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Soft body morphology, dissection and slide-preparation of Ostracoda: a primer

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Abstract: Most commonly used techniques for treating ostracod soft body for taxonomical purposes with optical microscopy are described with emphasis on the order Podocopida. A variety of procedures for pre-treatment, storage, recovery of dried specimens, dissection, temporary and permanent mounting, and staining methods are presented and evaluated. General morphology and terminology of the ostracod appendages are also summarised.

Key Words: Ostracoda; Dissection; Mounting; Staining; Appendages; Morphology.

1. Introduction

One of best diagnostic and most conspicuous trait of Ostracoda is a bivalved carapace that may completely envelop the whole animal body with limbs. Ostracodologists usually refer to calcified valves as “hard parts”, whereas to the appendages and other internal organs as „soft parts”. The classification of Recent ostracods is based mainly on differences in the soft part morphology which is ordinarily of little use by palaeontologists. However, considering the close functional relationship often existing between the soft and hard parts, each expressing to a considerable extent the morphology of the other, even basic knowledge on the appendages without doubt should help palaeontologists to better understand and interpret various features displayed in the valves of the fossil ostracods. Examining living material collected from modern habitats enables palaeontologists also to become familiar with intraspecific variability or ecology, which could greatly facilitate both taxonomical and palaeoenvironmental studies. Therefore the morphology and terminology of the soft parts of ostracods is outlined (focusing on limbs) in this primer prior to the description of various laboratory techniques involved in the appendage preparation for optical microscopy. Analogously, one may advise a biologist (neontologist) to familiarise oneself with the details of the “hard parts” morphology and some basic laboratory techniques used routinely in micropalaeontological work.

Several laboratory techniques and chemicals used in the study of the soft body of ostracods are in many respects analogous to those applied to other aquatic animals of similar size. However, the presence of the overdeveloped calcified carapace in ostracods, has proved necessary to develop a number of special methods for treating both Recent and fossil specimens. The bivalved nature of the carapace coupled with the small size of most ostracods appear also to be an important obstacle and the reason of a disinclination to study this group by biologists, since acquiring the expertise to perform properly a full dissection can take sometimes several months.

The present introductory text was intended to be used primarily by palaeontologists and novices working mainly with hard parts, thus it was deemed not necessary to include in it a detailed description of the techniques typically pertaining the study of the valves. Both preparation techniques and general morphology of the ostracod soft body is presented and exemplified by the order Podocopida (or subclass Podocopa; classification scheme according to Horne et al. 2002) which is the most species-rich and diverse ostracod group at the present day as well as has the best fossil record (Horne et al. 2002).

For the practical knowledge concerning methods of sampling, sample processing, initial laboratory treatments and culturing of Recent ostracods the reader is referred to other publications in which such procedures are described in detail, of which the most recommendable are the following: Bronstein (1947, Engl. transl. 1988); Athersuch et al. (1989); Henderson (1990); Griffiths & Holmes (2000); Meisch (2000) and Danielopol et al. (2002), as well as those concerning meiofauna in general (e.g., Higgins & Thiel 1988; Galassi et al. 2002; Sommerfield et al. 2005 or Giere 2009).

2. General morphology and terminology of the soft body

Although several authors have recently attempted to unify terminology pertaining ostracod limbs and their chaetotaxy (e.g., Hartmann 1966–1989; Danielopol 1978; Broodbakker & Danielopol 1982; Martens 1987; Matzke-Karasz 1995; Hartmann & Guillaume 1996; Meisch 1996; Cohen et al. 1998; Martens 1998; Meisch 2000; Maddocks 2000; Horne 2005; Karanovic 2005; Smith & Tsukagoshi 2005; Smith et al. 2005; Tsukagoshi et al. 2006; Boxshall et al. 2010), there is still lack of consensus on this matter in the literature and many confusion exists about limb homologies with other crustacean taxa and even within Ostracoda. Here we adopted terminology of the general limb morphology after Horne et al. (2002).

Ostracods have a short compact body (Fig. 1) with no true segmentation as often recognisable in other crustaceans. A faint constriction of the body usually just in front of the centre (indicated by a dashed-line in Fig. 1) marks the indistinct boundary between two main parts, the anterior head (cephalon) and the posterior trunk (consisting of the reduced thorax and the rudimentary abdomen). The later portion shows in a few

taxa some external traces of segmentation, suggesting 4–7 (subclass Myodocopa) or 10–11 (subclass Podocopa) barely discernible postcephalic segments (Horne et al. 2002).

