

# OSTRACOD SOFT-PART MORPHOLOGY, DISSECTION AND SLIDE-PREPARATION

Tadeusz Namiotko <sup>1)</sup> and Dan L. Danielopol <sup>2)</sup>

<sup>1)</sup> University of Gdańsk, Department of Genetics, Laboratory of Limnology, Gdańsk, Poland

<sup>2)</sup> Commission for the Stratigraphical & Palaeontological Research of Austria, Austrian Academy of Sciences, c/o Institute of Earth Sciences (Geology & Palaeontology), University of Graz, Austria



## INTRODUCTION

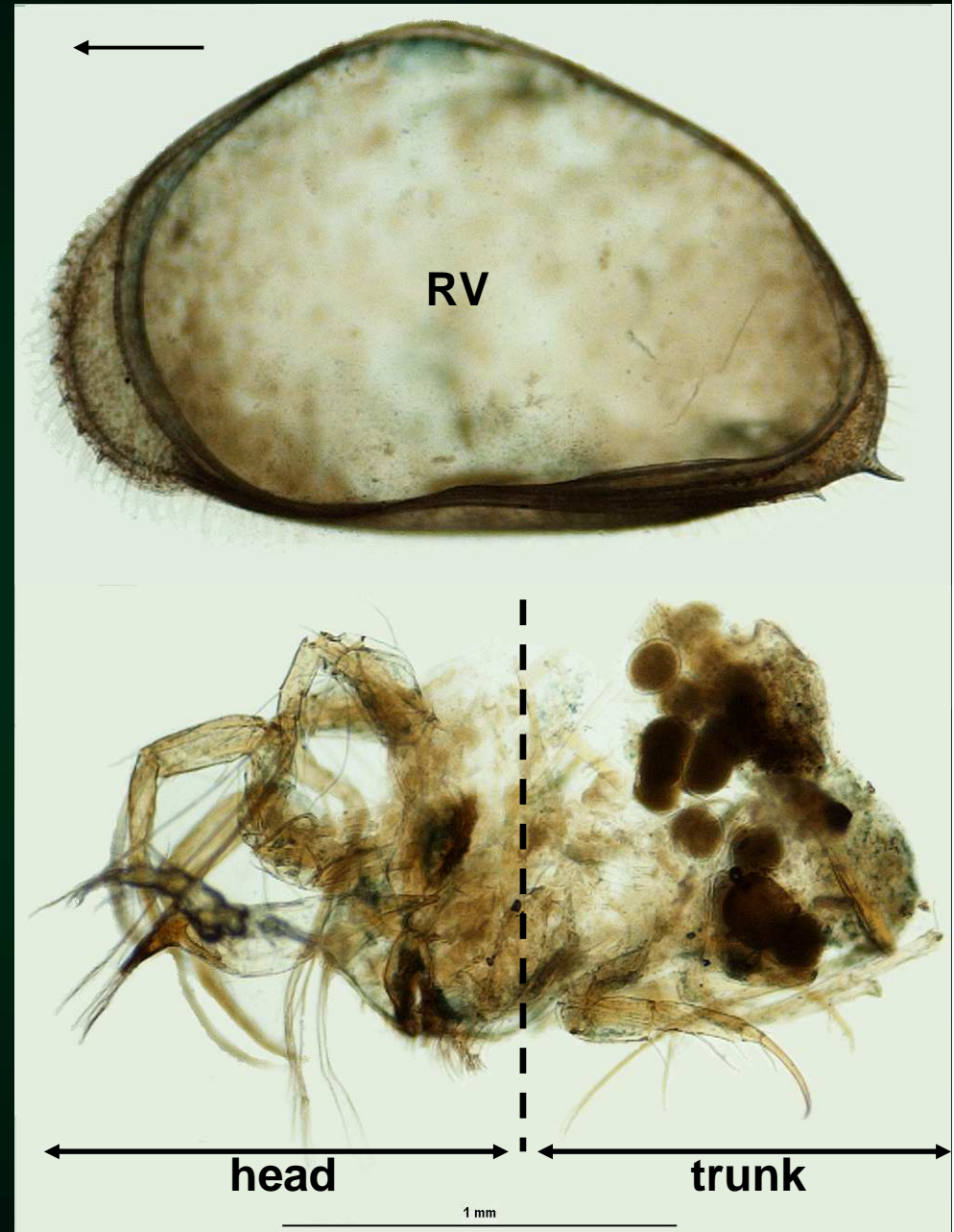
- General morphology and terminology of the ostracod appendages with emphasis on the order Podocopida
- Most commonly used techniques for treating ostracod soft body for taxonomical purposes with optical microscopy

# General morphology and terminology of the ostracod soft body

## Terminology of limb morphology after

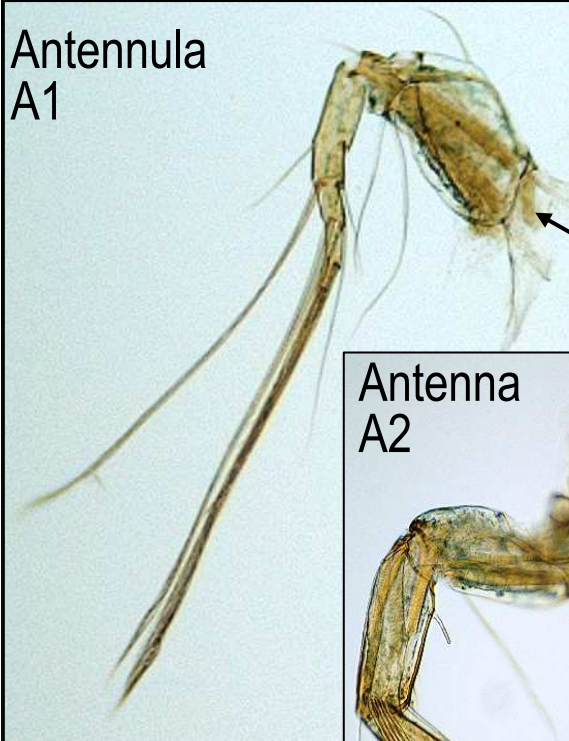
Taxonomy, Morphology and Biology  
of Quaternary and Living Ostracoda  
David J. Horne   Anne Cohen   Koen Martens  
The Ostracoda: Applications in Quaternary Research  
Geophysical Monograph 131  
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- short compact body with no true segmentation
- faint constriction in the centre marks indistinct boundary between two main parts:
  - anterior head (= cephalon)
  - posterior trunk (reduced thorax + abdomen)
- trunk in a few taxa with external traces of postcephalic segments:
  - 4-7 (subclass Myodocopa)
  - 10-11 (subclass Podocopa)
- up to 8 pairs of appendages (the fewest number of limbs of any crustaceans)



# General morphology and terminology of the ostracod soft body

Antennula  
A1



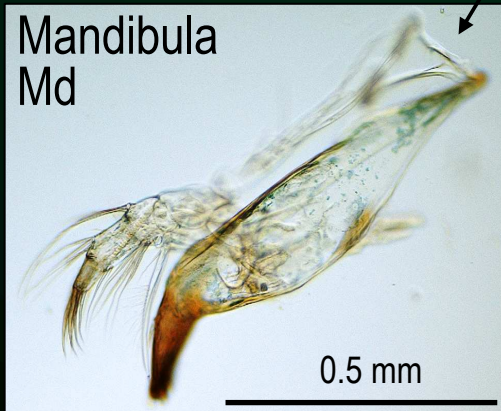
in Podocopa 4 pairs of limbs  
(=appendages) attached to cephalon  
(untypical for crustaceans)

