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# Female genital structures in several families of Centropagoidea (Copepoda: Calanoida)

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The female genital structures of 21 calanoid species belonging to the families Candaciidae, Centropagidae, Pontellidae, Sulcanidae, Temoridae and Tortanidae were studied using light and electron microscopy (scanning electron microscopy, transmission electron microscopy). Except the monotypic Sulcanidae, their organization conforms to a common pattern characterized by egg-laying ducts opening through paired adjacent gonopores into a small cavity, the genital atrium, covered by a genital operculum and opening to the outside through a distal atrial slit. No seminal receptacle is present and the seminal products must be stored for a brief period in the genital atrium.

The genital structures occupy a well delimited genital area, the main changes in which concern their position on the ventral face of the genital double-somite, and operculum morphology. Their organization and possible mode of operation are compared with those of other calanoid families. A possible correlation between absence of seminal receptacles and mating behaviour is discussed.

**Keywords:** Calanoida; female genitalia; morphology; reproductive biology

## 1. INTRODUCTION

In calanoid copepods, the female genital structures located in the genital double-somite are well-known only in the families Arietellidae (Ohtsuka *et al.* 1994) and Metridinidae (Cuoc *et al.* 1998), and in two species of the Diaptomidae, *Hemidiaptomus ingens* and *Mixodiaptomus kupelwieseri* (Cuoc *et al.* 1989*b*). Anatomical data on other families are incomplete or lack precision (Steuer 1923; Lowe 1935; Marshall & Orr 1955; Blades 1977; Hammer 1978; Blades-Eckelbarger & Youngbluth 1979; Vaupel-Klein 1982; Blades-Eckelbarger 1991), except in *Epilabidocera amphitrites* (Park 1966) and in the genus *Paraeuchaeta* (Geptner 1968), but even these data allow only limited functional interpretation.

The families Arietellidae and Metridinidae belong to the superfamily Arietelloidea which contains six other families (Augaptilidae, Discoidae, Heterorhabdidae, Lucicutiidae, Phyllopodidae and Hyperbionychidae). In both families, the genitalia have a basically paired organization, with separate copulatory pores and gonopores which correspond respectively to insemination openings of the two seminal receptacles and to the apertures of the two egg-laying ducts. These cuticular ducts, different from the membranous oviducts, are connected to the shell ducts coming from glands situated in the prosome. Many variations from this paired pattern have been observed, within this superfamily. Nevertheless, in all representatives, the genital area is open, meaning that the genital apertures, particularly the gonopores, are directly in contact with the exterior.

In this context it is interesting to study female genitalia to: (i) improve knowledge of the reproductive morphology and processes; and (ii) identify different evolutionary trends in these systems to enhance our understanding of phylogeny in the Calanoida.

This study is focused on the superfamily Centropagoidea, which comprises ten families, the representatives of which have colonized all aquatic biotopes, from marine to brackish and freshwater. A total of six are examined here: Pontellidae, Centropagidae, Temoridae, Candaciidae, Sulcanidae and Tortanidae. Acartiidae, Parapontellidae, Pseudodiaptomidae and the large freshwater family Diaptomidae will be the subject of separate works.

## 2. MATERIAL AND METHODS

### (a) *Material*

Material of the 21 species examined came from four institutions: Université de Provence, Laboratoire de Biologie Animale, Plancton, Marseille (UP), Muséum National d'Histoire Naturelle, Paris (MNHN), the Natural History Museum, London (NHM), and US National Museum of Natural History, Washington (USNM).

(i) *Candaciidae*

*Candacia ethiopica* (Dana, 1849), UP, Coast of Tripolitaine, Mediterranean Sea.
*C. armata* (Boeck, 1872), UP, Gulf of Marseille, Mediterranean Sea.

*C. bispinosa* (Claus, 1863), UP, Coast of Tripolitaine, Mediterranean Sea.
*C. simplex* (Giesbrecht, 1889), UP, Mediterranean Sea.

(ii) *Centropagidae*
*Boeckella bergi* Richard, 1897, USNM, no. 92968, Argentina.
*B. dentifera* Brehm, 1935, USNM, no. 79545, Argentina.
*B. poppei* (Mrazek, 1901), NHML, no.259–268.
*Centropages typicus* Kröyer, 1849, UP, Gulf of Marseille, Mediterranean Sea.
*Isias clavipes* Boeck, 1864, MNHN, Razouls collection.
*I. tropica* Sewell, 1924, USNM, Thailand.
*Limnocalanus johanseni* Marsh 1920, USNM, no. 204788, M. S. Wilson, Alaska, Skimo Lake.
*Osphranticum labronectum* Forbes, 1882, USNM, no.204798, Louisiana, St Landry Parish.

(iii) *Pontellidae*
*Anomalocera patersoni* Templeton, 1837, UP, Gulf of Marseille, Mediterranean Sea.
*Labidocera pavo* Giesbrecht, 1889, MNHN, Pacific, Razouls collection.
*L. wollastoni* (Lubbock, 1857), UP, Gulf of Marseille, Mediterranean Sea.
*Pontella mediterranea* (Claus, 1863), MNHN, Surroundings of Banyuls, Mediterranean Sea, Razouls collection.

(iv) *Sulcanidae*
*Sulcanus conflictus* Nicholls, 1945, NHML, Swan River estuary, West Australia.

(v) *Temoridae*
*Eurytemora velox* (Lilljeborg, 1853), UP, Camargue, France.
*Heterocope saliens* (Lilljeborg, 1862), NHML, no. 83–103.
*Temora stylifera* (Dana, 1849), UP, Gulf of Marseille, Mediterranean Sea.

(vi) *Tortanidae*
*Tortanus discaudatus* (I. C. Thompson & Scott, 1897), NHML, no.158–162, Vancouver, Is Canada.

### (b) *Methods*

(i) *Scanning electron microscopy (SEM)*

All species were prepared as described by Cuoc *et al.* (1997) for Metridinidae. For external morphology (figure 1), females were rinsed in distilled water, treated with 1–4% sodium hypochlorite to eliminate organic matter on the surface of the genital double-somite, rinsed again, dehydrated in acetone and critical-point dried. For internal morphology, a dorsal cut (as wide as possible) was made into the genital double-somite (figure 1). The urosome was isolated and treated with 1 to 4% sodium hypochlorite to eliminate all the soft parts, other than the muscles associated with the genital area. After rinsing in distilled water, samples were stained in an aqueous solution of chlorazol black, then dried as above. Samples were mounted on a stub and coated with gold or gold–palladium. Observations were done using a JEOL JSM 35C or a Hitachi 5 570 scanning electron microscope.

(ii) *Transmission electron microscopy (TEM)*

Internal morphology of genital structures was observed in *Centropages typicus* and *Temora stylifera*. Specimens collected in the Gulf of Marseille were prepared using methods previously described (Arnaud *et al.* 1988). Transverse thin sections of the genital double-somite were made by using glass knives, stained with an LKB staining device (Ultrastainer 2168) and observed in a Jeol 100C electron microscope.

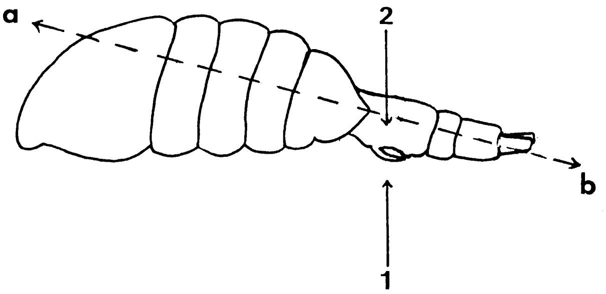


Figure 1. Schematic representation of a female calanoid showing the orientations of SEM observations. (*a*) External ventral view; (*b*) internal dorsal view, after removal of the dorsolateral cuticle (above plane a–b) and of the soft tissues inside the double-somite.

(iii) *Light microscopy (LM)*

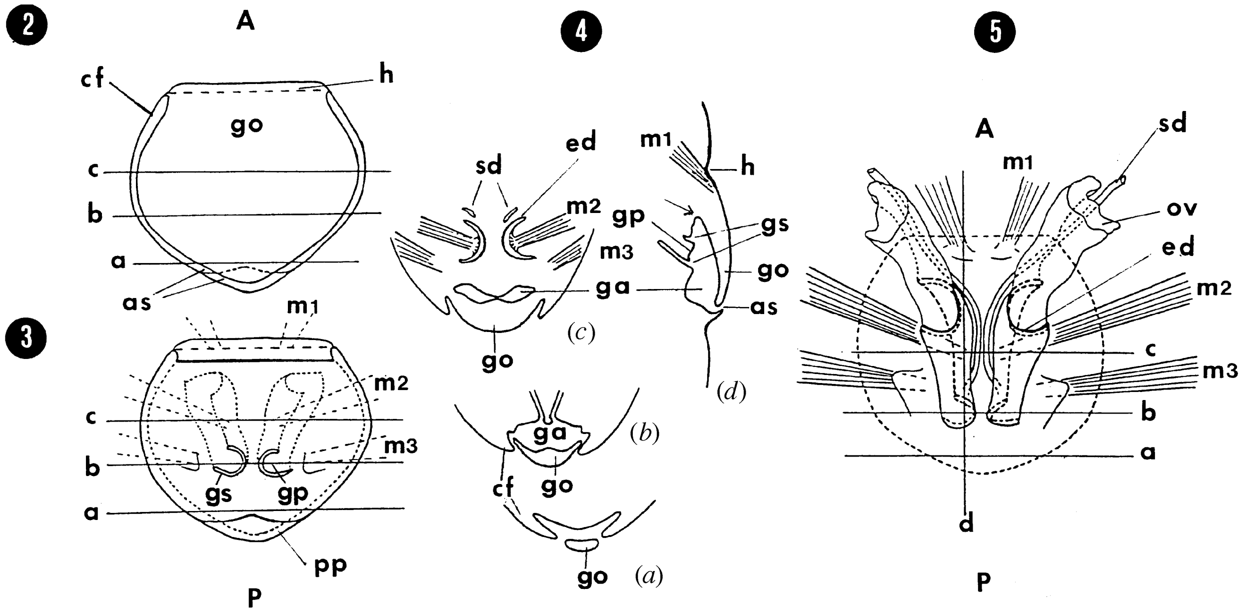
A total of nine species (*Candacia armata*, *C. ethiopica*, *C. simplex*, *Centropages typicus*, *Labidocera pavo*, *Pontella mediterranea*, *Sulcanus conflictus*, *Temora stylifera* and *Tortanus discaudatus*) were observed. Specimens were dehydrated and embedded in Epon. Semi-thin sections of the genital double-somite were cut using an LKB ultramicrotome and stained with Unna Blue.