Ostracod limbs (or appendages), excepting the antennula (or first antenna), are considered to derive from a generalised ancestral crustacean appendage composed of a basal protopod on which distally two rami are carried: an inner endopod (commonly larger) and an outer exopod (often strongly reduced). The rami (and sometimes protopod) are usually composed of a number of podomeres (articles or joints). From the protopod may arise medial endites and lateral exites (epipods if branchial in function). Adult ostracods possess up to eight pairs of functionally specialised limbs, including male copulatory appendages (Horne et al. 2002), which is the fewest number of limbs of any crustaceans (Brusca & Brusca 2003).

In Podocopa four pairs of limbs are considered to be clearly attached to the cephalon, which is untypical for crustaceans (for some details on a debate as to whether ostracods have four or five cephalic appendages see Meisch (2000) or Horne et al. (2002) and references therein). These are the following limbs from the anterior-most: antennula (A1), antenna (A2), mandibula (Md) and maxillula (Mx1) (Fig. 1). First two pairs (and the eye) are attached to the pre-oral forehead, while mandibula and maxillula are connected to the hypostome, constituting the ventral posterior portion of the cephalon, and forming the posterior edge of the mouth opening. The forehead, hypostome and upper lip (laying below the forehead and forming the anterior edge of the mouth) constitute parts of the complex chitinous framework of the cephalon. In the mouth region there are also food-rakes assisting with chewing food (MEISCH 2000).

The antennula (A1, Fig. 1) is uniramous (Boxshall et al. 2010), composed of 5-8 podomeres bearing a number of various setae and claws (up to c. 30 in Podocopa: Maddocks 2000; Smith & Tsukagoshi 2005), and has locomotory (swimming, crawling and/or burrowing) and sensory functions (served by chemo-sensorial setae or aesthetascs). It is usually long, flexible and moving upward and back. The chaetotaxy of A1 (the number and arrangement of various setae) as well as the number of podomeres differ in the various groups, providing useful but yet not fully exploited diagnostic characters. It is advised here to follow the antennular podomere notation of Smith & Tsukagoshi (2005), however, this notation can be applied successfully only if the ontogeny of A1 of a given species is sufficiently known.

The antenna (A2, Fig. 1) is biramous, however, the degree of development of the two rami, arising from usually one podomere-protopod, differs substantially in Myodocopa and Podocopa (Horne et al. 2002). In the former subclass the exopod is well developed (with at least 9 podomeres), while the endopod is reduced. On the contrary, in Podocopa it is the endopod which is better-developed ramus (with 3–4 podomeres armed with various setae, aesthetascs and terminal claws), whereas the exopodite is rudimentary (in a form of a small scale with short seta(-e) or of a long spinneret seta, except the order Platycopida where the exopod is developed almost as strongly as the endopod). The antennae are the most important locomotory appendages for ostracods,