Antenna  
A2

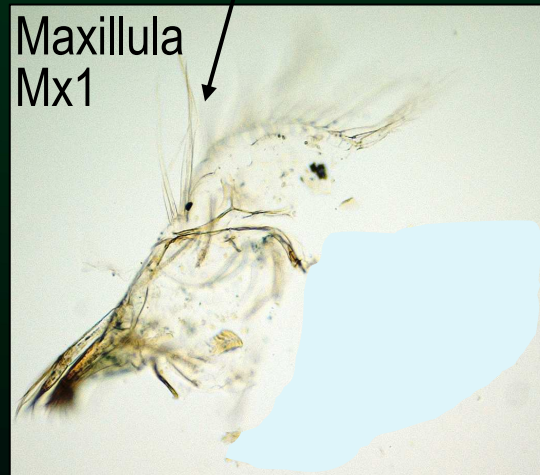


A1 and A2  
(and the eye)  
are attached  
to the  
pre-oral  
forehead

Mandibula  
Md



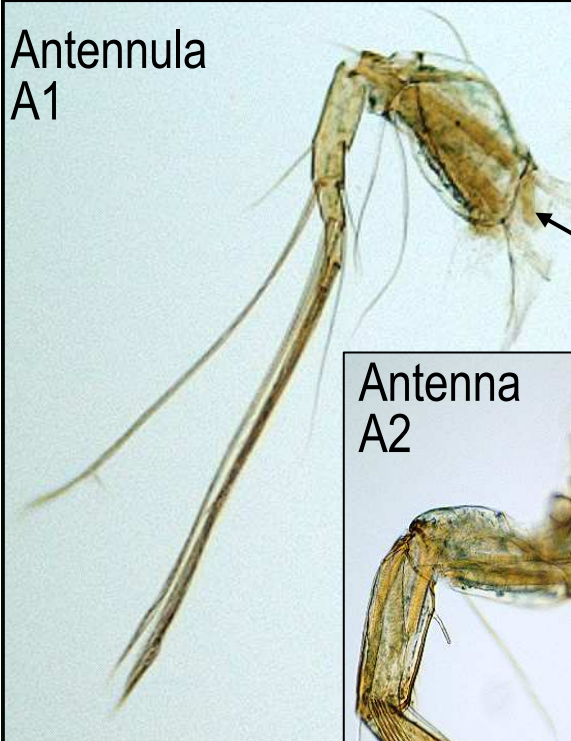
Maxillula  
Mx1



Md and Mx1 are connected to the hypostome

# General morphology and terminology of the ostracod soft body

Antennula  
A1



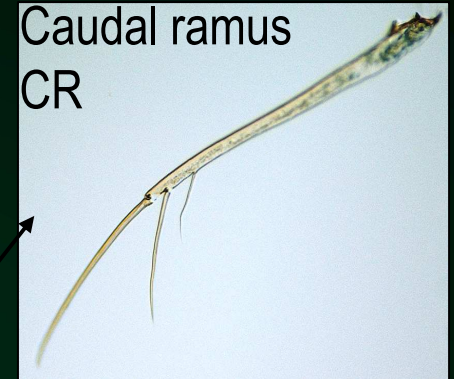
head limbs followed by:

- 3 pairs of trunk limbs
- (1 pair of male copulatory organs)
- 1 pair of caudal rami (or furcae)

Antenna  
A2



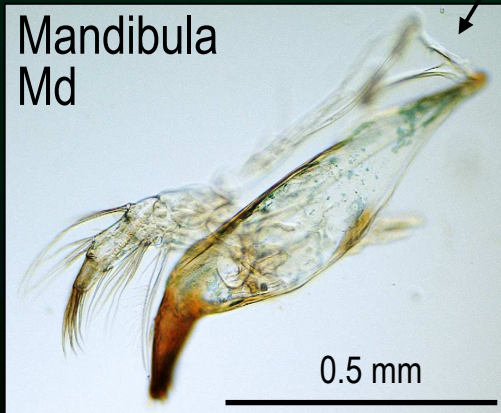
Caudal ramus  
CR



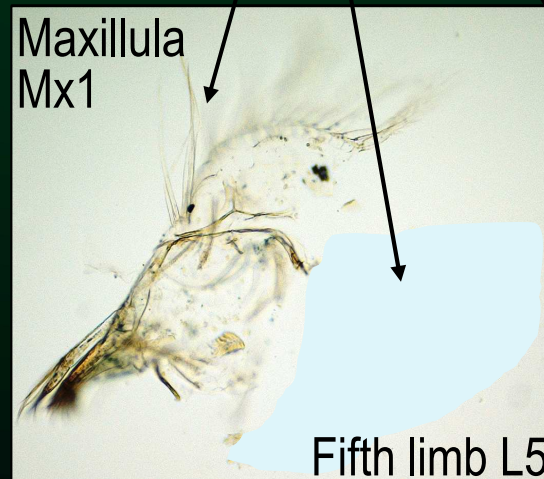
Seventh limb  
L7



Mandibula  
Md



Maxillula  
Mx1



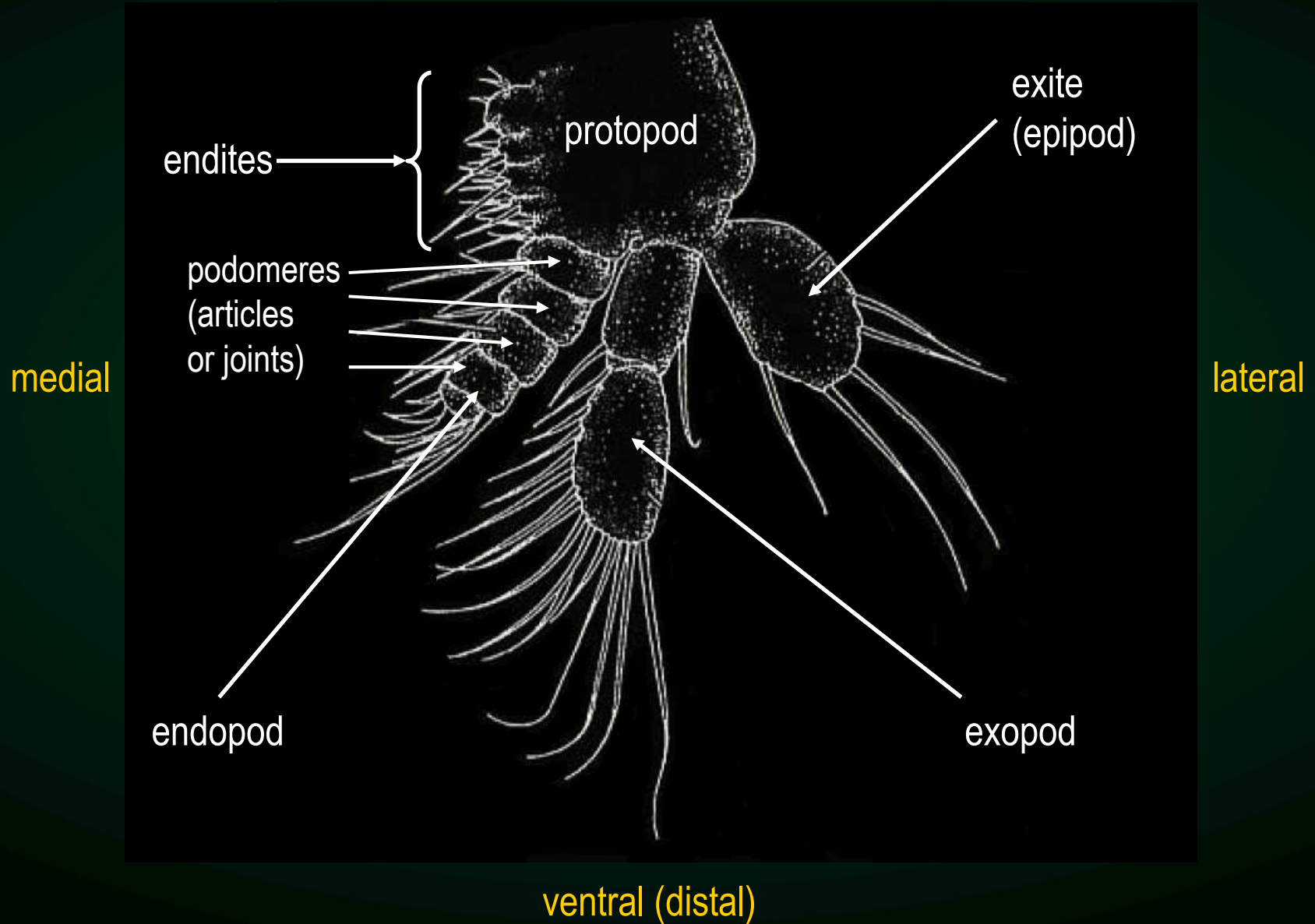
Fifth limb L5



Sixth limb  
L6

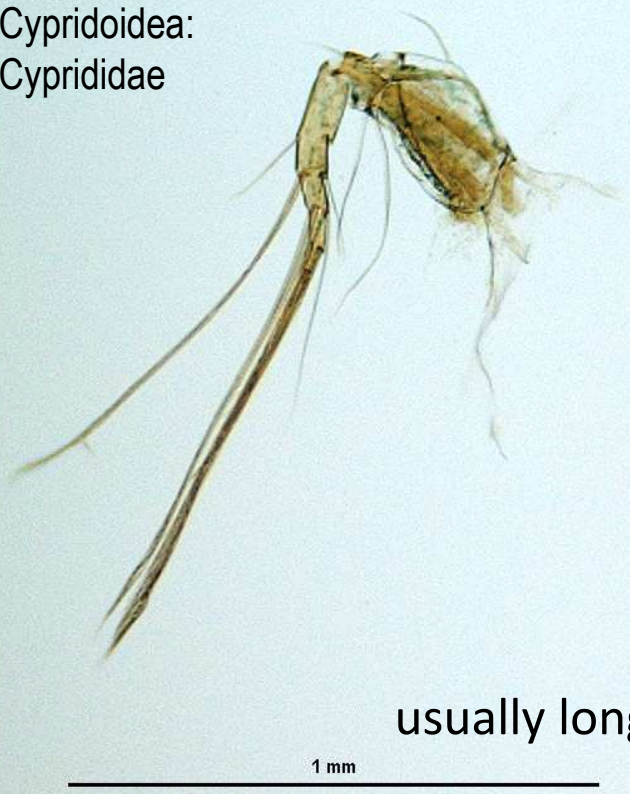
# Generalised ancestral crustacean appendage

dorsal (proximal)

