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The ontogeny and phylogeny of copepod antennules

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Comparative analysis of the development of antennulary segmentation and setation patterns across six orders of copepods revealed numerous common features. These features are combined to produce a hypothetical general model for antennulary development in the Copepoda as a whole. In this model most compound segments result from the failure of expression of articulations separating ancestral segments. In adult males, however, compound segments either side of the neocopepodan geniculation are typically formed by secondary fusion at the last moult from CoV (stage 5).

The array of segments distal to the articulation separating segments XX and XXI is highly conserved both in ontogeny and phylogeny: typically the distal segmentation of the adult female is already present in the CoI. A maximum of three setae is added to the distal array during the entire copepodid phase. This morphological conservatism is interpreted as evidence of the functional continuity of the distal setal array as a mechanosensory system providing early warning of approaching predators.

Sexual dimorphism typically appears late in development; the male undergoing modifications especially at the final moult to sexual maturity. These modifications include the formation of the neocopepodan geniculation at the XX to XXI articulation and, in some orders, the formation of a proximal geniculation at the XV to XVI articulation. A proximal geniculation is reported here from the Calanoida for the first time. The geniculations allow the male to grasp the female during any mate guarding and during spermatophore transfer. Particular setae on segments either side of the neocopepodan geniculation are modified as basally fused spines in at least some representatives of the Calanoida, Misophrioida, Cyclopoida, Harpacticoida and Siphonostomatoida.

The antennulary chemosensory system, comprising primarily the aesthetascs, is enhanced at the final moult in many male copepods. In planktonic copepods this enhancement may take the form of a doubling of the aesthetascs on almost every antennulary segment, as in the eucalanid calanoids, or of an increase in size of existing aesthetascs, as in the siphonostomatoid *Pontoeciella*, or of the transformation of possibly originally bimodal, seta-like elements into distally thin-walled, more aesthetasc-like elements, as in some calanoids, harpacticoids and poecilostomatoids. Enhancement of the chemosensory capacity of adult males appears to be linked with their mate-locating role. Copepods inhabiting the open-pelagic water column are more likely to exhibit enhancement of the chemosensory system than neritic or benthic forms. Enhancement may confer a greater sensitivity to chemosensory signals, such as pheromones produced by receptive females, which may retain their directional information at lower concentrations and, therefore, for longer periods, in oceanic waters than in more turbulent neritic waters.

Aesthetascs appear to be more evolutionarily labile than other setation elements, apparently being lost and regained within well-defined lineages. Caution is urged in the use of aesthetasc patterns in phylogenetic analysis. The ontogenetic analyses suggest that the timing of expression of intersegmental articulations during development may in future provide the most informative characters for phylogenetic study, rather than either segment numbers or the patterns of fused or undivided segments.

Keywords: copepod; antennule; development; phylogeny; functional morphology

1. INTRODUCTION

The multisegmented antennules of copepods show a segmentally repeated pattern of setation elements that has long been recognized (Lubbock 1853; Claus 1863). Giesbrecht (1892) showed that each segment typically carried two setae and one aesthetasc, which he referred to as a trithek. By using the posterior margin seta on segment XXIV as a reference, Giesbrecht compared antennules between groups of copepods by using a system of homologies based on 25 segments and this system was widely adopted. It was modified by Gurney (1931) who concluded that ‘the primitive antennule consisted of 27, and possibly of 28 segments’, a view supported by the discovery of female misophrioid (Boxshall 1983) and calanoid (Fosshagen & Iliffe 1985) copepods with 27-segmented antennules. After comparative study of all ten orders Huys & Boxshall (1991) proposed a detailed scheme of segmental homologies based on recognition of the 28 separate segments that are expressed within the copepods, even though no known copepod possesses this number.

Most copepods have sexually dimorphic antennules: those of the adult male typically being modified for grasping the female during mating. Male antennules typically have fewer expressed segments than those of females and the males of neocopepodan copepods are primitively characterized by the possession of geniculate antennules, although the geniculation is secondarily lost in some groups (Huys & Boxshall 1991).

Huys & Boxshall (1991) referred to all compound antennulary segments derived from more than one of the 28 ancestral segments, as 'fused segments'. Such compound segments can arise by two different processes: first, as a result of failure to separate during development, and second as a result of secondary fusion of segments that were separately expressed at an earlier ontogenetic stage (Karaytug & Boxshall 1996). The ontogenetic analyses in this paper indicate that most compound segments in copepods result from failure to separate rather than from secondary fusion. Fusion occurs primarily in adult males. Some highly metamorphosed parasitic copepods secondarily lose antennulary segmentation that was expressed earlier in development, some even lose the entire appendage. The general loss of segmental expression in later stages linked to parasitic specialization will be considered elsewhere.

Ontogenetic analyses are used here to build a model of antennulary development in particular copepod orders and in the Copepoda as a whole, that considers both segmentation and setation patterns. Such a model will permit the identification of any general patterns in antennulary sexual dimorphism and facilitate recognition of any exceptions to the general pattern.

Huys & Boxshall (1991) observed that the overwhelming evolutionary trend within the Copepoda is the reduction by fusion, failure to separate, or loss, of limb segments and their setation elements. They noted exceptions to this trend, however, particularly in the setation patterns of the antennules. As analysed by Giesbrecht (1892) some male calanoids possess two aesthetascs per segment giving a quadrithek arrangement (2 setae plus 2 aesthetascs) compared with the trithek (2+1) of the female. Huys & Boxshall (1991) speculated that the evolutionary doubling in the number of aesthetascs on the segments of the male antennules of certain calanoid copepods is correlated with the colonization of the open-pelagic environment where chemical cues are of paramount importance. The formulation of a general model of antennulary development will also allow examination of this speculation. The inclusion of analyses of development in the Misophrioida, Siphonostomatoida, Poecilostomatoida and Harpacticoida, each of which contains one or more lineages that have invaded the open pelagic, provides an independent perspective on such a correlation.

2. MATERIALS AND METHODS

Segmental homologies are identified by reference to the 28-ancestral-segment system proposed by Huys & Boxshall (1991) in which ancestral segments are identified by using Roman numerals, actual segments by Arabic numerals. Vertical tracking of segmentation through the series of copepodid stages (commencing with the adults and working backwards) was achieved primarily by using individual setation elements, or combinations of elements as reference points. Confirmation could be made in some cases by study of individuals preserved during moulting, as noted by Hulsemann (1991*a*). The unequivocal identification of segmental origins of setae added to compound segments in the earliest copepodid stages is possible only in calanoids and, to a lesser extent, in siphonostomatoids and misophrioids. When there is no clear morphological evidence from vertical tracking, setal derivations are made according to the following assumptions.

1. The time of first appearance of any setal element should match as closely as possible the pattern exhibited in the better resolved taxa, particularly the calanoids. This is based on the principle that maximum internal congruence should be sought in the data, and is equivalent to the parsimony principle in cladistic methodology.
2. Anterodistal setae appear first on any segment and anteroproximal setae can only be deemed to be present on a compound segment after the full complement of one anterodistal seta per incorporated segment is present.
3. Anterodistal setae are added first to the more distal incorporated segments in any compound segment. This is in accord with the general distal-to-proximal progression observed in both segmentation and setation features.

Material examined includes: *Platycopia orientalis* Ohtsuka & Boxshall, for locality data see Ohtsuka & Boxshall (1994); *Benthomisophria palliata* Sars, for locality data see Boxshall & Roe (1980); CoIV of *Archimisophria discoveryi* Boxshall, for locality data see Boxshall (1983); *Asterocheres echinicola* (Norman), collected from a sponge (*Halichondria panicea* (Pallas)) in the estuary of River Blackwater, Essex (registration number 1997.1208–1217); male of unidentified *Heterorhabdus* sp. from The Natural History Museum collections (registration number 1994.643–652); *Pachos punctatum* Claus, for locality data see Boxshall (1977); *Pontoeciella abyssicola* (Scott), for locality data see Boxshall (1979); and *Clytemnestra rostrata* (Brady) and *Candacia varicans* Giesbrecht (registration number 1994.3944–3948) from the collections of the Natural History Museum, London.

Copepodid stages I to V are denoted here by the abbreviations CoI to CoV. The sixth nauplius is denoted by NVI.

3. RESULTS

(a) *Antennulary development patterns*

(i) *Calanoids*

The antennules of calanoids through the copepodid phase have been well illustrated for several species including *Epischura massachusettsensis* Pearse (Humes 1955) and *Ridgewayia klausruetzleri* Ferrari (Ferrari 1995). The pattern of segment subdivision and setal addition shown schematically in figure 1 is based on these two species. The articulation between segments XX and XXI is expressed at CoI and is an important reference point, because it develops into the specialized geniculation in the grasping antennules of adult male neocopepodan

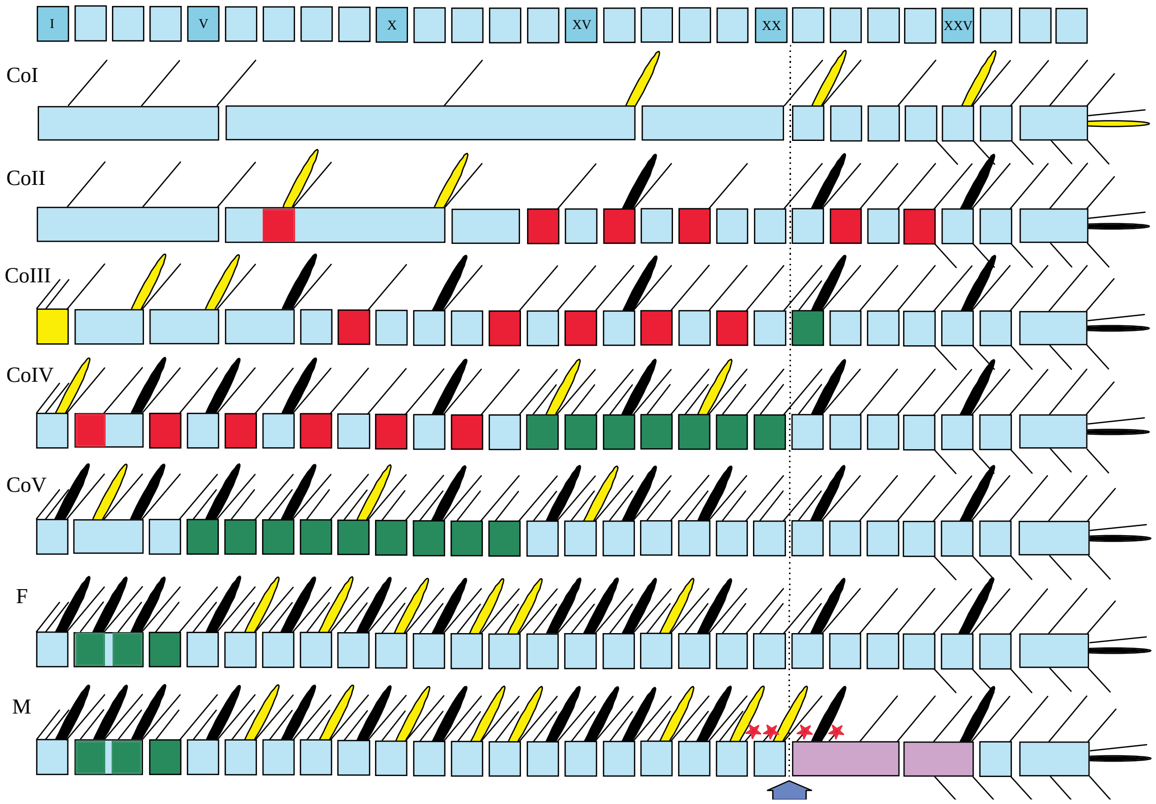

Figure 1. Schematic showing development of segmentation and setation through the copepodid stages of Calanoida, based on *Epischura massachusettsensis* and *Ridgewayia klausruetzleri* (Humes 1955; Ferrari 1995). The modified setae (red asterisks) around the geniculation in males are based on *Epischura* as are the distal fusions. The scale at the top indicates the presumed 28 segments of the ancestral copepod (see Huys & Boxshall 1991). Elements are shown as setae, spines or aesthetascs. A segment carrying a seta not present at the preceding Co stage is shown in red if the newly added seta is the anterodistal member of a trithek, in green if it is the anteroproximal member. The basal segment (I) is shown in yellow when its second and/or third setae first appear. Compound segments derived by secondary fusion in adult males are shown in lilac. The plane of the XX to XXI articulation is indicated by a vertical dotted line. Aesthetascs are shown in yellow when they first appear, then in black. The location of the neocopepodan geniculation in the adult male is indicated by the arrow. Abbreviations: CoI to CoV indicate copepodid stages I to V; F, female; M, male.