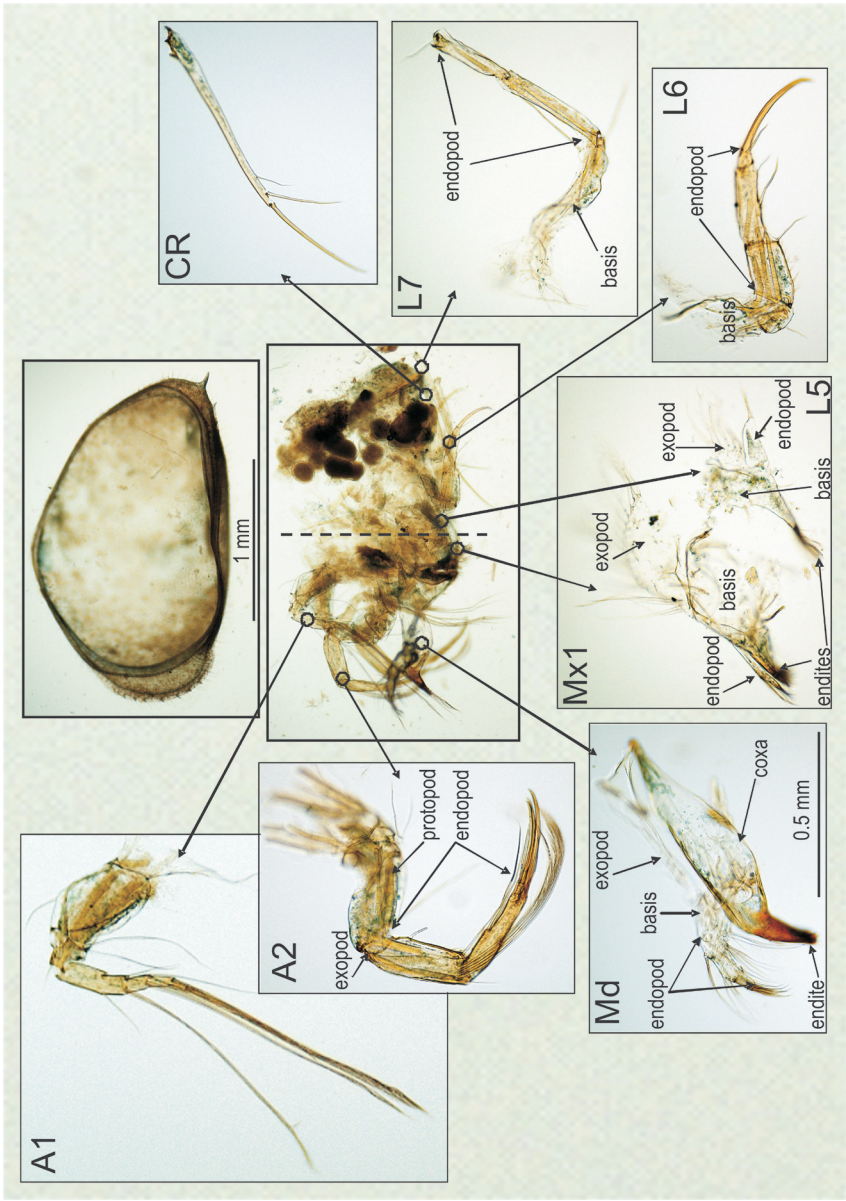

Fig. 1: Morphology of a female of *Cypris pubera* O.F. MÜLLER as an example of a cypridoidean ostracod (Podocopa: Podocopida: Cypridoidea: Cyprididae). Right valve in internal lateral view, the whole body removed from the valves and dissected individual limbs: A1 – antennula, A2 – antenna, Md – mandibula, Mx1 – maxillula, L5 – fifth limb (maxilliped), L6 – sixth limb (walking leg), L7 – seventh leg (cleaning leg), CR – caudal ramus.

with long natatory setae in swimming forms and/or chelate claws for crawling and burrowing. The complex chaetotaxy of A2 (often sexually dimorphic) is a significant character in ostracod taxonomy, sometimes also allowing distinction of particular larval stages. It is strongly recommended to consult the A2 chaetotaxic schemes and terminology for the detailed taxonomical study (Broodbakker & Danielopol 1982; Martens 1987; Meisch 2000 for the superfamily Cypridoidea, and Rossetti & Martens 1996, 1998 for Darwinuloidea, as well as Kaji & Tsukagoshi 2010 for homology of the antennal chaetotaxy among all podocopid superfamilies).

The mandibula (Md, Fig. 1) functions mostly as a feeding organ and typically corresponds well to the general structure of the ancestral crustacean biramous appendage, having both rami developed in addition to the protopod (Horne 2005). The protopod is composed of two podomeres, the large and heavily sclerotized coxa ventrally bearing strong endite (teeth), and the basis, which bears the exopodial branchial plate (often greatly reduced) and constitutes the first segment of a mandibular palp, consisting also of the endopod. The endopodal part of the palp is armed with various setae, the number and structure of which proved to be important diagnostic traits for some groups (e.g., the subfamily Candoninae). For the terminology of the mandibular chaetotaxy of the superfamily Cypridoidea see Broodbakker & Danielopol (1982).

The maxillula (Mx1, Fig. 1) is the fourth pair of the cephalic limb, usually modified to a great extent, having masticatory and respiratory functions. In the subclass Podocopa Mx1 consists of a) the single-podomere protopod bearing three endites, b) the endopod constituting a palp with up to three podomeres and c) the exopod forming usually a well-developed, large branchial plate, morphology of which seems to offer useful diagnostic and phylogenetical features (Horne 2005; Smith et al. 2005). Myodocopa do not possess large exopodial branchial plates, instead, they have them on the fifth limb, while on Mx1 small epipodial (arising from the protopod) branchial plates may be observed in some taxa (Horne 2005).