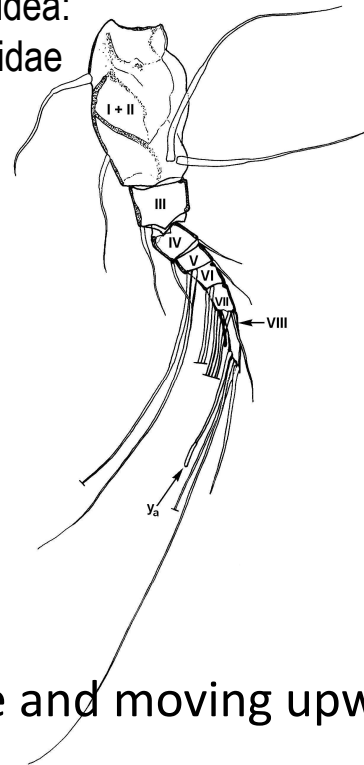


# A1 Antennula

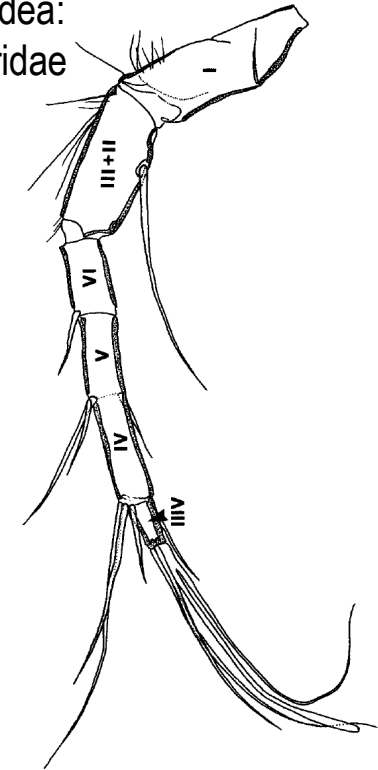
Cypridoidea:  
Cyprididae



Cypridoidea:  
Candonidae



Cytheroidea:  
Cytheruridae

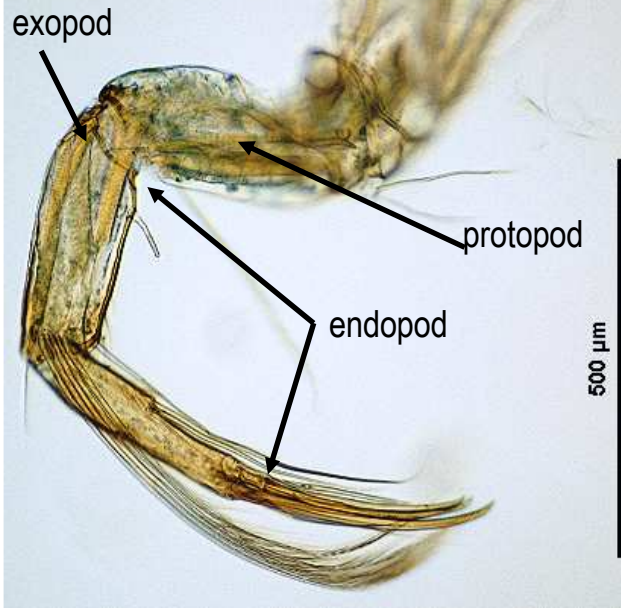


usually long, flexible and moving upward and back

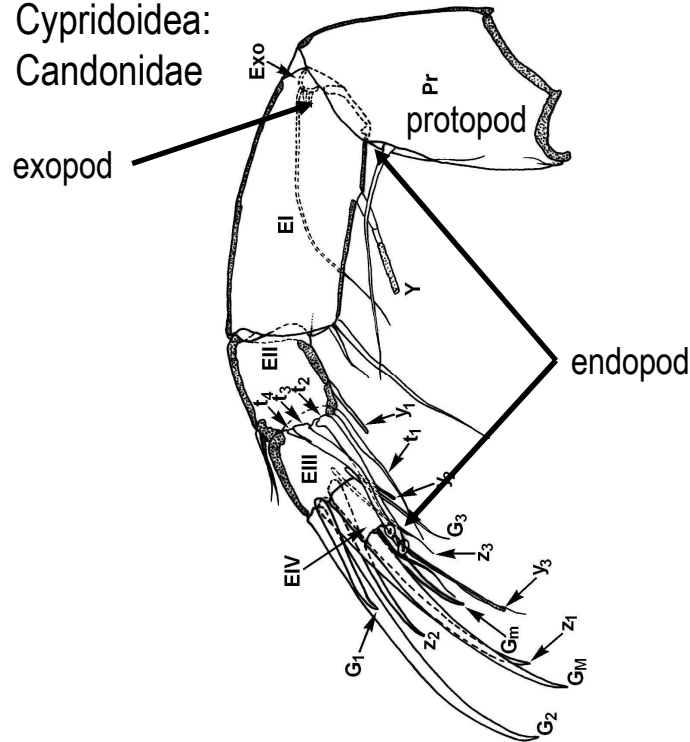
- uniramous, composed of 5-8 podomeres with up to 30 setae and claws
- function:
  - locomotory (swimming, crawling and/or burrowing)
  - sensory (served by chemo-sensorial setae or aesthetascs)
- chaetotaxy and number of podomeres useful but yet not fully exploited diagnostic characters

## A2 Antenna

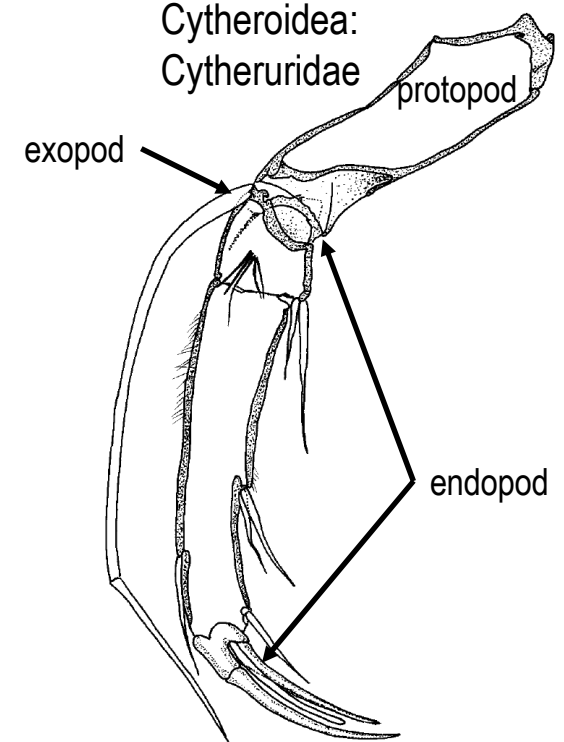
Cypridoidea:  
Cyprididae



Cypridoidea:  
Candonidae



Cytheroidea:  
Cytheruridae

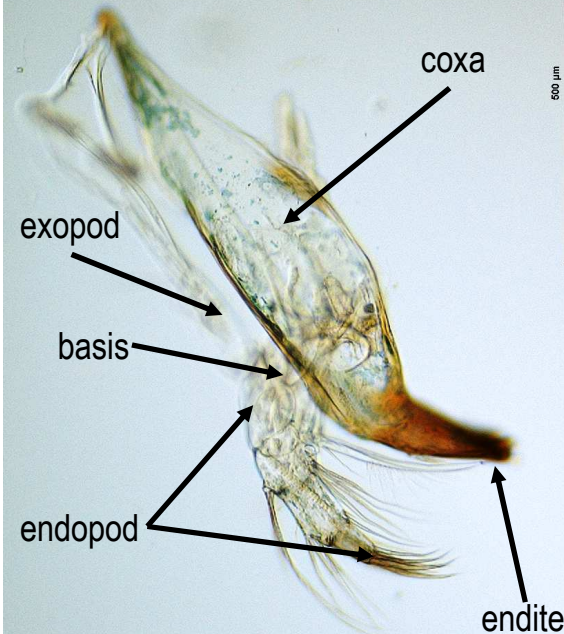


- biramous
  - in Myodocopa exopod well developed ( $\geq 9$  podomeres), endopod reduced
  - in Podocopa endopod well developed (3-4 podomeres), exopod rudimentary (in Platycopida exopod developed almost as strongly as endopod)
- most important locomotory appendages with long natatory setae in swimming forms and/or chelate claws for crawling and burrowing
- complex chaetotaxy is significant character in taxonomy (consult literature on chaetotaxic schemes and terminology for detailed taxonomical study)

