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Water uptake in terrestrial hermit crabs: a morpho-functional analysis

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Abstract
Coenobita hermit crabs succeeded in their adaptation to land thanks to their shell and to the possibility of carrying water within it. Such a water reserve may play several roles: to compensate for dehydration, to keep the gills and abdomen surface sufficiently wet to allow gas exchange, to reduce temperature through evaporation and to facilitate osmoregulation. The mechanism through which water is absorbed and then imbibed or stored within the shell was studied using transparent shells and dyes. We were able to show that there are two pathways involving different organs. Drinking relies on setae on the 3rd maxillipeds while shell refilling involves the setae of both claws, as well as setae of the 3rd maxillipeds, and inverted scaphognatite beating activity. Shell refilling and drinking are alternated and both are interrupted by grooming activity. Thus, refilling the entire shell takes a variable period of time. The absorbing setae of both the maxilliped and the claw show a particular twisted shape, strongly resembling in both structure and density the absorbing setae at the base of the 3rd and 4th walking legs of the Ocypodidae.

Keywords: Coenobita, *land crabs, terrestrial adaptation, water absorption, hermit crab limbs*

Introduction

Terrestrial hermit crabs all belong to the family Coenobitidae, and number about 20 species that are widely distributed across all tropical shores where they typically inhabit sandy beaches and dunes (Hartnoll 1988). They spend most of the daytime when it is not raining under debris or in the sand, and emerge at night time (De Wilde 1973; Vannini 1975a, 1975b). Their nocturnal habits help them to reduce dehydration. Even so, water loss occurs and the crabs are therefore obliged to take up water with some regularity. Water is available from two different sources: directly from the sea or from the sand when it has been moistened by the sea and/or rain.

Water uptake not only has to counterbalance water loss due to metabolic processes similar to other terrestrial decapods (respiration, transpiration, excretion and faeces production), but also has to refurnish the water supply permanently kept within the shell, which is probably used to breathe, via abdominal vascularization and a thin abdominal cuticle (Farrelly & Greenaway 2001; Greenaway 2003; Innocenti et al. 2004), as well as for local ammonia excretion as deduced from pH variations in the shell water (C. Becchi, personal observation).

Among the coastal Indo-Pacific species, migration takes place during nocturnal low tide leading the crabs toward the sea. Some species stop at the sea's edge where they have access to free water (*Coenobita cavipes*, Vannini 1976a, 1976b) while others, avoiding the water's edge, stop among the stranded detritus on the upper part of the beach and extract water from the wet sand (*Coenobita rugosus*, Vannini 1976b). However, in both cases, water is acquired in a manner known to be typical for this genus. *Coenobita* claws are bordered by setal tufts, and they are thought to be able to absorb water just by touching the water surface or wet sand with their claws (De Wilde 1973; Hartnoll 1988).

Since this water uptake process has never been investigated in detail, we decided to conduct

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systematic observations on some Indo-Pacific species, which were kept alive at the University of Florence.

Materials and methods

Most of the animals were collected with the permission of the local authority in Kenya (*Coenobita rugosus*, *C. cavipes* and *C. brevimanus*, from Kanamai and Watamu) and in Zanzibar (*C. rugosus*, from Unguja). Additional specimens of a fourth species came from Malaysia (*C. violascens*, from Besar Island). Animals were brought to the University of Florence laboratory and reared there. They were kept in a terrarium (80 × 60 cm) with food (fruit and vegetables, dry biscuits, clams) and water available (both fresh and sea water). Testing was performed during 2003–2004.

Before testing, animals were extracted from their shells by breaking them. After that, a glass shell of adequate size was offered, which was usually worn within a few minutes. Crabs were then kept without food or water for 3–4 days and then tested. Testing consisted of offering fresh water in a glass Petri dish (20 × 40 cm), which allowed observation of the crabs from every side, including from beneath. In one case, the left branchiostegite was removed from a single *C. cavipes* to allow direct observation of the path followed by the water within the gill chamber. All the observations, carried out in a dark room illuminated by a red light on single specimens, were videotaped.

