



Larval development and emigration behaviour during sea-to-land transition of the land hermit crab *Coenobita brevimanus* Dana, 1852 (Crustacea: Decapoda: Anomura: Coenobitidae) under laboratory conditions

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(Received 11 May 2013; accepted 30 October 2013; first published online 27 February 2014)

To determine the early life history of the land hermit crab *Coenobita brevimanus* Dana, 1852, larvae were cultured individually in the laboratory. The zoeal and the megalopal stages are described and illustrated. The larvae developed through four planktonic zoeal stages to the megalopal stage. The major differences in the zoeal characters between *C. brevimanus* and other described *Coenobita* species were found in the armature of the pleomeres, whereas the character of pleomeres of *C. brevimanus* zoeae is the same as that of the coconut crab *Birgus latro*, a different genus in the same family. Morphological similarity was also found in segmentations of antennules and antennae in megalopae between *C. brevimanus* and the coconut crab. Megalopae of *C. brevimanus* were cultured in containers holding seawater and a hard substrate. These crabs migrate from the sea to land after developing a habit of acquiring gastropod shells.

Keywords: land hermit crab; zoea; megalopa; morphology; behaviour

Introduction

The land hermit crabs, genus *Coenobita* Latreille, 1829 and the coconut crab *Birgus latro* (Linnaeus, 1767) belong to the family Coenobitidae; they mainly occur in subtropical and tropical coastal regions (Hartnoll 1988; Drew et al. 2010). *Coenobita* species and the coconut crab have been exploited as an ornamental animal (Pavia 2006) and for human consumption (Brown and Fielder 1991), respectively.

Although adult coenobitid crabs are fully terrestrial, their eggs hatch into the sea and their larvae develop through several planktonic zoeal stages to the megalopal stage. Larval development has been described and illustrated from laboratory-reared material for eight of the approximately 16 coenobitid species: *Coenobita cavipes* Stimpson, 1858 (Shokita and Yamashiro 1986; Nakasone 1988a), *Coenobita clypeatus* (Fabricius, 1787) (Provenzano 1962), *Coenobita compressus* H. Milne-Edwards, 1836 (Brodie and Harvey 2001), *Coenobita purpureus* Stimpson, 1858 (Nakasone 1988a), *Coenobita rugosus* H. Milne-Edwards, 1837 (Shokita and Yamashiro 1986; Nakasone 1988a), *Coenobita scaevola* (Forskål, 1775) (Al-Aidaros and Williamson 1989),

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Coenobita variabilis McCulloch, 1909 (Harvey 1992) and the coconut crab (Reese and Kinzie 1968). Further, three coenobitid species – *C. compressus*, *C. variabilis* and the coconut crab – have been cultured successfully through the megalopal stage to the first crab stage (Reese and Kinzie 1968; Reese 1968; Harvey 1992; Brodie 1999, 2002; Brodie and Harvey 2001; Hamasaki et al. 2011; Hamasaki, Yamashita et al. 2013). After settlement, the megalopae recognize and co-opt gastropod shells; then, they migrate onto land and metamorphose to the first crab stage.

Coenobita brevimanus Dana, 1852 is widely distributed in the Indo-West Pacific (Nakasone 1988b). However, little is known about its life history. Here, we successfully reared larvae of this species from hatching to juvenile crabs under laboratory conditions. We report the developmental durations and body sizes of larval and juvenile stages, with information on emigration behaviour such as acquiring shells, landing and burrowing during the sea-to-land transition. In addition, we describe and illustrate the zoeal and the megalopal stages of *C. brevimanus* from laboratory-reared material and compare them with those of other coenobitid species.

All coenobitid crabs in Japan are recognized collectively as a Natural Monument, to promote their conservation. This study was conducted under the permission of the Agency for Culture Affairs, Ministry of Education, Culture, Sports, Science and Technology of Japan.

Material and methods

Larval source

Ovigerous females of *C. brevimanus* were captured by hand during early July in 2008 and late June in 2010 on Ishigaki Island (24°27' N, 124°07' E), Okinawa Prefecture, Japan. They were transported to the laboratory at Tokyo University of Marine Science and Technology (TUMSAT), Tokyo, by air and maintained in tanks until hatching occurred at ~28°C in air at 85% relative humidity according to the method of Hamasaki (2011). Females hatched their eggs into the seawater in tanks at night and the newly hatched larvae were collected from the tank using a 1-l beaker in the early morning. After the larvae had hatched, all the female crabs were released back into the habitats from which they were captured.

Culture of zoeae

The larvae hatched from two females were cultured as described previously (Hamasaki, Sugimoto et al. 2013, Hamasaki, Yamashita et al. 2013). To obtain larvae used for morphological descriptions and body size measurements, 60 newly hatched larvae were housed individually in plastic beakers (200 ml seawater) in the early morning on 31 July 2008 (brood 1) or in the wells of 10 six-well cell culture plates (10 ml seawater in each well) in the early morning on 13 July 2010 (brood 2). Culture medium was artificial seawater (~28°C, ~34‰ salinity; SEALIFE, Marinetechn Ltd., Tokyo, Japan). *Artemia* sp. and the rotifer *Brachionus plicatilis* species-complex were fed to the larvae. The larvae of each zoeal and megalopal stage (five specimens from each brood) were fixed with 5% neutral formalin for 1 day and then preserved in 70% ethanol. In addition, to obtain megalopae for culturing to the

crab stage, larvae from brood 2 were reared in three 1-l beakers (30 individuals l^{-1}). Mean larval rearing temperature (\pm SD) was $28.0 \pm 0.1^{\circ}\text{C}$ and $27.9 \pm 0.4^{\circ}\text{C}$ for broods 1 and 2, respectively; these conditions were similar to the natural sea-surface temperatures in summer around Ishigaki Island.

Culture of megalopae and juvenile crabs

Individuals of *C. brevimanus* after metamorphosing to megalopae were cultured according to the method of Hamasaki et al. (2011) and Hamasaki, Yamashita et al. (2013). The animals were individually housed in transparent plastic containers (8 cm wide \times 20 cm long \times 6.5 cm high), equipped with an inclined simulated land surface (250 ml of coral sand; grain diameter ~ 0.5 mm) and seawater areas ($\sim 28^{\circ}\text{C}$, $\sim 34\text{‰}$ salinity), as illustrated by Hamasaki et al. (2011). The container was covered with a 0.9-mm mesh-size plankton net to prevent the animals escaping. The photoperiod (~ 13 h light : 11 h dark) and temperature ($28.3 \pm 0.7^{\circ}\text{C}$) in the culture room approximated the summer environment on Ishigaki Island. The relative humidity in the containers was $83.4 \pm 5.8\%$.

It is not known when megalopae migrate ashore. In the present study, two situations were considered: (1) megalopae might access land within 24 h after metamorphosing (group 1) or (2) they migrate ashore when they can walk steadily while wearing shells (group 2). In group 1, 15 naked megalopae on the day of moulting (0 days old) were transferred individually into the sea area in the culture containers using a large-mouthed pipette. Two gastropod shells [small (S) size, 4.0–4.1 mm in shell length (SL); large (L) size, 4.9–5.0 mm SL] were placed in the seawater and one shell [medium (M) size, 4.5–4.6 mm SL] was placed on land in each container. In group 2, 15 naked megalopae (0 days old) were placed individually in the wells of six-well cell culture plates at $\sim 28^{\circ}\text{C}$ and $\sim 34\text{‰}$ salinity. Each well contained 10 ml seawater with a sandy bottom (grain diameter ~ 0.5 mm) and three gastropod shells (S, M and L sizes). The megalopae were fed frozen larvae (first zoeae) of the coconut crab daily after two-thirds of the culture seawater in each well had been renewed. When the megalopa had walked steadily while wearing a shell for three successive days, it was carefully transported to the seawater in the culture container using small forceps. Two empty shells remaining in each culture well were moved to the test container for the designated place (S-size shell or L-size shell, seawater; M-size shell, land). The S- and L-size gastropod shells were *Littoraria undulata* (Gray, 1839) and the M-size shell was *Littorina brevicula* (Philippi, 1844).

Cultured animals were observed during the daytime once a day until the age of 60 days, for shell use (wearing or not), type of shell worn (S, M or L), location (seawater or land), burrowing in sand or not, feeding or not, survival and moulting. Five frozen larvae (first zoeae) of the coconut crab and a piece of freeze-dried feed (Hikari Bio-Pure FD Blood Worms, Kyorin Co. Ltd., Himeji, Japan) were given to the cultured individual animals each day as food in seawater and on a small plastic circular sheet (5 mm in diameter) on land, respectively. We considered that the cultured animal had fed when the number of given larvae was decreased and/or the freeze-dried feed was not found in the container before supplying the new foods. Uneaten foods were removed from the containers. Moulting can be determined by the presence of a cast exoskeleton (exuvia).