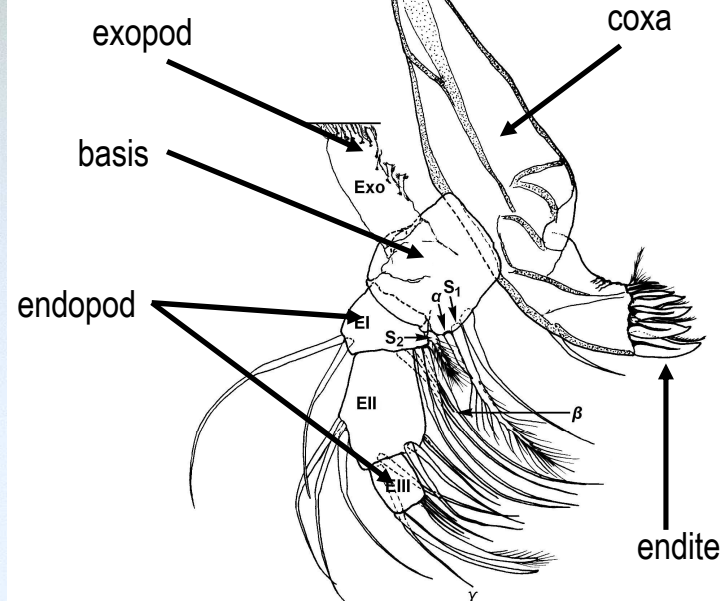


## Md Mandibula

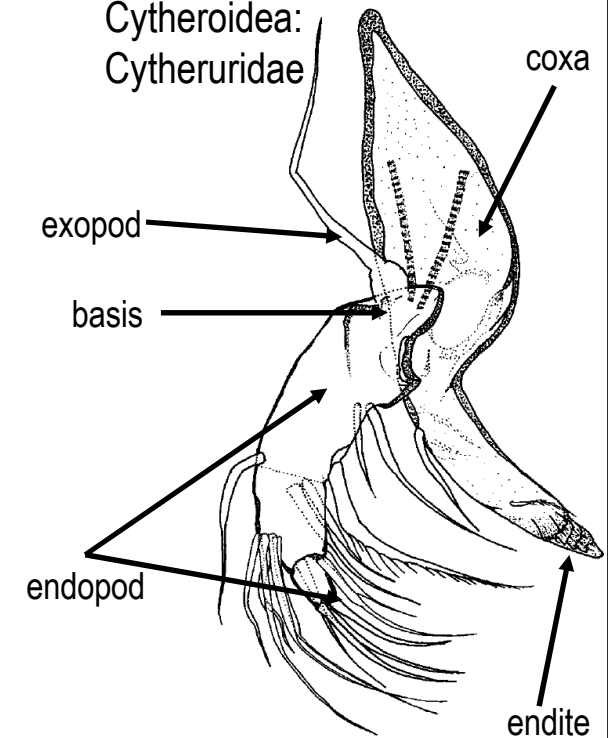
Cypridoidea:  
Cyprididae



Cypridoidea:  
Candonidae



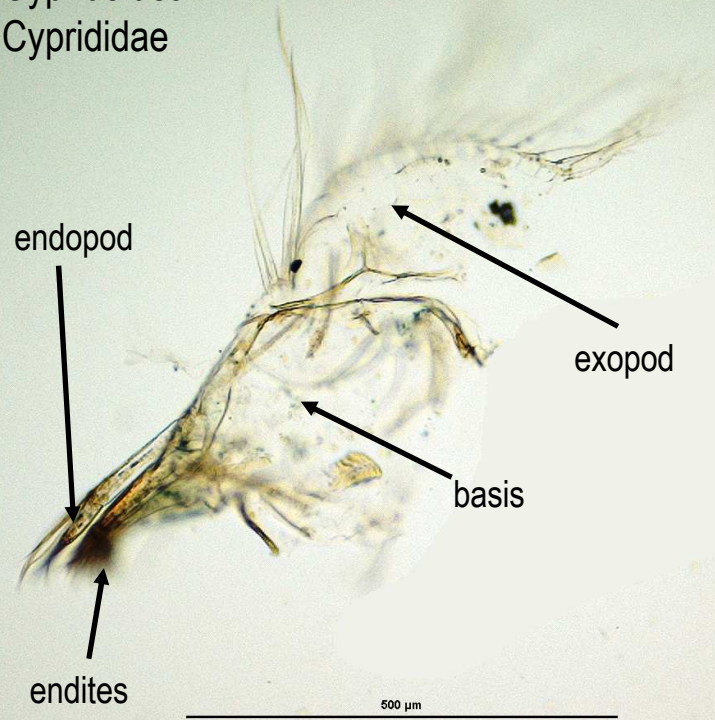
Cytheroidea:  
Cytheruridae



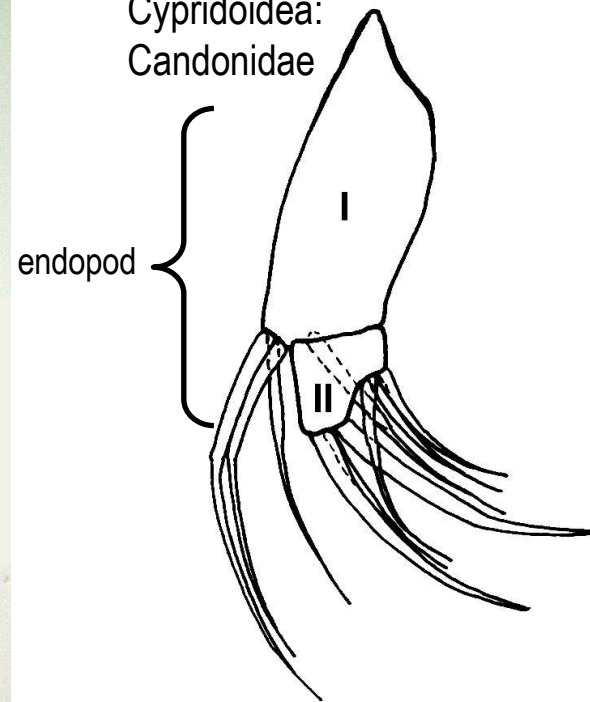
- biramous, both rami well developed:
  - exopod as branchial plate (often reduced)
  - endopod constitutes terminal part of mandibular palp (Mdp)
- protopod composed of two podomeres:
  - large and heavily sclerotized coxa with ventrally strong endite (teeth)
  - basis with exopod, constitutes 1st podomere of Mdp
- functions as feeding organ
- endopod chaetotaxy is important diagnostic trait