Observations were made to identify the way water was absorbed by the chelipeds and distributed to different parts of the body. The observation of water circulation was facilitated by adding E131, a common blue human food colouring, to the water.

Drawings were made using the *camera lucida* technique for dead specimens preserved in alcohol. The chelipeds and maxillipeds were prepared following the standard protocol for the scanning electron microscope (SEM; Felgenhauer 1987), and the structure and shape of their setae were observed using SEM (Philips XL 20).

A diagram of a *Coenobita* (Figure 1) shows the cheliped asymmetry, the two pairs of stout pereiopods (2nd and 3rd), the 4th pereiopod used to hold the shell from inside and the specialized 5th pereiopod used for grooming the gill chambers and abdomen, as well for removing faeces from the inside of the shell (C. Becchi, personal observations).

Non-terrestrial hermit crabs (i.e. without any specific structure for water absorption) such as *Clibanarius eurysternus* and *Dardanus guttatus* from Kenya, *Pagurus prideauxi* from Elba Island (Italy), and *Ocypode quadrata* from an unknown Western Atlantic locality, were also used for comparison. *O. quadrata* is a species that uses a tuft of setae present on the coxae of the 2nd and 3rd pairs of ambulatory legs for absorbing water from the substrate. When necessary, setae tufts were removed by excising them from the claw surface with a razor blade. All these specimens were taken from the crustacean collection of the Zoological Museum of the University of Florence.

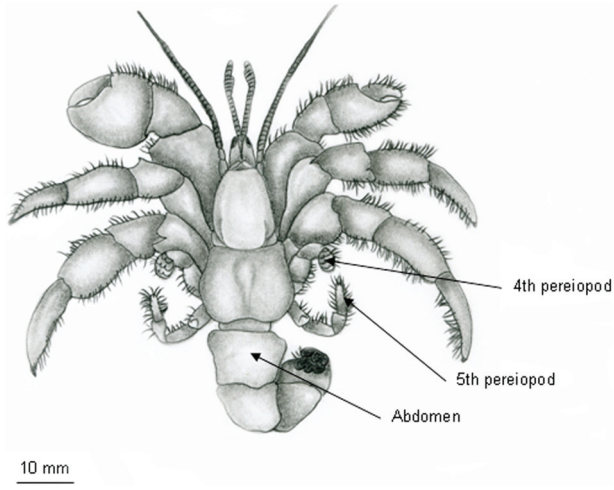

Figure 1. Diagram of a typical *Coenobita* sp. (redrawn from Hartnoll 1988).

Shell refilling time was measured on 22 animals belonging to three species (*C. rugosus*, *C. cavipes* and *C. violascens*). They were first kept with no access to water for 4 days (air relative humidity ranged between 55 and 70%; temperature ranged between 25 and 28°C). Since refilling activity is not continuous but consists of a series of bursts separated by grooming and immobility phases, refilling time was considered complete when the animals moved away from the water and started wandering and exploring the terrarium. This usually occurred within 1 h.

The volume and pH of the water within the shell were measured in 12 animals belonging to three species (*C. rugosus*, *C. cavipes* and *C. brevimanus*). The volume was measured by (1) weighing the crabs removed from their shell after 3 days of fresh water *ad libitum*, (2) weighing the crabs together with their new glass shell that was offered and immediately occupied and (3) weighing the crabs after they had spent 2.5 h with fresh water available. The water inside the shell thus corresponded to the water absorbed in the last 2.5 h before weighing, assuming that the hermit crab was already quenched.

Refilling activity usually took between 3 and 13 min, but the animals were left for 2.5 h in order to measure possible pH variation over a longer time span. A flexible pH paper strip was gently introduced between the shell edge and the carapace of the crab to determine pH.

