Introduction

1 Classification

Depending on the view of the higher classification of Crustacea, ostracods are classified as a subclass of the class Maxillopoda (Schram 1986; Brusca and Brusca 1990) or as a separate class (Forest 1994; Martin and Davis 2001) within the subphylum Crustacea. In the first case, ostracods are grouped together with tantulocarids, branchiurans, mystacocaridans, copepods, facetotectans, rhizocephalans, ascothoracidans, acrothoracicans, and thoracicans. McKenzie et al. (1983) classify ostracods into Entomostraca, together with Branchiopoda, Cirripedia, Branchiura, and Phyllocarida. Ostracods are here accepted as a separate class within Crustacea. According to Maddocks (1982) ostracods are divided into four orders: Myodocopida Sars 1866, Platycopida Sars 1866, Palaeocopida Henningsmoen 1953, and Podocopida Sars 1866. On the other hand, Martin and Davis (2001) and Horne et al. (2002) divide the class into subclass Myodocopa (with orders Myodocopida and Halocyprida) and Podocopa (with orders Platycopida, Podocopida, and Palaeocopida). Subclass Myodocopa has only marine representatives. Within the subclass Podocopa, Platycopida has almost only marine species (a very few brackish water species), Palaeocopida is known almost exclusively from fossils, and Podocopida has representatives in both fresh and marine environments. The Order Podocopida is treated in this book and its classification presented below (Table 1) follows Martens et al. (1998), Meisch (2000), and Horne et al. (2005). Letters after the names indicate the type of environment where representatives of a certain taxon can be found: “m” for marine species, and “f” for freshwater. Those with only marine representatives or commensal species are not considered further in the systematic part of the book, but, nevertheless, a key to all podocopid superfamiles as well as their general morphology is provided in this book.

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Table 1 Classification of the recent Ostracoda (only the podocopid lineages are listed below the suborder level)

Class Ostracoda Latreille 1802
- Subclass Myodocopa Sars 1866 m
  - Order Myodocopida Sars 1866
    - Suborder Myodocopina Sars 1866
  - Order Halocyprida Dana 1852
    - Suborder Halocypridina Dana 1852
    - Suborder Cladocopina Sars 1866
- Subclass Podocopa Sars 1866 m/f
  - Order Platycopida Sars 1866 m
  - Order Podocopida Sars 1866 m/f
    - Suborder Bairdiocopina Sars 1866 m
      - Superfamily Bairdioidea Sars 1866
      - Family Bairdiidae Sars 1866
      - Family Bythocyprididae Maddocks 1969
    - Suborder Cytherocopina Baird 1850 m/f
      - Superfamily Cytheroidea Baird 1850 m/f
      - Family Bythocytheridae Sars 1866 m
      - Family Cobanocytheridae Schornikov 1975 m
      - Family Cuneocytheridae Mandelstam 1959 m
      - Family Cushmanideidae Puri 1974 m
      - Family Cytherettidae Triebel 1952 m
      - Family Cytheridae Baird 1850 m
      - Family Cytherideidae Sars 1925 m/f
      - Family Cytheromatidae Elofson 1938 m
      - Family Cytheruridae Müller 1894 m
      - Family Entocytheridae Hoff 1942 f (living commensally on other crustaceans)
      - Family Eucytheridae Puri 1954 m
      - Family Hemicytheridae Puri 1953 m
      - Family Kliellidae Schäfer 1945 f
      - Family Krithidae Mandelstam 1960 m
      - Family Leptocytheridae Hanai 1957 m/f
      - Family Limnocytheridae Klie 1938a f
      - Family Loxoconchidae Sars 1925 m/f
      - Family Microcytheridae Klie 1938a m
      - Family Neocytheridae Puri 1957 m
      - Family Paracytherideidae Puri 1957 m
      - Family Paradoxostomatidae Brady and Norman 1889 m
      - Family Parvocytheridae Hartmann 1959 m
      - Family Pectocytheridae Hanai 1957 m
      - Family Psammocytheridae Klie 1938a m
      - Family Schizocytheridae Howe 1961 m
      - Family Trachyleberididae Sylvester-Bradley 1948 m
      - Family Xestoleberididae Sars 1928 f/m
      - Superfamily Terrestricytheroidea Schornikov 1969 m

(continued)

Table 1 (continued)

Family Terrestricytheridae Schornikov 1969
Suborder Darwinulocopina Sohn 1987 f
Superfamily Darwinuloidea Brady and Norman 1889
Family Darwinulidae Brady and Norman 1889
Suborder Cypridocopina Jones 1901 m/f
Superfamily Cypridoidea Baird 1845 m/f
Family Candonidae Kaufmann 1900a m/f
Family Cyprididae Baird 1845 f
Family Ilyocyprididae Kaufmann 1900a, f
Family Notodromadidae Kaufmann 1900a, f
Superfamily Macrocypridoidea Müller 1912 m
Family Macrocyprididae Müller 1912
Superfamily Pontocypridoidea Müller 1894 m
Family Pontocyprididae Müller 1894
Suborder Sigilliocopina Martens 1992c m
Superfamily Sigillioidea Mandelstam 1960
Family Sigilliidae Mandelstam 1960

2 Basic Morphology

As in many crustacean groups there is no standard terminology that is in universal use. Specialists working on Suborder Cytherocopina tend to have different terminology to the ones working on the Suborder Cypridocopina. Obviously in an overview, such as this book, a standard terminology needs to be adopted. Horne et al. (2002) already attempted to establish a consistent terminology for all ostracods. In this book, standard terms used for all other crustaceans describing a general structure of the crustacean appendage (endites, protopod, exopod, endopod, epipod, and segments) are used here as well. The terminology applied here for the ostracod limbs is a combination of the standard terms used by many modern authors in their publications. Descriptions of the limb chaetotaxy are based on the nomenclature proposed by the following authors: Broodbakker and Danielopol (1982), Martens (1987a), Meisch (1996, 2007), Rossetti and Martens (1996), and Karanovic (2007). Nomenclature used for the carapace surface structures follows Sylvester-Bradley and Benson (1971). However, not all terms defined by the latter authors are described here as they are applicable mostly for the marine taxa of the suborder Cytherocopina and are not developed on the shells of the freshwater species. General morphology and structure of the valves are the same as in Meisch (2000). In this chapter, each appendage is compared between the three superfamilies found in the freshwaters: Cytheroidea, Darwinuloidea, and Cypridoidea, with some remarks on other ostracods (Figs. 1–3).

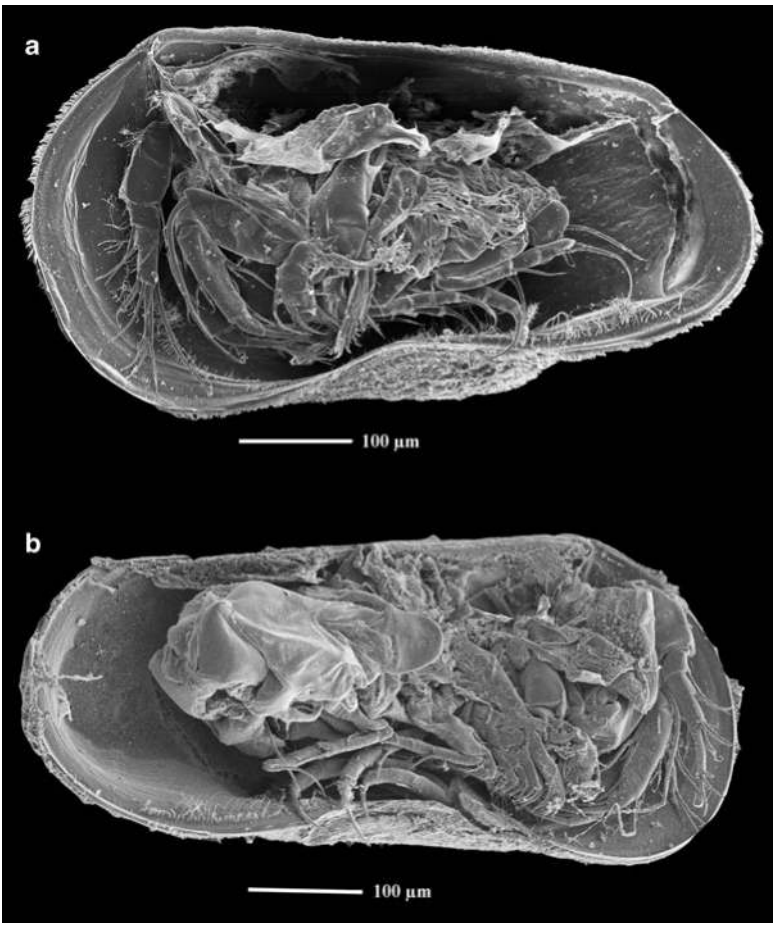

Fig. 1 *Paralimnocythere karamani* (Petkovski 1960a), SEM: (a) inside view of the adult ♀; (b) inside view of the adult ♂.

2.1 Carapace

The ostracod body is enclosed between two calcified valves that are connected in the dorsal part with simple chitinous, like in Cypridoidea, or complex calcite nonslip locking device (hinge), like in Cytheroidea. As in other crustaceans, the cuticle of the carapace is mineralized with low magnesium calcium carbonate in the form of calcite. The calcified shell consists of small crystallites embedded in a chitinous and protein matrix. The shell can be almost completely built of calcite crystals or composed of parallel chitinous lamellae together with a layer of crystallite. The carapace is an important functioning part of the ostracod anatomy, it encapsulates and protects the animal from predators, provides additional stability for the benthic way of life, and forms an integral part of the exoskeleton, providing anchorage

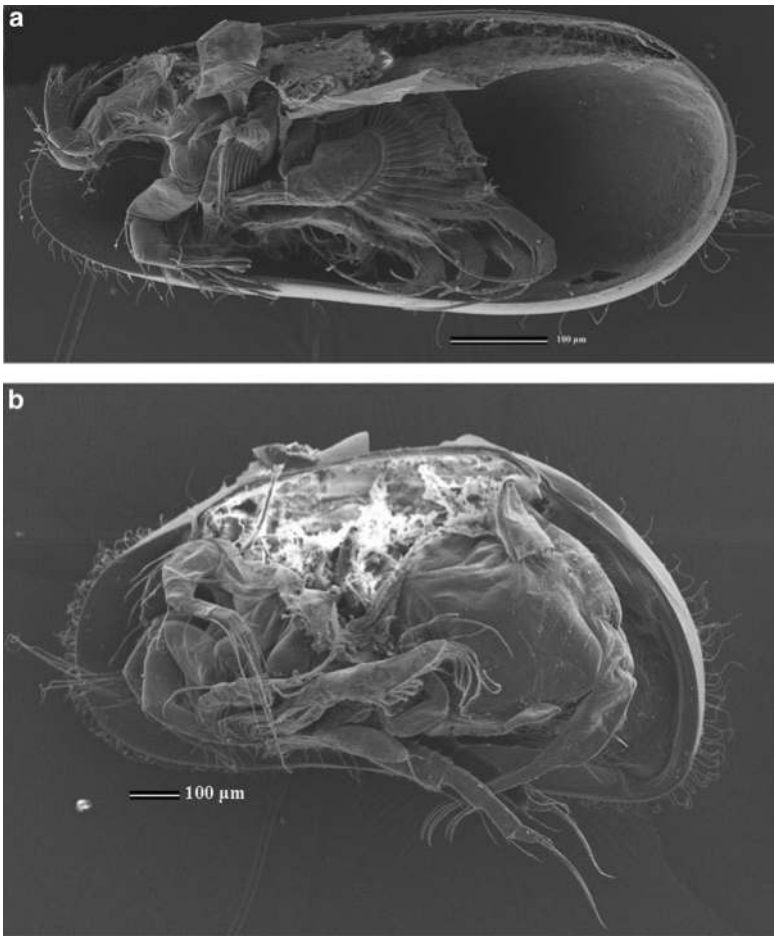

Fig. 2 *A, Darwinula stevensoni* (Brady and Robertson 1870), (a) inside view of the adult ♀; (b), *Candona lindneri* Petkovski 1969, inside view of the adult ♀.

points for some muscles of the limbs. Ostracods keep almost all their soft parts within the valves, but sometimes even substantial parts of uropodal rami, walking and cleaning legs, as well as the first and the second antenna, can be seen protruding through the valves (Fig. 4). Ostracods are enclosed in the carapace even in the first instar of their development. The carapace is formed by two lateral folds of the epidermis, originating dorsally in the head region. These folds or *duplicature* have an inner and an outer lamella. The space between the two lamellae is an extension of the body which in some taxa may house certain reproductive and digestive organs. The outer lamella is well calcified throughout, while the inner lamella has uncalcified and calcified parts (Fig. 5a). The calcified inner lamella is an extension of the calcified outer lamella, i.e., they are continuous with one another, and the only difference between the two lamellae can be found in the disposition of their cells and

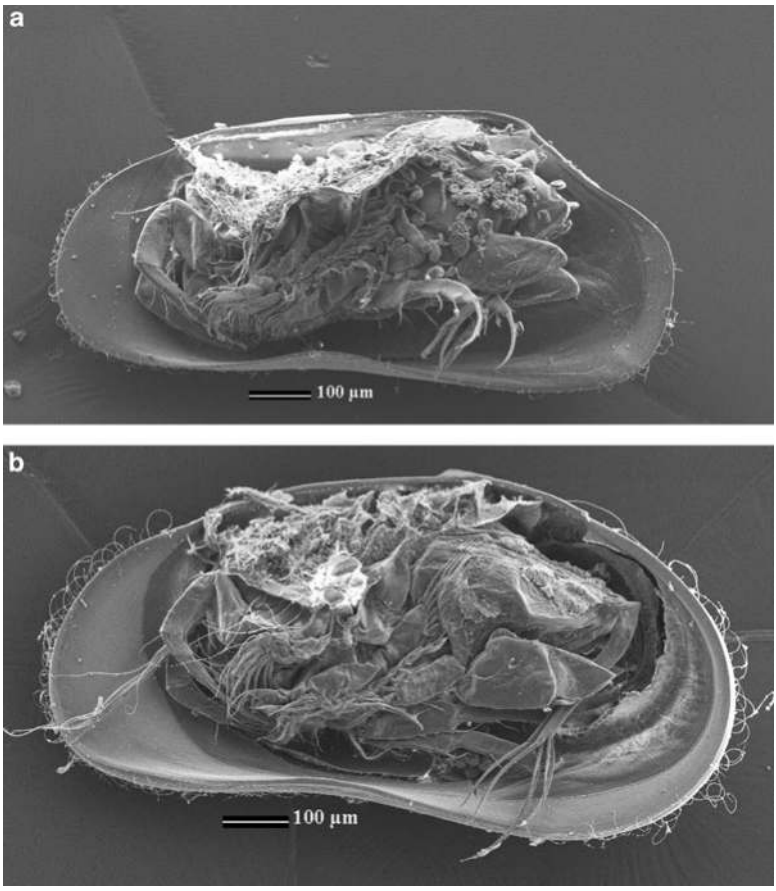

Fig. 3 SEM: *Candonopsis kingsleii* (Brady and Robertson 1870), SEM: (a) inside view of the adult ♀; (b) inside view of the adult ♂.

whether their cuticle fronts are on the outside, or face the body of the animal (Keyser 1990). The data available suggest that the inner lamella is the main organ in which osmoregulation takes place in freshwater ostracods (Keyser 1990). The calcareous components of the ostracod shell are arranged in at least two distinct layers: one thicker layer, composed of crystals of calcite with a foliated appearance, and a thinner laminated layer. The soft body of an animal is integral part of the duplicature and it is connected dorsally to the valves as well as laterally with the so-called adductor muscles, (Fig. 5b), which form a scar on the valves, and together with the mandibular scars, form central scar pattern. This is the first taxonomic character for distinguishing between different podocopid suborders as shown in Fig. 5c–f.

Looking at the carapace laying on its side, we can distinguish *anterior margin* and *anterior end*, *posterior margin* and *posterior end*, and *ventral* and *dorsal margin*. (Fig. 6a)

Fig. 4 *Trigonocypris globulosa* De Deckker 1978. Outside view from the right side, showing the protruding appendages. Photo: S. Halse.

On the inner lamella, we can recognize the following parts, whose structures bear important taxonomic information (Fig. 6a, c). Looking from the center toward the free margins, the first line we can see is called *inner margin* and it represents the line where inner lamella becomes calcified. After that, occasionally we can see several *inner lists* which can run continuously or only partly with the inner margin. Further on, on the *calcified inner lamella* (sometimes also called *duplicature* in the literature, but it is only a part of it and not synonymous), a zone where inner and outer lamella meet is called the *line of concrescence*, and the zone which follows and is usually transverse with canals is called the *fused zone*. The inner calcified lamella and the outer lamella may be fused throughout or there may be a space between them called *vestibulum*. *radial pore canals* are tubes, carrying nerves, passing through the fused zone between the calcified inner and outer lamellae. Sensillae protrude from pores. Sometimes radial/marginal pore canals start at the *line of concrescence* but do not run all the way through the *fused zone* (because they exit on the external surface of the valve before the outer margin), and in that case they are called *false radial/marginal pore canals*. The free extension of the calcified inner lamella is called *selvage*, and it can sometimes be inwardly displaced, in which case the free valve margin is formed by a more or less prominent extension of the outer lamella, called a *flange* (Fig. 6c).

The surface of the ostracod shell has many different features, which may have important taxonomic value. Pores appear to be the termination of pore canals which penetrate the shell and in live animals may bear a sensilla (Fig. 7), and on the surface they are called *normal pores*. In Cytheroidea many of the pores are partially closed by *sieve plates* (Fig. 7c, d). Pits on the surface of the carapace may be in the form of *punctae* (Fig. 7e) and *fossae* (Fig. 7f). Difference between punctae and fossae is in the fact that fossae are connected with walls or *muri*, which together form a *reticulum*. There can also be a "second order reticulation" inside

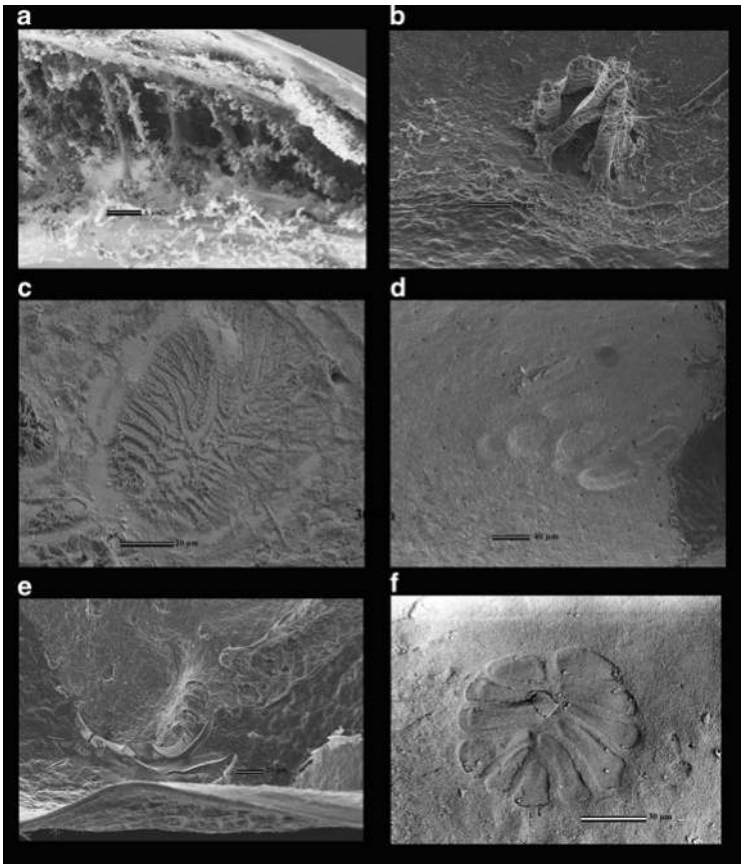

Fig. 5 SEM: (**a**, **f**) *Darwinula stevensoni* (Brady and Robertson 1870); (**b**) *Plesiocypridopsis newtoni* Brady and Robertson 1870; (**c**) *Acocypris capilatta* (Vávra 1895); (**d**) *Psychrodromus fontinalis* (Wolf 1920); (**e**) *Paralimnocythere karamani* (Petkovski 1960a): (**b**) adductor muscle attachment to the shell; (**c**–**f**), imprints of the adductor muscle scars (CMS). (**f**) Photo: D. Keyser

the fossae or even walls. *Sulcus* (plural: *sulci*) is a term describing any kind of groove on the exterior of the carapace (Fig. 8a). *Tubercles* or *nodes* (Fig. 8b) are another type of carapace ornamentation. They can be simple and rounded, or they can be additionally covered with warty expressions. Observation on *Cyprideis torosa* (Jones 1850), an animal which can be found in many different salinity levels, has shown that the noding on the surface of the shell in this species is directly connected with the osmoregulation the animal employs during the molting (Keyser 2005). Special kinds of tubercles are also *clavae* (Fig. 9b). A much smaller ornament in the shape of a prickle is called *papillae* (Fig. 9a). Clavae can sometimes be enlarged and called *carinae* (or *costae*) (Fig. 9c). Wing-like expansions (both thin and pointed and fat and rounded) are called *alae* (Fig. 9d).

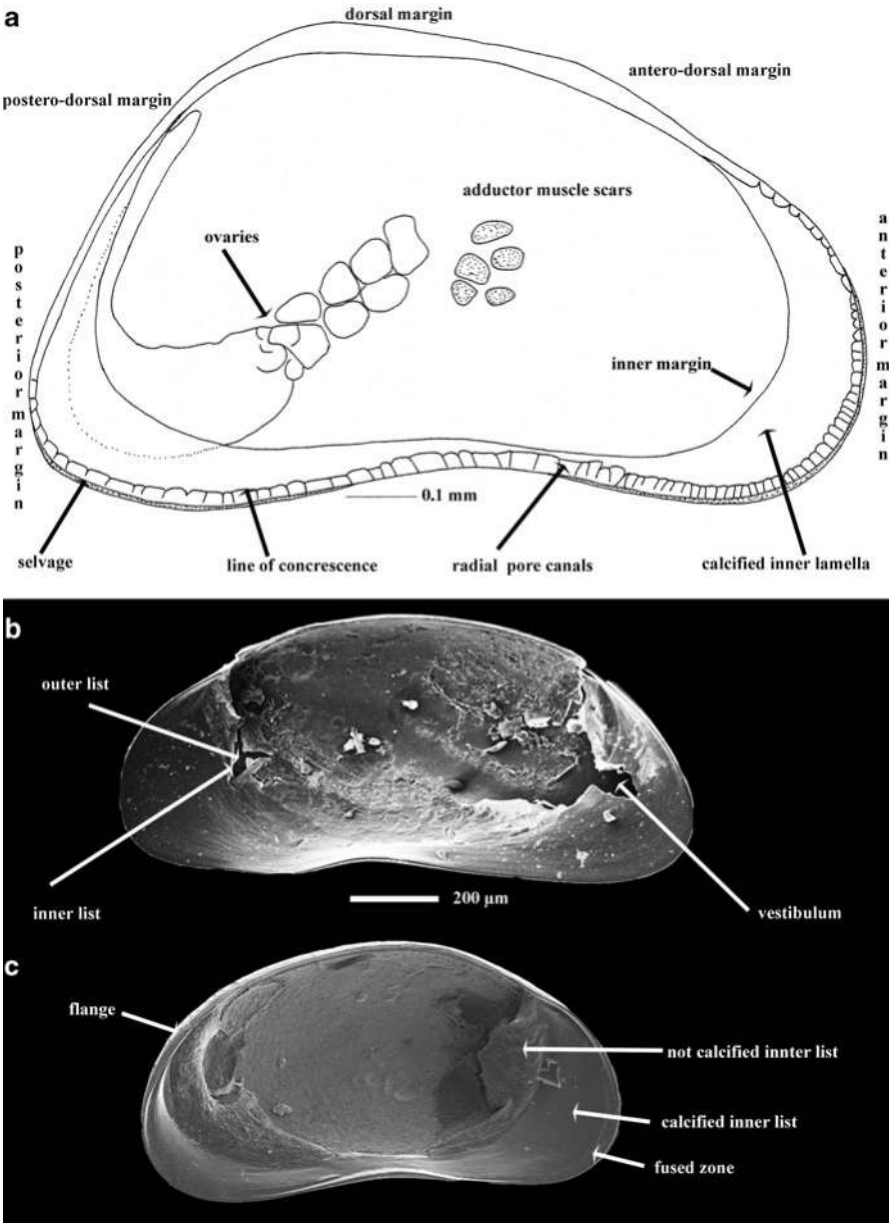

Fig. 6 (**a**) line drawings, (**b**, **c**) SEM. (**a**) *Candona sp*.; (**b**) *Humphcypris subterranea* (Hartmann 1964); (**c**) *Psychrodromus fontinalis* (Wolf 1920): (**a**) schematic view of the interior of the LV; (**b**) inside view of the RV; (**c**) inside view of the LV. (**b**) Photo: D. Keyser