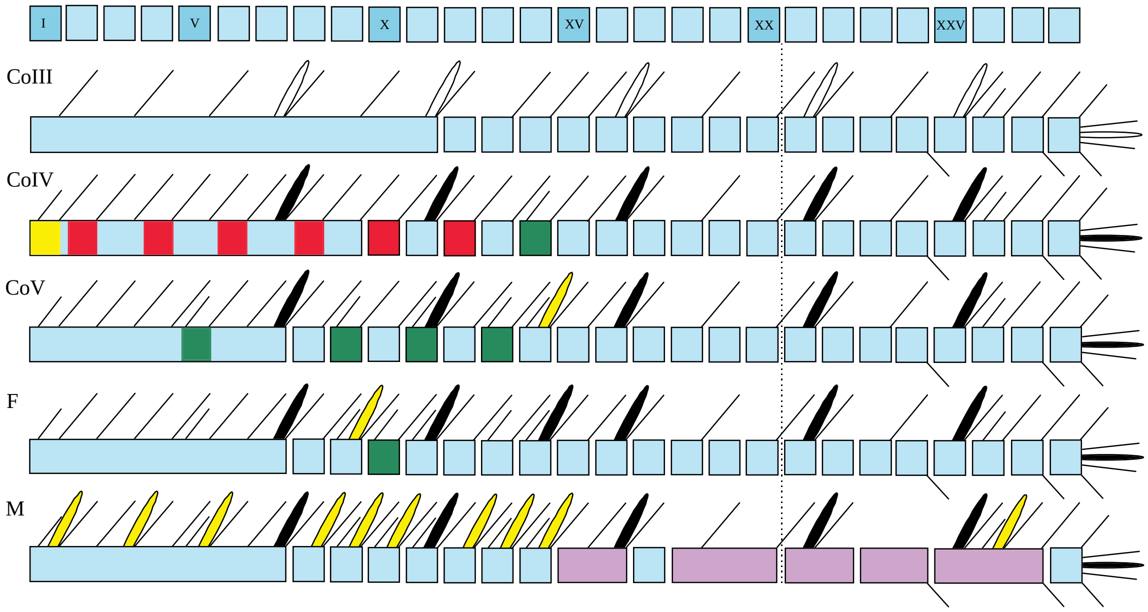

Figure 2. Schematic showing development of segmentation and setation through the later copepodid stages of Platycopioida, based on *Platycopia orientalis* (slightly modified from Ohtsuka & Boxshall (1994)). See figure 1 legend for interpretation. Aesthetascs are shown uncoloured in CoIII because CoI and CoII are unknown.

copepods (Huys & Boxshall 1991). In both species all segments distal to the XX–XXI articulation expressed in the adult female are already expressed at CoI. There is no change in distal segmentation of the female (figure 1) but in adult males of both species there is distal fusion, XXI–XXIII, on the geniculate antennule, and fusion of XXIV–XXV in *Epischura* only.

During the copepodid phase the three proximal segments of the CoI antennule subdivide, gradually giving rise to the adult female segmentation pattern. Adult segments XIV to XX are expressed at the moult to CoII, segments I and VIII to XIII at the moult to CoIII, and the full adult female segmentation pattern is typically expressed by CoIV stage. The only remaining compound segments in the CoIV to adult are the double apical segment (XXVII–XXVIII), a characteristic of all neocopepodan orders, and the double segment II–III which is typical of all calanoids with the exception of *Erebonectes nesioticus* Fosshagen (Huys & Boxshall 1991). The distal fusion XXI–XXIII on the geniculate antennule occurs at the CoV to adult male moult.

Setae are added in a regular pattern through the copepodid phase. At the moult to CoII, setae are added anterodistally to segments XIV, XVI, XVIII, XXII and XXIV. One seta is added to the undivided second segment (the precursor of segments VI to XI) and it is identified as derived from segment VII because of its association with an aesthetasc, the origin of which can be confirmed at CoIV. At the moult to CoIII, setae are added anterodistally to segments IX, XIII, XV, XVII and XIX. Also at this moult the second seta of the trithek (the anteroproximal seta, positioned midway along the anterior margin, proximal to the existing distal seta) first appears on segment XXI only. Another two additional setae appear on the basal segment (I) at this moult, completing its unique, 3-setae complement.

At the moult to CoIV anterodistal setae appear on a set of alternate segments i.e. II, IV, VI, VIII, X and XII. By this stage every segment (I to XXVI) has its anterodistal seta. Also appearing, but in a block of segments, are the anteroproximal setae on segments XIV to XX inclusive. At the moult to CoV the anteroproximal setae appear on the block of segments from V to XIII inclusive, and at the final moult to adult the anteroproximal setae appear on the block of segments from II to IV inclusive. This pattern is consistent in both species and has been confirmed by checking against other calanoids, such as *Drepanopus forcipatus* Giesbrecht (Hulsemann 1991*b*).

The appearance of aesthetascs seems to be variable between species but some of the variation may be accounted for by inadequate observation as they are thin-walled, often relatively small and easy to overlook. The presence of four aesthetascs in CoI is typical, located on segments XVI, XXI, XXV and XXVIII. The aesthetasc on segment XVI is unusual as it appears one moult before its associated anterodistal seta. Aesthetascs typically appear on segments VII and XI at CoII and on segments III and V at CoIII. The pattern of appearance of aesthetascs on the remaining segments appears variable, but a common scheme is for the aesthetascs on segments I, XIV and XVIII to appear at CoIV. The aesthetascs on the other proximal antennulary segments typically appear at the last two moults.

(ii) *Platycopioids*

The later developmental stages, from CoIII to adult, of *Platycopia orientalis* were described by Ohtsuka & Boxshall (1994). The antennules are 18-segmented in CoIII, 20-segmented in CoIV, 22-segmented in the CoV of both sexes and in the adult female and 15-segmented in the adult male. The ontogenetic pattern of changes in segmentation and the pattern of addition of setation elements are shown schematically in figure 2 using the 28-segment baseline of Huys & Boxshall (1991).

The addition of segments between CoIII and CoV is the result of subdivision of the large proximal segment: there being no subdivision of the already-expressed, distally located segments. The moult from CoV to adult male is marked by several fusions in the distal part. Segments expressed at CoV fuse in the adult to form the following compound segments: XV–XVI, XVIII–XX, XXI–XXII, XXIII–XXIV, XXV–XXVII. The apical segment, XXVIII, is expressed as a distinct segment at every stage from CoIII to adult in both sexes, a feature unique to the Platycopioida. CoI and CoII are unknown.

Noteworthy features of the setation pattern include: segments XVII, XIX and XXII lack setation elements at all stages; segment XXIV lacks an anterior margin seta at all stages; segment XXI possesses one seta plus an aesthetasc at all stages from CoIII onwards. A feature unique to platycopioids is the location of the two setae anteriorly on segment XXVI, rather than one anteriorly and one posteriorly as in the neocopepodan orders. The adult male loses the anterior seta on segment XXIII at the last moult.

(iii) *Misophrioids*

The only available account of the ontogeny of misophrioids is based on the bathypelagic genus *Benthomisophria* Sars (Boxshall & Roe 1980), but the full setation pattern was not illustrated. The copepodids of *Benthomisophria palliata* were re-examined even though this misophrioid exhibits reduced antennulary segmentation in the adult. In *B. palliata* the antennule is 6-segmented in CoI (figure 3*a*), 9-segmented in CoII (figure 3*b*), 12-segmented in CoIII (figure 3*c*), 15-segmented in CoIV (figure 4*a*), 17-segmented in CoV (figure 4*b*), 18-segmented in the adult female and indistinctly 18-segmented in the adult male (figure 4*c*). At the moult to adult male the following segments are formed by fusion of previously separate segments: XV–XVI, XIX–XX, XXI–XXIII and XXIV–XXVIII.

The origins of the expressed segments are shown in figure 5. The adult segmentation of the antennule distal to articulation XX–XXI is complete at CoI, apart from compound segment XXI–XXII which is divided into separate segments by CoII. The pattern of subdivisions of the proximal segments is different from that of calanoids (figure 1) although in *Archimisophria discoveryi* it is presumably more similar because the adult female segmentation, i.e. 27 defined segments, is already present at CoIV (Boxshall 1983).

At the moult to CoII the anterodistal setae are added to segments XXII and XXIV. Additionally, five setae appear along the undifferentiated proximal segment (precursor to segments I to XVIII). One of these, at the distal corner, presumably represents that present on segment XVIII at

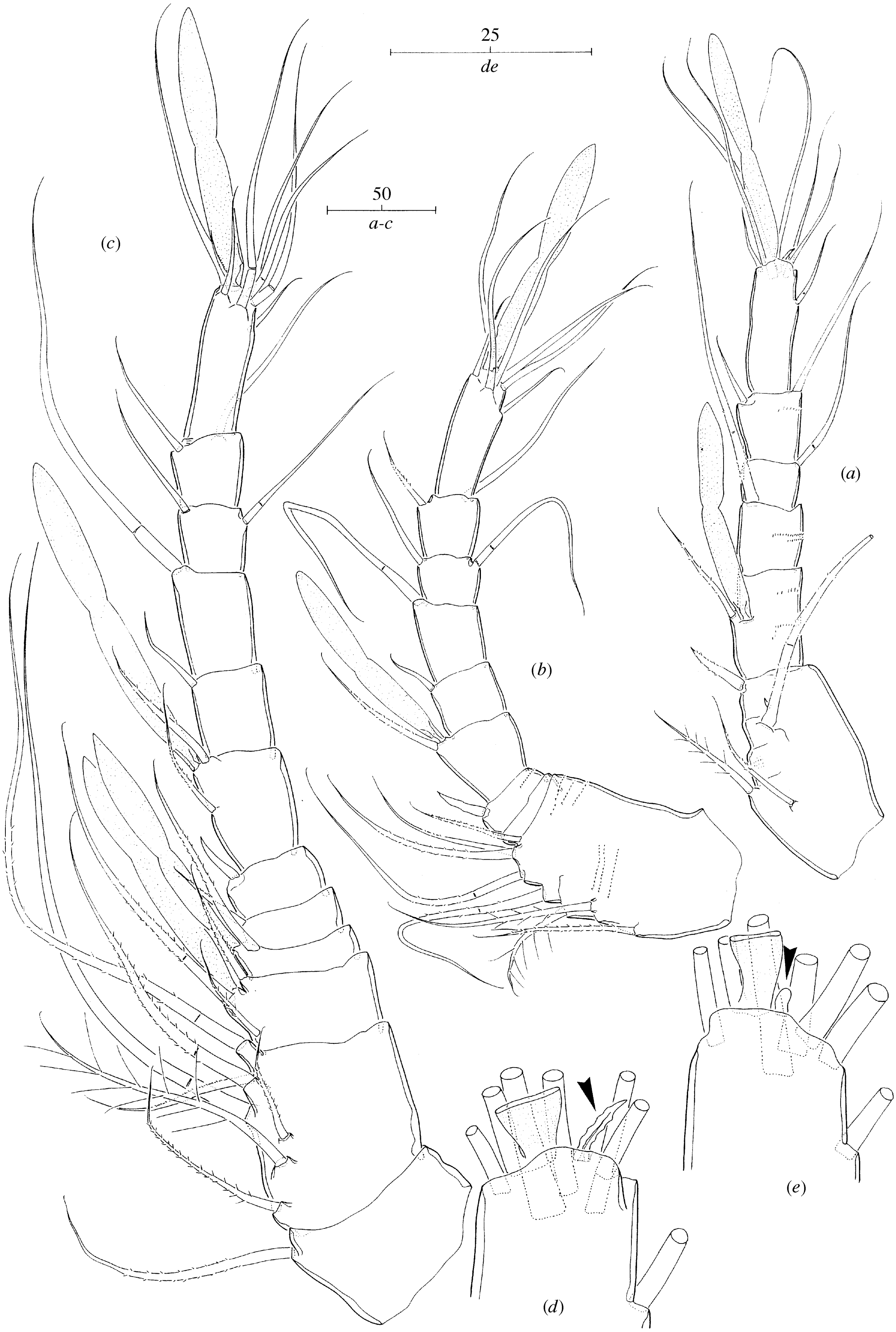

Figure 3. Antennules of early copepodid stages of *Benthomisophria palliata*. (*a*) CoI ; (*b*) CoII ; (*c*) CoIII; (*d*) tip of apical segment of CoI, arrowhead indicates spiniform element that degenerates in later stages; (*e*) tip to apical segment of CoII, with vestige of spiniform element arrowed. Scale bars in μm.