Results

Absorbing structures – claws and maxillipeds

Water absorption from pools and wet sand seems to occur in a similar way. The East African *Coenobita* species studied showed the typical setal tuft bordering the medial upper edge of the propodus of both claws, with the exception of *C. brevimanus*, where tufts are present on the minor claw only (Figure 2). The hairy areas were studied by removing all setae from the internal claw surface. The bare surface showed a similar pattern in all species on both claws, except for the major claw of *C. brevimanus* which was devoid of setal tufts. Three different areas were identified: the first bordering the distal edge of the propodus, the second made by several separated spots on the proximal edge of the propodus, and the third bordering the distal margin of the carpus (Figure 3). Setal tufts are also present on the medial surface of the 3rd maxilliped on the exo- and endopodite (Figure 4).

A study of the setal distribution (Figure 5) showed a similar pattern in all three species. If separate setal zones were identifiable on the bare surfaces of both the chelipeds and the maxillipeds, they were visible on intact organs as a single hairy patch on the chelipeds and as a double row on the 3rd maxillipeds. Furthermore, on the 3rd maxilliped, the distinction between the exo- and endopodite was inconspicuous and all tufts appeared as a single hairy row.

The absorbing setae of the claws, observed at different magnifications (Figure 6a–c), appeared very thick, long and loosely spiralled. Their surface was smooth right up to the tip (Figure 6d) and their shape and diameter appeared quite different from the cuspidate setae, which were present on limb segments (Figure 6b). No difference was observed among species.

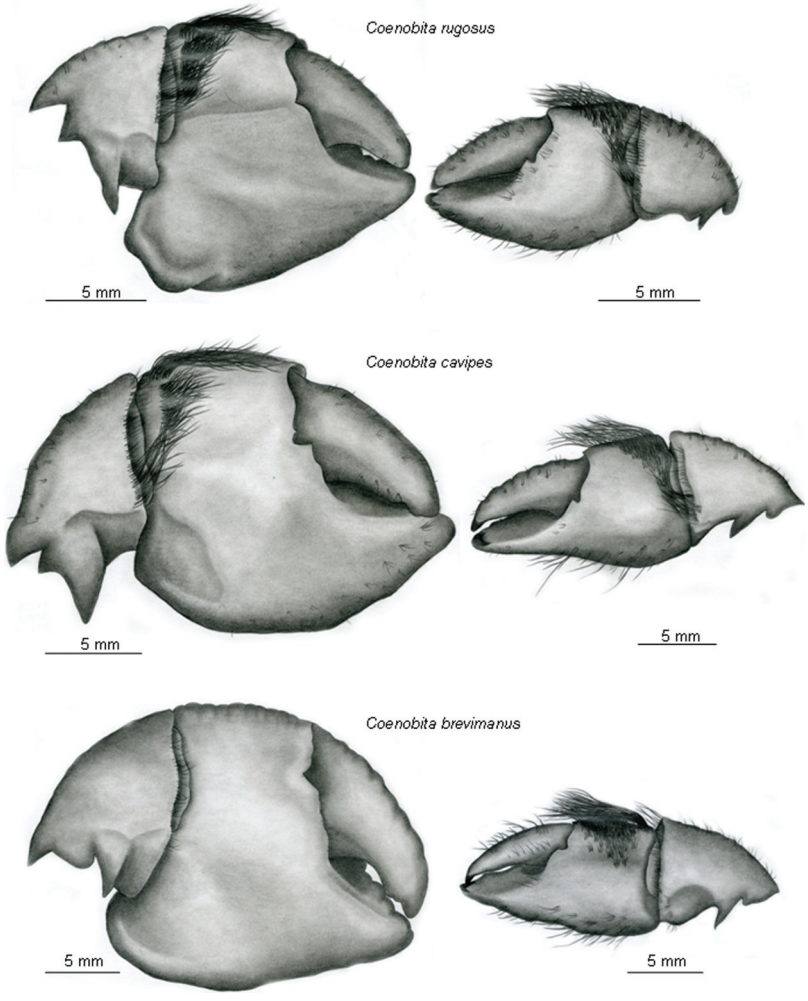

Figure 2. Internal face of the major claws (left) and minor claws (right) of three East African *Coenobita* spp.