However, the exuviae were not found in many cases in the present study, probably because the animals ate them; this behaviour was recorded earlier for juveniles and adults of the coconut crab (Fletcher et al. 1990; Fujita and Ito 2008). Alternatively, we estimated the moulting events as follows. Megalopae did not feed for several days before moulting to the first crabs, as is generally known for decapod crustacean species (Anger 2001). Therefore, we considered that the cultured animals had moulted when they reinitiated feeding after a non-feeding period. Furthermore, the first crabs of *C. brevimanus* have stouter and longer antennules than the megalopae (personal observation).

Larval measurements and descriptions

Measurements were made for five larvae of each zoeal stage and megalopal stage from each brood with an ocular micrometer. Total length (TL) was measured from the tip of the rostrum to the midpoint of the telson, excluding the telson processes. Carapace length (CL) was measured from the tip of the rostrum to the posteromedial margin of the carapace. The CL was also measured for the first and second crabs that survived at the end of culture experiments excluding the damaged specimens (one individual of the first crab stage in group 1 and one individual of the second crab stage in group 2).

The descriptions below were based on six zoeae of each stage and six megalopae from the two different broods. Specimens were dissected in 70% ethylene glycol. Larval dissections, drawings and measurements were done under a Nikon stereomicroscope (MZ-800; Nikon Corp., Tokyo, Japan) and a Nikon compound microscope (OPTIPHOT-2), both equipped with a camera lucida. Setal armature is described from proximal to distal segments. Rarely observed variations of setal number are reported in parentheses. Samples of each larval stage were deposited at the Museum of Fisheries Science in TUMSAT under the registration number MTUF-Ar0006–Ar0010.

Results

Development to the megalopal stage

The larvae of *C. brevimanus* developed through four planktonic zoeal stages to the megalopal stage (Figure 1). A few zoeae died in the cultures of brood 1 (one second zoea and one third zoea) and brood 2 (one first zoea, two second zoeae and one fourth zoea). The larval developmental periods of brood 2 were significantly longer than those of brood 1 at all stages (Welch's *t*-test, $p < 0.0001$). The mean inter-moult periods were ~3 days (brood 1) and ~3–4 days (brood 2) at the first three zoeal stages, and the period increased slightly to 5 days (brood 1) and ~6 days (brood 2) at the fourth zoeal stage (Table 1). The mean total times required from hatching to reach the megalopal stage were ~14 days (brood 1) and ~16 days (brood 2). The zoeae grew from 2.72 mm (brood 1) and 2.90 mm (brood 2) TL at hatching to 4.60 mm (brood 1) and 4.43 mm (brood 2) TL at the fourth stage; the megalopae were slightly over 3 mm TL (brood 1, 3.28 mm; brood 2, 3.31 mm) (Table 2).

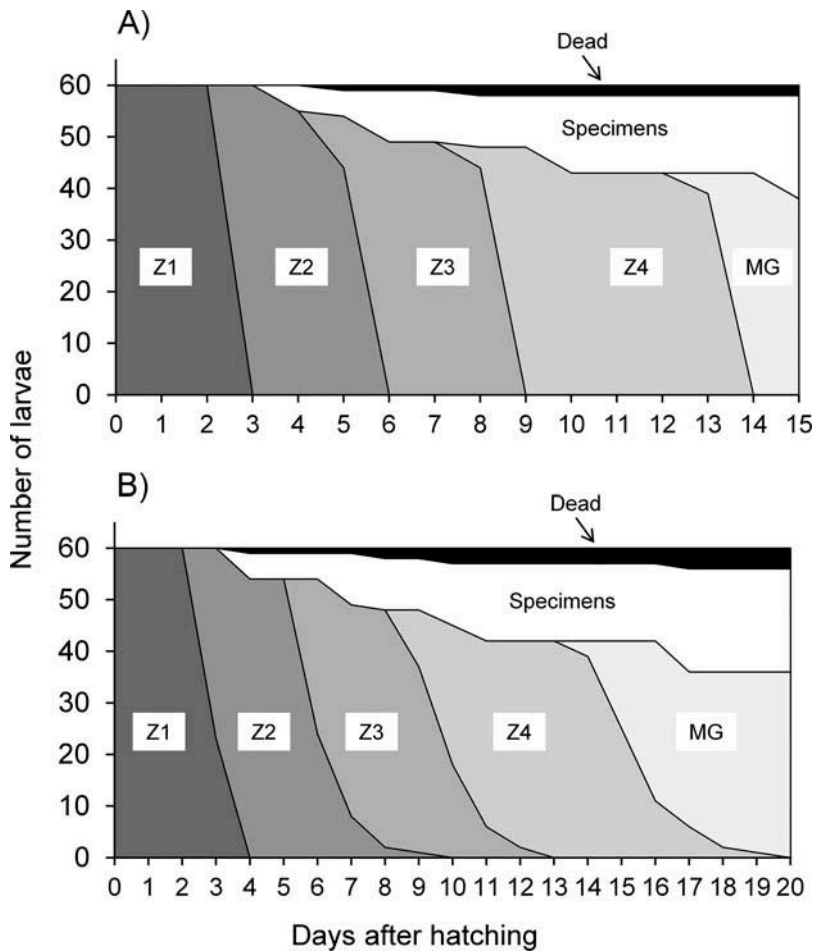


Figure 1. Numbers of larvae of *Coenobita brevimanus* reared individually in the laboratory. (A) Brood 1, hatched on 31 July 2008; (B) brood 2, hatched on 13 July 2010. Z1–Z4, first to fourth zoeae; MG, megalopa. Five specimens of each zoeal and megalopal stage were sampled from each brood.

Description of the larvae

First zoea

Carapace (Figure 2A, F). Rostrum tapered, long, extending beyond the tips of antennules, faintly carinate; posterolateral margins of carapace rounded; eyes sessile.

Antennule (Figure 3A). Uniramous, unsegmented, with three terminal aesthetascs, two to three terminal setae and a long subterminal seta.

Antenna (Figure 3B). Biramous; protopod with a serrated spine distally at the base of the exopod; endopod unsegmented, fused to protopod, with two long and one short

Table 1. Duration of larval and first crab stages of *Coenobita brevipimanus*.

Stage	Brood 1		Brood 2	
	n	Duration	n	Duration
		(days)		(days)
Z1	60	3.0 ± 0.0 (3)	59	3.4 ± 0.5 (3–5)
Z2	54	2.8 ± 0.4 (2–3)	52	3.2 ± 0.4 (3–5)
Z3	48	3.0 ± 0.0 (3)	47	3.7 ± 0.7 (3–5)
Z4	43	5.0 ± 0.0 (5)	41	5.6 ± 0.6 (5–7)
Z1–MG	43	13.9 ± 0.3 (12–14)	41	16.1 ± 1.3 (14–20)
MG			25	28.9 ± 4.4 (23–41)
C1			20	25.3 ± 5.9 (18–37)

Notes: Z1–Z4, first to fourth zoeae, respectively; MG, megalopa; C1, first crab. Duration; mean ± standard deviation (min–max).

Table 2. Body size measurements of larval and juvenile stages of *Coenobita brevipimanus*.

Stage	Brood 1			Brood 2		
	n	TL (mm)	CL (mm)	N	TL (mm)	CL (mm)
Z1	5	2.72 ± 0.02	1.17 ± 0.04	5	2.90 ± 0.08	1.24 ± 0.01
Z2	5	3.38 ± 0.13	1.49 ± 0.06	5	3.23 ± 0.03	1.40 ± 0.04
Z3	5	4.07 ± 0.09	1.77 ± 0.01	5	3.74 ± 0.24	1.66 ± 0.11
Z4	5	4.60 ± 0.11	2.04 ± 0.03	5	4.43 ± 0.11	2.00 ± 0.07
MG	5	3.28 ± 0.05	1.26 ± 0.03	5	3.31 ± 0.13	1.30 ± 0.05
C1				4		1.26 ± 0.07
C2				19		1.44 ± 0.05

Notes: Z1–Z4, first to fourth zoeae; MG, megalopa; C1, first crab stage; C2, second crab stage. TL, total length; CL, carapace length; mean ± standard deviation.

terminal setae; exopod (scaphocerite) with 10 setae on the mesial and distal margins, spine on the outer distal margin and fine setules on the inner margin.

Mandible (Figure 3C). Asymmetrical dentate; no palp bud.

Maxillule (Figure 3D). Coxal endite with 5–7 setae; basal endite with two strong denticulate spines and 2 setae; endopod three-segmented, distal segment with 2–3 terminal setae.

