Two new methods for opening closed carapaces of preserved Ostracoda (Crustacea)

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Scharf, B., F. A. Viehberg & C. Meisch, 2016. Two new methods for opening closed carapaces of preserved Ostracoda (Crustacea). *Bulletin de la Société des naturalistes luxembourgeois* 118 : 149-153.

Abstract. Specific identification of ostracods (Crustacea, Ostracoda) usually relies on the study of the characters of both the carapace and appendages. As preserved specimens often possess closed carapaces, removal of the soft body necessitates the destruction of one or even both valves which are then lost for further studies. Two methods for the gentle opening of closed carapaces with preservation of both valves are here presented. In a first step, the ostracod is picked up from the preservation liquid (i.e., alcohol) and exposed to the air. By evaporation, a gas bubble rapidly builds up in the closed carapace. In a second step, the animal is exposed to lowered air pressure, which causes the expansion of the gas bubble and therefore the opening of the carapace. Two procedures for the production of lowered air pressure are proposed: the first one uses a camping air pump connected to a desiccator, the second one a syringe associated with a vial covered with a cap that includes a central membrane. Both methods do not cause any damage to the valves nor the soft body of the ostracod.

Key words. Ostracoda, closed carapace, carapace opening, low pressure.

1. Introduction

Ostracods are small, mostly around 1 mm long crustaceans the body of which is totally enclosed within a carapace consisting of two dorsally hinged valves. Only the tips of the appendages stick out of the ventral opening of the carapace when the animal moves over or within the substrate or swims within the water column.

Although carapace shape, ornamentation and colour permit easy recognition of a number of species, the identification of others relies on the examination of the structure of the appendages and internal characteristics of both valves (Meisch 2000). To accomplish the preparation, the soft body of the animal needs to be removed from the carapace for dissection and examination in the light microscope.

For their study, ostracods are usually killed and subsequently stored in aqueous alcoholic (ethanol or isopropanol) solutions of 70 % (Meisch 2000), which concentration entails partially open but often also closed carapaces. At higher concentrations the animals mostly die with tightly closed carapaces (observations of the present authors). It should be noticed that for genetic analyses, such as DNA barcoding, it is recommended to kill and store the animals in 95-100 % ethanol (Smith & Horne 2016).

When dealing with closed carapaces the ostracod taxonomist is facing some sort of dilemma, being forced to break at least one of the two valves in order to extract the soft body from the carapace. The entire carapace or at least one valve is therefore lost for analytic studies or for images to be taken in the scanning electron microscope. This is



Fig. 1. Equipment of the camping air pump method.

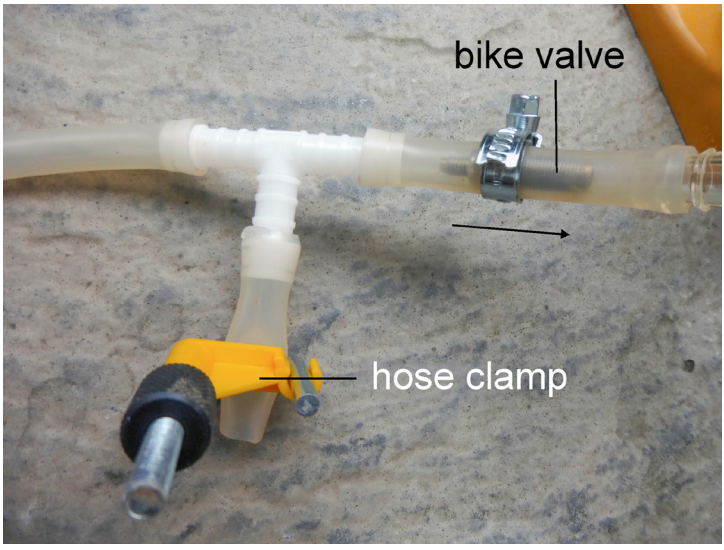


Fig. 2. Detail of Fig. 1, with the bike-valve in place.

particularly annoying when the type material of species is studied or when only a few specimens are available for examination.

When living animals are available, the following procedures may be applied.

Klie (1938: 17) recommends to introduce the living ostracods into boiling water for ½ - 1 minute, which results in open carapaces.

More recently, a number of contributors to OSTRACON, the discussion list on the Ostracoda on the Internet, have suggested a number of alternative methods (OSTRACON 2005, 2007):

(1) The ostracods are introduced into a solution prepared with 3 parts of methanol and 1 part of chloral hydrate crystals. The opening of the carapaces takes about 20 minutes.

