

Functional morphology and taxonomic relevance of the female genital structures in Acartiidae (Copepoda: Calanoida)

R.-M. Barthélémy

UPRES Biodiversité, Laboratoire de Biologie Animale, Plancton, Université de Provence, 3 Place Victor Hugo,
13331 Marseille Cedex 3, France. E-mail: bioplank@newsup.univ-mrs.fr

Observations of female genital structures using light and scanning electron microscopy in 25 species of Acartiidae (Copepoda: Calanoida) show the presence of paired gonopores and egg-laying ducts with a typical semicircular configuration in all specimens. In the genus *Acartiella*, there is no seminal receptacle and the external genital area serves as storage site of the spermatophoral products forming an external mass. Unlike in the other acartiids, the genital structures present a complex organization with paired adjacent gonopores and copulatory pores. In almost all the species, the seminal receptacles exhibit characteristic loop-shaped seminal ducts which connect them to the egg-laying ducts. The functional morphology and taxonomic relevance of genital structures are discussed. The present results do not justify the Steuer's subgenus division of the genus *Acartia*, the very predominant one of the family.

INTRODUCTION

In calanoid copepods, the female genital structures are located on the genital double-somite. They play the greater part in the storage of seminal products, fertilization and release of glandular secretions. In spite of their importance in the biology of the reproduction, they were the subject of few works only (Lowe, 1935; Marshall & Orr, 1955; Park, 1966; Blades, 1977; Hammer, 1978; Blades & Youngbluth, 1979; Vaupel-Klein, 1982), the most complete being recent (Cuoc et al., 1989; Blades-Eckelbarger, 1991; Ohtsuka et al., 1994; Cuoc et al., 1997). In Acartiidae, a family with a worldwide distribution and a large number of species, typical from coastal and brackish zones, the only current data are those of Gruber (1879) and especially Steuer (1923), obtained from observations on females *in toto*. For a correct anatomical and functional interpretation of the genital elements, a detailed study of the external and internal structures has been undertaken on several calanoid families using light and electron microscopy techniques. Our investigations in Candaciidae, Centropagidae, Pontellidae, Sulcanidae, Temoridae, Tortanidae (Barthélémy et al., 1998a) and Ridgewayiidae (Barthélémy et al., 1998b) are part of this study. The results obtained on the genital structures of 25 species of Acartiidae are presented here. The organization of these structures belonging to two main types is compared to that of other families and their role and function during the different phases of reproduction is discussed. Finally, the genital structures are examined as taxonomic criteria to precise the phylogenetical relationships between the members of this family, the systematic position of which still remain unclear and subject to discussion.

MATERIALS AND METHODS

Materials

Among the 25 species studied, 20 were provided by the following institutions: Laboratoire de Biologie Animale, Plancton, Université de Provence, Marseille (UP); Muséum National d'Histoire Naturelle, Paris (MNHN); The National Museum of Natural History, Washington, USA (USNM); The Natural History Museum, London (NHML). Female specimens of 3 species were given by Dr Haridas (*Acartiella keralensis*, *A. gravelyi* and *A. southwelli*), one by Dr Ghirardelli (*A. italica*) and Dr Belmonte (*Paracartia josephinae*).

Classification of the studied species is based on that of Razouls (1995)

Genus *Acartiella* Sewell, 1914

Acartiella gravelyi Sewell, 1919; *A. keralensis* (Weller-shaus, 1969); *A. major* Sewell, 1919, NHML, no. 35–49; *A. nicolae* Dussart, 1985, MNHN, River Mahakam, Borneo, B. Dussart collection; *A. sewelli* (Steuer, 1934), USNM, Bangladesh, Meghna River, near Matlab, no. 378445; *A. sinensis* Shen & Lee, 1963, USNM, no. 216287.

Genus *Acartia* Dana, 1846

Acartia amboinensis Carl, 1907, UP, Red Sea; *A. biflosa* (Giesbrecht, 1881), USNM, Elson Lagoon surface, Alaska, no. 204748; *A. chilkaensis sittangi* Steuer, 1934, USNM, Burma, Sittang River, near Matlab, no. 378445; *A. clausi* Giesbrecht, 1889, UP, coast of Marseille, Mediterranean Sea; *A. danae* Giesbrecht, 1889, UP, Mediproduct 4, Station

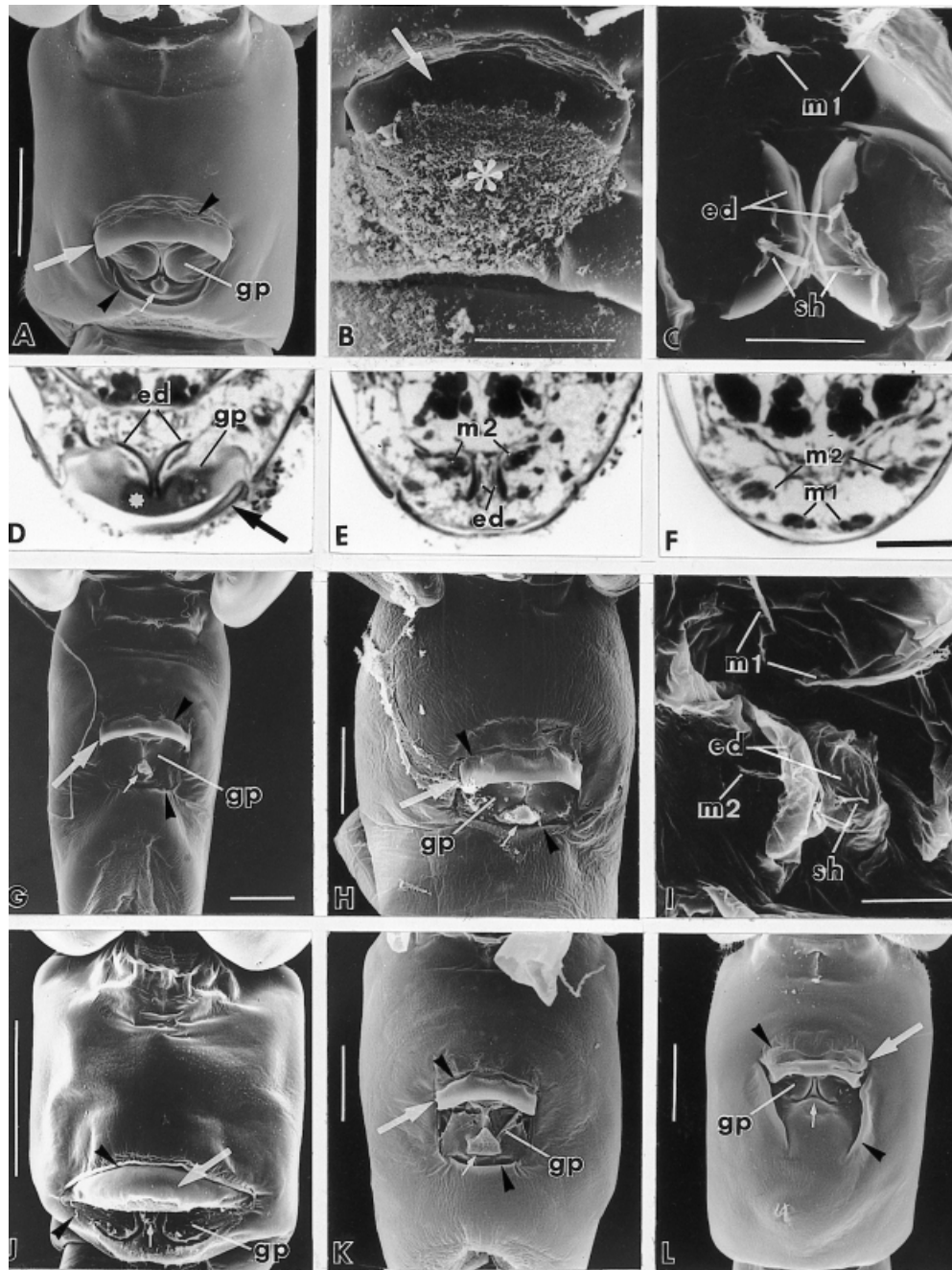


Figure 1. Scanning electron micrographs (A–C, G–L) and photomicrographs (D–F) of female genital structures in the genus *Acartiella*. (A–F) *A. keralensis*: (A) external ventral view of genital double-somite; (B) detail of external genital area in inseminated female. Note the spermatophoral products (asterisk) covering the gonoporal plates under the opercular pad (large arrow); (C) internal genital area; (D–F) serial transverse sections of genital double-somite (D, posteriormost; F, anteriormost). Note the spermatophoral products (asterisk) in D. (G) *A. gravelyi*, external ventral view of genital double-somite; (H & I) *A. major*; (H) external dorsal view of genital double-somite; (I) internal dorsal view of genital area. (J–L) external ventral view of genital double-somite of: (J) *A. nicolae*; (K) *A. sewelli*; and (L) *A. sinensis*. Abbreviations: ed, egg-laying duct; gp, gonoporal plate; m1, muscle of opercular pad; m2, muscle of egg-laying duct; sh, shell duct. Symbols: arrowhead, cuticular folds limiting the genital area; large arrow, opercular pad; small arrow, cuticular excrescence on the septum. Scale bars: A, G–H & J–L, 30 μ m; B & D–F, 20 μ m; C & I, 10 μ m.