## 3. RESULTS

### (a) *General pattern of female genital area*

In all species, the genital area is located on the ventral face of the genital double-somite and presents a similar pattern in its organization. In external view (figures 2–4), the area, rounded to ovoid in shape, is delimited by a cuticular fold (cf) and covered by a cuticular flap, the genital operculum (go), articulated by a hinge (h) along the anterior margin. This operculum is free posteriorly and covers a small cavity, the genital atrium (ga), which communicates externally via the atrial slit (as). In the dorsal roof of this cavity, two crescent-shaped slits, opposed by their convex part, are visible. These gonoporal slits (gs) delimit two cuticular plates: the gonoporal plates (gp). On both sides, the surface of the cuticle is more or less undulating symmetrically. The cuticular fold, bulging into a more or less prominent pad (pp), defines the posterior margin of the genital area.

In dorsal internal view (figure 5), in the median part of the area, two egg-laying ducts (ed) are present, each forming a crescent-shaped structure of cuticular origin, resulting from folding into a flattened tube, the double wall of which delimits a narrow space corresponding, in external view, to the gonoporal slit. Proximally, these cuticular ducts are connected to the thin oviductary epithelium (ov). There are two shell ducts (sd), arising from glands located laterally in the last prosomal somite, which pass adjacent to the convex surface of the egg-laying ducts before fusing with them more



Figures 2–5. Schematic organization of the genital area. Figure 2. External ventral view showing genital operculum. Figure 3. External ventral view after elimination of genital operculum. Figure 4. Transverse sections (*a*, *b*, *c*) and longitudinal section (*d*) corresponding to the planes a, b, c and d indicated on figures 2, 3 and 5. In figure 4*d*, note angle between internal face of operculum and atrium roof located posterior to hinge (arrow). Figure 5. Internal dorsal view. Abbreviations as follows: A, anterior; as, atrial slit; cf, cuticular fold; ed, egg-laying duct; ga, genital atrium; go, genital operculum; gp, gonoporal plate; gs, gonoporal slit; h, hinge; m1, muscle of the genital operculum; m2, muscle of the egg-laying duct; m3, muscle of the genital atrium; ov, oviduct; P, posterior; pp, posterior pad; sd, shell duct.

distally. Each egg-laying duct has a strong muscle (m2) inserted in its concave wall that originates latero-dorsally in the body. There are two other muscle pairs that insert at the level of the hinge of the genital operculum (ml) and laterally in the distal part of the atrium (m3). There is no special structure in the genital area for the storage of the seminal products (sp). This function is performed by the genital atrium which fulfils the role of seminal receptacle. The atrium is empty in non-inseminated females whereas, in inseminated females, it is filled with seminal products enveloped by a protective layer of amorphous material. Variations on this basic pattern have been observed particularly in the Candaciidae and Sulcanidae.

### (b) *The genital area in Pontellidae, Temoridae, Tortanidae and Centropagidae*

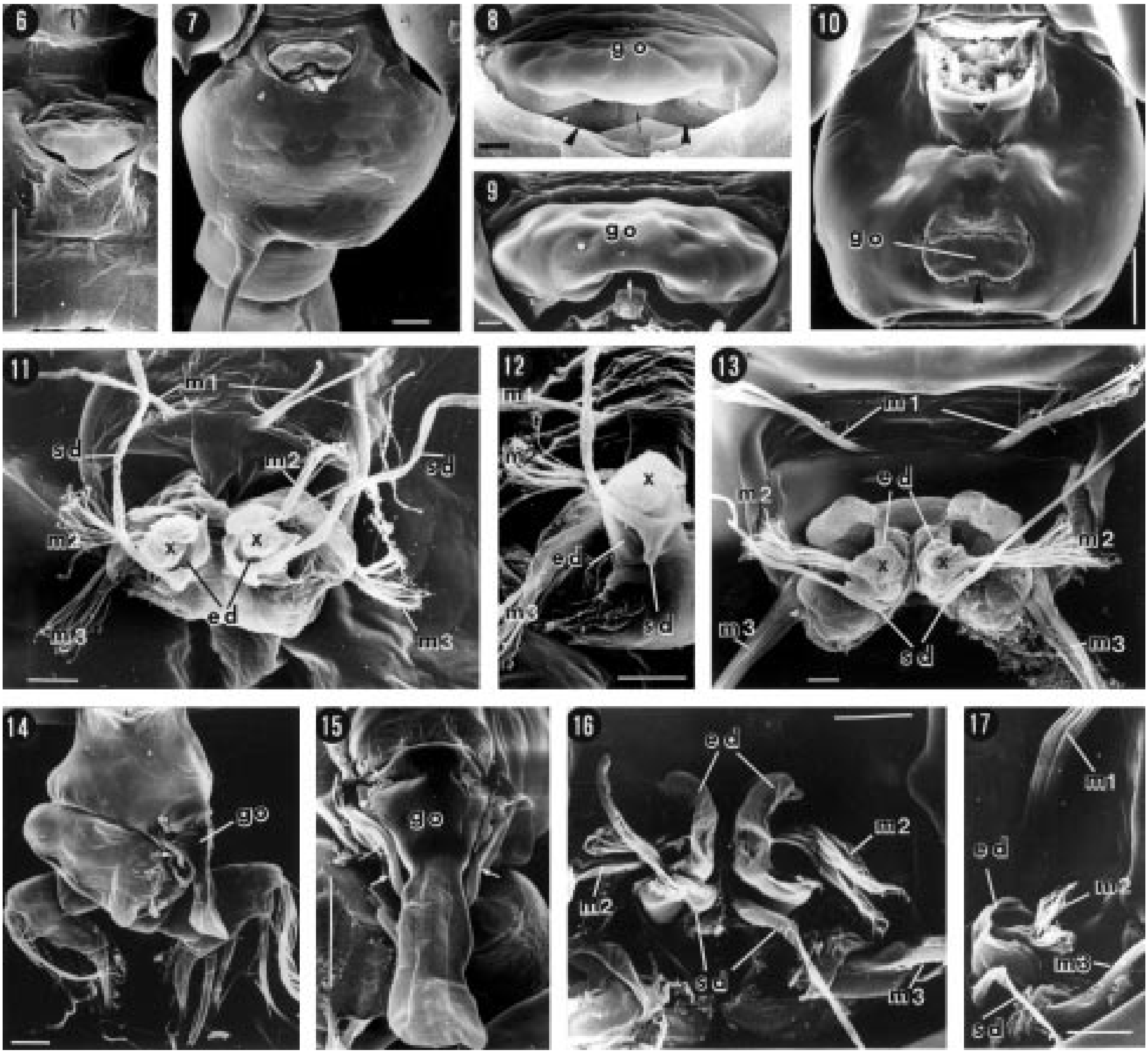
#### (i) *Pontellidae: description*

External genital area. The genital area is located medio-ventrally, in the anterior part of the double-somite, in *Pontella mediterranea* (figures 6 and 8) and *Anomalocera patersoni* (figures 7 and 9), posteriorly in *Labidocera wollastoni* (figure 10). In the first two species, it is located in an inclined plane compared with the antero-posterior axis of the somite, because of the strongly bulging ventral face in this part. It is completely covered by a genital operculum which is oval in *Pontella mediterranea* and *Anomalocera patersoni,* but subrectangular and bilobed in *Labidocera wollastoni*. In *Labidocera pavo* (figures 14–15), the genital area is displaced to the left posterior margin of the double-somite. It resembles a duck-bill, the superior part being the genital operculum, extending posteriorly in a spatula-like form and lying, proximally, on the concave distal part of the genital area.

Internal genital area. In all four species, internal structure conforms to the general pattern described above (figures 11–13, 16 and 17). The long shell ducts coming from the prosomal glands are dorsal to the egg-laying ducts. The latter are short, not exceeding 10 μm in *Pontella mediterranea.* In this species, the genital atrium is open to the exterior only at its most posterior part, within a short distance the edges of the genital operculum fuse with the cuticular folds delimiting the genital area. The genital atrium tends to be elongated anteriorly into paired pockets, on either side of the egg-laying ducts (figures 4 and 55). The atrial muscles insert at the level of these pockets. Sections in *Labidocera pavo* show a similar morphology of the genital atrium, which was full of seminal products in both examined females. The muscles of the atrium, egg-laying ducts and operculum all originate on the latero-dorsal wall of the double-somite.

#### (ii) *Temoridae: description*

External genital area. In *Temora stylifera* (figure 18), the genital area is oval, wider than long and located posteriorly on the double-somite. In inseminated females (figure 19), the seminal products form a plug locking the genital atrium and pushing the operculum anteriorly. Partial removal of this plug allows observation of the remains of these products, adhering to the operculum and gonoporal plates. They are visible in semi-thin section (figure 56) and comprise spermatozoa only, as confirmed by TEM observation of thin sections. In *Eurytemora velox* (figure 20) and *Heterocope saliens* (figures 21 and 22), the genital area occupies most of the medio-ventral region of the double-somite. In *E. velox,* there is an important anterior pad, and the operculum is longer than wide. In *H. saliens,* the area is trapezoidal, with a