(2) A few drops of a solution of 10 % pentobarbitone (Nembutal®) are added to the dish containing the ostracods. More drops should be added over the next few hours until the specimens relax and open their valves.

(3) A few drops of a solution of 96 parts of water, 3 parts of 95 % ethanol and 1 part of propylene-phenoxetol®. Relaxation with open carapaces should take 30–40 minutes.

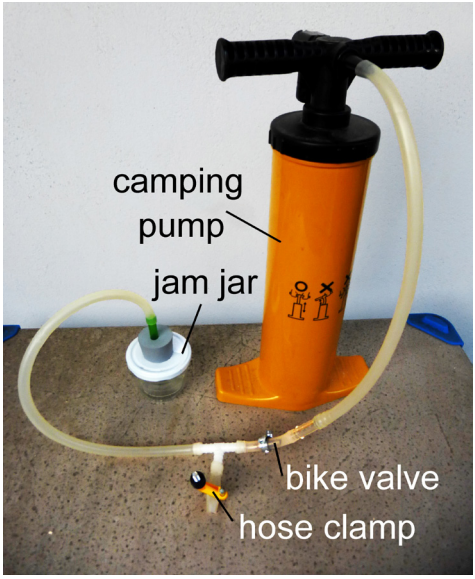


Fig. 3. Camping air pump method: the desiccator is replaced with a jam jar.

(4) A cigarette is soaked in water until the latter discolours, then a few millilitres of the solution are added to the water containing the living ostracods.

(5) A few drops of 96 % or 100 % ethanol are added to the water containing the living ostracods. Once the animals stop to react vividly, they are transferred into 70 % ethanol for permanent preservation. Most of the animals die with open carapaces. One of us (B.S., unpubl.) has worked successfully with this method up to now.

(6) It is possible to open the carapace by progressively heating up the 70% ethanol to boiling temperature. The increase in temperature produces gas within the carapace, which will result in its opening, sometimes explosively so. However, a boiling retardation may occur and the animal will be ejected from the test tube and therefore be lost.

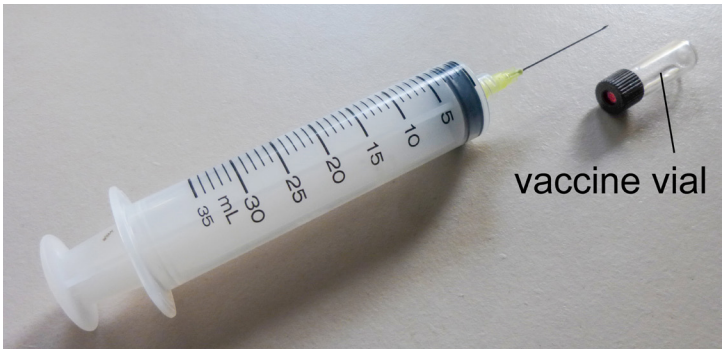


Fig. 4. Equipment of the syringe method.

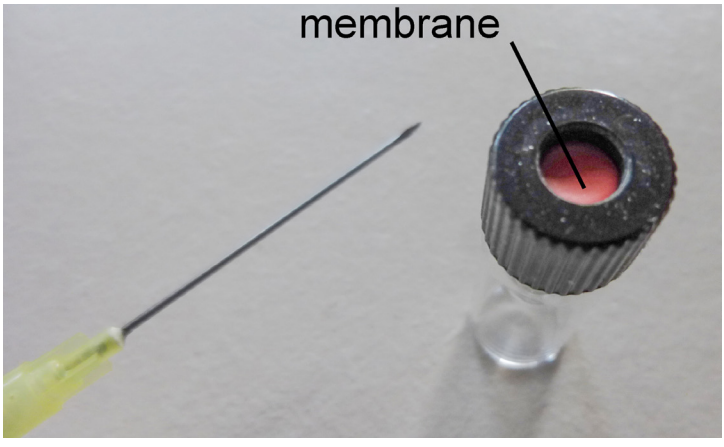


Fig. 5. Detail of Fig. 4: the syringe needle and the vaccine vial.

If a collection of preserved ostracods with closed carapaces is studied, the methods described above cannot be applied. One possible solution is to introduce the preserved ostracods into boiling water, after which the carapaces will open explosively (D. Keyser, Hamburg, pers. comm.). The boiling-point for water is 100 °C, that of ethanol is 78 °C, therefore the ethanol within the carapace expands abruptly into the gaseous phase and this will cause the opening of the carapace.

Here we describe two new techniques for the controlled, gentle opening of closed carapaces of ostracods previously preserved in c. 70 % or higher ethanol concentrations.

