

## Larval Development in *Peltogasterella* Studied by Scanning Electron Microscopy (Crustacea: Cirripedia: Rhizocephala)

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**Abstract.** SEM and light microscopic studies on the larvae of the rhizocephalan barnacles *Peltogasterella gracilis* and *P. sulcata* reared in the laboratory revealed the presence of 5 naupliar instars, the newly found stage being instar 3. It resembles instar 2 but is larger and has a body slightly tapering towards the posterior end, a reduced subapical (preaxial) seta on the antennule, and both transverse grooves and a specific denticle ornamentation on the hind body of female larvae. In cultures, the larvae of both species develop into cyprids within 4–5 days after release. *Peltogasterella* nauplii have a size comparable to that seen in *Sacculina* and *Peltogaster*, but they are much smaller than the larvae of the cold-water *Briarosoccus*. The nauplii of *Peltogasterella* are lecithotrophic and have limb setation and other feeding structures highly reduced. *Peltogasterella* nauplii have a cuticular flotation collar encircling the body, but it is very small, delicate, and almost smooth. It therefore differs from the homologous, but much larger, and heavily ornamented flotation collars seen in nauplii of *Peltogaster* and *Briarosoccus*. A characteristic feature in *Peltogasterella* nauplii is the single subterminal seta at the distal end of each frontolateral horn, whereas nauplii of *Briarosoccus* and *Peltogaster* have two such setae. The antennular basipod carries a short additional seta, which represents the only remaining rudiment of gnathobases. Nauplii of *Briarosoccus* and *Peltogaster* have a homologous, but significantly longer seta, whereas *Sacculina* nauplii lack this seta altogether. The nauplii of *P. gracilis* and *P. sulcata* are very similar in size and morphology, but those of *P. sulcata* differ by somewhat longer frontolateral horns and furcal spines. Another distinctive feature of *P. gracilis* nauplii is the ventral ornamentation of the hind body that shows different pattern at different stages of development and reflects an internal development of segments. This ornamentation is only weakly pronounced in *P. sulcata* nauplii. Comparison with larval development in *Briarosoccus tenellus* leads to the conclusion that the rhizocephalan ground pattern comprises six naupliar stages just as in other cirripedes (e.g., Thoracica). Comparison of lattice organ morphology indicates a sister group relationship between the families Peltogastridae and Lernaeodiscidae. Our study highlights the value of SEM revealed larval characters and of characters other than appendage setation as being important in rhizocephalan phylogeny.

**Key words.** Nauplius, cypris, larvae, morphology, SEM, lecithotrophy, cirripedes, rhizocephalans, parasitic barnacles.

### 1. INTRODUCTION

The Cirripedia Rhizocephala (parasitic barnacles) is a sister group of free-living Cirripedia Thoracica. In the basic development pattern ('ground pattern'), both taxa have a pelagic larval development comprising six naupliar instars and a terminal cypris larva, although some species have secondarily lost the nauplii. Numerous papers have focussed on naupliar development in the Thoracica and some general evolutionary patterns can now be discerned (ANDERSON 1994; GRYGIER

1994; KORN 1995; NEWMAN & ROSS 2001). In contrast, there exist only a few observations on larval development in the Rhizocephala (e.g., ICHIKAWA & YANAGIMACHI 1957; YANAGIMACHI 1961a, b; HAWKES et al. 1985; WALKER 1988; COLLIS & WALKER 1994; WALOSZEK et al. 1996; KORN et al. 1999).

The use of scanning electron microscopy is necessary to give a complete morphological description of small crustacean larvae. Several studies have recently used this technique to study cyprids over a wide range of rhizocephalans (WALKER 1985; GLENNER et al. 1989;

JENSEN et al. 1994a, b), but SEM based descriptions of naupliar stages exist only for *Sacculina carcinis* Thompson, 1836 (Sacculinidae) and *Briarosaccus tenellus* Boschma, 1970 (Peltogastridae) (WALKER 1985; COLLIS & WALKER 1994; WALOSSEK et al. 1996). The numerous differences between the nauplii of these two species from separate families and the studies on cyprids show the potential of larval characters in analysing the phylogeny and adaptive radiation of the Rhizocephala. To further expand the database on rhizocephalan larvae, we use light and scanning electron microscopy to examine the development in two congeneric species of Peltogastridae, *Peltogasterella gracilis* (Krüger, 1912) and *P. sulcata* (Lilljeborg, 1859), both parasitizing hermit crabs (Decapoda: Paguroidea) and widely distributed in the Pacific and Atlantic respectively.