Figure 4. (*a*–*c*) Antennules of late copepodid stages and adults of *Benthomisophria palliata*. (*a*) CoIV; (*b*) proximal segments of female CoV, showing additional segment; (*c*) adult male, ventral; (*d*) segments 5–11 of adult male, dorsal. Scale bars in µm.

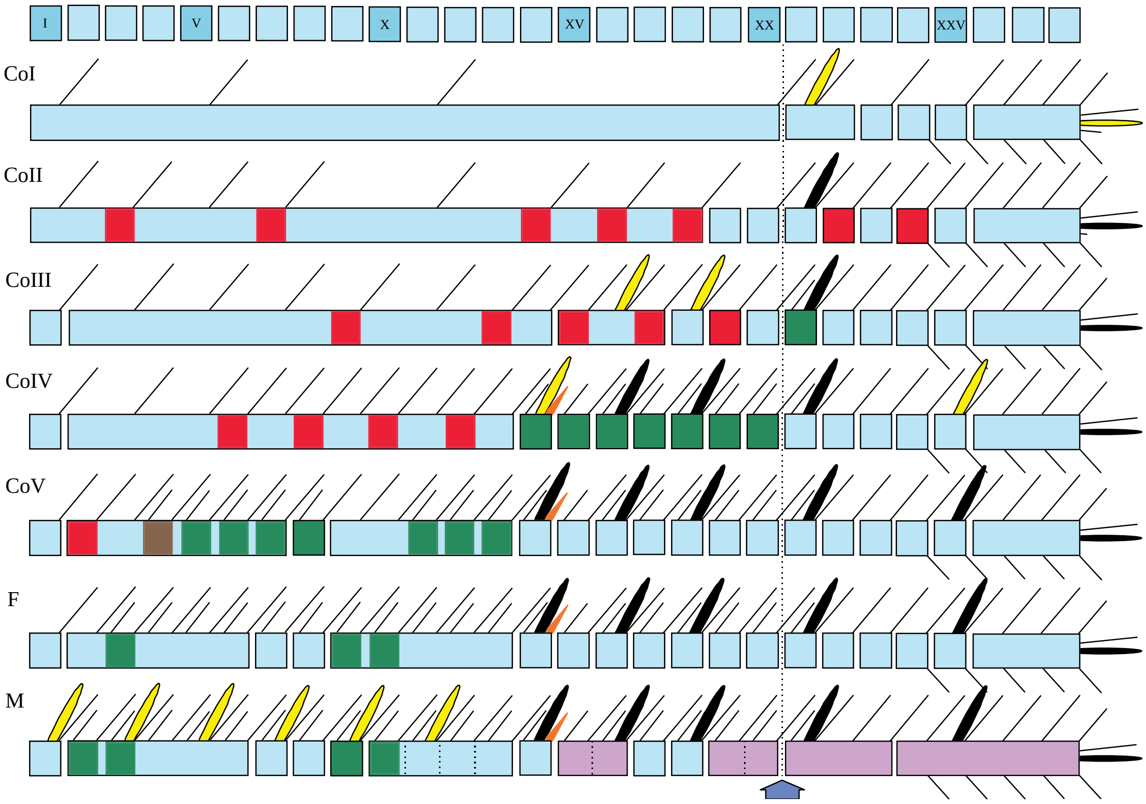

Figure 5. Schematic showing development of segmentation and setation through the copepodid stages of Misophrioida, based on *Benthomisophria palliata*. See figure 1 legend for interpretation. If anteroproximal and anterodistal setae are added simultaneously the segment is shown in brown. The anterodistal seta (indicated in orange) on segment XIV is modified as a spine from CoIV to adult. Incompletely expressed articulations are shown as dotted lines subdividing compound segments.

the next moult. The segmental origins of the other four are tentatively identified as III, VII, XIV and XVI. At the next moult, to CoIII, the anteroproximal seta is added to segment XXI, completing the trithek on this segment, and the anterodistal seta is added to segment XIX. A total of two setae is added to the triple segment XV–XVII, probably representing one each to XV and XVII from their locations either side of the aesthetasc originating on XVI. Additionally two setae are added to the elongate segment (precursor to II–XIV), presumably to segments IX and XIII.

At the moult to CoIV the anteroproximal setae appear on a block of segments from XIV to XX inclusive, as in calanoids (see figure 1). Simultaneously, four more setae appear on segment II–XIII. Segmental origins are proposed for these elements plus those added over the next two moults (figure 5), by application of the criteria outlined above. The anterodistal seta on segment XIV is transformed into a spine at the moult to CoIV.

The Misophrioida comprises two main lineages, the *Misophria*-lineage which is primarily hyperbenthic and bathypelagic, and the *Archimisophria*-lineage which is primarily found in anchialine caves or crevicular habitats (Boxshall 1989). Only in the *Misophria*-lineage is there a significant sexual dimorphism in the number of aesthetascs in the adults, with males carrying aesthetascs on up to seven of the proximal segments that lack aesthetascs in the female. The pattern of aesthetasc appearance is clear in *B. palliata*, a representative of the *Misophria*-lineage. Aesthetascs are present on only XXI and XXVIII in CoI, those on segments XVI and XVIII appear at CoIII, and those on XIV and XXV at CoIV. Only these six aesthetascs are present in the adult female. The additional aesthetascs present proximally in males appear at the final moult from CoV. Adults of both sexes of *A. discoveryi* carry five aesthetascs, on segments III, VII, XI, XVI and XXVII–XVIII. All five are already present in CoIV.

(iv) *Siphonostomatoids*

The siphonostomatoid antennule is often shortened with reduced segmentation but in *Asterocheres echinicola* the adult female has 21 segments. The CoI antennule is indistinctly 4-segmented (figure 6*a*), the CoII indistinctly 9-segmented (figure 6*b*), and the CoIII indistinctly 13-segmented (figure 6*c*). The distal articulation expressed at CoI is that between segments XX and XXI. The segmentation of the section distal to this XX to XXI articulation almost achieves its adult pattern by CoIV (figure 6*d*) in the female although articulation XXIII to XXIV is incompletely expressed. The adult pattern (figure 7*a*) in the proximal section appears only at the final moult. The CoV is sexually dimorphic (cf. figures 6*e*,*f*) and the schematic (figure 8) is based on the female CoV. The adult male antennule is 17-segmented (figure 7*b*) with four compound segments, XV–XVI, XIX–XX, XXI–XXIII and XXIV–XXVIII, that are not found in the female. Double-segment XV–XVI remains undivided from male CoV and in earlier stages but the three other compound

Figure 6. Antennules of early copepodid stages of *Asterocheres echinicola*. (*a*) CoI; (*b*) CoII; (*c*) CoIII; (*d*) CoIV; (*e*) female CoV; (*f*) distal section (segment VIII to apex) of male CoV. Scale bar in μm.

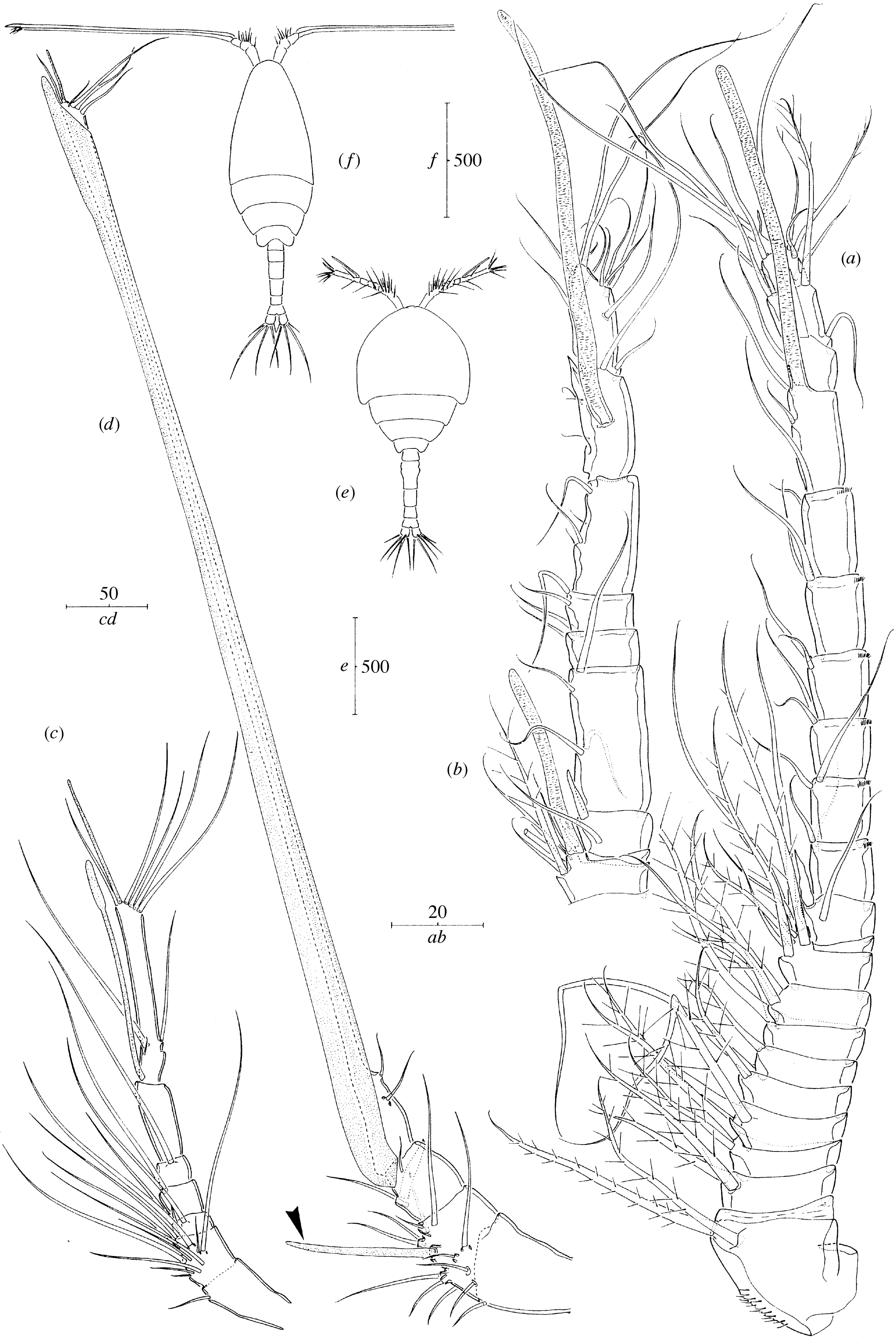

Figure 7. (*a*, *b*) Antennules of adults of *Asterocheres echinicola*. (*a*) female; (*b*) distal section (segment IX to apex) of male antennule. (*c–f*) *Pontoeciella abyssicola*. (*c*) Adult female antennule; (*d*) adult male antennule, with additional aesthetasc arrowed; (*e*) female, dorsal view; (*f*) male, dorsal view. Scale bars in µm.