2. Methods

The following two techniques for the opening of carapaces of preserved ostracods are described. The first method uses a desiccator (or a jam jar) and a camping pump (Figs 1-3). The second method uses a vaccine vial and a syringe.

2.1. The camping air pump method (Figs 1-3)

Camping air pumps are commonly used for inflating air mattresses. The proposed procedure is as follows.

(a) Pick up the ostracods with closed carapaces from the ≥ 70 % ethanol solution. Let the animals dry up in a small Petri dish (e. g., 5.5 cm in diameter). It can be seen in the stereomicroscope that a relatively large gas bubble builds up within the carapaces within about 1 minute (occasionally, however, this will take a bit longer).

(b) Add a few drops of ethanol over the animals and close the Petri dish with its cover.

(c) Introduce the Petri dish into the desiccator and close the latter.

(d) Generate low pressure with the camping air pump within the desiccator. It should be noticed that the low pressure produced by the usage of a simple water aspirator as commonly used in chemistry and biology laboratories, is not powerful enough to cause the opening of the carapaces.

(e) Open the hose clamp to reduce the vacuum. Normal air pressure is now re-established in the desiccator.

(f) Remove the dish from the desiccator and check the open carapaces in the stereomicroscope.

Alternatively, the desiccator may be replaced with a jam jar. However, according to our experience the jar does not stand as stable as a desiccator. If only possible a glass instead of a plastic desiccator should be used, this because ethanol is liable to damage the plastic material.

2.2. The syringe method (Figs 4-5)

This method uses a syringe and a 2 ml so-called vaccine vial, i.e. a vial with a membrane incorporated in the screw cap (figs 4 and 5). Both devices are commonly used in medical practice. Begin with step (a) as above. The animal with the air bubble within its carapace is then introduced into the vial together with some drops of ethanol before the membrane screw cap is tightly in place. The needle of the syringe is pushed vertically from the top through the membrane without touching the ethanol surface. Finally, the syringe is filled to produce reduced pressure within the vial.

The value of the lowered pressure within the vial may be calculated by comparing the volumes before the generation of the vacuum with those of the lowest pressure obtained. The normal atmospheric pressure of c. 1013 hPa is reduced to c. 500 hPa with the first method and down to c. 50 hPa with the syringe-method (depends on the syringe volume).

3. Results and discussion

Both methods were tested with closed carapaces of a number of both relatively large and small ostracods (approximate lengths after Meisch 2000): *Eucypris virens* (c. 2.0 mm), *Bradleystrandesia fuscata* (c. 1.5 mm), *Pseudocandona albicans* (c. 0.8 mm), juveniles of *Candona candida* (< 0.8 mm), *Cypria ophtalmica* (c. 0.6 mm), and *Cyclocypris ovum* (c. 0.5 mm). After each of the

two treatments, all carapaces were open, with the exception of those of *P. albicans*. The test with *P. albicans* was repeated with a larger gas bubble within the carapace, and was then successful. In general, the two methods proposed here produce similar results. In all cases the opening of the carapaces was wide enough to permit the introduction of dissecting needles into the inner carapace.

Both methods rely on the fact that gas expands its volume unlike liquids (incompressible) under lowered pressure. The enlarged gas volume opens the carapace sufficiently to permit the introduction of the dissecting needles in order to separate the valves and remove the soft body of the ostracod.

Both methods lead to the rupture of the adductor muscles – their contraction closes the carapace – during the opening process, which rupture greatly facilitates the separation of the valves.

Once the air bubble has built up within the carapace in the dry Petri dish, we recommend adding a few drops of ethanol to the animal. This makes ethanol instead of air to be sucked into the carapace during the opening process. As a result, the carapace will not rise to the surface of the ethanol, where separation of the valves is uneasy.

According to our experiments neither the valves nor the soft parts were damaged by the two techniques.

Both methods produce excellent results in less than five minutes. It is possible to treat several animals simultaneously. Sometimes the building up of a sufficiently large gas bubble in the carapace takes a little more than one or two minutes.

Important advantages of the two methods described here are the low-cost equipment and, above all, the gentle opening of the carapaces in a short time without any damage inflicted to valves and soft parts.

After experimenting with both methods we recommend the preferential usage of the syringe method. Indeed, the equipment is small enough to be permanently placed on the laboratory bench close to the microscope, the usage is easy and this method produces a lower pressure than the first one.

Acknowledgments

We thank Renate Matzke-Karasz (University of Munich) for drawing our attention to the discussion on Ostracon about the open carapace killing of ostracods. We are grateful to Dietmar Keyser (University of Hamburg) for discussing the new methods proposed here.

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