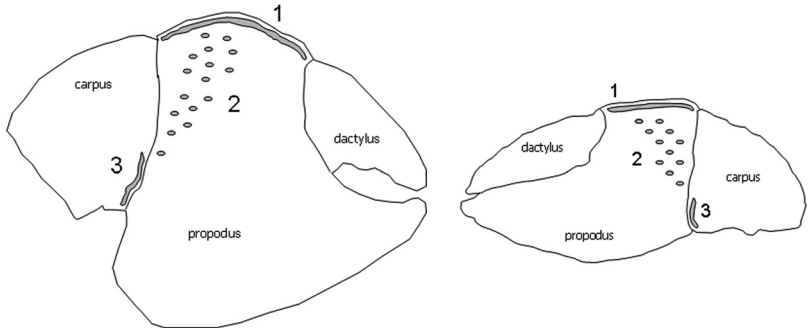

Figure 3. Internal surface of the major claw of *Coenobita rugosus* (setae removed). The grey areas indicate the three distinct hairy zones (1–3).

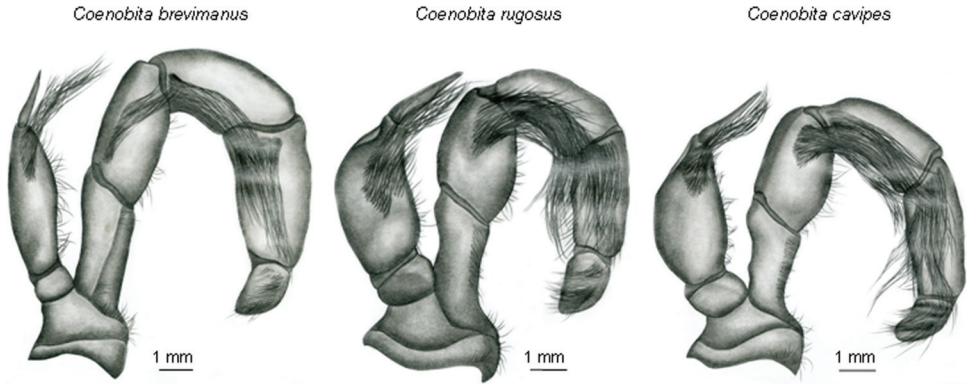

Figure 4. Internal face of the left 3rd maxilliped of three East African *Coenobita* spp.

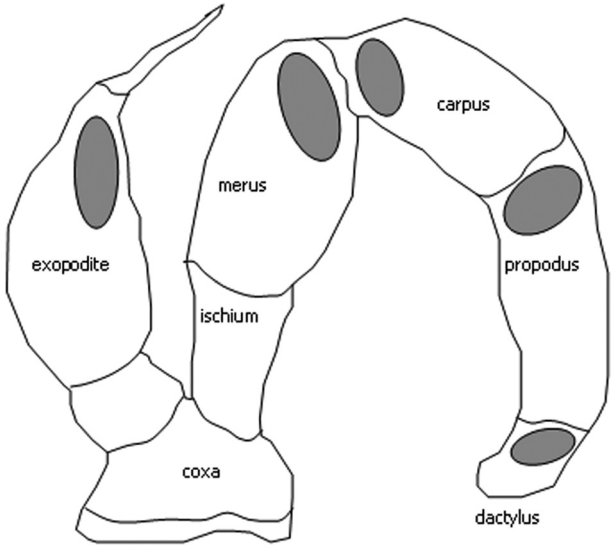

Figure 5. Internal surface of the 3rd maxilliped of *Coenobita rugosus* (setae removed). The grey areas indicate the five distinct hairy zones.