## 2. MATERIAL AND METHODS

We collected hermit crabs of the species *Pagurus ochotensis* Brandt, 1851 and *P. middendorffii* Brandt, 1851 parasitized with adult *P. gracilis* during August 1996 and 1998 in Vostok Bay, Sea of Japan. The closely related European species, *P. sulcata*, was collected from parasitized hermit crabs *Pagurus cuanensis* Thompson, 1843 and *Anapagurus chiroacanthus* (Lilljeborg, 1856) on the western coast of Sweden.

We carefully removed mature externae from the abdomen of the hosts and placed them into Petri dishes filled with filtered sea water. When larvae emerged we transferred them into 1–2 liter glass vessels filled with filtered sea water and reared them at 20 °C with a change of water every day. The development of *P. gracilis* lasted about four days (88 hours) from hatching to the cypris stage. When using these 1–2 liter vessels, we succeeded in rearing the larvae with negligible mortality even if we completely omitted water changes. In contrast, we failed in all our attempts to rear either isolated single or small (less than 10) lots of nauplii in Petri dishes with the intention to reveal the exact number of moults. Under these conditions, which are standard procedure for the Thoracica, the moults occurred asynchronously and usually about a half of the larvae died.

We sampled the nauplii of *P. gracilis* immediately (within 1 h) after hatching and thereafter at 24 h intervals. The nauplii of *P. sulcata* were sampled on the day of hatching (day 0) and on days 2, 3, 4, and 5. For light microscopic (LM)

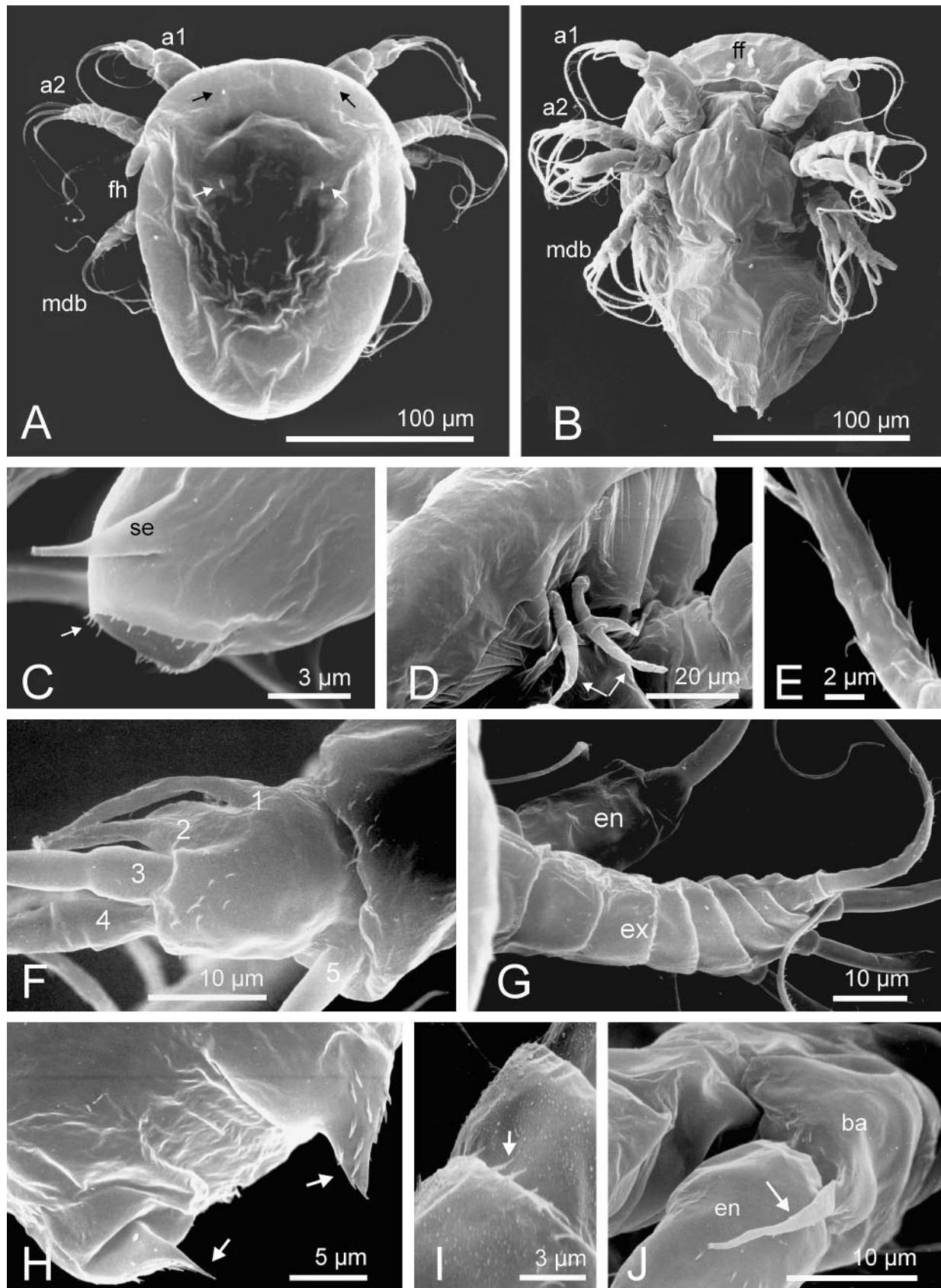
observations, we fixed the larvae in 4% formalin. For most SEM studies we used 2.0% glutaraldehyde dissolved in cacodylate buffer, but we also used some of the formalin samples. The larvae were rinsed, postfixed in OsO<sub>4</sub>, dehydrated in acetone, and critically point dried in CO<sub>2</sub>. We used JEOL JSM-840 microscopes at the Zoological Museum, University of Copenhagen (ZMUC) and the Royal Danish Agricultural University (RDAU) for observation and photography. The SEM stubs used in this study are deposited as voucher specimens in the ZMUC. At the ZMUC, we stored photos directly as digital files on a Win '95® based PC using a 'Semaphore' software and hardware interface to the SEM. The negatives obtained at the RDAU were digitized with a Microtek 5™ scanner. We constructed the final photo plates from the digitized SEM photos using Corel PhotoPaint® and Corel Draw® software. The body outlines of various developmental stages were drawn with use of a camera lucida. The measurements were with LM or using SEM photos. We measured the total length of larvae from the anterior margin to the ends of furcal spines (the maximum length), the width of the head (cephalic) shield behind the frontolateral horns (the maximum width), the length of the frontolateral horns, and furcal spines. The terminology used here follows those of WALOSSEK et al. (1996) and WALOSSEK & MÜLLER (1997).