21, 35°30'N 04°40'W; *A. fossae* Gurney, 1927, USNM, Tahiti, no. 107946; *A. hudsonica* Pinhey, 1926, USNM, Maizuru Bay, Japan, no. 231001; *A. italica* Steuer, 1910, Adriatic sea, Dubrovnik; *A. japonica* Mori, 1904, USNM, north Pacific Ocean, Wakasa Bay, Japan, no. 231030; *A. levequei* Grice, 1964, USNM, south Pacific Ocean, Galapagos Islands, no. 377398; *A. lilljeborgi* Giesbrecht,

1889, USNM, Punta Salinas, Ecuador, Guagaquil, no. 246748; *A. longiremis* Lilljeborg, 1853, MNHN-Cp1128, Rose collection, Polar Sea; *A. negligens* Dana 1849, USNM, North Atlantic Ocean, no. 269250; *A. omorii* Bradford, 1976, USNM, Maizuru Bay, Japan, no. 231001; *A. southwelli* Sewell, 1914; *A. tonsa* Dana, 1849, MNHN, no. 5–25 and R. Gaudy collection.

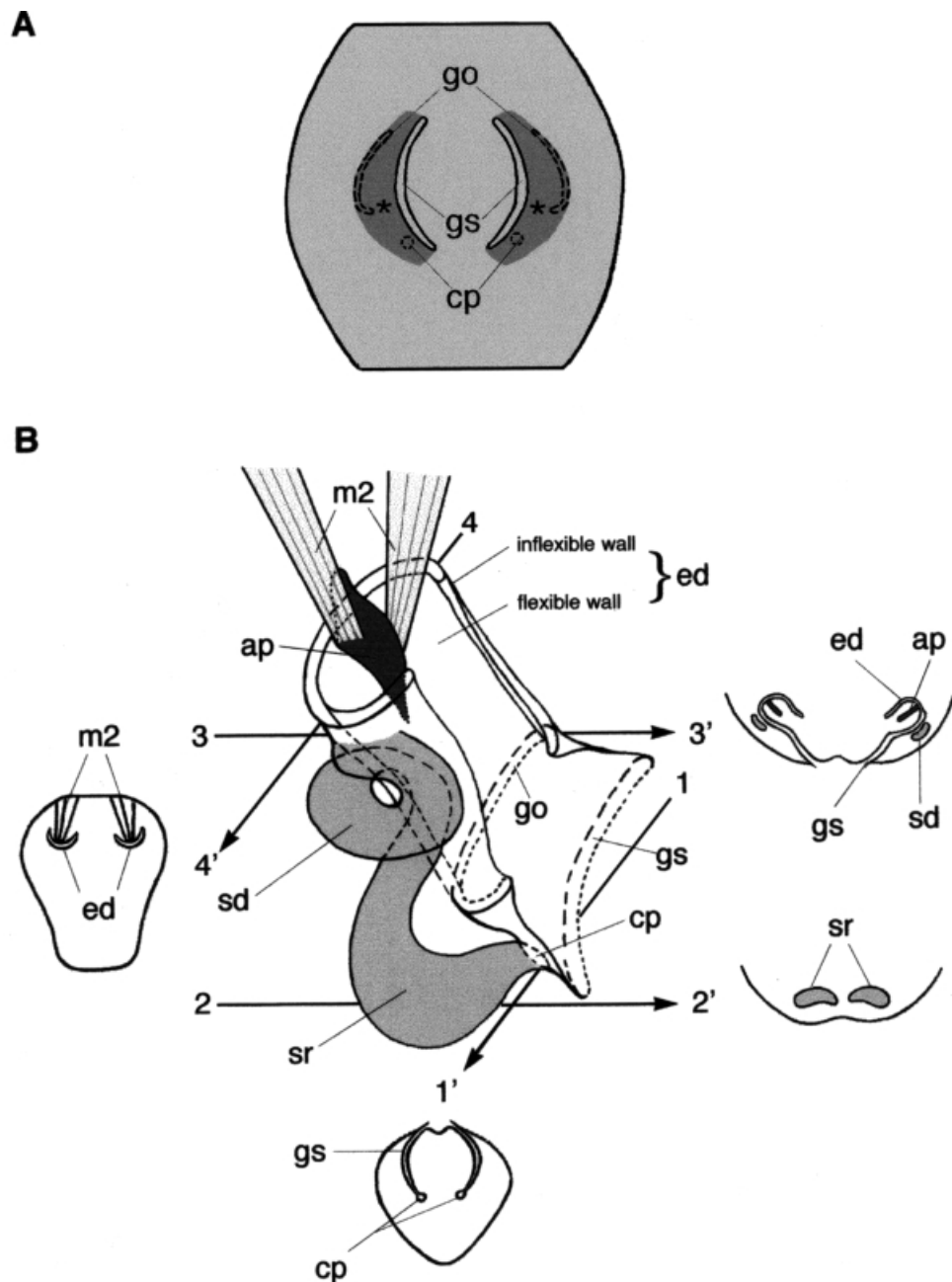


Figure 2. Schematic organization pattern of the genital area in *Acartia*. (A) External ventral view; (B) internal dorsal view of the left side and schematized frontal (1–1', 4–4') and transverse (2–2', 3–3') sections of the left and right genital structures. Note the opening position (arrowhead) of the seminal duct into the egg-laying duct. Abbreviations: ap, apodeme; cp, copulatory pore; ed, egg-laying duct; go, gonoporal slit; gs, genital slit; m2, muscle of the egg-laying duct; sd, seminal duct; sr, seminal receptacle. Symbols: *, lateral pad.

Genus *Paracartia* T. Scott, 1894

Paracartia josephinae Crisafi, 1974, Acquatina Lake, Italy;
P. grani Sars, 1904, MNHN, no. 361 and NHML, no. 27–47.

Genus *Paralabidocera* Wolfenden, 1908

Paralabidocera antarctica (Thompson, 1898), NHML, no. 82–91.

Methods

The specimens were studied using scanning electron microscopy (SEM) and light microscopy (LM).

Scanning electron microscopy

The specimens were prepared according to Cuoc et al. (1997). Female specimens were treated with 2% sodium hypochlorite to eliminate the organic remains on the surface of the

genital double-somite (external morphology), or to remove the soft parts, other than the genital structures after a dorsal cut into the genital double-somite (internal morphology, see Barthélémy et al., 1998a); then, they were rinsed, stained in an aqueous solution of chlorazol black, dehydrated in acetone and critical-point dried. Samples were then mounted on a stub and coated with gold or gold-palladium. Observations were carried out using a JEOL JSM 35C, or a Philips XL 30 ESEM scanning electron microscope.

Light microscopy

Specimens for light microscopy 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.