The 3rd maxilliped setae (Figure 7a) also appeared thick, long and loosely spiralled, but somewhat less spiralled than those on the claws. In contrast, their distal surface was quite different as it was covered by rows of fringed scales (Figure 7b). Once again, there was no difference among species. The spiralled structure of *Coenobita* setae can be better appreciated if compared with the setae of a marine hermit crab (Figure 8), where the setae are illustrated from a zone equivalent to zone 3 (see Figures 3 and 6). An analogous difference can be found by comparing the setae of the 3rd maxilliped of the above species as well.

The function of *Coenobita* setae in water absorption can be inferred by comparing their structure and their density with those of many Ocypodidae, known to allow ghost crabs (*Ocypode*) and allied species to extract water directly from wet sand (Vannini 1976b; Hartnoll 1988). Although they are a little less twisted, the setae of *Ocypode quadrata* (Figure 9) show a density and a structure similar to those on *Coenobita* claws and maxillipeds.

Carrying structures – the role of scaphognatite and thoracic limbs

Observations made using dye added to the water showed that water reached the mouth mostly via the

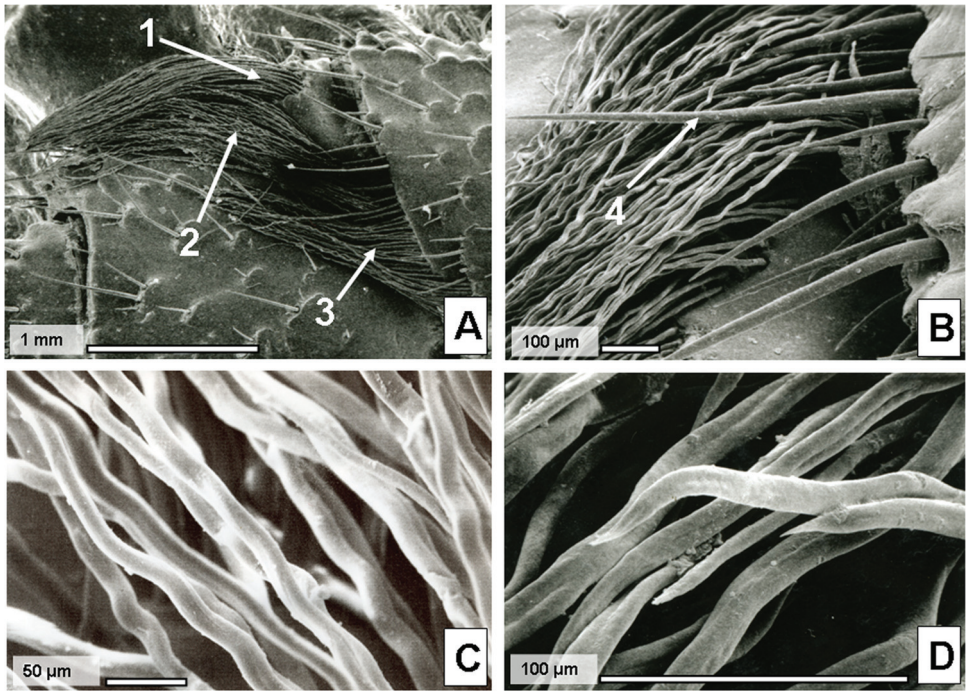

Figure 6. (a–b) The right minor claw of *Coenobita rugosus* showing (1, 2, 3) the three zones of absorbing setae and (4) the cuspidate non-absorbing setae. (b) Enlarged view of the cuspidate non-absorbing setae. (c) Enlarged view of the absorbing setae of zone 2 of right minor claw of *C. rugosus*. (d) Tip of the absorbing setae of zone 2 of the left major claw of *C. cavipes*.