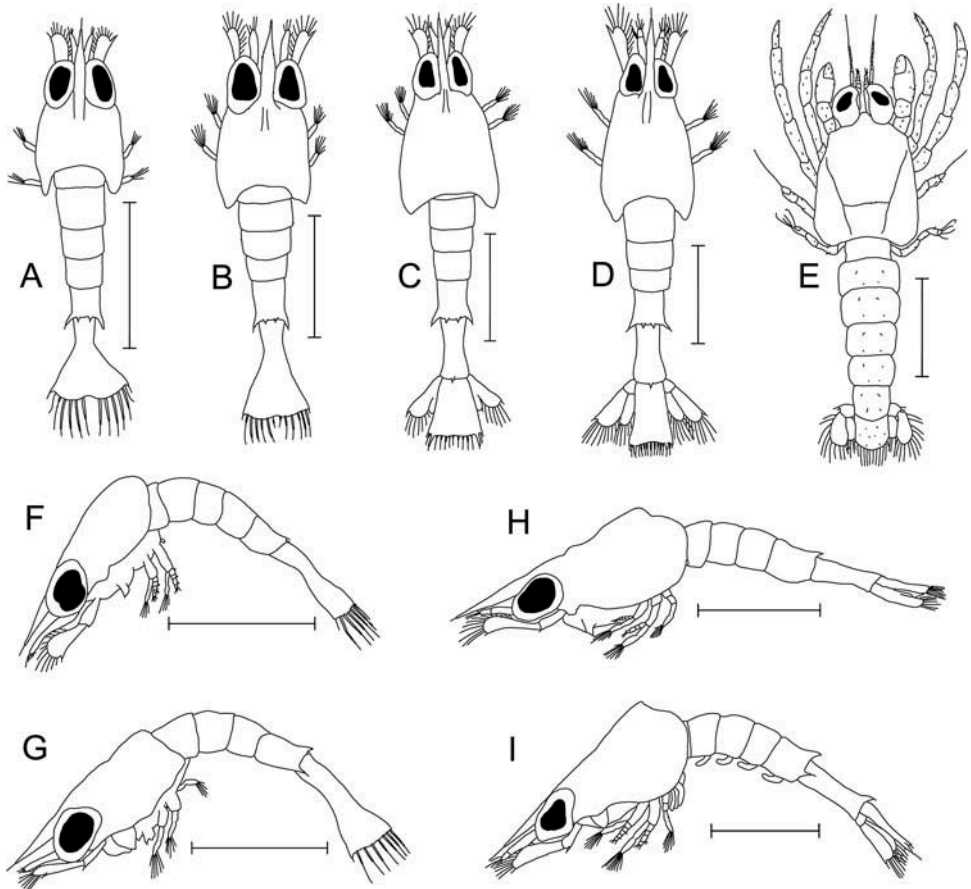


Figure 2. *Coenobita brevimanus*, whole animals. Dorsal view: (A) first zoea; (B) second zoea; (C) third zoea; (D) fourth zoea; (E) megalopa. Lateral view: (F) first zoea; (G) second zoea; (H) third zoea; (I) fourth zoea. Scale bar: 1.0 mm.

Maxilla (Figure 3E). Coxal endite bilobed, with 6–8 + 3–4 setae; basal endite bilobed, with 4–5 + 4 setae; endopod bilobed, with 2–3 + 3–4 setae and fine setules on outer margin; exopod (scaphognathite) margin with 4–5 plumose setae and fine setules on inner margin.

First maxilliped (Figure 3F). Coxa naked; basis with a hook-like process plus 2 fine setae at proximal end of the inner face and with 6–8 ventral setae arranged 2 + 2–3 + 2–3; endopod five-segmented, with 2–3, 2 (1), 1, 2, 4 (3) + I setae (roman number denoting dorsolateral seta), first three segments with some setules; exopod incompletely two-segmented, with 4 terminal natatory plumose setae.

Second maxilliped (Figure 3G). Coxa without seta; basis with 3 setae arranged 1 + 2; endopod four-segmented, with 2, 2, 2 (1), 4 + I setae, second and third segments with some setules; exopod as in the first maxilliped.

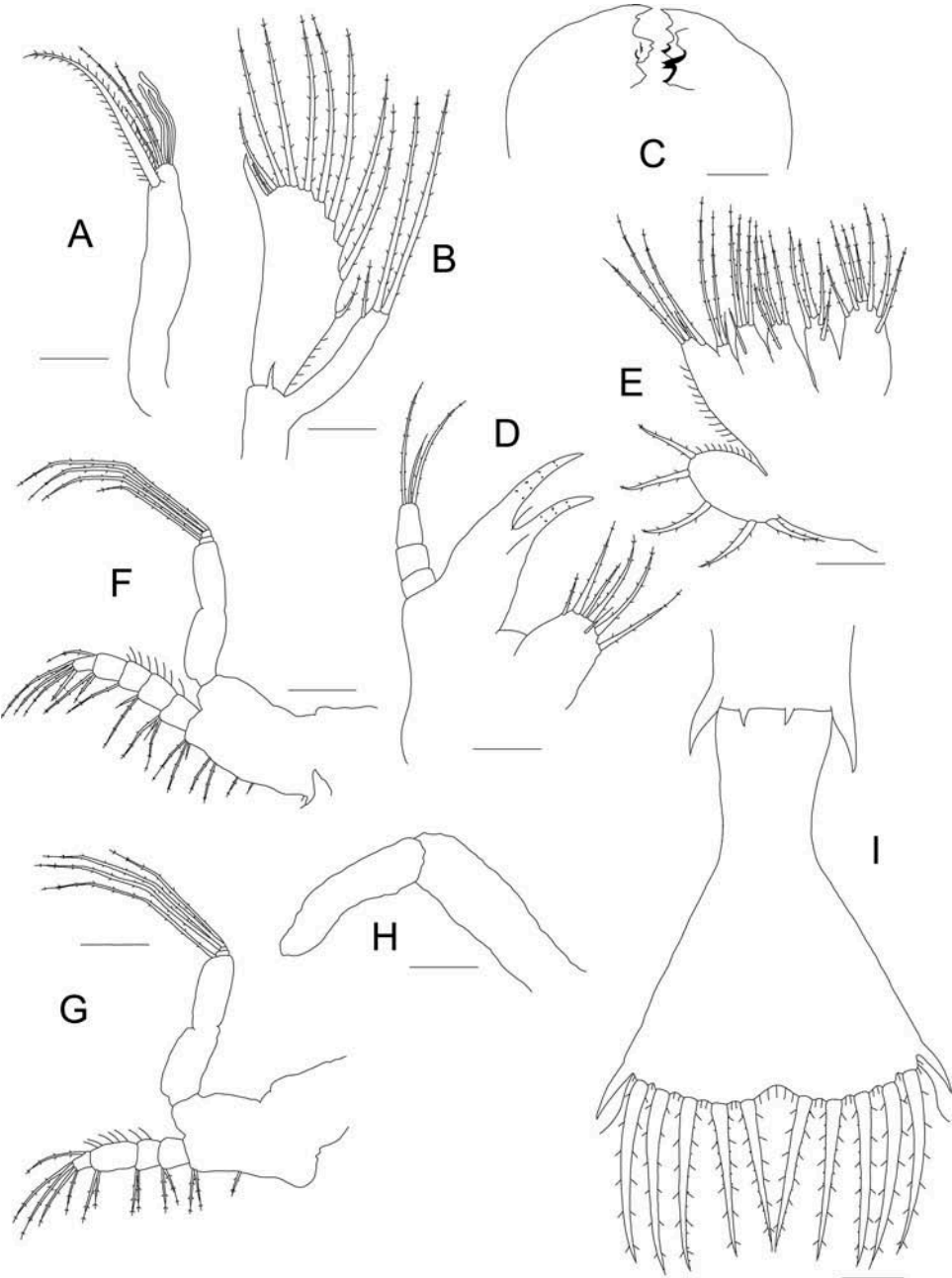


Figure 3. *Coenobita brevimanus*, first zoea: (A) antennule; (B) antenna; (C) mandibles; (D) maxillule; (E) maxilla; (F) first maxilliped; (G) second maxilliped; (H) third maxilliped; (I) telson. Scale bars: 100 µm (A–C, F, G, I), or 50 µm (D, E, H).

Third maxilliped (Figure 3H). Two-segmented uniramous bud.

Pleon (Figure 2A, F). With five pleomeres; pleomeres 1–4 naked; pleomere 5 with two mid-dorsal spines and a pair of large posterolateral spines.

Telson (Figure 3I). Posterior margin straight, median gap slightly notched; 7 + 7 (6 + 6) marginal processes, outermost (first) fixed spine, second fine hair, third through seventh (sixth) articulated setae; very short marginal setae between processes and on the median gap.

Second zoea

Carapace (Figure 2B, G). More noticeable rostral carina; eyes stalked; otherwise unchanged.

Antennule (Figure 4A). Uniramous, with 3 terminal aesthetascs, 4 terminal setae and 1 long and 3 short subterminal setae.

Antenna (Figure 4B). Protopod with a small spine near the junction of the scaphocerite; otherwise unchanged.

