Vol. 32: 43–54, 2023
https://doi.org/10.3354/ab00760

AQUATIC BIOLOGY
Aquat Biol

Published September 14

Novel, complex burrow structure and burrowing behavior of the mud-dwelling octopus *Octopus minor* (Sasaki, 1920)

Qi-Kang Bo[1,2], Jin-Hai Wang[1], De Xing[1], Yao-Sen Qian[3], Min-Peng Song[1], Xiao-Dong Zheng[1,*]

[1]Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Yushan Road 5, Qingdao 266003, PR China
[2]Tianjin Fisheries Research Institute, Jiefang Nan Road 442, Tianjin 300202, PR China
[3]Ganyu Institute of Fishery Science, East Huanghai Road 310, Lianyungang 222100, PR China

ABSTRACT: The strategies employed by octopuses for predator avoidance and escape, which are adapted to their structurally simple habitat, have been the subject of much research. In the present study, the shapes and structural characteristics of *Octopus minor's* burrows were investigated and the burrowing behavior was also observed to reveal the dynamics of burrow formation. From 2012 to 2017, 85 plasticized cement burrow models were measured. The burrow, a complex project, includes 7 interconnected structural parts: digging holes (DH), digging channel (DC), horizontal channel (HC), lounge (LG), breathing channel (BC), breathing holes (BH), and breathing hole heap (BHH), with each part having its own cross-sections of special shape and size. The diameters of all parts were very significantly different and had extremely significant correlations with other factors, except for the weight of the occupants. The burrows have 1–2 DHs, 1–4 BHs and 1 BHH, with DHs and BHs distributed at opposite ends of the burrow. The burrows were categorized into 7 types according to the number of DHs, and burrows with 2 BHs accounted for a distinct type. The diameters of LG, DC and HC and the occupants' weight decreased as the number of BHs increased. The process of excavating a burrow involved 5 steps: creating a DH, inserting arms into the DH, burrowing, excavating the BC, and creating the LG. Abandoned burrows could be reoccupied by other octopuses, regardless of whether they were larger or smaller than the previous occupants. As an adaptation to structurally simple environment, *O. minor* seems to use a particular skill in digging more complex burrows as shelters.

KEY WORDS: Cephalopod · *Octopus minor* · Mud-dwelling · Burrowing behavior · Burrow

1. INTRODUCTION

Octopuses, a specialized and highly evolved group of mollusks (Richter et al. 2016), are found in a variety of coastal habitats and, lacking external shells, nearly all octopuses must rely on shelters to avoid predators and competition (Aronson 1986). Roofing, burying and burrowing are common life strategies for benthic octopus species in soft sediments, where camouflage is often less suited to these structurally simple habitats (Trueman & Ansell 1969, Katsanevakis & Verriopoulos 2004, Montana et al. 2015), although camouflage has become an emblematic behavior of cephalopods for predator avoidance (Hanlon et al. 2009). Previous studies have identified several types of shelters used by octopuses in soft-sediment environments. In addition to using man-made objects (e.g. empty bottles, pipes, cans, plastic

*Corresponding author: xdzheng@ouc.edu.cn

Publisher: Inter-Research · www.int-res.com

cups and tires) (Anderson 1997, Katsanevakis & Verriopoulos 2004, Guerra et al. 2014) or naturally occurring cavities (e.g. coconut shells, empty shells and holes or crevices in stones or reefs) (Mather 1982, Anderson 1997, Katsanevakis & Verriopoulos 2004, Finn et al. 2009, Guerra et al. 2014), sometimes they dig and modify dens in sandy or muddy substrates. In previous studies, 4 types of den in soft-sediment substrates have been described: (1) wells, where the octopus digs a vertical hole and reinforces the inner wall with shells and other solid materials (Fig. 1A), such as those of *Octopus insularis* (Leite et al. 2009) and *O. vulgaris* (Katsanevakis & Verriopoulos 2004, Guerra et al. 2014); (2) holes, where the octopus digs or modifies holes underneath solid materials (Fig. 1B), such as those of *O. vulgaris* (Katsanevakis & Verriopoulos 2004, Guerra et al. 2014), *O. briareus* (Aronson 1986) and *O. tetricus* (Anderson 1997); (3) mucus-lined subterranean dens, where the octopus utilizes special sand fluidization and adhesive mucus for sediment manipulation and creates a subsurface cavity with a respiratory chimney (Fig. 1C), such as that of *O. kaurna* (Montana et al. 2015); and (4) burrows (see below, this paragraph, for a definition of 'burrows'), in which the octopus digs deep tunnels connecting a digging hole (DH) with one or more breathing holes (BHs) (Fig. 1D), in which octopuses hide themselves under the seafloor (Yamamoto 1942), such as those of *O. minor* (Sasaki, 1920) (the species studied in this paper, sometimes referred to as *O. variabilis*; Dong 1988). In the first 2 types of dens, wells and holes, the octopus generally collects and deposits solid materials, such as stones and shells, around the edge of the hole to fortify the den, and sometimes partially or completely barricades its entrance (Anderson 1997, Katsanevakis & Verriopoulos 2004). The fourth type of den (burrow), a more complex architecture compared to the other 3 types, has multiple horizontal or sloping tunnels with multiple openings, suggesting that the construction of these burrows requires more effort and energy. This type of shelter has multiple openings, similar to the burrows of the European rabbit *Oryctolagus cuniculus* L. (Kolb 1985); therefore, we use the term 'burrow' to describe this complex excavation in our study.

Yamamoto (1942) provided a preliminary description of the burrow structure of *O. minor*, including the length and depth of their burrows, the diameter of the holes (DHs and BHs) and the number of BHs. However, detailed and specific measurements of structural features and the relationship between occupant's size and burrow parameters were lacking, and worryingly, in the process of exposing the internal structure of these burrows, human excavation destroyed these structures, which in turn led to inaccurate reports by researchers. Studying den