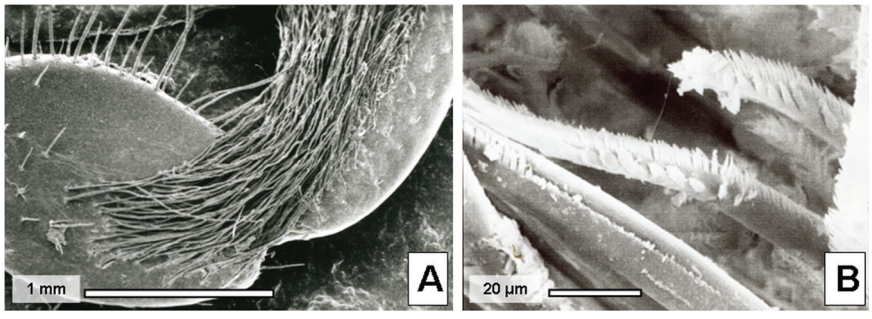

Figure 7. (a) Setae from the left 3rd maxilliped of *Coenobita cavipes*. (b) Setae from the left 3rd maxilliped of *C. rugosus* at greater magnification.

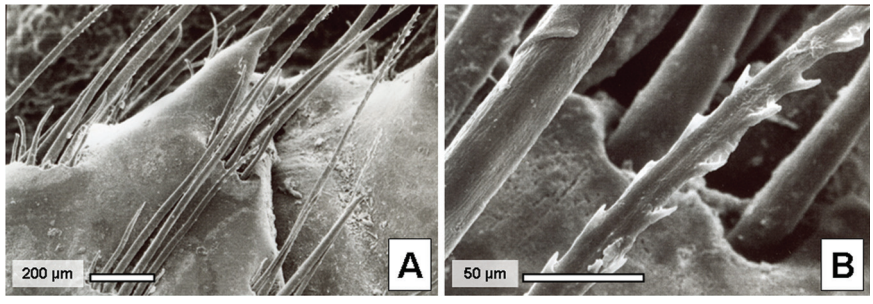

Figure 8. (a) Setae from the left cheliped propodus (distal internal margin) of the marine hermit crab *Clibanarius eurysternus*. (b) Those setae at a greater magnification.

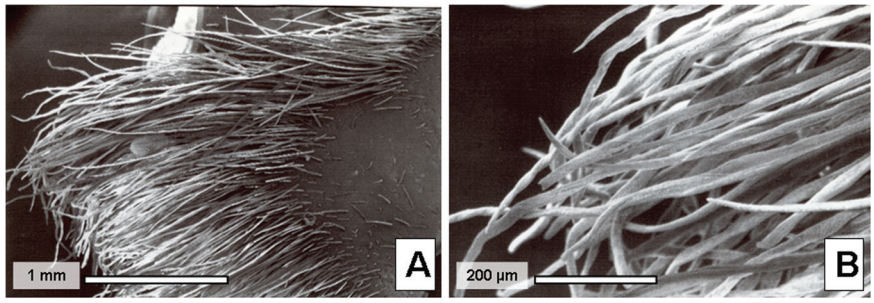

Figure 9. (a) Setae from the space between walking legs 3°–4° of the semiterrestrial crab *Ocypode quadrata* (Ocypodidae). (b) Those setae at a greater magnification.

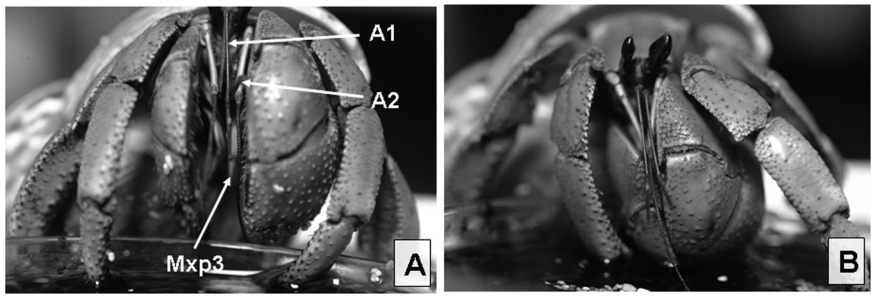

Figure 10. *Coenobita violascens* while (a) drinking and (b) refilling its shell.

