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# Water transport mechanism through open capillaries analyzed by direct surface modifications on biological surfaces

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**Some small animals only use water transport mechanisms passively driven by surface energies. However, little is known about passive water transport mechanisms because it is difficult to measure the wettability of microstructures in small areas and determine the chemistry of biological surfaces. Herein, we developed to directly analyse the structural effects of wettability of chemically modified biological surfaces by using a nanoliter volume water droplet and a hi-speed video system. The wharf roach *Ligia exotica* transports water only by using open capillaries in its legs containing hair- and paddle-like microstructures. The structural effects of legs chemically modified with a self-assembled monolayer were analysed, so that the wharf roach has a smart water transport system passively driven by differences of wettability between the microstructures. We anticipate that this passive water transport mechanism may inspire novel biomimetic fluid manipulations with or without a gravitational field.**

Water is important for maintaining life and activity. Land animals have evolved diverse water transport mechanisms depending on the habitat and body structure of animals that are actively driven by mechanical energies or passively driven by surface energies, including the extraction of moisture from food[1], absorption through the skin[2] or by oral/anal intake in most cases[3–5]. Most animals use suction or drinking, which requires mechanical energy to draw water into their mouths. Smart water transport systems based on a combination of active and passive mechanisms have been reported. Adult cats acquire water by lapping, thus exploiting the inertial energy of ascending columns based on water adhesion to the dorsal side of its tongue[3]. Shorebirds transport water using a capillary ratchet induced by repetitive opening and closing of their beak[4].

On the other hand some small animals only use passive water transport mechanisms. Insects found in the Namib Desert collect water droplets from the morning fog using hydrophilic–hydrophobic microstructures on their backs before transportation to their mouth[6]. Australian lizards transport water to their mouth using the capillary system of their grooved skin[7,8]. Desert sandgrouses transport water to their young by wetting their feathers, which are densely covered with wettable hydrophilic fibres[9]. The wharf roach transports water through the open capillaries of the caudal legs[10]. In comparison to active transport, little is known about passive transport because it is difficult to measure the wettability of microstructures in small areas and characterize the chemistry of biological surfaces.

In this article, we determined how the wharf roach transports water via open capillaries on its legs. In general, water moves up an open capillary using the interfacial free energy resulting from the surface chemical composition and microstructures. Firstly we investigated the surface chemical composition of the wharf roach using conventional surface chemical analysis such as energy dispersive X-ray, infrared and Raman spectroscopies. However, elucidation of surface chemistry was difficult because of considerable individual variability and heat damage. Secondly, we tried to prepare duplicated open capillaries by template and photolithographic methods.

However, exact duplicates of the legs could not be obtained because of the complicated microstructures. Herein, to solve these big problems of investigation of smart water transport systems of the wharf roach possessing complicated biological microstructures and undefined surface chemistry, we developed a novel method including direct modifications of biological microstructures and wettability analysis of small areas.

## Results

**Open capillaries on the legs of wharf roach.** The wharf roach *Ligia exotica* (Crustacea, Isopoda, Fig. 1a) is a fast-moving arthropod found on rocky seashores with seven pairs of legs used to escape the surging surf and drowning. However, water is essential for its existence[11], especially for gaseous exchange through its gills during respiration[12]. It possesses a smart water transport system[10] that transports water from the wet surface of the seashore using a pair of caudal VI and VII legs (Fig. 1b). Vertical water transport was observed on the wet surface of the VI leg, from the lower end of the $2^{nd}$ podite to the upper end of the $5^{th}$ podite (Fig. 1c and see Supplementary Movie S1 online). Scanning electron microscope (SEM) observations revealed that open capillaries are present from the $2^{nd}$ to the $5^{th}$ podite, except the $6^{th}$ podite on the VI leg (Fig. 1d). On the VII leg, open capillaries are found only on the $6^{th}$ podite. Water is transported to the gills via these open capillaries when VI and VII legs are firmly apposed[10].