## Mx1 Maxillula

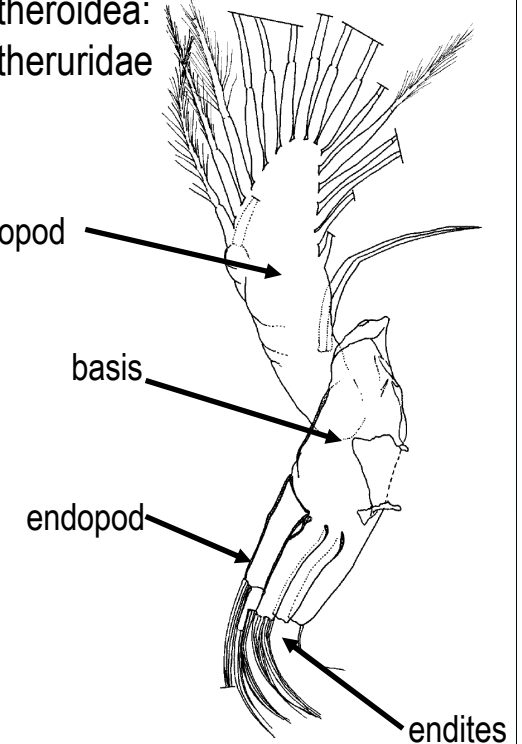
Cypridoidea:  
Cyprididae



Cypridoidea:  
Candonidae



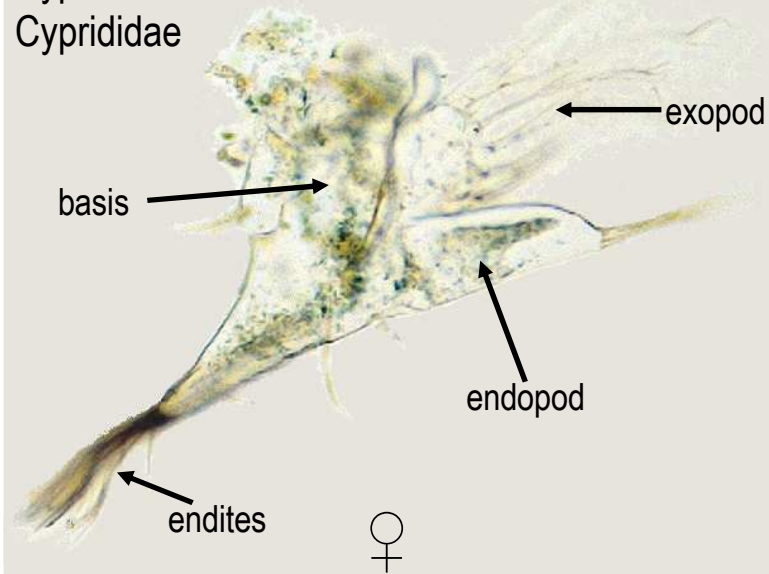
Cytheroidea:  
Cytheruridae



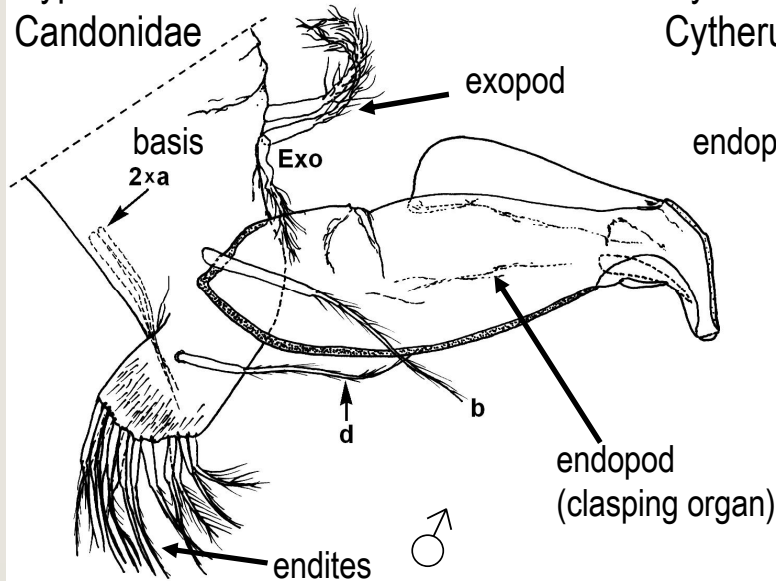
- usually greatly modified, in Podocopa consists of:
  - single-podomere protopod (basis) with 3 endites
  - endopod constituting a palp with up to 3 podomeres
  - exopod as usually large branchial plate
- in Myodocopa small epipodal branchial plates instead of exopodal plates
- masticatory and respiratory functions
- exopod offers useful diagnostic traits

## L5 Fifth limb

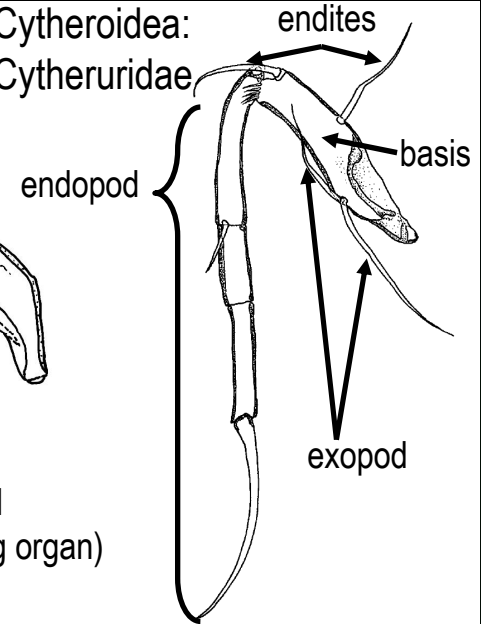
Cypridoidea:  
Cyprididae



Cypridoidea:  
Candonidae



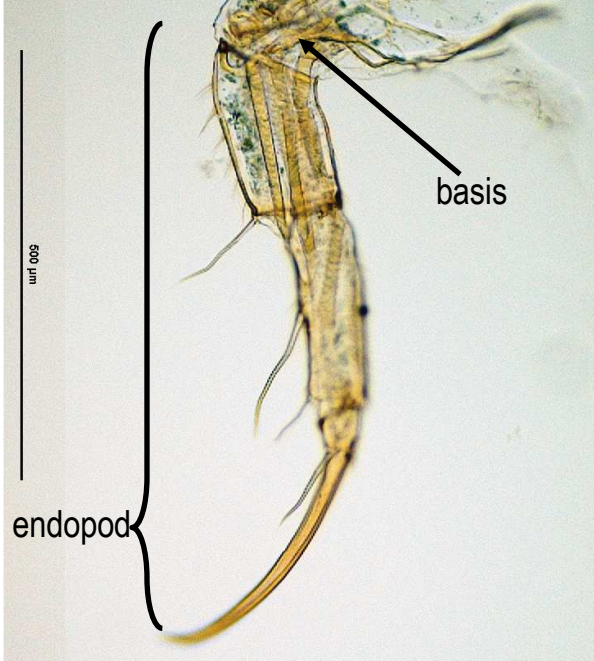
Cytheroidea:  
Cytheruridae



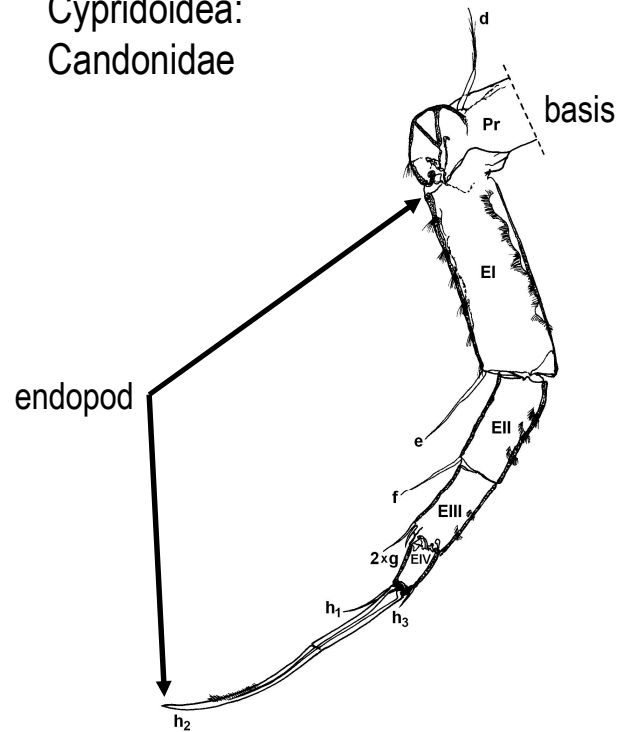
- differs in structure depending on function
- locomotory appendage (e.g. in Cytheroidea or Bairdioidea): walking leg with one protopod podomere, up to 4 podomere endopod, and reduced exopod represented by 1 seta (rarely well-developed plate)
- used for feeding (e.g. in Cypridoidea): maxilliped with one protopod podomere bearing endite setae, palp(leg)-like endopod, and small or totally lacking exopodial branchial plate
- in males of Cypridoidea endopod transformed into clasping organ used for holding the female during copulation
- respiratory (and/or filter feeding) appendage (in Myodocopa) with large epipodial branchial plate
- important in classification in several groups