3rd maxillipeds. When offered water, dehydrated crabs could not fill both their mouths and their shells simultaneously. When filling the mouth, the dye added to the water clearly indicated that the maxillipeds were involved and claws were kept apart (Figure 10a). The maxillipeds were gently dipped into the water or pressed against the sand and then carried to the mouth, leaving the claws uncoloured by the dye. During this phase, no water was seen entering the shell. In contrast, when the hermit refilled its shell, the maxillipeds were extended and touching each other while being embraced within the claws, which were also touching each other (Figure 10b). In this instance, the dyed water was clearly visible over all of the setal areas.

Although drinking and shell refilling were performed alternately, it was difficult to separate the two behaviours visually. Consequently, in one case, a crab that had been refilling its shell for about 10 minutes without apparently carrying any water to the mouth was frozen in liquid nitrogen. After dissection it was possible to verify that both the mouth and stomach were not at all coloured by the dye, indicating that the water had passed directly from the cheliped to the shell.

Removing a branchiostegite to observe the gill chamber through the glass shell allowed us to examine the way the water was transferred from the cheliped setae to the shell (Figure 11). The bases of the limb are close to the exhalant gill chamber orifice. When the scaphognatite (exopod of the 2nd maxilla) began beating actively, acting as a pump, water was sucked into the gill chamber. It was difficult to demonstrate that the scaphognatite had reversed its beating movements, pumping the water out, but this conclusion was strongly supported by the direction of the water flow and the immediate appearance of dye within the gill chamber. About half a minute later, the first droplets of coloured water were seen within the shell. Specifically, the water flows from the gills into the shell, following a deep furrow, which separates the abdomen from the coxae of the 5th pereiopods (Figure 12). The water is probably confined to the furrow by a few rows of setae on the 5th coxae and the surface tension of the water. The coxal rows of setae are kept much closer to each other and to the abdomen when the animal is refilling the shell, but additional detail on setal positioning is difficult because the zone was not visible through the glass shell and dye traces were present throughout the area.

Shell water content and pH

Water content varied from 0.5 to 2.5 g and was clearly related to the size of the crab ($r = 0.866$; $df = 10$; $P < 0.001$). No difference was detectable

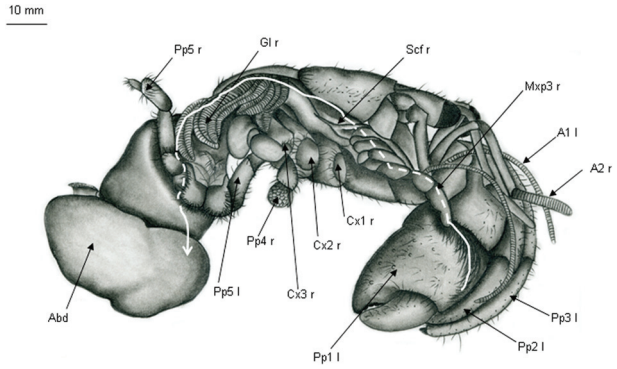

Figure 11. *Coenobita cavipes* (female) with right branchiostegite and 1–3 right pereiopods removed. (A1 l) and (A2 r), 1st left and 2nd right antennae; (Abd), abdomen; (Cx1 r), (Cx2 r) and (Cx3 r), coxae of 1st, 2nd and 3rd right pereiopods, respectively; (Gl r), right gills; (Mxp1-2 r), basal segments of 1st and 2nd right maxillipeds; (Mxp3 r), 3rd right maxilliped; (Pp1 l), 1st left pereiopod (= cheliped); (Pp2 l), 2nd left pereiopod); (Pp3 l), 3rd left pereiopod; (Pp4 r), 4th right pereiopod; (Pp5 r) and (Pp5 l), 5th right and left pereiopods, respectively; (Scf r), right scaphognatite. White line: water path from claw setae to the shell in which the abdomen is embedded. Dashed line: here the water flows along the hidden face of the limb.