## 3. RESULTS

### 3.1. Description of the larvae

For *Peltogasterella gracilis* we provide a complete description of all five naupliar instars and the cypris. For *P. sulcata* we focus mainly on those naupliar features that differ from *P. sulcata*. GLENNER et al. (1989) and JENSEN et al. (1994a, b) used SEM to study cyprids of *P. sulcata* and the morphology does not significantly deviate from that described and illustrated here for *P. gracilis*. As elaborated in the discussion, some previous studies of rhizocephalan development have overlooked certain stages, and therefore existing numbering systems can be misleading. To avoid confusion, we use the neutral term "instar" throughout this paper. Some structures, e. g., the flotation collar, are only described in that stage where they first appear. Where features are nearly identical in the two species, we sometimes illustrate them only from the one where we obtained the best micrographs.

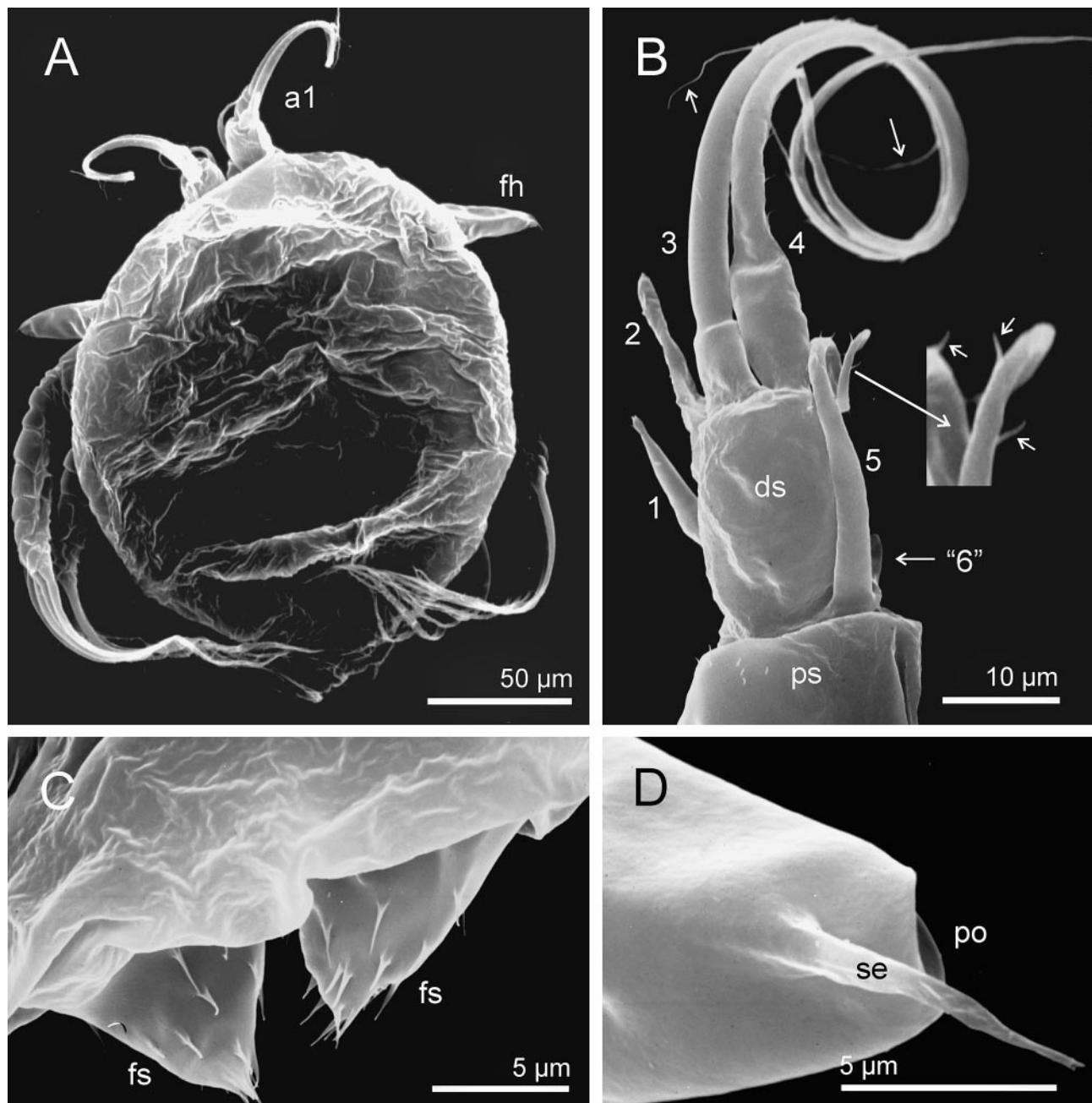
**Fig. 1.** *Peltogasterella gracilis*, nauplius 1. **A.** Dorsal view; black and white arrows point to setae on head shield. **B.** Ventral view. **C.** Tip of frontolateral horn, rimmed with few very small fringes. **D.** Frontal filaments, anterior view. **E.** Short, setulated seta (5) on postaxial side of distal (third) antennulary segment. **F.** Antennule, penultimate (second) and distal (third) segment, preaxial view. **G.** Antenna, preaxial view, annulation of exopod detailed in I. **H.** Furcal rami. **I.** Antennary exopod, denticles on outer, distal edges of annuli. **J.** Antenna, single short seta on protopod close to origin of endopod. 