## L6 Sixth limb

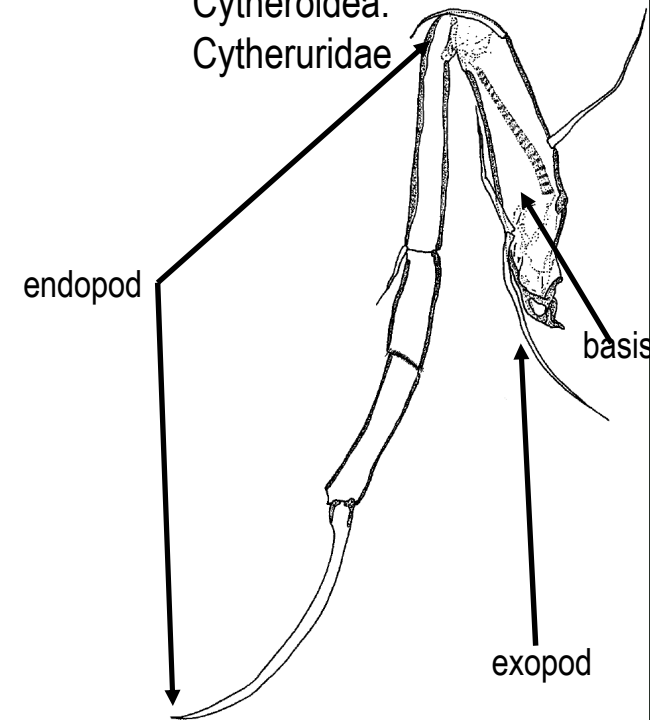
Cypridoidea:  
Cyprididae



Cypridoidea:  
Candonidae



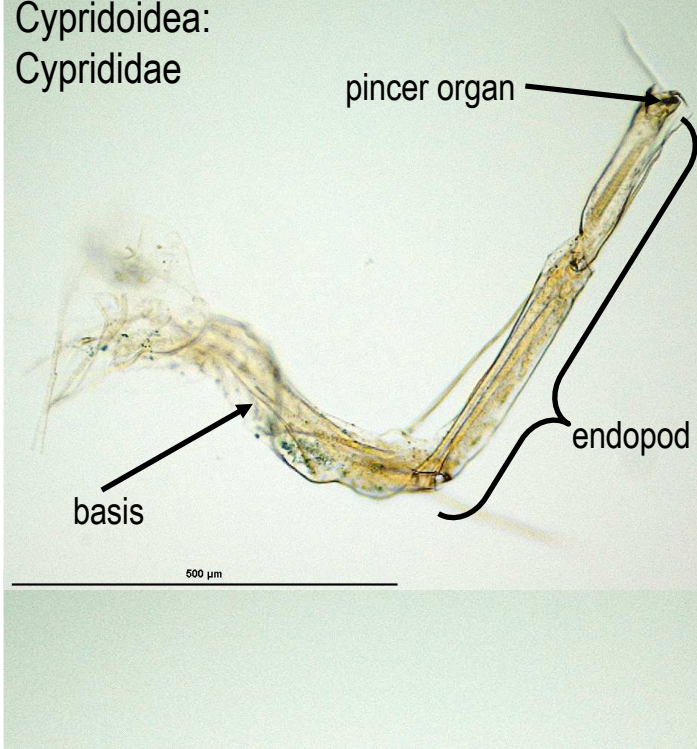
Cytheroidea:  
Cytheruridae



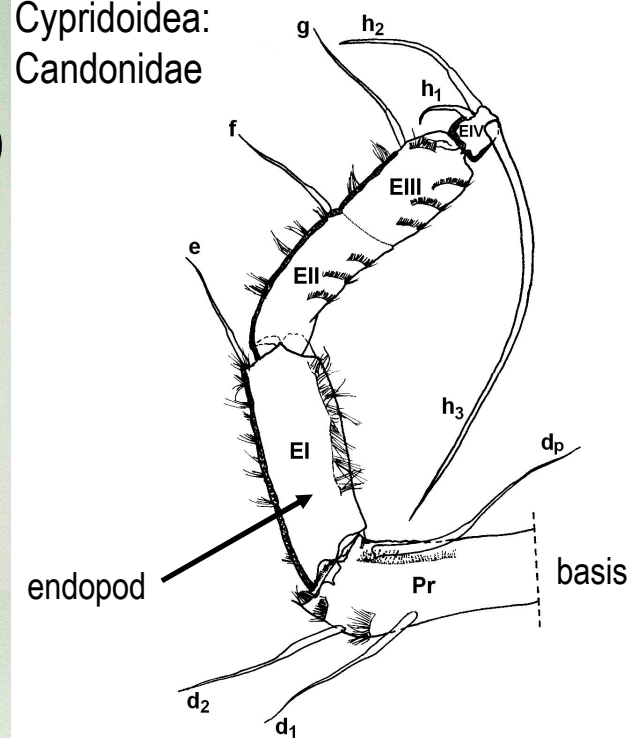
- in most Podocopa uniramous walking leg with protopod and up to 4 podomere long endopod, armed distally with strong claw
- in other taxa:
  - walking leg with epipodial branchial plate (suborder Halocypridina)
  - lamelliform (order Myodocopida)
  - modified into claspers in ♂♂ and rudimentary in ♀♀ (order Platycopida)
  - absent (suborder Cladocopina)

## L7 Seventh limb

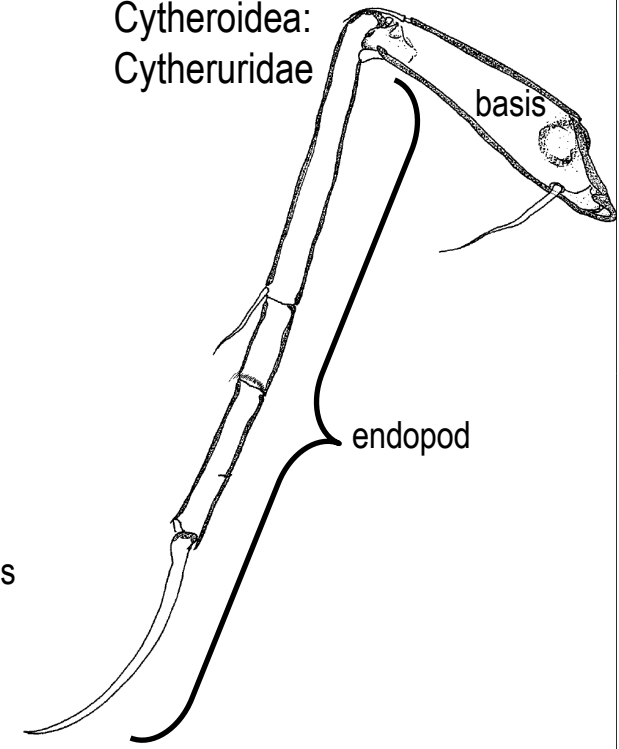
Cypridoidea:  
Cyprididae



Cypridoidea:  
Candonidae



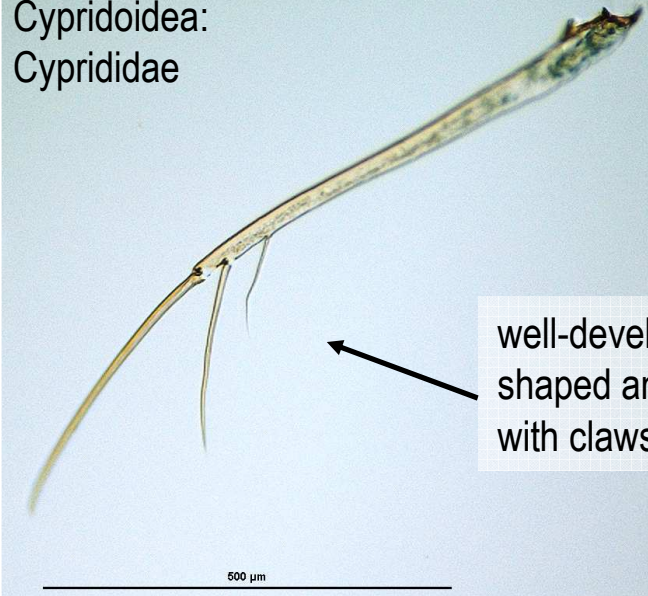
Cytheroidea:  
Cytheruridae



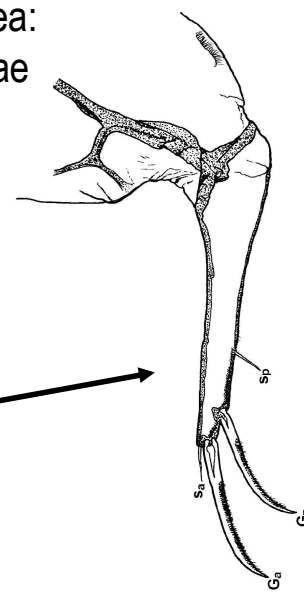
- in Podocopa:
  - walking leg similar to L6 (in order Palaeocopida or suborders Cytherocopina and Darwinulocopina of the order Podocopida)
  - cleaning leg, directed upwards used for removing foreign material from interior of valves (suborder Cypridocopina)
  - completely lacking (order Platycopida)
- in Myodocopa:
  - long, vermiform, flexible with cleaning function (order Myodocopida)
  - greatly reduced or totally absent (order Halocyprida)