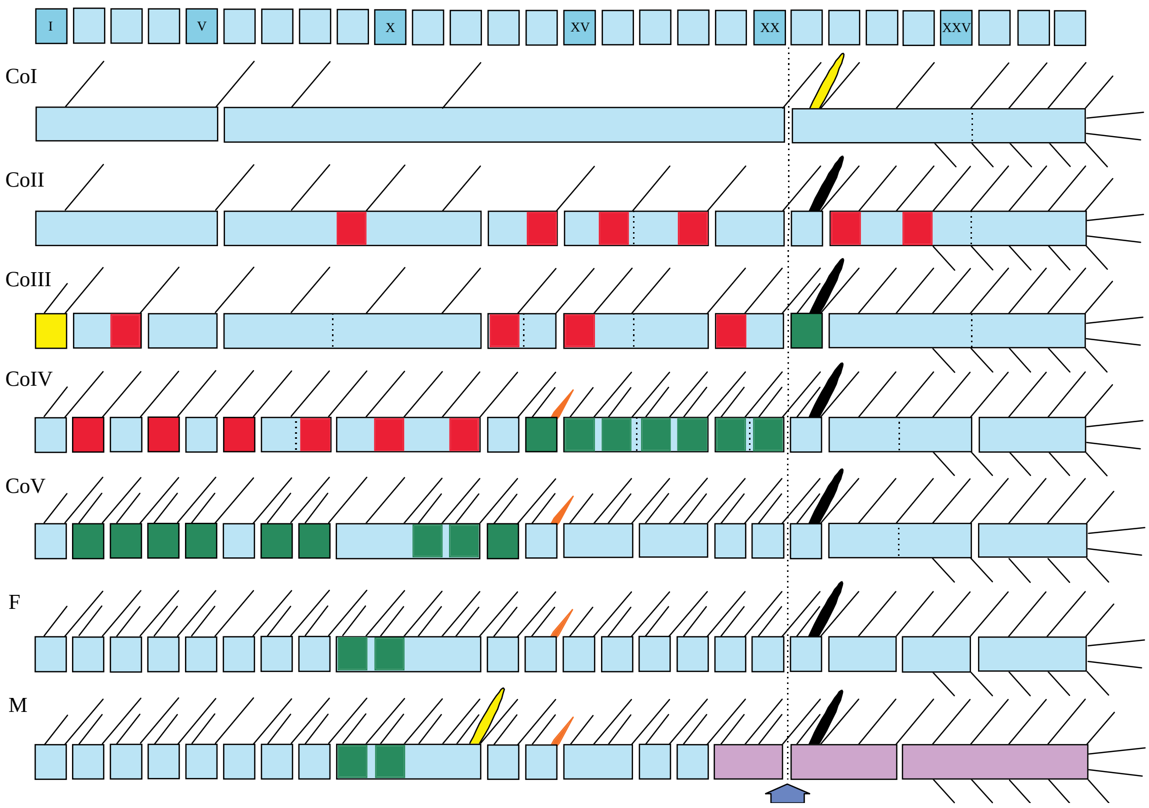

Figure 8. Schematic showing development of segmentation and setation through the copepodid stages of Siphonostomatoida based on *Asterocheres echinicola*. See figure 1 legend for interpretation. The anterodistal seta (indicated in orange) on segment XIV is modified as a spine from CoIV to adult. Incompletely expressed articulations are shown as dotted lines subdividing compound segments. The CoV is sexually dimorphic in *A. echinicola*; the schematic is based on the female CoV.

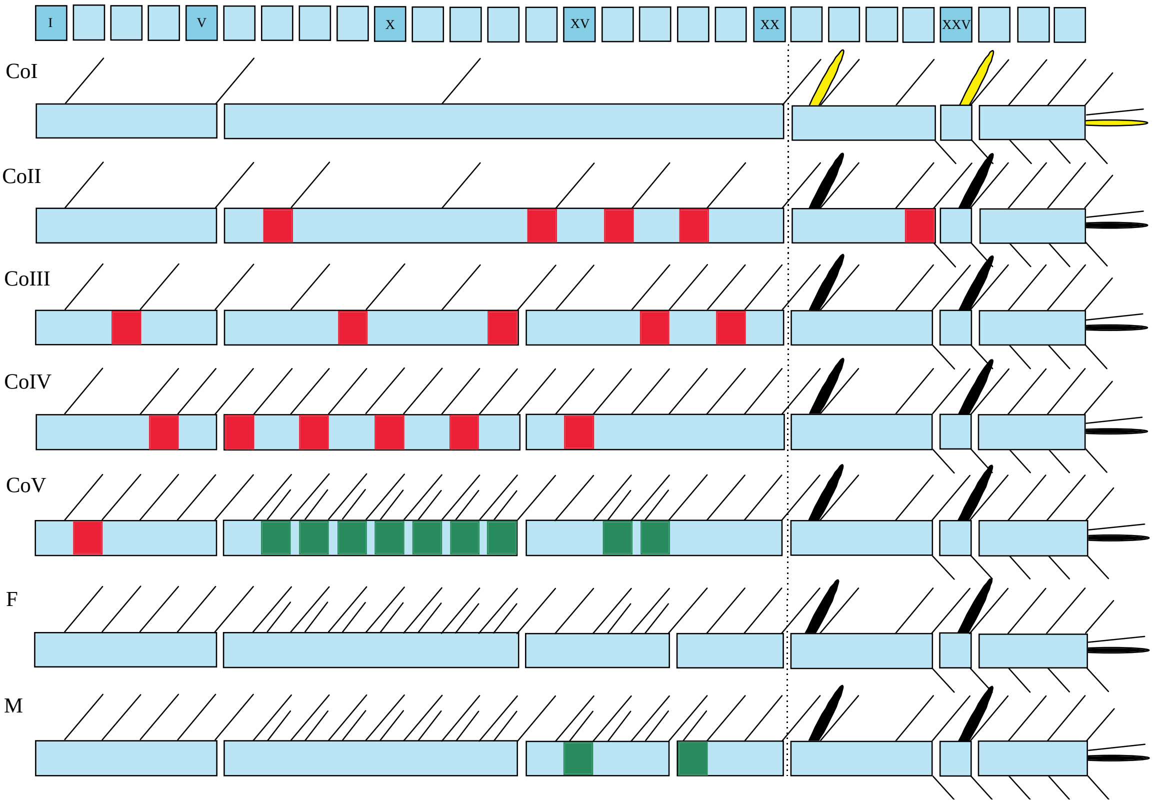

Figure 9. Schematic showing development of segmentation and setation through the copepodid stages of Poecilostomatoida, based on *Hemicyclops ctenidis* (Kim & Ho 1992), *Ergasilus briani* (Alston *et al*. 1996), *Taeniacanthus lagocephali* (Izawa 1986*a*) and *Leptinogaster major* (Humes 1986). See figure 1 legend for interpretation.

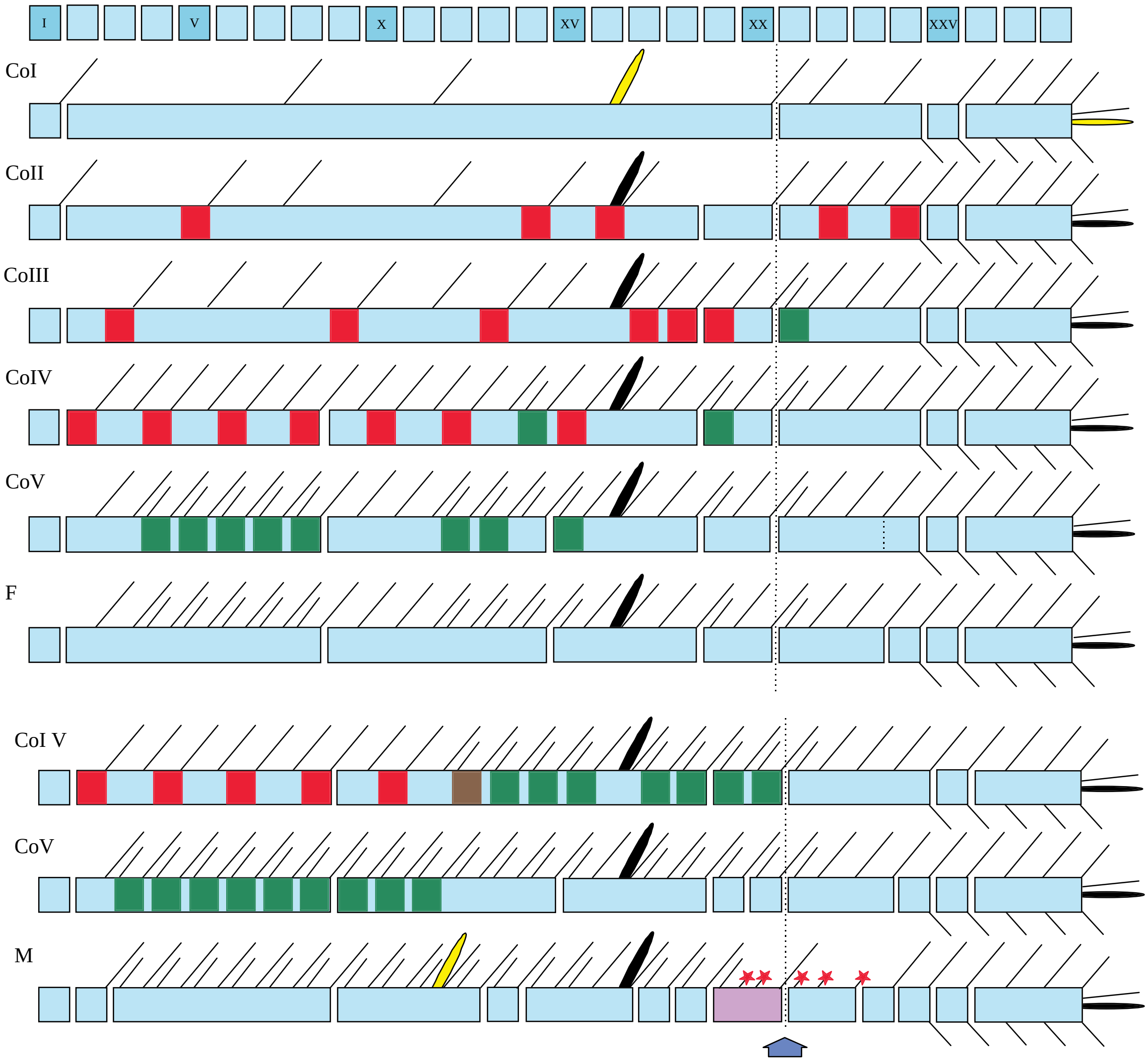

Figure 10. Schematic showing development of segmentation and setation through the copepodid stages of Harpacticoida, based on *Ambunguipes rufocincta* (R. Huys, unpublished data). Female copepodid development (upper part) is shown in full together with male CoIV to adult (lower part). See figure 1 legend for interpretation. If anteroproximal and anterodistal setae are added simultaneously the segment is shown in brown.

segments form secondarily at the moult from CoV (figure 6*f*). This is shown schematically in figure 8.

The development of the setation is similar to that of calanoids and misophrioids. In addition two setae appear on the distal segment (XXII–XXVIII) at CoII stage and these are identified as originating on segments XXII and XXIV after comparison with their positions in later copepodids. A total of four setae also appears on more proximal segments and these are identified as originating on segments IX, XIV, XVI and XVIII after similar comparisons. At the next moult the anteroproximal seta appears on segment XXI, as in calanoids and misophrioids, and anterodistal setae appear on segments III, XIII, XV and XIX. A second seta appears on the basal segment (I) at this moult.