1–5 = setae, a1 = antennule, a2 = antenna, ba = basis; en = endopod, ex = exopod, ff = frontal filaments, fh = frontolateral horn, mdb = mandible, pr = protopod, se = seta.



### 3.2. Development

In *Peltogasterella gracilis* development from hatching and release until the cypris stage lasted ca. four days (88 hours) at 20 °C. Instar 1 has an extremely short duration. Instar 2 nauplii begin to appear a few minutes after release, and within 1 h all the larvae in a culture will have moulted into this stage. Instar 3 occurred on

the second day of cultivation, and instars 2–4 each lasted 12 hours. Thus instar 5 appeared on the fourth day of cultivation and lasted two days. Cyprids appeared on day 5, 4 days after release. *P. sulcata* had a similar schedule of development, but our sampling program was not as complete. Instar 2 appeared at 1 hour after hatching. Instar 3 occurred on



**Fig. 2.** *Peltogasterella sulcata*, nauplius 1. **A.** Dorsal view. **B.** Antennule; oblique lateral view of the distal, third segment (ds); seta 3 and 4 with long setules (arrows), seta 5 with many short setules (inset). **C.** Furcal spines (fs); closer together than at similar stage of *P. gracilis* (Fig. 1H). **D.** Tip of frontolateral horn, with subterminal seta (se), but no fringes around terminal pore (po). 1–6 = antennulary setae, a1 = antennule, ds = distal antennulary segment, fh = frontolateral horn, fs = furcal spine, po = pore, se = seta, ps = penultimate antennulary segment.

the second day, instar 4 on the third, while instar 5 occurred on the fourth and fifth day. In both species, instars 2–5 had either a flotation collar or a distinct collar attachment ridge testifying to accidental loss of this structure.