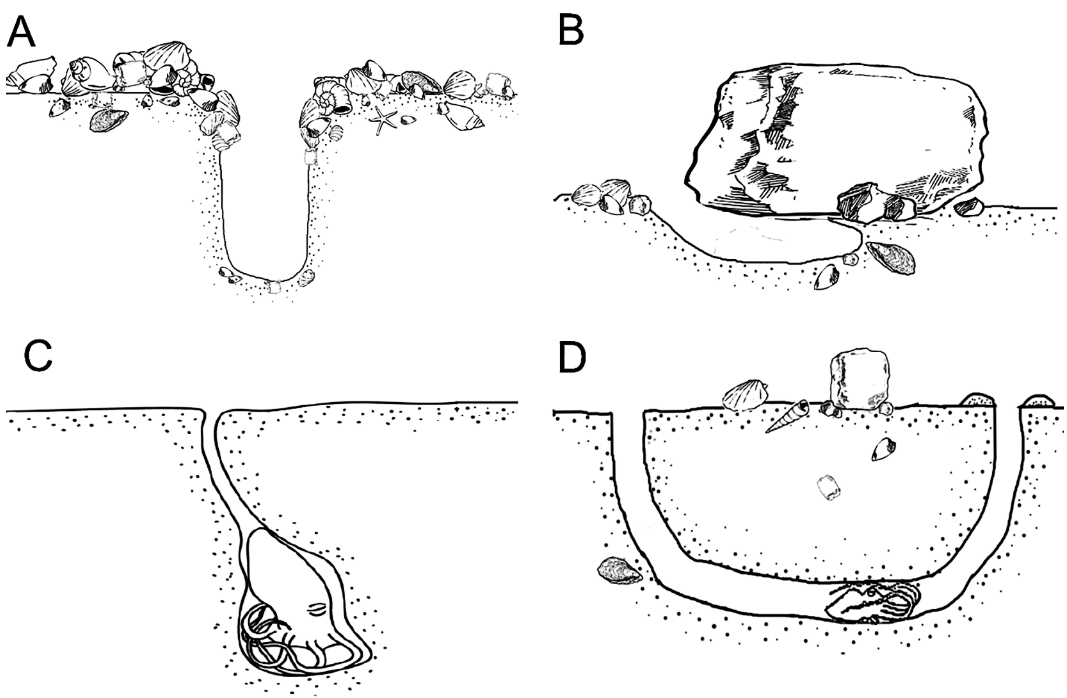

Fig. 1. The 4 types of dens excavated in the soft-sediment substratum: (A) well, (B) hole (modified from Katsanevakis & Verriopoulos 2004), (C) mucus-lined subsurface den (from Montana et al. 2015), (D) burrow (modified from Yamamoto 1942)

architecture without disturbance is challenging; therefore, a novel research method is needed to maintain the integrity of the original internal structure in future studies, similar to the method used by Boletzky (1996), where the den cavities were filled with a gelatinous preparation to measure the cavity volume. In addition to determining the structural characteristics of *O. minor* burrows, observations of burrowing behavior are needed to understand the dynamics of burrow formation.

Moreover, studying the ecological structure and behavioral aspects of *O. minor* burrow creation will facilitate the selection of appropriate artificial shelters and the innovation of novel artificial shelters for artificial breeding of octopuses; this is important because appropriate shelters can reduce the frequency and intensity of inter-individual conflicts in this bellicose species. This will prevent the octopuses from expending too much energy and getting injured, and energetic females will subsequently complete ovarian development in temporary culture under artificial ripening, thus becoming high-quality stock with markedly high reproductive performance. In addition, the study of den structure and behavioral characteristics of *O. minor* is critical to understanding the ecology of this species and to fisheries management (Cochrane & Garcia 2009), as den availability, size preferences, and the characteristics of the seabed are thought to be the main factors influencing octopus density and distribution patterns (Hartwick & Thorarinsson 1978, Leite et al. 2009). Similarly, studying their burrow structure and behavioral traits is important for understanding the natural living conditions of *O. minor* in the NCAAR (the National Conservation Area for Aqua-germplasm Resources of *O. minor*), an area in Moon Lake (Shandong Province, Northeast China) established by the Chinese Ministry of Agriculture in December 2012. It will also make it easier to assess the role and impact of the sanctuary in preserving this commercially important species, whose populations have declined sharply due to overfishing (Kim et al. 2008, Bo et al. 2016).

Octopus minor Sasaki, 1920, a coastal and sedentary species, is generally found in soft sediments of the seabed from the waters of Russia and northern Japan to those of southern coastal China (Dong 1988). This species has also been identified as ecologically important because it is a generalist predator (Bo et al. 2020). Artificial propagation that release individuals back into the wild has been conducted to mitigate the sharp decline in *O. minor* population (Bo et al. 2016); by 2019, nearly 2.3 million juvenile octopuses had been reintroduced into the coastal waters of Shandong (Sauer et al. 2021). Despite the strong desire for successful artificial breeding and intensive attention given to the conservation of natural resource, few studies have focused on the ecology and behavior of *O. minor*. To better understand their burrow ecology, the objectives of the present study were to (1) investigate the shapes and structural characteristics of burrows by obtaining plasticized-cement burrow models *in situ* (by pouring quick-setting cement into the burrow cavity, similar to the method of concrete pouring) and determine if there are other functional parts of burrows that have not been previously described; (2) elucidate the relationship between different burrow dimensions and occupant size; and (3) observe the burrowing behavior of *O. minor* and thereby describe the dynamics of burrow formation.

2. MATERIALS AND METHODS

2.1. Study site

The study area was Moon Lake, a 5 km^2 semi-enclosed lagoon (37° 21′ 00″ N, 122° 35′ 00″ E) with a soft-sediment substrate without large rocks or coral reefs (sandy loam, according to the standards of Soil Survey Staff 2010; the proportions of sand, silt and clay are 72.91, 23.14, and 3.95%, respectively; Fig. 2). The average water depth is 2 m, the deepest water depth does not exceed 3 m, and the tidal variation is synchronous with that of the open sea. This lagoon is an essential habitat for *O. minor* and supports a high-density population of this species (Bo et al. 2016), with an average annual yield of 6226 kg from 2006 to 2014. Burrows were identified and measured from mid-September to late November, from 2012 to 2017, when the sediment surface was exposed at maximum ebb tide. Over the course of 6 yr, 85 burrows were investigated.

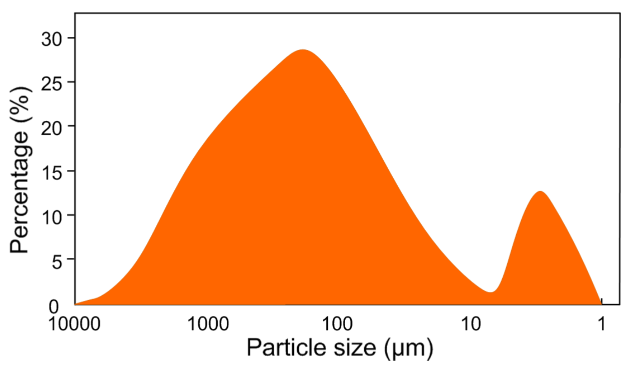

Fig. 2. Particle size distribution curve of the soft-sediment substrate in Moon Lake according to laser diffraction method

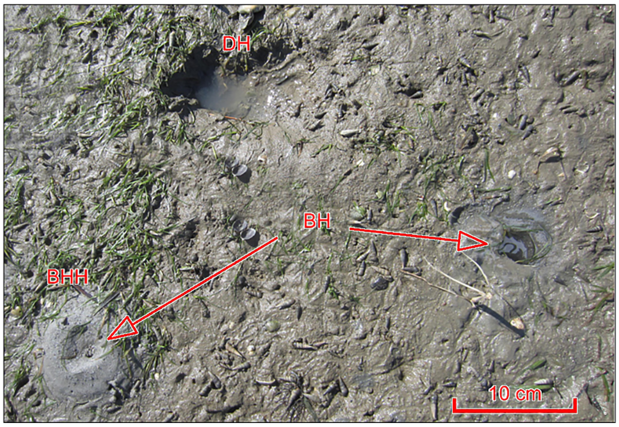

Fig. 3. Burrow openings of *Octopus minor* on the sediment surface. BH: breathing hole; BHH: breathing hole heap; DH: digging hole

2.2. Locating burrows

As described by Yamamoto (1942), uplifted mounds of fresh sediment surrounding the thin BHs of burrows are usually visible on the sediment surface when the water uncovers the sediments at low tide (Fig. 3, Fig. A1 in the Appendix), similar to magma accumulating around a volcanic crater. Near the BHs (about 20–60 cm in diameter), 1 or 2 larger DHs (ranging from 2 to 5 cm in diameter) can be found, with no accumulation mounds. When the occupant octopus remains undisturbed, synchronized with the gill ventilation of the resident octopus, water intermittently wells up from the BHs; after the water wells up, water from the surrounding sediment seeps into and replenishes the burrow, and the water level in the DHs slowly rises, with the effect that the water level in the DHs slowly rises and falls. Sometimes the octopus extends its arm tips out of the BH or DH (Fig. A1B). These unique characteristics are easily recognizable and can help distinguish burrows of *O. minor* from those of other macrobenthos. The number and diameters of BHs and DHs and the linear distance between each tunnel hole (the BHs and DHs) were measured on-site in the field. To accurately record the diameter of each irregular hole, we calculated the average of the maximum and minimum diameters.