The four pairs of the head limbs are followed by three pairs of the trunk limbs, one pair of the male copulatory appendages and a pair of caudal rami (or furcae). The trunk limbs vary in structure and function among taxa.

The fifth limb (L5, Fig. 1) differs in the structure in various taxa depending on the function it performs and is regarded important in classification in several groups. It may serve as a locomotory appendage (for instance in the superfamilies Cytheroidea or Bairdioidea) and then it has a form of a walking leg with a single protopod podomere and up to four endopod podomeres (the distal one armed with a strong claw), while the exopod is reduced, represented usually just by one seta or rarely by a well-developed branchial plate (Horne 2005). This limb may be also modified for feeding (e.g., in the superfamily Cypridoidea) and forms a maxilliped with a single protopod podomere (provided with setae used in feeding), a leg-like or palp-like endopod, and a small or totally lacking exopodial branchial plate. In males of Cypridoidea the endopod is transformed into an often unsymmetrical clasping organ used for holding the female during copulation.

In several Myodocopa the fifth limb serves as a respiratory (and/or filter feeding) appendage as it bears a large epipodial branchial plate (Horne 2005; Cohen et al. 2007). Between or just in front of the fifth limbs the so-called brush-shaped organs, considered vestiges of an additional pair of appendages of an unknown function, are found in males of several representatives of the order Podocopida (e.g., the suborder Cytherocopina; Horne et al. 2002).

The sixths limb (L6, Fig. 1) in the majority of the representatives of the subclass Podocopa is a uniramous walking leg with the (usually undifferentiated) protopod and up to four podomere long endopod, armed distally with a strong claw. In other taxa it may be a walking leg with an epipodial branchial plate (suborder Halocypridina), lamelliform (order Myodocopida), modified into claspers in males and being rudimentary in females (order Platycopida) or absent (suborder Cladocopina; Cohen et al. 1998, 2007; Horne et al. 2002; Horne 2005).

The seventh limb (L7, Fig. 1) in the Podocopa is either a walking leg similar to the L6 (as in e.g., the order Palaeocopida or the suborders Cytherocopina and Darwinulocopina of the order Podocopida) or a cleaning leg, directed upwards, often terminated with a set of complex pincers and used for removing foreign material from the interior of the valves (as in Cypridocopina) or it is completely lacking (as in Platycopida; Horne et al. 2002). In the Myodocopa this limb has the cleaning function, being long, vermiform and flexible (as in the order Myodocopida) or it is greatly reduced or totally absent (as in the order Halocyprida; Cohen et al. 2007).

The male copulatory appendages (or hemipenes), located in front of or attached to the caudal rami and usually paired, may be regarded as the transformation and integration of 3–5 pairs of thoracic appendages (Martens & Horne 2009). These are often large and complex structures, varied in various taxa and considered very important taxonomic characters. The detailed internal structure of the copulatory organs is often very difficult to study and needs much practice to obtain satisfactory results. For more details on morphology and terminology pertaining to the hemipenis the reader is referred to McGregor & Kesling (1969), Danielopol (1969, 1978), Martens (1990, 1998), Meisch (2000), Smith et al. (2006) and Smith & Kamiya (2007).

The paired caudal rami or furcae (CR, Fig. 1) are attached to the posteroventral end of the ostracod body, however, their position relative to the anus differs fundamentally in Myodocopa and Podocopa. In the former subclass they are situated posterior, while in the latter – anterior to the anus (Cohen et al. 2007). When fully developed they are plate- or rod-shaped structures with setae and/or claws and have essentially a locomotory function (Myodocopa, Palaeocopida, Platycopida, most Podocopida). In some groups they are reduced to various extent, being represented in extreme case just by minute setae (as in e.g., Cytheroidea or Darwinuloidea). Caudal rami and their attachment are of systematic importance (Horne et al. 2002; Meisch 2007).