### 3.2.1. Instar 1 (Figs. 1–2)

***P. gracilis*.** This short-lived instar has an ovoid shape (Figs. 1A, B). The smooth head shield carries only a single pair of large setae. The margin of the head shield is swollen, but the stage lacks both a flotation collar and its attachment ridge. The frontolateral horns are not subdivided; they point ventrolaterally and are closely applied to the body immediately after hatching (Fig. 1A). Each horn terminates in the opening of the well known frontal horn gland (WALKER 1973), here of oval shape and fringed with a few, very short processes (Fig. 1C). A single long and thick seta inserts slightly proximal to the distal pore. It projects a little beyond the tip of the horn and remains present in all succeeding naupliar instars. The frontal filaments are shorter than in subsequent stages (Fig. 1D). There is a minute labrum and a depression that might be considered as the rudiment of the mouth. The furcal spines are short, widely spaced, and covered by small denticles (Fig. 1H). An anus is absent both from this and all subsequent stages.

The antennule (Figs. 13, 14) consists of 3 segments (or portions, sensu WALOSSEK et al. (1996): a short ring-shaped proximal segment, a slender rod-shaped middle segment, and a short cone-shaped distal segment. The latter arises from the middle segment in a central position and carries five setae (Figs. 1F, 13). One short and smooth seta (1) with a characteristic bifid tip inserts on the preaxial side. Three setae (2–4) arise at the top of the segment; one of these is a rather short (2) with a smooth wavy surface and could represent an aesthetasc, while setae 3 and 4 are much longer. Finally, the postaxial side of the distal segment carries a shorter and distinctly setulated seta (5), which inserts so close to the base that it can appear to arise from the middle antennular segment when viewed with LM. A spine adjacent to, but clearly separate from, seta 5 might represent a rudimentary seta 6 (Fig. 2B for *P. sulcata*).

The antenna and the mandible have well developed subdivisions (Fig. 2G for *P. sulcata*). The antennary coxa and basis are about the same size (Fig. 13). The basis carries a single short seta close to the origin of the weakly two-divided endopod (Figs. 1J, 13). The exopod consists of seven annuli, but only the distal five bear long setae. The two most terminal setae appear to arise from terminal annuli (Fig. 1G). The endopod bears three setae (Figs. 13, 14).

The mandible (Figs. 13, 14) closely resembles the antenna but its exopod consists of only six annuli and

carries but four setae; the proximal two annuli lacking setae. The endopod carries two setae. Irregularly distributed, minute denticles adorn the outer distal edges of the annuli in both the antennal and the mandibular exopods (Fig. 1I).

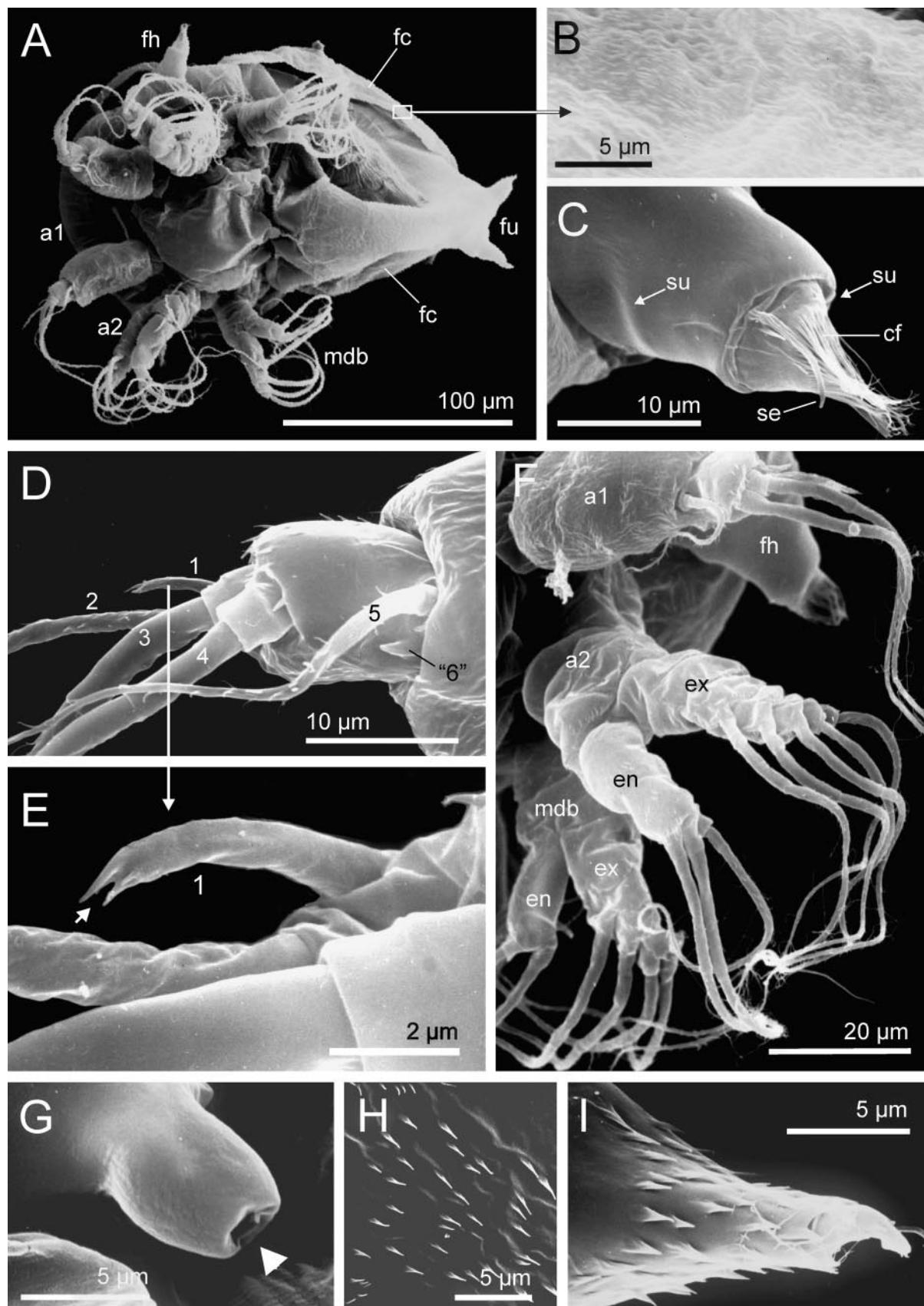
***P. sulcata*.** The frontolateral horns (Fig. 2A) point more laterally than in *P. gracilis*. Each frontolateral horn bears a subterminal seta, but has an absolutely smooth margin around the terminal pore (Fig. 2D). The minute labrum has a terminal pore. The furcal spines (Fig. 2C) are longer and closer to each other than in *P. gracilis*. The antennule has both structure and setation identical to that in *P. gracilis*. The distal segment has one subapical seta (1) on the preaxial side, three terminal (2–4) setae, and one postaxial (5) seta; a rudimentary seta 6 inserts near the base of the seta 5 (Fig. 2B).