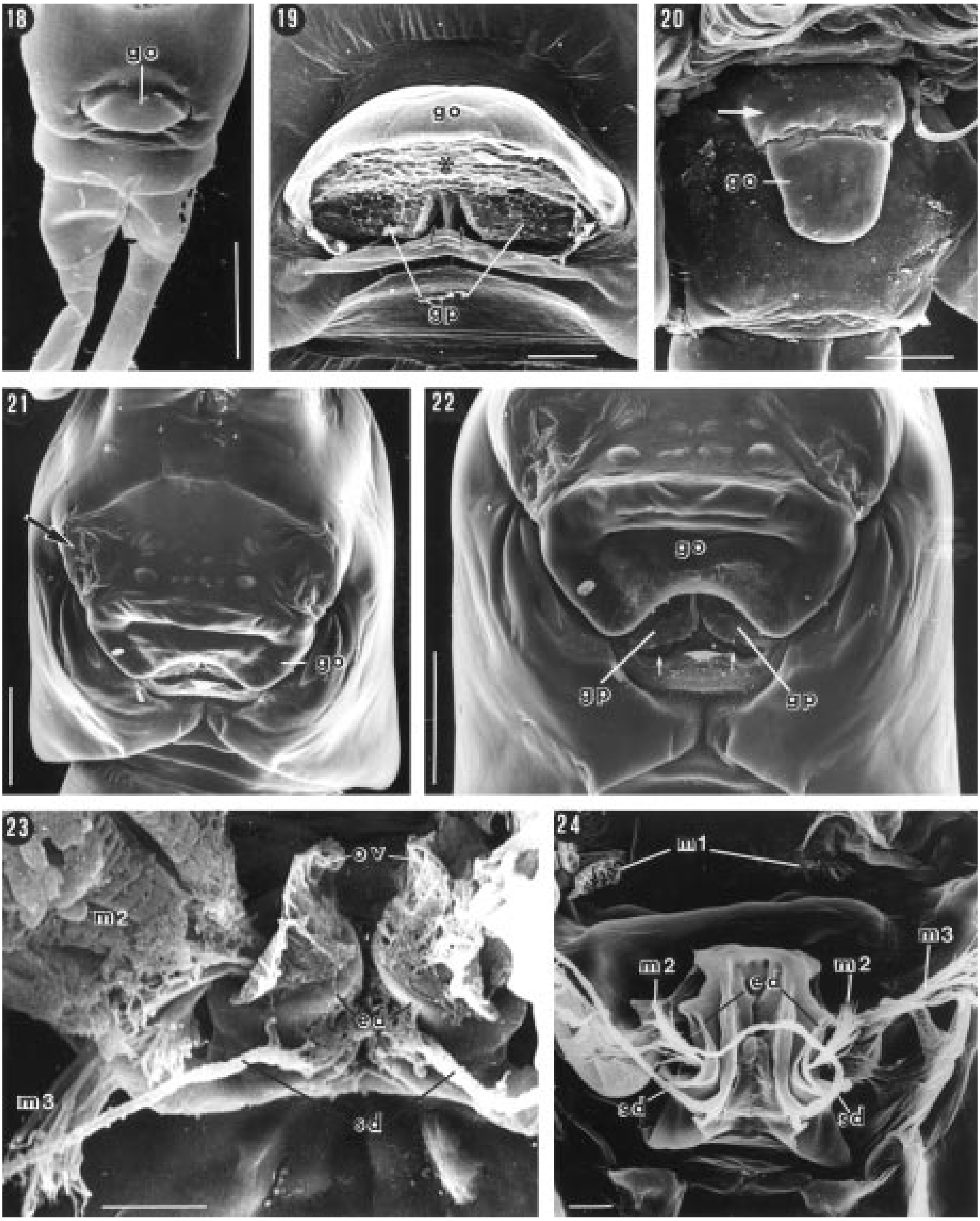
Figures 6–17. Scanning electron micrographs of genital area of Pontellidae. Figure 6. External ventral face of genital double-somite of *Pontella mediterranea*. Figure 7. Same for *Anomalocera patersoni*; scale bars 50 µm. Figure 8. Detail of external genital area of *Pontella mediterranea*. Figure 9. Same for *Anomalocera patersoni*. Note genital operculum covering genital atrium (arrows) with visible gonoporal plates (arrowheads) in *Pontella mediterranea*; scale bars 10 µm. Figure 10. External ventral face of genital double-somite of *Labidocera wollastoni*. Note posterior bilobate edge (arrowhead) of genital operculum; scale bar 50 µm. Figure 11. Internal dorsal view of genital area of *Pontella mediterranea*. Figure 12. Detail of left side. Figure 13. Internal dorsal view of genital area of *Anomalocera patersoni*. Note remnants of oviducts concealing proximal edge of the egg-laying ducts (cross); scale bars 10 µm. Figure 14. External ventral view of urosome of *Labidocera pavo*. Figure 15. Detail of genital area of *Labidocera pavo*. Note position of genital area at left posterior corner of double-somite and its duck-billed form with a very long operculum overlying gutter-like distal part of genital area (arrows); scale bars 50 µm. Figure 16. Internal dorsal view of genital area of *Labidocera pavo*. Figure 17. Detail of right side; scale bars 10 µm. Abbreviations as for figures 2–5.

strong anterior pad which extends into a bilobed operculum and two latero-posterior thickenings. The operculum does not completely cover the posterior part of the genital atrium, allowing the gonoporal slits and plates (figure 22) to be seen.

Internal genital area. In *Heterocope saliens* (figure 24), the organization is typical, with paired shell ducts connected to the egg-laying ducts and three pairs of muscles. In *Eurytemora velox* (figure 23), both pairs of ducts, the muscles of the egg-laying ducts and the atrial muscles are clearly visible. In *Temora stylifera*, the muscles of the egg-laying duct only were identified. Semi-thin sections showed, however, the presence of opercular muscles (figure 56) but atrial muscles were not observed.

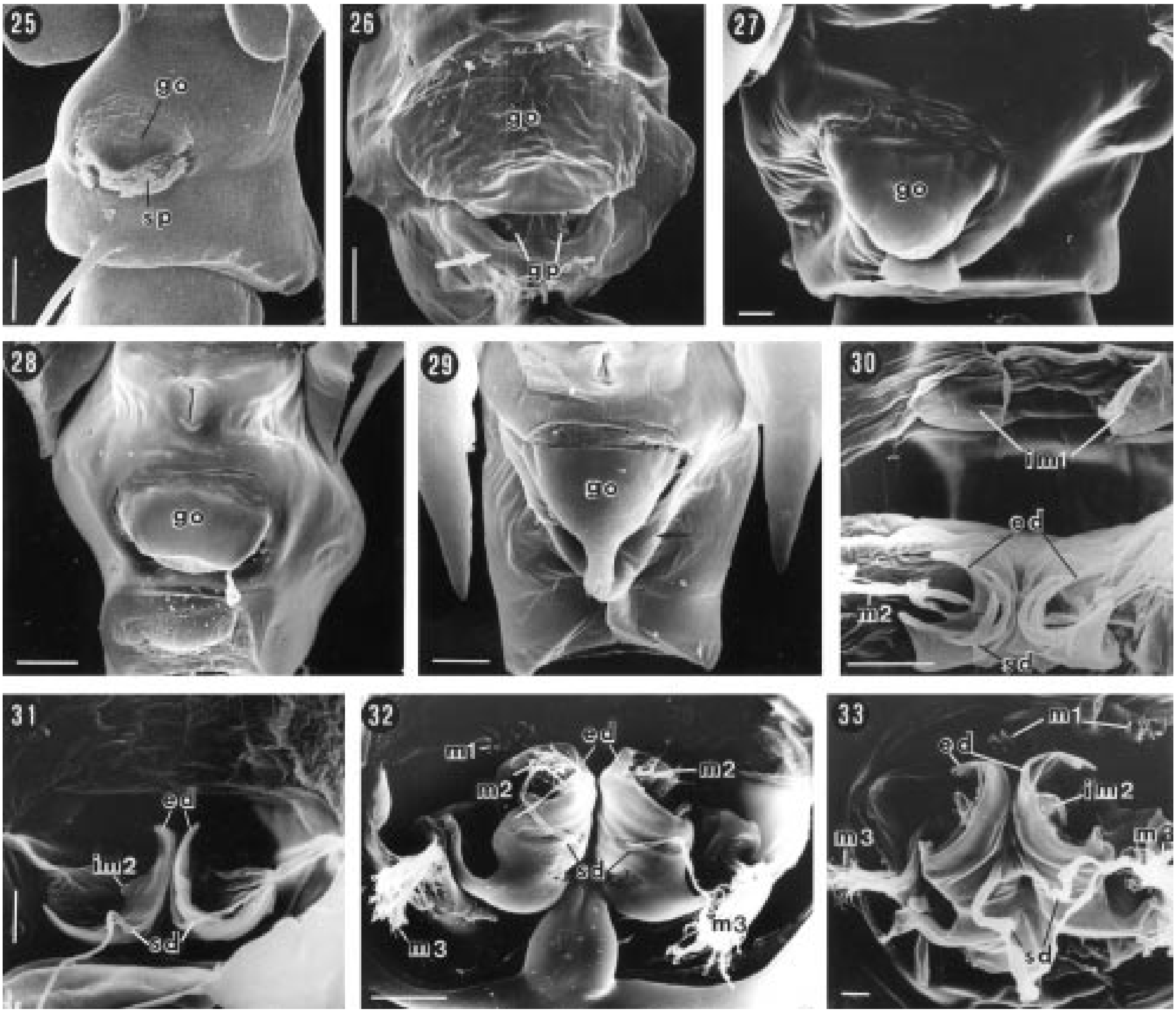
### (iii) *Centropagidae: description*

External genital area. It is located on medio-ventral axis in all species. Each species shows special characteristics. In *Centropages typicus* (figure 25) the area is medio-ventral, slightly displaced to the left; it is covered by a triangular genital operculum, small in size and not prominent except in inseminated females in which it is displaced



Figures 18–24. Scanning electron micrographs of genital area of Temoridae. Figure 18. External ventral view of urosome of *Temora stylifera*; scale bar 50 µm. Figure 19. External ventral view of genital area of inseminated female of *Temora stylifera*. Note genital operculum raised by seminal products (asterisk), plus the gonoporal slits (arrows) and plates; scale bar 10 µm. Figure 20. External ventral face of genital double-somite of *Eurytemora velox*. Figure 21. Same for *Heterocope saliens*. Note anterior thickening (arrow) of genital area forming pad above operculum; scale bars 50 µm. Figure 22. Detail of external genital area of *Heterocope saliens*. Note short bilobate operculum overlaying incompletely the gonoporal slits (arrows) and plates; scale bar 50 µm. Figure 23. Internal dorsal view of genital area of *Eurytemora velox*. Figure 24. Same for *Heterocope saliens*; scale bars 10 µm. Abbreviations as for figures 2–5.