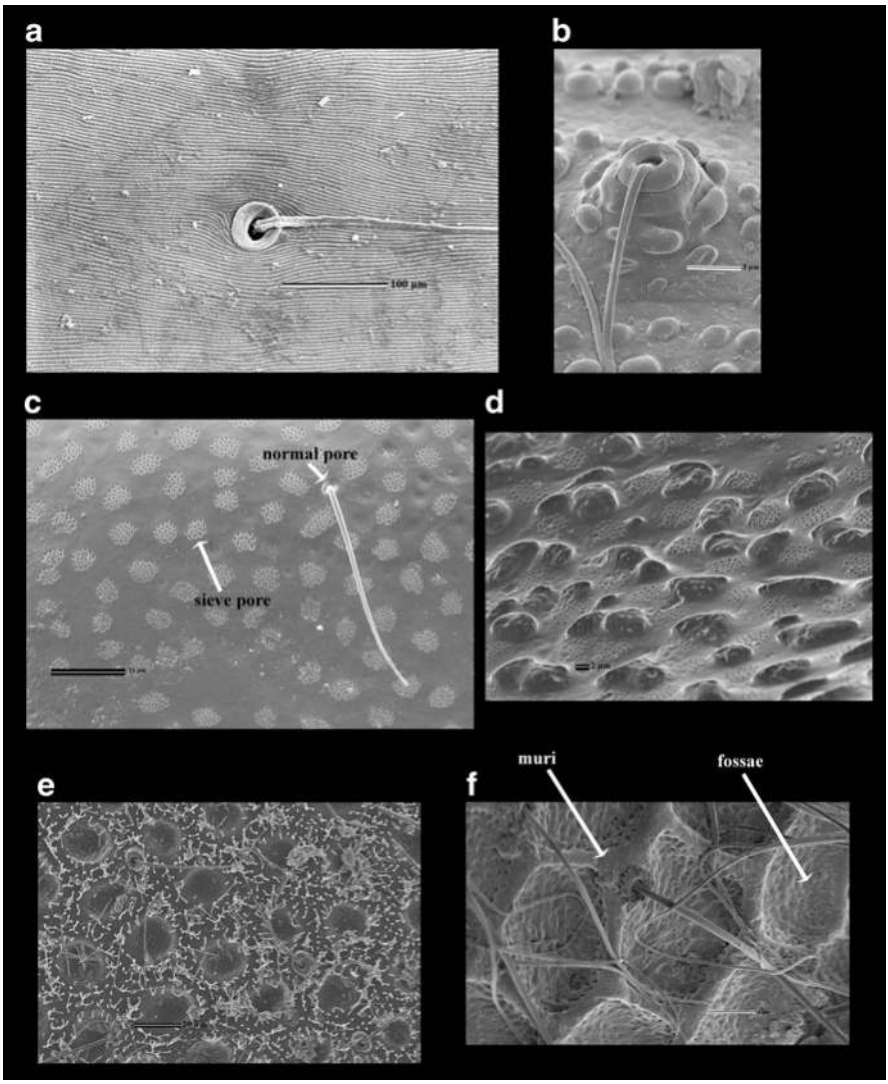

Fig. 7 SEM: (**a**) *Ilyodromus viridulus* (Brady 1886b); (**b**) *Meridiescandona facies* Karanovic 2003c; (**c**) *Gomphodella quasihirsuta* Karanovic 2009; (**d**) *Gomphodella aurea* Karanovic 2009; (**e**) *Ilyocypris brady*, Sars 1870; (**f**), *Humphreyscandona waldockae* Karanovic and Marmonier 2003: (**a**) normal pore, (**b**) detail of the surface, showing a normal pore; (**c**, **d**) detail of the surface; (**e**) detail of the surface showing rounded pits; (**f**) detail of the surface showing primary and secondary ornamentation. (**a**) Photo: D. Keyser

Ostracod shells have many different shapes. Most common shapes in the freshwater ostracods in lateral view are “kidney” or “bean” shape (Fig. 10a), elliptical (Fig. 10b), trapezoidal (Fig. 10c, g), triangular and subtriangular (Fig. 10d, e, h), or elongated (Fig. 10f), or any variation of the previous shapes. In dorsal view, the

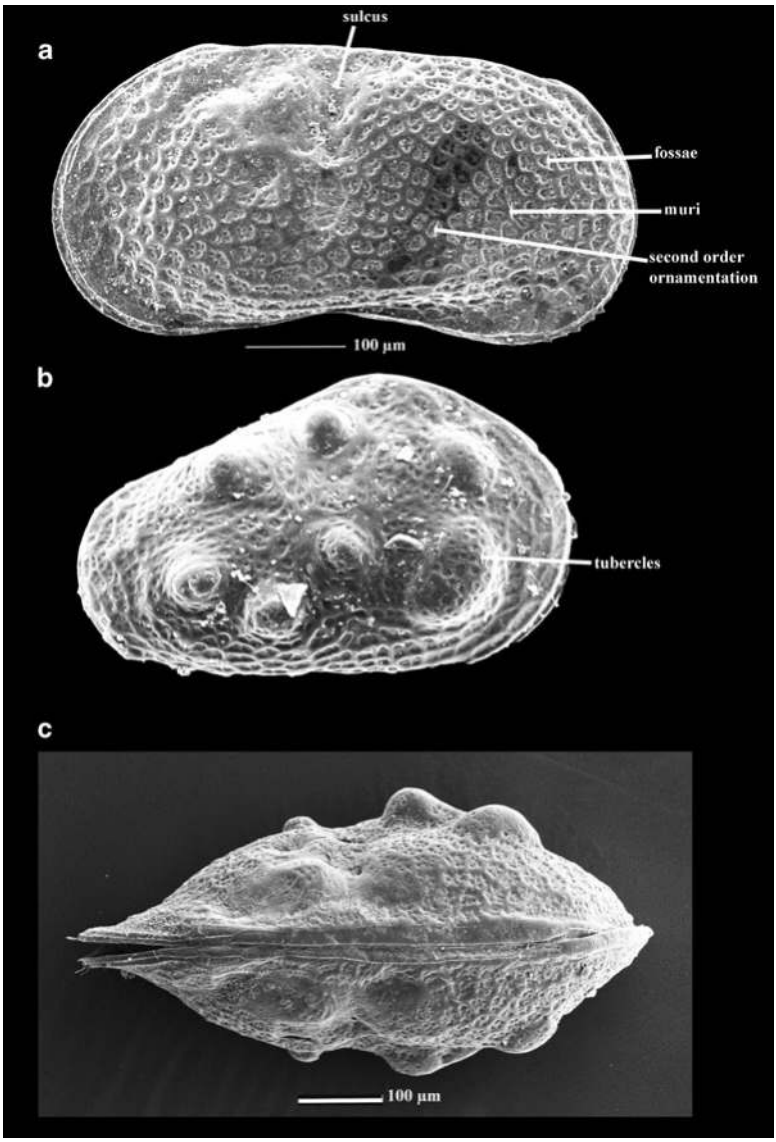

Fig. 8 SEM: (**a**) *Limnnocythere inopinata* (Baird 1843a, b); (**b**) *Cytherissa lacustris* (Sars 1863); (**c**) *Limnnocythere inopinata* (Baird 1843a): (**a**, **b**) lateral view; (**c**) dorsal view. Photos: D. Keyser

shape can be ovoid or globular (Fig. 11a), laterally compressed (Fig. 11d), kite-like (Fig. 11e), or just oblong (Fig. 11b, c).

The coloration of the shell varies from being white, as in many subterranean species (Fig. 12d), to being very vividly colored (Fig. 12a, b), sometimes with very characteristic patterns. The color is usually provided by pigments deposited within

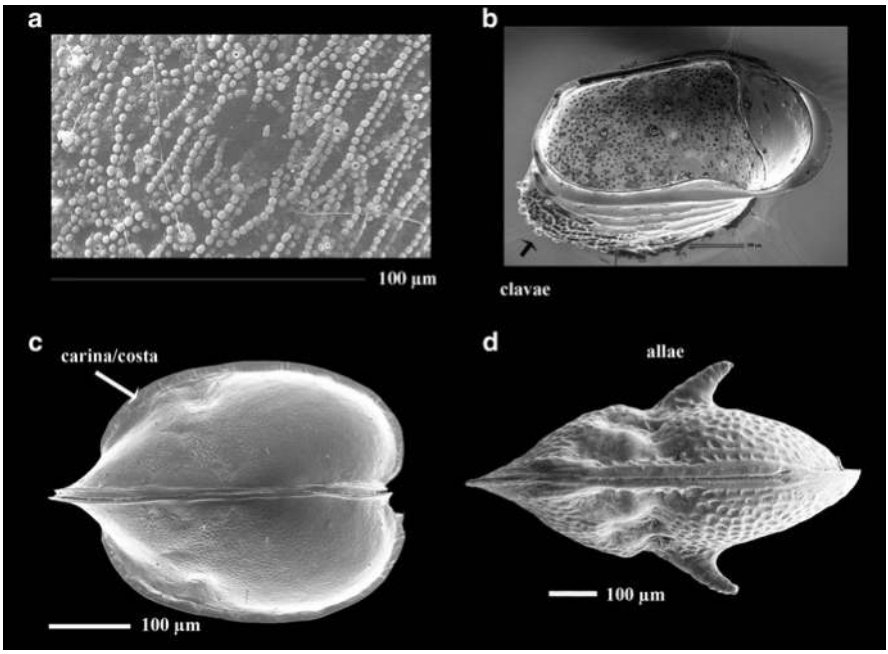

Fig. 9 SEM: (**a**) *Meridiescandona lucerna* Karanovic 2003c; (**b**) *Gomphodella martensi* Karanovic 2009; (**c**) *Gomphodella aura* Karanovic 2009; (**d**) *Limnocythere scutariense* Petkovski 1961: (**a**) detail of the surface showing papillae; (**b**) lateral view of the LV from inside; (**c**) dorsal view; (**d**) dorsal view. (**d**) Photo: D. Keyser

the epidermis of the calcified outer lamella. The color may vanish with prolonged preservation, e.g., in alcohol.

In some species, it is very difficult to distinguish between male and female carapace shape, but in others this is very easy. In some Cypridoidea, males are obviously larger than females and have an enlarged posterior chamber to accommodate a copulatory organ, while females are small and have quite a different shape. In most freshwater Cytheroidea, it is also very easy to distinguish males from females (Fig. 13a, b). In some lineages, females are much more robust, because of the brooding chamber in the posterior part of the body, where they keep eggs and early instars. Even if the brooding chamber does not exist, there is a clear difference between male and female carapace (Fig. 13c–f).

2.2 Body Segmentation

The usual division of an arthropod body, into head (cephalon), thorax, and abdomen, is not clearly recognizable in ostracods. Nevertheless, some authors (e.g., Tsukagoshi and Parker 2000) believe that Podocopid ostracods have a maximum

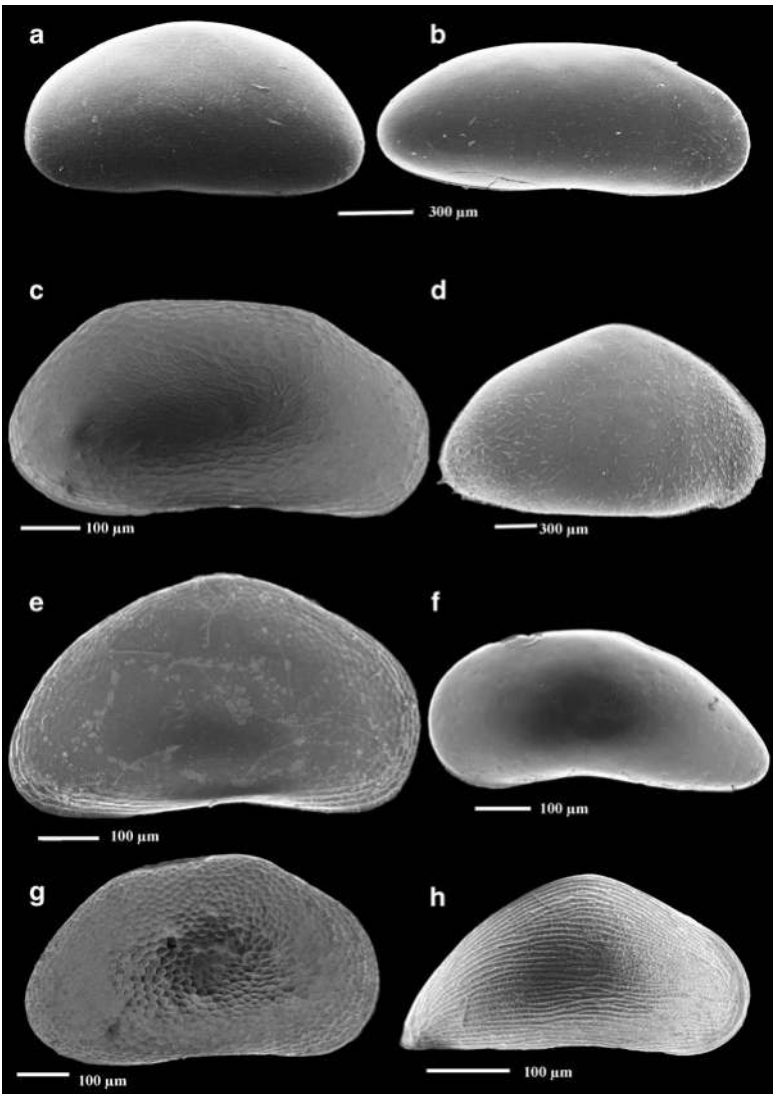

Fig. 10 SEM: (**a**) *Ilyodromus sp.*; (**b**) *Stenocypris major* (Baird 1859b); (**c**) *Meridiescandona facies* Karanovic 2003c; (**d**) *Cypris pubera* Müller 1776; (**e**) *Humphreyscandona fovea* Karanovic and Marmonier 2003; (**f**) *Origocandona inanitas* Karanovic 2005b; (**g**) *Humphreyscandona waldockae* Karanovic and Marmonier 2003; (**h**) *Pilbaracandona eberhardi* Karanovic and Marmonier 2003: (**a**) LV, outside view; (**b**, **c**, **d**, **e**, **g**, **h**) RV, outside view; (**f**) LV, outside view. (**a**, **b**, **d**) Photos: D. Keyser

of 11 trunk segments (thorax and abdomen). This is based on the observation of body segmentation in eight podocopine families (one belonging to Bairdocopina and seven to Cytherocopina). Among these families a maximum number of body segments have been found in the, presumably, most primitive of the examined lineages (Tsukagoshi

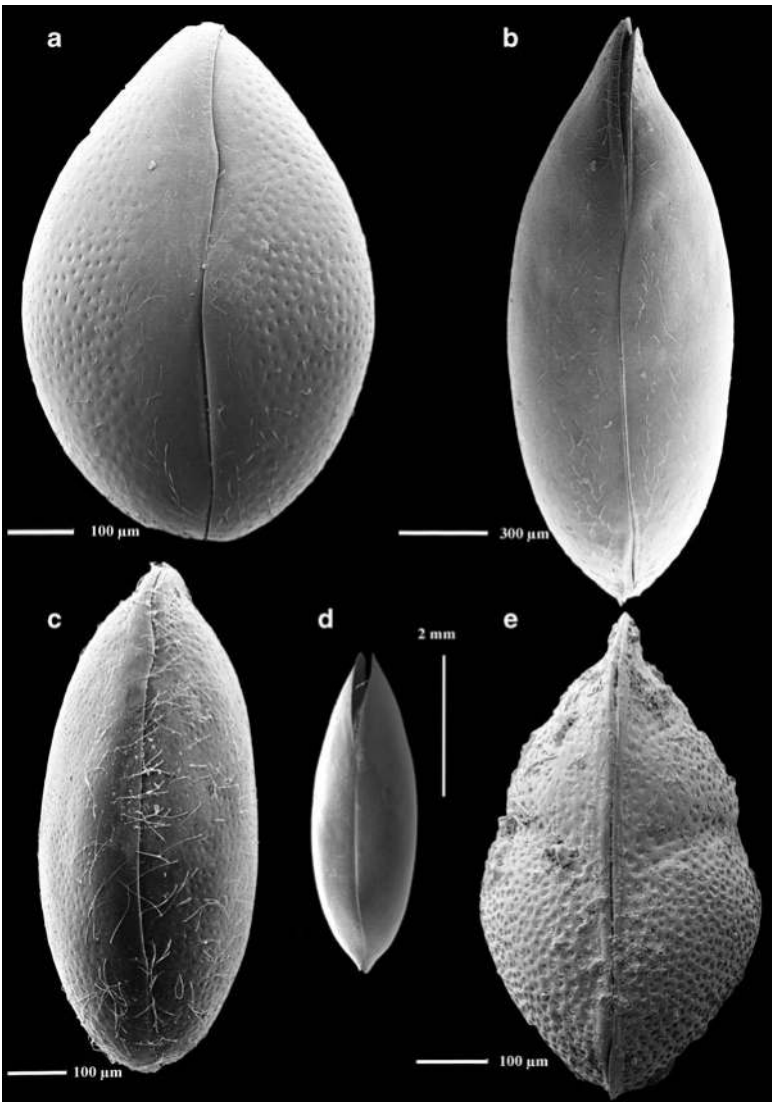

Fig. 11 SEM: (**a**) *Cypridopsis vidua* (Müller 1776), (**b**) *Trajancypris sp.;* (**c**) *Pseudocandona sp.;* (**d**) *Repandocypris austinensis* Halse and McRae 2004 (e) *Gomphocythere sp.*: dorsal views. (**a**, **b**, **c**, **e**) Photos: D. Keyser; (**d**) Photo: S. Halse

and Parker 2000), namely in the family Leptocytheridae. The number of trunk segments becomes less in more derived taxa. The same number (11) of trunk segments occurs in Platycopida (Schulz 1976). The supposed segmentation of the trunk region in all these taxa is sometimes marked by cuticular folds and/or assemblages of spines and setae (Fig. 14). It is also believed that in these lineages copulatory appendages in females are derived from segments associated with the fifth thoracic region, while those of the males with the tenth one. On the other hand, Matzke-Karasz and Martens

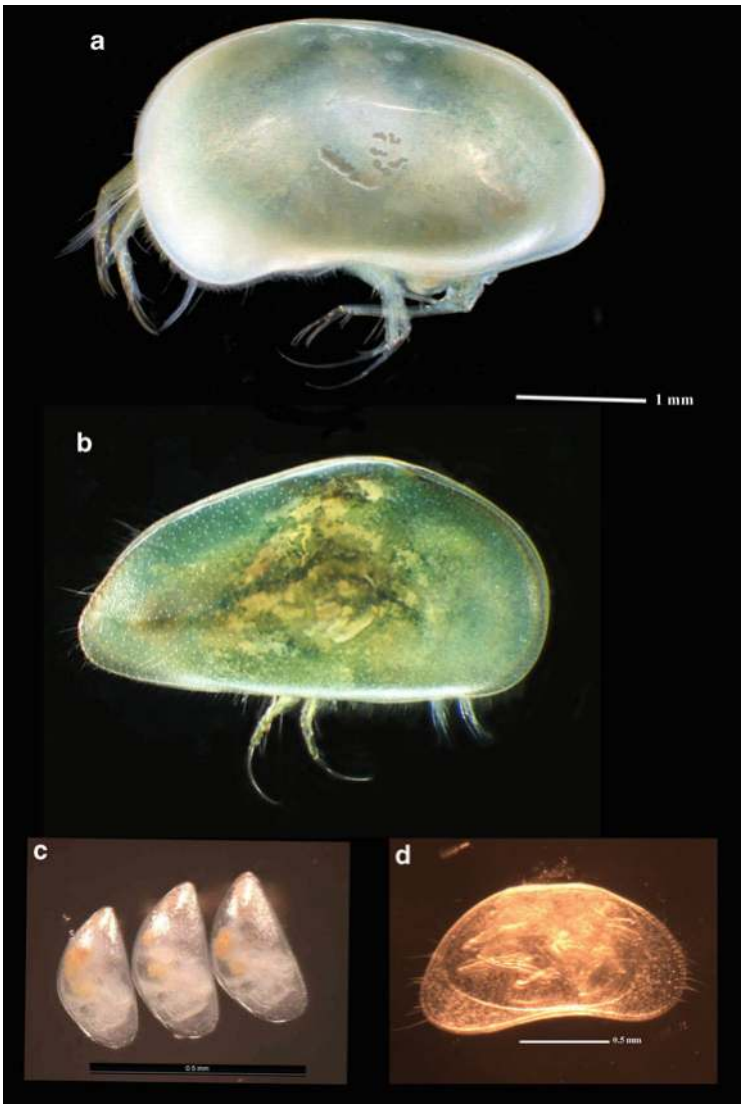

Fig. 12 Microscopic photographs: (**a**) *Australocypris beaumonti* Halse and McRae 2004; (**b**) *Lacrimicypris kumbar* Halse and McRae 2004; (**c**) *Areacandona sp.*; (**d**) *Candonopsis sp.*: (**a**) lateral view from the left side; (**b**, **d**), lateral view form the right side; (**c**) three individuals laying on the right side. (**a**, **b**) Photos: S. Halse; (**c**, **d**) Photos: T. Karanovic

(2005, 2007) consider that the female copulatory appendage is derived from more than one segment. They have based their conclusions on the morphology of a representative of the family Cyprididae, namely giant ostracods from the subfamily Liocypridinae. Females of these species have several (three, five, or six) paired appendage-like structures associated with the female genital field. Within the Myodocopa, some members of the Cladocopina have definite indications of dorsal trunk segmentation,

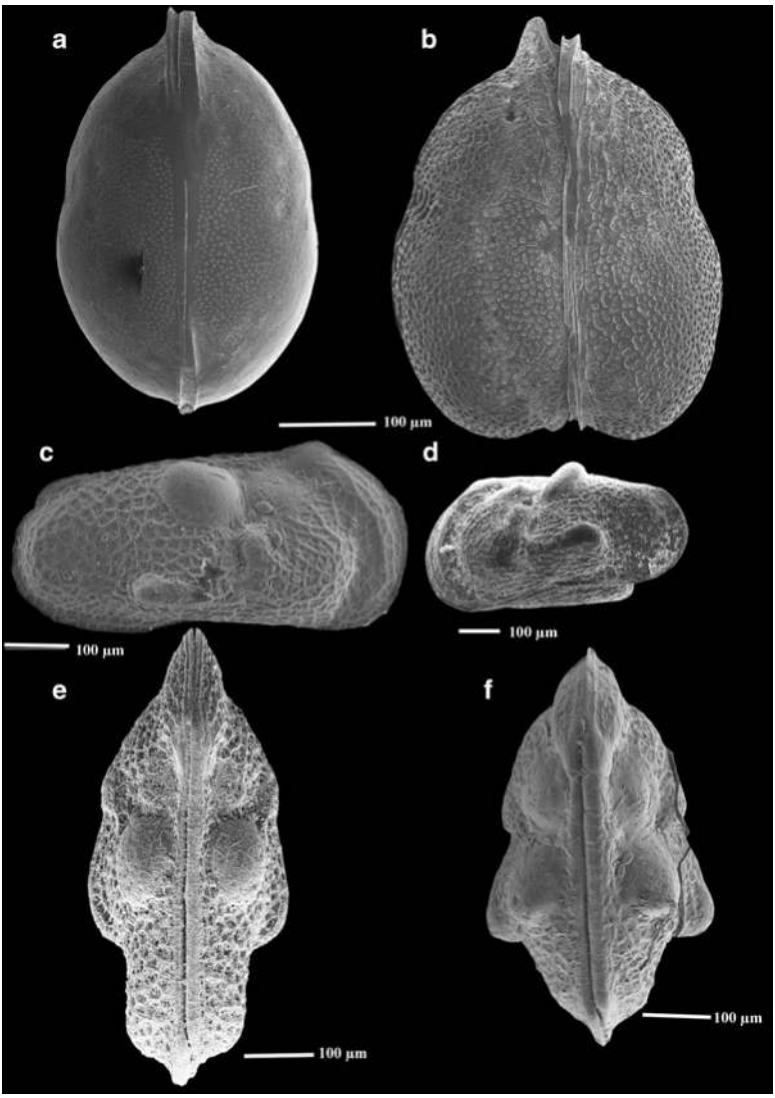

Fig. 13 SEM: (**a**, **b**) *Gomphodella sp.*; (**c**, **d**, **f**) *Paralimnocythere karamani* (Petkovski 1960a); (**e**) *Paralimnocythere sp.:* (**a**, **e**) ♂, dorsal view; (**b**, **f**) ♀, dorsal view; (**c**) ♂ lateral view form the right side; (**d**) ♀ lateral view from the left side. (**e**) Photo: D. Keyser

and remnants of dorsal trunk segmentation are visible in a few of the Halocyprid species (Cohen et al. 1998). Nevertheless, in most species of Podocopid ostracods body segmentation is completely reduced, some think as a result of paedomorphic evolution (Tsukagoshi and Parker 2000), and there are at the most seven pairs of appendages, four (or five) cephalic ones, and three (or two) thoracic ones, and no abdominal appendages. The body terminates in paired uropodal rami.

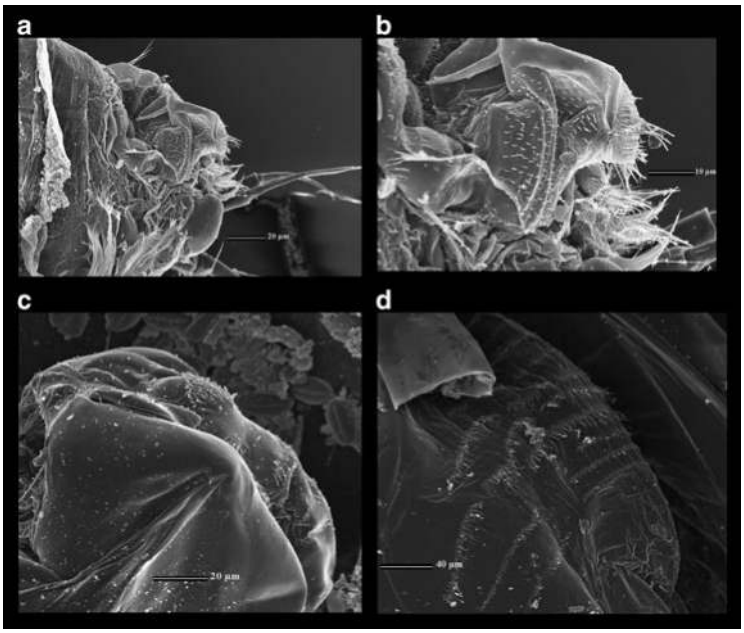

Fig. 14 SEM: (**a**, **b**) *Gomphodella quasihirsuta* Karanovic, 2009; (**c**) *Paralimnocythere karamani* (Petkovski 1960a); (**d**) *Candona lindneri* Petkovski 1969b: (**a**, **b**, **d**, ♀) posterior end of the body; (**c**, ♂) posterior end of the body

2.3 Antennula

This is the first appendage, situated in the head region. It is also called the first antenna, or "A1" (Fig. 15). It is used for locomotion and it also has a sensory function, since it is equipped with some sensory setae – aesthetascs. It is believed that this appendage is uniramous, and that it does not have any traces of the biramous appendage. This is also believed to be true for all other crustaceans (Boxshall et al. 2010). However, the first (two) segments of the antennula in Cypridoidea have sometimes been regarded as constituting a "protopod," and the rest of the limb as an "endopod," In Darwinuloidea, this goes even further, some authors labeling the group of setae on the second segment of the antennula as the "exopod" (Rossetti and Martens 1996, 1999). Karanovic (2005e) proposed a new terminology for the antennula, acknowledging the presence of all three rami on this appendage, and trying to underpin the homologous structures on the antennula between the three freshwater lineages, namely, Cypridoidea, Cytheroidea, and Darwinuloidea. This approach was not received enthusiastically among carcinologists and other ostracod workers, as it undoubtedly challenges the homology of this appendage with other crustaceans and arthropods in general, and it is strongly suggested that it should be abandoned (Boxshall and Jaume 2009; Smith and Kamiya 2008; Boxshall et al. 2010). Maddocks (2000) provided a comparative analysis of the antennula among the podocopid lineages, labeling setae numerically

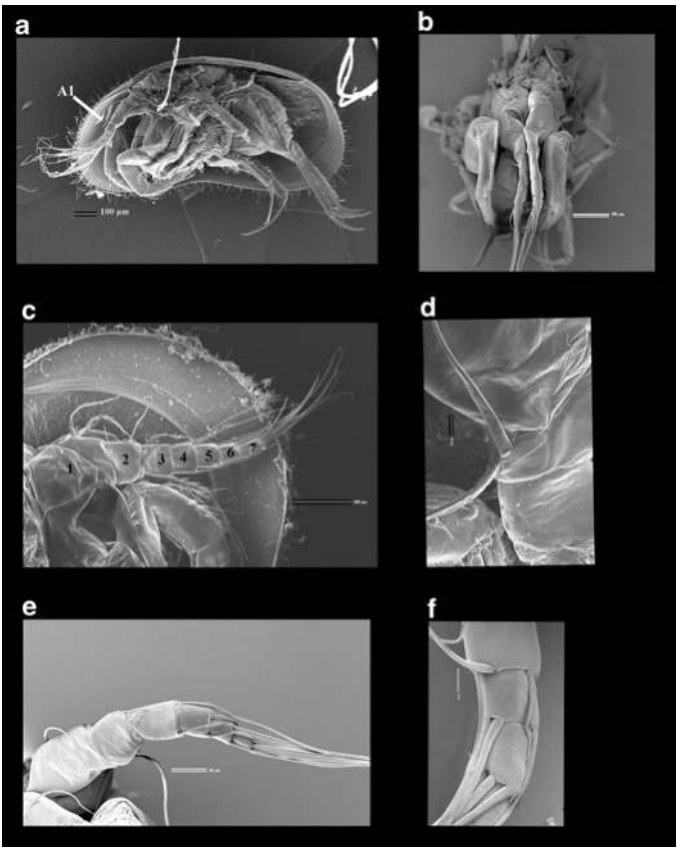

Fig. 15 SEM: (**a**) *Psychrodromus olivaceus* (Brady and Norman 1889); (**b**, **e**, **f**) *Heterocypris incongruens* (Ramdohr 1808); (**c**, **d**) *Ilyocypris brady* Sars 1870: (**a**) adult ♀ inside view; (**b**) ♀, anterior view; (**c**, **e**, **f**) A1; (**d**) postero-dorsal setae on the first segment of the A1

from the proximal to distal. Although this system may be helpful in determining the homologous structures, it is very complicated and difficult to use. In this book, the labeling of setae and segments is abandoned and only a standard description of setae according to their actual position of the appendage is applied. There is no sexual dimorphism in the morphology of the antennula in any of the Podocopan lineages. In Myodocopa, on the other hand, males usually have many transformed setae.