Mandible (Figure 4C). Incisor and molar processes with more teeth than in the previous stage.

Maxillule (Figure 4D). Coxal endite with 7 setae; basal endite with 4 strong denticulate spines and 2 setae; endopod three-segmented, second segment with 0–1 setae and distal segment with 3 (2) setae.

Maxilla (Figure 4E). Coxal endite with 7 (6) + 4 setae; basal endite with 5 (4) + 4 setae; endopod with 2–3 + 4 (3) setae; scaphognathite with 5–7 marginal plumose setae.

First maxilliped (Figure 4F). Basis with 7–8 setae arranged 2 + 3 + 2–3; endopod with 2–3 + I, 1–2 + I, 1 + I, 2, 4 + I setae; exopod with 6 terminal natatory plumose setae.

Second maxilliped (Figure 4G). Basis with 2–3 setae arranged 1 (0) + 2 (1); endopod with 2, 2 + I, 2 + I, 4 + I setae; exopod as in the first maxilliped.

Third maxilliped (Figure 4H). Endopod bud with a terminal seta; exopod two-segmented, with 5 terminal natatory plumose setae.

Pereiopods. Four pereiopod buds visible.

Pleon (Figure 2B, G). Unchanged.

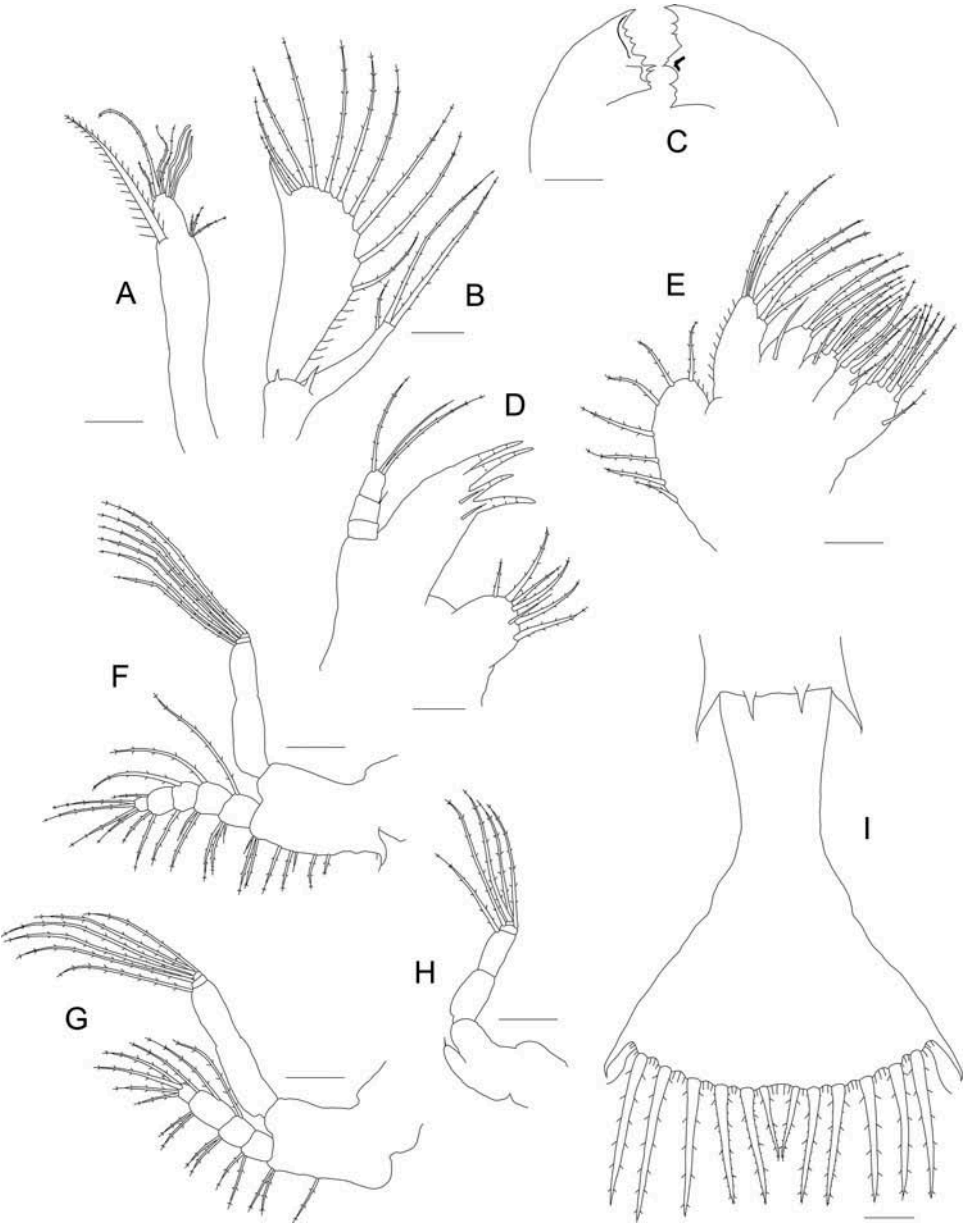


Figure 4. *Coenobita brevipennis*, second zoea: (A) antennule; (B) antenna; (C) mandibles; (D) maxillule; (E) maxilla; (F) first maxilliped; (G) second maxilliped; (H) third maxilliped; (I) telson. Scale bars: 100 μ m (A–C, F–I) or 50 μ m (D, E).

Telson (Figure 4I). Posterior margin slightly convex, with 8 + 8 processes with the addition of a short median pair of setae.

Third zoea

Carapace (Figure 2C, H). Essentially unchanged.

Antennule (Figure 5A). Protopod with 2 (3) long setae at the base of the endopod and 3–4 setae on the opposite side; endopod as a small bud with a long seta; exopod with 2–4 terminal aesthetascs and 3–4 terminal setae.

Antenna (Figure 5B). Endopod articulated with the protopod, bearing a terminal seta; scaphocerite with 11–13 setae; otherwise unchanged.

Mandible (Figure 5C). Essentially unchanged.

Maxillule (Figure 5D). Coxal endite with 7–8 setae; otherwise unchanged.

Maxilla (Figure 5E). Coxal endite with 8 (9) + 4 setae; basal endite with 5 (4) + 4 (3) setae; endopod with 3 (2) + 4 setae; scaphognathite with 8–9 marginal plumose setae on its distal lobe; proximal lobe distinct, elongate, often with 1–3 proximal setae.

First maxilliped (Figure 5F). Endopod with 3 + I, 2 + I, 1 + I, 2 (1), 4 + I setae; otherwise unchanged.

Second maxilliped (Figure 5G). Basis with 3 setae arranged 1 + 2; exopod with 7 (6) terminal natatory plumose setae; otherwise unchanged.

Third maxilliped (Figure 5H). Exopod with 6 terminal natatory plumose setae; otherwise unchanged.

Pereiopods. Four obvious buds.

Pleon (Figures 2C, H). With 6 pleomeres; armature of pleomeres 2–5 unchanged; pleomere 6 with a mediodorsal spine.

Uropods (Figure 5I). Exopod, endopod and protopod fused; endopods naked, much smaller than the exopod; exopod with 8 (9) setae along the posterior and inner margins and fine setules on the mesial margin.

Telson (Figure 5I). Posterior margin nearly straight, with 8 + 1 + 8 processes; a short median seta flanked by eight processes; outermost (first) fixed spine, second fine hair, third articulated seta, fourth larger fixed spine, fifth through eighth articulated setae.

Fourth zoea

Carapace (Figure 2D, I). Unchanged.

Antennule (Figure 6A). Protopod with sometimes 2 mesial setae, 4 long setae at the base of the endopod and 4 setae on the opposite side; endopod bud developed; exopod with 2–4 terminal and often 2 subterminal aesthetascs and 4 terminal setae.