At CoIV the anteroproximal setae appear on a block of segments from XIV to XX inclusive, exactly as in calanoid (figure 1) and misophrioid (figure 5) patterns. In addition, anterodistal setae appear on segments II, IV, VI, VIII, X and XII, and the anterodistal seta present on segment XIV since CoII is transformed into a stout spine. This spine, appearing at CoIV, serves as a useful reference point in the study of antennule development in this order and appears to have a homologue in the Cyclopoida, as noted by Giesbrecht in 1899 and in the Misophrioida (figures 4*a* and 5). The five setae located on segments around the neocopepodan geniculation that are modified into basally fused spines in *Epischura* (figure 1) are all reduced in size at the moult to adult male (figure 7*b*).

The aesthetasc arrangement in siphonostomatoids is specialized, with only those present on segments XXI and XXVIII being retained in most species. The apical aesthetasc is often lost as in *A. echinicola*. Rarely, an aesthetasc is present more proximally (on segment XI) as in male *A. echinicola* (figure 7*b*). This extra aesthetasc appears only at the final moult. In females the pattern of aesthetascs typically does not change during development. In those species with sexually dimorphic aesthetasc arrangements, the additional aesthetascs of the adult males probably appear at the last moult from CoV, as reported in *Cancerilla* by Carton (1968).

(v) *Poecilostomatoids*

There have been numerous studies on development in this order. The schematic (figure 9) is based on *Hemicyclops ctenidis* Ho & Kim (Kim & Ho 1992), *Ergasilus briani* Markevitch (Alston *et al.* 1996), *Taeniacanthus lagocephali* Pearse (Izawa 1986*a*) and *Leptinogaster major* (Williams) (Humes 1986). The system of ancestral segmental homologies is based partly on the analysis of Marchenkov & Boxshall (1995), but differs from that proposed by Huys & Boxshall (1991) only in the position of the proximal articulation rather than in both of the first two articulations.

The CoI antennule is typically 5-segmented. Throughout development from CoI to adult there are typically three segments distal to the XX to XXI articulation. Proximal to that articulation the number of segments increases from two to four by the adult (figure 9).

At the moult to CoII the anterodistal seta appears on segment XXIV. This is the only element added to the array of segments distal to the XX to XXI articulation during the entire copepodid phase of development in poecilostomatoids. The long second segment (precursor to segments VI to XX) gains a total of four setae. At the moults to CoIII and CoIV anterodistal setae are added to the three proximal segments. At the moult to CoV one anterodistal seta is added to the first segment and anteroproximal setae are added to the second and third segments. At the moult to adult female the third segment divides but no setal additions occur, however in the adult male one seta is added to each of the newly separated segments XIV–XVII and XVIII–XX.

Poecilostomatoids typically have three aesthetascs at CoI, originating from segments XXI, XXV and XXVIII. Occasionally a fourth is present, originating from a more proximal segment (probably XVI), as in CoI of the myicolid *Panaietis yamaguti* Izawa (Izawa 1986*b*). The penultimate antennulary segment (XXV) of all copepodid stages of almost every poecilostomatoid thus far described is expressed as a distinct segment and carries a seta and an aesthetasc anterodistally and a seta posterodistally. This free segment represents a characteristic signature for the Poecilostomatoida. Some poecilostomatoids have additional aesthetascs in adults, up to ten aesthetascs are expressed within the order (Marchenkov & Boxshall 1995), but no relevant data are available on the sequence of appearance of these additional aesthetascs during development.

(vi) *Harpacticoids*

Developmental changes in antennulary segmentation and setation are variable in harpacticoids (Dahms 1989). The schematic (figure 10) is based on unpublished data of Huys from *Ambunguipes rufocincta* (Brady). The antennule of CoI is 5-segmented. The number of segments expressed distal to articulation XX to XXI increases from three in CoI to four in the CoV (incompletely defined) and adult female, and to five in the adult male. The first segment (I) is separate throughout the copepodid phase, the second (II–XX) divides into four segments (II–VIII, IX–XIV, XV–XVIII and XIX–XX) by the adult female. In male CoV and adult the segmentation pattern proximal to the XX to XXI articulation differs from that of the female CoV and adult (see figure 10). Unusual changes occur at the final moult in males: two previously expressed articulations (XIV to XV and XIX to XX) are lost and five are gained (XII to XIII, XIII to XIV, XVI to XVII, XVII to XVIII and XXII to XXIII). There is also a secondary fusion of segments XIX and XX associated with the formation of the neocopepodan geniculation.

The development of the setation in *A. rufocincta* shares some features with calanoids, siphonostomatoids and misophrioids. At CoII two setae appear on segment XXI–XXIV, here interpreted as the anterodistal setae belonging to segments XXII and XXIV. At the moult to CoIII another seta is added to the same compound segment (XXI–XXIV), interpreted here as the anteroproximal seta of segment XXI. Throughout development setae added on the more proximal segments are attributed to particular ancestral segments using assumptions outlined above (see § 2). It is interesting to note that the seta associated with the large aesthetasc (on segment XVI) appears at CoII, one moult after the aesthetasc, as in calanoids (figure 1).

A total of two aesthetascs (XVI and XXVIII) is present in the copepodid phase in the female. In *A. rufocincta* males have a third aesthetasc, identified as derived from segment XI, that appears at the final moult to adult. In other harpacticoids there is often a noticeable decrease in size of setae and increase in diameter of aesthetascs at the final CoV to adult male moult (Dahms 1988).

Sexual dimorphism is particularly strongly expressed in harpacticoids. Male and female antennules differ in segmentation by CoV and setation differences are already apparent by CoIV in *Ambunguipes*. Anteroproximal setae are added within the block of segments XII to XX at the moult to CoIV in males, but only within the block XIV to XIX in females. This is the only known example in the Copepoda of the appearance of anteroproximal setae proximal to segment XIV before the moult to CoV. Anteroproximal setae are added at the moults to CoV in both sexes (figure 10). At the moult to adult male five setae (shown by red asterisks in figure 10) on segments around the geniculation are transformed into basally fused spines.

The basal segment (I) is expressed from CoI in harpacticoids and it can bear up to three setae (Dahms 1992). In polyarthrans these setae are retained through to the adult but in oligarthrans two are lost at the moult to CoII, so that a single seta at most is present from CoII onwards. The early appearance and subsequent loss of two of these setae is unique to harpacticoids as the full complement of three setae on the basal segment occurs only in calanoids and, then, only in CoIII. The presence of three setae on the basal segment might indicate a derivation from more than one original segment.

(b) ***Generalized pattern of copepod antennulary development***

Comparison between the developmental patterns identified above for six of the ten copepod orders reveals some common features which may be attributable to a shared ancestor, possibly of the Copepoda as a whole. This hypothetical scheme (figure 11) is based primarily on the typical pattern of development found in calanoids (figure

1), modified by separation of segments XXVII and XXVIII throughout the copepodid phase (as in platycopioids, see figure 2) and by separation of segments II and III from CoIV to adult (as in siphonostomatoids, see figure 8, and the misophrioid *Archimisophria discoveryi*).

Huys & Boxshall (1991) referred to all compound antennulary segments as 'fused segments'. Karaytug & Boxshall (1996) showed that compound segments can arise as a result of failure to separate during development, and by secondary fusion of segments that were separately expressed at an earlier stage. This analysis (figures 1–10) shows that most compound segments arise by failure to separate. The two compound segments in adult female *Ridgewayia klausruetzleri* (II–III and XXVII–XXVIII), for example, were never separate during development, neither were segments IX–XII, XXII–XXIIII, XXIV–XXV and XXVI–XXVIII during the development of *Asterocheres*. In the generalized developmental pattern of females (figure 11) there are no hypothesized changes in the segmentation of the distal part of the antennule, from segment XXI to XXVIII, during the copepodid phase.

True fused segments are most often found in adult males, especially in the distal part of the antennules, and they typically appear at the last moult. Segments that were separately expressed in the male CoV often fuse at the last moult. Such fusions are usually, but not exclusively, associated with the development of the neocopepodan geniculation, as some occur in the Platycopioida which primitively lack the geniculation (figure 2). In *Platycopia orientalis*, for example, the fused segments XV–XVI, XVIII–XX, XXI–XXII, XXIII–XXIV and XXV–XXVII were all formed secondarily at the final moult (Ohtsuka & Boxshall 1994). In the Neocopepoda, apart from these secondary fusions associated with the development of the geniculation at sexual maturity, the adult segmentation pattern for the distal array of segments is usually already established by the CoI stage in males, as in females. The harpacticoids and *Asterocheres* are the exceptions here, as subdivision of the distal array of segments is delayed in both sexes, not being complete until the adult stage (figures 8, 10).

The setation of this distal array changes little during ontogeny. In typical female development only three setation elements are added: an anterodistal seta each to segments XXII and XXIV at the moult to CoII, and an anteroproximal seta to segment XXI at the moult to CoIII. These additions were remarkably conservative: if these elements were present in the adult they invariably appeared at these moults, never at any other moult. No other elements were added to the distal array of segments XXI to XXVIII in any of the species studied. The setation of this array, in the females at least, was complete by CoIII. In some orders one or more of these three elements do not appear. In poecilostomatoids, for example, the anterior seta on segment XXIV appears at CoII but those on segments XXI and XXII never appear. In platycopioids all three setae are absent from CoIII to adult and it is predicted here that they will be absent in the as yet unknown CoI and CoII stages of *Platycopia*.

The distal array of segments (XXI to XXVIII) is represented by the distal segment of the 3-segmented naupliar antennule (Oberg 1906), and segments I to V and VI to XX by the first and second naupliar segments, respectively (figure 11). The apical antennulary segment (XXI–XXVIII) in the last nauplius stage of calanoids carries up to 17 setae, four of which are lost at the moult to CoI (Hulsemann 1991*a*). The four lost elements appear to include posterior setae from segments XXII and XXIII and, possibly, two setae from apical segment XXVIII. Other setae from the apical segment (XXVIII) are sometimes lost during the copepodid phase; for example, in *Benthomisophria* an apical seta degenerates during CoI and CoII (figure 3*d*,*e*), and is lost by CoIII. The presence of six setae attributed to the apical segment at the calanoid NVI stage perhaps indicates that the apical segment (XXVIII) is derived from more than one segment and that, as Gurney (1931) speculated, 'the primitive antennule may have consisted of 30 or 31 segments'.

The earliest timing for the appearance of anteroproximal setae on segments XIV to XX is CoIV and, with one exception, the earliest appearance of these setae on the proximal block of segments (II to XIII) is CoV. The exception is the male of *Ambunguipes* (figure 10). In the male of this harpacticoid, but not the female, anteroproximal setae attributed to segments XII and XIII are present at CoIV. The early appearance of the setae on these two segments is interpreted as an accelerated character, perhaps linked to the strong sexual dimorphism expressed relatively early in development in harpacticoids.

Antennulary development shows a distinct distal-to-proximal pattern. The articulations separating the more distal segments are typically expressed earlier than those separating the more proximal ones and this process is complete by CoIV. Similarly, segmental setation is typically complete earlier on more distally located segments than on proximal ones, although the pattern of appearance of anterodistal setae on alternate segments is very marked.

The timing of expression, i.e. the first appearance, of a particular articulation may be accelerated or delayed. Delayed expression relative to the hypothetical scheme proposed in figure 11 is common, especially in those parasitic or benthic free-living copepods that have short antennules with few segments. In these forms many articulations are never expressed, perhaps as a result of having been delayed beyond the terminal moult (post-displacement). The articulation V to VI, for example, is expressed in calanoids, siphonostomatoids and poecilostomatoids in CoI but this articulation is never expressed in *Platycopia* or harpacticoids. It does not appear in *Benthomisophria*, although it is expressed in the isolated CoIV of *Archimisophria*. In cyclopoids, this articulation can be expressed relatively late, in the adult only of *Paracyclops* (Karaytug & Boxshall 1996), for example, but it appears earlier, at CoIV, in the parasitic cyclopoid *Lernaea* L. (Boxshall *et al.* 1997).