Detailed observations showed that open capillaries of the VI leg comprised two different cuticular protrusions, a hair-like protrusion (HLP) and a paddle-like protrusion (PLP). In the $4^{th}$ and $5^{th}$ podites, HLPs and PLPs are located at the edge and centre of open capillaries, respectively (Fig. 1e). Joints of legs contained HLPs (Fig. 1f). In the $2^{nd}$ and $3^{rd}$ podites, the entire area of open capillaries comprised only HLPs bending slightly towards the direction of oncoming water (Fig. 1i) and each protrusion is approximately 50-µm long (Fig. 1j). PLPs were approximately 100-µm high and 200-nm thick and were aligned parallel to the water flow (Fig. 1g). The outsides of open capillaries were covered with small curved plates (ca. 10-µm high; Fig. 1h), so that the outside roughness was lower than that of open capillaries.

**Surface chemical modification of open capillaries.** Legs of *L. exotica* were cut, rinsed and freeze dried[13] to maintain fine structures, which were physically modified by gold sputtering (Fig. 2a). The interfacial free energies of the gold-sputtered legs were chemically modified using hydrophilic and hydrophobic compounds[14,15]. The same modification was performed on flat mica substrates to verify the relationship between the molar ratio and wettability. To quantify the narrow area in open capillaries (Fig. 2b), water contact angles ($\theta$) were measured using 1.0 nanolitre droplet (Fig. 2c). We plotted $\theta$ of the chemically modified flat mica substrates ($\theta_f$), HLP ($\theta_h$) and PLP ($\theta_p$) areas and the outside of open capillaries ($\theta_o$) against the molar ratio of the hydrophobic thiol compound ($r$) (Fig. 2d and see Supplementary Movie S2–S4 online). $\theta_f$ decreased gradually from $108°$ (hydrophobic) to $13°$ (hydrophilic) as $r$ decreased. $\theta_o$ also decreased gradually from $124°$ to $48°$ like $\theta_f$, but it
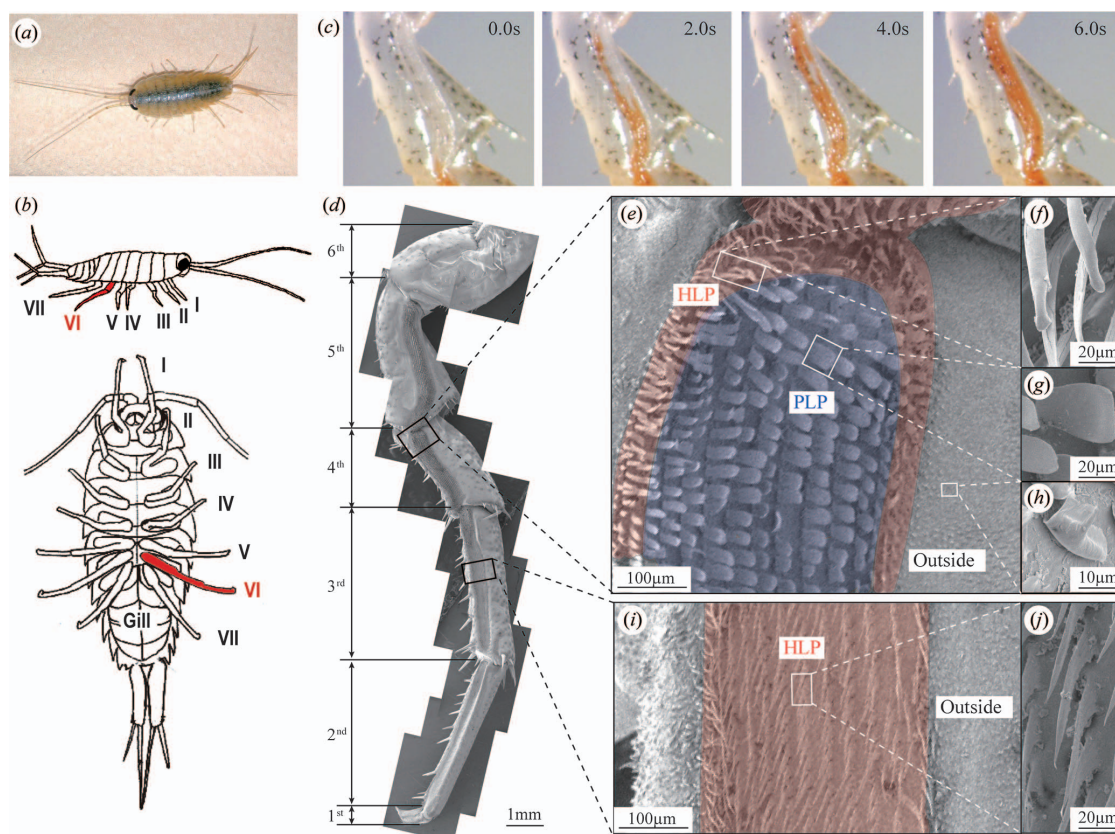