## CR Caudal ramus

Cypridoidea:  
Cyprididae

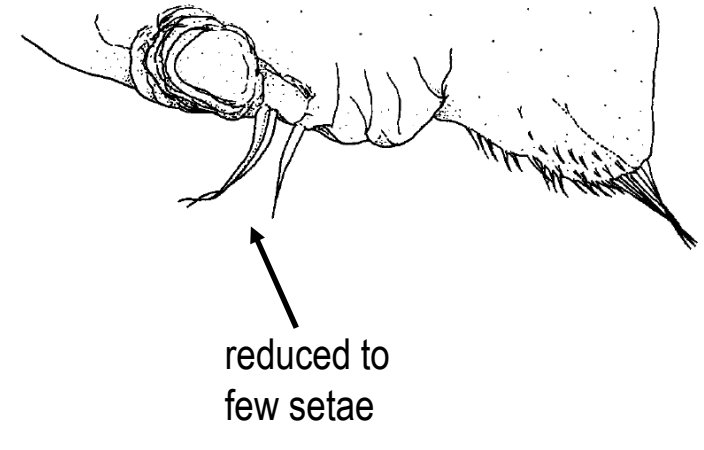


Cypridoidea:  
Candonidae



well-developed, rod-  
shaped and armed  
with claws and setae

Cytheroidea:  
Cytheruridae



reduced to  
few setae

- attached to posteroventral end of body
  - in Myodocopa posterior to anus
  - in Podocopa anterior to anus
- plate- or rod-shaped structures with locomotory function (Myodocopa, Palaeocopida, Platycopida, most Podocopida)
- reduced to various extent, in extreme case just a few minute setae (e.g. Cytheroidea or Darwinuloidea)
- CR and their attachment are of systematic importance

## HP Hemipenis

Cypridoidea:  
Ilyocyprididae



Cypridoidea:  
Candonidae



Cytheroidea:  
Leptocytheridae



- located in front of or attached to CR and usually paired
- regarded as transformation and integration of 3-5 pairs of thoracic appendages (Martens & Horne 2009)
- often large and complex, varied in various taxa
- very important taxonomic characters
- detailed internal morphology difficult to study and needs much practice
- consult relevant literature for details on HP morphology and terminology: McGregor & Kesling (1969), Danielopol (1969, 1978), Martens (1990, 1998), Meisch (2000), Smith et al. (2006), Smith & Kamiya (2007)

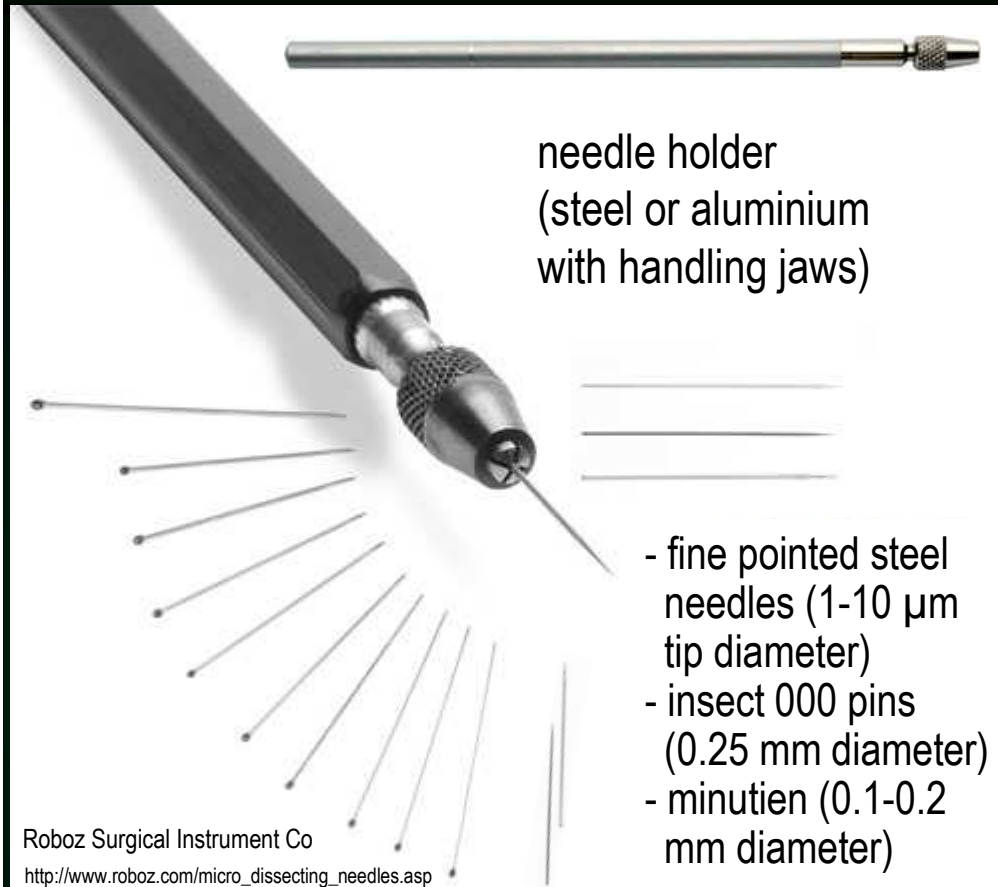
# Dissection

It is important to have all necessary materials on hand prior to dissection:





# Dissection



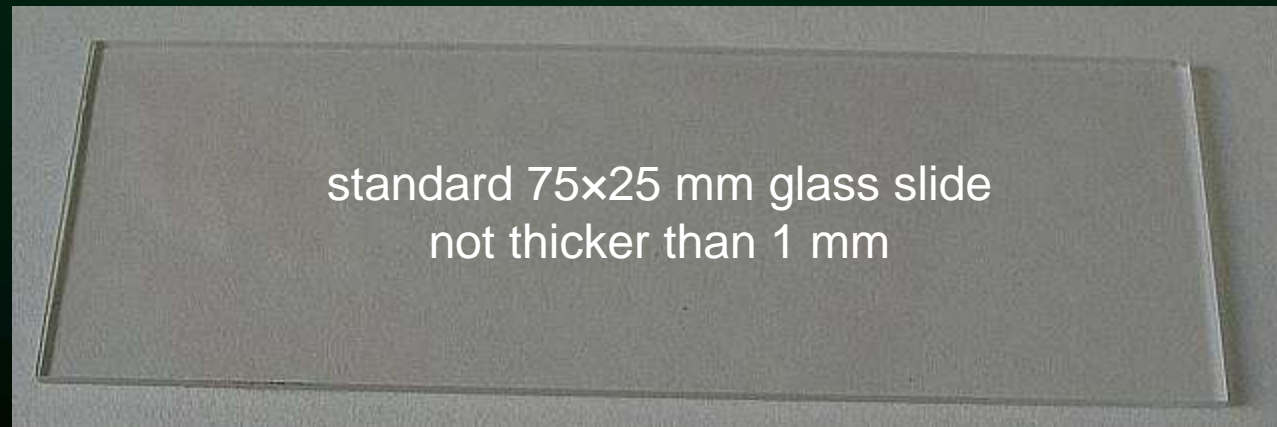
needle holder  
(steel or aluminium  
with handling jaws)

- fine pointed steel needles (1-10  $\mu\text{m}$  tip diameter)
- insect 000 pins (0.25 mm diameter)
- minutien (0.1-0.2 mm diameter)

Roboz Surgical Instrument Co  
[http://www.roboz.com/micro\\_dissecting\\_needles.asp](http://www.roboz.com/micro_dissecting_needles.asp)



cover slip  
18-20(24) mm square  
0.13-0.17 mm thick



standard 75x25 mm glass slide  
not thicker than 1 mm

## Dissection

Without experience ostracod dissection is a difficult task and often results in damage or loss of specimens

If enough material for study:

- examine several specimens and select larger and well-preserved ones with open valves
- retain intact voucher specimens in ethanol for verification of identification or for use in other studies
- deposit surplus specimens in any recognisable collection (if valuable material, potentially new species...)



## Dissection

Dissection requires two steps:

1. Opening and disarticulation of valves and separating soft body from valves
2. Separating appendages from the body

If live ostracods are being killed for dissection, use:

- dilute ~ 30% ethyl alcohol
- narcotics used for anaesthesia or euthanasia in veterinary practice allows animals to die with open valves
- easier removal of soft parts from carapace !