### 3.2.2. Instar 2 (Figs. 3–4)

***P. gracilis*.** The body is longer than in the first instar and with an elongate ovoid outline. The dorsal surface now carries a total of seven pairs of setae and this pattern remains unchanged in all succeeding naupliar instars (Fig. 12). Counting from the anterior end, the second pair is by far the largest, is terminally pored, and corresponds to the single pair of pored setae found in the first instar. A pair of much smaller setae is inserted laterally, but very close to these two large setae. Four additional pairs of small setae are inserted more posteriorly. At least the two anteriormost pairs, including the large ones, represent setal precursors of the lattice organs of the cypris (RYBAKOV et al., in press). The possible relationship between the more posterior setae and cypris lattice organs remains uncertain.

Unlike instar 1, nauplius instars 2–5 are all surrounded by a flotation collar in the shape of a hollow torus attached to the body along its entire inner perimeter (Fig. 3A). The collar is circular in cross section, but so small that it cannot easily be seen with LM. The morphology of the collar remains unchanged in the succeeding nauplius instars. It consists of a very thin cuticle with a smooth or only slightly wavy surface pattern (Fig. 3B). If broken free, a lost collar leaves a fine double ridge encircling the entire head shield from the frontal margin, above the bases of the frontolateral horns and posteriorly to the furcal spines. We found this “collar attachment ridge” in all instar 2–5 specimens without a collar (Fig. 5A). *Briarosaccus tenellus* nauplii have 3 pairs of pores located in close contact with this ridge (WALOSSEK et al. 1996), but we found no such pores in any instars of *Peltogasterella* Krüger, 1912, including the first instar.

Two sutures subdivide the frontolateral horns into three portions. The distal suture is very well expressed,



but the proximal one is much weaker and looks like only a slightly pronounced groove. The horns point laterally and are somewhat longer than in instar 1. A distinct crown of thin long fringes encircles the terminal gland opening of the horn in both this and all succeeding nauplius instars (Fig. 3C).

The frontal filaments are small, curled, and taper towards the distal ends. A large pore is located between the bases of the filaments and forms the exit of an anterior unicellular gland (unpublished observation based on sectional series). It may be homologous to the unicellular, but somewhat more dorsally exiting gland found by WALLEY (1969: fig. 6 gl) in the nauplii of *Semibalanus balanoides* (Linnaeus, 1767). The minute, cylindrical labrum points posteriorly and terminates in a pore (Fig. 3G). The supposed nonfunctional mouth is a small hole hidden under the labrum. The furcal spines are covered with numerous denticles (Fig. 3I), except for their proximal portion. Irregularly distributed denticles also cover the dorsal and ventral surfaces of the hind body (Fig. 3H).