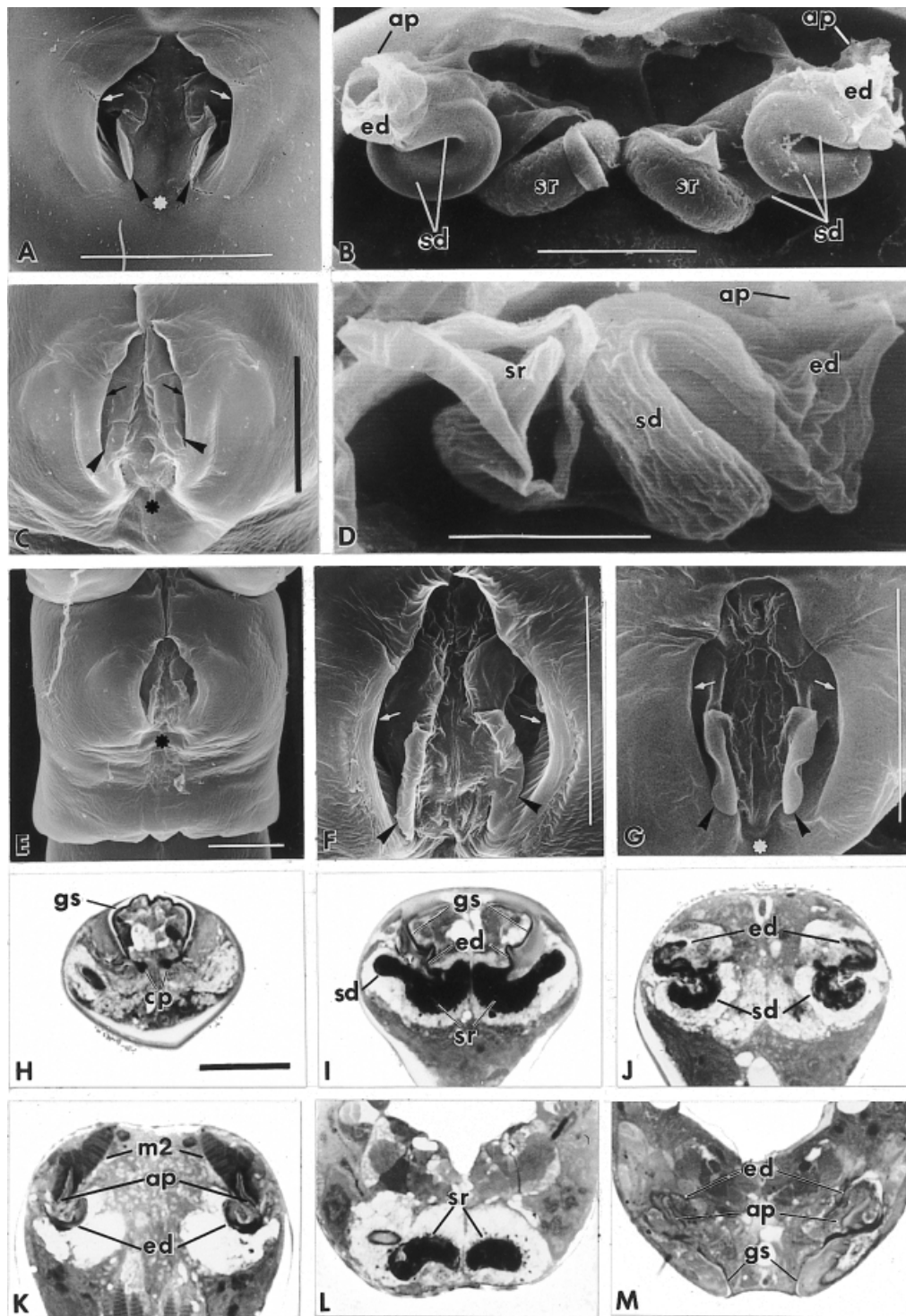


Figure 3. Scanning electron micrographs (A–G) and photomicrographs (H–M) of the female genital structures in subgenus *Acartiura*. (A–G) Genital area of: (A–B) *A. clausi*; (C–D) *A. hudsonica*; (E–F) *A. omorii*; and (G) *A. longiremis*. (A, C, E–G) External ventral view. Note the two lengthened genital slits (small arrows) each protected by a lamellar flap (arrowheads) and the medioventral position of genital area in *A. omorii* (E). (B, D) Internal dorsal view. Note the characteristic loop-like form of the seminal duct (sd). (H–M) Frontal (H–K) and transverse (L & M) serial sections of genital double-somite of *A. clausi* (H, ventralmost; K, dorsalmost; L, posteriormost; M, anteriormost). Abbreviations as for Figure 2. Symbols: *, fixation site of the spermatophore. Scale bars: A, C & E–M, 30 μm ; B, 20 μm ; D, 10 μm .

RESULTS

Genus Acartiella

This genus currently comprises ten species. This study focuses on six of them.

External genital area

The genital area is always located on the ventral face of the genital double-somite. It is situated on the posterior edge in *Acartiella keralensis* (Figure 1A) and *A. nicolae* (Figure 1J), and in medioventral position in *A. graveyi*

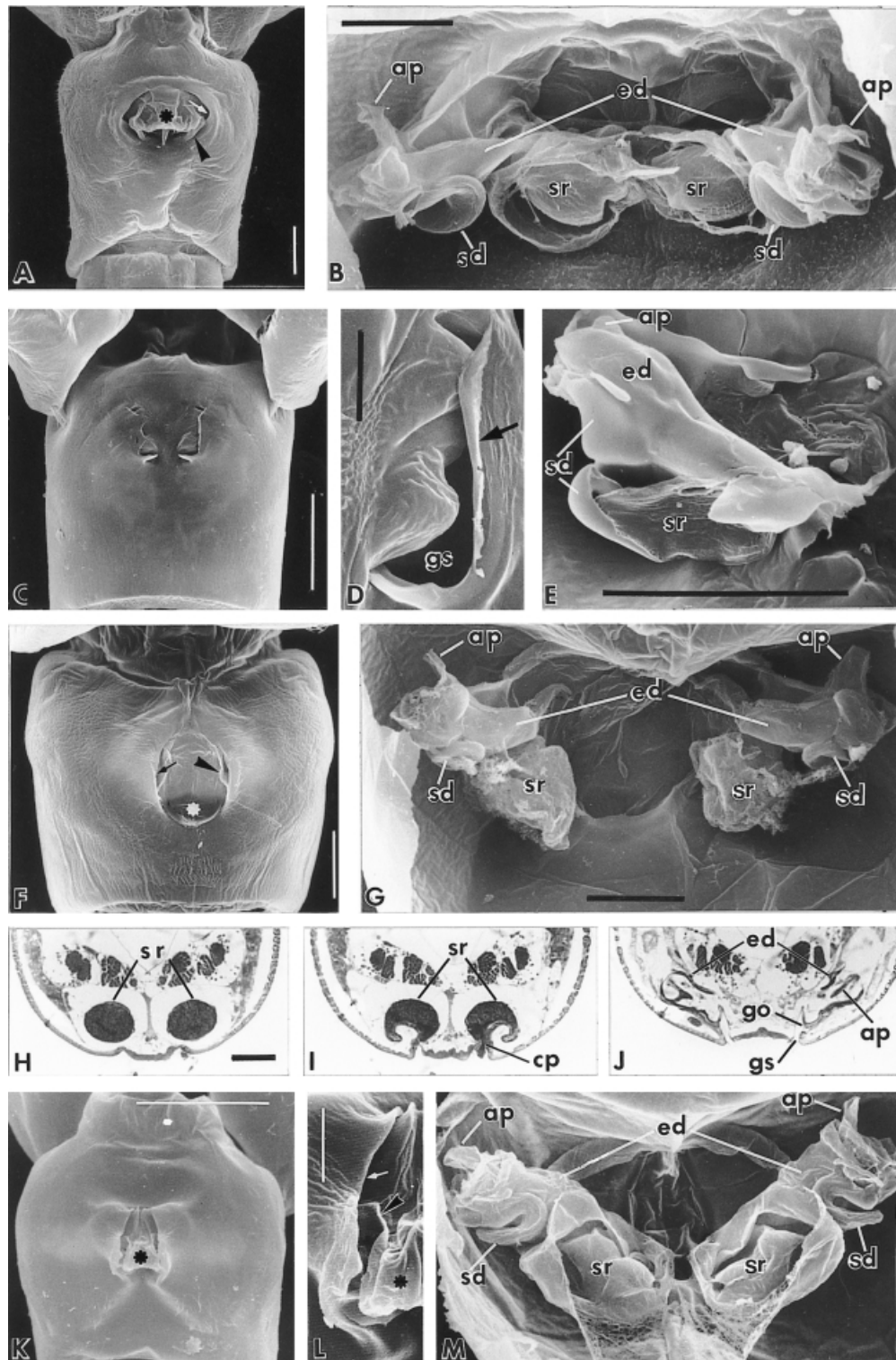


Figure 4. Scanning electron micrographs (A–G, K–M) and photomicrographs (H–J) of the female genital structures in subgenus *Acanthacartia*. (A–B) *A. biflosa*: (A) external ventral view of genital double-somite; (B) internal genital area. (C–D) *A. chilkaensis sittangi*, genital double-somite (C) with detail of the left genital slit (D). Note the epicuticular structure (large arrow) masking partially the genital slit (gs). (E) *A. italica*, internal genital area. (F–J) *A. levequei*, (F) ventral face of genital double-somite; (G) internal genital area; (H–J) transverse sections of genital double-somite (H, posteriormost; J, anteriormost). (K–M) *A. tonsa*, genital double-somite (K) with detail of the right genital slit (L), and internal genital area (M). Abbreviations as for Figure 2. Symbols: *, cuticular protuberance; arrowhead, lamellar flap; small arrow, genital slit. Scale bars: A, C, F & K, 30 μ m; B, E, G–J & M, 20 μ m; D & L, 5 μ m.