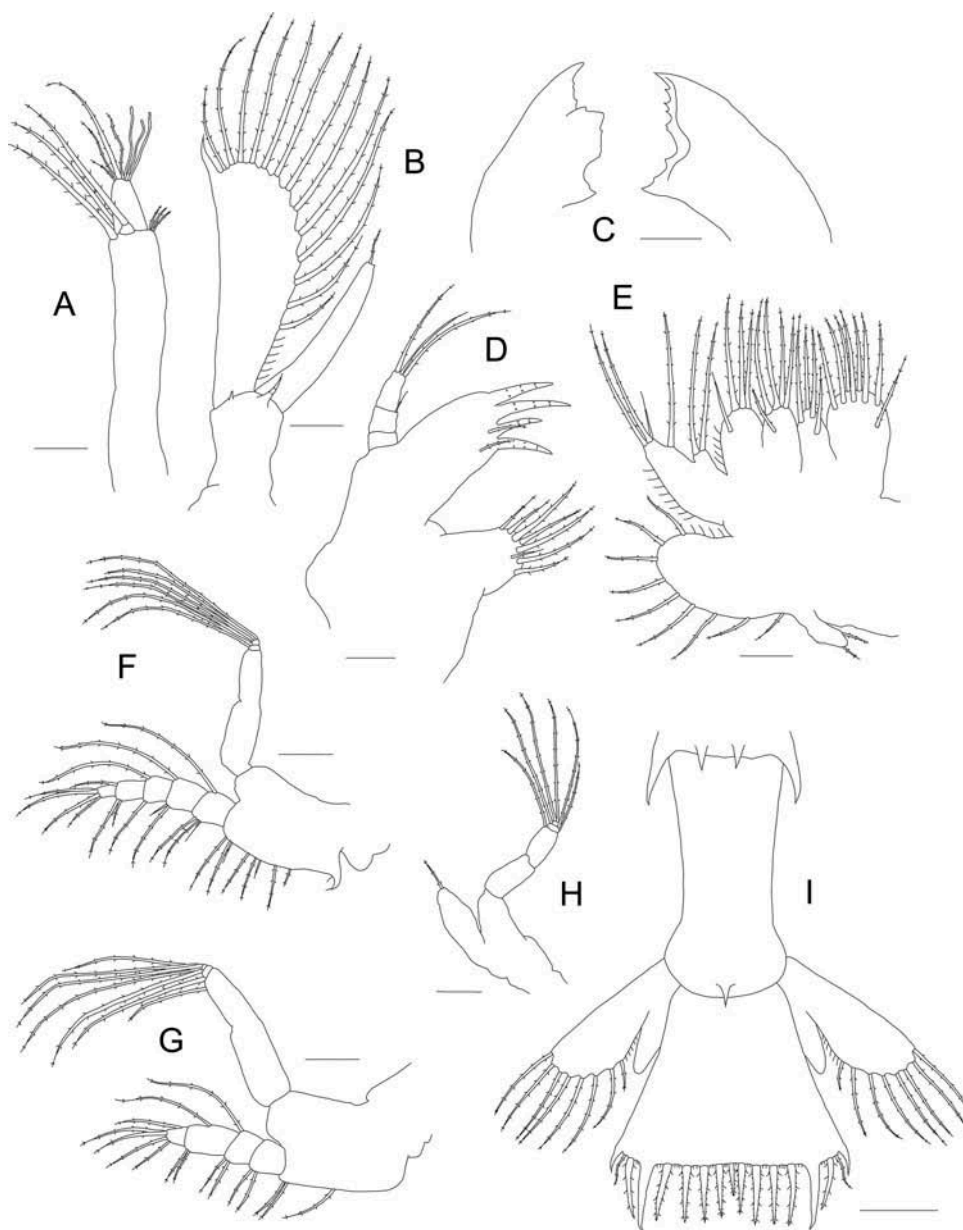


Figure 5. *Coenobita breviparus*, third zoea: (A) antennule; (B) antenna; (C) mandibles; (D) maxillule; (E) maxilla; (F) first maxilliped; (G) second maxilliped; (H) third maxilliped; (I) telson. Scale bars: 100 μ m (A–C, F–I) or 50 μ m (D, E).

Antenna (Figure 6B). Endopod two-segmented, almost as long as the exopod; scaphocerite with 14–16 setae; otherwise unchanged.

Mandible (Figure 6C). Palp bud present.

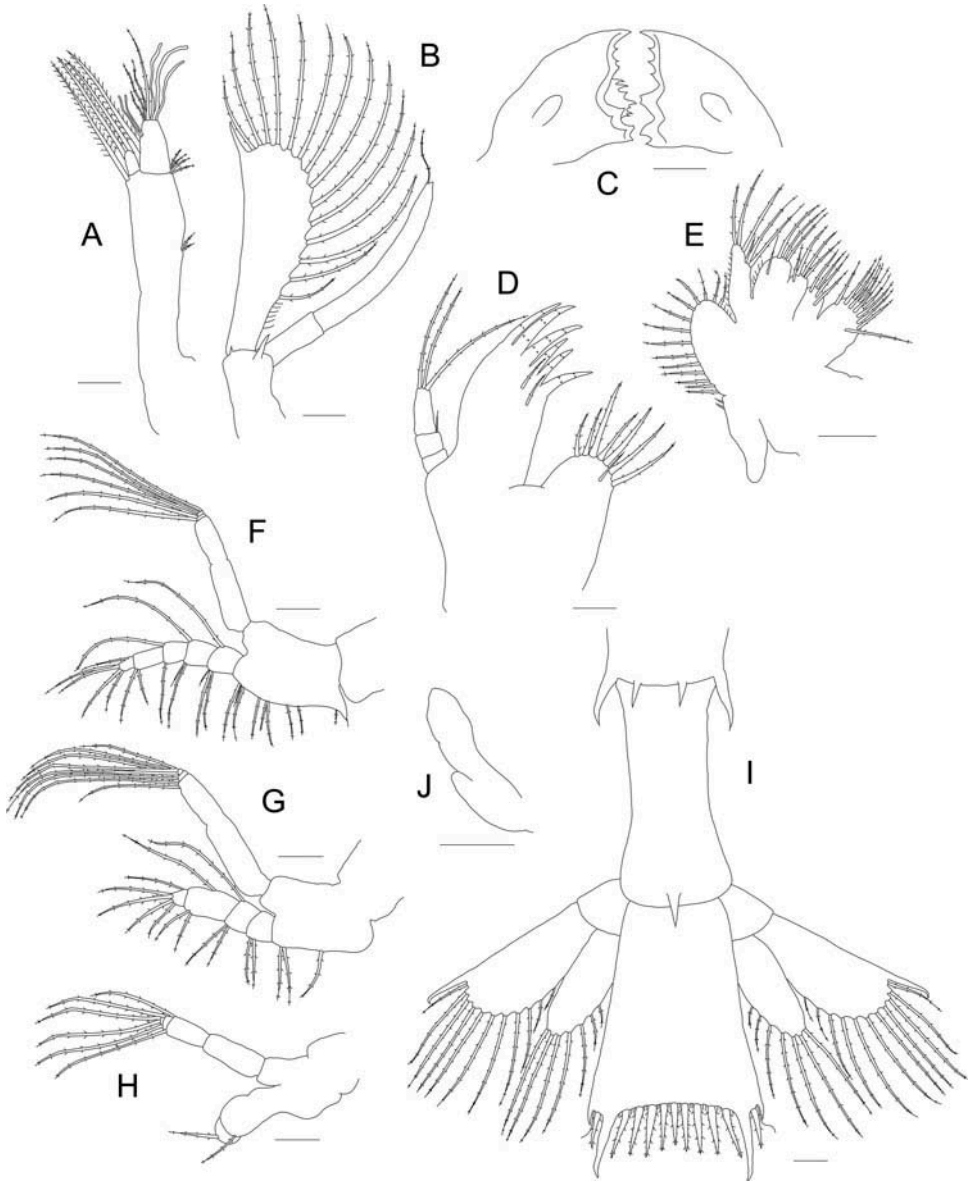


Figure 6. *Coenobita brevimanus*, fourth zoea: (A) antennule; (B) antenna; (C) mandibles; (D) maxillule; (E) maxilla; (F) first maxilliped; (G) second maxilliped; (H) third maxilliped; (I) telson; (J) pleopod. Scale bars: 100 μ m (A–C, E–J) or 50 μ m (D).

Maxillule (Figure 6D). Coxal endite with 6–8 setae; basal endite with 6 (5) strong denticulate spines and 2 setae; endopod three-segmented, second segment with 1 (0) seta and distal segment with 3 setae.

Maxilla (Figure 6E). Coxal endite with 10–12 + 4 setae; basal endite with 4–6 + 4 setae; endopod with 3 + 4 (3) setae; scaphognathite with 11–15 marginal plumose setae on the distal lobe; proximal lobe with often two distal setae on the outer margin.

First maxilliped (Figure 6F). Endopod with 3 + I, 2 + I, 1 + I, 2, 4 + I setae; otherwise unchanged.

Second maxilliped (Figure 6G). Basis with 3 (2) setae arranged 1 + 2 (1); exopod with 7–8 terminal natatory plumose setae; otherwise unchanged.

Third maxilliped (Figure 6H). Endopod two-segmented, each segment with a seta terminally; otherwise unchanged.

Pereiopods: Five buds present.

Pleon (Figure 2D, I). Pleomeres 2–5 with pleopod buds (Figure 6J); otherwise unchanged.

Uropods (Figure 6I). Exopod, endopod and protopod articulated; endopod with 6–7 setae and inner marginal row of fine setules; exopod with a sharp posterolateral marginal process, 11–12 setae along the posterior and inner margins and fine setules on inner margin.

Telson (Figure 6I). Unchanged.