2.3.1 Superfamily Cypridoidea

The antennula has a maximum of eight segments (Fig. 15c). The first two segments are only partly subdivided and here they are counted together as the first segment. This segment bears a maximum of four setae: two situated on the anterior side, and the

other two situated on the postero-dorsal side (Fig. 15d). The more proximal of the two anterior setae is often transformed into a sensory organ, called the Wouters organ (Smith and Matzke-Karasz 2008). This is the case in most of the representatives of the family Cyprididae and the subfamily Paracypridinae (family Candonidae). The other two subfamiles of Candonidae – Candoninae and Cyclocypridinae – do not have this organ. Instead the seta is present in this place. In many subterranean Candoninae one of the two setae is missing. Two setae on the dorsal side of the first antennular segment originate almost from the same spot and are often very long. It is very rarely that one of these setae is missing. The following segment is jointed with the previous one and it is usually the shortest of all the segments, carrying one seta anteriorly which is rarely long. Posteriorly, on the dorsal side there may be another sensory organ, called Rome organ. This organ has different shapes (Fig. 16) and it is usually present in most Cypridoidea, except for Candoninae. The third segment is also Jointed with the previous one, and it is usually the longest of all the antennular segments. All the other segments are not jointed with each other. The third segment usually only carries one seta anteriorly and one posteriorly. The fourth, fifth, and seventh segments carry a maximum of four setae each: two anterior and two posterior setae. Anterior setae are usually very long, longer than the posterior ones. Reduction in the number of setae is common, and this occurs on the posterior side rather than the anterior side. Reductions are especially common among the subterranean animals. Posterior setae also tend to transform into more claw-like structures. The sixth segment may have two anterior and two posterior setae, and, in addition, one small seta which is situated on the antero-dorsal side of the segment, called the alpha seta. This seta is often missing. With the exception of the alpha seta and the two posterior setae on the first antennular segment,

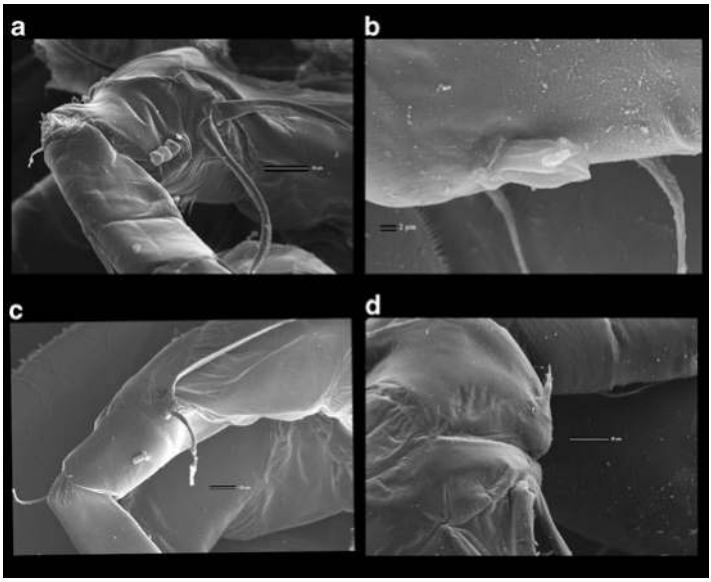

Fig. 16 SEM of Rome's organ on A1: (a) *Sarscypridopsis ochracea* (Sars 1924); (b) *Psychrodromus olivaceus* (Brady and Norman 1889): (c) *Candonocypris sp.*; (d) *Diacypris whitei* (Herbst 1958).

all the other setae on the antennula are situated on the ventral side of the appendage. The terminal segment carries a total of four setae as well, and the most posterior is always the shortest one. The most anterior seta is always transformed into aesthetasc "ya." The length of setae varies among the species, while the number is quite often used as a generic character. The segments also may fuse with each other, most usually from the third toward the seventh, but there can also be fusions between the first and the second segment. In the case of a segment's reduction by fusion, the setae are often still present on the fusion line. Setae on the antennula are used for locomotion, and are often feathered. The length ratio of the segments is a useful taxonomic feature.

2.3.2 Superfamily Cytheroidea

Here the antennula is much more stout and has shorter setae (Fig. 17a–d). There is no evidence of the fusion on the first segment, which is usually very long and it never carries any seta. The first segment is jointed with the following one, which can carry up to three setae, but in freshwater lineages there is most usually one posterior seta, situated medially on the dorsal side, or quite distally, on the border between this and the following segments (Fig. 17e). In contrast to Cypridoidea, this segment is elongated in Cytheroidea. It is really rare that the antennula in the superfamily Cytheroidea has seven segments, especially among the freshwater groups. There is usually a fusion between non-jointed segments and there is a maximum of six, but more often five segments altogether. Nevertheless, the setae are still present on the point of the segment fusions. The third segment is also jointed with the second one, while all the other segments are not. The third segment carries only one anterior seta. The two following segments are most often fused and they carry one or two setae antero-medially and one seta postero-medially. Distally there are also three setae anteriorly and one seta posteriorly. The terminal segment has two setae which are free, and two which are fused together proximally. The length of this fusion is an important taxonomic feature at the subfamily and tribal levels. One of the fused setae is normally developed, while the other is transformed into a chemical receptor, or aesthetasc. All setae on the antennula in this ostracod group are relatively short and often transformed into strong claws (Fig. 17f).

2.3.3 Superfamily Darwinuloidea

There is only a joint between the first and second segment, and there is a maximum of six segments altogether (Fig. 18). All of the segments are robust. The first one may carry one or two setae antero-medially. The second segment carries up to three setae postero-distally, which sometimes exit from a protrusion. The second segment has only one seta anteriorly. The third and fourth segments have one posterior and one anterior seta each. The penultimate segment has two anterior and two posterior setae, while the last segment has two setae and an aesthetasc. In some species, there is one

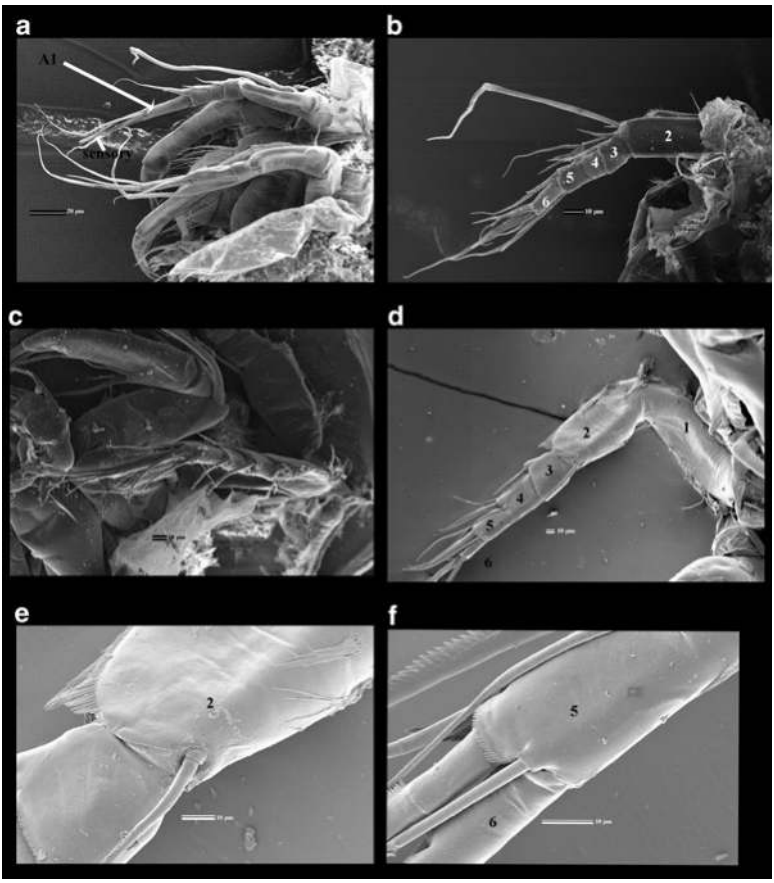

Fig. 17 SEM: (**a**, **b**) *Gomphodella quasihirsuta* Karanovic 2009; (**c**) *Paralimnocythere karamani* (Petkovski 1960a); (**e**, **f**) *Limnocythere dorsosicula* De Deckker 1982c: (**a**) position of the A1 and A2; (b, d), A1, (c, e, f), A1, details

small seta (called alpha seta) anteriorly on the fourth and fifth segments. These two setae, as well as the group of posterior setae on the second segment, are situated dorsally on the appendage, while other setae are situated ventrally. There is often reduction in the posterior and sometimes anterior setae, but never in the number of segments. Setae on the antennula are usually stiff and a couple of them transformed into claws.

2.4 Antenna

The antenna, or A2, is the second appendage originating behind the antennula (Fig. 19a, b). It has locomotory and sensory functions. Unlike the antennula, the antenna is a clearly biramous appendage in all ostracods. Nevertheless, the exopod

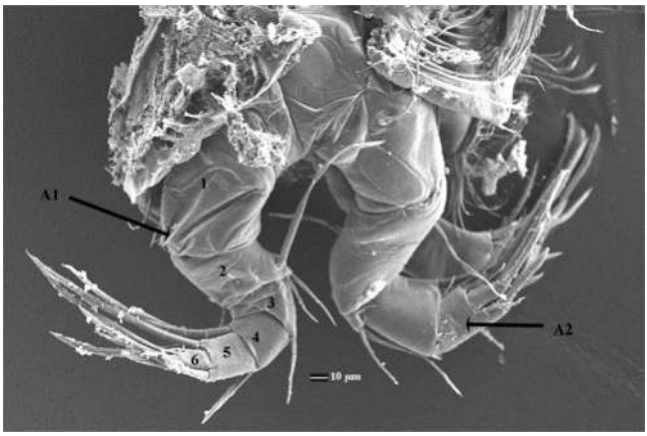

Fig. 18 SEM: *Darwinula stevensoni* (Brady and Robertson 1870): position of the A1 and A2

is much reduced in all representatives of Podocopida, while in Platycopina the exopod is almost as developed as the endopod. In Myodocopa the exopod is much larger than the endopod. It has numerous segments and long swimming setae, while the endopod is reduced to two- or three-segmented ramus with short and often transformed setae. In some myodocopid and podocopid lineages, this ramus is also sexually dimorphic. The antenna offers many taxonomically important characters and its study is essential for a proper identification on the species level.

2.4.1 Superfamily Cypridoidea

The first protopodal segment has three setae: one more proximal, situated on the external side, and two more distal setae, situated postero-laterally. The second segment on the antenna (this one is usually called the first segment in many publications) has one seta situated on the inner side of the segment. Attached to this segment is an exopod (Fig. 19c) which consists of a small plate and up to three setae: the most anterior one is the longest, followed by two considerably shorter setae. The number and length of these setae can vary in the subfamily Candoninae, especially in subterranean species, and there are cases with all three setae being short or even only two setae present. The first endopodal segment carries postero-medially an aesthetasc "Y" (Fig. 20) which can be of variable length and it is much longer in the subterranean animals than in those living in surface waters. Distally on the first endopodal segment there may be a group of up to six setae, called swimming setae. They are situated on the inner side of the appendage, and the most anterior one is always much shorter than the rest of the setae. The length of the swimming setae varies from being very long and by far exceeding the tips of the distal claws, to being very short and barely visible. If these setae are long they are usually feathered.

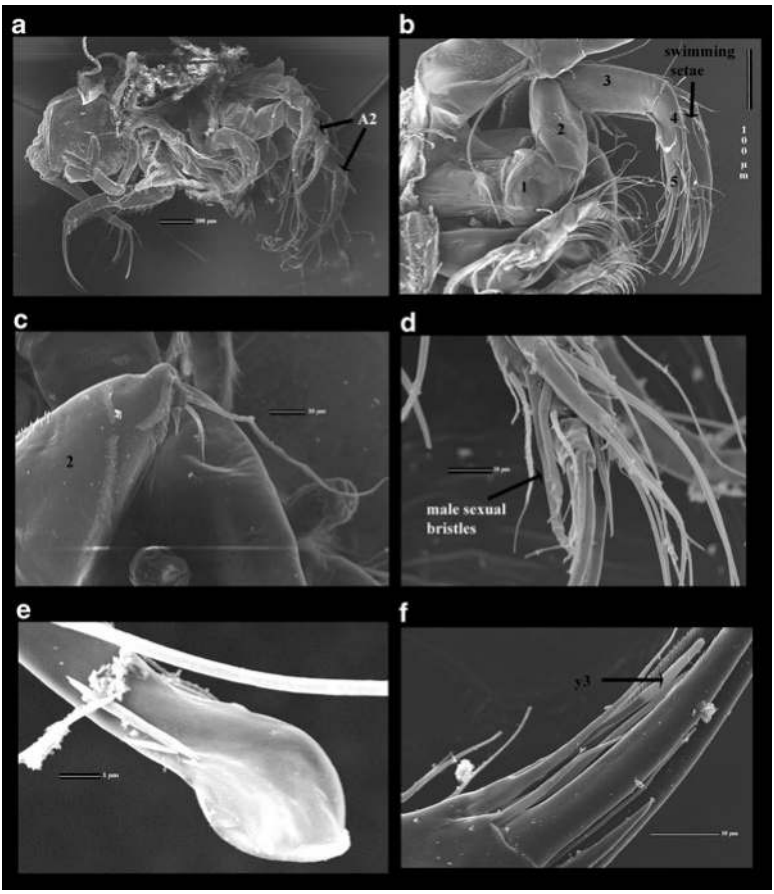

Fig. 19 SEM: (**a**) *Heterocypris reptans* (Kaufmann 1900a); (**b**) *Psychrodromus olivaceus* (Brady and Norman 1889); (**c**) *Candonopsis kingsleii* (Brady and Robertson 1869); (**d**, **e**) *Trapezicandona sp.*; (**f**) *Herpetocypris brevicaudata* Kaufmann 1900b: (**a**) view of the soft body, (**b**) A2, (**c**) exopod on the A2; (**d**) ♂ sexual bristles on the A2; (**e**) detail of the ♂ sexual bristle on the A2; (**f**) terminal segment on the A2 and "y3" aesthetasc

In some lineages of the family Cyprididae, these setae are absent, but this is really a rare case, while in the subfamily Candoninae none of the species has these setae present. The length of the swimming setae is an important taxonomical feature, and their number is a good indicator of the instar stage during the development. The first endopodal segment also carries one or two setae postero-distally, one of which is most often considerably longer than the other. The second endopodal segment in females has up to two setae situated antero-medially on the external side and up to four setae postero-medially on the inner side of the segment, called "t"-setae. Distally, on the external side this segment carries up to three setae, called "z"-setae, and up to three claws, "G1" and "G3" situated on the inner side of the

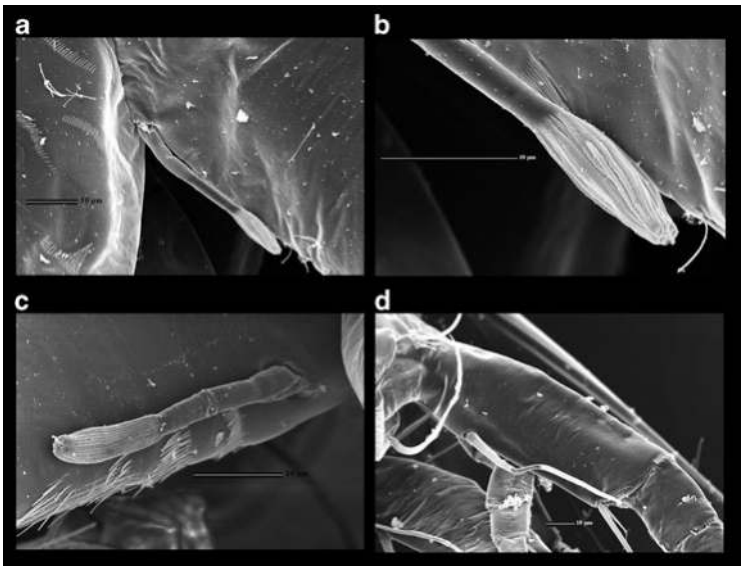

Fig. 20 SEM: (**a**, **b**) *Potamocypris fulva* (Brady 1868); (**c**) *Sarscypridopsis ochracea* (Sars 1924); (**d**) *Trapezicandona sp*.: aesthetasc "Y" on the first endopodal segment of the A2

appendage, and G2 situated laterally (Fig. 21). The first "z"-seta, "z1" may be a little bit more strongly developed than the other two. The length and number of the postero-medial setae and of the "t" setae can vary and it may be a useful taxonomic character. In males of the family Candonidae, the second endopodal segment is subdivided and two of the "t"-setae, namely "t2" and "t3," can be transformed into the male sexual bristles (Fig. 19d, e). The posterior setae on the same segment in males are often more strongly developed than the anterior ones, and sometimes even claw-like. In males of most Cypridoidea, one or two of the "z"-setae can be transformed into long claws, while claws "G2" and "G3," long in females, are reduced in males (Fig. 21a). The level of transformation in males, as well as the length of all the "z" setae and claws on the penultimate segment, in both males and females, is a very important taxonomic feature. The second endopodal segment also carries two sensory setae situated posteriorly, one medially on the segment, and the other distally. The last segment on the antenna is very short and it has two claws and three additional setae. The claw situated on the inner side of the segment is called "GM" and it is long in females, and the one situated on the exterior side is short and is called "Gm." In males, the claw on the inner side is short and the one on the exterior side is long. One of the accompanying setae on the terminal segment is a sensory seta, "y3" (Fig. 19f), which can be very long in subterranean species. The claws are usually heavily serrated and this can also be a sexually dimorphic feature. The length ratio between the segments on the antenna is a useful taxonomic feature as well. The entire appendage is often covered with fine short pseudochaetae.

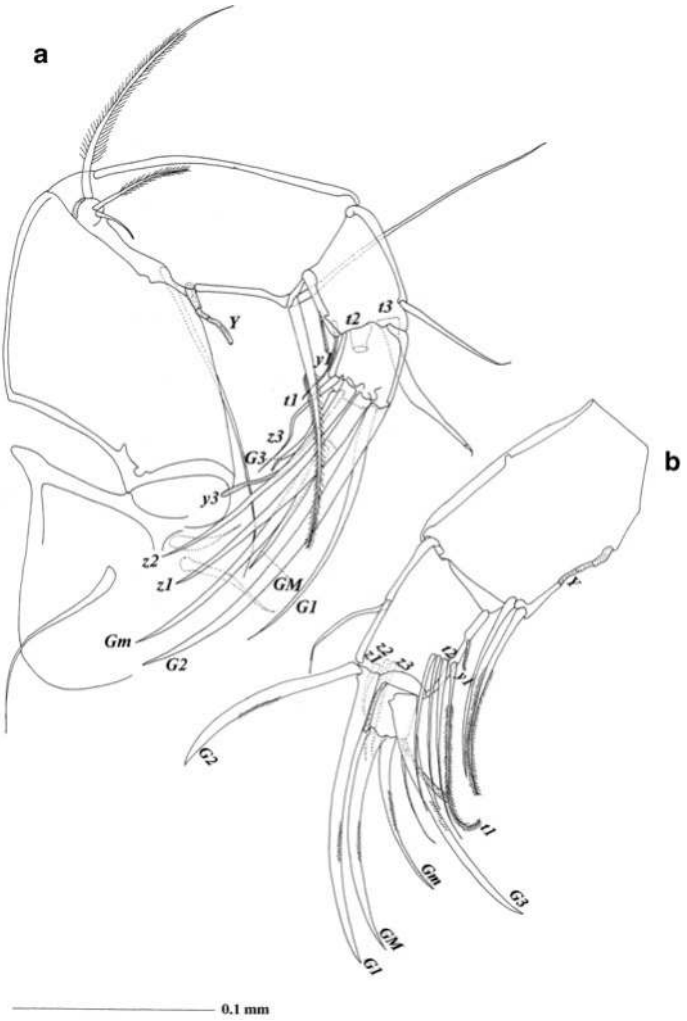

Fig. 21 Line drawings of the A2: (**a**) *Candona piercei* Turner 1895; (**b**) *Candona sigmoides*, Sharpe 1897: (**a**, ♂); (**b**, ♀)

2.4.2 Superfamily Cytheroidea

In this lineage the exopod forms a long spinneret seta (Fig. 22a), consisting of up to three segments. The protopod does not carry any setae. The endopod is three segmented and the first segment carries only one seta postero-distally. The following segment has up to two setae antero-medially and up to three setae postero-medially, one of which is an aesthetasc. The second endopodal segment has one or two setae postero-distally. These setae are usually more strongly developed. The terminal segment is very short and it carries two or three claws, and this segment

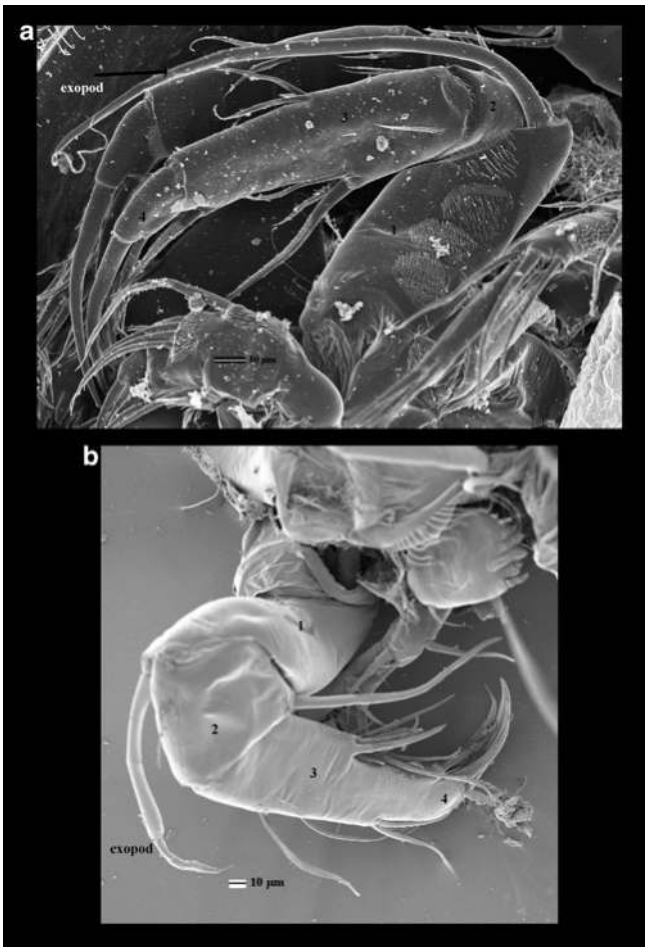

Fig. 22 SEM: (**a**) *Paralimnocythere karamani* (Petkovski 1960a); (**b**) *Limnocythere dorsosicula* De Deckker 1982c: A2

may be sexually dimorphic. The number of claws is an important taxonomic feature on the family level. The second antenna is also often covered with fine pseudochaetae, and the length ratio of the segments is an important taxonomic character. There are no swimming setae.

2.4.3 Superfamily Darwinuloidea

The antenna in darwinulids is much more similar to the one in Cypridoidea than to the one in Cytheroidea (Figs. 18 and 23). The first segment may carry up to two setae posteriorly: one (more proximal) situated on the exterior side of the appendage, and the other (more distal) on the interior side. On the anterior side of this

segment, almost medially, there may be a hook-like structure. The second protopodal segment has one seta, situated medio-distally on the interior side of the segment, and a group of sensory setae situated close to this seta. The exopod in darwinulids also consists of a plate (but longer than in Cypridoidea) and a maximum of three setae (Fig. 23a, c), one of which is small and the other two subequally long. The length and number of these setae are important taxonomical features. The first endopodal segment has one or two setae postero-distally. There are no swimming setae in darwinulids. The second endopodal segment lacks the postero-medial setae, but does have the antero-medial, or "t"-setae. There is only one "z"-seta present distally on the segment, while all three claws "G1," "G2," and "G3" are usually well developed. The second endopodal segment also has two aesthetascs ("y1" and "y2"). The last segment has two claws, "GM" (the long one) and Gm

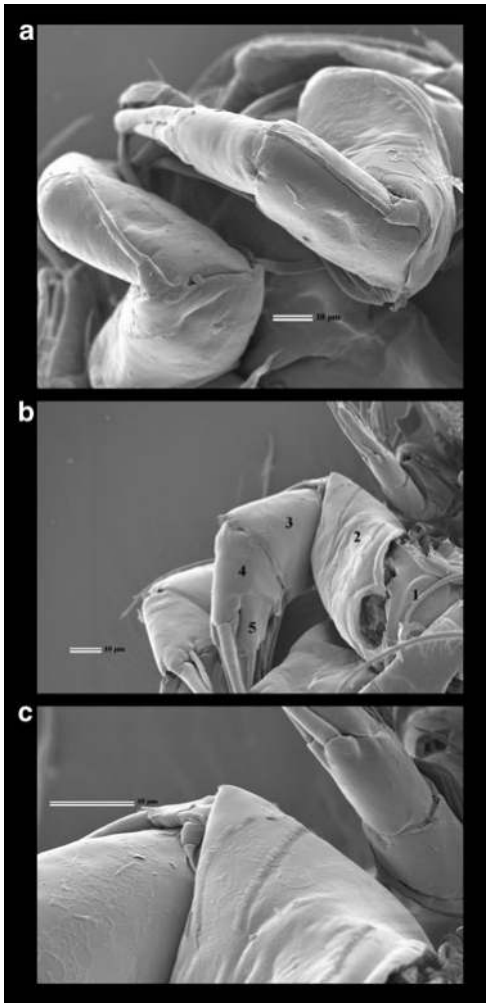

Fig. 23 SEM: *Penthesilenula brasiliensis* (Pinto and Kotzian 1961): (**a**) A2, dorsal view; (**b**) A2 lateral view; (**c**) exopod on the A2

(the short one) and the aesthetasc "y3." The male antennae have six segments compared with five in the female and a series of putative chemical receptors originating at the extra segment boundary. In males, there are four "t" setae. Seta "t1" is transformed to a flattened disc-shaped projection. Setae "t2" and "t3" are short and broad; seta "t4" is thin. The claw "G3" is also much shorter than in females and transformed into a broad structure.