(Figure 1G), *A. major* (Figure 1H), *A. sewelli* (Figure 1K) and *A. sinensis* (Figure 1L). A cuticular fold, more or less prominent, limits the genital area on all, or part of its periphery. On the anterior edge, this fold constitutes the

hinge of the anterior opercular pad which is lenticular in *A. nicolae*, trapezoidal in *A. sinensis* and subrectangular in others. This pad does not completely cover the genital area, so the gonoporal slits and plates are easily visible

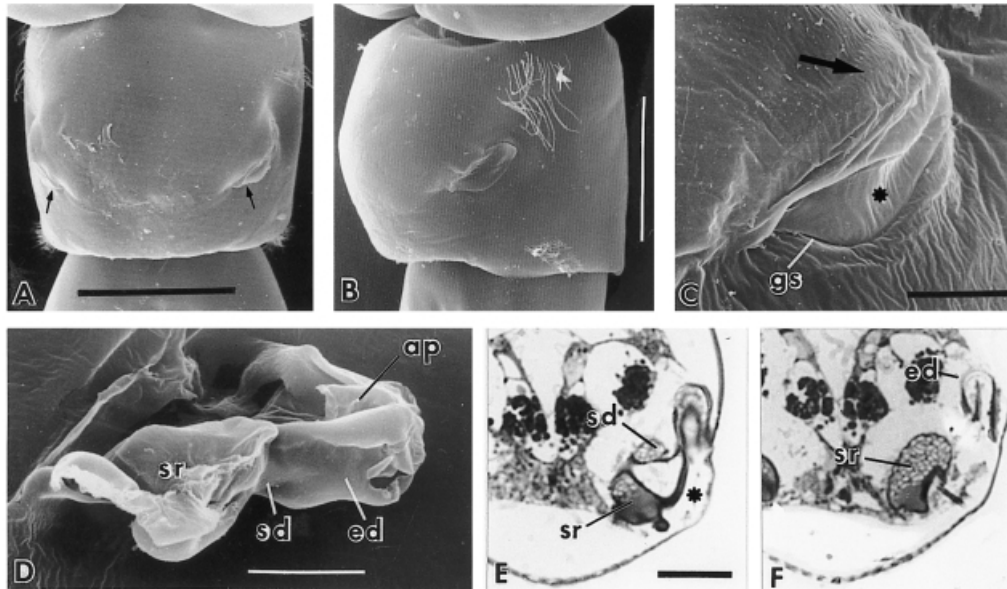


Figure 5. Scanning electron micrographs (A–D) and photomicrographs (E–F) of the female genital structures in subgenus *Acanthacartia* (*A. fossae*). (A–B) External ventral (A) and left lateral (B) views of genital double-somite. Note (A) the fragmented genital area with lateral genital structures (arrows). (C) Detail of the left genital structure. Note the anterior thickening (arrow) prolonged by a small cuticular flap similar to an operculum (*). (D) Internal dorsal view. Note the seminal duct (sd) opening in the egg-laying duct (ed) distally. (E–F) Transverse sections of genital double-somite (E, posteriormost; F, anteriormost). Abbreviations as for Figure 2. Scale bars: A & B, 50 μm ; E & F, 20 μm ; C & D, 10 μm .

except in inseminated females in which they are masked by the mass of spermatophoral products (Figure 1B & D). A small cuticular excrescence is always present between gonoporal plates on the septum.

Internal genital area

The egg-laying ducts are rather short, slightly cuticularized and very widened at their proximal extremity. Two small shell ducts arrive to the level of the convex wall of the egg-laying duct (Figure 1C & I). Two pairs of muscles are present, one inserted in the concave wall of the egg-laying duct and the other in the opercular pad (Figure 1C, E, F & I). There is no seminal receptacle.

Genus *Acartia*

This genus comprises more than 50 species; thus the great majority of the family representatives are commonly distributed in six subgenera. Sixteen species, representing five of these subgenera (we cannot obtain specimens belonging to the subgenus *Hypocartia*), were studied. In most of them, the genital area is located on the ventral face of the genital double-somite and presents a complex organization, but homogeneous and consistent with a common general pattern.

General pattern

In external ventral view (Figure 2A), the genital area is medioventral. Only two genital slits resulting from deep folds of the cuticle are visible. These lateral folds form two pads, the width of which corresponds to the depth of the genital slits. In the bottom of each genital slit is the posteriorly positioned copulatory pore, and anteriorly the gonopore corresponding to the gonoporal slit.

In internal dorsal view (Figure 2B), the area is characterized by paired seminal receptacles connected to two

egg-laying ducts (ed), in which the oviducts open. Each egg-laying duct forms a gutter-shaped structure with a double wall, a flexible one fused to the other which is inflexible. The inflexible wall is, partly, in direct continuity with the cuticle of the fold. The flexible wall is in continuity, under this fold, with the cuticle situated in the middle of the genital area. These two walls limit a narrow space corresponding to the gonoporal slit (go) that distally opens in the genital slit (gs) visible in external ventral view. Each egg-laying duct presents, on the concave part of its flexible wall, a lamellar apodeme (ap) on which a strong dilator muscle (m2) is inserted. The other extremity of this muscle is fixed on the antero-lateral wall of the genital double-somite. Each seminal receptacle forms a tubular sac more or less flattened and twisted, which goes along the egg-laying duct and opens in the genital slit by a copulatory pore (cp). This pore gives access to the most bulged part of the sac forming the so-termed receptacle, covered by a thin and slightly cuticularized wall. There are no well differentiated intermediary copulatory ducts. This receptacle is prolonged laterodorsally by a remarkable loop-shaped structure which constitutes a large seminal duct (sd), the wall of which is very cuticularized; the seminal duct opens in the proximal part of the egg-laying duct. The receptacles and seminal ducts are filled with seminal products after insemination.

The main variations of this general pattern reside in the relative disposition of the genital slits, either close (compact genital area), or distant (fragmented area), and in the shape more or less twisted of the seminal ducts.

Subgenus *Acartiura* Steuer, 1915

External genital area. It is compact and occupies a large medioventral zone, spherical in *A. clausi* (Figure 3A), oval in *A. hudsonica* (Figure 3C), *A. omorii* (Figure 3E & F) and