3. Dissection, staining and mounting

Although an experienced ostracodologist (zoologist) is sometimes able to identify ostracod specimens to the genus or species level just by the external morphology of the carapace and can dispense with the examination of the soft body, for the accurate identification and description of specimens a complete dissection of the soft parts is usually necessary. Without experience, dissecting appendages is a difficult task, and often, especially in case of small ostracods, it is not possible to avoid damage. Hence, if there is enough material for study, it is recommended for practice that an initiate should examine several specimens and select larger and well-preserved specimens with open or at least not tightly closed valves (Fig. 2).

Fig. 2: If there is enough material for study, larger and well-preserved specimens with open valves should be selected for dissection (indicated by an arrow).

The first step is to open and disarticulate the valves, and then separate valves and the soft body. If live ostracods are being killed for dissection, it is vital to use dilute c. 30 % ethyl alcohol, as this causes animals to die with more or less open valves, making removal of the soft parts from the carapace easier. Alternatively, similar results may be obtained by killing animals by adding narcotics used for anaesthesia or euthanasia in veterinary practice, as e.g., urethane (or ethyl carbamate) (Henderson 1990) or MS-222 (or tricaine methanesulfonate) (Schmit & Mezquita 2010) but these may be toxic and the present authors have no experience with such chemical compounds. Details on other chemicals which may be potentially used in the ostracod studies and are commonly used as pre-preservation anaesthetising agents in other marine and fresh water animals when material must be preserved in a relaxed condition may be found in Green (2001).

Prior to removal the animal soft body from the carapace, the examination of the shape and general surface features as well as measuring of the complete carapace has to be done. Opening the valves of freshly killed or preserved specimens is best carried out on a standard 3×1 inch microscope glass slide in a drop of glycerine under a stereoscopic binocular microscope at a magnification of c. 20–60×. As finally the transmitted light and high magnification will be used for examining fine morphological de-

tails of the dissected appendages, the glass slide should not be thicker than 1 mm and the volume of the glycerine drop should be just enough to fill the area under a cover-slip (see also below). For larger specimens a depression slide, a watch glass or an embryo dish may be used to separate valves from the soft body, and 96 % ethanol or water instead of glycerine as a dissecting medium, if valves are to be used for SEM or geochemical analyses. Two dissecting needles are necessary, the finest entomological pins (no. 000) fitted in dissecting-needle handles (or fixed just to any convenient grip, as e.g., a matchstick) are most suitable. Pasteur pipettes, small flexible forceps, fine brushes and tiny wire loops may also be useful while dissecting, and it is important first to have all necessary materials on hand (Fig. 3).

Fig. 3: A set of materials necessary for dissection and slide-preparation of ostracods: dissecting needles, pipettes, forceps, fine brushes, an embryo dish, nail varnish, mounting medium (Hydro-Matrix®), an aluminium holder for a glass slide and/or cover slips, a three-well embryo slide, a micropalaeontological slide, self-adhesive paper labels, a standard glass slide, depression glass slides, cover slips and a small Petri dish.

If the carapace valves are not tightly closed, the needles should be put between the valves allowing valves to be slightly open (Fig. 4A–C) so that one is able to insert one needle between one valve and the body, and holding the specimen in the place to pry this valve off the body with the second needle, cutting the central adductor muscle and dorsal connection of the body to the valves (Fig. 4D–F). Finally, one should remove the body from the other valve freeing it also from the adductor muscles (Fig. 4G).

Specimens with firmly closed valves may be opened up in a numbers of ways. The most straightforward way is to place one needle in the middle of the ventral margin of