Opening the valves:

- on standard glass slide  
(depression slide, watch glass or embryo dish for larger specimens)
- in a glycerine drop of volume just enough to fill area under cover-slip  
(96% ethanol or water for SEM or geochemical analyses)
- under stereoscopic microscope at 20-60× magnification

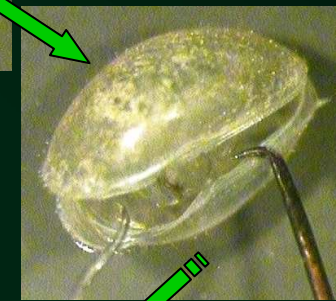
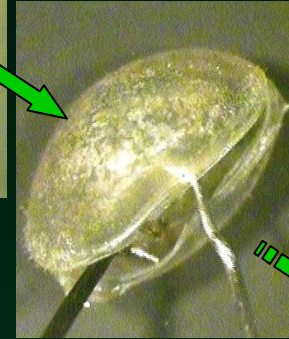


## Opening the valves

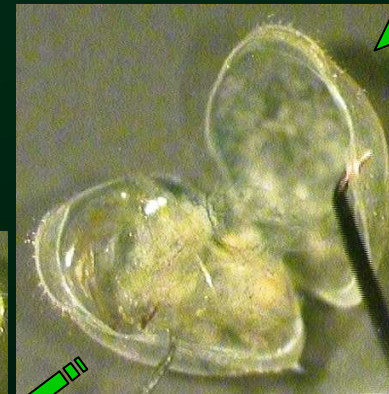
- remove body from other valve freeing it also from adductor muscles



- put needles between the valves allowing them to be slightly open



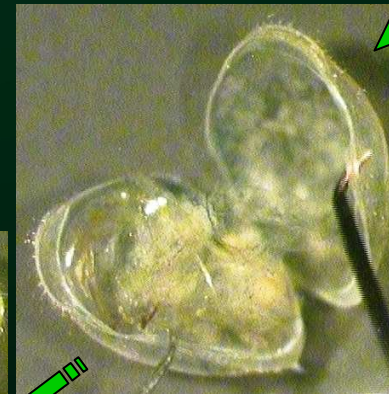
- insert one needle between one valve and body



- cut central adductor muscle and dorsal connection of body to valves



- holding the specimen in the place, pry this valve off the body with second needle



## Opening the valves

Specimens with firmly closed valves may be opened in a numbers of ways:

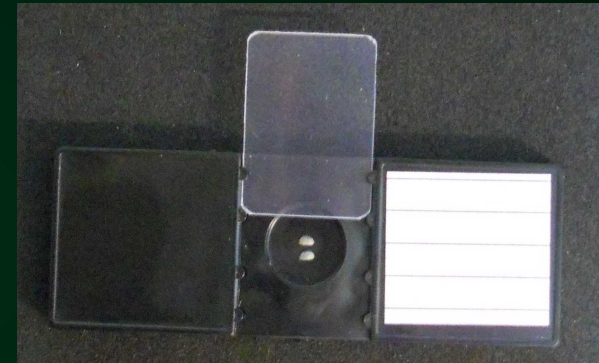
- place one needle in the middle of carapace ventral side and put pressure on its dorsal part by the second needle
- transfer alcohol fixed specimens to water
- breach one valve in the middle of ventral margin (where mutilation is the least severe) to prise the valves apart
- press a specimen in glycerine drop on glass slide by cover-slip to crush the carapace (appendages are examined as undissected smashed body)
- repeated heating and cooling in water
- gluing one valve to a slide and prising off the other

Opening valves and removing soft body is one of most frustrating part of ostracod preparation and only much practice can provide satisfactory results

## Opening the valves

Valves separated from the soft body:

- remove from glycerine
- transfer to petri-dish or watch glass with distilled water or alcohol and wash to get rid of the glycerine
- dry in air and place in micropalaeontological slide (mount with water-soluble gum tragacanth adhesive if necessary)



Valves can also be stored in:

- Euparal or glycerine jelly on depression slide (allows observation in transmitted light)
- 70-80% ethyl alcohol in a vial

Decalcification of valves can be minimised using pure or buffered ethanol

## Dissection of appendages

Continue dissection of appendages in glycerine on the same glass slide where in first step valves were separated from the soft body

- orient animal, and sketch general shape and position of appendages before separating them from the body (if you are novice)
- (➤ start dissection by dividing body into anterior and posterior part inserting the needles in the middle of dorsal side and cutting body along transverse dorsoventral axis between Mx1 and L5)
- (➤ divide halves of body along sagittal plane into right and left portions)
- remove subsequently all appendages with needles (→ video)

### Notes:

- in some taxa Mx1 and L5 are attached and have to be first removed from the body together and then teased apart
- small limbs or reduced caudal rami of some taxa are removed together with adjacent parts and not separated
- dissection of hemipenes requires more practice and patience
- take care that no air bubbles remain in glycerine or are attached to pieces of body causing them to float at glycerine surface

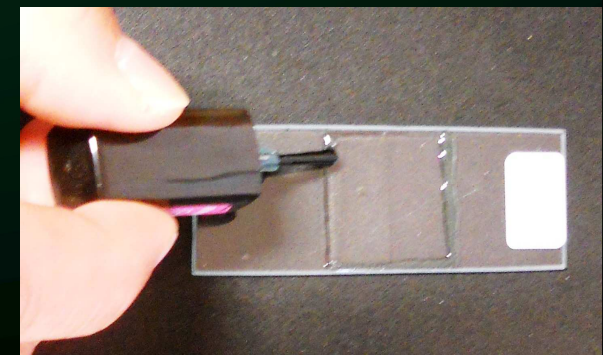
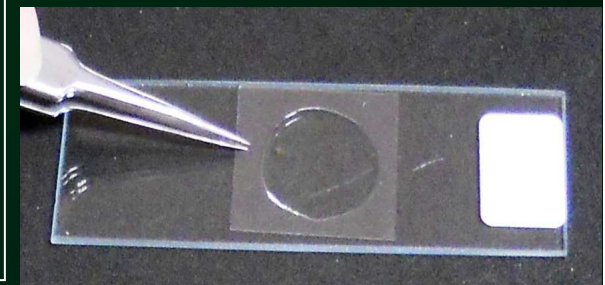
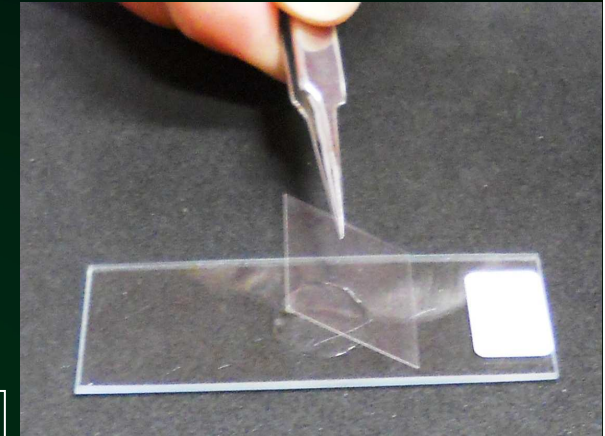
## Slide preparation

Dissected appendages are placed in centre of glycerine drop and covered carefully with round or square cover-slip:

- lower cover-slip over glycerine drop at an angle, with one edge touching the glass slide first
- allow glycerine to spread slowly out between the glass slide and the cover-slip without applying pressure

Take some practice to determine how much glycerine to use. If too much is placed on slide, cover-slip floats on thick glycerine layer and limbs spread out to edges of cover-slip. If too little glycerine is used, layer is too thin, not extending to the edges of the cover-slip and appendages may be squashed.

- seal preparation with nail polish, mark with label and keep flat and undisturbed in dust free area





## Mounting

For detailed taxonomic examination or slide-preparation for museum collections other mounting media are often used:

- polyvinyl lactophenol (PVL)
- Hydro-Matrix®
- glycerine jelly
- Euparal
- Canada balsam



Select mounting medium depending on clearing effect, purpose of the mount, type of microscopy employed or preservation time

Specimens can be dissected:

- in glycerine and then transferred to the eventual mounting medium
- directly in permanent medium if dissection can be completed before mountant becomes dry (transferring dissected pieces may result in their loss)

**Warning!**

Some mountants may be harmful, special care must be taken and preparation has to be carried out in properly ventilated laboratories.

## Slide examination and staining

Appendages are best observed in transmitted light at magnifications of 100-400×

For examination of details (e.g. minute setae) use:

- oil immersion (magnification of 1000×
- phase or differential (Nomarski) interference contrasts
- staining (either before dissection or in ultimate mounting medium)

Methylene Blue



Lignin Pink



Chlorazol Black



simply mix with mounting medium