2.3. Obtaining plasticized-cement models of burrows

In order to study the exact underground structure of the burrow, we obtained plasticized-cement models of the burrows by pouring quick-setting cement into the burrow cavity. To protect the octopuses from asphyxiation due to cement caking, the resident octopuses were forced out of burrows from the DH by dropping several drops of ethyl alcohol into the BH, and the occupant octopuses were weighed before they were released into the environment in the same place. Quick-setting cement (Jintang Bulou JT-55, Sofino New Building Materials, Tianjin, China) was slowly poured into the entrance of a DH until the cement overflowed from the BHs. One day later, the plasticized-cement model was dug out from the sediment (Fig. A1C–D). The depth of the tunnel was measured from the sediment surface to the central axis of the deepest channel. The 3-dimensional plasticized-cement models were then transported to the laboratory for further measurements, including measuring the diameter of the channel below the sediment surface and identifying the different functional parts of the burrow by determining the structural features shared by the models. For the underground channel, we measured the maximum and minimum diameters at 3 different locations in each part and averaged them to obtain the dimensions.

2.4. Laboratory experimental design

Live *O. minor* individuals (wet weight = 119.2–155.0 g, n = 30) were collected as soon as they were caught in Moon Lake by local fishermen and were maintained in indoor cement ponds (5.7 × 2.7 × 1.5 m) with shelters (tiles and polyvinyl chloride pipes). The ponds had a depth of 80 cm, and a filtered recirculating water system was installed to maintain constant conditions of approximately 13°C, salinity 28.5‰, and pH 8. Prior to behavioral observations, the octopuses were acclimated for 1 wk and fed live clams *Plicatula philippinarum* and crabs *Hemigrapsus sanguineus* ad libitum.

To examine burrowing behaviors, 12 captive *O. minor* (wet weight = 137.35 ± 2.73 g) were individually placed in 12 specially designed 'ant-farm' aquariums (L × W × H = 90 × 10 × 60 cm; Fig. 4) as described by Montana et al. (2015), in which a thin layer of soft-sediment substrates from the wild burrow surroundings were sandwiched between the front and back glass walls and the octopuses burrowed next to the glass such that their burrow and burrowing activity were visible. Two tests were designed to observe burrowing behavior. In Test 1, soft sediment was piled up to a level of 40 cm in the center of the test aquarium and a 4 cm wide gap was left between the edge of the soft-sediment substrates and the glass walls on the left

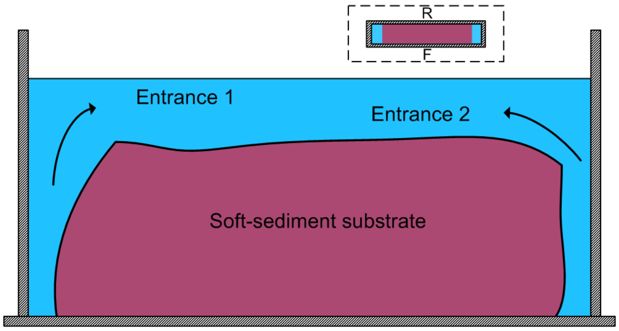

Fig. 4. Front view and plan view (in the dashed box, F: front; R: rear) of the specially designed 'ant-farm' aquariums

and right sides of the aquarium (Fig. 4); the gaps were used as entrances to induce burrowing behavior. This trial lasted 5 d and was designed to observe burrowing behavior. Test 2 was designed to determine whether octopuses would reuse the burrows of previous occupants. The creator of the burrow was removed as it came out in search of food, and another octopus was placed in the aquarium. During the observation period, a surveillance camera (LKD-HKZL02, Shenzhen Laikedi Technology, Shenzhen, China) was placed in front of the tank to record the behavior of *O. minor*. Filtered recirculating water kept the water in the aquariums oxygenated and fresh, and an escape net covered the opening of each aquarium. The octopuses in each aquarium were fed 6 clams per day. Shade was provided for the octopuses.

2.5. Statistical analysis

Normality and homogeneity of variances were tested by Kolmogorov-Smirnov and Bartlett-Box *F* tests, respectively. When data did not meet the assumptions of 1-way ANOVAs and *t*-tests, the non-parametric ANOVA equivalent (Kruskal-Wallis test) and Mann-Whitney *U*-test were used to determine significant differences. Data were analyzed by ANOVA when comparing multiple groups ($k > 3$) and when the data met the assumptions of normality and homogeneity of variance, Tukey's test was used. The significance of linear regression coefficients was determined by Pearson's correlation analysis when the data were normally distributed and homogeneous in variance; when these assumptions were not met, Spearman's correlation analysis was used. Differences in distribution were tested using the chi-squared goodness of fit test (χ^2). Statistical analyses were performed with IBM Statistical Package for the Social Sciences (SPSS) Statistics version 20.

3. RESULTS

3.1. Interior structure of *O. minor* burrow

In the present study, 85 octopus burrows were measured. The burrows had an average depth of 21.60 ± 3.19 cm (mean ± SD). We obtained plasticized-cement models of the burrows (Fig. 5) and found that the internal structure of the burrows consisted of 7 parts, including DHs (where the octopus drilled into the burrow), BHs breathing hole heaps (BHHs, fresh sediment mounds surrounding the BHs), and underground channels connecting the holes (Figs. 5 & 6). According to the direction and function, the underground channels included the digging channel (DC), the horizontal channel (HC) and the breathing channel (BC). Moreover, at the base of the BC and the end of the HC, we found an enlarged section of the whose diameter was significantly larger than that of the HC and DC (1-way ANOVA, $F_{2,324} = 164.59$, $p \le 0.001$; Fig. 7); we called this section the lounge (LG, Figs. 5 & 6), a place where the octopus could rest or reside. The BC tapered sharply until it generally became a thin line (Fig. 6).

The diameter of the HC was significantly larger than that of the DC (34.9 ± 6.1 mm) (Tukey's test, $p < 0.001$; Fig. 7), indicating that the channel gradually widened from the DC to the LG. The diameters of LG, HC and DC strongly correlated with each other ($n = 109$, Pearson's coefficients (r) for DC-HC, DC-LG and HC-LG were 0.0832, 0.633 and 0.594, respectively; all $p < 0.001$; Fig. 8). Differently shaped cross-sections of the underground channel were present: the DC had nearly circular cross-sections and the BC, HC and LG had elliptical cross-sections. However, the LG had a flatter underside, and the BC had compressed elliptical cross-sections (Fig. 6). The DH was nearly circular and occasionally irregular. Only one BHH was found in each burrow.