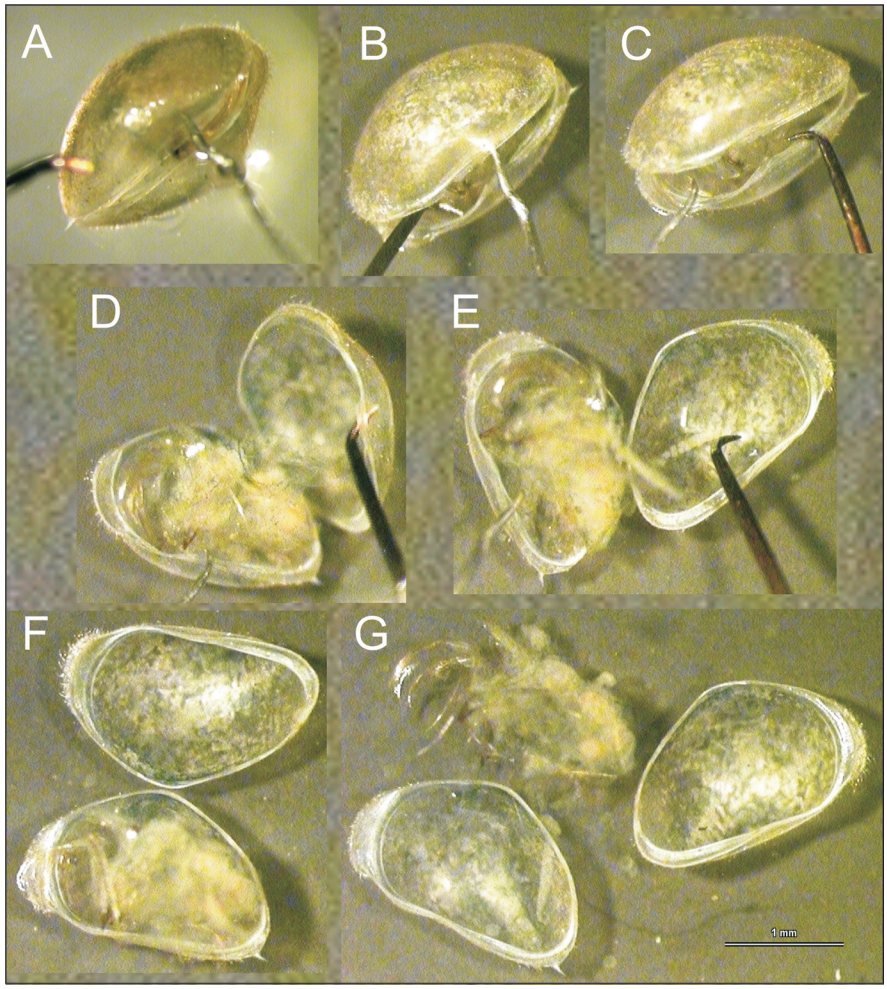

Fig. 4: Successive stages of a separation of the valves and the soft body. Consult text for details.

a specimen immersed in a drop of glycerine on a glass slide and attempt to put a gentle pressure on its dorsal part by the second needle. If this action successfully causes the valves slightly open, the procedure described above can be followed. Sometimes, alcohol fixed specimens may open when simply transferred to water (F. VIEHBERG, pers. comm.). If unsuccessful, sometimes it is necessary to breach one valve a little or more (preferably in the middle of the ventral margin where the mutilation is the least severe) in order to prise the valves definitely apart. As a last resort (particularly when sufficient

material is available for study), specimens with tightly closed valves (especially those minute and with ball-shaped carapaces) can be pressed when in a glycerine drop on a glass slide by a cover-slip to crush the carapace. The appendages (particularly those well-chitinized) may be farther examined either after removing the cover-slip and cleaning the broken valve remains off the body or just as undissected smashed body.

For stubborn specimens when there is enough material for study, other more complex procedures may be also employed which consist of repeated heating and cooling in water or gluing one valve to a slide and prising off the other (see for details Van Morkhoven 1962; Athersuch et al. 1989). The opening of the valves and removing the soft body is one of the most frustrating part of ostracod preparation not only for a novice and only much practice could provide satisfactory results.

As both opening of valves and subsequent dissection of appendages sometimes result in damage and finally partial loss or unavailability of some specimens or parts thereof for further studies, it is always recommended that whenever possible one should retain additional intact voucher specimens preserved in ethanol and deposit these in any recognisable collection for a verification of the identification or for a use in other studies.

When the valves and soft body are separated, the valves have to be removed from glycerine, transferred to a small petri-dish or better to a watch glass and washed thoroughly by immersing in distilled water or alcohol to get rid of the glycerine. Cleaned valves should then be dried in air and finally placed and mounted with a water-soluble gum tragacanth adhesive in labelled cardboard or plastic cavity slides (micropalaeontological slides) for storing (Fig. 5A). Meisch (2000) advocates also to mount valves for permanent preservation in Euparal or glycerine jelly on a microscopic depression slide, which allows observation in transmitted light. The valves can also be stored in a vial of 70–80 % ethyl alcohol. However, sometimes in the long run both Euparal and alcohol cause some decalcification, thus not all workers are fully satisfied with this method. Decalcification of valves can be avoided or minimised when using ethanol which is buffered, absolute (analytically pure) or denatured by butanone or methyl ethyl ketone (F. Viehberg, pers. comm., later method according to B. Scharf).