**Figure 1 | Open capillaries on the legs of *Ligia exotica*.** (a) An digital photograph of *Ligia exotica* used in this study taken by Dr. Horiguchi. (b) Illustrations of *Ligia exotica* depicted by Dr. Horiguchi; side and ventral views. (c) Sequence of images showing water transport through open capillaries on the $5^{th}$ podite of a freshly isolated VI leg. (d) Combined SEM image of the VI leg comprising the six podites: the dactylopodite ($1^{st}$ podite), the propodite ($2^{nd}$ podite), the carpopodite ($3^{rd}$ podite), the mieropodite ($4^{th}$ podite), the ischiopodite ($5^{th}$ podite) and the basipodite ($6^{th}$ podite). (e–j) SEM images of open capillaries; (e) the two different cuticular protrusions were observed in the open capillaries of the $4^{th}$ podite, (f) a hair-like protrusion (HLP) located at the edge of the jointand (g) a paddle-like protrusion (PLP) located at the centre. (h) The outsides of the open capillary were covered with small curved plates. (i,j) The entire area of on the $3^{rd}$ podite was covered with HLP. The outsides of open capillaries were also covered with small curved plates.
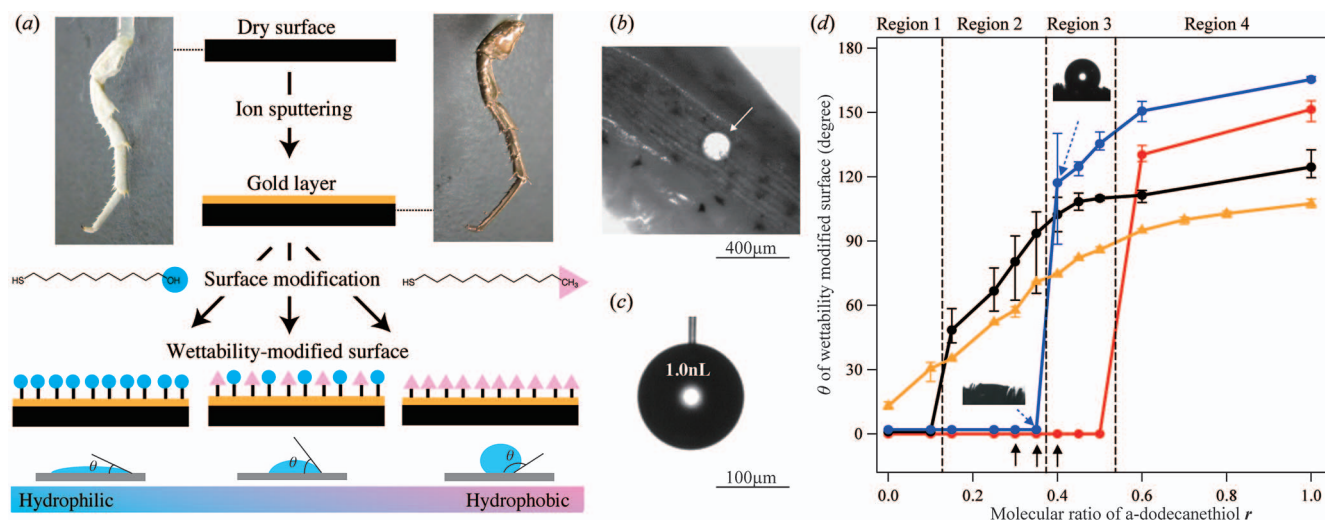
**Figure 2 | Surface chemical modification of open capillaries.** (a) Schematic illustrations of surface physical/chemical modifications of the VI leg. Cut legs were physically modified by gold sputtering. The gold-sputtered legs were chemically modified using by hydrophilic and hydrophobic compounds. (b) Top view of measurement area of wettability analysis. (c) Projection view of the small volume droplet by a 1,000 fps high-speed camera in wettability analysis. (d) Water contact angles of the chemical-modified flat mica substrates ($\theta_f$, yellow), the HLP area ($\theta_h$, red) the PLP area ($\theta_p$, blue) and the outside of open capillaries ($\theta_o$, black) of the 4th podite plotted against the molar ratio of the hydrophobic thiol compound ($r$). Solid arrows indicate the samples shown in water transport analysis (Fig. 3).