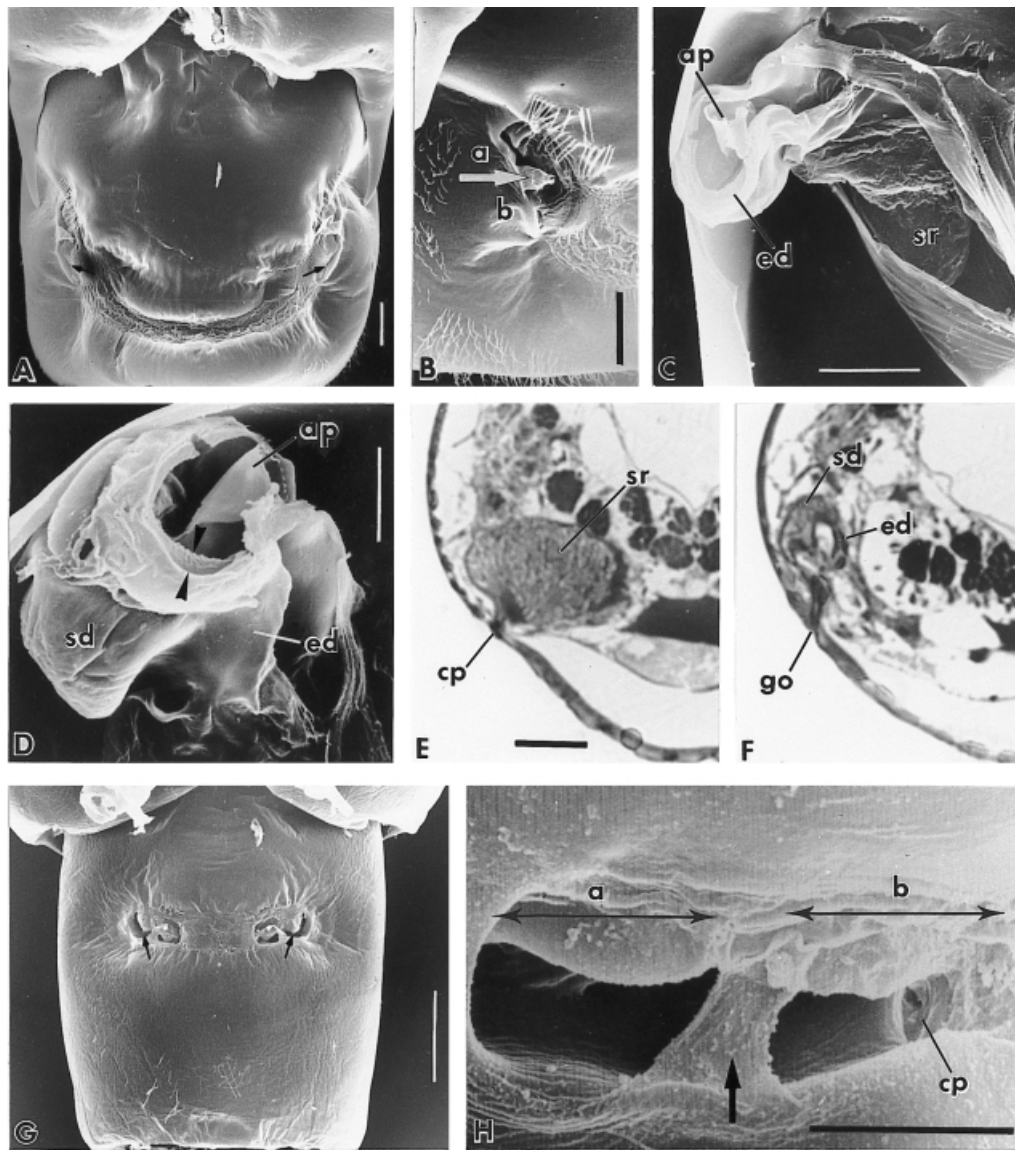


Figure 6. Scanning electron micrographs (A–D, G–H) and photomicrographs (E–F) of the female genital structures in subgenus *Acartia* (*Planktcartia*). (A–F) *A. danae*: (A) external ventral view of genital double-somite. Note the position of the genital structures (arrows); (B) detail of the right lateral genital structure. Note the thin cuticular lamella (arrow) dividing the genital slit in two fields; (C) internal genital area, left side. The seminal duct is not visible; (D) other detailed internal view of genital area (left side) showing the seminal duct (sd) very distended at this level and the two walls (arrowheads) of the egg-laying duct (ed); (E–F) transverse sections of genital double-somite (E, posteriormost; F, anteriormost). (G–H) *A. negligens*: (G) ventral face of the genital double-somite with lateroventral genital structures (arrows); (H) detail of the right genital slit with a median cuticular lamella (arrow) and copulatory pore (cp). Abbreviations as for Figure 2. a, gonoporal field; b, copulatory field. Scale bars: E–G, 20 μm ; A–C, 10 μm ; D & H, 5 μm .

A. longiremis (Figure 3G). It is bordered on almost all its periphery by a large and deep cuticular fold limiting two long genital slits, slightly curved. Each slit is protected on its laterointernal margin by a small lamellar expansion of the cuticle. Sections made in *A. clausi* show that copulatory pores (Figure 3H) are situated in the prolongation of the gonoporal slits at the distal extremity of genital slits. In all species examined under the genital area, a depression of the cuticle of the genital double-somite corresponding to the attachment site of the spermatophore was observed.

Internal genital area. Its organization is similar to that of the general pattern, with a central position of the seminal receptacle and a clearly divergent orientation of egg-laying ducts connected to the seminal ducts which form a

very pronounced loop (Figure 3B & D). Semi-thin sections, especially in *A. clausi* (Figure 3H–M), show that in inseminated females the seminal products fill the receptacles and seminal ducts (Figure 3I–J & L); they are also observed in the narrow lumen of each egg-laying duct (Figure 3J & K). Paired strong muscles (Figure 3K) inserted both on the lamellar apodeme of the flexible wall of each egg-laying duct (Figure 3B, D, K, M), and the anterolateral wall of the genital double-somite are present. In SEM, only the insertion site of these muscles on the ducts is visible (Figure 3B & D).

Subgenus Acanthacartia Steuer, 1915

External genital area. Its organization shows two variants. In the first, observed in five of the six species studied, the

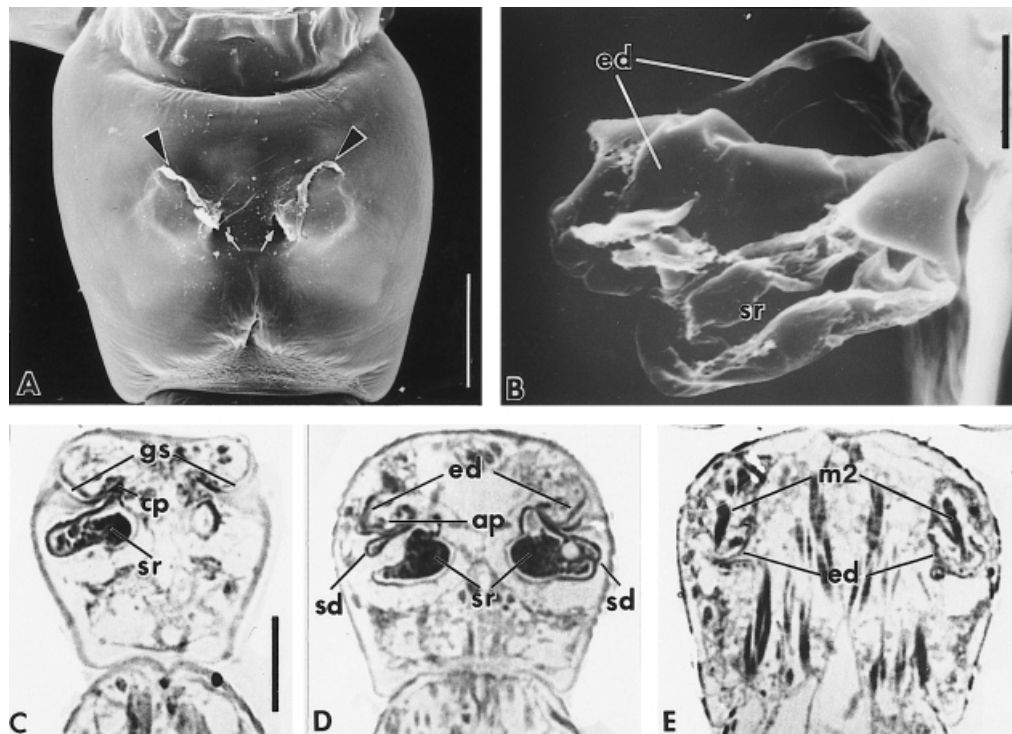


Figure 7. Scanning electron micrographs (A–B) and photomicrographs (C–E) of the female genital structures in subgenus *Euacartia* (*A. southwelli*). (A) External ventral view of genital double-somite. Note the epicuticle (arrowheads) covering the genital slits except the copulatory field (arrows). (B) Internal genital area, left side. (C–E) Frontal sections of genital double-somite (C, ventralmost; E, dorsalmost). Abbreviations as for Figure 2. Scale bars: A & C–E, 20 μm ; B, 5 μm .

compact genital area occupies a medioventral zone, with a limit conspicuous in *A. biflosa* (Figure 4A), *A. levequei* (Figure 4F) and *A. tonsa* (Figure 4K & L), and less definite in *Acartia chilkaensis sittangi* (Figure 4C) and *A. italica*, in which an epicuticular structure partially covers the genital slits (Figure 4D). In the three first species, a more or less thin small lamellar expansion protects the genital slits. Distally, between the genital slits, the cuticle forms a protuberance under which the spermatophore is fixed. The latter is marked in *Acartia biflosa* and *A. tonsa*, but rather discreet in *A. levequei*. In the second variant, only observed in *A. fossae*, the fragmented genital area is constituted by two lateroventral structures (Figure 5A & B), each formed with an arch-shaped pad, prolonged by a small cuticular flap in operculum-like form (Figure 5C), the free posterior rim of which limits the genital slit.

Internal genital area. In *Acartia biflosa* (Figure 4B), *A. chilkaensis sittangi*, *A. italica* (Figure 4E), *A. levequei* (Figure 4G & H) and *A. tonsa* (Figure 4M), the internal genital area presents a similar configuration to the general pattern, with voluminous seminal receptacles and seminal ducts in a well-individualized loop-like form, but with a diameter smaller than in species of the subgenus *Acartiura*. The copulatory pores and gonoporal slits are only visible in semi-thin sections (Figure 4I & J). In *A. fossae* (Figure 5D), the anatomical relationships between egg-laying duct and receptacle are slightly different and modify the configuration of the genital area (Figure 5D–F). Indeed, contrary to the previous cases, the seminal ducts distally fuse with the egg-laying ducts, just before their opening in the genital slit. These seminal

ducts are therefore very short and not twisted as in the other species.

Subgenus Acartia Dana, 1846 (=Planktarticaria Steuer, 1915; see Wilson 1966)

External genital area. It presents a fragmented configuration in the two species studied. Genital slits are lateroventral with a vertical orientation in *A. danae* (Figure 6A & B), and ventral with a horizontal orientation in *A. negligens* (Figure 6G & H). In the two species, a thin cuticular superficial lamella separates the genital slits in two fields, the gonoporal and the copulatory fields. In *A. negligens*, the copulatory pore is localized at the extremity of the copulatory field.

Internal genital area. In the two species, it differs from the general pattern by the morphology of the seminal ducts. Indeed, the latter, slightly twisted, does not form a marked loop but a simple bend. Furthermore, their distal part is dilated in a pocket in large contact with the proximal part of the egg-laying duct (Figure 6D & F). The seminal receptacles form voluminous sacs, conical in SEM and ovoid in transverse section (LM) in *A. danae* (Figure 6C & E).

Subgenus Euacartia Steuer, 1915

External genital area. In *A. southwelli*, one of the two species of this subgenus, the area is fragmented and located on the ventral face of the genital double-somite (Figure 7A). Genital slits are almost entirely covered by an epicuticle. Only an aperture at the level of the copulatory pores is visible.