Dissection of the soft body parts is routinely performed from specimens wet preserved in alcohol (or formalin). If dried, the specimens may be sometimes effectively restored to the state suitable for studying appendages but it requires additional treatments. Athersuch et al. (1989) recommend gradual moistening with water or immersing in 10 % aqueous solution of TSP (trisodium phosphate) for 24 hours, after which the specimens can be washed and returned to alcohol or further dissected (see also Wechsler et al. 2001).

In a day-to-day practice, the dissection of appendages is found easiest to be continued in glycerine on the same glass slide where in the first step valves were separated from the soft body. If that step was done thoroughly, the compact unfragmented body of an animal can be seen under a stereomicroscope at about 20–60×. If you are unfamiliar with the ostracod soft body morphology, it is advisable first to orient the animal,

and sketch the general shape and position of the appendages before separating them from the body. Although most ostracods show no external evidence of segmentation, a slight constriction of the body near the middle indicates the boundary between the anterior head and the posterior trunk (see above). It is recommended to start the dissection of the appendages by dividing the body into these two parts inserting the needles in the middle of the dorsal side and cutting the body along the transverse dorsoventral axis between the fourth and fifth limbs (indicated by a dashed-line in Fig. 1). Then the halves of the body should be divided along the sagittal plane into the right and left portions, and subsequently all appendages gently removed with the needles. In some taxa (representatives of the superfamily Cypridoidea) the fourth and fifth limbs are attached and have to be first removed from the body together and then teased apart (see Fig. 1). Small limbs or reduced caudal rami typical for some taxa are, with a little practice, difficult to localise and dissect, therefore they sometimes are removed together with adjacent parts and not separated.

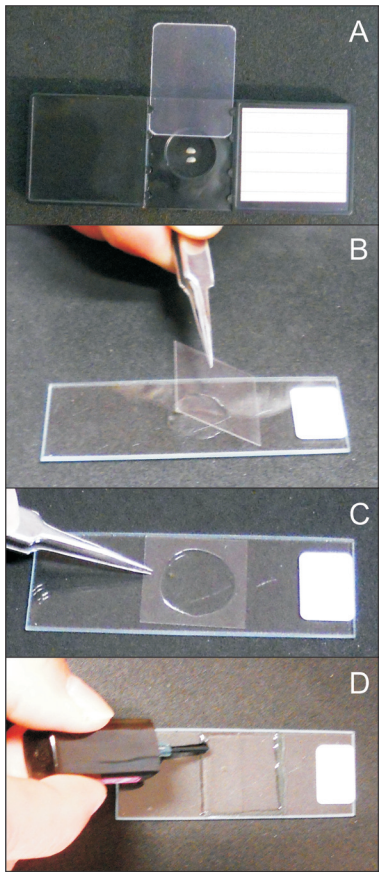

Fig. 5: Cleaned valves placed and mounted in a labelled micropalaeontological slide (A). Covering dissected appendages by a cover-slip (B and C) and final sealing by a nail polish (D).

Anatomical dissection of the hemipenes requires more practice and patience (some details may be found in Danielopol 1982). Care should be taken that no air bubbles remain in glycerine or are attached to pieces of the body causing them to float at the glycerine surface.

Once the appendages are dissected and placed in the centre of the glycerine drop, they are covered carefully with a 15 mm round or 18–20 mm square and 0.13–0.17 mm thick cover-slip as follows. Lower the cover-slip over the drop at an angle, with one edge touching the glass slide first (Fig. 5B). Allow the glycerine to spread slowly out between the glass slide and the cover-slip without applying pressure (Fig. 5C). It takes some practice to determine just how much glycerine to use. If too much is placed on the slide, the cover-slip will float, creating a glycerine layer that is too thick and causing the appendages spread out to the edges of the cover-slip. If too little glycerine is used, the layer is too thin, not extending to the edges of the cover-slip and appendages may be squashed. Finally, the preparation is sealed with a nail polish (Fig. 5D), marked with the label and kept flat and undisturbed in a dust free area. It is also advisable to label slides permanently with a diamond needle in addition to the paper labels.