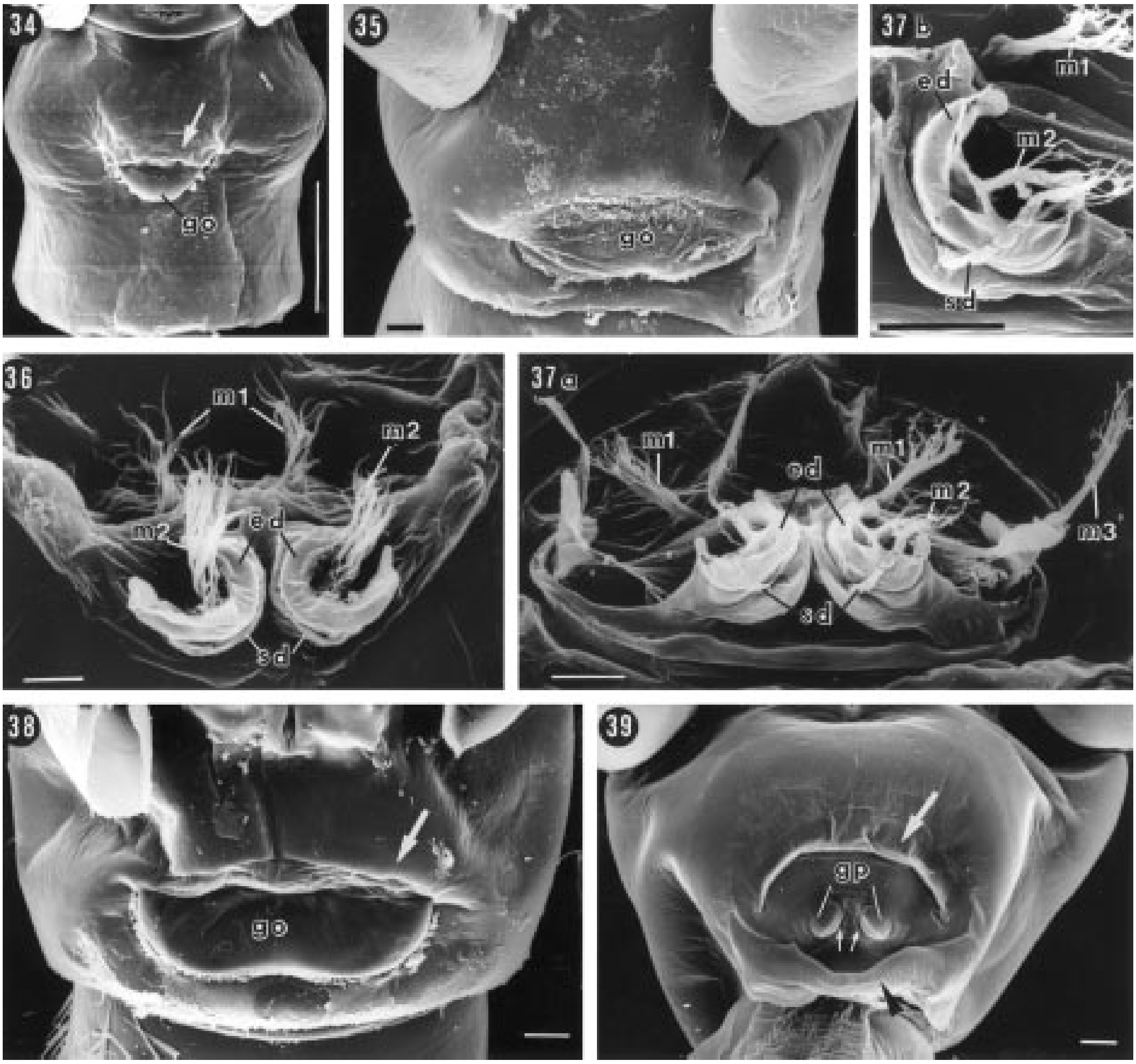
by the seminal products filling the genital atrium. In *Isias tropica* (figure 26), it is similar in shape, but larger: it occupies almost all the posterior half of the double-somite, and is limited distally by a strong pad. The operculum does not completely cover the genital atrium, so the gonoporal slits and plates are visible. In *Boeckella*, the area is in a central position in *Boeckella bergi* (figure 28) and *B. dentifera* (figure 29), but posterior in *B. poppei* (figure 27). It is not



Figures 25–33. Scanning electron micrographs of genital area of Centropagidae. Figure 25. External ventral view of genital double-somite of *Centropages typicus* (inseminated female). Figure 26. Same for *Isias tropica*. Figure 27. Same for *Boeckella poppei*. Figure 28. Same for *B. bergi*. Figure 29. Same for *B. dentifera*. Note genital operculum raised by seminal products in *Centropages typicus*, funnel-shape of genital operculum in *B. dentifera*, and the posterior pad (arrow) in *Isias tropica*, *Boeckella poppei*, *B. dentifera*; scale bars 25 μm. Figure 30. Internal dorsal view of genital area of *Centropages typicus*. Figure 31. Same for *Isias tropica*. Figure 32. Same for *Boeckella bergi*. Figure 33. Same for *B. poppei*; scale bars 10 μm. Abbreviations as for figures 2–5, except im1, zone of insertion of m1; im2, zone of insertion of m2; sp, seminal products.

prominent in *B. bergi* and is limited by the trapezoidal operculum. In *B. dentifera* the area is prominent, triangular, with a funnel-shaped operculum. Its morphology is similar to that observed in *Labidocera pavo* (figures 14 and 15). *Boeckella poppei* is similar to *B. dentifera* but the genital operculum is larger, as wide as long, with a regularly rounded distal edge, and the posterior pad forms a bilobed expansion. In *Limnocalanus johanseni* the genital area is central and resembles that of *Centropages typicus* in the shape and size of the operculum, the main difference being the anterior thickening (figure 34). Finally, in *Osphranticum labronectum* (figure 35) the area is posteriorly located, clearly wider than long, with a marked thickening on the anterior margin.

Internal genital area. The genera *Boeckella* and *Osphranticum* (figures 32, 33 and 37) have strong egg-laying ducts. In *Centropages typicus, Isias clavipes* and *I. tropica* only the shell ducts, egg-laying ducts and their muscles were identified internally (figures 30 and 31), although in *Centropages typicus,* the insertions of the opercular muscles can be observed (figure 30) and these muscles are visible in semi-thin sections. Atrial muscles were not observed in these three species, nor in *Limnocalanus johanseni.* In this last species, only the muscles of the operculum and egg-laying ducts are clearly visible (figure 36). In *Centropages typicus,* sections of inseminated females revealed that the atrium is filled by a strongly staining mass of seminal products, isolated from outside by an amorphous envelope (figure 57). As in *Temora stylifera,* TEM observations showed this mass to be composed only of spermatozoa. The atrium tends to form two antero-lateral pockets as in *Pontella mediterranea* (figure 55), but is largely open because the operculum and the atrium are fused only along their antero-lateral edges.



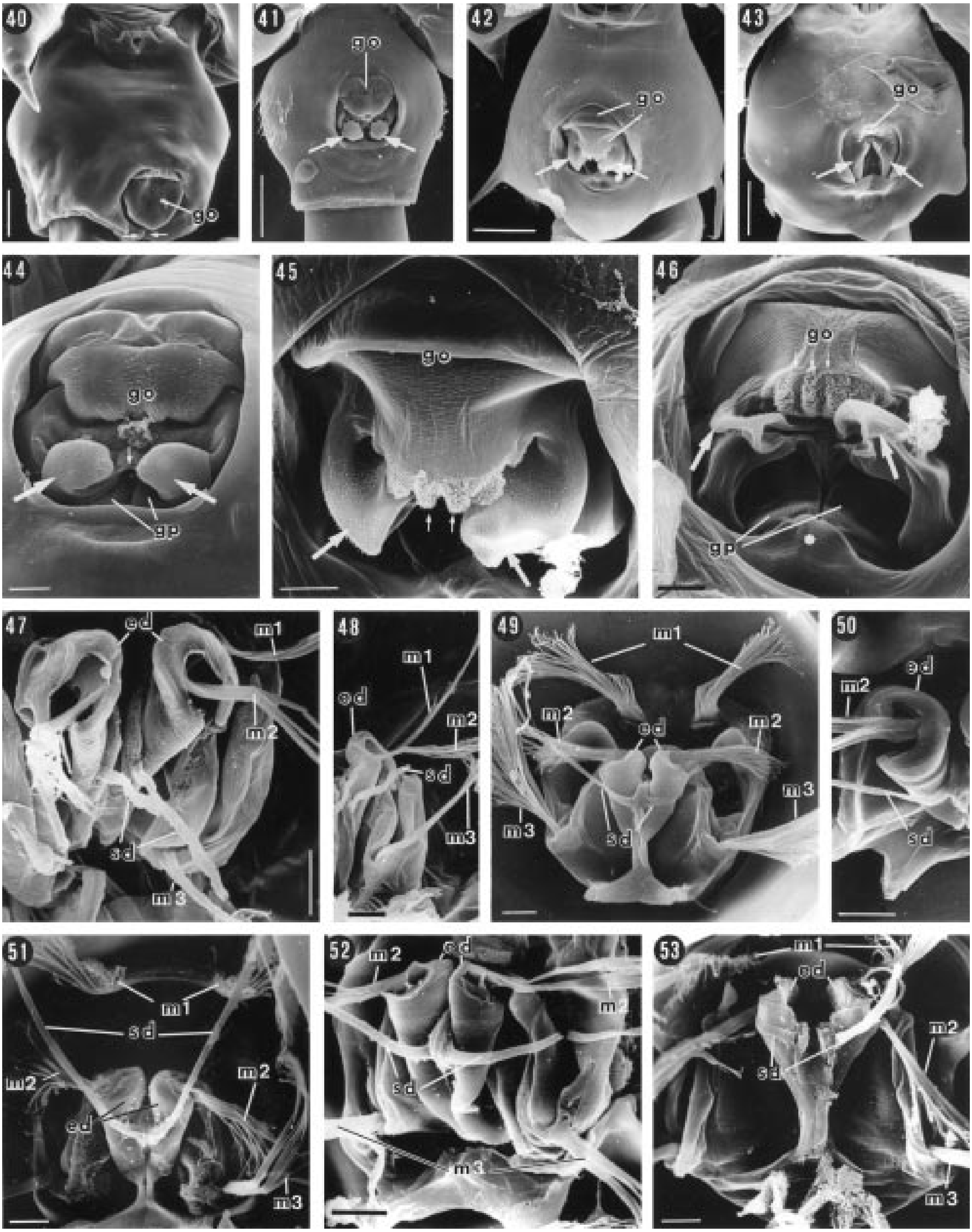
Figures 34–37. Scanning electron micrographs of genital area of Centropagidae. Figure 34. External ventral face of genital double-somite of *Limnocalanus johanseni*. Figure 35. Same for *Osphranticum labronectum*. Note thickening (arrows) of anterior edge of genital area; scale bars 100 µm (figure 34) and 10 µm (figure 35). Figure 36. Internal dorsal view of genital area of *Limnocalanus johanseni*. Figure 37*a*. Same for *Osphranticum labronectum*, with detail of right egg-laying duct (figure 37*b*); scale bars 10 µm. Abbreviations as for figures 2–5. Figures 38–39. Scanning electron micrographs of genital area of Tortanidae and Sulcanidae. Figure 38. External ventral view of genital double-somite of *Tortanus discaudatus*. Note thick anterior pad (arrow) of genital area; scale bar 10 µm. Figure 39. External ventral view of genital double-somite of *Sulcanus conflictus*. Note genital area with prominent pad (arrow), gonoporal slits (small arrows) and plates, and thick posterior pad (arrowhead); scale bar 10 µm. Abbreviations as for figures 2–5.