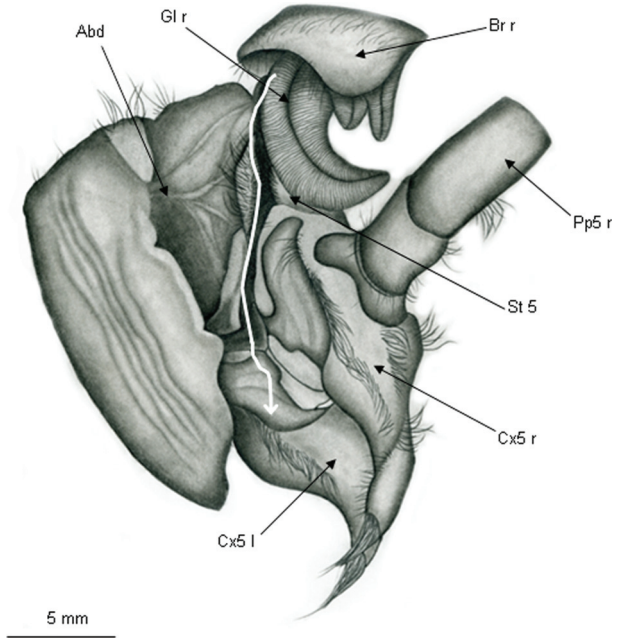

Figure 12. *Coenobita rugosus* (male). (Abd), abdomen; (Br r), right branchiostegite; (Cx5 l) and (Cx5 r), coxae of 5st pereiopods, left and right respectively; (Gl r), right gills; (Pp5 r), 5th right pereiopod; (St5), 5th thoracic sternite. White line: water path from gill to the shell in which the abdomen is embedded.

among the three species studied (F = 1.92; df = 2, 9; P = ns). Refilling time ranged between 3 and 13 minutes, showing a good correlation with the size of the crab (r = 0.712; df = 20; P < 0.001), but was not different among the species (F = 1.43; df = 2, 19; P = ns).

The pH of the shell water was measured only once, 2.5 h after the shell refilling, in 12 specimens belonging to three species. The pH varied from 7.7 (the value of the offered freshwater) to 8.5 (SE = 0.04) but showed no relationship with the specific species (F = 0.93; df = 2, 9; P = ns).

Discussion

The *Coenobita* hermit crabs have succeeded in their adaptation to land thanks to their shell and the ability to carry part of the marine environment within it. The water reserve that these crabs are able to carry with them has a multipurpose function: to compensate for dehydration, to keep the gills and abdomen surface sufficiently wet to allow gas exchanges, to reduce temperature through evaporation and to facilitate osmoregulation (De Wilde 1973; McMahon & Burggren 1979; Greenaway 2003).

Our observations of the mechanism by which water is absorbed and distributed within the gut and/or the shell indicate that there are two differing pathways that involve different organs. Drinking relies on the 3rd maxillipeds alone: their setae touch the water or wet sand and are then carried to the mouth. In contrast, shell refilling involves a complex chain of organs and events. At first, the setae of the claws and 3rd maxillipeds are involved; then a negative pressure, probably due to the reversed beating of the scaphognathite, causes water to enter the gill chamber; finally, water surface tension and the multilamellar gills are probably responsible for water accumulation within the gill chamber. This process ends about half a minute after the beginning of sucking a continuous stream of excess water into the shell.

In all species studied, shell refilling and drinking are alternated and both are interrupted by grooming activity. Hence, complete shell refilling takes a variable period of time (from 3 to 13 minutes), while the amount of water stored in the shell varies from 0.5 to 2.5 g; both values positively correlate with the animal's size. A preliminary test indicated that after refilling, the shell water becomes progressively more alkaline, supporting the view that the thin abdominal cuticle may play a role in ammonia (NH_3) excretion (McMahon & Burggren 1979).

The particular shape of the twisted setae, which are similar in structure and density to the absorbing setae found in Ocypodidae, is clearly the powerful instrument from which the whole chain of water absorption starts, and this mechanism is a basic instrument in the land adaptation of this

genus. The twisted shape probably creates capillary spaces between the setae and, at the same time, the surface tension of the water prevents setal collapse.

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