Megalopa

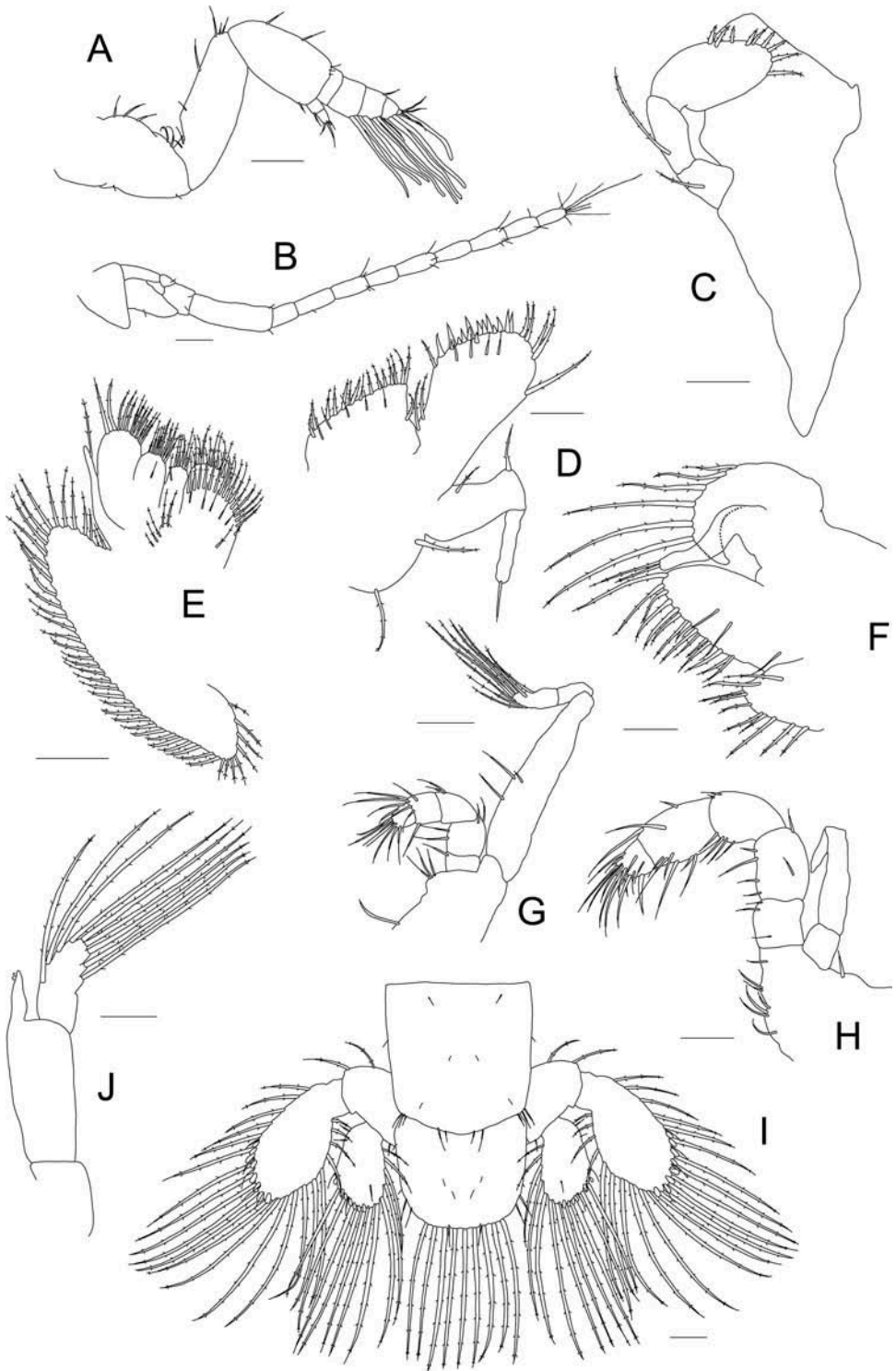
Carapace (Figure 2E). Shield more than half the total carapace length, slightly longer than broad; rostrum prominent, rounded; ocular peduncles reach to the base of the ultimate segment of the antennular peduncle.

Antennule (Figure 7A). Biramous; peduncle three-segmented, each segment with some setae; endopod two-segmented, proximal segment with 1 seta and distal segment with 4 setae; exopod four-segmented, second through ultimate segments with a total of 8 (9) aesthetascs, penultimate segment with 1–4 setae and ultimate segment with 3 setae.

Antenna (Figure 7B). Peduncle five-segmented, each segment with some setae; supernumerary segment present; acicle rounded knob with a dorsal seta; flagellum with 9 articles, setal formula 0, 1 (0), 3–5, 0, 3–6, 0 (1), 4–5, 2, 7–9.

Mandible (Figure 7C). Reduced and simplified; palp three-segmented, first segment with a seta, second segment with a seta and distal (third) segment with 8–10 setae.

Maxillule (Figure 7D). Coxal endite with 18–22 setae; basal endite with 11–12 setae and 14–15 spinules; endopod unsegmented, with well-developed, recurved external lobe sometimes bearing a terminal seta, internal lobe with 2 setae on the inner margin; 2 setae near the lower base of the endopod.



Maxilla (Figure 7E). Coxal endite with 26–34 + 8–11 setae; basal endite with 8–13 + 11–13 setae; endopod unsegmented, with a terminal seta; scaphognathite with 45–53 marginal plumose setae.

First maxilliped (Figure 7F). Coxal lobe with 5–11 setae; basal lobe with 18–19 setae; endopod unsegmented, often with 1–2 terminal setae; exopod with a strong lateral constriction, 6–10 marginal and terminal plumose setae.

Second maxilliped (Figure 7G). Coxa with a seta; basis with 3 setae; endopod five-segmented, basal segment with a ventral setae; second segment with 2 ventral setae and 2 (1) distal submarginal setae; third segment with 2 distal submarginal setae; penultimate segment with 6 marginal and submarginal setae; ultimate segment with 9 marginal and submarginal setae; exopod three-segmented, with 3 (2) setae along the inner margin of the basal segment, distal segment with 6 (8) terminal plumose setae.

Third maxilliped (Figure 7H). Coxa and basis with several setae, respectively; endopod five-segmented, ultimate and penultimate segments heavily setose, remaining segments with scattered setae; exopod three-segmented, sometimes with 1–2 terminal setae.

Pereiopods (Figure 8A–E). Chelipeds similar; dactyl subequal to the palm in length, each segment with scattered setae; ambulatory legs with dactyl terminating in a corneous claw surrounded by setae, each segment with scattered setae; fourth pereiopod with scattered short setae on the coxa, basis and ischium; merus and carpus with longer setae on dorsal margin; propodus with short and long setae plus marginal and submarginal corneous scales; dactyl terminating in corneous claw, with scattered short setae and single long seta; fifth pereiopod with scattered short setae on its proximal segment; long dorsal and ventral setae on the merus and carpus; propodus and dactyl with several long, curved setae and corneous scales plus scattered short setae.

Pleon (Figure 2E). Six unarmed somites with scattered, laterally paired dorsal setae; biramous pleopods on pleomeres 2 through 5 (Figure 7J); exopod well developed, with 9 plumose setae; endopod a simple lobe with 2 small coiled subterminal setae.

Tail fan (Figure 7I). Telson slightly wider than long, with scattered, laterally paired setae on the dorsal surface; posterior margin with 9 long plumose setae and 2 short setae at its posterolateral angle; uropods biramous, symmetrical; exopod with 20–22 long plumose setae and 7–9 corneous scales marginally, plus 4 (3) submarginal short setae; endopod margin with 11–12 long plumose setae, 3–7 short marginal setae and 5–9 corneous scales; protopod with 2 setae anterior to the exopod base and 1 short seta between endopod and telson.

Figure 7. *Coenobita brevimanus*, megalopa: (A) antennule; (B) antenna; (C) mandible; (D) maxillule; (E) maxilla; (F) first maxilliped; (G) second maxilliped; (H) third maxilliped; (I) telson; (J) pleopod. Scale bars: 100 μ m (A, B, E, G–J) or 50 μ m (C, D, F).