### (iv) *Tortanidae: description*

The external genital area (figure 38) resembles that of *Osphranticum labronectum* (figure 35) in its posterior location on the double-somite, its shape and morphology. The internal genital area conforms to the general pattern. Sections of the genital double-somite (figure 58) indicate that the genital atrium is similar to that observed in *Pontella mediterranea* and *Centropages typicus* and it acts as a seminal receptacle in the same way.

### (v) *Candaciidae: description*

External genital area. The genital area is approximately round and entirely delimited by a cuticular fold, except in *Candacia simplex*. It is medio-ventral in *C. simplex* (figure 41), sub-median in *C. bispinosa* (figure 42) and *C. ethiopica* (figure 43), and posterior in *C. armata* (figure 40). *C. armata* only presents a morphology close to the general pattern, having an operculum with a small anterior thickening. However, it is characterized by the distal edge of the somite extending into two small median lobes, the free margins of which are contiguous and form the posterior pad (figures 40, 54 and 59*a*). In the other species, the operculum has a composite structure resulting from differentiation of several elements. In *C. simplex* (figures 41, 44 and 54) the operculum is wide proximally, slightly bilobed, and prolonged medially. The medial prolongation is ornamented with microfolded cuticle along its distal edge. Posteriorly, the operculum



Figures 40–53. Scanning electron micrographs of genital area of Candaciidae. Figure 40. External ventral view of genital double-somite of *Candacia armata*. Figure 41. Same for *C. simplex*. Figure 42. Same for *C. bispinosa*. Figure 43. Same for *C. ethiopica*. Figure 44. Detail of structure of operculum of *C. simplex*. Figures 45–46. Same for *C. bispinosa*. Note in *Candacia armata* the simple operculum covering genital area and the two posterior lobes joining together medioventrally (arrows), and in *C. simplex*, *C. bispinosa* and *C. ethiopica* the complex operculum with two more or less developed lateral expansions (arrows). Note also in *C. simplex* (figure 44) the posterior margin of the operculum (small arrow) and in *C. bispinosa* (figures 45–46), the lobate median part of the operculum with microfolded cuticle (small arrows), the lateral expansions (arrows) contiguous with the median part of the operculum, and the posterior pad (asterisk); scale bars 50 μm (figures 40–43) and 10 μm (figures 44–46). Figure 47. Internal dorsal view of genital area

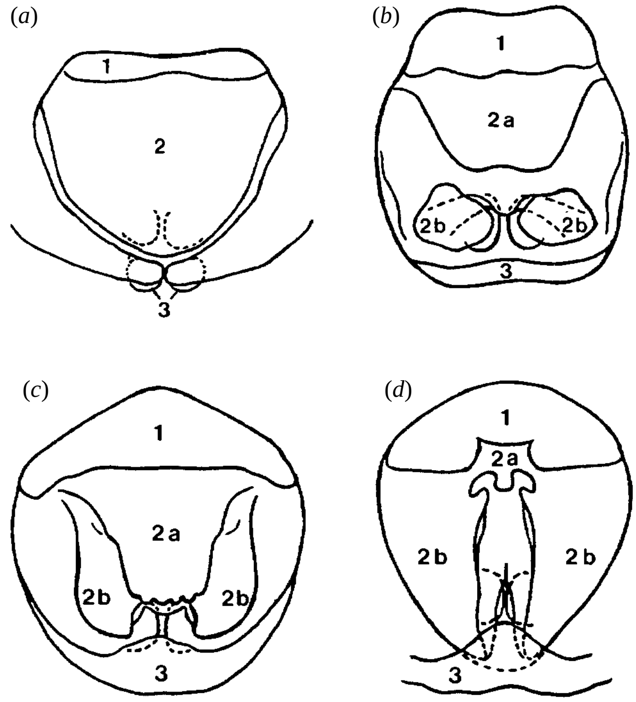


Figure 54. Schematic comparison of external genital area in (*a*) *Candacia armata*, (*b*) *C. simplex*, (*c*) *C. bispinosa*, and (*d*) *C. ethiopica*. Homologous structures have the same number: (1) anterior thickening of genital operculum; (2*a*) median part of genital operculum; (2*b*) lateral expansions of genital operculum; (3) posterior pad.

forms two lateral expansions directed towards the median axis and connected basally to the free lamellar margin of the operculum. The whole forms a complex cover protecting the anterior part of the genital atrium. The atrial cavity is, however, distally open, partly revealing the gonoporal slits and plates. In *C. bispinosa* (figures 42, 45, 46 and 54) the genital area is similar but the anterior part of the operculum is more prominent, and its median prolongation is multilobate and ornamented with microfolded cuticle. The lateral expansions partly covering the gonopores are better developed than in *C. simplex* and contiguous with the median part. In *C. ethiopica* (figures 43 and 54) the median part is almost entirely reduced while the lateral expansions represent the main part of the functional operculum. They are wing-shaped and their extremities fit beneath the thin posterior pad of the genital area. In semi-thin sections, the lateral expansions, of triangular shape, have thick internal edges forming the lateral walls of the genital atrium (figures 60*a*,*b* and 61*a*). The deepest part of the lateral walls progressively join, then fuse and divide the atrium into a narrow dorsal atrial cavity and a ventral atrial cavity (figures 60*c* and 61*b*,*c*). In *C. ethiopica* the genital atrium is closed distally by the posterior pad (figure 61*a*) and the genital atrium communicates with the outside only by a small opening (figures 54 and 61*b*,*c*).

Internal genital area. The internal organization corresponds to the general pattern in the four species (figures 47–53). The egg-laying ducts are long and joined distally. On each side, the cuticular fold forms an angular structure into which the muscles of the genital atrium insert. The three pairs of muscles originate laterally to latero-dorsally on the body wall (figures 59–61). Several additional details serve to distinguish *Candacia armata*. In this species, the concave sides of the egg-laying ducts are directed towards the latero-dorsal walls of the double-somite and the atrial muscles insert near each other on the distal internal edge of the atrial wall (figures 47, 48 and 59*b*). In the other *Candacia*, the egg-laying ducts have their concave side oriented towards the latero-ventral wall (figures 49–53). In *C. simplex* and *C. bispinosa* the egg-laying ducts and shell ducts are connected medio-dorsally by a small cuticular structure (figures 49–52). In inseminated females, the seminal products are stored in the genital atrium, whether it is of the general pattern as in *C. armata* (figure 59*b*–*d*), or relatively open as in *C. simplex*, in which the atrium is closed anteriorly at level of lateral expansions (figure 60*a*–*e*). In *C. ethiopica*, the atrium is similar in structure but closed distally by the posterior pad (figure 61*a*–*d*).

### (vi) *Sulcanidae: description*

The external genital area is different in the lack of a genital operculum. Located in the distalmost part of the genital double-somite, it is delimited partly by cuticular folds forming an anterior arch-shaped pad and a rather thick and prominent posterior pad. In the middle of the area, two gonoporal slits, of the usual shape, limit the gonoporal plates (figure 39).

The internal genital area differs from the general pattern only in the absence of the atrial muscles. The short, thick egg-laying ducts receive the shell ducts; the muscles of the anterior pad are well-developed, in spite of the absence of operculum (figure 62*a*–*c*).

## 4. DISCUSSION

### (a) *Variation in structure of the genital area*

The genital area is on the ventral face of the genital double-somite, and in many cases, forms a more or less marked bulge (figure 63). It is situated medially, slightly medio-ventrally, anteriorly or posteriorly. The exception is *Labidocera pavo* in which the genital area is completely displaced to the left latero-distal margin (figures 14, 15 and 63).

In all cases, the genital area is limited by a pronounced cuticular fold, but this fold is rarely uniformly thickened all around the area. In several species, it is only really visible on the lateral and distal margins of the genital area (figure 63). Thus, anteriorly, there is no discontinuity between the double-somite and the operculum in *Centropages typicus*, *Isias tropica* and *Candacia simplex*, and only slight discontinuity in *Boeckella bergi*. In contrast, in *Limnocalanus johanseni* and *Osphranticum labronectum*, the anterior edge is clearly thickened and forms a pad lightly separated

Figures 40–53 (*continued*). of *Candacia armata*. Figure 48. Right side of genital area; scale bars 10 μm. Figure 49. Internal dorsal view of genital area of *C. simplex*. Figure 50. Detail of left egg-laying duct; scale bars 10 μm. Figures 51–52. Internal dorsal view of genital area of *C. bispinosa*. Figure 53. Same for *C. ethiopica*; scale bars 10 μm. Abbreviations as for figures 2–5.