The wet weight of *O. minor* occupants ranged from 29.87 to 93.72 g with a mean of 54.52 ± 12.44 g according to the Pearson's correlation analysis ($n = 85$), and their weight was not significantly correlated with the diameter of the DC ($r = 0.168$, $p = 0.081$), or the HC ($r = 0.148$, $p = 0.125$) or LG ($r = 0.119$, $p = 0.22$).

3.2. Types of burrows

Our investigations showed that the number of DHs and BHs varied among burrows; usually there were 1–2 DHs and 1–4 BHs in a burrow. The burrows were categorized into 7 types according to the number of

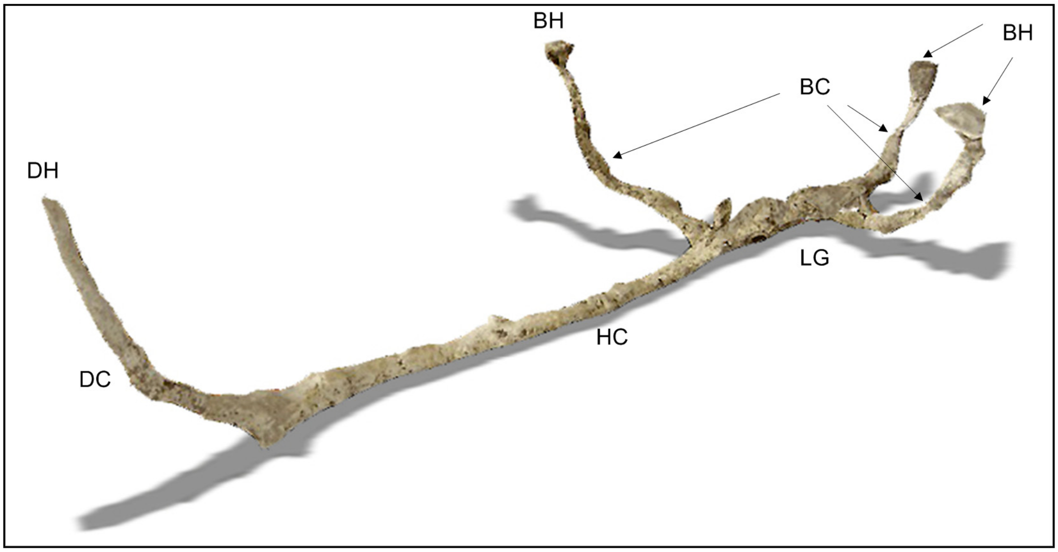

Fig. 5. Stereoscopic photographic display of plasticized-cement burrow model with one digging channel (DC) and 3 breathing holes (BH). BHs appear as inverted conical concrete blocks because cement infiltrated the spaces between the coarser sand grains and solidified with the coarser sand when quick-setting cement was poured into the burrows. BC: breathing channel; DH: digging hole; HC: horizontal channel; LG: lounge

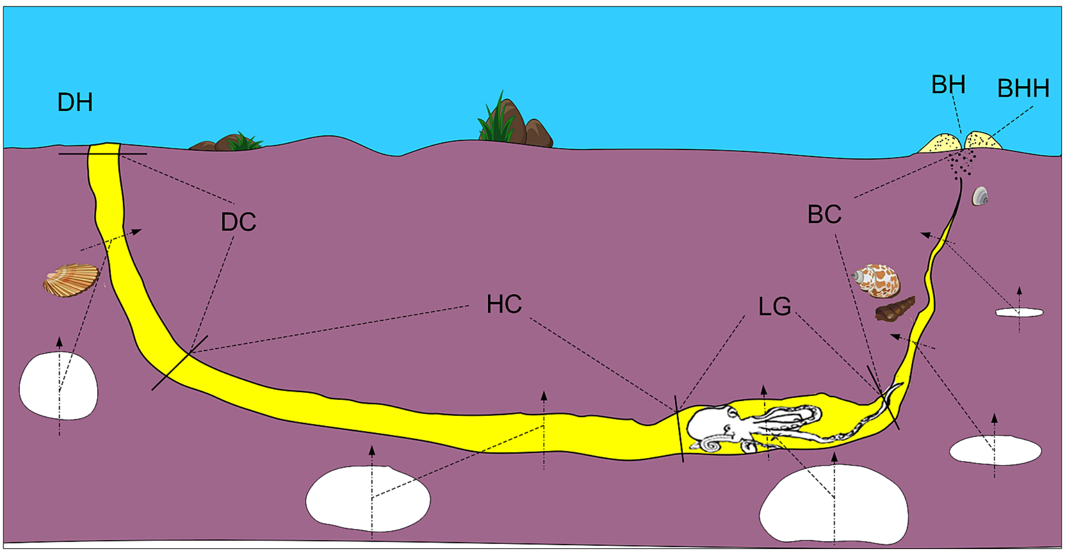

Fig. 6. Sketch of *O. minor* burrow (with one DH and one BH), showing the interior structure and cross-sections of the different parts. BHH: breathing hole heap; other definitions of abbreviations as in Fig. 5

DHs and BHs: 1DH1BH (burrows with one DH and one BH), 1DH2BH (burrows with one DH and 2 BHs), 1DH3BH, 2DH1BH, 2DH2BH, 2DH3BH and 2DH4BH. The proportions of these 7 types are shown in Fig. 9. Of the 85 burrows we observed, the most common type was 1DH2BH (23.53%) and the least common type was 2DH4BH with 2.35%.

The proportion of burrows with multiple BHs (71.75%) was significantly greater than that of burrows with a single BH (28.24%) (chi-squared fit test, $p \leq 0.001$), and burrows with 1 DH accounted for a substantial percentage of total burrows (62.35%) (chi-squared fit test, $p = 0.023$). Burrows with 2BHs accounted for a significant proportion (41.18%) (chi-squared fit test, $p < 0.001$), followed by burrows with 1BH or 3BHs (both at 28.24%) and 4BHs (2.35%), when burrows were grouped by the number of BHs.

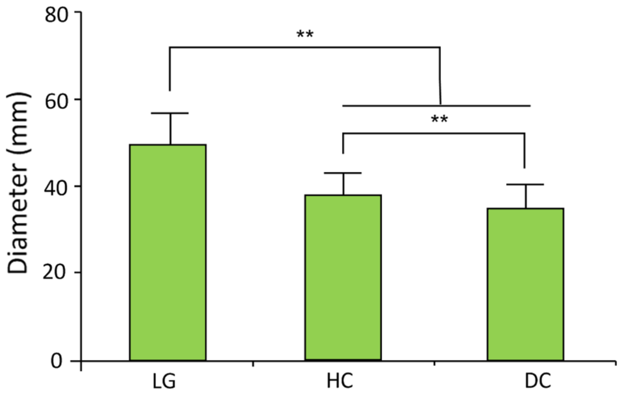

Fig. 7. Diameters of lounge (LG), horizontal channel (HC) and digging channel (DC) in the *O. minor* burrows. **p ≤ 0.01