The appendages are best observed in transmitted light at magnifications 100-200× but for the examination of fine structures magnifications of 400× and frequently 1000× (oil immersion) are necessary. To aid in the observation of details such as minute setae, etc., the systems of phase contrast or differential (or Nomarski) interference contrast as well as staining (see below) can be employed. However, staining is not recommended if the latter contrast is planned to be used.

As in the glycerine it is difficult to control the proper horizontal arrangements of the dissected appendages when covering them by the cover-slip and to avoid their sweeping to or out of the cover-slip edges, as well as considering that a nail polish is sometimes an unstable barrier against glycerine, resulting in the glycerine leaking out, other mounting media are often used and sometimes also other more complex techniques are suggested, if special detailed taxonomic examination is to be done and/or specimens are to be prepared for museum collections (see Danielopol 1982; Athersuch et al. 1989; Meisch 2000). Commonly used mounting media for such purposes include polyvinyl lactophenol (PVL), Hydro-Matrix® and glycerine jelly, or rarely Euparal and Canada balsam. It has to be warned that some of these agents may be harmful, therefore special care must be taken and the preparation has to be carried out in properly ventilated laboratories. The mounting medium should also be selected depending on the clearing effect, the purpose of the mount, the type of microscopy employed or the preservation time. Consult Koomen & Vaupel Klein (1995) for more details. Specimens can be dissected in glycerine (as described above) and then transferred to the eventual mounting medium. However, if the dissection can be completed within a few minutes, i.e., before a mountant becomes dry, it is always practical to dissect directly in the permanent medium, rather than to attempt to move small dissected pieces as it may result in their loss.

According to Athersuch et al. (1989), specimens can be dissected in a glycerine drop at one end of a glass slide and then dissected appendages should be transferred and carefully arranged in a sequence in a PVL film on a cover-slip placed on the other end of the slide. The appendage arrangement should be accomplished within 5–10 min before the medium becomes too viscous. Next, after allowing the mounting medium to dry slightly for a few minutes to fix the appendage positions, the cover-slip should be overturned and slowly laid with forceps over a drop or a streak of PVL placed in the centre of the glass slide. This should be done gently to avoid air bubble formation. Let the mounting medium extend to the edges of the cover-slip and stabilise. At room temperature the mounted appendages are usually ready for examination after a few days. PVL does not require sealing if stored horizontally in room temperature but it is recommended to use a clear nail polish or melted paraffin as sealing materials for longer storage or shipping of the slides.

Instead of PVL, Meisch (2000) recommends the use of Hydro-Matrix® which is likewise soluble in water and alcohol, but in contrast to PLV it is not toxic.

Danielopol (1982) describes the mounting method in glycerine jelly between two cover-slips fasten to an aluminium holder, which allows the observation of the dissected appendages from both sides of the mount. The appendages dissected in glycerine are transferred and arranged in melted (warmed to about 40 °C) glycerine jelly, spread as a thin film in one cover-slip (size 24 × 24 mm), which is first attached with plasticine to a glass slide. After a few hours of drying, the dissected appendages should be coated by a new film of warm jelly, and finally covered by the other cover-slip (size 18 × 18 mm) with small pieces of plasticine attached at the four corners, which prevents squashing of the thicker appendages. When glycerine jelly is dry, which takes usually 3–6 hours, the mount must be sealed by Canada balsam, Eukit or Murrayite for permanent storage. In the final step, the preparation should be unattached from the glass slide and fixed in an aluminium holder with the circular hole.

Although there are a number of various staining techniques available, some relatively simple which can be easily employed are recommendable to aid in the observation of fine details of the dissected appendages. Staining is especially advisable when a mounting medium with a strong clearing effect is used, i.e., lightening the chitin and eventually making dissected appendages difficult to see. Staining may be performed either before dissection or stains may be added to the ultimate mounting medium. Stains, which produce satisfactory results in ostracods include Methylene Blue, Lignin Pink or Chlorazol Black. The preferred stain is simply just mixed with the mounting medium (Danielopol 1982; Athersuch et al. 1989; Meisch 2000).

Finally, SEM is extremely useful in studying details of the finest soft body structures. However, appendages for SEM require special techniques, e.g., freeze-drying or critical-point-drying. As this is beyond the scope of this primer, for more details the reader is referred to Sandberg (1970), Athersuch et al. (1989), Matzke-Karasz (1995), Meisch (2000) or Danielopol et al. (2002) and references therein.

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