The appendages resemble those in instar 1 (Fig. 3F). The antennule has a somewhat thickened second segment. Distally the antennule carries five setae, and seta 1 has a bifurcated tip (Fig. 3E), just as in the first instar.

***P. sulcata*.** The frontolateral horns point laterally (Fig. 4A) and are longer than those in *P. gracilis*. The proximal part of the horn is weakly annulated, but it has a pronounced distal suture. As in *P. gracilis*, a crown of thin fringes encircles the terminal opening (Fig. 4H). The frontal filaments have the typical appearance (Fig. 4B). The labrum in some samples has two distal papillae (Fig. 4C). Furcal spines are longer than in instar 1 and also somewhat longer than in *P. gracilis* (Fig. 4I). All the appendages look very similar to those of *P. gracilis*. The antennule retains five setae. The central part of the distal segment is covered with spinules (Fig. 4D), which are absent in instar 1. The putative rudiment of seta 6 is now well pronounced and clearly set aside from seta 5 (Fig. 4E). In the antenna, Fig. 4F clearly illustrates how the enditic seta originates on the basipod close to the joint with the endopod.

The mandibular exopod has six distinct annuli, with the distal margins of annulus 2–6 bordered by small spines (Fig. 4G).

### 3.2.3. Instar 3 (Figs. 5–6)

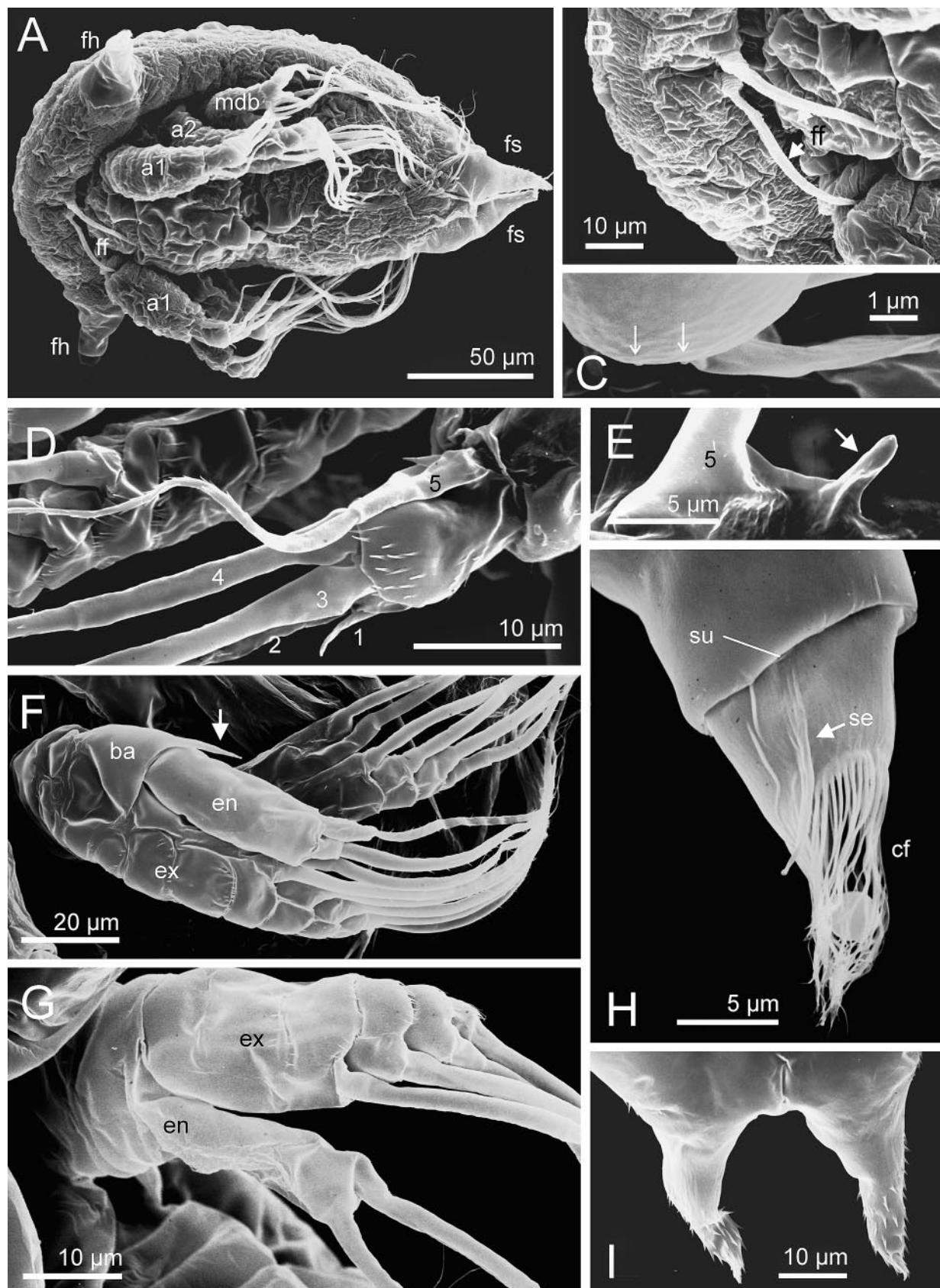
***P. gracilis*.** This nauplius has a body only slightly longer than in instar 2 and with a similar shape, except that the hind body is slightly more elongated and somewhat laterally compressed. Neither the frontal filaments nor the frontal horns have changed perceptibly. Most examined specimens of this stage had lost the flotation collar during processing (Fig. 5A). The antenular setation has changed and now retains only 4 setae, since seta 1 is reduced to a mere spine-like rudiment that, in the two subsequent naupliar stages, disappears completely (Figs. 5B, C). The antenna and mandible show no significant changes. Viewed with LM, the ventral side of the hind body in females shows a midventral longitudinal groove and a series of transverse grooves, whereas male larvae lack the transverse grooves (Fig. 12). The grooves represent evidence of the developing thoracopods of the cypris larva (WALOSSEK et al. 1996). In SEM, the ventral side of both sexes has a longitudinal groove and a characteristic longitudinal band consisting of transverse rows of small denticles (Fig. 5E), exactly as described by WALOSSEK et al. (1996) for *Briarosaccus tenellus*. The furcal spines have a somewhat longer and more slender shape than in instar 2.