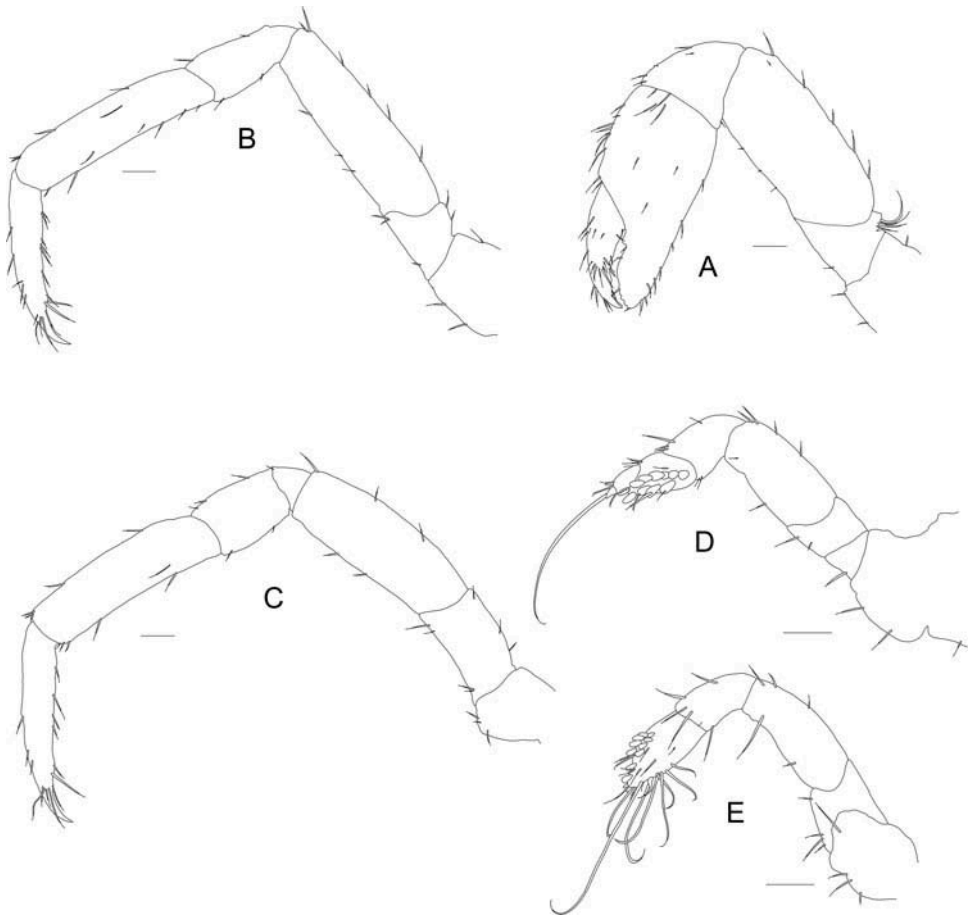


Figure 8. *Coenobita brevimanus*, megalopa: (A–E) first to fifth pereopods, respectively. Scale bar: 100 μm .

Emigration behaviour and development after the megalopal stage

In both culture groups, megalopae began to acquire shells at the ages of 3–4 days (Figure 9A, C). The proportions of animals wearing shells then increased linearly and almost all animals carried shells after ~10 days of age. In group 1, a small portion of megalopae (7–13%) migrated onto land without acquiring shells during days 2–7. The proportion of megalopae without shells on land decreased largely by day 11. The proportions of animals on land linearly increased during days 12–16 and then fluctuated around 80%. In group 2, megalopae carrying shells were transferred to the culture containers during days 10–20 (Figure 9D) and they migrated onto land. The proportion of animals on land increased linearly during days 11–18 and then fluctuated around 90%. In both groups, after migrating onto land, animals often moved between land and seawater.

Three and two animals died before moulting to the first crab stage in groups 1 and 2, respectively (Figure 9B, D). Ten animals moulted to the second crab stage in

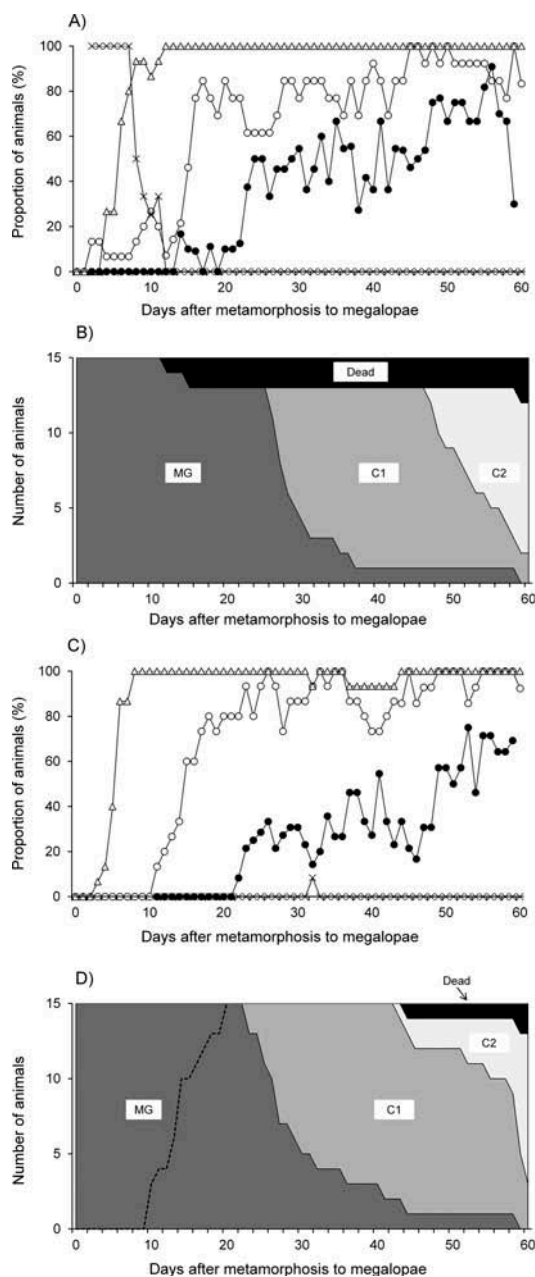


Figure 9. The proportion of all surviving megalopae and juveniles of *Coenobita brevimanus* that were on land (○), the proportion carrying shells (Δ), the proportion without shells on land (×) and the proportion of animals found on land that were buried in the sand (●) (A, C), and numbers of megalopae (MG) and juveniles (C1 and C2, first and second crab stages) (B, D) in the two culture groups. (A, B) Megalopae could access land within 24 h after metamorphosing (group 1). (C, D) Megalopae could access land when they walked steadily while wearing shells (group 2). (D) The cumulative number of megalopae that transferred to land (dashed line) is also included for culture group 2.

both groups. The proportion of animals that moulted to the first and second crab stages did not differ between groups 1 and 2 (Fisher's exact probability test, $p = 1$). Megalopae moulted to the first crabs on land during days 26–37 in group 1 and days 23–41 in group 2. Animals moulted to the second crabs during days 47–58 in group 1 and days 43–60 in group 2, respectively. No significant differences were found in the inter-moult periods of the animals between groups (Welch's t -test; megalopae, $p = 0.127$; first crabs, $p = 0.691$), and the mean inter-moult periods of the megalopae and the first crabs were 28.9 and 25.1 days, respectively (Table 1). The CL (1.26–1.30 mm) was similar between the megalopa and the first crab stage and grew to 1.44 mm at the second crab stage (Table 2). Statistical comparison was not conducted for the CL of the first crab stage because of a small sample size, but there was no significant difference in the body sizes of the second crab stage between groups (Welch's t -test, $p = 0.638$).

Before moulting, ~50% of megalopae and all the first crabs created a small cavity in the sand along or near the wall at a higher place in the container to hide themselves in for 1–5 days and the moulting occurred there. The proportions of animals burying themselves in the sand before moulting were not significantly different between groups 1 and 2 (Fisher's exact probability test, $p = 1$). They often used these burrows even after moulting. Hence, the proportions of animals burying themselves in the sand tended to increase to around 50% and 70% in group 1 and to around 30% and 60% in group 2 during the moulting periods to the first and second crabs stages, respectively (Figure 9A, C).

In both groups, animals appeared to prefer S- or L-size shells and their preferences changed with age (Figure 10). Animals sometimes changed their shells. Although some megalopae were interested in L-size shells (group 1) or M- and L-size shells (group 2) before landing, the proportions of animals wearing S-size shells increased from days 11–12 when the proportions of animals landing began to increase (Figure 9A, C). The shell preference changed after they moulted to the first crab stage so that the proportions of animals with L-size shells increased from the first to the second crab stages.

Discussion

The major differences in the zoeal characters between *C. brevimanus* (the present study) and other described *Coenobita* species (Provenzano 1962; Shokita and Yamashiro 1986; Nakasone 1988a; Al-Aidaros and Williamson 1989; Harvey 1992; Brodie and Harvey 2001) were found in the armature of the pleomeres. All zoeal stages of other *Coenobita* species possess a medial dorsal spine on the posterior margin of the second through fifth pleomeres and a prominent spine on each posterior lateral margin of the fifth pleomere, whereas all zoeal stages of *C. brevimanus* possess no spines on the second through fourth pleomeres but two mid-dorsal spines and a pair of large posterolateral spines on the fifth pleomere. Interestingly, the character of pleomeres of *C. brevimanus* zoeae is the same as that of the coconut crab, the genus *Birgus* (Reese and Kinzie 1968). This confirms the idea put forward by Reese and Kinzie (1968) that the coconut crab and *C. brevimanus* might share similar armature in zoeal pleomeres because ecologically and morphologically they appear to be the most closely related species.

