, Ishigakijima Island; ON, Okinawajima Island; YR, Yoronjima Island; OE, Okinoerabujima Island; TK, Tokunoshima Island; AO, Amami-Oshima Island; KK, Kikaijima Island; TA, Takarajima Island; YK, Yakushima Island; CC, Chichijima Island; HJ, Hachijojima Island; KS, Kushima; OT, Otsuki; SH, Shirahama; PM, Point Manazuru; OD, Odawara.



**Figure S2.** Seasonal air temperature fluctuations at seven representative localities in Japan. Climate normals, which are three-decade (1981–2010) averages of monthly (10-day interval) temperatures, are shown for Ishigakijima Island (24°20.2' N, 124°09.8' E), Okinawajima Island (26°35.6' N, 127°57.9' E), Chichijima Island (27°05.5' N, 142°11.4' E), Amami-Oshima Island (28°22.7' N, 129°29.7' E), Yakushima Island (30°23.1' N, 130°39.5' E), Kushima (31°27.9' N, 131°13.2' E), and Odawara (35°16.6' N, 139°09.3' E). Temperatures were measured at the meteorological station in each locality. Data are derived from the Japan Meteorological Agency (<http://www.data.jma.go.jp/obd/stats/etrn/index.php>).



**Figure S3.** Changes in feeding rates in relation to temperature when the low-temperature tolerance limits were evaluated for early juveniles of six terrestrial hermit crab species, *Birgus latro*, *Coenobita brevipanus*, *C. cavipes*, *C. purpureus*, *C. rugosus*, and *C. violascens*. Experiments were conducted using two or three lots with 10 or 15 juveniles (a total of 30 animals) from two broods of each species in 2012–2014, but the feeding incidents were observed in the experiments conducted in 2013 and 2014. Feeding rate was calculated as (number of crabs fed)/(number of crabs survived)  $\times$  100. Different colours represent the different culture lots in each brood of the species.



**Figure S4.** Changes in survival rate in relation to temperature when the low-temperature tolerance limits were evaluated for early juveniles of six terrestrial hermit crab species, *Birgus latro*, *Coenobita brevimanus*, *C. cavipes*, *C. purpureus*, *C. rugosus*, and *C. violascens*. Experiments were conducted using two or three lots with 10 or 15 juveniles (a total of 30 animals) from two broods of each species. Different colours represent the different culture lots in each brood of the species.