2.5 Mandibula

Mandibula, or Md, is the third head appendage used mainly for feeding (Fig. 24). In some myodocopid lineages (order Myodocopida), this appendage actually resembles a lot the podocopid antenna, while in others (order Halocyprida) it is very similar to the actual mandibula of the podocopid lineages.

2.5.1 Superfamily Cypridoidea

The appendage consists of three clear parts (Fig. 24d): coxa, branchial or vibratory plate, and the palp. The coxa is a robust part, distally equipped with strong teeth, forming a masticatory process (Fig. 24c), which is used for processing food. These strong teeth accompanied by setae. Slightly more proximally from the largest tooth, there is a single, short, and strong seta, which is also densely covered with small setules. The first segment of the palp is actually the basis of the appendage and it carries a branchial or vibratory plate (Fig. 24a, b, e). The vibratory plate represents an exopod and it is used for circulation of the fluids in the domiciliar space. There are usually around seven long pappose setae (also called rays), and only in some subterranean lineages there is a strong reduction, in which case there are only one or two setae at the most. The first segment of the palp carries posteriorly a group of four setae: two "S" setae, one short "alpha" seta, and one unnamed seta. In the family Cyprididae, one of the "S" setae is particularly long and usually bent. Both "S" setae carry a row of long setules along the inner margin. The length of the "alpha" seta may be an important taxonomic feature. The first endopodal segment, or the second segment on the palp, has a maximum of three setae anteriorly. Posteriorly on the segment there is a group of up to seven setae distally, of which the shortest one is usually plumed and is called the "beta" seta. One seta originates more medio-distally on the segment and is clearly separated from the group of three to five setae. The appearance of the "beta" seta and the number of setae in the group is an important taxonomic feature. Most of the setae are heavily pappose. The third segment on the palp has a maximum of four setae antero-distally, situated more on the exterior side of the segment. On the interior side of the segment, along the distal margin there is a row of up to four setae, the most anterior one is called the "gamma" seta; its morphology (length and presence/absence of the small setae)

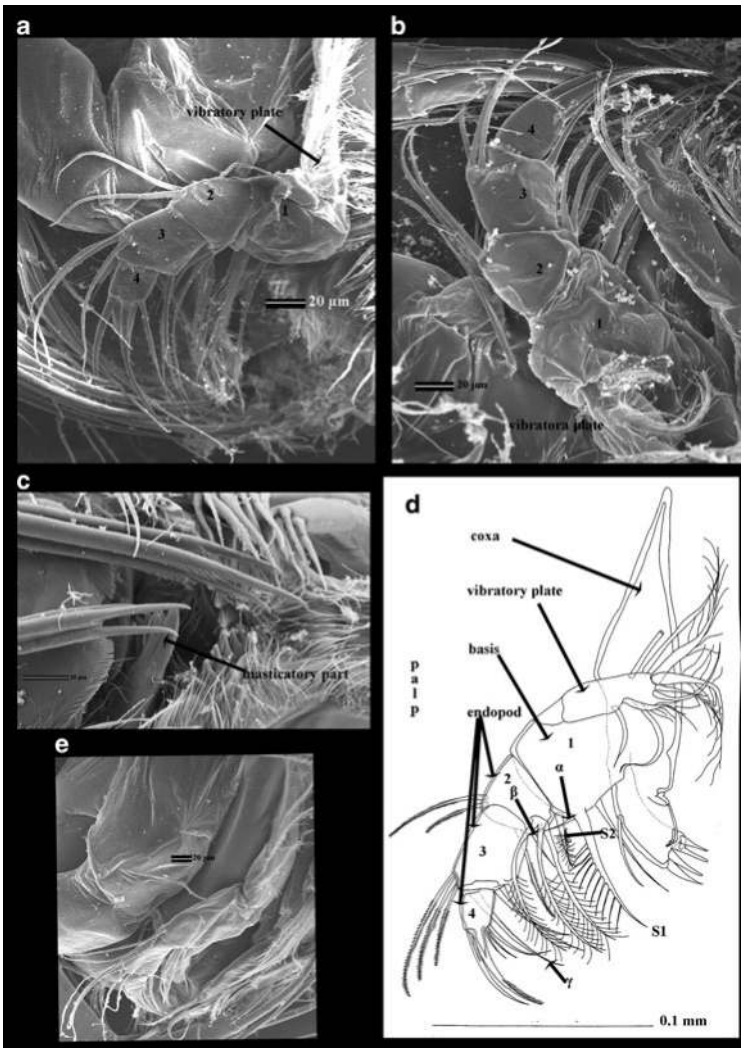

Fig. 24 (**a**, **b**, **c**, **e**) SEM; (**d**) Line drawing: (**a**) *Candona neglecta* Sars 1887; (**b**) *Candona lindneri* Petkovski 1969b; (**c**, **e**) *Psychrodromus olivaceus* (Brady and Norman 1889); (**d**) *Typhlocypris parvula* (Sars 1926): (**a**, **b**, **d**, **e**) Md; (**c**) detail of the Md from the ventral side of the body

being an important taxonomic character. On the most posterior end, there is additionally one long and one very short seta. The most distal segment of the palp has a central claw which can have a broad (like in some Candoninae lineages), or a narrow basis. This claw is accompanied on both sides with up to four setae, sometimes two of them being transformed into claws as well. The length ratio of the segments on the palp is an important taxonomic feature, especially the length of the terminal segment.

2.5.2 Superfamily Cytheroidea

In this superfamily the mandibula is similar to Cypridoidea (Fig. 25b). However, there are some clear differences. The division between the segments of the palp is often not clear, and also re are only two setae on the first segment posteriorly, and they setae are never as pappose as long as in Cypridoidea. The anterior side of the second segment also carries only one or two setae, and the group of setae posteriorly on the same segment is not as clear. There is no clear homology between the setae; therefore, "alpha," "beta," and "gamma" setae are not recognizable. The terminal segment carries only two or three claws and is never long. Some of the setae on the posterior side of the palp may be transformed and this can be an important taxonomic feature. The number of setae on the exopod varies and it can be reduced to only one seta in some lineages.

2.5.3 Superfamily Darwinuloidea

In this superfamily the coxa is very short and stout (Fig. 25a). The vibratory plate (exopod) has very short filaments. The palp is only three-segmented, of which the first segment belongs to the protopod and only two remaining ones to

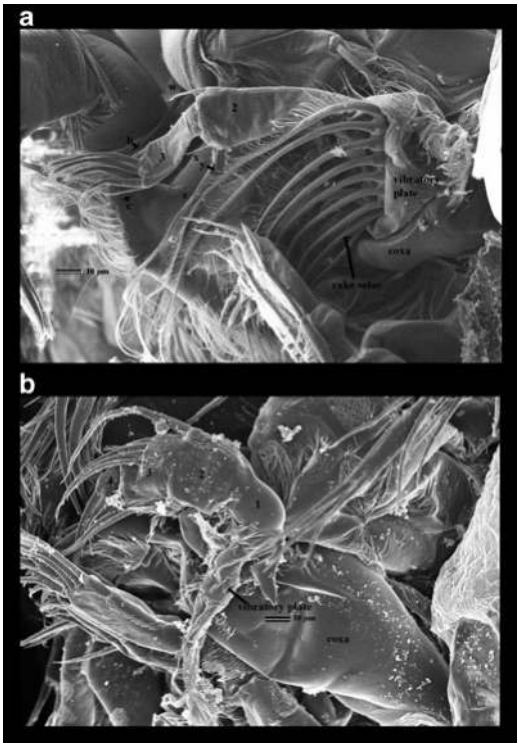

Fig. 25 SEM: (**a**) *Darwinula stevensoni* (Brady and Robertson 1870); (**b**) *Paralimnocythere karamani* (Petkovski 1960a): Md

the endopod. On the posterior side of the first segment of the palp, there is a group of setae, aligned in a row, and called rake setae. The number of these setae is indicative for the larval stage, as it increases during ontogeny after every molt; only the adults have all eight setae present. Besides the rake setae there are two additional setae on the posterior side of the first segment. The second segment of the palp may have up to two setae antero-distally (named setae "y" and "z"), one seta medially (seta "x") and one postero-distally (seta "w"). The terminal segment is very characteristically curved and it has up to seven setae: the more posterior one is called "b" seta, and the most anterior one "c" seta. The length and number of setae on the palp are important taxonomical features.

2.6 Maxillula

Maxillula, first maxilla or Mxl, is the fourth appendage on the body (Fig. 26). Its function is almost completely in respiration and feeding. It has a relatively large vibratory plate (or branchial plate) with numerous heavily feathered setae (also known as rays), enabling the transport of fluids through the domicilar space inside the carapace. The branchial plate is missing in all Myodocopa. While in some podocopid lineages, branchial plates are also present on some post-maxillular appendages the one on the maxillula is always the largest (Smith et al. 2005). Most of the setae on the branchial plate are orientated posteriorly, with an exception of the group of clearly separated setae which are situated distally on the plate and which are orientated anteriorly; the latter setae are called "reflexed setae" and are an important taxonomic character at higher taxonomic levels. Besides the branchial plate, there are three endites and an endopod, which is usually two-segmented. The three endites and the endopod terminate in claws which have a feeding role. The branchial plate is thought to be an exopod (Horne 2005).

2.6.1 Superfamily Cypridoidea

In this superfamily, the number of rays on the branchial plate is between 20 and 30, with two to six being reflexed setae (Fig. 26a). The endopod is always two segmented (Fig. 26b). The first segment carries most of the setae antero-distally, with one seta situated more medially than the others. This seta is sometimes missing. There is a maximum of eight setae altogether on this segment and they are usually pappose. The second segment varies in shape and this is an important taxonomic feature at the genus level in the families Cyprididae and Candonidae. It can be elongated, rectangular, or spatula-like. Distally this segment carries also a different number of claws and setae, but most usually there are up to three claws and three setae. The number of claws/setae is often reduced in subterranean representatives. Of the three endites the most anterior one carries the strongest claws and there can be two to six such strong claws, accompanied with some

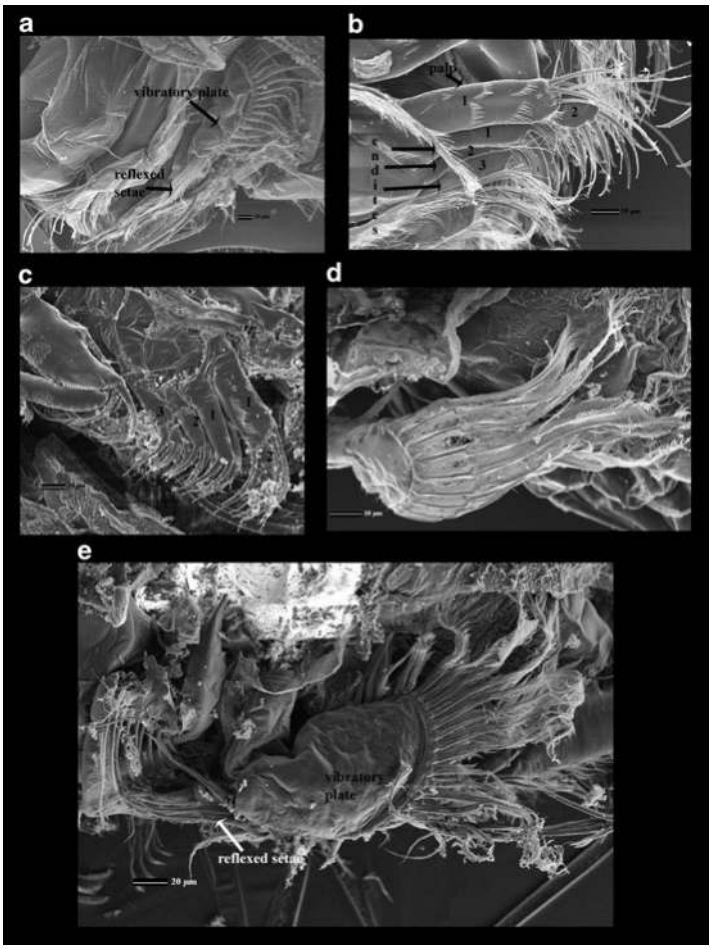

Fig. 26 SEM: (**a**, **b**) *Psychrodromus olivaceus* (Brady and Norman 1889); (**c**) *Paralimnocythere karamani* (Petkovski 1960a); (**d**) *Gomphodella quasihirsuta* Karanovic, 2009; (e) *Darwinula stevensoni* (Brady and Robertson 1870): (**a**, **d**, **e**) Mxl, showing the vibratory plate; (**b, c**) Mxl palp and endites

setae. The claws can also be feathered or serrated or smooth, and this may be a useful taxonomic feature on the species level. The other two endites carry up to eight claws, which are less developed than the ones on the first endite.

2.6.2 Superfamily Cytheroidea

In this superfamily the branchial plates are usually much smaller and there are less than 20 rays on the plate (Fig. 26d), one or two being reflexed setae. The palp can be

one- or two-segmented (Fig. 26c). The three endites carry five or fewer weak claws, and there are no such well-developed claws as in Cypridoidea.

2.6.3 Superfamily Darwinuloidea

Darwinulidae often have more than 30 rays on the branchial plate, four being reflexed setae (Fig. 26e). The palp is two-segmented with five to six setae on the first segment, and two claws and two to three setae on the second segment. On the first two endites there are two or three strong claws and up to six setae. The last endite carries usually only setae, or weakly developed claws.

2.7 First Thoracopod/The Fifth Limb

This appendage has the greatest number of different names: fifth limb, first thoracopod, second maxilla, or maxilliped, and abbreviations used are T1 or L5. There are two opinions regarding whether this limb belongs to the head region or to the thoracic one. Cohen and Morin (1997) consider fourth and fifth limbs as head limbs, because in Ostracoda the maxillary excretory gland, if present, is always associated with the fifth limb. Thus, the fourth limb is termed the first maxilla and the fifth limb the second maxilla. This is certainly true for the subclass Myodocopa, but doubtful in Podocopa lineages. Smith and Martens (2000) provide several arguments for this: the fifth limb is not attached to the ventral head plate ("sternum") in Sigilliocopina, Bairdiocopina, and Cytherocopina; in many podocopids (Bairdiocopina and Cytherocopina and some Cypridocopina, like *Macrocypris),* the fifth to seventh limbs are clearly homologous walking legs with homologous segments and chaetotaxy (Meisch 1996); and during the larval development of Cypridocopina, the fifth limb changes from a walking leg in the A-4 instar to feeding appendages in the A-3 instar. However, even among Myodocopa, the appearance of the fifth limb is not the same: in Myodocopida there is a large branchial plate, three endites, and multisegmented exopod, while in Halocyprida this leg is biramous with coxa and basis, and both exopod and endopod, the second one being multisegmented. There is also a small branchial plate. Branchial plate on the fifth limb is present in some Podocopa lineages, but, again, Horne (2005) considers these to be exopods, while in Myodocopa they are epipods. In Podocopa, this leg is being used for walking and feeding and even plays a role in copulation behavior in males.

2.7.1 Superfamily Cypridoidea

In this superfamily the fifth limb is sexually dimorphic. In females, it consists of a protopod, endopod, and a small exopod (Figs. 27a, b and 28b). The exopod is a branchial plate with a maximum of six, usually strongly pappose rays. The number of

rays is often reduced, and may represent an important taxonomic feature. However, because this part easily falls off during the dissection, loses some rays, or becomes folded, the number of rays is often difficult to interpret, and should be taken with caution. The endopod consists of a single segment in Notodromadidae, Cyprididae, and Candonidae, while it is two- or three-segmented in Ilyocyprididae. The protopod bears proximally two setae, called "a" and "a'" setae, and often one of them is missing. There are three more setae, situated more distally, "b," "c," and "d" setae, the presence of which is an important taxonomic feature in the family Cyprididae. The protopod terminates anteriorly with numerous pappose setae, arranged more or less in a row, representing the endites. In the males the endopod (or "palp") is transformed into a clasping organ (Figs. 27c and 28a), while in the females it is a simple elongated segment (sometimes two- or three-segmented) terminating with a couple of setae. Males use the palp for grabbing and handling the female during the copulation. In males it can be one- or two-segmented. It consists of one segment in Candoninae. The proximal part is called the body, and the distal, often curved and elongated, part is

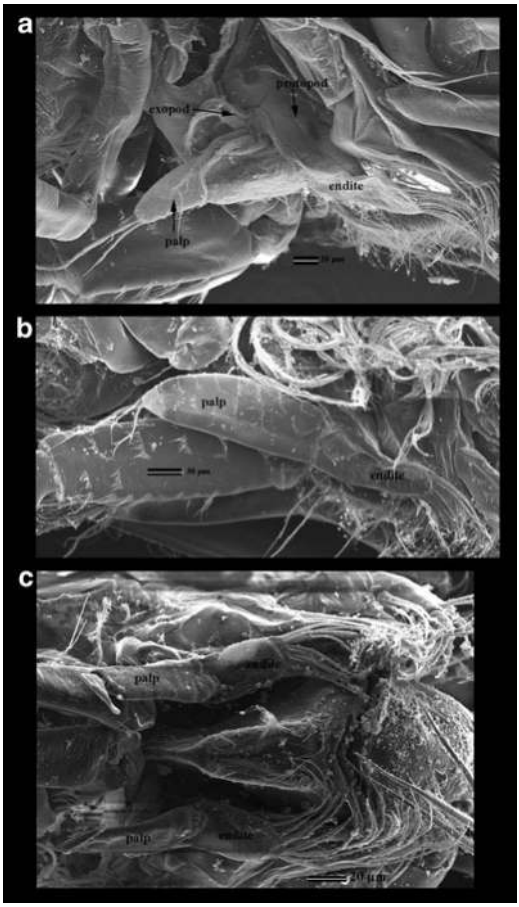

Fig. 27 SEM: (**a**) *Heterocypris reptans* (Kaufmann 1900a, b, c); (**b**) *Candona lindneri* Petkovski 1969b; (**c**) *Plesiocypridopsis newtoni* Brady and Robertson 1870: (**a**, **b**) L5, ♀; (**c**) L5, ♂, showing the position of the prehensile palps

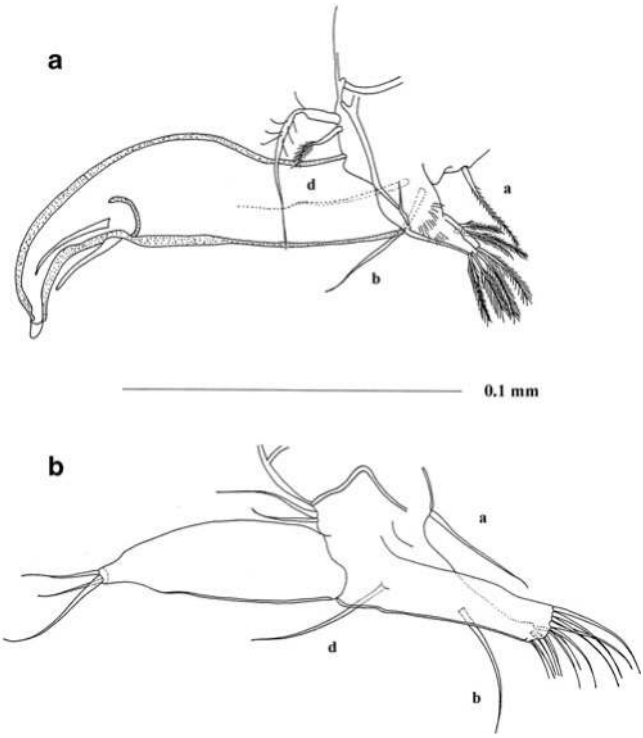

Fig. 28 Line drawings; (**a**) *Candona crogmaniana* Turner 1894; (**b**) *Typhlocypris fluviatilis* (Hoff 1942): (**a**) L5, ♂; (**b**) L5, ♀

called the finger. There are two additional structures on the point where the body and finger join, and they can be quite elaborately developed, or very thin, and often one is missing. The tip of the finger also has an additional structure, very similar to the one between the body and finger. The palps are often asymmetrical, and their appearance is a very important taxonomic character on the species level.

2.7.2 Superfamily Cytheroidea

The fifth limb in this group is a walking leg, not much different from the more posterior legs (Figs. 29a and 31a). It is, however, the smallest of all three pairs of the thoracopods and easily recognizable by the presence of two, instead of one apical seta anteriorly. On the anterior side of the protopod there are two more setae, both attached proximally. Posteriorly, there may also be one seta, attached medially or more distally, and it is often pappose. This seta is often interpreted as an exopod. The endopod is three-segmented, each segment carrying one seta anteriorly, and the apical segment terminates in a claw. In some families, this appendage may be sexually dimorphic.

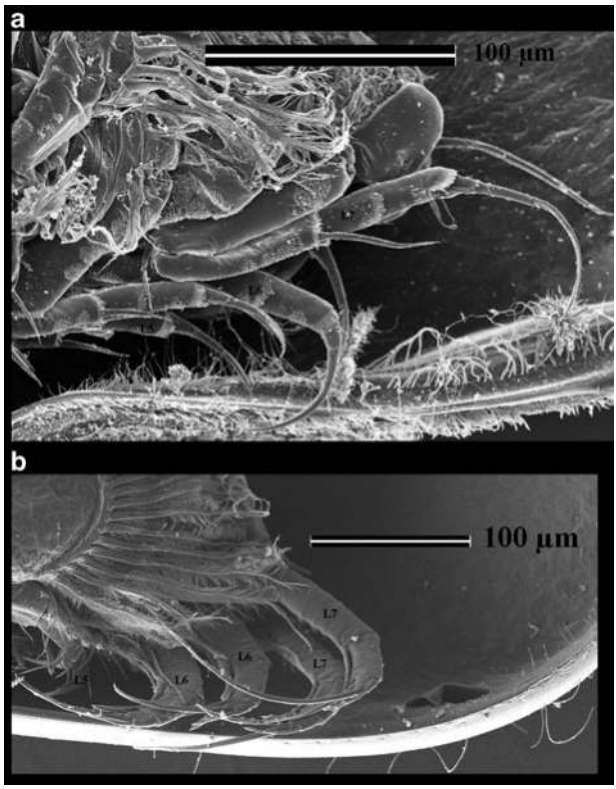

Fig. 29 SEM: (**a**) *Paralimnocythere karamani* (Petkovski 1960a); (**b**) *Darwinula stevensoni* (Brady and Robertson 1870): posterior end of the body, showing thoracopods

2.7.3 Superfamily Darwinuloidea

In this lineage, the fifth limb is sexually dimorphic as in Cypridoidea. In both males and females there is a protopod and a small branchial plate attached to it. The protopod terminates with endites, similar to Cypridoidea. The endopod is a walking leg in females, consisting of three segments and terminating with a claw (Figs. 29b and 31d, e). In males, the endopod is a clasping organ, similar to that in Cypridoidea.

2.8 Second Thoracopod/The Sixth Limb

This appendage is a walking leg in all representatives of the order Podocopida, with the branchial plate, if present, reduced to only one seta. In Platycopida males have the endopod transformed into a clasping organ, and there is a branchial plate with

five to seven rays. In females belonging to this lineage there is only the branchial plate present, all other parts being completely reduced. Cladocopina lack the second thoracopod, while this appendage is a walking leg with a three-segmented endopod, and a branchial plate in Halocypridina. In all other Myodocopa the sixth appendage has a large branchial plate, and four endites, and no walking-like structures are present.

2.8.1 Superfamily Cypridoidea

There are two incompletely separated basal segments, each bearing one seta antero-distally, "d1" and "d2." The second segment has a knee joint with the endopod. One or both setae are sometimes missing (Fig. 30a), which is an important character at the genus level, as well as is the length ratio between these two setae. The endopod is four- or three-segmented (Fig. 30a, b). The first segment is the longest and it carries one seta antero-distally, the "e"-seta. This segment is jointed with the following one. The following two segments are sometimes fused. There is usually one seta antero-distally on the second segment, the "f"-seta, and two setae on the fourth segment, the longer one called the "g"-seta. The terminal segment is short and it most usually carries one long claw and two setae, or very rarely two claws and one seta. The most anterior one is called "h1," the claw is "h2," and the most posterior one is "h3." The claw is often well serrated and heavily sclerified. Length ratios between the claw and the endopodal segments, as well as the length ratios between the segments, are important taxonomic features. The appendage is often covered with pseudochaetae, especially at the joints of segments or from where the setae originate.

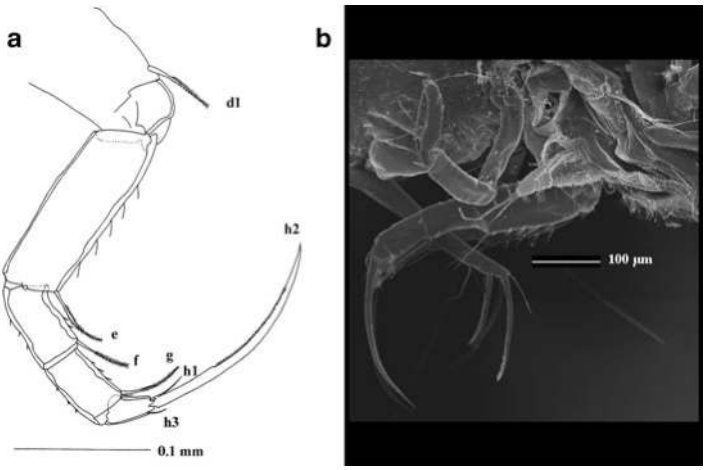

Fig. 30 (**a**) Line drawing; (**b**) SEM: (**a**) *Typhlocypris elliptica* (Furtos 1933); (**b**) *Heterocypris reptans* (Kaufmann 1900a, b): L6

2.8.2 Superfamily Cytheroidea

This appendage is very similar to the previous one in the same superfamily, with the exception that there is only one seta most distally on the protopod and that it is longer than the first thoracopod (Figs. 29a and 31b). The (knee) joint is present only between the protopodal and the first endopodal segment. The appendage is often covered with pseudochaetae, especially at the joints of segments or from where the setae originate.