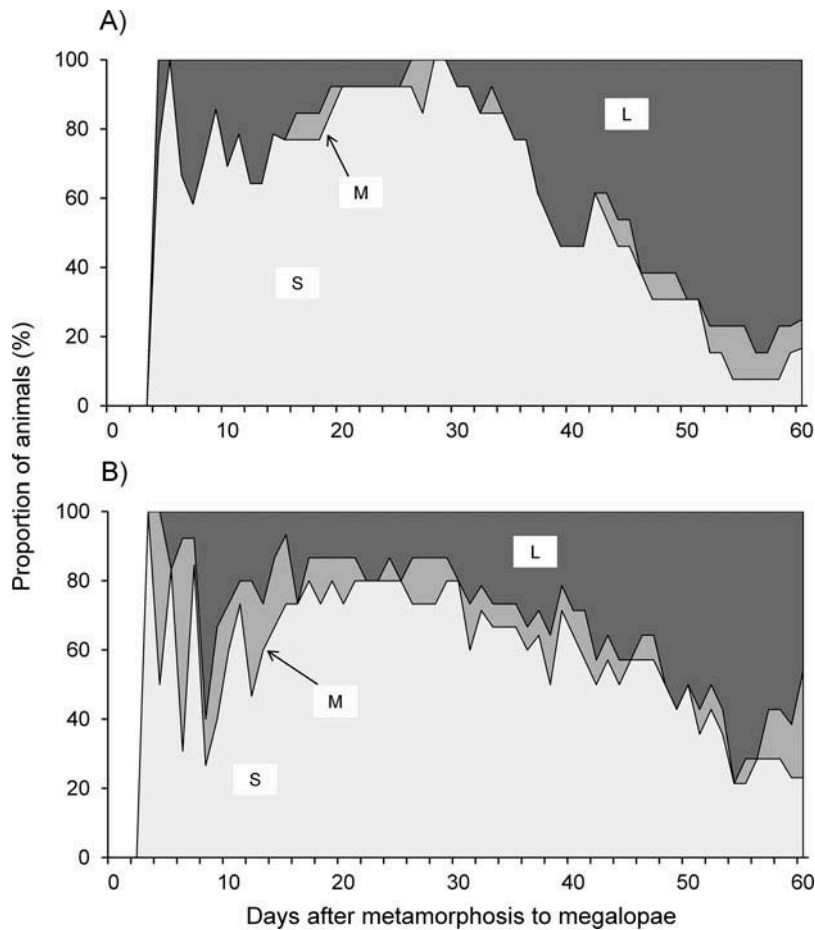


Figure 10. The proportions of *Coenobita brevimanus* carrying three different sizes of gastropod shells. The small (S) and large (L) gastropod shells were *Littoraria undulata* and the medium (M) size shell was *Littorina brevicula*.

The other zoeal characters are generally similar among the described coenobitid species although the number of zoeal stages differs among species and the minor differences are found in the setation of some appendages, as indicated by several authors (Shokita and Yamashiro 1986; Nakasone 1988a; Harvey 1992; Brodie and Harvey 2001). The general future development patterns of the megalopae are also similar among the coenobitid crabs. However, the most apparent differences in the megalopae have been found in segmentations of antennules and antennae. The exopod and the endopod of the antennule are unsegmented in previously described *Coenobita* species, but megalopae of *C. brevimanus* have antennules with four-segmented exopods and two-segmented endopods as does the coconut crab. Furthermore, the antennal flagellum of the coconut crab megalopae consists of 11 articles, whereas that of *Coenobita* species consists of five to seven articles. Megalopae of *C. brevimanus* showed the intermediate character in the number of articles of the antennal flagellum between the coconut crab and other *Coenobita* species: they have nine articles.

Our results provide the first evidence that *C. brevimanus* larvae are morphologically similar to the genus *Birgus* rather than the other *Coenobita* species in the Coenobitidae. Further descriptive studies of larval development and molecular phylogenetic studies are required to clarify the phylogenetic relationship among the coenobitid species.

Intraspecific variability in the number of zoeal stages is known in many decapod crustaceans (Knowlton 1974; Gore 1985; Anger 2001). The causes of the variability have been attributed to genetic and maternal factors and to environmental and nutritional conditions (Anger 2001). The numbers of zoeal stages of coenobitid species based on laboratory rearing are as follows: *C. cavipes* passes through five stages (Shokita and Yamashiro 1986; Nakasone 1988a), *C. clypeatus* through four to six (mainly five) (Provenzano 1962), *C. compressus* through four to five (mainly five) (Brodie and Harvey 2001), *C. purpureus* through five (Nakasone 1988a), *C. rugosus* through five (Shokita and Yamashiro 1986; Nakasone 1988a), *C. scaevola* through seven (Al-Aidaros and Williamson 1989), *C. variabilis* through two (Harvey 1992) and the coconut crab through three to five (mainly four) (Reese and Kinzie 1968; Wang et al. 2007; Hamasaki et al. 2009). Brodie and Harvey (2001) suspected that the reported lack of variability in the number of zoeal stages in four species – excluding *C. variabilis*, which exhibits abbreviated larval development – might be artefacts of low survivorship to the megalopal stage (*C. scaevola*) or of group rearing (*C. cavipes*, *C. purpureus* and *C. rugosus*). In the present study based on two culture experiments, larval mortality was low and larvae showed no variations in the number of zoeal stages (four stages) although the zoeal durations of *C. brevimanus* varied between the two broods. Larval culture studies under the various environmental and nutritional conditions are needed to evaluate the flexibility in the developmental pathway of *C. brevimanus* zoeae.

In this study, *C. brevimanus* migrated from the sea to the land at the megalopal stage after developing their habit of acquiring gastropod shells as previously reported for *C. variabilis* (Harvey 1992), *C. compressus* (Brodie 1999) and the coconut crab (Reese and Kinzie 1968; Reese 1968; Hamasaki et al. 2011). Hermit crabs select shells according to shell species, sizes (internal volume and weight) and/or shape (e.g. Osorno et al. 2005; Sallam et al. 2008; Caruso and Chemello 2009). Our results might suggest that *C. brevimanus* megalopae and juveniles avoided the medium size shells (*Littorina brevicula*) and that their shell utilization pattern changed ontogenetically from small to large shells (*Littoraria undulata*) after moving to land. However, our experimental design confounded three variables, i.e. shell size, shell species and shell-placement microhabitat (water and land), so it did not allow us to distinguish whether the crabs selected shells based on size, species or microhabitat.

For land hermit crabs dwelling in nature in a potentially desiccating medium, carrying shells must play an important role to reduce evaporative water loss from the body surface (Greenaway 2003). Land hermit crabs can carry water within their shells, and this is used as a reservoir to replace evaporative losses (De Wilde 1973; Greenaway 2003), but megalopal and adult land hermit crabs without shells desiccate rapidly (Herreid 1969; De Wilde 1973; Brodie 2005). Cultured megalopae and juveniles of *C. brevimanus* often returned to the sea area after moving to land, as shown by the proportion of land-dwelling animals that fluctuated around 80–90%. This behaviour is also known for *C. compressus* (Brodie 1999) and the coconut crab (Hamasaki et al. 2011; Hamasaki, Yamashita et al. 2013). Hamasaki, Yamashita

et al. (2013) suggested that this behaviour might be associated with acquiring seawater to avoid desiccation in air because the coconut crabs could not survive after moulting to the crab stage when the animals were unable to access seawater.

Megalopae of *C. compressus* (Brodie 1999; Brodie and Harvey 2001) and *C. variabilis* (Harvey 1992) remain underground for 1–6 days and 1–2 days, respectively, before re-emerging as juvenile crabs. This behaviour appears to protect the animals from desiccation and predation during moulting. The burrowing behaviour was also observed for ~50% of megalopae and all the first crab stages in *C. brevimanus* for 1–5 days before moulting. Furthermore, the shell-carrying megalopae and juveniles of the coconut crab are also known to exhibit burrowing behaviour, which is only weakly relevant to moulting (Hamasaki, Yamashita et al. 2013). Hence, the ecological significance of the burrowing behaviour might differ between coenobitid species. The microhabitat of megalopae and early juveniles in nature might reflect the differences in their behaviour patterns in the laboratory. Comparative studies under controlled conditions should be useful for understanding the early life ecology of the coenobitid crabs during the cryptic stage.

Acknowledgements

We thank Okinawa Prefectural Board of Education for permission to culture the land hermit crabs. We are grateful to the two anonymous reviewers for their constructive comments and suggestions, which have improved the manuscript. This study was supported in part by Grants-in-Aid for Scientific Research (B) (24310171) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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