(c) *Male geniculations*

Apart from the Poecilostomatoida, all neocopepodan orders have geniculate antennules in the males of at least some representatives that are used for grasping the female during precopulatory and postcopulatory mate guarding, and during mating (Boxshall 1990; Dürbaum 1995). In adult males, articulation XX–XXI is expressed as a specialized geniculation which facilitates grasping the

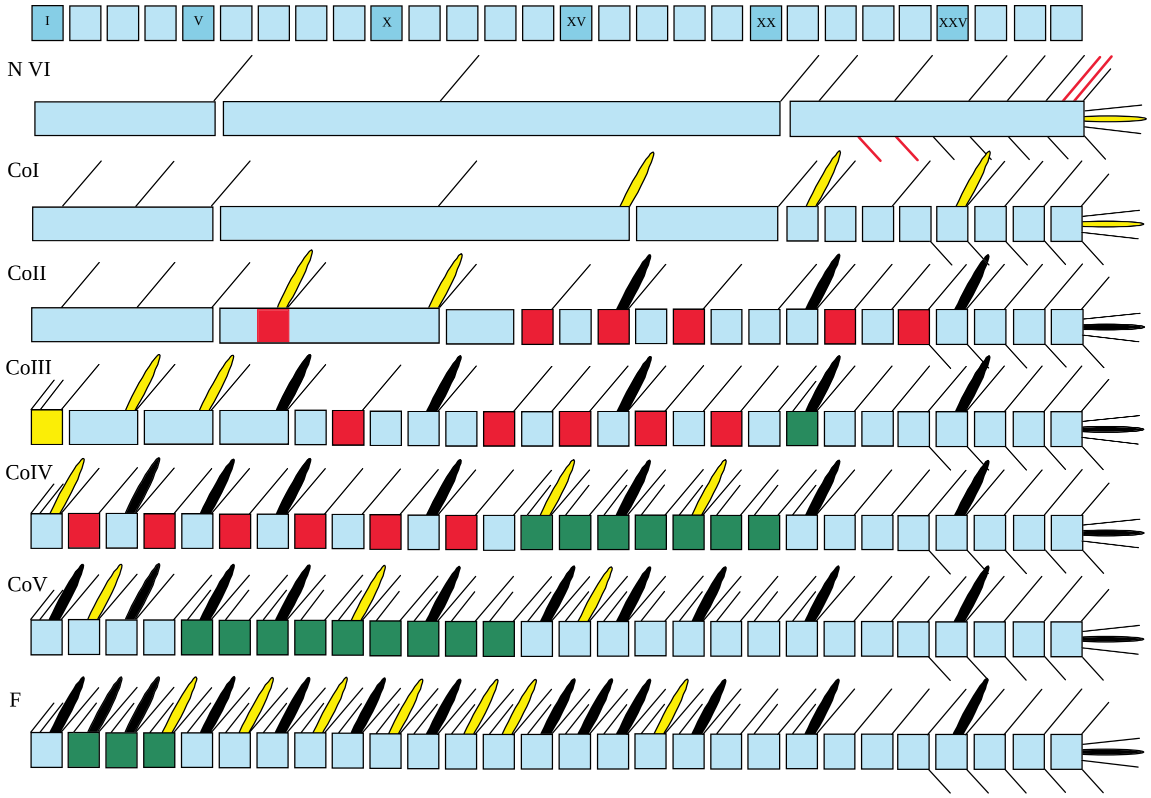

Figure 11. Schematic showing hypothetical ancestral developmental pattern for segmentation and setation of copepod antennules, based primarily on calanoids (figure 1), modified by separation of segments XXVII and XXVIII throughout the copepodid phase as in platycopioids (figure 2) and by separation of segments II and III from CoIV to adult (as in figure 8). See figure 1 legend for interpretation. Naupliar setae indicated in red are lost at the moult from NVI to CoI (Hulsemann 1991*a*,*b*).

body or appendages of the female. It has an area of arthrodial membrane posteriorly and an anteriorly located hinge-line about which adduction of the distal part takes place. Adduction–abduction is effected by opposing muscles which originate proximally in the antennule and insert on the rim of segment XXI. The geniculation differs from the telescopic articulation present in the female at this position in the restriction of flexure to a single plane only, by the presence of a hinge-line, and in the reduction of the extent of arthrodial membrane present. There are typically segmental fusions either side of the geniculation (XIX to XX and XXI to XXII), but these are apparently absent in the geniculate antennules of males of the misophrioids *Speleophriopsis* Jaume & Boxshall and *Huysia* Jaume, Boxshall & Iliffe (Jaume *et al.* 1998). Such fusions can be difficult to assess because in some taxa a suture line separates segments that are functionally fused (i.e. unable to articulate against each other), however, they should be considered as a subsequent addition to the complex of character states that comprises the neocopepodan geniculation.

A further aspect of the neocopepodan geniculation is the modification of setation elements on segments around the geniculation. In adult males with geniculate antennules setae on segments XIX, XX, XXI and XXII are typically modified into basally fused, plate-like or spine-like structures, usually with a pore and often with surface ridges. The modified elements are the distal seta on segment XIX, the proximal seta on XX, and both setae on XXI. The seta on segment XXII is also modified in some groups but it may be reduced in size rather than fully transformed and fused to the segment. These setae are modified in some male calanoids (figure 12), cyclopoids (Karaytug & Boxshall 1996), misophrioids (Jaume *et al.* 1998), harpacticoids (Huys & Boxshall 1991: fig. 2.4.4) and siphonostomatoids (Boxshall & Huys 1994: fig. 9B). All five of these orders, however, also contain representatives in which these setae are unmodified. In calanoids, for example, these setae are unmodified in some species of *Ridgewayia* (Ferrari 1995), but modified in *Epischura* (Humes 1955). These elements are unmodified in male poecilostomatoids which primitively lack geniculate antennules. Modification of these elements typically occurs at the last moult from CoV to adult male. In the cyclopoid *Paracyclops*, for example, these elements are unmodified setae in the male CoV (Karaytug & Boxshall 1996), but in *Epischura* they are already partly modified in the CoV (Humes 1955).

A second geniculation, proximal to the neocopepodan geniculation, is present in the antennules of male misophrioids and cyclopoids, and a vestige of this geniculation remains in gelyelloids (Huys & Boxshall 1991). The proximal geniculation is a modification of the articulation between segments XV and XVI. Segment XV typically carries a large sheath-like, distal extension that partly encloses segment XVI. In male cyclopoids (Karaytug &

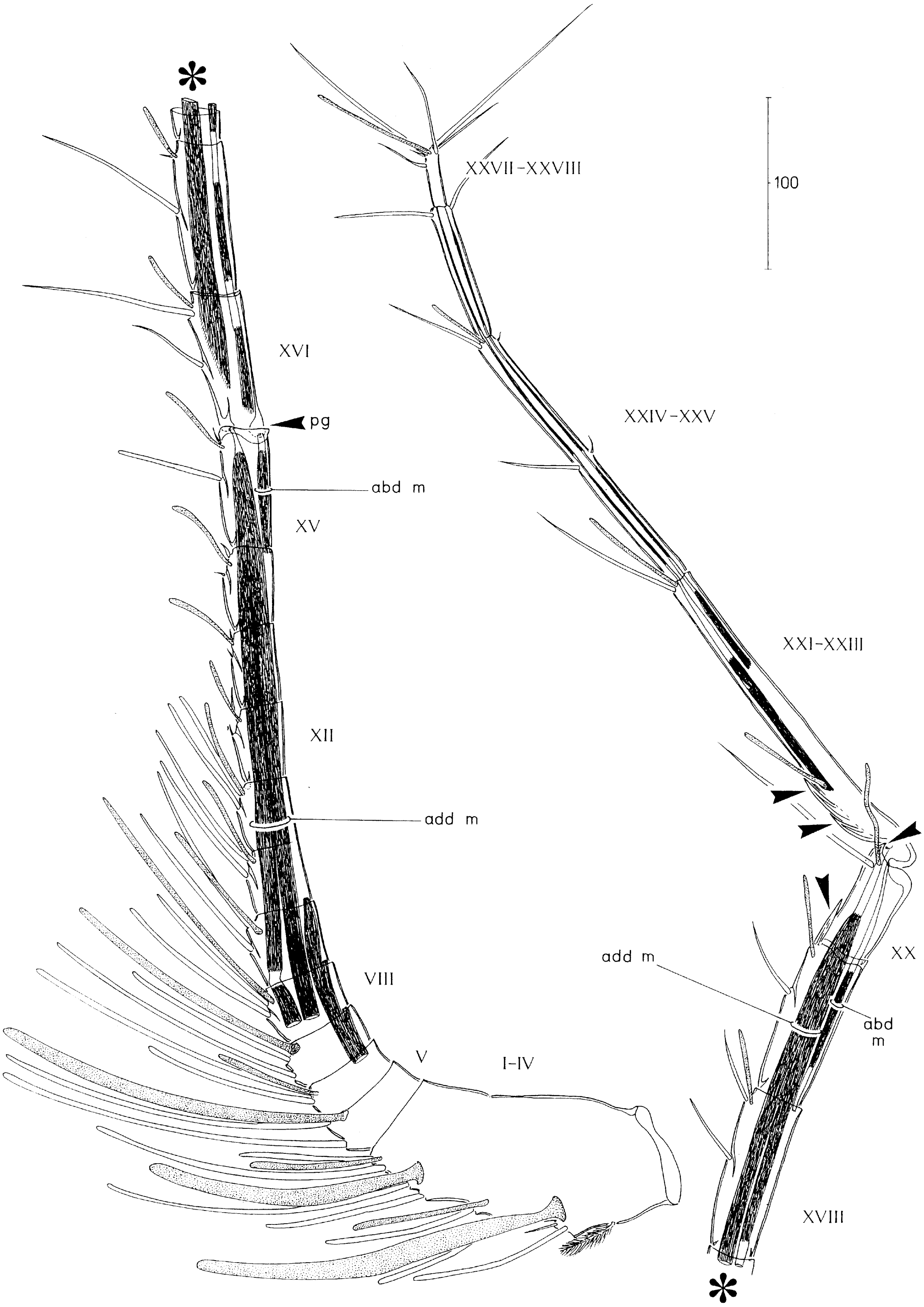

Figure 12. Left antennule of male *Heterorhabdus* sp. showing intrinsic adductor and abductor muscles at both geniculations; drawn in two sections as indicated by asterisk. Roman numerals show ancestral segment numbers; modified setae associated with neocopepodan geniculation indicated by unmarked arrowheads. Scale bar in μm. Abbreviations: add m, adductor muscle; abd m, abductor muscle; pg, proximal geniculation.

Boxshall 1996) the transformations of both articulations into geniculations occurs at the moult from CoV to adult.

A modified proximal articulation is noted here in some males of the calanoid family Heterorhabdidae. No sheath is present but the articulation between segments XV and XVI is modified: there is a strong posterior hinge-line and reduced arthrodial membrane dorsally and ventrally. These features tend to restrict flexure to a single plane and adduction–abduction motions are effected by opposing muscles (figure 12).

The adult male of the plesiomorphic platycopioid *Antrisocopia prehensilis* Fosshagen has symmetrical geniculate antennules. The geniculation occurs in a unique position, between segments XXIV and XXV (Huys & Boxshall 1991), and involves a large moveable spine located on segment XXIV. This is a third kind of geniculation within the Copepoda.