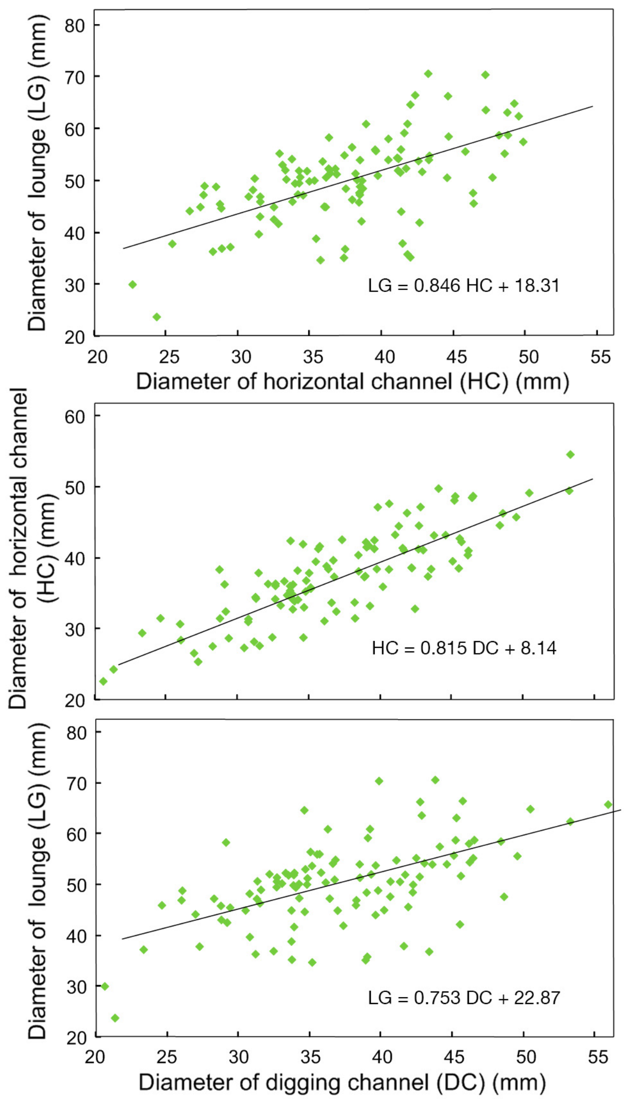

Fig. 8. Relationship of the diameters of digging channel (DC), horizontal channel (HC) and lounge (LG)

The linear distance between burrow holes was measured, and the linear distance between DH and BH (median = 38.61 cm) was significantly larger than that between DHs (DH–DH, median = 17.73 cm) (Kruskal-Wallis test, $p < 0.001$; Fig. 10) and that between BHs (BH–BH, median = 14.36 cm) (Kruskal-Wallis test, $p < 0.001$), indicating that DHs and BHs were concentrated at opposite ends of the burrow. The linear distance between DHs was significantly larger than that between BHs (Kruskal-Wallis test, $p < 0.001$), indicating that the distribution of BHs was more concentrated.

Spearman correlation analysis (n = 85) revealed no significant correlation between the weight of *O. minor* occupants and DH–BH ($\rho = 0.131$, $p = 0.233$), or DH–DH ($\rho = 0.011$, $p = 0.954$) or BH–BH ($\rho = 0.112$, $p = 0.273$) distances.

When the burrows were divided into 2 groups by the number of DHs (1DH group, with 1 digging hole; and 2DH group, with 2 digging holes), there was no difference in the weight of the occupants and the diameters of the LG, DC and HC between these 2 groups. However, when the burrows were divided into 3 groups according to the number of BHs, the occupant weight of group 1BH (burrows with 1 BH, n = 24) was significantly greater than that of group 2BH (burrows with 2 BHs, n = 35) (Tukey's test, $p = 0.005$) and group 3BH (burrows with 3 BHs, n = 24) (Tukey's test, $p = 0.004$), and the diameters of the DC and HC in group 3BH were significantly smaller than those in group 1BH and group 2BH (Tukey's test, $p < 0.01$; Fig. 11). Due to the small number of burrows (n = 2), the parameters of group 4BH (burrows with 4 BHs) were not compared with those of the other groups.

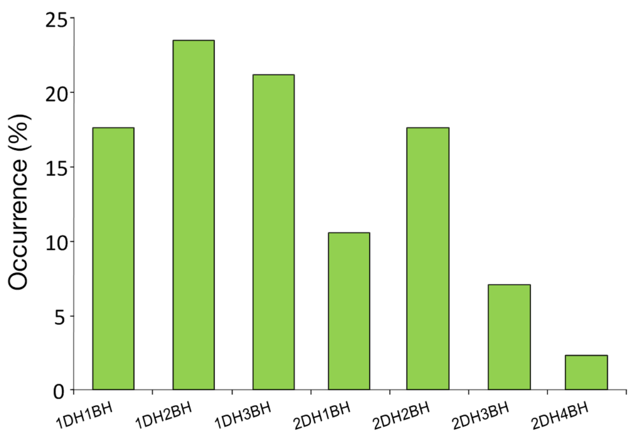

Fig. 9. Proportion of occurrences of the 7 burrow types of *O. minor*. BH: breathing hole; DH: digging hole; 1DH1BH: burrows with one DH and one BH

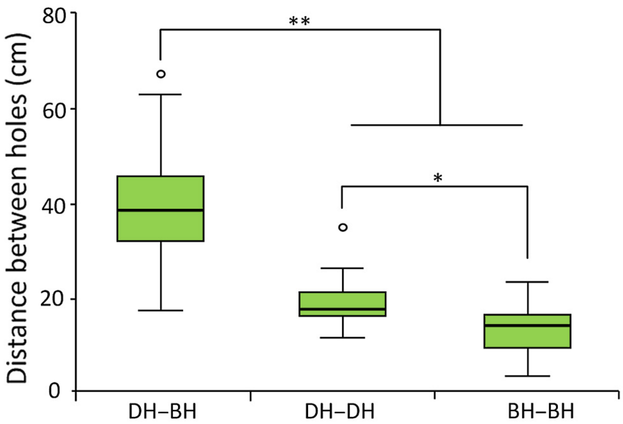

Fig. 10. Box plots of the distance between openings of *O. minor* burrow. The boxes indicate lower and upper quartiles with medians (bold black line) inside the boxes, the whiskers indicate maximum and minimum values of the data batch and the small circles indicate outliers. DH–BH: distance between digging hole (DH) and breathing hole (BH); BH–BH: distance between BHs; DH–DH: distance between DHs. **$p \leq 0.01$; *$p \leq 0.05$

3.3. Burrowing behavior in the laboratory

Our findings indicated that *O. minor* can excavate burrows in soft sediments. In Test 1, all 12 octopuses burrowed to varying degrees over the 5 d. Of them, 7 octopuses excavated complete burrows that resembled wild burrows over an average of 3.8 d, and the rest of the burrows were relatively small underground cavities. Upon being placed in the water, the octopuses immediately opened their arms to search, and upon locating the gap created to promote burrowing, they quickly moved to the gap and