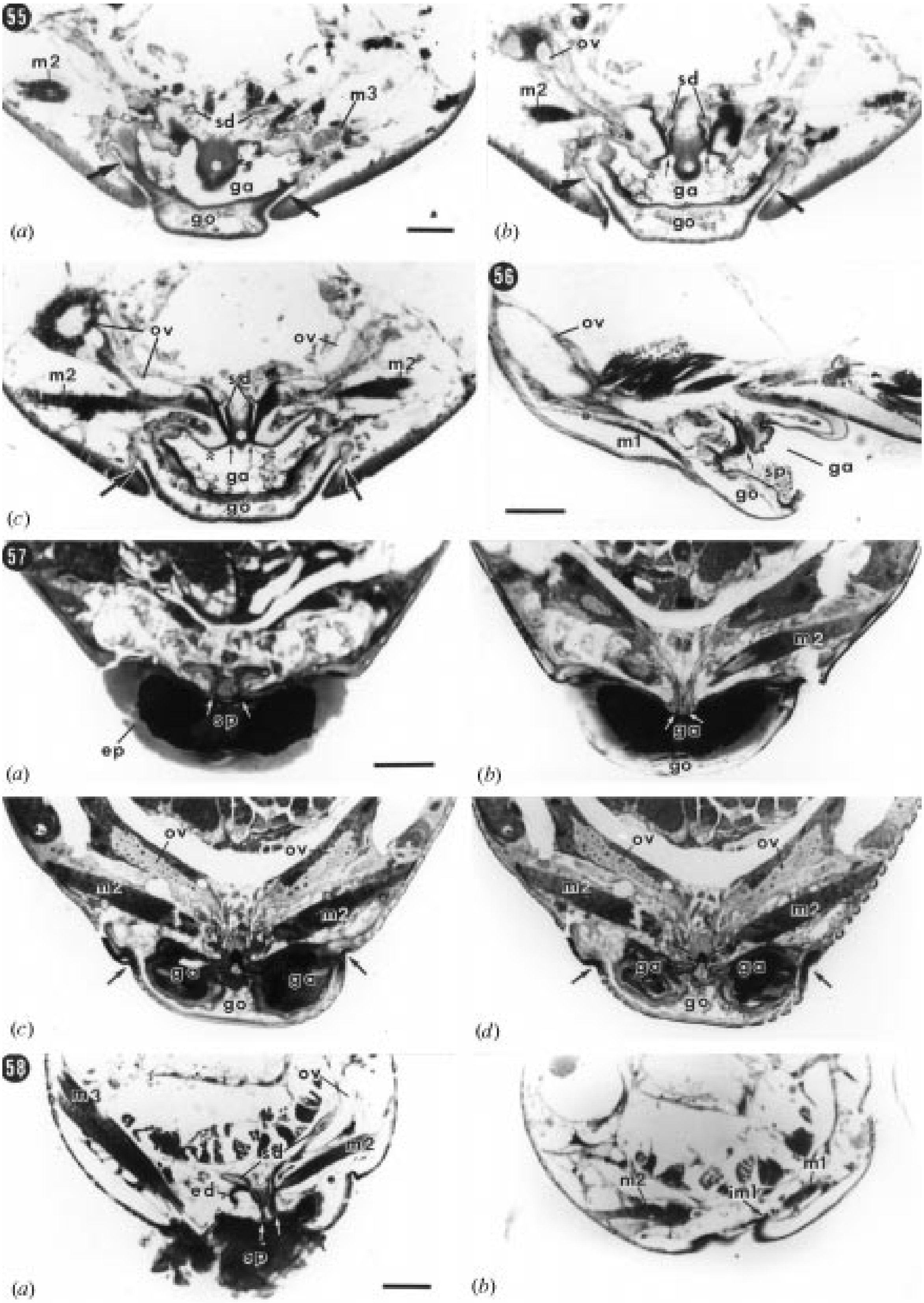


Figure 55. Photomicrographs of transverse sections of genital double-somite of *Pontella mediterranea* (section (*a*) is posteriormost, (*c*) anteriormost). Note shell ducts and genital atrium closed laterally by fusion of edges of genital operculum with cuticular folds (arrows) surrounding genital area (*a*, *b*, *c*), the gonoporal slits (small arrows) (*b*, *c*) on both sides of rod (asterisk), more prominent in posterior part of atrium. Note also the gonoporal plates (double chevrons) are continuous with the wall of the egg-laying ducts (*b*, *c*); scale bar 20 µm. Abbreviations as for figures 2–5. Figure 56. Photomicrograph of a longitudinal section of genital double-somite of *Temora stylifera*. Note gonoporal slit (arrow), gonoporal plate (double chevrons) and seminal products under the genital operculum; scale bar 20 µm. Abbreviations as for figures 2–5 and 25–33. Figure 57. Photomicrographs of serial transverse sections of genital double-somite of inseminated female of *Centropages typicus* (section (*a*) posteriormost, (*d*) anteriormost). Note seminal products isolated from outside by amorphous envelope (*a*), genital operculum fused antero-laterally to weak cuticular folds surrounding

from the operculum. According to Mazzocchi *et al.* (1995), *Centropages bradyi* presents the same morphology. *Temora stylifera*, *Boeckella dentifera*, *B. poppei* and *Tortanus discaudatus* also have a weakly marked thickening, in contrast to *Eurytemora velox* and *Heterocope saliens* in which this pad is well developed. In the Pontellidae, the morphology is similar but the cuticular fold surrounds the whole area, separating it from the rest of the double-somite, as in *Pontella mediterranea* and *Anomalocera patersoni*. In both these species there is no clear thickening of the anterior edge of the area. In the genus *Labidocera*, the anterior cuticular fold constitutes a hinge line between the operculum and the anterior pad. It protrudes in *L. pavo* and is flattened and lightly marked in *L. wollastoni*, in which the configuration of the genital area is similar to that of *L. aestiva* (Blades-Eckelbarger & Youngbluth 1979; Blades-Eckelbarger 1991). In the Candaciidae, a well-marked cuticular fold delimits the almost circular genital area without a particular anterior thickening. Except in Tortanidae, species in other families show, in addition, some more or less pronounced thickenings on the lateral and/or posterior margins as in the pontellids *Pontella mediterranea*, *Anomalocera patersoni* and *Labidocera pavo*, in the temorid *Heterocope saliens*, and in the centropagids *Isias tropica*, *Boeckella dentifera* and *B. poppei*. *Sulcanus conflictus* has no operculum nor opercular hinge but is characterized by a well-developed posterior pad which appears to join the extremities of the anterior pad laterally (figure 63).

The operculum is an expansion of the anterior margin of the genital area that it covers. The genital atrium is located beneath, on the dorsal roof of which are located the gonopores. The distal aperture, formed where the operculum is free, is the atrial slit. The anterior proximal margin of the operculum constitutes the hinge with the genital double-somite. Paired muscles insert around this hinge (figures 2–5), giving the operculum mobility and allow the atrium to open. There are three types of hinge, depending on the morphology of the anterior pad. The hinge may be ill-defined because there is no cuticular interruption between somite and operculum (*Centropages typicus*, *Isias tropica*, *Boeckella bergi* and *Candacia simplex*). It is clearly marked when the anterior margin forms a thickened pad (*Labidocera wollastoni*, *L. pavo*, *Temora stylifera*, *Eurytemora velox*, *Heterocope saliens*, *Boeckella dentifera*, *B. poppei*, *Limnocalanus johanseni*, *Osphranticum labronectum* and *Tortanus discaudatus*). It is concealed in an anterior cuticular fold in *Pontella mediterranea*, *Anomalocera patersoni*, *Candacia armata*, *C. bispinosa* and *C. ethiopica*.

Except in *Candacia simplex*, *C. bispinosa*, *C. ethiopica* and *Sulcanus conflictus*, the operculum forms a simple flap taking the exact shape of the genital area and protecting the atrial cavity (figure 63). Typically the atrium forms a single cavity occupying just the distal half of the area. The atrial cavity is more or less bilobed because of latero-anterior extensions (figures 55–57) and it is likely that the paired spermathecae mentioned in the pontellids *Epilabidocera amphitrites* (Park 1966) and *Labidocera aestiva* (Blades-Eckelbarger & Youngbluth 1984) correspond to atrial extensions.

The complex opercula of *Candacia simplex*, *C. bispinosa* and *C. ethiopica* appear to form a transformation series from the simple type of *Candacia armata* (figure 54). The atrial cavity is complex, divided in two chambers, one antero-dorsal, near the gonopores, the other, ventral, opening to the outside by a small atrial orifice.

In *Sulcanus conflictus* there is no operculum but the muscles inserting on the anterior cuticular fold could be considered homologues of the opercular muscles. It is likely that the operculum has completely regressed resulting in a completely open atrium. The position and morphology of the gonoporal slits and plates are clearly visible and are similar to most other studied species.

In all species the egg-laying ducts show the same gutter-like construction in the closed state, as in some other calanoids. The closed state corresponds with the crescentic shape of the external gonoporal slits. The ducts vary in width and length from species to species. These ducts are opened by a pair of muscles. There are two other pairs present: the opercular muscles inserting at level of the hinge in all species and atrial muscles inserting on the atrial wall, on each side of the egg-laying ducts. The study of a unique example of the genus *Isias* did not permit identification of the opercular and atrial muscles. It is unlikely that the former are absent in both species studied, which have a well-developed operculum. According to Park (1966), the pontellid *Epilabidocera amphitrites* has similar genitalia with the same musculature. The only important variation found is the loss of atrial muscles in *Centropages typicus*, *Limnocalanus johanseni*, *Temora stylifera* and *Sulcanus conflictus*. In the same way as mentioned before, the absence of atrial muscles in *Limnocalanus johanseni* has to be verified.

Although this analysis of female genitalia covers only 21 species compared with the total (several hundreds) in the families studied, the following generalizations can be made: (i) internal organization is homogeneous except in the absence of atrial muscles in a few species; (ii) external morphology is highly variable. However, in all species, the genital area is delimited by a cuticular fold, at least laterally and posteriorly and is, with one exception, covered by an operculum articulated with the somite by an anterior hinge. Other characters such as the extent of peripheral thickenings or modifications of the operculum, vary with species, in the different families. Their diversity confers to the genital area a morphology characteristic of the species, that is useful in species level systematics, as in *Boeckella* and *Candacia* species (figure 54).

### (b) *Relationships between organization patterns of the female genital structures in Calanoida*

In the Centropagoidea, the genital area is well-known only in two species of Diaptomidae, *Hemidiaptomus ingens*

Figures 55–58 (*continued*). genital area (arrows) (*c*, *d*), genital atrium constituting two pockets anteriorly (*b*, *c*, *d*), gonoporal slits (small arrows) (*a*, *b*); Scale bar 20 µm. Abbreviations as for figures 2–5 and 25–33 except ep, amorphous envelope. Figure 58. Photomicrographs of oblique transverse sections of genital double-somite of inseminated female of *Tortanus discaudatus* (section *a* posteriormost, *b* anteriormost). Note connecting of shell ducts with egg-laying ducts and gonoporal slits (small arrows) in genital atrium containing seminal products (*a*). Note also insertion site of genital operculum muscle (*b*); Scale bar 20 µm. Abbreviations as for figures 2–5 and 25–33.