(d) *Enhancement of the chemosensory system in pelagic copepods*

The aesthetascs of copepods have been widely regarded as putative chemosensors, although direct neurophysiological evidence is lacking. They are thin-walled, sometimes with a large apical pore (Boxshall 1982: fig. 3), rather flaccid and innervated by numerous dendrites (Gresty *et al*. 1993). Setae are rigid, sclerotized structures and are typically innervated by one or two dendrites (Gresty *et al*. 1993). Setae are demonstrably mechanosensory in function (Yen & Nicoll 1990; Yen *et al*. 1992) and are typical arthropodan mechanosensors in the presence of scolopale bodies around their ciliary bodies. Bimodal elements with both mechanosensory and chemosensory function have also been reported from copepod antennules (Hull & Laverack 1993) and may be more widely distributed than hitherto realized.

Giesbrecht (1892) noted that calanoid males in some families possess two aesthetascs per segment giving a quadrithek arrangement (2 setae plus 2 aesthetascs) compared with the trithek (2+1) of the female. Quadritheks occur on one or more segments of the male antennules in many families of calanoids, but the number of segments bearing a quadrithek varies greatly between genera within a family and between families within a superfamily (Huys & Boxshall 1991: table 3.16.1). In some members of the Eucalanidae double aesthetascs are present along almost the entire length of the male antennules (to segment XXIV in *Eucalanus attenuatus* (Dana)) (Giesbrecht 1892) whereas in other calanoid families, when present, they are restricted to the proximal part of the antennule. In the Metridinidae and Augaptilidae double aesthetascs are present in males on segments up to and including XIII. In these two families only double aesthetascs can be present on all segments within the block from II to XIII. In all other families that have double aesthetascs in males they are restricted to segments within the range II to XI only. In the Aetideidae double aesthetascs may be found on each segment from III to IX and on XI (Giesbrecht 1892), but in most clausocalanoidean families they are more often present on odd-numbered segments III, V, VII, IX and XI, than on even-numbered segments. The concentration of extra aesthetascs in the proximal section of the male antennule is marked in most families.

Rarely quadritheks occur on the antennulary segments of females of the family Calanidae (Fleminger 1985). Fleminger interpreted the quadrithek-bearing females as the product of sex change by the larger-sized late juvenile potential males. They are not further considered here.

Analysis of the distribution of quadritheks in male calanoids revealed a clear phylogenetic pattern (G. A. Boxshall & R. Huys, unpublished observations). They are absent from members of the two most plesiomorphic superfamilies: the Pseudocyclopoidea and the Epacteriscoidea. Quadritheks on male antennules occur in the Arietellidae (but only in *Arietellus aculeatus* (Scott)) (Ohtsuka *et al*. 1994) but are common in genera of some families within the superfamily Arietelloidea, including particularly the Metridinidae and Augaptilidae (Giesbrecht 1892), and absent from others, such as the Heterorhabdidae. Families within the large superfamily Centropagoidea lack sexually dimorphic quadritheks. Some species in the Candaciidae, such as *Candacia varicans* are exceptional in having two aesthetascs on segment VII but they are present in both sexes. Quadritheks are present in at least some members of all other valid calanoid superfamilies except in the poorly known Ryocalanoidea. (The recently established calanoid superfamily Fosshagenioidea (Suárez-Morales & Iliffe 1996) is not accepted as valid by us.)

Enlargement of aesthetascs, rather than an increase in aesthetasc numbers, can also be observed in male pelagic copepods. The planktonic siphonostomatoid *Pontoeciella abyssicola* carries just two aesthetascs in the adult female (figure 7*c*,*e*), presumably representing those from ancestral segments XXI and XXVIII. The male carries one additional aesthetasc located proximally (arrowed in figure 7*d*), but the homologue of the proximal aesthetasc of the female (XXI) is grossly enlarged. In the female this aesthetasc is 142 µm long and lies parallel to, but free from, the compound apical segment, whereas in the male it is 1118 µm long and fused to the elongate compound apical segment for most of its length. This pair of aesthetascs spans more than 2.2 mm (figure 7*f*) and provides an enormous increase in area compared with that of the female. The antennulary setae of male *P. abyssicola* are mostly shorter than those of the female, but this is probably because of the non-feeding status of the adult male, which has an atrophied oral cone and mouthparts (Boxshall 1979). Reductions in antennulary setal size have previously been reported in other pelagic copepods with non-feeding males (Boxshall *et al*. 1998).

Comparison of antennules from a variety of pelagic copepods has revealed many examples of the apparent transformation of setation elements. In males of various calanoids, including heterorhabdids such as *Heterorhabdus* and *Disseta*, eucalanids such as *Rhincalanus gigas*, and pelagic poecilostomatoids, such as *Pachos*, setae have undergone transformation into thin-walled aesthetasc-like elements. In *Heterorhabdus* males, for example (figure 12), most of the setae on the proximal segments (homologous with ancestral segments I to XI) have a more rigid basal part and a thin-walled, distal part with a rounded apex. The true aesthetascs are readily distinguishable from these transformed elements. The transformed elements may originally have been bimodal.

Giesbrecht (1892) described the antennules of *Pachos* males as armed with numerous additional aesthetascs on proximal segments. The antennule of male *Pachos punctatum* (figure 13*c*) carries many relatively thin-walled, mid-length elements which have rounded tips and resemble aesthetascs. They are here interpreted as transformed setae because the typical aesthetascs are clearly identifiable on segments 2, 4, 5, 6 and 7. In female *Pachos* fewer elements are present on the proximal segments but all are seta-like, i.e. rigid with pointed tips. In the male unmodified setae are retained only on the distal segments. In the planktonic poecilostomatoid family Lubbockiidae one setal element on the second antennulary segment is aesthetasc-like in female *Lubbockia* Claus whereas it is an unmodified seta in all other genera (Huys & Böttger-Schnack 1997).

In the planktonic harpacticoid *Clytemnestra rostrata* the chemosensory system is enhanced in both sexes by transformation of setae into aesthetasc-like elements. The adult female carries aesthetascs only on segments 4 and 6 but each aesthetasc is accompanied by a transformed seta to which it is fused at the base (figure 13*a*). The transformed seta has a minutely spiniform tip. A similarly transformed seta is present on segment 3. The male antennule (figure 13*b*) is similarly provided with transformed elements but carries an additional aesthetasc on segment 3. This extra aesthetasc is the homologue of the additional male aesthetasc of *A. rufocincta* which appears on segment XI at the final moult (figure 10).

4. DISCUSSION

(a) *Ontogenetic patterns*

The general model of copepod antennulary development proposed here, after comparison of ontogeny across six orders, is largely based on inference, using the positions of particular setation elements along the antennule as reference points from one moult to the next and working backwards from the adults in which segmental homologies are more readily identifiable. The adult female segmentation distal to the XX to XXI articulation is usually fully expressed in CoI. However, there are exceptions, especially in non-planktonic groups such as the parasitic Siphonostomatoida, in which the adult distal segmentation pattern is established later in development. Changes in distal segmentation through the copepodid phase are relatively rare in females. In males, changes in the distal part from CoI to CoV are similarly rare except for the segmental fusions that commonly appear around the geniculation at the final moult from CoV.

The highly conservative pattern of appearance of setation elements along the antennule is also remarkable, especially in the distal array of segments from XXI to the tip. A maximum of three setae is added to this distal array throughout the copepodid phase: the anterodistal seta on segment XXII, that on segment XXIV, and the anteroproximal seta on XXI. This underlying conservatism of the distal array of setae and segments through ontogeny indicates a requirement for functional continuity in these sensory elements. Electrophysiological studies by Yen *et al.* (1992) of the antennulary setae in five calanoid genera representing four different families demonstrated that they are mechanosensors. More specifically, ablation of the distal tip of the antennule in *Pleuromamma* and *Euchaeta* deprives the copepod of its rapid escape response to mechanical signals (Lenz & Yen 1993). It is likely that the distal array of setae is involved in detecting approaching predators, forming the main component of the mechanosensory early-warning system of all copepods.

As soon as the copepod enters the water column it represents a potential prey item and the possession of a functional approaching-predator warning system would be highly advantageous. The setae of the distal array are already present in late nauplii of planktonic calanoids (Hulsemann 1991*a*) as well as throughout the copepodid phase. Clearly this mechanosensory system is operational very early in ontogeny and functional continuity is maintained throughout the copepodid phase. The twin giant fibres present in the ventral nerve cord of copepods (Park 1966) mediate the escape response to the musculature of the swimming legs and we speculate here that they should be closely connected, probably within the protocerebrum, with the axons innervating the distal array of the antennules.

The functional constraints on the antennule ensure that it must maintain its capacity to detect food, approaching predators and potential mates. The balance of priorities between these three roles during development affects the configuration of the sensory array. The mate-detection role is performed by the adult male, therefore it is to be expected that the mate-detection system develops relatively late but is fully functional by the adult stage.

The food-detection system is required by all feeding stages, but adult males of many pelagic copepods have adopted a non-feeding strategy. In the non-feeding males of *Euchaeta rimana* Bradford it is noteworthy that setae along the length of the antennule are reduced in size. The reduction of the proximal setae associated with prey location in feeding stages (female and male CoV) is correlated with the non-feeding status. Males no longer detect prey and shed the prey-detection system (Boxshall *et al.* 1998). The associated reduction in size of the setae of the distal array in adult males indicates that predator escape responses may be compromised as the balance of the male sensory system shifts towards mate-location behaviour. Similarly the non-feeding males of *Pontoeciella* (order Siphonostomatoida), and the non-feeding male in the order Mormonilloida (Huys *et al.* 1992), also have reduced antennulary setae. An interesting parallel is that members of the poecilostomatoid family Sapphirinidae are visual predators but in one genus, *Copilia* Dana, the males are non-feeding and these lack elaborate naupliar eyes, their primary prey-location system. In parasitic copepods with a planktonic infective stage the long-range food (equals host) location system may be functional only for a limited time, until a host is found, although mobile parasites that change hosts must retain a functional food-particle (equals host) detection system. The pressure on maintaining a functional approaching-predator detection system is common to all stages inhabiting the water column, hence the stability in the distal array in both sexes and all copepodid stages. The dynamics of these changing role priorities may be reflected both in the segmentation and the setation of the antennules in both sexes during development and the differing roles of the sexes are also reflected in the sexual dimorphism, particularly of the adults.