2.8.3 Superfamily Darwinuloidea

In this lineage no branchial plate (like that on the first thoracopod) is present on the second thoracopod. There are three setae on the protopod anteriorly (two apically and one medially). The (knee) joint is present only between the protopodal and the first endopodal segment. The endopod is four-segmented and it has three distal setae on the first segment, one on the second, while the terminal segment is very similar to Cypridoidea (Figs. 29b and 31d, e).

2.9 Third Thoracopod/The Seventh Limb

This appendage is absent in the order Platycopida (Podocopa) and Cladocopina (Myodocopa). In all Podocopida, except for Cypridocopina, this is a walking appendage. In Cypridocopina this leg turns dorsally and often has a terminal pincer. It serves for cleaning the posterior domicilar space, and therefore is often referred as the "cleaning leg." In Myodocopida (Myodocopa) the leg is multisegmented and worm-like, carrying few setae on some of the segments and terminating in a rather complex structure. In Halocypridina (Myodocopida) the leg is very small, consisting of a cylindrical ramus and two terminal setae.

2.9.1 Superfamily Cypridoidea

The basal segment has two setae anteriorly: "d1" and "d2," and one posteriorly – "dp" (Fig. 32a, b). One or more of these setae are often missing, which is an important taxonomic character at the genus level. This segment is joint-connected with the endopodal segments. There are up to four endopodal segments, the first one also having a joint connection with the proceeding segment. The first endopodal segment carries one seta anteriorly – seta "e"; the following two segments are often fused and each carries one seta antero-distally: "f" and "g". One of these three setae is often missing, which is an important taxonomic character at both generic and specific levels. The terminal segment may be normally developed, but nevertheless shorter than the preceding segments (Fig. 32d–f), or it can be almost fused with the

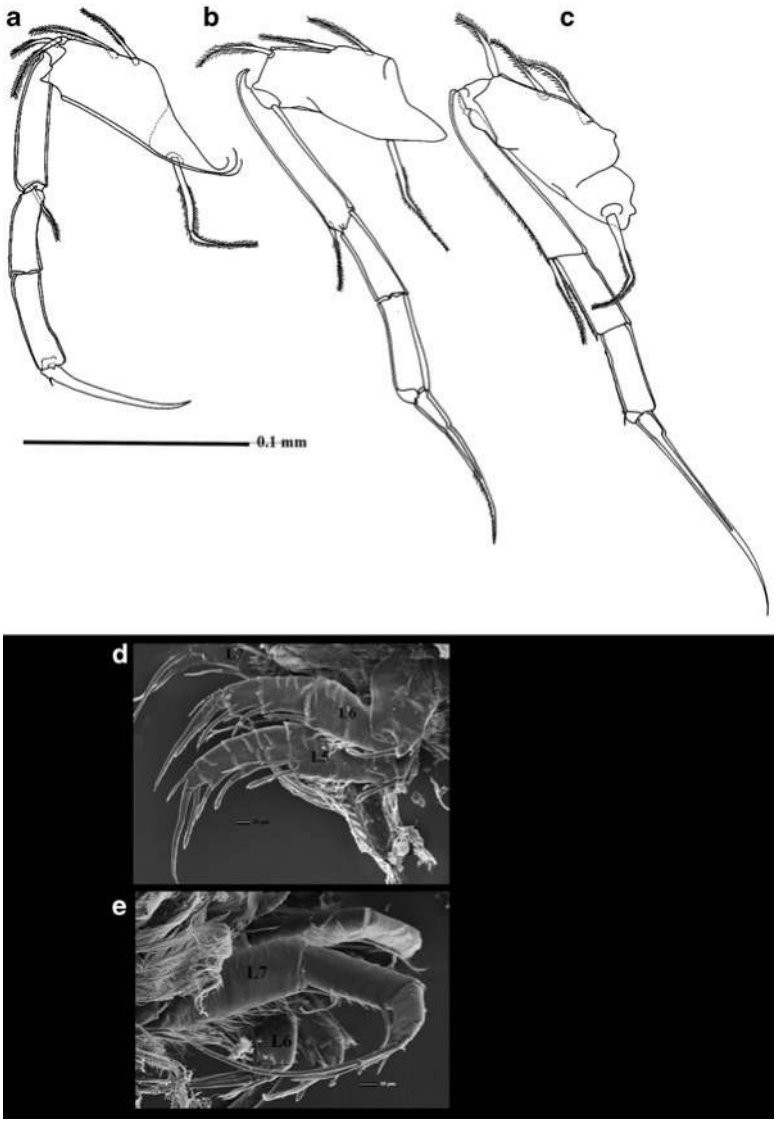

Fig. 31 (**a**–**c**), Line drawings; (**d**, **e**) SEM: (**c**) *Gomphodella quasihirsuta* Karanovic, 2009; (**d**) *Penthesilenula brasiliensis* (Pinto and Kotzian 1961); (**e**) *Darwinula stevensoni* (Brady and Robertson 1870): (a) fifth limb, (b) sixth limb; (c) seventh limb, (d,e) thoracopods.

previous segment in a structure called the "pincer" organ (Fig. 32c). On both normally developed and transformed segments, the three setae, "h1," "h2," and "h3", can be recognized. The length ratio between these setae is an important generic feature. The appendage is often covered with pseudochaetae, especially at the joints of segments or from where the setae originate.

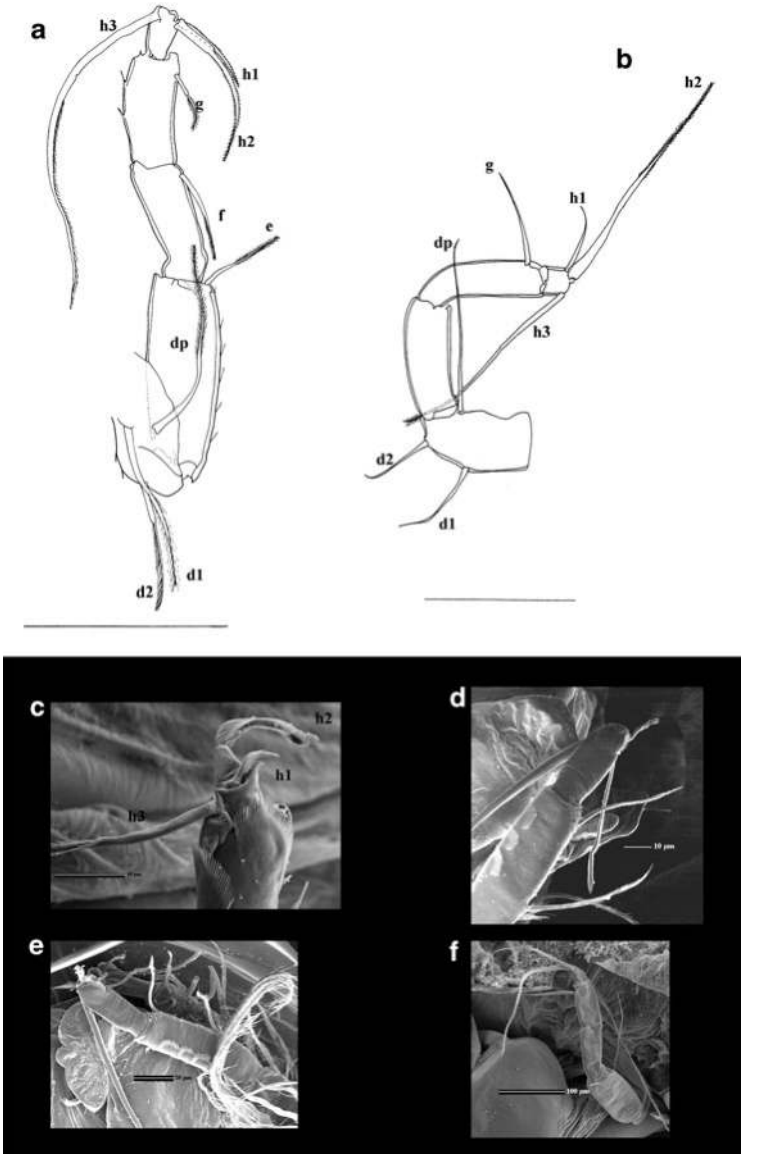

Fig. 32 (**a**, **b**) Line drawings; (**c**–**f**), SEM: (**a**) *Latinopsis patagonica* Karanovic and Datry 2009; (**b**) *Typhlocypris punctata* (Furtos 1933); (**c**) *Sarscypridopsis ochracea* (Sars 1924); (**d**, **e**) *Cyclocypris ovum* (Jurine 1820); (**f**) *Candona neglecta* Sars 1887: (**a**, **b**, **e**, **f**) L7; (**c**) pincer organ; (**d**) terminal segment of the L7

2.9.2 Superfamily Cytheroidea

In this superfamily, the seventh limb is a walking leg very similar to the sixth limb, terminating with a claw (Fig. 31c), or in some lineages the terminal segment may carry a long seta. This appendage may also be sexually dimorphic.

2.9.3 Superfamily Darwinuloidea

As in the previous superfamily, this leg is used for walking and it differs from the sixth limb only in number of setae on the protopod (two, instead of three), and the first endopodal segment (one, instead of three) (Figs. 29b and 31d, e).

2.10 Uropodal Ramus

The posterior end of the ostracod body is equipped with a pair of unarticulated appendages called uropodal rami, furca or caudal rami. Recently Meisch (2007) argued that ostracod uropodal processes are not analogous with the furca of other crustaceans as they do not originate from the telson. Instead, Meisch (2007) considers that the most posterior appendages are actually modified uropods, arising from the vibratory plates of the ancestral appendage. Their position in relation to the anal opening differs between ostracod subclasses: in Myodocopa they are positioned posterior to the anus, and in Podocopa anterior. In Myodocopa and Platycopida, these appendages look like strong plates with claw-like or seta-like appendages. Therefore, Meisch (2007) uses the term "uropodal plates/lamellae" for the most posterior appendages of these two lineages. In Podocopida, the most posterior body appendages are transformed into two elongated rami with a maximum of two/three distal claws and one to six setae. For this group, Meisch uses the term "uropodal rami," which is accepted in this book. This appendage is used mostly for walking, but in Myodocopa and Platycopida also for feeding.

2.10.1 Superfamily Cypridoidea

In this superfamily, the uropodal rami consist of two rod-shaped structures (rami), each with two claws – one anterior and one posterior, and two setae – one anterior and one posterior (Fig. 33a). Often a number of reductions occur, and the morphology of the uropodal rami is a very important taxonomic feature on every taxonomic level in this superfamily (Fig. 33b). In some subfamilies, it is reduced to a short, whip-like ramus, or it may be completely missing. In the family Cyprididae there is often asymmetry of the two rami. Sexual dimorphism is very rare. The length ratios between the claws, between claws and rami, and between setae and claws are very important taxonomic characters. There is often a seta dorsal to the basis of the ramus, called caudal seta.

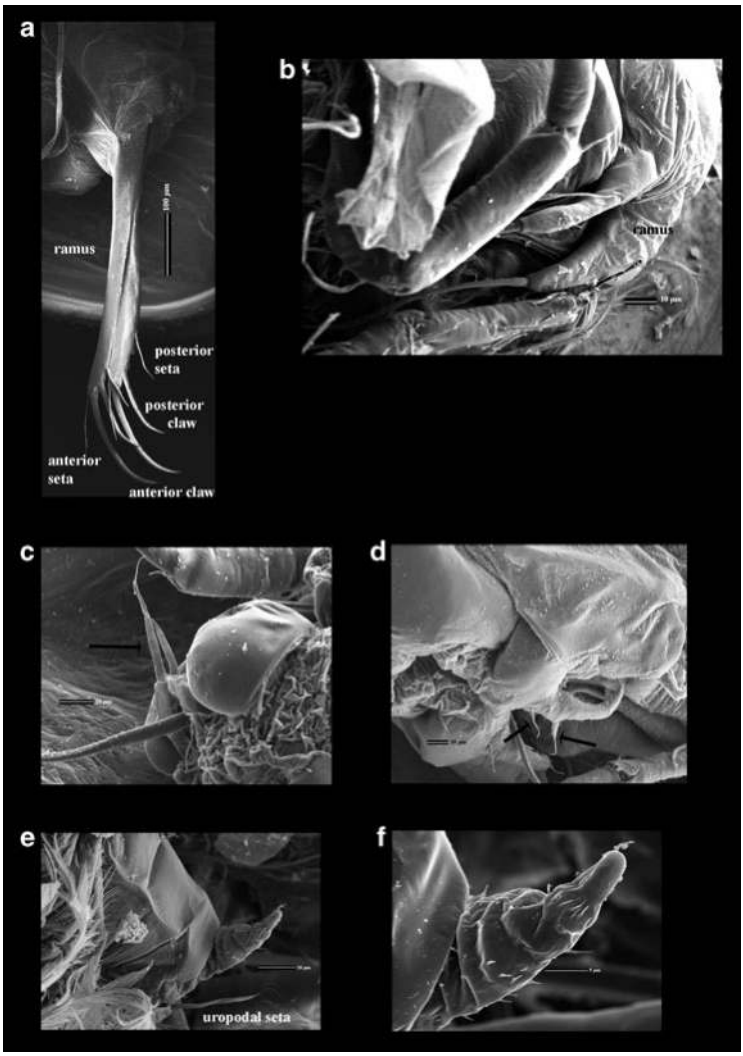

Fig. 33 SEM: (**a**) *Candonocypris sp.*; (**b**) *Sarscypridopsis ochracea* (Sars 1924); (**c**, **d**) *Paralimnocythere karamani* (Petkovski 1960a); (**e**, **f**) *Darwinula stevensoni* (Brady and Robertson 1870): (**a**–**c**) uropodal ramus; (**d**) uropodal ramus setae incorporated in hemipenis; (**e**, **f**) most posterior end of the body

2.10.2 Superfamily Cytheroidea

In this superfamily, the uropodal ramus is reduced to a short ramus and couple of setae (Fig. 33c). In males, the uropodal rami are incorporated in the hemipenis (Fig. 33d).

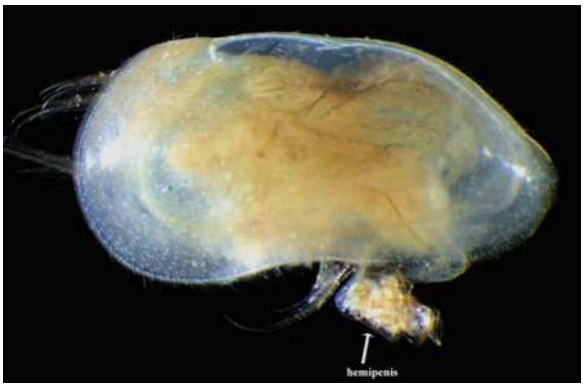

Fig. 34 Microscopic photograph: *Repandocypris austinensis* Halse and McRae 2004. Photo: S. Halse.

2.10.3 Superfamily Darwinuloidea

This appendage is also reduced to a short ramus with a distal seta, or only a seta (Fig. 33e), or completely missing in some females of Darwinuloidea. In a single male described so far, the uropodal ramus is not incorporated in the hemipenis and it is similar to the female, except being shorter. The body terminates with a so-called abdominal process (Fig. 33f).

2.11 Copulatory Appendages

In males the copulatory organ (Fig. 34) is called hemipenis and it is a paired appendage in all ostracod lineages except for Halocyprida. It is a very complex apparatus, especially in Podocopida, and it is believed to arise from several appendages fused together. The hemipenis is situated in front of the uropodal ramus and it sometimes constitutes more than a third of the ostracod body size. In Cypridocopina it is dorsally connected with the Zenker Organ (Fig. 35a). Although taxonomically very important, the structure of the hemipenis is the least understood of all the appendages, and it is still difficult to identify the homologous structures in different ostracod lineages. This may be because in different lineages the hemipenis may have arisen from a different number of body segments and therefore involved different appendages.

2.11.1 Superfamily Cypridoidea

In this superfamily one can recognize two distinct parts on the hemipenis: the chitinous "shell" called the peniferum and the internal parts (Fig. 35c, d). The chitinous shell has two or three lobes called: outer, middle, and inner lobe. The outer lobe is on

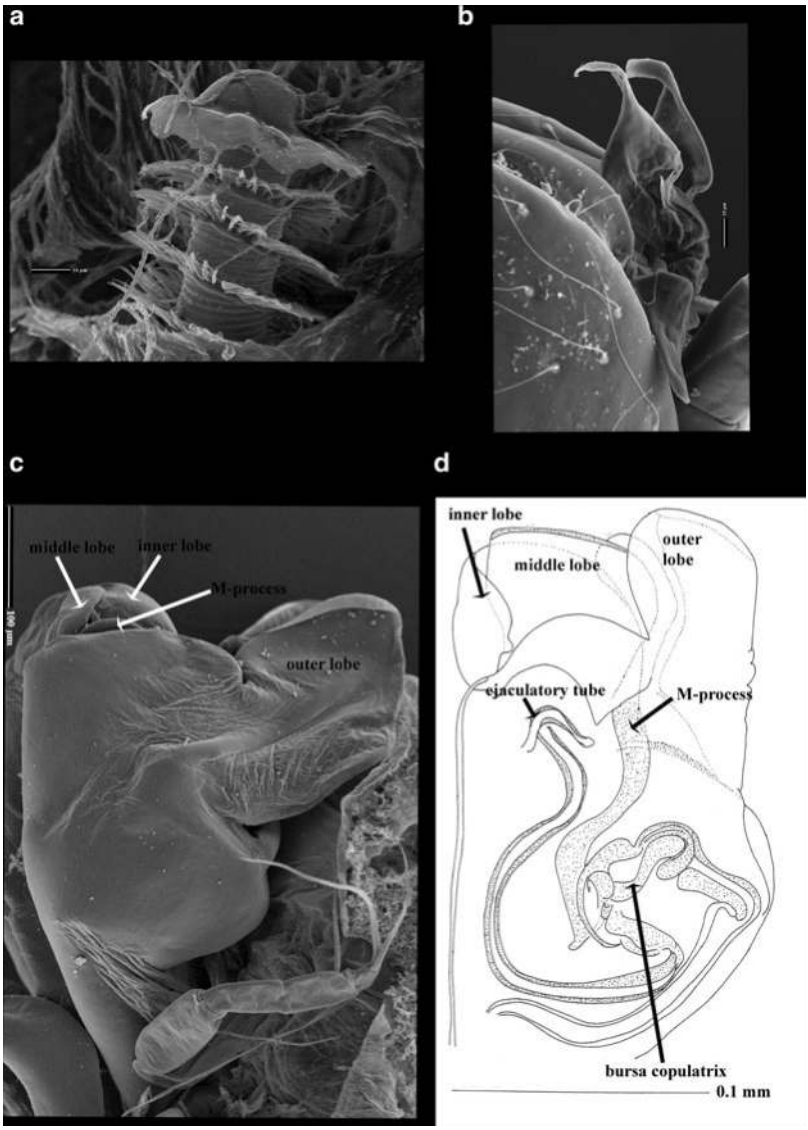

Fig. 35 (**a**–**c**) SEM; (**d**) Line drawing: (**a**, **b**) *Cypria karamani* Petkovski 1976; (**c**) *Candona neglecta* Sars 1887; (**d**) *Typhlocypris punctata* (Furtos 1933): (**a**) Zenker organ, top part; (**b**) hemipenis is erection; (**c**, **d**) hemipenis

the curved part of the hemipenis, while the inner lobe is on the more straight part of this organ. Of the internal structures the following parts can be recognized: spermiducts and the *bursa copulatrix*. Spermiducts are often coiled and making a complex labyrinth internally. Their walls are often well-chitinous as well. The spermiducts terminate distally with an ejaculatory tube. The *bursa copulatrix* is situated proximally and it is

bowl-shaped. It often has a well-chitinous process, called the "M" process. Structures and shapes of both the peniferum and the internal parts are very important taxonomic features. Because the hemipenis changes shape drastically during the erection, the proper way to observe it is at rest.

2.11.2 Superfamily Cytheroidea

In Cytheroidea there is no Zenker organ and the sperm is pumped with the aid of a muscular hemipenis (Fig. 36). It is still difficult to homologize parts of the hemipenis between Cytheroidea and Cypridoidea, and different terminology is often used in the literature. Nevertheless, there is a peniferum which consists of distal and lateral lobes and there is a complex clasping organ (Fig. 36b), which is protruded during the copulation. This piece has an upper ramus, a lower ramus, as well as the

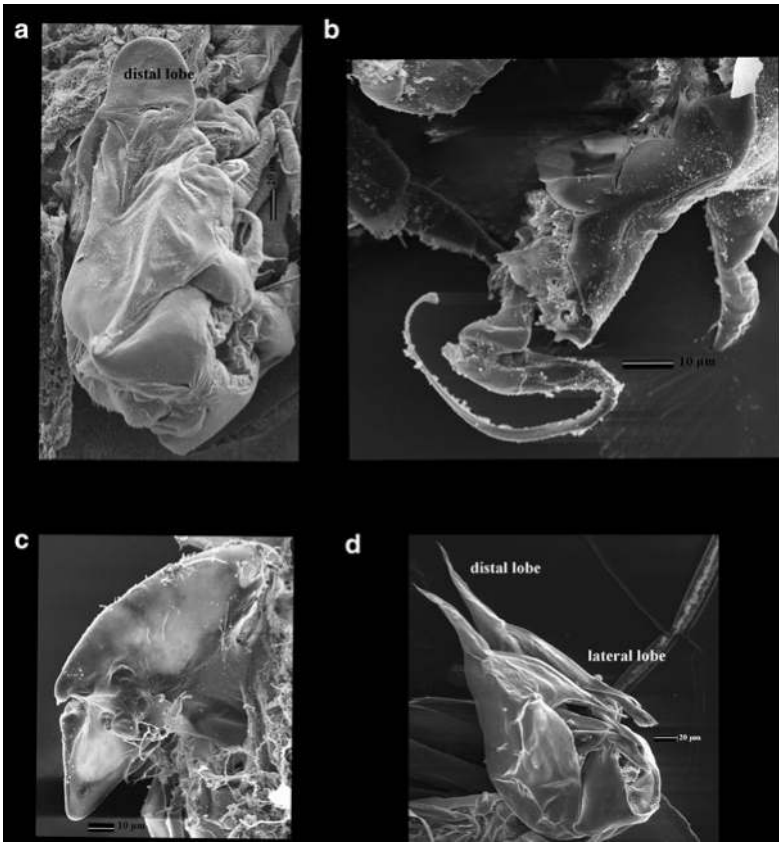

Fig. 36 SEM: (**a**, **b**) *Paralimnocythere karamani* (Petkovski 1960a); (**c**) *Gomphodella quasihirsuta* Karanovic, 2009; (**d**) *Leptocythere sp.*: (**a**, **c**, **d**) hemipenis; (b), clasping organ. (**d**) Photo: D. Keyser

copulatory process. The shape and structure of the peniferum and the clasping organ are both very important taxonomic characters.

2.11.3 Superfamily Darwinuloidea

According to the only male ever recorded, the hemipenis is more similar to Cypridoidea than Cytheroidea. There is also a dorsal structure outside the hemipenis, which may be homologous with the Zenker organ. The hemipenis also consists of three lobes and internal system of canals but no labyrinth as in Cypridoidea.

3 Anatomy of Ostracods

3.1 Exoskeleton

The head region is supported by the chitinous framework connected by a thin membrane. The following parts can be recognized (Fig. 37a–d): forehead, upper lip, and lower lip (this is in the ostracod literature often referred to as hypostome, but it is not homologous with the hypostome of other arthropods). Antennula, antenna, and eyes are situated on the forehead. Between the posterior margin of the upper lip and anterior margin of the lower lip, lies the mouth opening. Rake organ, a paired chitinous structure used for food processing, is situated in the mouth region and it is attached to the lower lip. Mandibula and maxillula are connected to the upper margin of the lower lip with chitinous supports. Other appendages in the body are also connected to the body and with each other by a network of chitinous rods. Attachment of the uropodal ramus is by far the most apparent of all other attachments in the body, and is often used in the taxonomy of freshwater ostracods (Fig. 37e, f).