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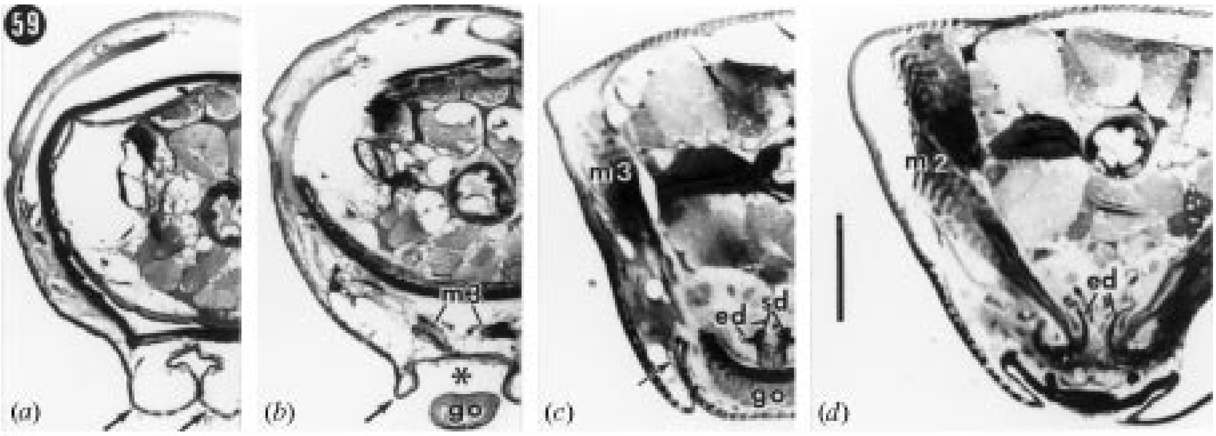

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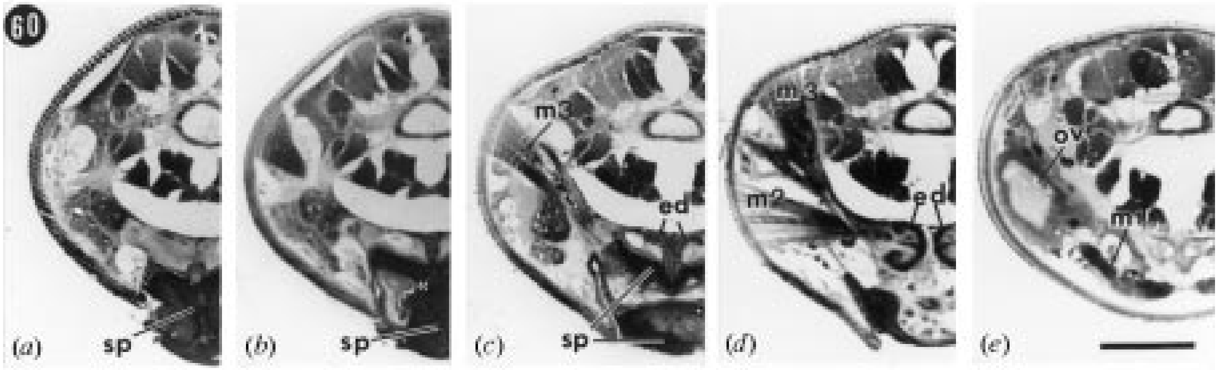

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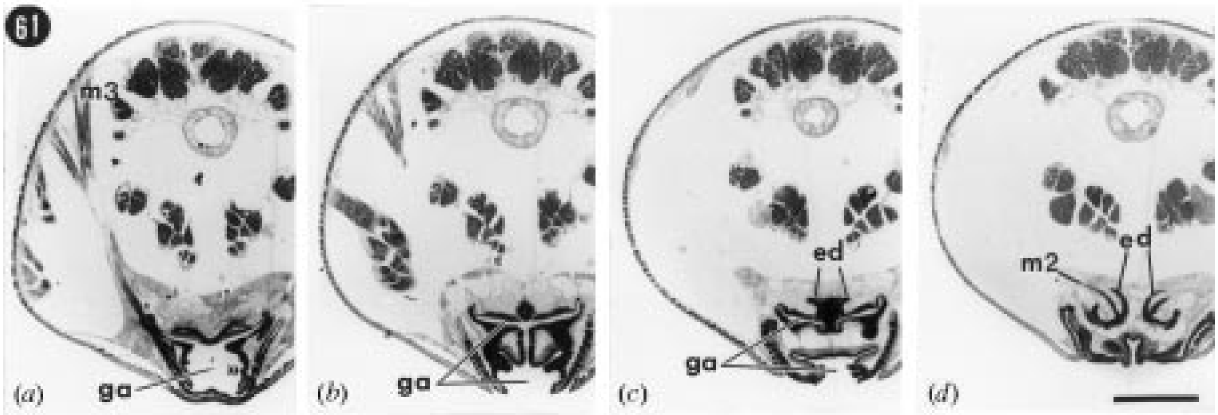

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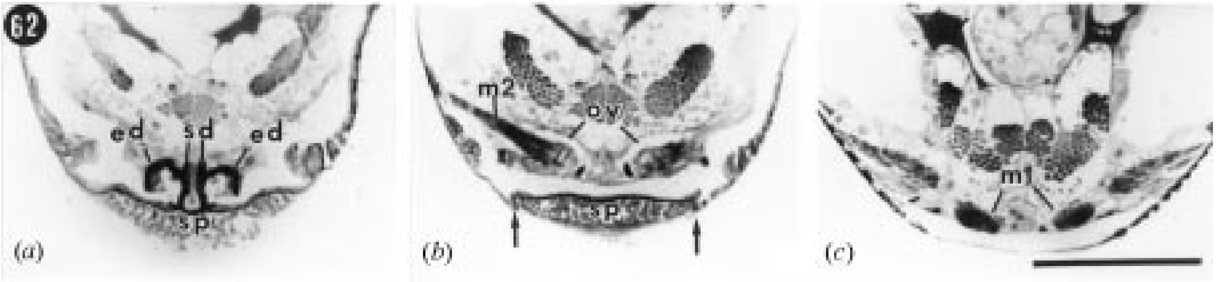

Figure 59. Photomicrographs of serial transverse sections of genital double-somite of *Candacia armata* (section (*a*) posteriormost, (*d*) anteriormost). Note two posterior lobes joining together medioventrally (arrows) (*a*, *b*), genital atrium (asterisk) closed anteriorly by fusion of operculum with cuticular folds of genital area (arrow) (*c*), egg-laying ducts and shell ducts (*c*, *d*); scale bar 50 µm. Abbreviations as for figures 2–5. Figure 60. Photomicrographs of serial transverse sections of genital double-somite of inseminated female of *Candacia simplex* (section (*a*) posteriormost, (*e*) anteriormost). Note genital atrium containing seminal products with single cavity posteriorly (*a*, *b*) and two cavities anteriorly (*c*) caused by fusion of lateral expansions (double chevrons) (*b*); scale bar 50 µm. Abbreviations as for figures 2–5 and 25–33. Figure 61. Photomicrographs of serial transverse sections of genital double-somite of female *Candacia ethiopica* (section (*a*) posteriormost, (*d*) anteriormost). Note genital atrium comprising a single cavity posteriorly (*a*), then anteriorly divided into a dorsal cavity and an open ventral cavity (*b*, *c*); scale bar 50 µm. Abbreviations as for figures 2–5, and <<, lateral expansion of genital operculum. Figure 62. Photomicrographs of serial transverse sections of genital double-somite of inseminated female of *Sulcanus conflictus*. Note fusion of shell ducts with egg-laying ducts (*a*). Note also cuticular folds of genital area (arrows) and seminal products (*a*, *b*); scale bar 50 µm. Abbreviations as for figures 2–5 and 25–33.

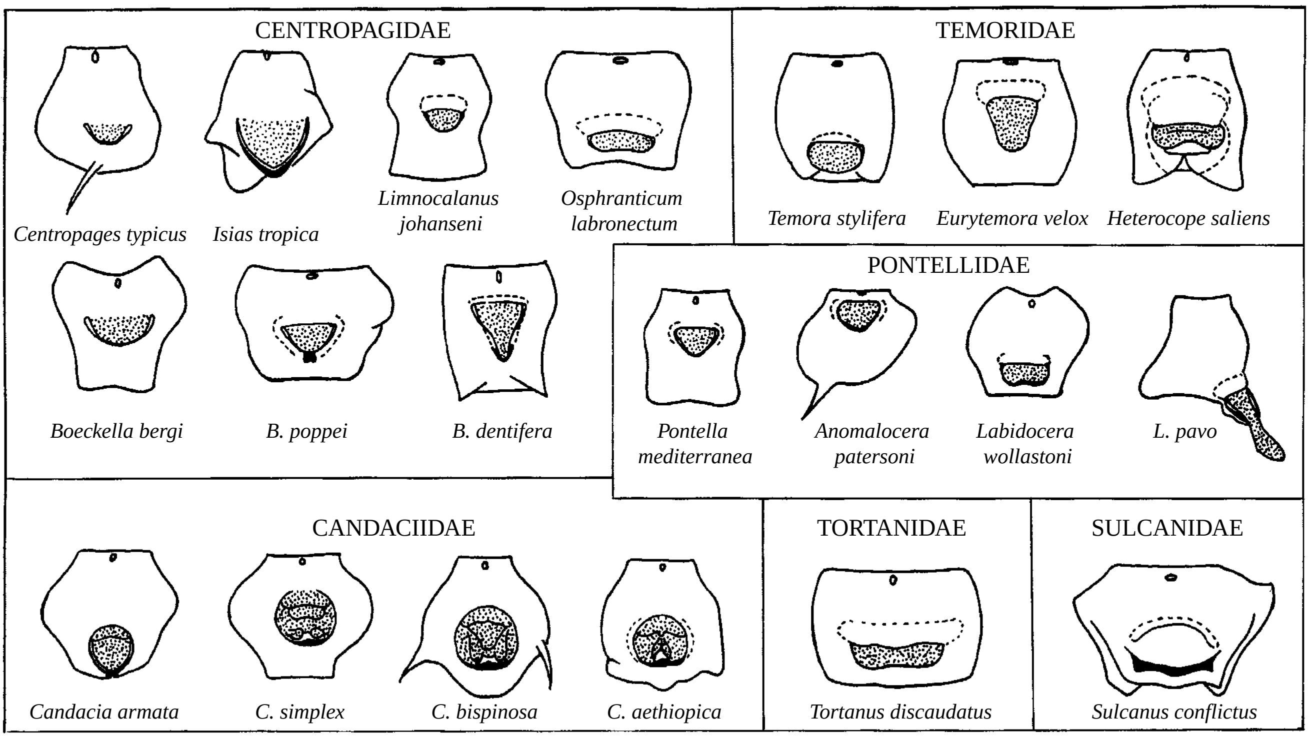

Figure 63. Schematic representation of external genital area in the species studied. Dashed line, limit of the anterior pad and lateral thickenings; shading, posterior pad; dots, genital operculum.

and *Mixodiaptomus kupelwieseri* (Cuoc *et al.* 1989*b*). Its internal structure is similar to that described here, with two cuticular gutter-like egg-laying ducts widely different from the membranous secretory oviducts (Cuoc *et al.* 1989*a*,*b*), two shell ducts, three paired muscles and no seminal receptacle. The external area is delimited by a cuticular fold and with a more or less thickened posterior border ('bourrelet dorsal' of Cuoc *et al.* 1989*b*). It differs particularly in the reduction of the operculum to a prominent anterior bar ('bourrelet ventral' of Cuoc *et al.* 1989*b*), provided with strong lateral lobes. The reduction of the operculum is observed only in the subfamily of Diaptominae, not in the Paradiaptominae (R.-M. Barthélémy and C. Cuoc, unpublished results). In Diaptominae, the atrium is consequently open, revealing the gonoporal slits and plates, as in *Sulcanus conflictus*.