was slightly higher than $\theta_f$ ($r \gtrsim 0.15$). $\theta_h$ and $\theta_p$ decreased abruptly to 0° (superhydrophilic) at $r = 0.50$ and 0.35, respectively. According to the Cassie–Baxter wetting theory[16], a rough surface containing small air pockets is more hydrophobic than a flat surface. On the hydrophobic-modified leg ($r = 1.0$), $\theta$ followed the order $\theta_p > \theta_h > \theta_o > \theta_f$. SEM images (Fig. 1e) clearly show that the roughness and amount of air pockets in PLP and HLP areas were greater than those on the outside. According to the Wenzel wetting theory[17], when $\theta_f$ is smaller than 90°, a rough surface is more wettable than a flat surface. $\theta_h$ drastically decreased at $r \leq 0.6$ ($\theta_f \leq 90°$). However, high hydrophobicity in the PLP area was maintained even when $\theta_f$ reached 72° ($r = 0.40$; Cassie–Baxter wetting). This wetting type was defined by roughness and the amount of air pockets, which depended on the shape of the surface microstructures in open capillaries.

The wettability-modified legs were classified into the following four regions according to the relationship between $r$ (i.e. $\theta_f$) and $\theta$ of the three microstructures (Fig. 2d). Region *1* indicated that all areas were modified to become superhydrophilic ($\theta_h = \theta_p = \theta_o = 0°$). In region *2*, open capillaries ($\theta_h = \theta_p = 0°$) were superhydrophilic, whereas the outside was not wettable. In region *3*, only the HLP area of open capillaries was wettable ($\theta_h = 0°$). Strong water repellency of all areas was observed in region *4*.

**Water transport through open capillaries of the wettability-modified legs.** Water transport from the 4th to 5th podite of the wettability-modified legs was observed using green coloured water to make a colour contrast with the gold background. Water accumulated in region *1* of the wettability-modified legs in open capillaries and spread to the outer area of each podite. However, water transport was not observed in region *4* because of its strong water repellency. Two types of water flow through open capillaries were observed in region *2*, (i) water leaked from the joint between each podite and spread to the outside (Fig. 3a and see Supplementary Movie S5 online; $r = 0.30$, $\theta_f = 62°$) and (ii) water flowed only through open capillaries (Fig. 3b and see Supplementary Movie S6 online; $r = 0.35$, $\theta_f = 71°$). Water leaked readily to the outside when $\theta_o$ was low in region *2*. However, water transport via open capillaries was only achieved efficiently when $\theta_o$ was high. In region *3*, water was not transported to the 5th podite (Fig. 3c and see Supplementary

Movie S7 online; $r = 0.4$, $\theta_f = 78°$). These results indicate that water only climbed upward via open capillaries when they were superhydrophilic compared with the outside ($\theta_p = 0°$ and $\theta_p \ll \theta_o$). Hydrophobicity of the outside had an important role in preventing water leakage from open capillaries.