3.2 Digestive System

This system starts with the mouth opening (Fig. 38a). The food is handled first with the mandibula, maxillula, and the first thoracopod, although the breaking of the food is most usually achieved by the mandibula. The ball of food is pushed with the help of mandibula and the rake organ into the oral cavity. Then, via the esophagus, the food passes into the anterior intestine. The front part of the anterior intestine is called stomach, and it is lined with secretory cells, and it receives the products of the paired digestive glands called the hepatopancreas. Following the anterior intestine, the food passes into the posterior intestine which is a straight tube leading

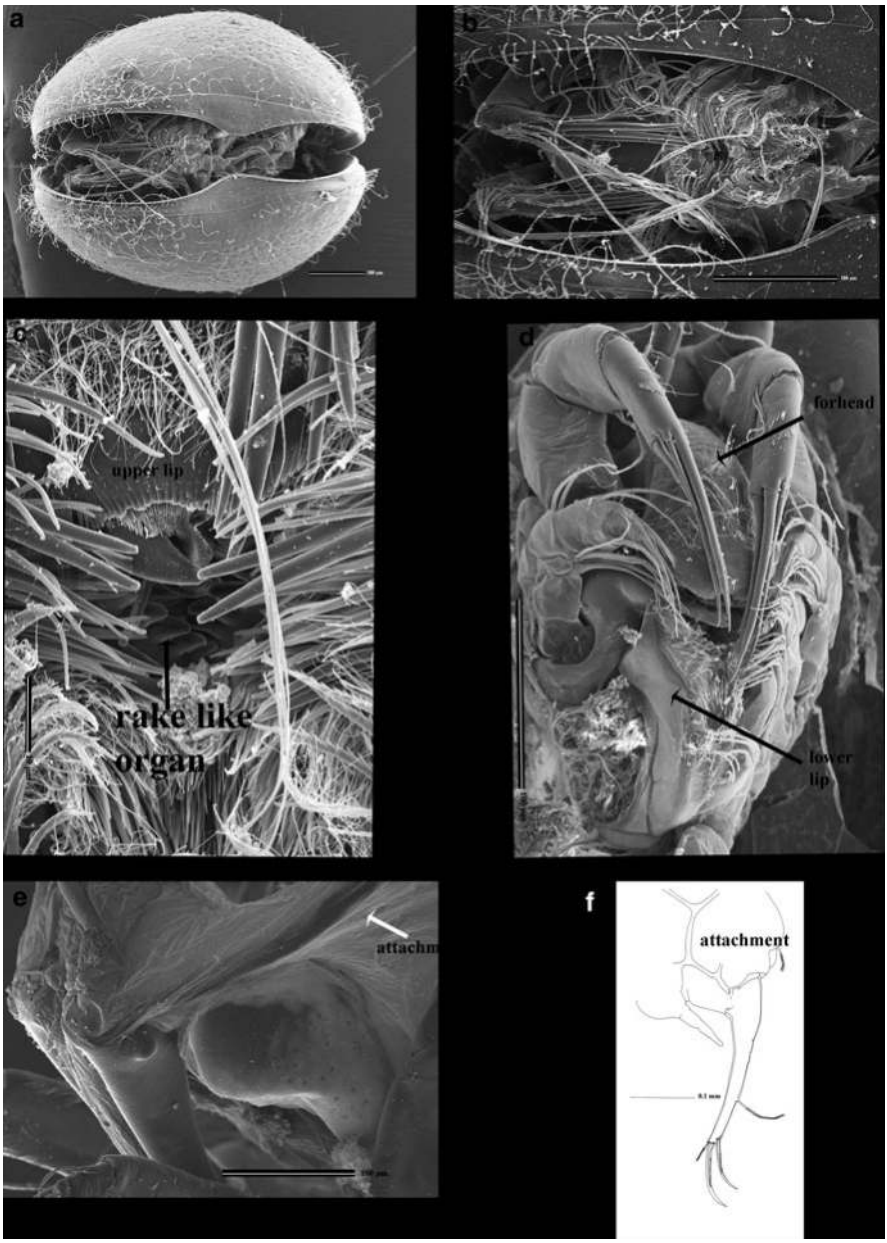

Fig. 37 (**a**–**e**) SEM; (**f**) Line drawing: (**a**–**c**) *Cypridopsis vidua* (Müller 1776); (**d**) *Psychrodromus olivaceus* (Brady and Norman 1889); (**e**) *Eucypris cf. virens* (Jurine 1820); (**f**) *Candona crogmaniana* Turner 1894: (**a**) ventral view, showing the position of the mouth; (**b**) mouth opening; (**c**) rake-like organ; (**d**) mouth region from the anterior; (**f**) attachment of the uropodal ramus; (**f**) uropodal ramus

to the anus. Peristaltic movement can often be observed in the living ostracods (McGregor 1967), by feeding ostracods with dyed food particles. Gut content is often visible through the shell (Fig. 38g). Observation of the feeding of the myodocopids (Vannier et al. 1998) reveals that these animals use the fourth limb and the uropoda lamellae in coordination to abrade and eventually tear open the protective integument of living/dead prey such as annelids. The mandibular palps are used mainly to hold the food. Food is transferred to the mouth by the fourth and the fifth limb and is passed to the esophagus by the endites (mandibles, and fourth and fifth limbs). Food is subsequently pumped up to the stomach by peristaltic contractions of the esophagus.

3.3 Respiration and Blood Circulation

Ostracods belonging to the subclass Podocopa do not have gills and the respiration is carried out across the entire body surface, with the aid of the branchial plate of the mandibula, maxillula, and the fifth limb, which, by constant fanning, provide a flow of oxygenated water and remove the CO_2. Epidermal cells of the inner lamella of the carapaces are also an important part in respiration and osmoregulation (Yamada et al. 2004). Podocopids have no heart and the circulation of the body fluids is probably achieved by a rhythmic contraction of the muscles in the body wall and gut or by general movement of the animal. Myodocopid ostracods have a circulatory system which consists of a single-chambered dorsal heart (pericardium, and myocardium with two ostia), efferent vessels (aorta and secondary arteries), and an integumental afferent network of sinuses radiating from the adductor muscle area to a peripheral channel leading to heart. The heartbeat and the linear velocity of hemolymph in sinuses range from 0.5 to 6 times s^{-1} and 200 to 1,000 μm s^{-1}, respectively. Hemocytes of irregular shapes occur within the circulating hemolymph. This typical open circulatory system is found in most myodocopid ostracods and other crustaceans (Abe and Vannier 1995). However, the dorsal heart, the internal vessels, and the anastomosing network of sinuses running through the carapace are not the only circulatory/respiratory organs known to occur within the group. For instance, cylindroleberidid ostracods develop paired "gill-like structures," so-called because of overall resemblance to the gills of larger malacostracans (Vannier et al. 1996).

3.4 Nervous and Sensory System

The central nervous system consists of three parts: cerebrum, a circum-esophagal collar of fused ganglia, and a ventral chain of ganglia running antero-posteriorly (Rome 1947b; Hartmann 1967). Rome (1947b) divides the circum-esophagal collar into a protocerebrum into which the optic nerves pass, deuterocerebrum which

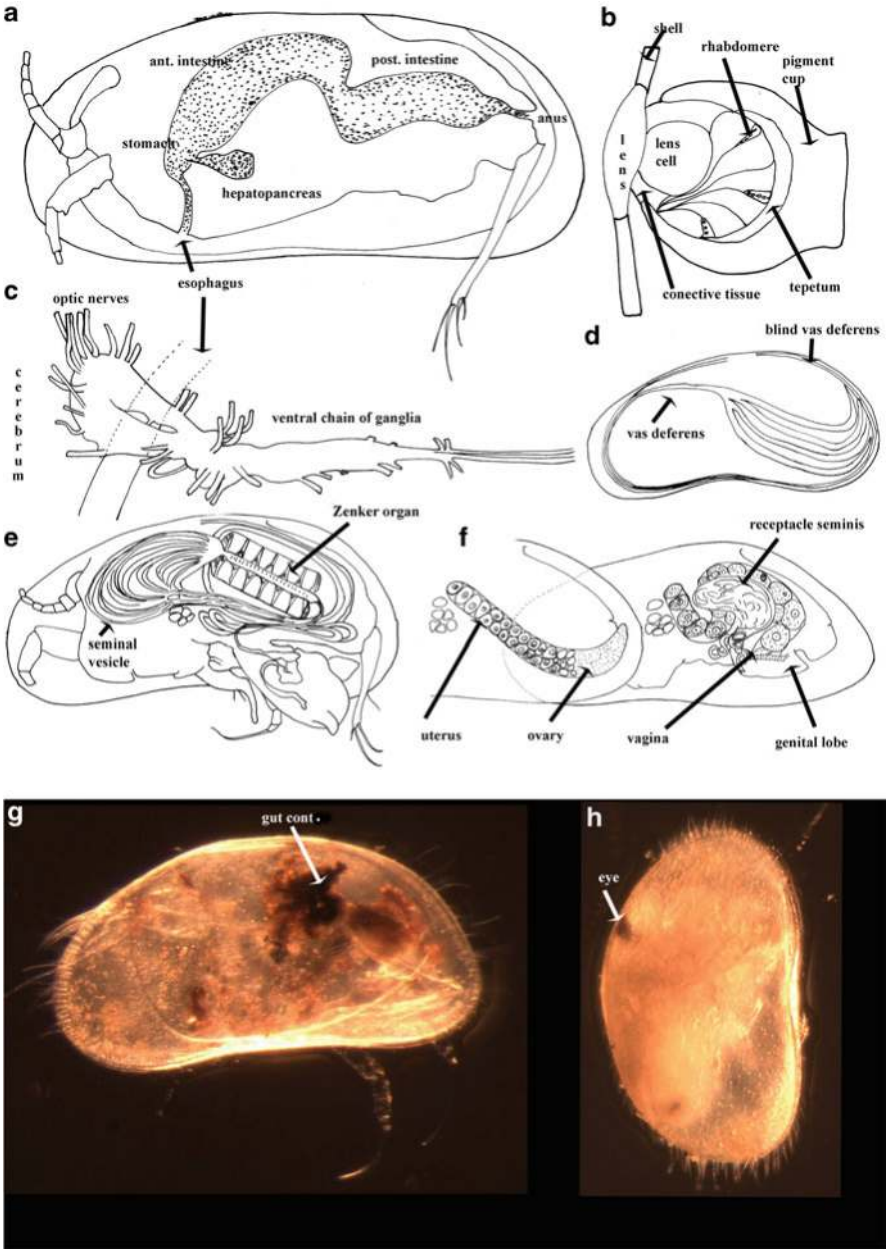

Fig. 38 (**a**–**f**) Line drawings; (**g**, **h**) Microscopic photographs: (**g**) *Candonopsis sp*.; (**h**) *Sarscypridopsis ochracea* (Sars 1924): (**a**) digestive tract; (**b**) frontal eye; (**c**) nerve system; (**d**, **e**, **f**) reproductive system; (**g**) gut content; (**h**) eye. (**a**) Modified after Henderson (1990); (**b**) modified after Tanaka (2006); (**c**) after Rome (1947b); (**d**–**f**) modified after Meisch (2000). (**g**, **h**) Photo: T. Karanovic

receives the antennula nerves, and triterocerebrum which receives the antenna nerves. Nerves from other limbs run into the chain of large ganglia (Fig. 38c).

The eyes are placed near the base of the antennulae. Some members of the subclass Myodocopa have both compound and frontal (naupliar) eyes, and Podocopa only have frontal eyes (Fig. 38h). The frontal eyes are more developed in the podocopid ostracods. The frontal eyes are tripartite: two lateral ocelli and a single ventral ocellus. Each lateral ocellus sometimes attaches to the valve and develops a cuticular lens on the valve itself (Bonaduce and Danielopol 1988). The pigmented cup is connected with the cuticular lens by the connective tissues (Fig. 38b). The three frontal eyes are situated on top or dorsal to the brain. Each of the three cups is lined with tapetal cells (tapetum), which contain the reflecting material formed as crystal-like plates. The sensory cells are usually few, varying between two to five. In addition to the sensory cells each cup has two fairly large lens cells, which bulge halfway out, from the cups. Some podocopids lack the lens cells. In podocopid ostracods, the light-gathering ability of the eye is dominantly affected by the thickness and curvature of the outer surface of the lens (Tanaka 2006). The nerves from each of the cups have separate courses to the brain. In some of the deep-sea myodocopids, the frontal eyes have reached their most elaborate structure and function in ostracods (Elofsson 2006).

Many setae play a sensory function in the ostracod body. Some of the setae on the surface of the carapace may play this role. On the soft parts, there are a number of specially modified setae in podocopid ostracods, called aesthetascs, especially on the antennula and antenna (Danielopol 1971), and they can be especially long in the species living in the subterranean waters (Danielopol 1973) (Fig. 20). Similarly, myodocopid ostracods are also well equipped with different sensory setae on their appendages, most of all on the antennula.

3.5 Reproductive Organs

In Podocopida the reproductive organs are paired in both sexes. Gonads are paired in all Myodocopa, except Cladocopida. In females there are two ovaries, and oviducts lead from ovaries to the uterine opening situated near the inner middle of the genital lobe. This lobe often bears some additional appendage-like structures which can be very helpful in taxonomy (Fig. 39c). The vaginal opening lies in front of the uterine opening and usually has well-chitinous rings. This opening is connected to the *receptaculum seminis* with a long and often coiled canal (Fig. 38f). There is no evidence for the internal connections between the seminal receptacle and the uterus; it is believed that the sperm pass from the vagina directly to the uterus to fertilize the eggs. Imprints of the ovaries can be visible on the shell (Fig. 39b).

The testes of Cypridoidea consist of four long coiled tubes on either side of the body (Fig. 39a, d). These tubes unite to form the *vas deferens*. Ventrally the *vas deferens* divides into the blind section which passes under the testes and runs

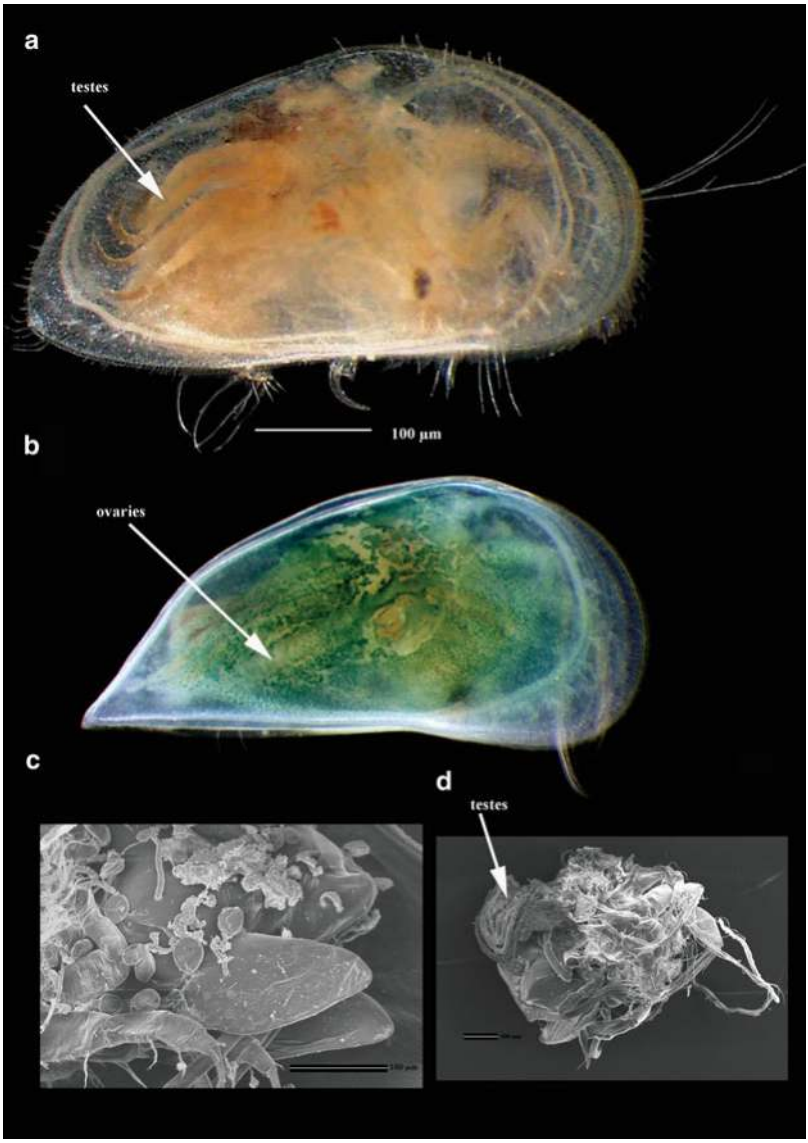

Fig. 39 (**a**, **b**) Microscopic photographs; (**c**, **d**) SEM: (**a**) *Lacrimicypris kumbar* Halse and McRae 2004; (**b**) *Mytilocypris mytiloides* (Brady 1886b); (**c**) *Fabaeformiscandona fabaeformis* (Fischer 1851); (**d**) *Notodromas persica* Gurney 1925: (**a**, **b**) lateral view showing the imprints of gonads on the carapace: (**c**) genital field in ♀; (**d**) testes tubes in ♂. (**a**, **b**) Photos: S. Halse

toward the dorsal side where it terminates. The main tube leads from the ventral side to the dorsal where it enters the body. The v*as deferens* forms many coils embracing various organs within the body before it enlarges into a *seminal vesicle* and forms the Zenker organ (Fig. 38d, e). This organ serves as a pump for

spermatozoa and it consists of a number of chitinous whorls of spines and muscles. The Zenker organ is connected with a tube to the hemipenis. Wingstrand (1988) recognized eight types of sperm in ostracods. Some podocopids have proportionally the largest sperm in the animal kingdom, being 10 times longer than the entire body. Cytheroidea and Myodocopa do not have Zenker organs.

4 Biology of Ostracods

4.1 Ostracod Habitats, Endemism, and Radiation

Ostracods can be found in many different water habitats. They live in both marine and freshwater interstitial waters, temporary and permanent freshwater bodies, large and small lakes, springs (including thermal springs), streams, rivers, pools, semiterrestrial habitats, and deep subterranean waters. The marine species live from shallow waters to abyssal depths. There is no type of aquatic ecosystem on Earth, both marine and freshwater, from where ostracods have not been recorded. There are many factors influencing the ostracod biodiversity and distribution in a certain ecosystem. Physical factors that control the distribution of ostracods are temperature, substrate, sediment type, vegetation, bottom topography, depth and transparency of water, and bottom currents. Examples of relevant chemical factors are salinity, pH, total phosphate, and dissolved oxygen. Biological elements influencing the distribution of ostracods are food supply and competition with other animals. Other factors that play an important role in zoogeography of ostracods are dispersal ability, mode of reproduction, morphological adaptation, and barriers (Puri 1966; Smith and Horne 2002).

The most investigated zoogeographical region is the Palearctic, with more than 700 non-marine species, followed by the Nearctic with 300 species. The Holarctic holds half of the known non-marine ostracod biodiversity. The Afrotropical region has 450, while the Neotropical around 280. The Oriental region has approximately 200 species, while from the Australian region only 176 species are recorded so far (Martens et al. 2007) (Fig. 40). As shown in Table 2, the Afrotropical and Australian regions have the highest percentages of endemic species. All these numbers are greatly misleading as most of the other regions, excluding Holarctic, have been poorly studied and large territories are still to be surveyed for many invertebrate groups, including ostracods.

At the species level, nearly all freshwater families have an endemic rate of around 90%, meaning that only about 1/10 of all species have intercontinental distribution, and further on, calculating over all known species, close to 94% of them are known from one zoogeographical region only (Martens et al. 2007). At the genus level, around 60% of genera occur in one zoogeographic region only, while endemism on the suprageneric level is rare and known only for certain tribes of subterranean Candoninae.

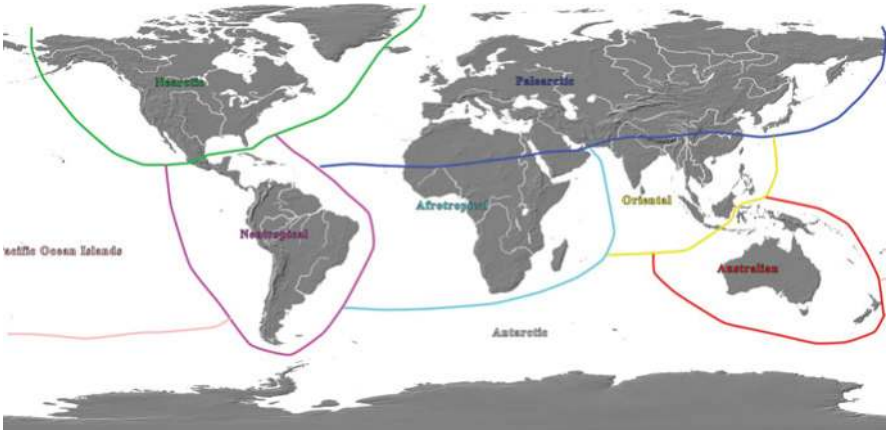

Fig. 40 Map of the zoogeographic regions of the world

Table 2 Numbers of non-marine species and endemisms in the zoogeographical regions

Zoogeog.regions	Holarctic		Afrotropical	Neotropical	Oriental	Australian
	Palearctic	Nearctic				
No of species (%of endemics)	700 (80)	300 (71)	450 (93)	280 (84)	200 (83)	176 (92)

Subterranean waters are ecosystems that often have an endemic fauna. There are a few models proposed for colonization of the subterranean habitats (Danielopol and Rouch 2004), the most common are animals escaping harsh environmental conditions on the surface (climate-relict model), animals actively colonizing the environment and adapting to it (the active colonization model), animals escaping epigean predators and/or strong competitive pressure, and various passive colonization models from animals being transported from epigean to hypogean environments during the courses of floods, to animals passively colonizing continental subterranean waters from marine interstitial during the regressive phases and progressively adapting to the inland non-marine subterranean waters. There is probably more than one factor influencing ways of colonization, subsequent (or pre-) adaptation, and the present day distribution of subterranean ostracods. Probably the way of colonization differs between ostracod lineages. Ostracod species, as any other living being, spread their area of distribution as much as possible, and colonize different environments. Even the "true" surface water ostracod, such as *Cypridopsis vidua* (Müller 1776) (Roca and Danileopol 1991), actively explores and colonizes the interstitial habitats. Ostracods are very diverse in subterranean waters, especially in some parts of the world, such as Australia, where the biodiversity in certain parts (such as arid Western Australia) is greater than in the surface waters (Karanovic 2007). Subterranean biodiversity is also well documented in the European karstic systems (Danielopol 1976, 1979, 1980a, b; Rogulj et al. 1994) and much less documented in other parts of the world (Danielopol and Hartmann 1986);

nevertheless, investigations of these less explored regions should reveal more ostracod taxa, as happened when investigations of the Australian underground waters started some 10 years ago. Subterranean ostracods display a number of morphological adaptations, such as reduction of size, loss of carapace pigmentation, and eye pigmentation; elongated segments and claws (for better gripping on the surface); strong reduction of the swimming setae on the second antenna; and increased development of sensory setae (Danielopol 1978a, b, c; Marmonier and Danilopol 1988; Marmonier et al. 1989). Some groups of ostracods are more often found in the subterranean waters than the others. The subfamily Candoninae (of the superfamily Cypridoidea) is the most common inhabitant of the subsurface waters, while other groups, such as the family Cyprididae (of the same superfamily), are only rarely found in such ecosystems, and very few species are stygobionts – i.e., species living in subterranean water. Subterranean species have more restricted distribution than the surface water species, and often are endemic to small karstic areas. Endemisms of ostracods in subterranean waters are sometimes present on taxonomic levels above the species, suggesting the old age of these faunas and their extended period of isolation. Such is the case with subterranean Candoninae of Australia (Karanovic 2007), Africa (Martens 1992c), and Central and South America (Broodbakker 1983c).

Hyporheic zones are boundaries between the shallow groundwater and the surface waters in streams and rivers and they typically house species that live in either the ground water below, or surface water above, and the species diversity is usually not very high. Biodiversity in springs and flowing water (streams and rivers) is also not very high, as ostracods generally tend to crawl on the surface of either the bottom or aquatic plants, and flowing water is a disturbing factor. In such habitats ostracods can be found in areas of rivers and streams where water stagnates and around the vegetation, especially in moss. Many species living in and around springs and spring-connected ecosystems have reduced swimming setae on the second antenna, and very powerfully developed claws on the antenna, walking leg, and uropodal ramus.

Lakes can hold a great ostracod biodiversity and species live from shallow waters around shores to the deep zones. All three non-marine lineages, Darwinulocopina, Cytherocopina, and Cypridocopina have many representatives in the continental lakes. Species of Cypridoidea living in lakes are usually good swimmers and have well-developed, long swimming setae on the second antenna, as well as long, often plumose setae on the first antenna. As for many other animal groups, ancient lakes are places which hold a large biodiversity and high endemism. Ancient lakes, mostly appearing around 7–10 million years, are deep (from 70 m (Lake Victoria) to 1.7 km (Lake Baikal)), allowing a great variety of ecological niches. The percentage of endemic species in different ancient lakes varies, but is usually very high (94% in Lake Tanganyika) and it often includes a relatively high percentage of the endemic genera (16% in Lake Tanganyika) (Martens 1994a, 1997b; Schön and Martens 2004).

Small water bodies such as rock pools, fed only by rainwater, are important temporary water habitats for ostracods all over the world, but especially in Africa (Jocque et al. 2006), and even there, some endemisms among species can be found, especially among the gigantic ostracods (Martens 1998a). Ostracods are very often

associated with root systems of floating plants (pleuston) in flood plains in South America (Higuti et al. 2007). Certain groups of ostracods are known from the semiterrestrial habitats, such as leaf litter, bromeliad cups, or floating fen soil (Danielopol and Vespremeanu 1964) and are more common in South America than anywhere else in the world (Pinto et al. 2003, 2004, 2005a, b, 2008).

4.2 Reproduction

Marine ostracods most commonly reproduce sexually (Cohen and Morin 1990). All three lineages of non-marine ostracods started off from sexual ancestors. In some lineages (Cypridoidea for example) the invasion of continental waters was accompanied by an increasing importance of asexual reproduction; in Cytheroidea, parthenogenesis might have originated later (Martens 1998b). Bisexual reproduction allows for mutation, recombination, and hybridization and creates a flexible and diverse gene pool, but invests around 50% of its biomass in the production of males, these being lost for direct reproduction. Parthenogenesis, on the other hand, ensures genetic diversity by mutation and polyploidy and is less genetically plastic, but invests 100% of the biomass into direct reproduction (Martens 1994a).

Parthenogenesis is indeed a very common way of reproduction in ostracods. Until very recently it was believed that Darwinuloidea have been reproducing parthenogenetically for the last 100 Mya. In that way this lineage was often regarded as "ancient asexual", one of the oldest on Earth (Butlin et al. 1998; Martens, 1998b; Martens et al. 2003). Smith et al. (2006) reported first males from Japan. It is not quite certain though if those males are reproductively active. In the case of *Limnocythere inopinata* (Baird 1843a), some rare, unfunctional males have been reported in otherwise parthenogenetic populations (Geiger et al. 1998). Horne (1983) calculated that of 286 species of Cypridoidea, known from Europe at that moment, 57% reproduce only parthenogenetically, as no males have yet been recorded. In Cytheroidea, of 50 European species 28% reproduce parthenogenetically. Many species belonging to Cypridoidea (most Cyprididae and some Candonidae) and some freshwater Cytheroidea have mixed reproduction, meaning that they have completely parthenogenetic populations, populations consisting of parthenogenetic females as well as sexual males and females, and bisexual populations. Another phenomenon in mode of reproduction is "geographical parthenogenesis", i.e., some species reproducing in one part of their area of distribution parthenogenetically and in others sexually. This is known for many European non-marine species. In most cases, parthenogenetic populations are distributed in northern parts of their area, while sexuals are limited to the southern parts. There are two hypotheses for geographical parthenogenesis. One is post-glacial recolonization hypothesis: after the glaciations in Europe, parthenogenetic reproduction was favored over sexual as this ability gives a species an advantage over sexual lineages - there is no necessity for males, and a single egg is enough to start a viable population, so that parthenogens were able to colonize the newly available

habitats more quickly than the sexuals. The other hypothesis is quite contrary, that in fact a stable climate during the Holocene sustained parthenogenetic populations rather than sexual (Horne and Martens 1999). The basic idea is that environmental/climatic stability favors parthenogens since they can reproduce more than the sexuals; unstable or fluctuating environmental/climatic conditions favor sexuals since with genetic mixing a population can adapt to the changing conditions. This is based on the fossil record of some bisexual populations found in the Northern Europe during the Late Glacial period and early Holocene. Nevertheless, stability of all types of aquatic habitats leads to predominance of sexuality in their faunas, such as in ancient lakes and subterranean waters (Martens 1998b).