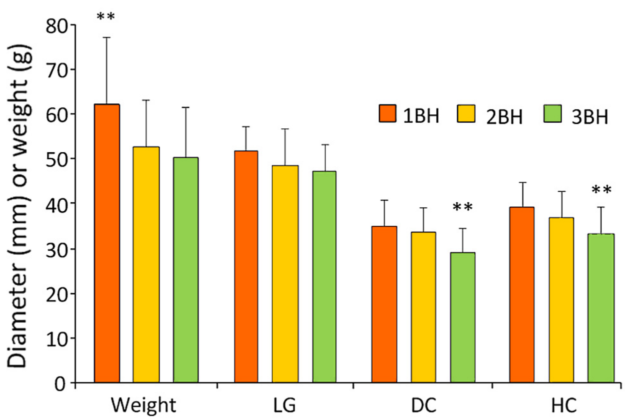

Fig. 11. Diameters of lounge (LG), digging channel (DC), horizontal channel (HC) and occupant weight of the 3 groups according to the number of breathing holes (BH). 1BH, 2BH, 3BH: burrow groups with 1, 2 and 3 breathing holes, respectively. **$p \leq 0.01$

occupied that area, remaining on the bottom of the test aquarium while acclimating to the new environment. The process of digging a complete burrow can be divided into 5 stages. Stage 1, creating a DH: after acclimating, the octopus anchored itself to the sediment surface with outstretched arms and, by several strong jetting actions of the mantle and siphon, stirred up sufficient amounts of particles to create a hole in the sediment under the arm crown, which was considered as the DH. Stage 2, inserting the arms into the DH: along with injecting water into the sediment, the octopus withdrew its arms in the order fourth to first pair, bending them backward and inserting the base of its arms as deeply as possible into the hole. The above 2 stages are similar to the first 2 steps of *O. kaurna* burrowing behavior, as described by Montana et al. (2015). Stage 3, burrowing: the octopus opened the arm crown and forced the bent arms apart, compressing the mud to the periphery and widening the hole laterally, while maintaining its mantle and siphon above the sediment surface to continue the jetting action; then the basal part of the arms (near the arm crown) gradually moved forward into the hole; after the arms almost entered the hole, the anterior half of the first 2 pairs of arms stretched and stuck backward out of the hole, rubbing the inner wall and bringing the soil out of the hole. At this point, the excavation of the DC had begun. By repeating these processes, the octopus continuously excavated a DC, simultaneously with forward movement, and quickly sheltered itself. Stage 4, excavating a BC: once the octopus had managed to draw itself entirely into the shelter of the DC (Fig. 12A), it was in no hurry to continue excavating the DC; the jetting action became weak and seemed to change from the function of displacing sediment particles to the completion of gill ventilation. Instead, the octopus took full advantage of the long length of the first pair of arms, alternately stretching those arms to excavate a thinner and longer channel, which was regarded as the BC; and other arms attached to the inner surface of the channel to anchor the body (Fig. 12B). Stage 5, creating an LG: after creating the BC, the octopus compressed the mud to the periphery or eroded the inner walls with the arms and jetting action, renovating the downstream part of the DC as a spacious LG where it spent most of its time (Fig. 12C). This stage marked the completion of the burrow, as by this point the burrow had all the functional structures of burrows in the wild, lacking only the BHH that would be created on the sediment surface.

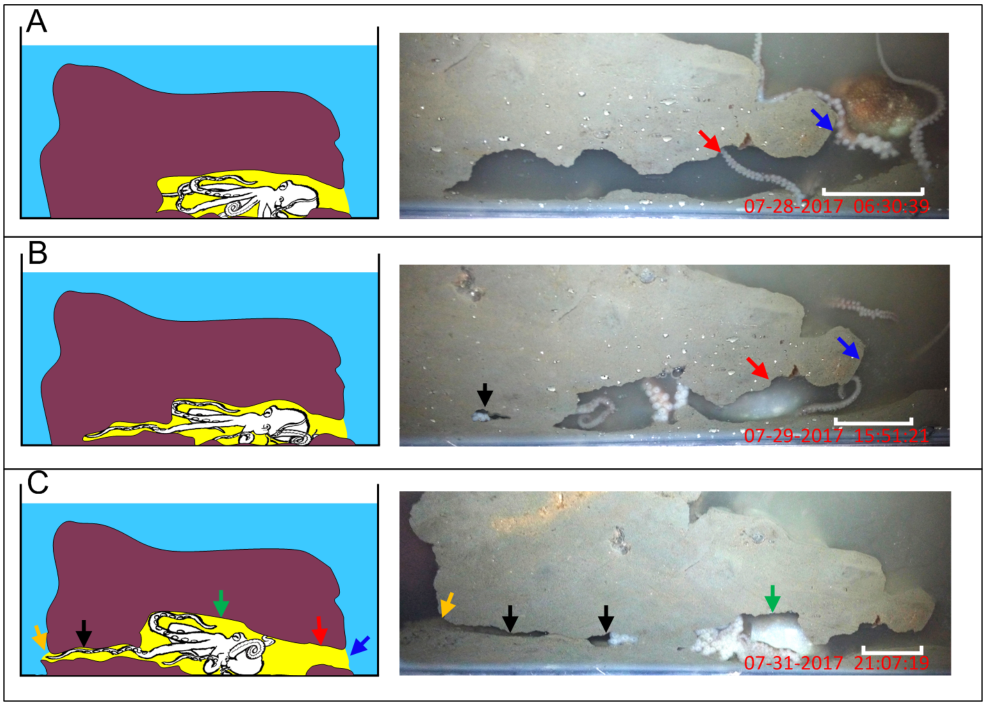

Fig. 12. Drawings and photographs of *O. minor* burrowing behavior: (A) octopus burrowing a digging channel, (B) octopus excavating a breathing channel, (C) formation of a spacious lounge. Colored arrows indicate specific parts — blue: digging hole; red: digging channel; green: lounge; black: breathing channel; orange: breathing hole. Scale bars: 100 mm

During the breathing process, light clay particles were observed to be carried into the DC and ejected from the BC by the generated water flows, indicating that octopuses inhaled fresh seawater from the DH and then discharged the water and feces to the seabed surface through the BH, completing an efficient breathing process.

In Test 2, the original occupant that created the burrow was removed when it left the burrow at night. On the second day, another octopus was placed near the DH of the burrow. The new octopus immediately opened its 8 arms to search for and find the DH of the burrow, and then stretched its first pair of arms into the burrow. After discovering that the burrow was unoccupied, the octopus quickly entered the burrow, usually by bending its first pair of arms in the middle with the arm tips being oriented backward. After its first 3 pairs of arms entered the burrow, the octopus pulled in its head and mantle. In the 12 test aquariums, all octopuses were observed to reuse the existing burrows as shelter. When octopuses were similar to or smaller than the previous occupant octopuses, they reoccupied the burrow immediately, whereas larger octopuses had to drill hard into the burrow to widen the entrance and channel.

4. DISCUSSION

There are few field studies on the ecology of octopus burrows, and much less on detailed, specific measurements of structural features and the relationship between octopus' size and burrow parameters. Mather (1982) found that *Octopus joubini* selected the smallest entrance size up to a lower limit on aperture size where the octopus could not get in. Hartwick et al. (1978) found a positive relationship between *Enteroctopus dofleini* size and den cavity volume, but Mather (1982) found that there was a range of cavity volumes acceptable for *O. joubini* dens, while volumes that were larger or smaller than this range were apparently rejected. In this present study, there was no significant correlation between the weight of *O. minor* occupants and channel aperture, and no significant correlation between wet weight and burrow length. These 2 findings may be due to reoccupation of the burrow by an octopus that does not match the size of the original occupant. Guerra et al. (2014) found no significant differences in den diameter for 3 size-classes of octopuses (small, medium and large); some old and well-constructed 'permanent' dens on the soft bottom of the Rodas Inlet were used successively by several generations of octopuses. In the present study, we observed that octopuses occupied an empty burrow regardless of body weight in a laboratory observation experiment, similar to Aronson (1986) who found that in addition to a larger intruder driving smaller *O. briareus* occupants and forcibly occupying the den, a smaller intruder could win the use of den in the wild in rare cases. A similar result to Aronson (1986) was also found by Yarnall (1969) for *O. cyanea* under artificial pond conditions. Nevertheless, Hartwick et al. (1978) found that *E. dofleini* usually were size-matched with the previous occupant in the reoccupancy experiment.