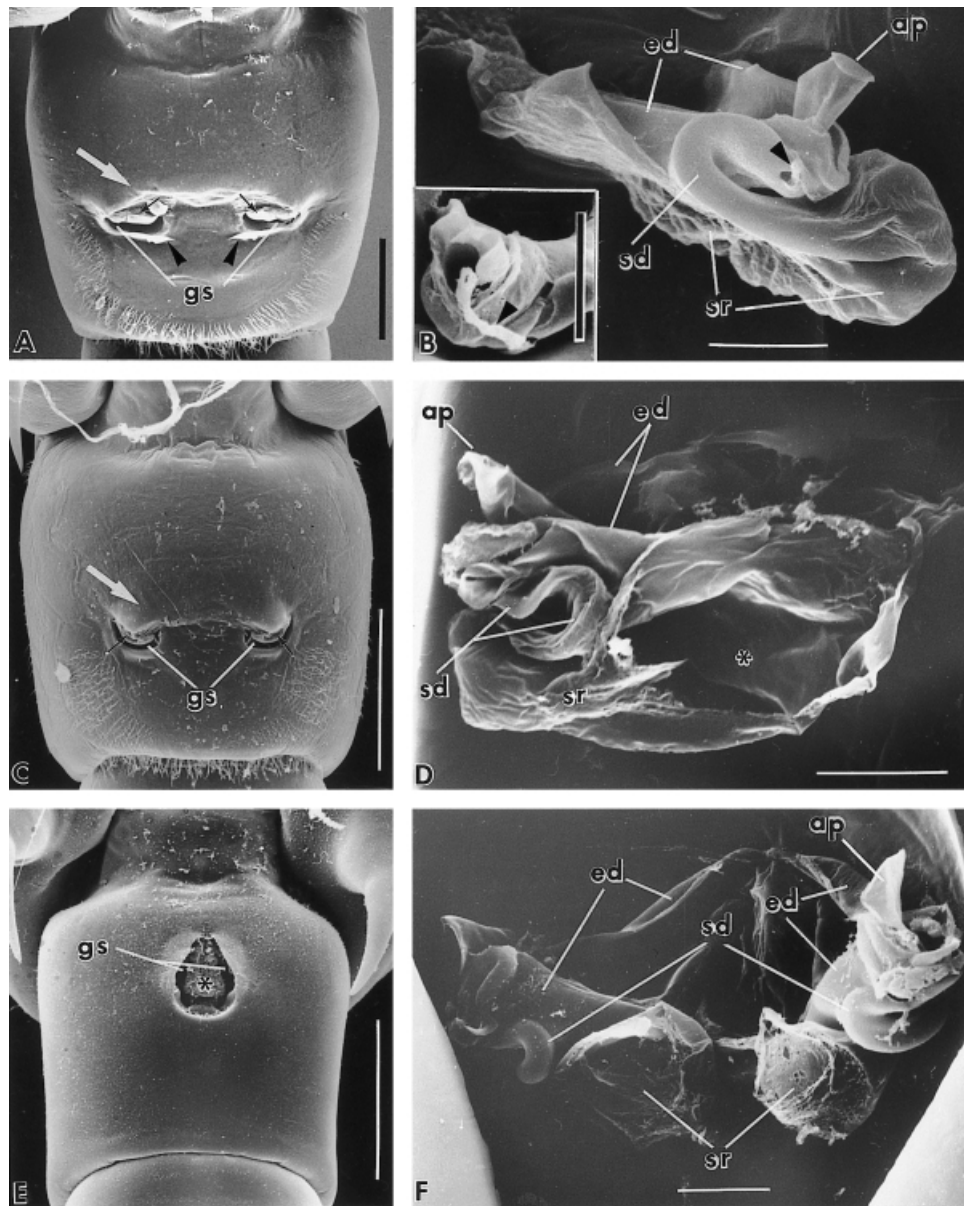


Figure 8. Scanning electron micrographs of the female genital structures in subgenus *Odontacartia*. (A–B) *A. amboinensis*: (A) genital double-somite. Note the thin cuticular lamella under each genital slit (arrowhead); (B) internal genital area (right side) with detail of the proximal part of the egg-laying duct (inset) showing a small duct (arrowhead) similar to a shell duct. Note the typical bend-shaped seminal duct (sd). (C–D) *A. japonica*: (C) genital double-somite; (D) internal genital area (left side). Note the ventral wall (*) of the rended seminal receptacle (sr). (E–F) *A. lilljeborgi*: (E) genital double-somite. Note the cuticular protuberance (*) between the genital slits; (F) internal genital area. Abbreviations as for Figure 2. Symbols: large arrow, anterior pad; small arrow, opercular pad. Scale bars: A, C & E, 50 μm ; B, D & F, 10 μm .

Internal genital area. The seminal receptacles are clearly prolonged distally (Figure 7B–D) and their seminal ducts, only identified on semi-thin sections, are rather flattened and seem to open in the proximal part of the eggs-laying ducts (Figure 7D). The apodeme of the egg-laying ducts, although not observed in SEM, is very visible with its associated muscles in semi-thin sections (Figure 7E).

Subgenus Odontacartia Steuer, 1915

External genital area. It occupies an almost medioventral position in the three species studied. In *A. lilljeborgi* (Figure 8E), it is compact, and well-delimited all around its periphery by a deep cuticular fold; the genital slits are separated from each other by a median triangular cuti-

cular protuberance. In *A. amboinensis* (Figure 8A) and *A. japonica* (Figure 8C), the genital area is fragmented, but the two genital slits are limited by a common anterior pad that extends to a small opercular flap at the level of each of them. In addition, in *A. amboinensis*, a thin cuticular lamella is situated under each genital slit in the neighbouring of the copulatory pores.

Internal genital area. Its organization is identical to the general pattern in *A. lilljeborgi* (Figure 8F), with a characteristic loop-shaped structure of the seminal duct as in subgenus *Acartiura*. In *A. amboinensis* (Figure 8B) and *A. japonica* (Figure 8D), the egg-laying duct—seminal receptacle complex appears clearly stretched. Each seminal receptacle goes up laterodorsally at the level of

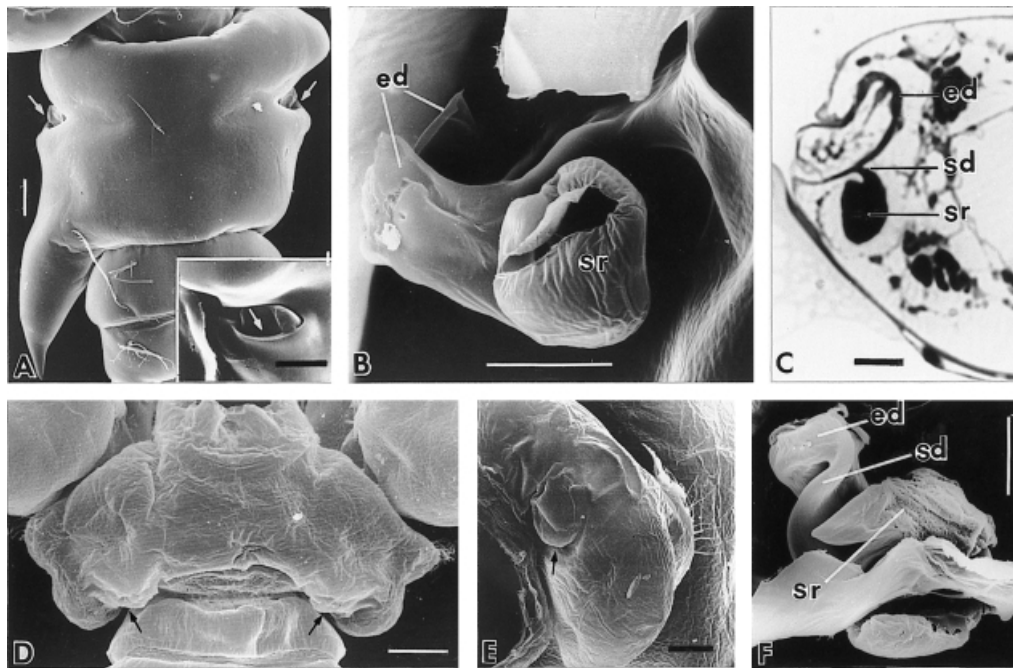


Figure 9. Scanning electron micrographs (A–B, D–F) and photomicrograph (C) of the female genital structures in genus *Paracartia*. (A–C) *P. josephinae*: (A) external ventral view of genital double-somite showing lateral position of genital slits (arrows) and detail of the left one (inset); (B) left side of the internal genital area. (C) Transverse section of genital double-somite (left side). Note the short seminal duct opening in the egg-laying duct distally. (D–F) *P. grani*: (D) ventral face of the genital double-somite showing lateroposterior position of the genital slits (arrows); (E) an other view of the left genital slit; (F) internal dorsal view of the left side of the genital area. Abbreviations as for Figure 2. Scale bars: D, 20 μm ; A–C & E–F, 10 μm .