As yet there is no clear evidence for reversals from asexual to sexual reproduction, i.e., the production of functional males from asexual females (Smith et al. 2006), and also the other way round. Studies of the karyotype of the parthenogenetic females show very unstable chromosomal structures (multiplication of sex chromosomes, and fusion of chromosomes) which may provoke the suppression of male production through chromosome disjunction or loss and explain how asexuals derive from sexuals (Schön and Martens 1998). There are many environmental factors, known in other parthenogenetic crustaceans, that may influence the switch from one mode of reproduction to another, such as temperature, availability of food, endosymbiotic microorganisms, etc. In some species, like *Metacypris cordata* (Brady and Robertson 1870), sex ratio is known to vary dramatically with a season, males being present only for a month or two in the summer (Meisch 2000), and in some species even then being very rare (Smith et al. 2006).

4.3 Reproductive Behavior

The mating process in ostracods can be divided into four phases (Horne et al. 1998): mate recognition, copulation, insemination, and the post-mating phase. The first phase can be recognition at a distance (chemical and optical sensors) or by a direct contact (by males grabbing the females with the second antenna or/and prehensile palps), provoking the females to accept the male. Six different mating positions have been recognized for podocopid ostracods so far. The copulation lasts from a few seconds to 30 min, during which time males extends the posterior extremity of the body toward ventro-posterior part of its own carapace (in some species the hemipenis rotates for 180° clockwise (McGregor and Kesling 1969)). Once a male and a female are connected, the transmission of spermatozoa into the genital organs of the female is initiated, in Cypridocopina by contraction of the Zenker organ. During the post-mating phase males and females remain active. After a few seconds, the hemipenis starts to retract inside the carapace. One male can inseminate a couple of females during a relatively short period of time (30 min). In some nektobentonic myodocopids, after copulation, females lose their swimming setae and become restricted to the benthic way of life, so that they cannot attract other males (Cohen and Morin 1990).

4.4 Ontogeny

In the subclass Myodocopa, all representatives of Myodocopida have brood care – carrying embryos within the postero-dorsal cavity of the carapace, releasing offspring as first instars. On the other hand, only a single genus of Halocyprida is known to have brooding care of embryos, while all the others release eggs directly into the sea. In Podocopa, brooding of embryos is less common and only representatives of Darwinulocopina and some families of Cytherocopina brood embryos in the posterior cavity of the shell. The number of embryos and eggs that can be retained at the same time in the brood chamber varies among species. For example, in the case of Darwinuloidea, *Darwinula stevensoni* Brady and Robertson 1885 can contain up to 10 or 15 juveniles at the same time in the brood chambers, and it releases its juveniles in the third instar, while *Penthesilenula brasiliensis* (Pinto and Kotzian 1961) carries up to six juveniles and releases them after the second instar (Pinto et al. 2007). Development in most brooders is slower than in depositors (Cohen and Morin 1990).

Ostracod growth is characterized by a number of molts which happen on the transition between the instars. The instars are usually described as first, second, third, etc., or as A, A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, "A" meaning an adult. The number of larval instars is fixed for certain lineages, and most of the podocopids have nine instars (eight juveniles and one adult). The first instar is already enclosed between valves and usually has three pairs of appendages. In some species, the carapace needs more than 100 h to complete its full calcification (around one week) from the time the nauplius hatches (Yamada and Keyser 2010). Prior to molting the ostracods begin producing shells by storing a huge amount of calcium phosphate granules together with chitin precursors in the outer epidermal cells. These granules release their contents into the extracellular space directly outside the epidermal cells. This material is transformed into small platelets, now made of calcium carbonate. These small platelets disintegrate into small granular structures, which are in fact amorphous calcite. This granular substance then forms the crystals, which, in connection to the chitin and proteins, build the shell of ostracods (Keyser and Walter 2004).

In Myodocopa, the ontogenic development comprises four to seven juvenile instars and a single adult instar. In contrast to Podocopa, the first instar in Myodocopa has five or even six appendages, being at the stage of metanauplius. For an easier comparison, different developmental stages for the subclass Podocopa are presented in Table 3. Ontogeny of Cypridocopina follows the model of *Eucypris virens* (Jurine 1820) described by Smith and Martens (2000). Development of *Darwinula stevensoni* (Brady and Robertson 1870), described by Smith and Kamiya (2008), is an example of the Darwinulocopina ontogeny. Ontogeny of three representatives of the suborder Cytherocopina, namely *Loxoconcha japonica* Ishizaki 1968 (Superfamily Cytheroidea, Family Loxoconchidae), *Uncinocythere occidentalis* (Kozloff and Whitman 1954) (Superfamily Cytheroidea, Family Entocytheridae), and *Terrestricythere elizabethae* Horne et al. 2004 (Superfamily

Table 3 Comparative ontogeny of the subclass Podocopa

	C	A1	A2	Md	Mxl	Mxp/L5	L6	L7	CR	other	C	A1	A2	Md	Mxl	Mxp/L5	L6	L7	CR	other
Podocopida/Cypridocopina/Cypridoidea/Cyprididae											Podocopida/Darwinulocopina/Darwinuloidea									
A-8/first instar	13%	4-sg	No ss	3-sg pl	Miss.	Miss.	Miss.	Miss.	Miss.		20%	5-sg	SA	Cx+3-sg pl 1 rs	Miss.	Miss.	Miss.	Miss.	Miss.	
A-7/second instar	20%	4-sg	No ss	SA	Anl.	Miss.	Miss.	Miss.	Anl.		25%	5-sg	SA	2 rs	Anl	Miss.	Miss.	Miss.	Anl.	
A-6/third instar	24%	4-sg	No ss	SA	usg pl	Miss.	Miss.	Miss.	Anl.		39%	5-sg	SA	3 rs	usg pl	Miss.	Miss.	Miss.	Anl.	CS
A-5/fourth instar	29%	4-sg	1 ss	SA	usg pl	Anl.	Miss.	Miss.	Anl.	rake	55%	5-sg	SA	4 rs	sg pl	Anl.	Miss.	Miss.	Anl.	
A-4/fifth instar	36%	5-sg	2 ss	SA	sg pl	4-sg WL	Anl.	Miss.	SA		45%	5-sg	SA	5 rs	SA	4-sg WL (SA)	Anl.	Miss.	Red.	
A-3/sixth instar	46%	6-sg	3 ss	SA	SA	Mxp 3-sg pl	4-sg	Anl.	SA		54%	6-sg (SA)	SA	6 rs	SA	SA	4-sg WL	Anl.	Red.	
A-2/seventh instar	58%	7-sg (SA)	4 ss	SA	SA	Mxp usg pl	5-sg (SA)	3-sg CL (SA)	SA		64%	SA	SA	7 rs	SA	SA	5-sg WL (SA)	4-sg WL	SA	
A-1/eight instar	80%	SA	5 ss	SA	SA	SA	SA	SA	SA	Cop.Anl	81%	SA	SA	8 rs (SA)	SA	SA	SA	5-sg WL (SA)	SA	Cop. Anl
Adult	100%	A	6 ss	A	A	A	A	A	A	Cop.	100%	A	A	A	A	A	A	A	A	Cop.
Podocopida/Cytherocopina/Cytheroidea/Loxoconchidae											Podocopida/Cytherocopina/Cytheroidea/Enthocytheridae									
A-8/first instar	18%	5-sg	SA	usg pl+2 claws	Miss.	Miss.	Miss.	Miss.	Anl.		/	/	/	/	/	/	/	/	/	/
A-7/second instar	23%	5-sg	SA	2-sg pl	Anl.	Miss.	Miss.	Miss.	Anl.		45%	6-sg	SA	cx+usg pl	Miss.	Miss.	Miss.	Miss.	Anl.	
A-6/third instar	29%	5-sg	SA	2-sg pl	usg pl	Miss.	Miss.	Miss.	Anl.		53%	6-sg	SA	cx+usg pl	Anl.	Miss.	Miss.	Miss.	Anl.	
A-5/fourth instar	33%	5-sg	SA	4-sg pl	sg pl	Anl.	Miss.	Miss.	Anl.		59%	6-sg	SA	SA	sg pl	Anl.	Miss.	Miss.	Anl.	
A-4/fifth instar	42%	5-sg	SA	SA	SA	3-sg WL	Anl.	Miss.	Red.		63%	6-sg	SA	SA	SA	3-sg WL	Anl.	Miss.	Anl.	
A-3/sixth instar	50%	6-sg	SA	SA	SA	3-sg WL	3-sg WL	Anl.	Red.		70%	6-sg	SA	SA	SA	3-sg WL	3-sg WL	Anl.	Red.	
A-2/seventh instar	40%?	6-sg	SA	SA	SA	4-sg WL (SA)	4-sg WL (SA)	3-sg. WL	SA		80%	6-sg	SA	SA	SA	4-sg WL (SA)	4-sg WL (SA)	Anl.	Red.	Cop. Anl.
A-1/eight instar	80%	6-sg	SA	SA	SA	SA	SA	4-sg WL (SA)	SA	Cop. Anl.	92%	7-sg (SA)	SA	SA	SA	SA	SA	4-sg WL (SA)	Miss.	Cop. Anl.
Adult	100%	5-sg	A	A	A	A	A	A	A	Cop+bo in ♂	100%	A	A	A	A	A	A	A	Miss.	Cop.
Podocopida/Cytherocopina/Terrestricytheroidea											Podocopida/Bairdiocopina/Bairdoidea									
A-8/first instar	27%	SA	SA	cx+sg pl	Miss.	Miss.	Miss.	Miss.	Anl.	Hook-like organ	/	/	/	/	/	/	/	/	/	/
A-7/second instar	31%	SA	SA	SA	Anl.	Miss.	Miss.	Miss.	Anl.	Hook-like organ disappears	15%	5-sg	4-sg	3 pm	Anl.	Miss.	Miss.	Miss.	Anl.	
A-6/third instar	35%	SA	SA	SA	sg pl	Miss.	Miss.	Miss.	Anl.		20%	5-sg	5-sg	cx+3-sg pl	SA	Miss.	Miss.	Miss.	Anl.	
A-5/fourth instar	43%	SA	SA	SA	SA	Anl.	Miss.	Miss.	Anl.		26%	5-sg	5-sg	cx+3-sg pl	SA	Anl.	Miss.	Miss.	Anl.	
A-4/fifth instar	50%	SA	SA	SA	SA	4-sg WL (SA)	Anl.	Miss.	SA		33%	5-sg	5-sg	cx+4-sg pl (SA)	SA	4-sg WL	Anl.	Miss.	SA	
A-3/sixth instar	60%	SA	SA	SA	SA	SA	4-sg WL	Anl.	SA		45%	7-sg (SA)	6-sg (SA)	SA	SA	4-sg WL	4-sg WL	Anl.	SA	
A-2/seventh instar	71%	SA	SA	SA	SA	SA	5-sg WL (SA)	4-sg WL	SA		56%	SA	SA	SA	SA	4-sg WL	5-sg WL (SA)	4-sg WL	SA	
A-1/eight instar	86%	SA	SA	SA	SA	SA	SA	5-sg WL (SA)	SA		80%	SA	SA	SA	SA	5-sg WL (SA)	SA	5-sg WL (SA)	SA	Anl. ♂ bo, Anl. Cop.
Adult	100%	A	A	A	A	A	A	A	A	Cop.	100%	A	A	A	A	A	A	A	A	Cop.
Platycopida/Cytherelloidea																				

A-8/first instar	38%	5-sg	1-sg ex 3-sg en	4 pm	Anl.	Miss.	Miss.	Miss.	Anl.	–	–	–	–	–	–	–	–	–	–	–
A-7/second instar	44%	5-sg	1-sg ex 3-sg en	4 pm	Anl.	Miss.	Miss.	Miss.	SA	–	–	–	–	–	–	–	–	–	–	–
A-6/third instar	50%	5-sg	1-sg ex 3-sg en	4 pm	SA	Anl.	Miss.	Miss.	SA	–	–	–	–	–	–	–	–	–	–	–
A-5/fourth instar	56%	5-sg	2-sg ex 4-sg en (SA)	4 pm	SA	Anl.	Miss.	Miss.	SA	–	–	–	–	–	–	–	–	–	–	–
A-4/fifth instar	64%	5-sg	SA	cx, bs, 2-sg pl (SA)	SA	Anl.	Anl.	Miss.	SA	–	–	–	–	–	–	–	–	–	–	–
A-3/sixth instar	72%	6-sg (SA)	SA	SA	SA	bs, ex, usg en	Anl.	Anl.	SA		–	–	–	–	–	–	–	–	–	–
A-2/seventh instar	83%	7-sg (SA)	SA	SA	SA	bs, ex, usg en	Anl.	Anl.	SA	Cop. Anl.	–	–	–	–	–	–	–	–	–	–
A-1/eight instar	87%	SA	SA	SA	SA	Sexually dimorphic	Sexually dimorphic	disappears	SA	Cop. Anl.	–	–	–	–	–	–	–	–	–	–
Adult	100%	SA	SA	SA	SA	A	A	/	A	Cop.	–	–	–	–	–	–	–	–	–	–

A adult; *Anl*. anlage; *bo* brushed-shape organs; *bs* basis; *CL* cleaning leg; *Cop*. copulatory appendage; *CS* caudal seta; *cx* coxa; *ep* endopod; *ex* exopod; *Miss*. missing; *pm* podomeres (meaning that there is no distinction between coxa, basis, exopod, and endopod); *pl* palp; *pp* protopod; *rake* rake-like organ; *rs* rake seta; *SA* similar to adult (meaning that some additional setae might be added, or setae and segments change their proportions, but the number of segments and/or parts is accomplished); *sg* segmented; *ss* swimming setae; *usg* unsegmented; *WL* walking leg

Terrestricytheroidea) are presented after Smith and Kamiya (2003, 2005) and Horne et al. (2004). Development of *Neonesidea oligodentata* (Kajiyama 1913) is an example for the Suborder Bairdiocopina (Smith and Kamiya 2002). Finally, ontogeny of the Order Platycopida follows Okada et al.'s (2008) description for *Keijcyoidea infralittoralis* Tsukagoshi et al. 2006. Platycopida have the first instar (A-8) already with four appendages (antennula, antenna, mandibula, and maxillula) plus the uropodal ramus, and the fifth appendage appears already at the second instar (A-7). Podocopida, Cypridocopina, and Darwinulocopina have eight juvenile instars and the anlage of the fifth appendage appearing on the fourth (A-5) (Cypridocopina), or the third (A-4) (Darwinulocopina) instar. A common characteristic of these two suborders is the absence of the anlage of the uropodal ramus on the first instar, which is present in Cytherocopina and Bairdocopina. The development of the Suborder Cytherocopina is not uniform. In most of the superfamilies there are eight instars. The only exception is the family Entocytheridae, with only seven instars. Also, in Terrestricytheroidea the fifth appendage appears already at the second instar, and in all the other Cytherocopina at the fourth. Bairdiocopina (Podocopida) also have only seven instars, and it is postulated that there is an additional instar that molts before the larva hatches (Okada et al. 2008). This suborder also has the first instar already with the anlage of the maxillula, and if there is indeed one instar before the hatching, then the anlage of the maxillula appears on the second instar.

Development time of ostracods is very closely linked to the temperature (Cohen and Morin 1990). At higher temperatures, the animals grow faster but have shorter life span. This was observed for *Heterocypris incongruens* (Ramdohr 1808; Latifa 1987), and also if they grow in lower temperatures, animals tend to become bigger (Martens 1985a). It is also known from the experiments on *Herpetocypris brevicaudata* Kaufmann 1900b that the content of calcium in the shells which have been calcified in lower temperature is lower as well (Roca and Wansard 1997). The impact of salinity on the growth of ostracods differ among different lineages and, for example, in the case of *Mytilocypris henricae* (Chapman 1966) (Cyprididae), specimens tend to be more elongated in lower salinities (Martens 1985a). van Harten (1975) found a negative correlation between mean length of *Cyprideis torosa* (Jones 1850) (Cytherideidae) and environmental salinity.

Juveniles of ostracods appear to behave like adults and occupy similar habitats, except that the swimming is limited.

4.5 Food and Predation

All freshwater and most of the marine ostracods are deposit feeders, and only a few lineages (Myodocopida, Family Cylindroleberididae and Podocopida, Family Cytherellidae) are filter feeders.

In the natural environment freshwater ostracods are defined as generalists, feeding on algae, organic detritus, dead and living plant material, invertebrate

feces, and bodies of the dead animals. There are relatively few papers published on the topic, but certain species do prefer certain kind of food. Most commonly in the laboratory experiments, algae (living or dry) and commercial fish food is chosen for successful survival and growth of many ostracods (Otero et al. 1998; Mezquita et al. 1999a; Baltanás et al. 2000). Although mostly benthic organisms, laboratory observations suggest that ostracods can feed on periphyton (Roca et al. 1993). Some species (*Cypridopsis vidua* (Müller 1776)) even actively look for *Chara* beds to feed on the periphyton and to hide from predators. *Notodromas monacha* (Müller 1776) is a primarily neuston feeder (Kiss 2004). Based on the laboratory experiments, biofilms can be an important part of the ostracod diet (Lawrence et al. 2002). Cyanobacteria, like *Tolypothrix tenuis*, are a preferred food for *Eucypris virens* (Jurine 1820) over other food offered (such as other small crustaceans and some vegetables) in the experimental conditions (Schmit et al. 2007). On the other hand, some gas-vacuolate euplanctonic blue-green algae (such as *Microcystis, Ananbena, and Oscillatoria*) may have a devastating effect on ostracods, causing death within 24 h, depending on the strain (Mills and Wyatt 1974).

Fish eat ostracods, sometimes in great numbers. However, experiments with the ostracod *Cypridopsis vidua* showed that 26% of specimens eaten by small bluegill sunfish passed the intestine alive and unharmed by tightly closing their shells (Vinyard 1979). Eggs of *Heterocypris incongruens* (Ramdohr 1808) have been shown to pass the digestive tract of goldfish unharmed (Kornicker and Sohn 1971). On the other hand, the presence of ostracod can be quite disturbing for other animals. For example, presence of *Cypridopsis vidua* may drastically damage a snail culture, causing snails to retreat in their shells, while the ostracods are attacking them (Lo 1967).

4.6 Commensalism/Parasitism

The family Entocytheridae (Cytherocopina) is a diverse group which lives on other crustaceans. It has over 170 species. Species are very small and they have narrow shells, well adapted to living in the tiny spaces on the gills and thoraxes of crayfish, amphipods, and crabs. They have specially adapted thoracic limbs and antennae to grab hold of their hosts. Although the exact relationship between the entocytherids and their hosts is not clear, it is thought that they live commensally, the hosts taking benefit from the ostracods helping to keep the bodies of their hosts clean (Hart and Hart 1974). Nearly all the members of Cypridocopina are thought to be free-living, and only some genera of the marine family Pontocyprididae have been known to include commensal species living on sponges and echinoids (Maddocks 1979). In the suborder Cytherocopina, representatives of the marine family Paradoxostomatidae have highly modified mouth parts, and at least a few representatives of this group are commensal on invertebrate hosts (Horne and Whittaker 1985).

4.7 Parasites on Ostracods

A number of parasites and epibionts have been reported from freshwater ostracods, including cestodes, protozoans, helminthes, peritrichs, and acanthocephalans (Griffits and Evans 1994). In the case of a particular peritrich, *Nuchterleinella cornelia* Matthes 1990, the parasite clusters around the genitalia and uropodal ramus, but it is unknown if the presence of this particular parasite affects the viability of an ostracod. Some marine ostracods of the subclass Myodocopa are often inhabited by small crustacean ectoparasites. Vannier and Katsumi (1993) observed that these parasites cling on to the dorsal region, close to the heart of the species. However, the ostracods do not seem to remove these parasites even though they are feeding on the food debris off the host body surface or tissue fluid excretion.

4.8 Dispersal Abilities and Strategies

Ostracods are relatively low-mobile and their long distance dispersal is passive. One of the major factors for a successful dispersal and new colonization is the possession of dry-resistant eggs. An egg that is capable of remaining viable despite long-term desiccation and freezing is a major asset. It can be wind transported and survive extremely low temperatures of high altitudes. Such eggs are common in the family Cyprididae (Cypridocopina), and less common in other lineages. Martens (1989a) raised a species belonging to the family Limnocytheridae (Cytherocopina) from dried mud, opposing the long-standing assumption that no Cytherocopina lays dry-resistant eggs. Darwinulocopina do not produce such eggs. Dry-resistant eggs can remain viable up to 50 or even 100 years, and are well suited for passive dispersal (Martens 1994a, b). Ostracods use many means for passive dispersal: humans, birds, amphibians, fishes, insects, plants (floating vegetation), stratospheric air currents, and water currents. Birds are a very common way of transport for freshwater ostracods, especially for those that do not lay dry-resistant eggs, such as *Cytherissa lacustris* (in Sywula 1990). The ostracods are usually transported by being attached to the bird's feathers and legs. Due to human activities, such as rice cultivating, many tropical species of ostracods can be found today in the European rice fields. Many species disperse in a torpid (dehydrated) state (Horne 1983), a strategy used by some Cypridocopina which do not lay dry-resistant eggs. Both adults and juveniles can pass unharmed through the gut of fish, and some species have even been recorded from the lower digestive tracts and the feces of ducks (Proctor 1964). McKenzie and Hussainy (1968) carried out several experiments in order to test viability of eggs/adults of some Cytheroidea after exposure to dry conditions, and they concluded that after prolonged exposure to dry conditions (in this case 9 days was a limit), adults did not revive a normal activity after being returned to the water, but juveniles contained in the brooding chamber of these

animals were still active. This experiment points out the importance of brooding for dispersal of ostracods. Brooding is therefore a main asset for a successful dispersal for all darwinulids (since all are brooders) and many cytheroids. The other strategy for a successful stretching of the area of distribution is parthenogenetic reproduction, known for many ostracods in all three freshwater lineages. In this way, the species can rely only on a single fertile female to start a new population. Many of the widely distributed freshwater species reproduce parthenogenetically. However, the species has to have a wide range of ecological tolerance to be able to survive in the new environment, especially if a new habitat has a considerably different temperature, salinity, oxygen dissolution, and other physical and chemical factors influencing the viability of an ostracod population. Danielopol et al. (1994) suggest that the present day distribution of freshwater subterranean ostracods is a result of both active and passive dispersal. They hypothesized that, due to their ecological flexibility, these ostracods can resist brief period of transportation in surface waters. Sometimes, subterranean ostracods actively disperse over large distances and within the sediments, constantly exploring their surroundings, and proliferating in suitable habitats.

The best example of rapidity of ostracod dispersal into newly available niches under favorable conditions is the European fauna. Ostracods have occupied (or reoccupied) niches that were ice-covered only a few thousand years ago (McKenzie 1971a).

Looking into paleozoogeography and present day distribution of non-marine lineages, we see that representatives of the superfamily Cypridocopina are by far the most successful, both in the aspects of their biodiversity and the number of ecological niches they have occupied. From the uppermost Jurassic (Mesozoic) to the present day they have dominated ostracod faunas in almost every non-marine environment and they are often the sole constituents of such faunas (Whatley 1990). The great rapidity of their dispersal is attributed to their ability to reproduce parthenogenetically and to lay desiccation and freezing-resistant eggs. Darwinulids are certainly the oldest lineage of non-marine ostracods, appearing in Devonian (Paleozoic), and always living in non-marine waters, but their dispersal strategies are obviously far less in numbers than in Cypridocopina. Similarly, Cytherocopina also have relatively few freshwater lineages, being, on the other hand, very diverse in marine environments, which does not require dessication resistant eggs. So, from the Triassic (Mesozoic) when they first appear in non-marine environments, the lineage was not particularly successful both in the evolution of species and niches colonization.

5 Phylogeny

The position of ostracods within Crustacea and Arthropoda, in general, is a debated question. They have been often associated with tantulocarids, branchiurans, mystacocaridans, copepods, facetotectans, rhizocephalans, ascothoracidans,

acrothoracicans, and thoracicans, forming the class Maxillopoda. The definition of this class is rather broad and it includes crustaceans with five cephalic, six thoracic, and four abdominal segments, plus a telson, but reductions of this basic plan are common; thoracopods are variously fused with cephalon thoracic segments with biramous (sometimes uniramous) appendages, lacking epipods; abdominal segments lack typical appendages; carapace present or reduced; and with both simple and compound eyes, the latter being unique and defined as a "maxillopodan eye." As far as ostracods are concerned, there are many more exceptions to the definition of Maxillopoda than there are arguments in favor, so they have been excluded from the class Maxillopoda (Martin and Davis 2001; Horne 2005; Horne et al. 2002, 2005; Regier et al. 2005; Newman 2005, etc.). Ostracods are now considered one of the basal groups in the subphylum Crustacea, closely related to other "primitive" crustaceans such as Branchiopoda, Cephalocarida, Remipedia, and Mystacocarida (Newman 2005). On the other hand, data obtained from DNA cluster ostracods together with Branchiura (parasitic crustaceans), being the most basal on the phylogenetical tree of the pancrustacea (crustacea + hexapoda) (Regier et al. 2005). Ostracods have been related to Phosphatocopida (another Cambrian group of bivalved arthropods).The last group has only four pairs of head appendages, and some authors do not consider Phosphatocopida to be Crustaceans at all (Hou et al. 1996; Walossek and Müller 1998; Shu et al. 1999). But it is still a question of whether the fifth limb in podocopids belongs to the head or thoracic region. Many authors prefer the first opinion (e.g., Athersuch et al. 1989; Smith and Martens 2000). Besides being a "problematic" group, difficult to relate with other crustaceans, the ostracods are as much problematic in their own systematics and phylogeny.