## Discussion

In the 2nd and 3rd podites, water flowed homogeneously along HLP, while in the 4th and 5th podites, water flowed along the HLP area initially, before filling the PLP area (Fig. 1c). To evaluate the wetting dynamics of open capillaries, penetration times of a 1-nL water droplet in HLP ($t_h$) and PLP ($t_p$) areas were measured using a high-speed camera (Fig. 4a, 4b). $t_h$ and $t_p$ of the wettability-modified legs at r = 0.35 were 17 ms and 66 ms, respectively. In region *2*, $t_h$ was always smaller than $t_p$, suggesting that the HLP area was more wettable than the PLP area. Water flow in the 4th and 5th podites comprised two steps (Fig. 4c), an initial slow infiltration into the HLP area and its spread into the PLP area in the centre of open capillaries without any leakage. The features of water flow in the
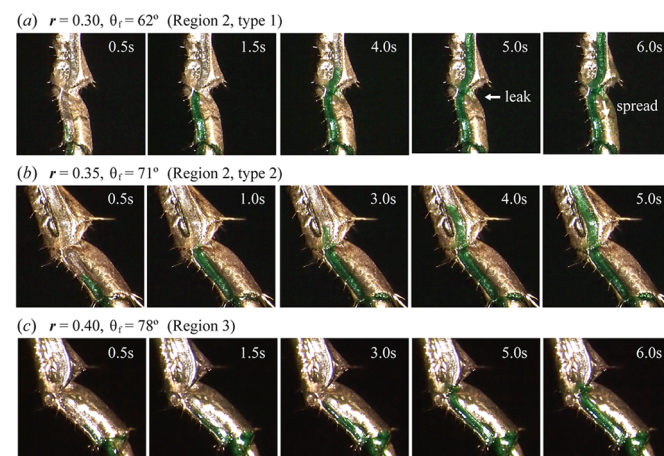


**Figure 3 | Water transport by open capillaries of the wettability-modified legs.** (a–c) Image sequence of water transport in open capillaries of the wettability-modified legs at (a) $r = 0.30$, (b) $r = 0.35$ and (c) $r = 0.40$.
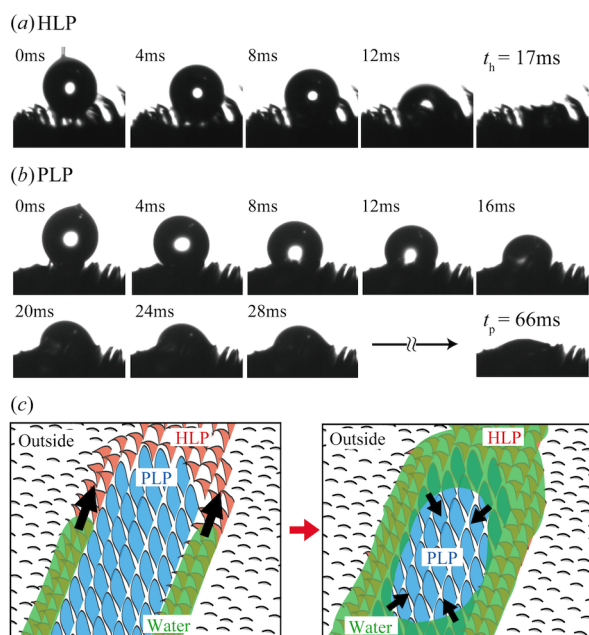
**Figure 4 | Mechanism of water transport by open capillaries.** (a,b) Sequence of images showing the penetration of a 1-nL water droplet in (a) HLP and (b) PLP areas of the wettability-modified legs at $r = 0.35$ captured using a high-speed camera (1,000 frames per second). (c) Schematic illustration of the water transport mechanism by open capillaries on the 4th and 5th podites. Water initially rapidly infiltrated into the HLP area, before spreading to the PLP area in the centre of open capillaries without any leakage. The PLP area in the centre of open capillaries played an important role as a water reservoir.

wettability-modified leg at $r = 0.35$ ($\theta_f = 71°$) resembled those of freshly autotomized legs (Fig. 1c) whose $\theta_h$ ($t_h$), $\theta_p$ ($t_p$) and $\theta_o$ were $0°$ (20 ms), $0°$ (150 ms) and $68 \pm 19°$, respectively. The PLP area in the centre of open capillaries on the 4th and 5th podites played an important role as a water reservoir. The results showed that the water roach adsorbed water using a passive mechanism produced by three different wettability areas comprising surface microstructures, i.e. the HLP, PLP and outside.