The different shapes and sizes of the cross-sections of the underground channel within a burrow seemed to be explained by the intensity of the occupant's breathing movements. We suspect that it was the unrestricted breathing activity that made the diameter of LG significantly larger than that of the HC and DC. Observations in the laboratory showed that the DC was used only as a passageway, and the diameter of the DC was only as large as necessary to allow the soft body to enter or exit the burrow. The small and circular cross-section of the DC also meant that the octopus had to crawl through the DC with a contracted body and barely breathe in the DC. As the cross-section became wider and more elliptical in the HC, the breathing process probably recovered, but mantle movement was still restricted, so that the digger sometimes had to leave the burrow for a while to breathe (Fig. 12A). However, there must be a space in the burrow where the minimum diameter allows the occupant to freely inhale fresh water into the mantle for effective gill ventilation. Our observational experiments showed that the occupants were consistently found in an enlarged section at the end of the HC, which we labelled the LG. This chamber, which had the widest elliptical cross-section, suggested that the occupant's mantle bulge could reach its full amplitude within it. Undoubtedly, it is reasonable to speculate that the diameter of the DC is roughly equal to the mantle width of the digger's contracted body when drilling through the DC, and that the diameter of the LG is roughly equal to the mantle width of the digger's bulging body when a full indraft of fresh water is taken into the mantle. This explains the extreme difference in diameter between the LG and DC (and HC); and if the digger contracts its body and bulges its mantle as much as possible in a certain proportion, it is logical that there is very significant correlation between the diameter of the LG and DC (and HC). Although burrows can be reoccupied by larger octopuses, we observed that the successors will modify the burrow size according to their own body size. In addition, the likelihood that the tidal flow causes the much softer and looser sediment on the surface to collapse and flow toward the center of the DH contributes to the irregular opening of the DH; alternatively biotic interference, such as movement of macrobenthos and vegetation blockage, may also result in irregular openings.

Thus, given the unrestricted breathing, we speculate that *O. minor* generally resides in the spacious LG in the wild, as it did in the observation experiment. The burrowing models (Fig. 5) showed that the BCs emanated from the LG and extended up to the seabed surface, indicating that the octopus remains at the end of the DC, excavating the BC and breathing; it rarely extends the HC after forming the BC. This burrowing strategy is consistent with the behavioral observation experiment in which the octopuses stopped excavating the DCs once the BCs reached the outside (Stage 4 in the process of burrow digging); this strategy is destined to cause DHs and BHs to be distributed at opposite ends of the burrow, as described in the Results section.

Yamamoto (1942) described the tunnel-dwelling habits of this species (synonym, *O. variabilis*) in Korea. Yamamoto (1942) provided a preliminary description of the burrow structure but did not describe the LG, perhaps because of difficulties in keeping the structure intact during manual excavation to expose the internal structure. We compared the data recorded by Yamamoto (1942) with similar measurements made in the present study (Table 1). Substrate type, which depends on sediment particle size, is likely an important cause of these differences (Dorgan 2015).

As the water flow generated by the breathing process gushes out of the end of the BC, the relatively lighter and finer sands in the soft-sediment substrate around the end of the BC are generally expelled, leaving behind coarser sand, and then the soft-sediment substrate is deposited around the BH to form a BHH. Consequently, as observed in the present study, cement infiltrates into the cracks and solidifies with the coarser sand surrounding the BH to form an inverted conical concrete block (Figs. 5 & A1C–D) after quick-setting cement is poured into the burrows. Only one BHH was found in each burrow, although sometimes more than one BH were observed within a burrow, indicating that the occupant kept using only one BC for a period. Sometimes the occupant extended its arm tips out of the BH to dredge the BC. The cyclicality, frequency and reasons for altering the BC to breathe need further study.

Table 1. Summary of burrow features observed in the present study and in Yamamoto (1942). DH: digging hole; LG: lounge; BH: breathing hole; D_{DH} and D_{BH}: mean diameters of DH and BH, respectively. n = 85 (n = 25 for D_{BH}) for this study and n = 15 for Yamamoto (1942)

Category	Minimum–maximum (median) (cm) Depth	D_{DH}	D_{BH}	Difference between D_{DH} and D_{BH}	Presence of LG	Number of DHs
Yamamoto (1942)	5–100 (28.7[a])	2–6 (5[a])	2–6 (2[a])	No	No	1
Present study	8.9–23.4 (21.6[b])	1.8–10.5 (5.9 [b])	0.6–5.1 (1.6[b])	Highly significant	Yes	1–2

Note: Significant differences were tested using the Mann-Whitney *U*-test. Data in the same column with different superscript letters are significantly different ($p \le 0.05$)

We found that octopuses smaller than 29.8 g do not excavate burrows. Bo et al. (2014) found that smaller octopuses often seem to prefer rocks/stones and shells as shelter when available. This could be explained by the inability of small octopuses to expropriate and/or excavate burrows, or it could be a matter of convenience that saves smaller octopuses of high growth rates from continuously needing larger dens (Katsanevakis & Verriopoulos 2004). However, the results of the present study showed that smaller individuals were more likely to occupy burrows with multiple BHs (Fig. 11), which was surprising given that larger individuals require more ventilation. We speculate that a higher number of BHs facilitate the ease of escape because the occupants sometimes drilled out of the burrow from a BH when we attempted to force the occupant out of the burrow before the quick-setting cememt was poured in.

5. CONCLUSIONS

In this study, we investigated the burrows of the mud-dwelling octopus species *O. minor*. We obtained models by pouring quick-setting cement into the burrows. These burrows were not simple underground dens but contained 7 distinct structural parts. At the base of the BC and at the end of the HC, a spacious chamber LG was found. In addition, we identified 7 types of burrows with varying numbers of DHs and BHs. Differences in length and diameter of these structural components were found. Additionally, we verified that this species is able to dig tunnels in the laboratory that are similar to those found in the wild, and the process of excavating a burrow by *O. minor* included 5 stages.

Acknowledgements. We thank Professor Chung-Cheng Lu for correcting the grammar and providing ideas for the article. We also thank the local fishermen of Mashan Group Co. Ltd. (Shan-Dong Province, China) for taking us to the seabed for sampling. This work was supported by the Natural Science Foundation of China (31672257, 32170536) and the Fundamental Research Funds for the Central Universities (201822022).