In the Arietelloidea data are available only for the Arietellidae (Ohtsuka *et al.* 1994), Hyperbionychidae (Ohtsuka *et al.* 1993) and Metridinidae (Cuoc *et al.* 1997). The basic organization of the genital structures includes egg-laying ducts and paired seminal receptacles. The receptacles and ducts communicate to the exterior by paired copulatory pores and by crescent-shaped gonopores which are not covered by an operculum nor grouped into a well-delimited genital area. The construction of the egg-laying ducts, studied in detail in the Metridinidae, is identical to that of the Centropagoidea and always involves a connection with the shell ducts. The presence of the latter in *Arietellus simplex* (Arietellidae) (Cuoc *et al.* 1997) suggests that they are also a constituent element of the genital area in the Arietelloidea.

Among other calanoids, the only interesting data concern the euchaetid *Paraeuchaeta* (Geptner 1968). In this genus the prominent and well-delimited genital area displays a complex structure and significant morphological variation. From Geptner's work, we can deduce that the external genital area is provided with an operculum ('genital valve') equipped with paired muscles, and covering the genital atrium ('genital cavity') bordered by a posterior integumental fold and prominent lateral pads. Internal structures include seminal receptacles, opening to the extremity of a 'structure in arch' associated with glandular ducts, undoubtedly representing the egg-laying ducts and shell ducts. The other muscles mentioned by Geptner are probably those of the egg-laying ducts. Our observations of *Paraeuchaeta gracilis* and *P. barbata* confirm this (R.-M. Barthélémy and C. Cuoc, unpublished results). Finally, despite the specialized morphology of the genital area, the organization of genitalia in *Paraeuchaeta* only differs from that of the Centropagoidea in the presence of receptacles connected to the egg-laying ducts or the atrial cavity.

Current knowledge suggests that the two basic patterns of genitalia, the Centropagoidea and Arietelloidea, represent two main evolutionary trends diverging from the hypothetical ancestral calanoid proposed by Huys & Boxshall (1991). This ancestor has medio-ventral genitalia, with adjoining gonopores and copulatory pores, connected respectively to paired oviducts and seminal receptacles. This ancestral pattern is conserved in Arietelloidea and only the relative arrangement of the components has been modified among the Hyperbionychidae, Arietellidae and Metridinidae (Ohtsuka *et al.* 1993, 1994; Cuoc *et al.* 1997). In contrast, the centropagoids studied up to now lack seminal receptacles and associated structures (copulatory pores, and copulatory and seminal ducts), but have the remaining structures concentrated in a well-delimited genital area covered by an operculum. It is proposed that these centropagoids have diverged from a basic-type with the following characteristics of the genita area: medio-ventral location on genital double-somite, latero-posterior edge limited by a cuticular fold, simple operculum with undifferentiated hinge and paired muscles, genital atrium with paired muscles, and paired egg-laying ducts with associated muscles and shell ducts. The main modifications involve the loss of the atrial muscles (in the Centropagidae and Temoridae), the location of the genital area, and the morphology of the operculum (in all the families). The most striking changes consisting of the regression of the operculum (Diaptominae, Sulcanidae). It is likely that future works will permit us to establish the link between the ancestral calanoid and the Centropagoidea radiation from this basic-type, which raises several questions especially concerning the following points.

1. The loss of the seminal receptacles. This transformation suggests a change in storage of seminal products from internal (seminal receptacle), to semi-external (atrium) or external (Sulcanidae, Diaptominae). The reproductive advantage of such a change is unclear.
2. The development of operculum, and opercular and atrial muscles. The hypothesis that the genital operculum is derived, at least partly, from the sixth pair of legs (lost in Calanoida) is attractive. Possible supporting evidence is provided by the Misophrioida, in which genital structures are associated with vestigial sixth legs operated by muscles that are possibly homologues of the opercular and atrial musculature in calanoids (Boxshall 1982, 1984; Huys & Boxshall 1991). Comparative study of the genital musculature of calanoids is necessary to test this hypothesis.

### (c) *Relationships between the functional morphology of the genitalia and reproductive strategies*

During insemination, the genital atrium serves as a storage site of the seminal products discharged from the spermatophore attached at the level of the atrial slit. Deposition of the seminal products can be facilitated by raising the operculum by contraction of the opercular muscles and by expansion of the atrial cavity caused by contraction of the atrial muscles. The regression of the operculum (Sulcanidae, Diaptominae) without regression of the associated muscles means that these muscles have probably another function during the mating and for the egg extrusion. The seminal products lie directly against the atrial wall, on the gonoporal slits and plates. In inseminated females the atrial cavity is completely filled with seminal products, effectively preventing further inseminations. In the Centropagoidea spermatophores with complex mating plates ('coupling device', Lee 1972) are common. Such structures, which more or less completely surround the genital double-somite and prevent any other access to the genital area, have been described in species of *Centropages* (Lee 1972; Blades 1977; Blades-Eckelbarger 1991) and *Labidocera* (Fleminger 1967, 1975; Blades & Youngbluth 1979).

Opening of the egg-laying ducts is presumably the same as in *Hemidiaptomus ingens* (Cuoc *et al.* 1989*b*) and in the Metridinidae (Cuoc *et al.* 1997). Contraction of the muscles of the egg-laying ducts opens the gonopores and completely retracts the gonoporal plates. Simultaneously, the opercular muscles probably raise the operculum and the atrial muscles dilate the atrial chamber. The combined action of all three muscles breaks the envelope surrounding the spermatozoa stored in the atrium. Oocytes are drawn into the egg-laying ducts by contraction of their muscles and, possibly, forced out into the atrium when the muscles relax. The oocytes are fertilized when they come into contact with the seminal products before being expelled from the atrium. The wide atrial orifice created either by the articulation of the operculum (*Temora stylifera*, *Centropages typicus*, figures 18 and 25), or by its regression (*Sulcanus conflictus*, figure 39), could explain the loss of the atrial muscles. The process of fertilization differs from that of the Metridinidae in which only the necessary fraction of seminal products stored in the receptacles is discharged via the seminal ducts, into the end of the egg-laying ducts, to fertilize the oocytes liberated simultaneously.

The eggs are expelled directly into water in Candaciidae and Pontellidae, in *Tortanus discaudatus*, *Centropages typicus*, *Isias clavipes*, *Temora stylifera* and *Heterocope saliens,* whereas they are laid in an egg-sac in *Hemidiaptomus ingens* and *Mixodiaptomus kupelwieseri* (a general feature of Diaptomidae), in *Osphranticum labronectum* and *Boeckella* species. In *Eurytemora velox* (Huys & Boxshall 1991; R.-M. Barthélémy, personal observations) the eggs are extruded into an agglomeration in cementary material. The sac or the cementary material are made of oviductary secretions expelled immediately before the ovocytes (Cuoc *et al.* 1989*a*,*b*). The timing and mode of ejection of the shell secretions are unknown, as is the role of these secretions.

One of the main consequences of the organization and functional morphology of the genital area in Metridinidae (Cuoc *et al.* 1997) is that a maximum of one or two matings, at the beginning of the reproduction season, provide the female with a sufficient store of seminal products in her receptacles to fertilize the successive clutches of eggs throughout its life. This ability to produce several viable clutches is probably also the case in Arietellidae and has been recorded in other calanoids provided with receptacles, such as *Calanus finmarchicus* and *C. pacificus* (Marshall & Orr 1952; Runge 1984) and some species of *Pseudocalanus* (Corkett & McLaren 1978). It is probably the same in *Eucalanus elongatus* and *Gaetanus pileatus* which display large receptacles (R.-M. Barthélémy, personal observations). In these populations, males are often numerous only at the beginning of the reproductive period, then become rare later. For many authors (Marshall 1949; Chapman 1969; Corkett & McLaren 1978; Hopkins 1982; Tande & Grønvik 1983; Bayly 1986), the short life span of males explains, at least in part, the relative abundance of females to males, observed in calanoids, such as in *Calanus helgolandicus* (Green *et al.* 1993; Kouwenberg 1993), *Pseudocalanus elongatus* (Digby 1950; Green *et al.* 1993), *Pseudocalanus minutus* (Marshall 1949) and *Clausocalanus* spp. (Kouwenberg 1993).

The morphology and inferred function in the Centropagoidea suggest that a copulation is necessary before each egg clutch to replace the spermatozoa store of the atrium, as in the marine species *Temora stylifera* (Ianora *et al.* 1989) and *Centropages typicus* (Ianora *et al.* 1992). In fresh- and brackish-waters, a same process has been demonstrated. In species of *Diaptomus*, Watras & Haney (1980), Watras (1983), Williamson & Butler (1987) observed production of non-viable eggs which are directly rejected into the water in the absence of copulation, whereas, after mating, the eggs are viable and laid in an egg-sac. The necessity of a copulation before each clutch of eggs has been also noted in *Eurytemora velox* (Gaudy & Pagano 1987) and *Eurytemora affinis* (Heinle 1970; Katona 1975). Data on the sex-ratio in populations of species without a seminal receptacle indicate near equality of males and females throughout the period of reproduction. Kouwenberg (1993) recorded a sex-ratio close to 1 in *Temora stylifera* and *Centropages typicus*, and noted in *Candacia armata* a less pronounced dominance of females than in *Calanus helgolandicus*. Comita (1956) found a sex-ratio close to 1 in *Limnocalanus johanseni*. Bayly (1962) in *Boeckella propinqua* noticed that more males than females were present in all collections made, whereas Hairston *et al.* (1983) observed a constant sex-ratio of about 1:1 in diaptomid populations. These data are in accord with the suggestion that centropagoids require a fresh supply of spermatozoa for each egg clutch, as this would necessitate a sex-ratio near unity.

However, according to Jamieson & Burns (1988) and Maly (1991), some freshwater centropagids belonging to the genera *Boeckella* and *Calamoecia* are able to produce multiple fertile clutches after a single insemination. In the study by Maly (1991), of seven species (*Boeckella minuta*, *B. pseudochelae*, *B. symmetrica*, *B. triarticulata*, *B. fluvialis*, *Calamoecia ampulla* and *C. lucasi*), two (*B. fluvialis* and *C. ampulla*) did not produce egg-sacs in the absence of males, whereas *B. pseudochelae* and *B. triarticulata* had only one more egg-sac. If the configuration of the genital area in these latter species is the same as this described here, we can speculate that viable seminal products remain in the atrial cavity after the first clutch and fertilize the second. Comparative anatomical study of the genital double-somite of inseminated females before the first clutch, appears necessary to explain these behavioural observations.

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