In this study, we determined the mechanism of a water transport system through open capillaries against gravity on the legs of an arthropod using a novel method that was readily applicable to biological surfaces. We conclude that the wharf roach transports water from a wet surface to the gills using a combination of two different micro-protrusions and the outside of open capillaries. The method of wettability modification of biological surfaces used in this study is applicable to other small creatures with passive water transport mechanisms. The results suggest that water can be transported through an open-capillary by means of differences in the interfacial free energy of structured surfaces even in a non-gravitational field such as cosmic space. Recently, an open air capillary system for use in a non-gravitational field was being researched at NASA's Ames Research Center[18]. We anticipate that this mechanism may be applied to open-air micro-fluidic devices and fluid manipulations with or without a gravitational field.

## Methods

**Animals.** Adult specimens of the wharf roach *Ligia exotica* were collected from the boulders of the littoral of Lake Hamanako (34°45_N, 137°35_E), a brackish lake in Shizuoka Prefecture, on the Pacific Ocean side of Japan. The animals were maintained in the laboratory in plastic tanks containing shallow amounts of filtered seawater and a broken piece of plant pots.

**Preparation of legs for scanning electron microscopy and gold spattering.** The body length of ca. 2 cm was used for this experiment. The legs of animals were easily autotomized by controlling their free movements with the help of a pair of fine forceps at the proximal end of basipodite. The 6th and 7th legs were pre-fixed overnight in 2% glutaraldehyde and 2% paraformaldehyde buffered with 0.1 M sodium cacodylate buffer adjusted to pH 7.2. The specimens were then rinsed several times in 0.1 M sodium cacodylate buffer solution, post-fixed for 2 h in 1% OsO$_4$ in phosphate buffered saline (0.13 M, pH 7.4) at room temperature, dehydrated through a graded series of ethanol solutions, and rinsed in 100% *t*-butylalcohol three times at 37°C. Then the specimens were freeze-dried (JEOL, JFD-300) for several hours at 2°C.

For scanning microscopy observations, dried specimens were coated with OsO$_4$ (Meiwa, Plasma multicoater PMC-5000) and observed with a scanning electron microscope (Hitachi S-3000N). For water flow observations, legs were coated with gold ion sputtering (5 mV, 225 s), in order to make them covered by same surface material. Various surface-modified legs with systematically changed interfacial free energies were made by surface treatment of hydrophilic and/or hydrophobic thiol compounds on gold-coated legs. The gold-coated legs were immersed in 1 mM ethanol solution including different molar ratios of hydrophilic thiol compound (11-mercapt-1-undecanol) and hydrophobic one (1-dodecane thiol) for 12 h. After the surface treatments, the chemical-modified legs were rinsed by ethanol and dried naturally for at least overnight. The same treatments were achieved by flat mica substrates to measure the surface wettability which is related with the interfacial free energy between the surface and water.

**Observation of water flow using isolated legs.** The law and wettability-modified legs were then attached to a fine plastic rod by dental wax to the autotomized proximal end. The other side of the rod was held in a manipulator (Narishige), so that the pereiopod could be stationed in its natural vertical position. The pereiopod was then immersed from the tip. The water was coloured with red/green water-soluble food dye (Tsukemoto Corporation) to facilitate observation of its flow with the help of a horizontally mounted stereomicroscope. To keep the position of pereiopod, the level of water was moved upward by a manipulator. The action was recorded on a digital camera (Olympus E-5, with video capture function).

**Measurement of the interfacial free energy.** The interfacial free energy was compared with surface wettability measured by the automatic microscopic contact angle meter (Kyowa MCA-3). A 1-nL microdroplet of water formed on the tip of a glass capillary having inner diameter of 5 μm was dropped on the surface with capturing of a projection view of the small volume droplet by a 1,000 fps high-speed camera. Water contact angles ($\theta$) were measured by the captured image when the water microdroplet did not change for 10 ms. If the $\theta$ was $0°$, in other wards the wettability of the surface was superhydrophilic, penetration times (t) of a 1-nL water droplet were measured using a high-speed camera.

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## Author contributions

H.H. collected animals and prepared dried samples; Y.H. performed surface modifications; D.I. collected SEM images and data and performed wettability analysis; Y.M., H.Y. and K.I. performed conventional surface chemical analyses; K.T. and T.S. discussed wettability; D.I., T.H. and M.S. designed the study and wrote the paper. All authors discussed the results and commented on the manuscript.

## Additional information

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