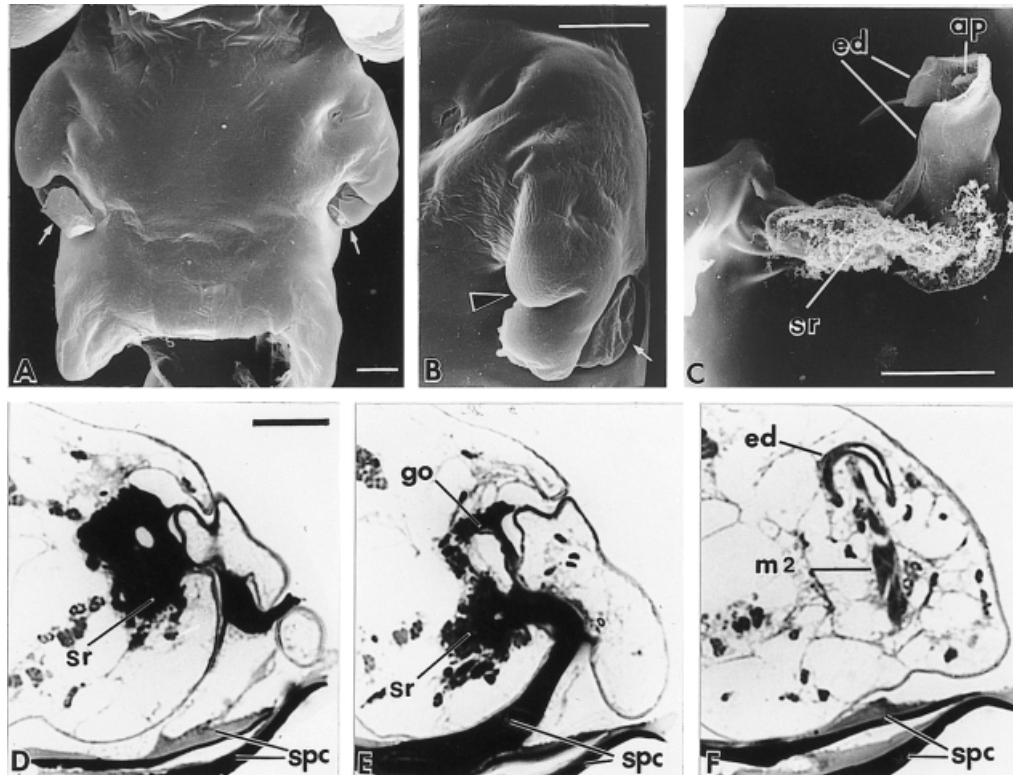


Figure 10. Scanning electron micrographs (A–D) and photomicrographs (E–F) of the female genital structures in genus *Paralabidocera* (*P. antarctica*). (A) External ventral view of genital double-somite showing lateral position of genital slits (arrows). (B) Left lateral view. Note position of the genital slit (arrow) under a bilobed expansion (arrowhead). (C) Internal dorsal view of the right side of the genital area. (D–F) Transverse sections of genital double-somite (D, posteriormost; F, anteriormost). Abbreviations as for Figure 2. spc, spermatophoral complex. Scale bars: 20 μm .

the egg-laying duct. Consequently, the seminal duct is shorter and simple bend-shaped. Finally, in *A. amboinensis* only, a small duct, probably corresponding to a shell duct, is connected to the egg-laying duct at the same level as the seminal duct (Figure 8B).

Genus *Paracartia*

Two species, *P. josephinae* and *P. grani*, are studied among the six of the genus.

External genital area

It presents a fragmented configuration. In *Paracartia josephinae* (Figure 9A), the genital slits are mediolateral. In *P. grani* (Figure 9D & E), they are located on the internal face of the lateroposterior extensions of the genital double-somite and closed by a small cuticular flap in operculum-like form.

Internal genital area

In *P. grani* (Figure 9F), it exhibits a similar configuration to the general pattern described for species of the genus *Acartia*. On the other hand, in *P. josephinae* (Figure 9B & C), the massive seminal receptacles are prolonged by short seminal ducts (Figure 9C) opening into the distal part of the egg-laying ducts.

Genus *Paralabidocera*

The study concerns *P. antarctica*, one of the three species of the genus.

External genital area

It is fragmented (Figure 10A & B). The mediolateral genital slits are lodged under bilobed cuticular expansions.

Internal genital area

It shows the most marked differences compared to the basic organization described in the genus *Acartia*. Indeed, no seminal duct was identified either in SEM (Figure 10C), or in semi-thin sections (Figure 10D–F), and the seminal receptacles appear to open directly into the distal part of the egg-laying ducts. The paired dilator muscles of the egg-laying ducts are very visible in transverse sections (Figure 10F).

DISCUSSION

Comparative functional morphology

The organization of the genital area in Acartiidae is related to two principal types: (1) the *Acartiella*-type characterized by a compact area, devoid of seminal receptacles; (2) the *Acartia*-type, characterized by either a compact or fragmented area, always provided with receptacles and paired copulatory pores.

In the *Acartiella*-type, the organization is identical to that previously described in the diaptomoids *Hemidiaptomus ingens* and *Mixodiaptomus kupelwieseri* (Cuoc et al., 1989) and in the sulcanid *Sulcanus conflictus*, characterized by loss of the opercular pad (Barthélémy et al., 1998a). It is also close to that described in other diaptomoids (Barthélémy et al., 1998a) and in some Ridgewayiidae (Barthélémy et al., 1998b), devoid of seminal receptacles,

but in which a genital operculum, a distal extension of the opercular pad of *Acartiella* (Figure 1A, G & H, J & K), completely protects the external area and limits a more or less deep space, the genital atrium. In the two cases, the external area serves as a storage site for the spermatophoral products, forming a mass either external in the non-operculated species, or semi-external raising the genital operculum in operculated forms. An operculated area, devoid of seminal receptacles, also characterizes all the diaptomids Paradiaptominae, but without storage of seminal products at its level (Defaye et al., unpublished data).

In the *Acartia*-type, the genital area presents an organization characterized by complex anatomical relationships between the structures implicated in laying and storage and transfer of seminal products. Seminal ducts joining the egg-laying ducts and seminal receptacles show a remarkable morphology that was used by Steuer (1923) in the description of the subgenera *Acartiura*, *Acanthacartia* and *Odontacartia*. However, Steuer noticed several transitional species between these subgenera (*A. plumosa*, *A. negligens* and *A. danae*) and SEM observations confirm these trends. This *Acartia* pattern may be compared to the Arietelloidea (Ohtsuka et al., 1994; Cuoc et al., 1997). In this superfamily, the fragmented area is provided with copulatory pores and seminal receptacles but it always represents an open area, i.e. the well-individualized gonopores and copulatory pores are never masked under a cuticular fold as in Acartiidae. Some variations are however observed in the relative disposition of the gonopores and copulatory pores in the Arietelloidea: they are either clearly separated in most species, according to different trends, or almost adjacent in the genus *Scutogerulus*, with a configuration close to the hypothetical ancestral pattern proposed by Huys & Boxshall (1991). In the Acartiidae of the *Acartia*-type, this adjacent disposition of the gonopores and copulatory pores is constant in the species studied. Indeed, this disposition is observed both in species with compact (ten species) or slightly fragmented (five species) areas and in the four only species with an area clearly fragmented attesting to an important lateral migration of the genital slits. This migration of the genital orifices corresponds to an evolutionary trend also occurring in the Arietelloidea. From a functional viewpoint, this configuration is associated with a simultaneous insemination of the two receptacles due to the bifid structure of the spermatophore (Steuer, 1923); its fixation site is situated between and under the copulatory pore fields, at the level of a well-delimited site in several species (Figures 3 & 4) and is probably easily recognized by the male partner. In the species with very lateral copulatory pores, the same modes of insemination exist due to an adequate coupling structure of the spermatophore that surrounds more or less entirely the genital double-somite (Steuer, 1923). These modes differ from those observed in metridinid arietelloids of the genus *Gaussia* and *Metridia* in which the insemination of the two receptacles implies two matings (Cuoc et al., 1997). As in the Metridinidae, the Acartiidae of the *Acartia*-type probably display the presence of a thin epicuticular membrane closing the copulatory pores, a typical character of non-inseminated females. Indeed, the thin residual membranes observed at the level of the genital slits of several species (Figures 4C

& D, 6B & H, 7A, 8A) could belong to membranes closing them in non-inseminated females.