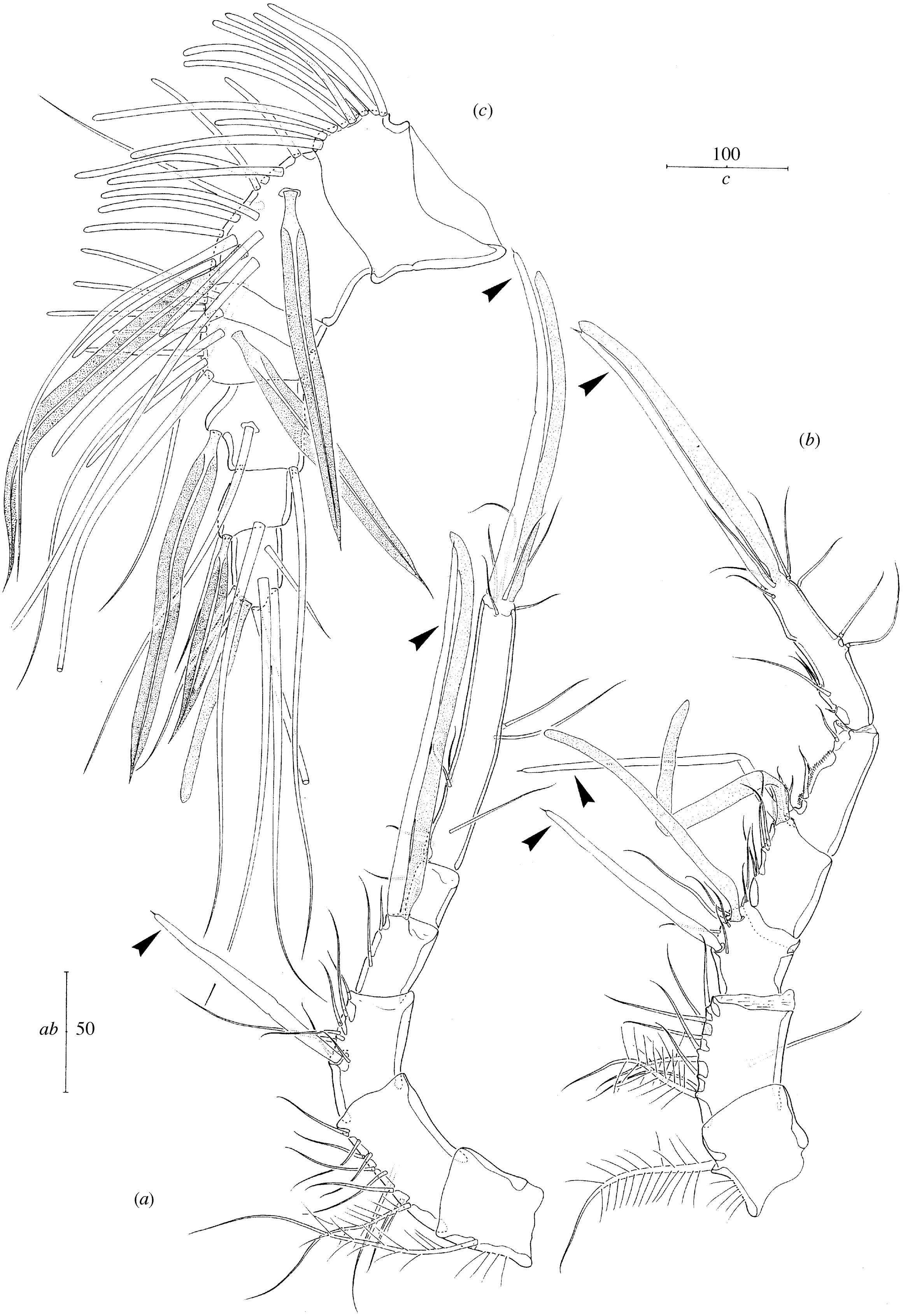

Figure 13. (*a*) Antennules of adult female *Clytemnestra rostrata*; (*b*) adult male; (*c*) antennule of adult male *Pachos punctatum*. Scale bars in µm. Arrows indicate modified setae in *Clytemnestra*.

The ontogenetic analyses suggest that it is the timing of expression of intersegmental articulations during development that may in future provide the most informative characters for phylogenetic study, rather than either segment numbers or the patterns of fused or undivided segments. Simple comparisons in the segmentation patterns of antennules in adults may conceal differences of ontogenetic pathways, similar to those noted for copepod swimming-legs by Ferrari (1988). The appearance of segment I at CoI, two moults earlier than in calanoids, is a feature unique to the polyarthran and oligarthran harpacticoids and its three setae appear at the same moult (CoI), also accelerated by two moults compared with calanoids. This constitutes an autapomorphy of the Harpacticoida and provides further evidence of accelerated development in this order.

Antennulary development follows a distal-to-proximal pattern. Articulations separating the more distal segments are typically expressed earlier than those separating the more proximal ones and segmental setation is typically complete earlier on more distally located segments than on proximal ones. Several genes, such as *Distal-less* (*Dll*), *Dachshund* (*Dac*), *Decapentaplegic* and *Wingless* have been identified that organize the proximal–distal axis within arthropod limbs (Cohen *et al*. 1989; Mardon *et al*. 1994; Lecult & Cohen 1997). The protein products of such genes may be expressed in discrete or overlapping domains along the proximal–distal axis of the limb. Copepod antennules with 28 basic segments are considerably more complex than the 9-segmented *Drosophila* legs and might offer a high resolution model for the study of the genetic mechanisms controlling the precise spatial domains of *Dac* and *Dll* expression in arthropods.

(b) *Geniculations*

Male antennules are typically specialized for grasping the female during mating, in having the articulation between segments XX and XXI forming a geniculation with associated secondary segmental fusions and setal modifications. Alternative expressions of this character exist within the copepods. Primitively the geniculate antennules are symmetrical but calanoids typically have a geniculation on one side only, either left or right according to the family. Very rarely there is asymmetry within a genus or species, with some males left-handed and others right-handed (Ferrari 1984). Many calanoid families, the clausocalanoidean families for example, have secondarily lost the neocopepodan geniculation and their males possess relatively unmodified antennules, similar to those of the female.

The presence of a proximal geniculation in calanoids is reported here for the first time. A proximal geniculation between segments XV and XVI was previously known only in the misophrioid–cyclopoid–gelyelloid lineage (Huys & Boxshall 1991). In cyclopoids, the double geniculation allows the male to firmly grasp the rami of the fourth swimming-legs of the female with both antennules during spermatophore transfer. The presence of a geniculation in precisely the same position in a heterorhabdid, a representative of a relatively plesiomorphic calanoid superfamily (Andronov 1974), may indicate that the proximal geniculation was an ancestral neocopepodan character rather than a novel acquisition in the misophrioid–cyclopoid–gelyelloid lineage. The elaborate nature of the proximal geniculation, in particular the presence of the sheath, remains an apomorphy of this lineage.

The proximal geniculation appears to be evolutionarily labile. Each of the large orders in which this geniculation is present also contains numerous forms without it. For example, it is absent in *Benthomisophria* (figure 4*c*) but present in the closely related genus *Misophria* Boeck. When the proximal geniculation has been secondarily lost segments XV and XVI are often fused and the original segmentation may be marked with a partial suture line, as in *Benthomisophria* and the parasitic cyclopoid *Lernaea* (Boxshall *et al*. 1997). However, the same orders contain forms which lack the proximal geniculation but have segments XV and XVI separately expressed in the male.

(c) *Enhancement of the chemosensory system*

The most marked change in antennulary setation of male calanoids is the acquisition of additional aesthetascs at the moult from CoV. The addition of aesthetascs at this moult, as expressed in male eucalanids, is more or less equivalent to a doubling of the aesthetascs present in the female. Virtually every segment with a single aesthetasc in the female has two aesthetascs in the adult male, as in *Eucalanus attentuatus* (Giesbrecht 1892). In many families this phenomenon of aesthetasc doubling is expressed in a secondarily reduced form (Giesbrecht 1892; Huys & Boxshall 1991: table 3.16.1; Boxshall *et al*. 1998). In the Euchaetidae, for example, double aesthetascs are present only on a few proximal segments (II and III in *Euchaeta rimana*), and these segments lack aesthetascs in the female. In adult males of *Euchaeta* aesthetascs, not present in the female, appear on segments 1 (I), 2 (IV) to 4 (VI), 6 (VIII) and 7 (IX), 8 (XII) and 9 (XIII), 11 (XV), 13 (XVII) to 16 (XX), 19 (XXIII) and 20 (XXIV) at the final moult.

The addition of aesthetascs at the last moult in males is a widespread phenomenon among pelagic calanoid copepods. The distribution of double aesthetascs in the Calanoida suggests that they have arisen at least three times independently. The plesiomorphic superfamilies Pseudocyclopoidea and Epacteriscoidea have only single aesthetascs in males. Members of these families are typically hyperbenthic. Within the superfamily Arietelloidea double aesthetascs on male antennules are present in the pelagic families Metridinidae and Augaptilidae. In the Arietellidae they are also present in *Arietellus* but not in the more plesiomorphic genera that inhabit the hyperbenthic zone (Ohtsuka *et al*. 1994). They are absent in other families such as the Heterorhabdidae, Lucicutiidae, Discoidae and Hyperbionychidae. The last of these families is hyperbenthic rather than pelagic. Double aesthetascs have arisen once, probably twice, within the Arietelloidea. The Centropagoidea is a large superfamily comprising mainly families that inhabit fresh waters, such as the Diaptomidae, or estuarine and coastal waters, such as the Acartiidae, Centropagidae, Pontellidae, Pseudodiaptomidae, Sulcanidae, Temoridae and Tortanidae. None of these families has double aesthetascs on male antennules. Within the superfamily double aesthetascs appear only in a few species of Candaciidae, a family of the open-pelagic water column. However, double aesthetascs are present in both sexes, suggesting an independent origin of the character in this family. Males of the remaining superfamilies, Megacalanoidea, Bathypontioidea, Eucalanoidea, Spinocalanoidea and Clausocalanoidea all have double aesthetascs in at least some representatives. Members of these superfamilies are predominantly inhabitants of the oceanic pelagic zooplankton. Double aesthetascs were presumably present in the common ancestor of the lineage comprising all these higher calanoid superfamilies (cf. Park 1986: fig. 1), although their presence in the superfamily Ryocalanoidea cannot yet be confirmed.

The independent appearance of sexually dimorphic double aesthetascs at least two and probably three times within the Calanoida, provides supporting evidence for Huys & Boxshall's (1991) speculation that 'the evolutionary doubling of aesthetascs on the segments of the male antennules is correlated with the colonization of the open-pelagic environment where chemical cues are of paramount importance'. Doubling of aesthetascs took place within the Arietelloidea in taxa that have colonized the open-pelagic zone. They are lacking within the large superfamily Centropagoidea which is largely neritic. Only the Candaciidae has successfully colonized the open-pelagic zone and only in this family do any species have double aesthetascs, but they are present in both sexes. Finally, the colonization of the open pelagic by the main lineage of the Calanoida appears to be coincident with the acquisition of double aesthetascs.

Calanoid families that dominate in the more turbulent, coastal waters, such as the Acartiidae and Temoridae, lack additional aesthetascs in males but chemosensory mate-detection is highly effective in these families, as shown by Doall *et al.* (this volume) for *Temora longicornis* (Müller). An increase in number of aesthetascs presumably provides a greater surface area for intercepting water-borne signal molecules and may permit the detection of pheromones at lower concentrations. This may be equivalent to allowing the male to detect older chemical tracks laid down by females. Pheromonal signals probably persist (i.e. retain their directional information component) longer in less turbulent waters and it seems likely that enhanced sensitivity to chemical signals could increase mate-location opportunities for copepods in open-pelagic waters compared with those of coastal waters where turbulence may destroy the directional component of the chemical signal faster.

An alternative strategy for enhancing the surface area of chemosensors is to enlarge existing aesthetascs rather than acquire additional aesthetascs. Such a strategy is found in the *Pontoeciella abyssicola*, the only member of the family Pontoeciellidae, which is one of only two families of siphonostomatoids that are truly planktonic. The enhancement of the chemosensory system in the non-feeding male of this species also supports the hypothesis that mate location by male copepods in the open pelagic is mediated by chemical signals.

Evolutionary changes in the setation patterns of all copepod appendages, including the antennules, appear to proceed predominantly by reductions and losses (Huys & Boxshall 1991). Exceptions to this empirical oligomerization principle are rare, with very few known examples of secondary gains in setal number from the presumed ancestral condition. However, our studies indicate that aesthetascs are an exception. They are much more labile than setae, being gained and/or lost along an evolutionary lineage with relative ease. Sexually dimorphic aesthetascs have been gained separately by three lineages within the Calanoida, but also by lineages within other orders. For example, the siphonostomatoid *Cancerilla*, a member of a highly derived family, the Cancerillidae, has rows of extra aesthetascs on several segments of the male antennules (Carton 1968) and the adult male of the parasitic cyclopoid *Haplostoma* Canu has paired rosettes of 120 or more additional aesthetascs located proximally on the antennules (Ooishi & Illg 1977). Caution should be exercised in the use of aesthetascs as characters in phylogenetic analysis although those that appear in the earliest stages (particularly CoI and CoII) appear to be relatively less labile.

Other, novel chemosensory systems have arisen independently in males of various copepod lineages, including the cephalosome flap organ of the planktonic genus *Oithona* Baird (Nishida 1986) and the cephalic pleural organ of the parasitic cyclopoid *Pachypygus gibber* (Thorell) (Hipeau-Jacquotte 1986). The appearance of such systems in adult male copepods testifies to the importance of chemical signals in the mating behaviour of the Copepoda as a whole.

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