A bivalved carapace enclosing the body and limbs may not be regarded as a synapomorphy of the two ostracod subclasses: Myodocopa and Podocopa. Such carapace occurs also in some lineages of Branchiopoda, and although in majority of Branchiopoda the carapace has growth lines, in Spinicaudata (Branchiopoda as well), the carapace lacks the growth lines like in ostracods. And even though in both ostracod subclasses the carapace is calcite, it shows some remarkable differences in morphology. Because of the rich fossil record much attention has been paid to the structure of the carapace in defining the phylogeny of ostracods. But as Horne et al. (2005) said, the carapace is at the same time rich in data and awash with homeomorphism. Most recently, Siveter et al. (2010) described a myodocopid ostracod from the Silurian deposits with preserved soft parts which indicate that this species belongs to the family Cylindroleberididae, but the appearance of the carapace relates it more to other families, especially Cypridinidae and Sarsiellidae, questioning the utility of the carapace alone in establishing the affinity of fossil ostracods. Some drastic discrepancies occur in carapace development and structure between present day and fossil ostracods. The species *Manawa staceyi* Swanson 1989 has recently been described as the only living representative of the otherwise extinct lineage, Palaeocopida (Podocopa). The larva of this species has a one-piece carapace (cephalic shield) and the adult has eight, instead of a maximum of seven appendages (excluding the copulatory ones and the uropodal ramus) in all other

podocopids. Further, Siveter et al. (2003) described and 3D-reconstructed one fossil myodocopid species and found that adults had a one-piece carapace as well (but not otherwise very like the Manawan "shield"). Studying the development of a myodocopid ostracod Wakayama (2007) showed that the carapace in this species forms from the two buds on each side of the embryo that grow and meet dorsally into a single piece carapace which becomes hinged during further ontogeny. As for embryonic development itself, it is known that myodocopids have no less than four (most usually five) appendages already on the first instar, but in podocopids this differs largely between the lineages: some having only three appendages, some four, but never five. Looking into the soft part morphology there is even more evidence for polyphyly of the ostracods. Based on studies of the morphology and musculature of the appendages Horne (2005) suggested that the podocopid branchial plates are exopods, while those in myodocopids are epipods. In Podocopida the protopods of the post-mandibular appendages appear to be undifferentiated, consisting only of the basis, while in myodocopids they have basis, coxa, and often a precoxa as well. Also, the position of the uropodal ramus is different in two lineages: in myodocopids it is posterior and in Podocopa it is anterior to the anus (Meisch 2007). It is indeed true that there are many differences between the appendages between the two ostracod subclasses, but there is also a question if we are dealing with the homologous appendages when comparing those lineages (Horne et al. 2002). Ostracods went through so many reductions after having developed the bivalved carapace, and it is highly possible that in different lineages different appendages became reduced, and ultimately disappeared. A cladistic analysis of the extant superfamilies of the two ostracod subclasses performed using only the morphology of the soft part shows a good resolution of the phylogeny of the myodocopid superfamiles, but not in the podocopids (Horne et al. 2005). Not only morphological data suggest the polyphyly of ostracods. Using different genes and different representatives, ostracods came out on the tree as polyphyletic. On the tree obtained using 18S rDNA, podocopids are associated with pentastomatids and branchiurans, while myodocopids with copepods (Spears and Abele 1997). Using three genes (elongation factor-1 alpha, the largest subunit of RNA polymerase II, and elongation factor-2) branchiurans cluster with podocopids and myodocopids: they group with podocopids by amino acids and with myodocopids by nucleotides (Regier et al. 2005). However, all three groups: myodocopids, podocopids, and branchiurans stand at the base of the crustacean trees. Yamaguchi and Endo (2003) and Oakley and Cunningham (2002) also used 18S as a marker to test phylogenetical relationships within ostracods, using outgroups Branchiura, Copepoda, Cirripedia, Mystacocarida, Malacostraca, Branchiopoda, Cephalocarida, Insecta, and Chelicerata in the first analysis, and only copepods and branchiopods as outgroups for the second analysis. The results confirm polyphyly, but they also show that the phylogenic relationships among ostracod superfamilies in both subclasses (Podocopa and Myodocopa) are not resolved. This may be because of the marker used, as 18S is used to test the crustacean relationships and it may not be suitable for lower level units.

6 Practical Methods in Studying Ostracods

Here, only the qualitative methods for collecting the recent freshwater ostracods are described. Quantitative methods are reviewed in Danielopol et al. (2002).

6.1 Collecting

Ostracods live in every type of the freshwater ecosystem and there are several different kinds of equipment that are used for their collecting. However, at the base of all equipment is a net of an appropriate mesh size. To ensure the collection of small ostracods, including juvenile stages the recommended mesh size is 75 μm. The net is sewed to a metal frame (Fig. 41a) which has a handle. It is recommended to have the net protected with a thick material not to be damaged by pulling over the sediments. Nets constructed in this way are used for collecting ostracods from small ponds, puddles, rivers, and springs. It is also useful to have a coarse mesh over the mouth of the net to stop large stones, bits of wood, etc., from getting in and damaging the net. Also, sediment and water can be extracted with a bucket and then poured through the net. This also works well with aquatic macrophytes, which can be placed immediately in a bucket held under the water, then extracted and torn up and/or agitated to dislodge animals, and the residue then poured through a sieve.

For collecting in larger water bodies from standing on the shore, nets with a larger diameter and longer handles are used. The net is pulled over the surface and also close to the bottom. Before collecting close to the bottom, the sediment should be disturbed. Ostracods are also collected close to the vegetation on the river banks or ponds, and by rinsing the moss in the springs. Sometimes, if the water body is particularly small a rubber pipette can be used to suck in water. This kind of pipette is also useful for collecting in caves from small water bodies and also for sucking the dripping water from stalactites. Samples from wells can be taken with the Cvetkov net (Cvetkov 1968) (Fig. 41b, c). It is attached at the top to a length of rope or cable by a link and is then lowered to the bottom of a well or borehole. Once on the bottom, the net is jerked up and down several times and the weight suspended from the bottom of the net disturbs the sediment causing it to become suspended in the water, along with any fauna that might be present. The net is then drawn rapidly up through the water column to capture the specimens. The net also has a detachable filter that screws into a collar, attached to the bottom of the net, enabling the easy removal of specimens. The Bou–Rouch pump (Bou and Rouch 1967) is used for collecting in interstitial waters. The equipment consists of a zinced iron pipe, 2–3 cm in diameter and 1.5–1.8 m in length. At one end is a spiked tip, with rows of holes above this. At the other end is a lip or thread. The pipe is driven into sediments with a hammer and once at the desired depth a piston pump is fitted on the end for pumping up the water, which is then either pumped into a storage tank or through a sieve/net. Another way to collect the animals from the interstitial waters is

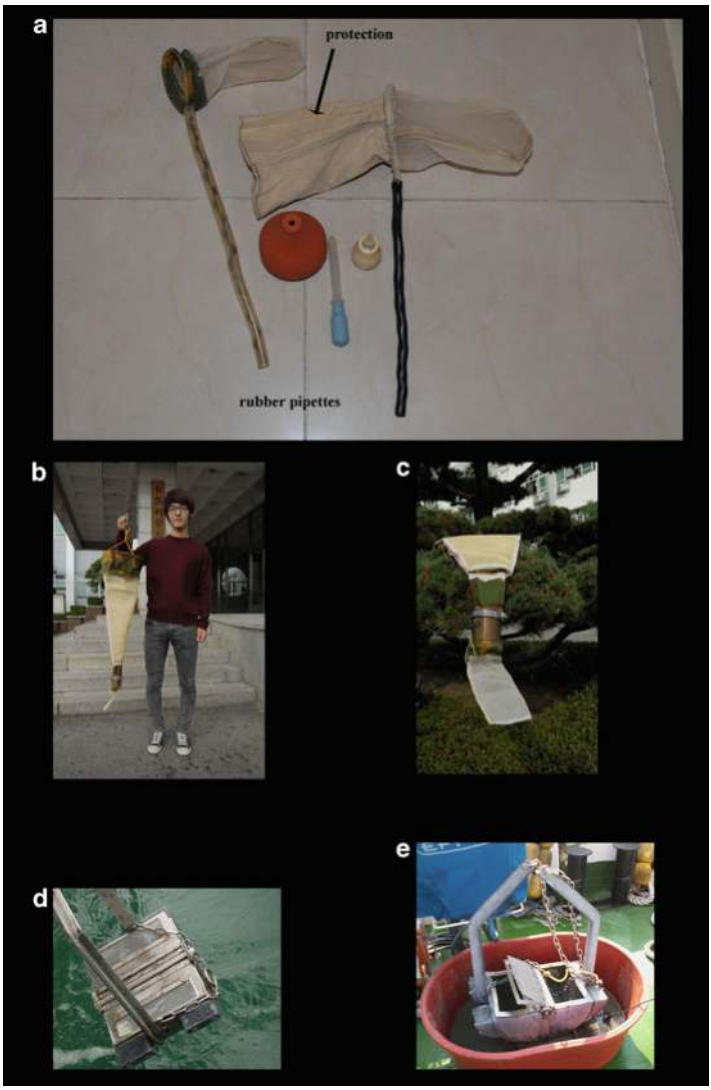

Fig. 41 Photographs of the collecting equipment: (**a**) plankton nets and pumps; (**b**) Cvetkov net; (**c**) lower part of the Cvetkov net, (**d**, **e**) Eckman drage. (**a–c**) Photos: T. Karanovic; (**d**, **e**) Photos: K.C. Kim

Karaman–Chappuis method (Karaman1935; Chappuis 1942). A hole is dug on the shore, until the water level is reached. Then the water which accumulates in it is collected with a dish and immediately passed though the net. For collecting from the larger water bodies such as lakes, besides a small net which is useful for collecting ostracods along the banks and close to the water vegetation, different equipment is used. The "Ekman Grab" (Fig. 41d, e) is used for soft bottoms

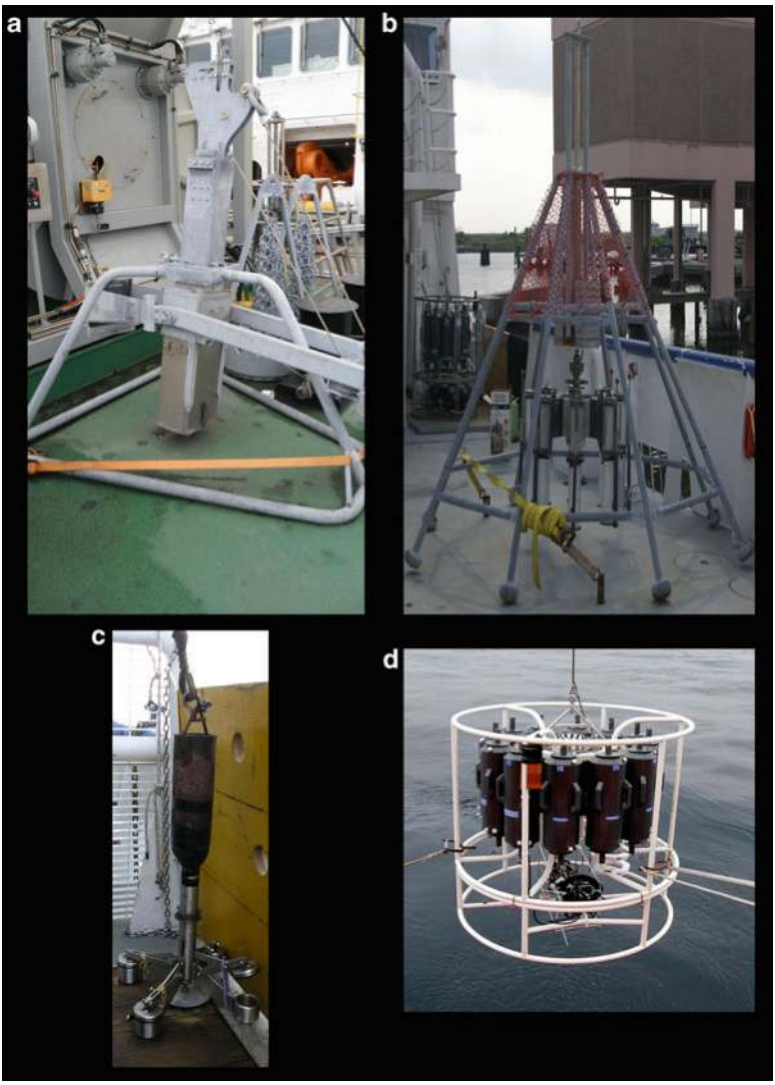

Fig. 42 Photographs of the collecting equipment: (**a**) box corer; (**b**) Multiple corer; (**c**) Kayak corer; (**d**) Niskin bottles. Photos: K. C. Kim

that are free of vegetation, such as sticks and decayed leaves (or with short, erect vegetation only) as well as intermixtures of sand, stones, and other coarse debris. Its specialized function is the taking of quantitative and qualitative samples. It is not recommended for rocky or sandy bottoms or moderate macrophyte growth because small pebbles or macrophyte stems prevent proper jaw closure. It consists of two halves of a relatively small box. The grab is placed onto the bottom sediment, then closed, and pulled up to retrieve a sample. A two-way mechanism prevents accidental

closure while the dredge is dropping. The impact of the dredge on the bottom surface triggers the spring-loaded release mechanism without using a messenger. After mechanism closure, the dredge is pulled up to retrieve the sample. There are a number of Box Core Samplers available for deep lake, which can be deployed up to 6,000 m (Fig. 42a). During descent, insertion, and sampling, the top of the sample tube remains open allowing a free flow of water. This prevents pressure buildup and following disturbance of the sediment surface. After landing on the bottom an automatic mechanism releases the closing shovel. The shovel is drawn into vertical position so that the bottom of the tube is closed and the sample is kept inside. On withdrawal from the bottom, the top of the sample tube is closed and sealed by a hinged flap with a soft rubber packing. Other types of corers are Kayak corer (Fig. 42c) and multicorer (Fig. 42b). The last has an advantage of taking several core samples at one time and it is usually equipped with computer measuring systems for the water temperature and quality. Niskin bottles are used for collecting water at specific depths (Fig. 42d). The bottles are lowered into the water on a wire cable to the specified sampling depth. A messenger is then sent down the wire to quickly close the top and bottom of the bottles, trapping water inside the bottles. The bottles are then brought to the surface where the water is let out through a plastic tube into other sample collection bottles. Sometimes it is useful to collect animals with the aid of a trap: jars or plastic bottles are left with a bait (fish, liver, and smelly (non-processed) cheeses) in place for 24 h and certainly not longer than 48 h, as the bait itself can become a pollutant in the water if left for too long.

6.2 Fixing and Sorting

The samples can be fixed in 75% ethyl alcohol or in 96% (non-denaturated, pure) ethyl alcohol in case the material is also needed for the DNA, or the ostracods can be picked while alive and then fixed. In the case that the sample is used for DNA extracting, it is best to place the sample in a freezer. For only morphological taxonomy it is helpful to fix the animals first in about 30% alcohol, so that they die with the valves gaping open (this makes opening for dissection later on much easier) and then store in 75%. Once the sample is ready for sorting, part of it is placed in a Petri dish and ostracods are separated under the dissecting/field microscope using a pipette. The samples are also sorted and preserved in 75/95% alcohol. It should be noted that glycerin or formaldehyde must never be used as they decalcifie the shell.

6.3 Studying

Ostracods are best observed on a cavity slide and in propylene glycol. This medium very slowly evaporates and does not damage the tissue in case it is needed for DNA analysis. Ostracods are first observed under the dissecting microscope and

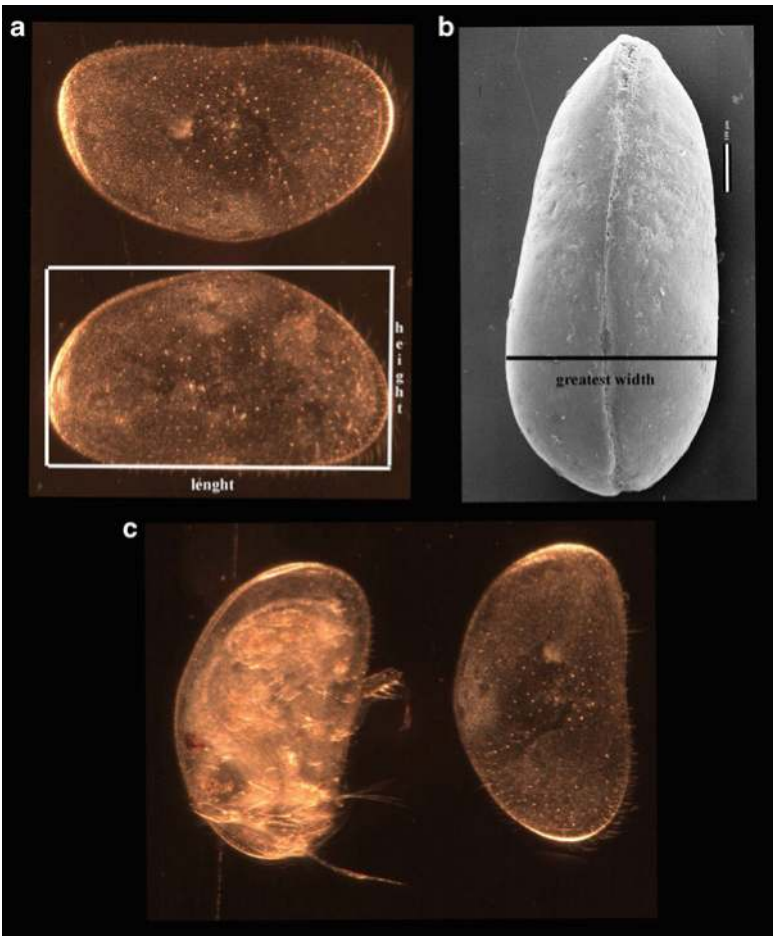

Fig. 43 (**a**, **c**) Microscopic photographs; (**b**) SEM: (**a**, **c**) *Sarscypridopsis ochracea* (Sars 1924); (**b**) *Cyprideis sp*.: (**a**) measuring length and height of an ostracod shell; (**b**) measuring width of an ostracod shell; (**c**) open shell. (**a**, **c**) Photos: T. Karanovic: (**b**) Photo: D. Keyser

measured with the aid of ocular micrometers. A specimen is then studied in detail. This is done with a compound microscope. The shell is first drawn from the outside (both with the animal laying on the left and right sides). It is also important to draw a dorsal and ventral view of the shell. This can be done by placing a very small amount of cotton wool on a slide in a drop of medium and then manipulating the shell and trying to place it between two cotton threads. All these aspects are necessary for measuring the length, height, and the width of an animal (Fig. 43a, b). After all external outlines are examined and drawn the shell needs to be opened. This can be achieved by inserting a very fine (usually entomological) dissecting needle (entomological pins mounted in pin chucks are very effective) ventrally between the valves. This is one of the most complicated stages in handling the animal as the valves can

sometimes be very tightly closed. When the valves are open, the soft parts need to be separated from the shell (Fig. 43c). Sometimes, the adductor muscle scars are quite strong, so they need to be broken on the other side of the shell as well. Once the soft body is out, the inside appearance of the valves needs to be examined and drawn as well. The shell can be stored on the micropaleontological slides, or kept in a separate vial in alcohol. For detail observation of the fine structures of the shell a Scanning Electron Microscope (SEM) can be used. Special preparation procedures are required for SEM work. Specimens must be metal-coated using standard SEM techniques, or if using SEMs with environmental chambers no coating is required. The soft body is dissected on a normal microscope slide, either in a drop of a permanent mounting medium or glycerin. If the soft body is dissected in the latter medium, after the examination the parts need to be mounted on a separate slide in a permanent medium. This can cause the loss of some appendages, so it is recommended to dissect the animal directly in a permanent medium. Among others the following media are used for making permanent slides: Fore's medium or Hydro Matrix. The soft body is

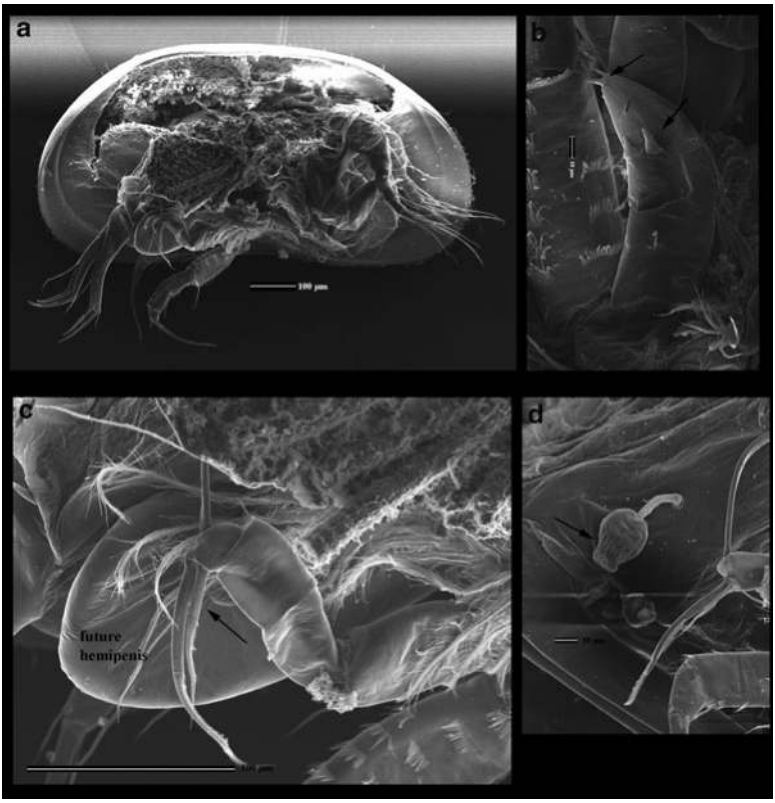

Fig. 44 SEM, (**a–c**) *Candona neglecta* Sars 1887; (**d**) *Candonopsis kingsleii* (Brady and Robertson 1870): (**a**) juvenile ♂ inside view; (**b**) prehensile palp in juvenile ♂; (**c**) setae on a juvenile; (**d**) epiphyte attached on the genital field

dissected with fine entomological needles. Dissected soft parts are covered with a coverslip. The soft parts are then observed under the high power microscope and drawn in detail with the aid of a camera lucida, or a drawing tube attachment. The soft body can also be observed with the SEM. For this purpose, animal goes thought the critical drying point (CDP) procedure. This can be done on the whole animal (while the soft parts are still in the shell), on the animal taken out from the shell but not dissected, or on dissected appendages. In the first two scenarios, animals need to be dissected when mounted on an SEM stub. The methods depend on the purpose of the SEM.

For a proper taxonomic identification it is necessary to ensure that one is dealing with adult specimens. To distinguish between juveniles and adults, the data in Table 1 can be used. In Darwinulids only the adult has all rake setae present on the Md. In species with swimming setae it is also easy to distinguish adults from juveniles, as only adults have all swimming setae present. In all ostracods, juveniles can be distinguished from adults by the special "plumed" appearance of setae (Fig. 44c) and also by incomplete development of the copulatory appendages and prehensile palps in males (Fig. 44a–c). Where the carapace is concerned, juveniles have much narrower calcified inner lamella than adults (Yamada 2007). When studying ostracods it is necessary to illustrate as much as possible, even if some characters do not appear to be taxonomically important. However, since we are still learning about the ostracod phylogeny and trying to identify homologous structures, it might be useful for future studies to collect as much information as possible from all investigated species.

7 Trends and Application of Ostracods

In the light of recent concerns regarding climate change issues, ostracods have been intensively used as palaeoecological and palaeoclimatological indicators of the past environmental conditions and climatic changes and events. Analysis of ostracod assemblages can yield important information regarding the environmental conditions of a certain deposit, biostratigraphy, taphonomic processes, paleoclimate, palaeoceanography, and palaeobiogeography (Griffiths and Evans 1992; Wansard et al. 1997; Frenzel and Boomer 2005; Boomer et al. 2003; Danielopol et al. 2008, etc.). Ostracods are also often used to indicate and reconstruct past human activity (Palacios-Fest 1997; Palacios-Fest et al. 2002). All this is possible thanks to the chemical composition of the ostracod shell. The utility of ostracod shells has been brought forward especially with the development of the physical and chemical analyses. Inductively Coupled Plasma Spectrometry opened opportunities such as analyzing the Mg/Ca ratio in the ostracod shell, as an indicator of palaeotemperature; Sr/Ca ratio as an indicator of salinity; oxygen isotopes for salinity and temperature; and carbon isotope analysis as an indicator of the ecosystem productivity. The proportion of the ^{14}C in the ostracod shell can provide information of the age of the assembly. Apart from its chemical composition, the ostracod shell provides ecological information based on the level of calcification and ornamentation as well.

Although to a lesser extent, the ecological studies involve not only palaeo-assemblages, but also recent ostracods as indicators of pollution or seasonal changes (Wansard and Mezquita 2001; Mezquita et al. 1999b, 2001, etc.).

Ostracods were one of the first aquatic invertebrate groups to be studied with the allozyme electrophoresis techniques (Sywula and Lorenc 1982; Sywula 1989, 1992; Sywula et al. 1991, 1995). More recently, DNA sequencing has also been applied to ostracods, but not with great success, comparing with other groups. But, nevertheless, some DNA work has been done with different approaches: reproductive modes, population genetics, phylogeny, evolution of vision, and biogeography (Schön and Martens 2003).

The number of ostracod taxonomists is constantly decreasing because the major funding requires applied research where there is little or no space for taxonomy based on morphology. Also, because the field of ostracodology is dominated by paleontologists the importance of the looking in the soft part morphology in order to identify taxa is underestimated. Nevertheless, the finding of the well-preserved soft parts in fossils is increasing interest in the taxonomy based on the soft part morphology. Some remarkable discoveries which underpin the discrepancy between the taxonomy based on shell and taxonomy based on shell and soft parts, when interpreting phylogeny and zoogeography, may push forward ostracod taxonomy again. The increasing concern about global biodiversity issues, such as the disappearance of habitats and species, can play a positive role in giving taxonomy a new impetus.

8 Key to Ostracod Subclasses, Orders, and Suborders

1. A2 biramous: endopod reduced (1–3 segments), exopod strongly developed (up to nine segments). UR posterior to the anus . . . . . subclass Myodocopa (2)

– A2 biramous: endopod strongly developed (three to four segments), exopod moderate or greatly reduced (1–3 segments). UR anterior to the anus . . . subclass Podocopa (4)

2. Anterior rostrum on the carapace well developed; Md without large teeth on the coxa; Mxl and L5 both short and not leg like; L7 vermiform with numerous; annulations; male copulatory organ paired . . . order Myodocopida/suborder Myodocopina

– Anterior rostrum on the carapace well developed or absent; Md coxa with well-developed teeth; L5 leg like; L7 reduced or absent; male copulatory organ not paired . . . . . suborder Halocypridina (3)

3. L5 and L6 leg like; L7 reduced . . . . . suborder Halocypridina

– L5 leg like; L6 and L7 missing . . . . . suborder Cladocopina

4. A2 exopod 2-segmented. . . . . .order Platycopida/suborder Platycopina

– A2 exopod strongly reduced. 5

5. Mxl with large branchial plate; only three postmaxillular appendages present (excluding UR and copulatory organ). . . . . .order Podocopida (6)

– Mxl without branchial plate, endopod leg like, there are four postmaxillular appendages (excluding UR and copulatory organ) . . . order Palaeocopida/suborder Kirkbyocopina

6. Zenker organ absent, sperm pump incorporated in copulatory organ. 7

– Zenker organ present 8

7. Branchial plate on L5 reduced and carrying maximum of four setae . . . suborder Cytherocopina

– Branchial plate on L5 well developed with many unreflexed and four reflexed setae. . . . . .suborder Bairdiocopina

8. L7 walking leg. 9

– L7 cleaning leg . . . . . suborder Cypridocopina

9. UR well developed, each ramus with three to four terminal claws and one seta . . . Suborder Sigilliocopina

– UR reduced to seta(e) or absent . . . . . .suborder Darwinulocopina