In the two types *Acartiella* and *Acartia*, the semicircular configuration of the egg-laying duct represents the constant characteristic of the genitalia in all the calanoids studied until now. This configuration corresponds to the closed state of the ducts, the opening of which is due to associated muscles that constitute the basic musculature of the genital structures. Indeed, as probably in all the Arietelloidea, these egg-laying duct muscles are the sole present in the *Acartia*-type, a significant character by comparison with the musculature of *Acartiella* and other calanoids provided with two (genera *Acartiella*, *Centropages*, *Sulcanus*, *Temora* in Diaptomoidea and *Ridgewayia* in Pseudocyclopoidea) or three pairs of muscles (genera *Anomalocera*, *Boeckella*, *Candacia*, *Eurytemora*, *Hemidiaptomus*, *Labidocera*, *Mixodiaptomus* and *Pontella*). The additional muscles are necessary for the mobility of the opercular structure and probably, in the second group, for the storage or the discharge of the seminal products in the atrial structure. Opening of the egg-laying ducts occurs only during the extrusion of oocytes, according to a specified pattern (Cuoc et al., 1989, 1997). Consequently, in *Acartiella*, in which the ovisacs are apparently absent, the fertilization and eggs dropping probably occur in the same timing as in most other diaptomoids (Barthélémy et al., 1998a). By contrast, in *Acartia*, opening of the egg-laying ducts causes a large communication with the seminal ducts and allows the oocytes-spermatozooids contact before extrusion of the oocytes out of the gonopores. This synchronization of egg-laying and seminal duct opening has already been noted in Metridinidae but, in this case, the contact oocytes-spermatozoa only occurs at the exit of the gonopores (Cuoc et al., 1997). As in this family, several successive clutches are fertilized by seminal products stored during initial insemination (Heinle, 1970; Ianora et al., 1996). Nevertheless, the remating is often considered necessary in *Acartia* to permit the realization of a female total reproductive potential (Parrish & Wilson, 1978; Uye, 1981). Indeed, considering the large contact zone between the seminal and egg-laying ducts, it is probable that an important quantity of the seminal products stored is carried away at the time of extrusion of the oocytes, hence requiring several matings. The last structure generally present in all the calanoids, i.e. the shell ducts arising from glands located in the last thoracic segment and opening in egg-laying ducts, was only observed in *Acartiella* and in *Acartia amboinensis* (Figure 8B). However, a study using transmission electron microscopy will be necessary to confirm or invalidate the absence of these shell ducts and their associated glands.

Genital structures and taxonomy

Previous studies (Ohtsuka et al., 1994; Cuoc et al., 1997; Barthélémy et al., 1998a) have shown that genital structures represent valid characters at different taxonomic levels and permit a more complete approach to the phylogenetic relationships. Our results confirm the systematic interest of these structures which must be examined with attention in the framework of the current classification of acartiids based on the previous investiga-

tions of Steuer (1915, 1923) that led to the revision of the genus *Acartia*. Indeed, Steuer included all the acartiids in the genus *Acartia* that he divided into two groups, the arostratae (rostral filaments absent) and the rostratae (rostral filaments present). Consequently, he considered the genera *Acartiella* Sewell (1914) and *Paracartia* T. Scott (1894) as subgenera and created six new subgenera: *Acartiura*, *Acanthacartia*, *Planktcartia*, *Euacartia*, *Hypoacartia* and *Odontacartia*. The subgenus *Acartiura* comprised all arostratae not belonging to the subgenus *Acartiella*, while the five others, *Acanthacartia*, *Planktcartia*, *Euacartia*, *Hypoacartia* and *Odontacartia* comprised all the rostratae (except *Paracartia*). The selection criteria of the five latter were essentially the antennule morphology and the shape and ornamentation of the last thoracic segment. Since then, this classification has often been criticized but never been really questioned. Especially, Gurney (1931) restored the two genus *Acartiella* and *Paracartia*, but preserved the six subgenera in the genus *Acartia*. Later, Bowman (1965) considered that Steuer's primary division could be abandoned because of the discovery of an arostratae population of *A. lilljeborgi* (subgenus *Odontacartia*). More recently, Madhupradap & Haridas (1994) also speculated about the validity of the six subgenera created by Steuer. Finally, we have referred to Razouls' classification (1995), which represents the most complete inventory of species of the family, preserving the genus *Acartiella* and the six subgenera in the genus *Acartia*. Our results provide supplementary elements in favour of a revision of the current classification.

The morphology of the external genital area and the absence of seminal receptacles very clearly isolate species of the genus *Acartiella* Sewell (1914), the validity of which is thus confirmed. These important differences between the female genital structures of the *Acartiella* and those of the other acartiids could appear as an element in favour of removing *Acartiella* from Acartiidae. Therefore, Bradford (1976) pointed out that *Acartiella* should remain in the Acartiidae especially because the exopods of swimming legs 2–4 are devoid of articulated outer edge spines, as in the other Acartiidae. Thus, taking into account these data, it seems necessary that the *Acartiella* species preserve their genus position, distinct from the other Acartiidae genera, and contrarily to the classification recently proposed by Mauchline (1998).

Among the majority of species of the genus *Acartia*, we can distinguish two main groups, based on the external morphology of the genital area on one hand, and the seminal duct morphology on the other. The first one unites ten species, viz., *A. clausi*, *A. hudsonica*, *A. omorii* and *A. longiremis* of the subgenus *Acartiura*, *Acartia bifilosa*, *A. chilkalensis*, *A. italica*, *A. levequei* and *A. tonsa* of the subgenus *Acanthacartia* and *A. lilljeborgi* of the subgenus *Odontacartia*, exhibiting a compact external area, with well-defined structure for each of them. Furthermore, all exhibit a very homogeneous configuration of their internal area with seminal ducts in characteristic loop-like form. According to Steuer (1923), this loop is visible in ventral view after clearing the specimens. The duct seems less voluminous only in the subgenus *Acanthacartia* species and thus it is slightly visible in lateral view (Steuer, 1923). The similitude in the organization of the genital structures of all these species confirms the close

relationships between the subgenera *Acartiura* and *Acanthacartia* noted by Steuer (1923) and more recently emphasized by Bradford (1976). Thus, contrarily to the data of these authors, the morphology of the genital complex does not justify the distinction of two subgenera. The second group comprises *Acartia danae*, *A. negligens* of the subgenus *Acartia* (*Planktacartia*), *A. amboinensis*, *A. japonica* of the subgenus *Odontacartia* and *A. southwelli* of the subgenus *Euacartia*; these species offer a fragmented external area with ventrolateral genital slits, and seminal ducts, simple bend-shaped opening in the proximal zone of the egg-laying ducts, as in the precedent group. Therefore, the genital anatomy does not justify the distinction between the different subgenera.

As for acartiids of the second group, *Paracartia grani* is characterized by a fragmented area but the internal area is provided with loop-shaped seminal ducts opening directly in the proximal zone of the egg-laying duct. This organization, at least for the external area with latero-posterior genital slits, is analogous to that observed in *Paracartia africana* and *P. latisetosa* (Steuer, 1923) and could constitute an important characteristic of the genus. In this case, the species *Paracartia josephinae* (with medio-lateral genital slits and an unlooped seminal duct with a distal opening into the egg-laying duct) would be removed from this taxon. It may be noted that Belmonte (1998) already observed the presence in this species of distinctive characters such as the asymmetry of the genital segment and the presence of resting eggs with typical spines.

In the genus *Paralabidocera*, the sole examined species also presents a fragmented area with two lateral genital slits, but internally there is no seminal duct and the receptacle seems to open distally in the egg-laying duct. This anatomical peculiarity is important; nevertheless, because of the absence of data on the internal genital area of the two other species of the genus (*Paralabidocera grandispina* Waghorn (1979) and *P. separabilis* Brodsky & Zvereva (1908)), we cannot evaluate its taxonomical value. By contrast, the presence of paired genital orifices situated on both side of the genital segment is a constant character of the genus. The three species of the genus *Paralabidocera* therefore present genital structures clearly differing from those of the Pontellidae. Thus, in spite of the resemblance with the genus *Labidocera* (Pontellidae) observed by Wolfenden (1908), the comparison with the genital structures in Pontellidae (Barthélémy et al., 1998a) confirms that the genus *Paralabidocera* must be referred to Acartiidae (Vervoort, 1951; Brodsky & Zvereva, 1976).

Finally, *Acartia fossae* (subgenus *Acanthacartia*), as *Paracartia josephinae*, is characterized by a fragmented area, with very lateral genital slits, and a short seminal duct without loop opening into the distal zone of the egg-laying ducts. According to these morphological characters, both species are close to *Paralabidocera antarctica* but differ from the latter especially by the structure of the fifth pair of female legs, biramous in *Paralabidocera*.

In conclusion, the comparative study of the genital area of the Acartiidae, the systematic of which is yet debated, makes it necessary to review this family. However, current research on the genital structures within the superfamily Diaptomoidea and closest relatives

might permit a proposal of their main evolutionary trends and relationships. Finally, the acquisition of new data using genetic markers (Bucklin et al., 1995; Cervelli et al., 1995), may give important information for a better comprehension of the phylogenetic relationships within this family.

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