LITERATURE CITED

Anderson TJ (1997) Habitat selection and shelter use by *Octopus tetricus.* Mar Ecol Prog Ser 150:137–148

Aronson RB (1986) Life history and den ecology of *Octopus briareus* Robson in a marine lake. J Exp Mar Biol Ecol 95: 37–56

Bo QK, Zheng XD, Wang PL, Bi KZ (2014) Basic growth relations in experimental rearing of newly hatchlings of *Octopus minor* (Sasaki, 1920). Oceanol Limnol Sin 45: 583–588 (in Chinese with English Abstract). http://qdhys.ijournal.cn/hyyhz/ch/reader/view_abstract.aspx?file_no=20140200056&flag=1

Bo QK, Zheng XD, Gao XL, Li Q (2016) Multiple paternity in the common long-armed octopus *Octopus minor* (Sasaki, 1920) (Cephalopoda: Octopoda) as revealed by microsatellite DNA analysis. Mar Ecol 37:1073–1078

Bo QK, Zheng XD, Chen ZW (2020) Feeding intensity and molecular prey identification of the common long-armed octopus, *Octopus minor* (Mollusca: Octopodidae) in the wild. PLOS ONE 15:e0220482

Boletzky Sv (1996) Cephalopods burying in soft substrata: agents of bioturbation? Mar Ecol 17:77–86

Cochrane KL, Garcia SM (eds) (2009) A fishery manager's guidebook. John Wiley & Sons Press, New York, NY

Dong ZZ (1988) Fauna sinica. Science Press, Beijing (in Chinese)

Dorgan KM (2015) The biomechanics of burrowing and boring. J Exp Biol 218:176–183

Finn JK, Tregenza T, Norman MD (2009) Defensive tool use in a coconut-carrying octopus. Curr Biol 19:R1069–R1070

Guerra Á, Hernández-Urcera J, Garci ME, Sestelo M and others (2014) Dwellers in dens on sandy bottoms: ecological and behavioural traits of *Octopus vulgaris.* Sci Mar 78:405–414

Hanlon RT, Chiao CC, Mäthger LM, Barbosa A, Buresch KC, Chubb C (2009) Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. Philos Trans R Soc Lond B Biol Sci 364:429–437

Hartwick EB, Thorarinsson G (1978) Den associates of the giant Pacific octopus, *Octopus dofleini* (Wulker). Ophelia 17:163–166

Hartwick EB, Breen PA, Tulloch L (1978) A removal experiment with *Octopus dofleini* (Wulker). J Fish Res Board Can 35:1492–1495

Katsanevakis S, Verriopoulos G (2004) Den ecology of *Octopus vulgaris* Cuvier, 1797, on soft sediment: availability and types of shelters. Sci Mar 68:147–157

Kim DH, An HC, Lee KH, Hwang JW (2008) Optimal economic fishing efforts in Korean common octopus *Octopus minor* trap fishery. Fish Sci 74:1215–1221

Kolb HH (1985) The burrow structure of the European rabbit (*Oryctolagus cuniculus* L.). J Zool 206:253–262

Leite TS, Haimovici M, Mather J, Lins Oliveira JE (2009) Habitat, distribution, and abundance of the commercial octopus (*Octopus insularis*) in a tropical oceanic island, Brazil: information for management of an artisanal fishery inside a marine protected area. Fish Res 98:85–91

Mather JA (1982) Choice and competition: their effects on occupancy of shell homes by *Octopus joubini*. Mar Behav Physiol 8:285–293

Montana J, Finn JK, Norman MD (2015) Liquid sand burrowing and mucus utilisation as novel adaptations to a structurally-simple environment in *Octopus kaurna* Stranks, 1990. Behaviour 152:1871–1881

Richter JN, Hochner B, Kuba MJ (2016) Pull or push? Octopuses solve a puzzle problem. PLOS ONE 11: e0152048

Sauer WHH, Gleadall IG, Downey-Breedt N, Doubleday Z and others (2021) World octopus fisheries. Rev Fish Sci Aquacult 29:279–429

Soil Survey Staff (2010) Keys to soil taxonomy, 11th edn. USDA Natural Resources Conservation Service, United States Department of Agriculture, Washington DC. https://nrcs.app.box.com/s/xi57bj6zyo601eokr7v715mkdpeaa81h/file/1020963161643

Trueman ER, Ansell AD (1969) The mechanisms of burrowing into soft substrata by marine animals. Oceanogr Mar Biol Annu Rev 7:315–366

Yamamoto T (1942) On the ecology of *Octopus variabilis typicus* (Sasaki), with special reference to its breeding habits. The Malacological Society of Japan [currently: Venus] 12: 9–20 (in Japanese with English Abstract). https://www.jstage.jst.go.jp/article/venusomsj/12/1-2/12_KJ00004338393/_article/-char/ja

Yarnall JL (1969) Aspects of the behaviour of *Octopus cyanea* gray. Anim Behav 17:747–754

Appendix.

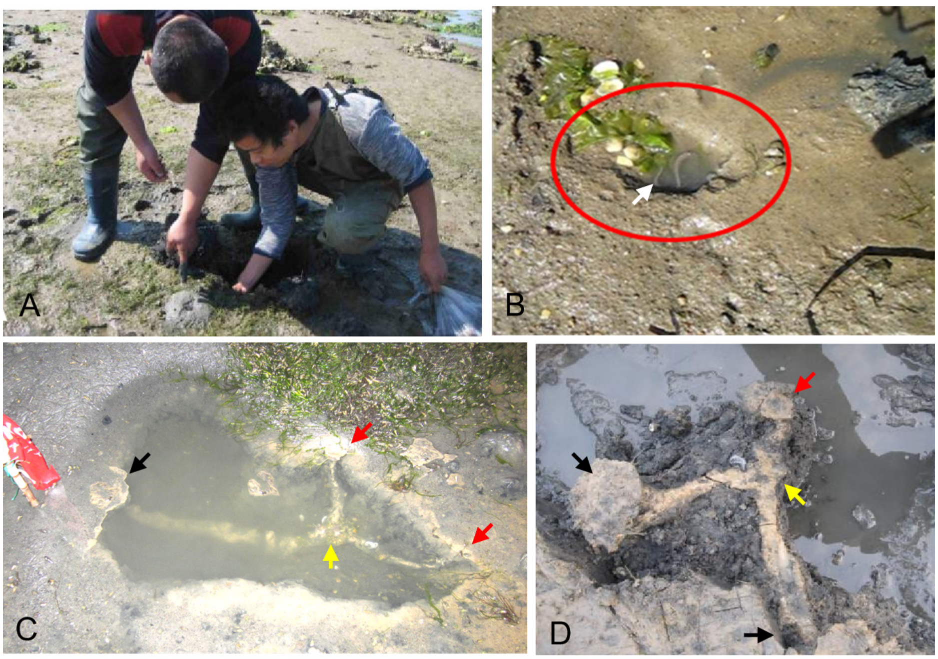

Fig. A1. (A) Locating burrows in the wild via breathing hole heap (BHH) and digging hole (DH) findings. (B) An octopus stretches its arm tips out of the DH. (C) Plasticized-cement model of an Octopus minor burrow with one DH and 2 breathing holes (BHs) removed from the sediment in situ. (D) Plasticized-cement model of a burrow with 2 DHs and one BH removed from the sediment in situ. The white, black, red and yellow arrows in (B–D) indicate the octopus arm tips, DH, BHs and lounges (LGs), respectively

Editorial responsibility: Roger Villanueva, Barcelona, Spain
Reviewed by: A. Guerra, Y. Ikeda

Submitted: February 17, 2023
Accepted: June 22, 2023
Proofs received from author(s): September 4, 2023