***P. sulcata*.** The frontolateral horns (Fig. 6B) are similar to those of *P. gracilis*. Also, as in *P. gracilis*, the antennule carries only four setae, because the subapical seta (1) is reduced to a small denticle close to the basis of seta 2 (Fig. 6C). The furcal spines (Fig. 6D) are thinner and considerably longer, both compared to the preceding stage and to instar 4 in *P. gracilis*. The ventral surface of the hind body has neither midventral longitudinal groove nor distinct transverse grooves like those present in *P. gracilis*, but the denticle ornamentation (medial band of denticles) is well pronounced (Fig. 12).

### 3.2.4. Instar 4 (Figs. 7–8)

***P. gracilis*.** Although only slightly longer, this instar differs significantly from instar 3 in both species (Figs. 7A, B). It has a pear-shaped body outline with a significantly narrower hind body distinctly set off from the anterior body region. The slightly arched head shield has a distribution of setae unchanged compared to ear-

**Fig. 3.** *Peltogasterella gracilis*, nauplius 2. **A.** Oblique ventral view; note small, but distinct flotation collar (fc). **B.** Surface of flotation collar, smooth, without reticulated rib pattern. **C.** Tip of frontolateral horn encircled by cuticular fringes (cf) and flanked by solitary seta inserted proximal to suture (su). **D.** Antennule, lateral view, setation of distal segment; note rudimentary seta “6” adjacent to seta 5. **E.** Detail of D; bifurcated seta 1. **F.** Oblique ventral view of left side appendages. **G.** Rudimentary labrum with distal gland opening. **H.** Ventral surface of hind body with irregularly distributed denticles. **I.** Furcal spine, with denticles. 1–“6” = setae, a1 = antennule, a2 = antenna, cf = cuticular fringes, en = endopod, ex = exopod, fh = frontolateral horn, mdb = mandible, se = seta, su = suture.



lier stages. The ventral surface is somewhat convex. Some larvae retain an intact flotation collar. The frontolateral horns point ventrolaterally. The labrum is small and papilla-like as in the preceding stages. The antennule differs from the preceding instar in having a more distended second segment and in lacking seta 1 completely (Fig. 7C, 13, 14). The antenna and mandible have a more regular denticulation along the distal margins of their exopodal annuli (Fig. 7E). The terminal setae of the antennae carry very long and thin setules (Fig. 7G). The hind body is conical, projects ventrally and tapers towards the posterior end. Its ventral side has a longitudinal furrow extending to the furcal spines and a fine posteromedially directed striation (Fig. 12). As in nauplii of *Briarosaccus tenellus*, this pattern may indicate the developing thoracopods (WALOSSEK et al. 1996), but unlike this species the hind body in *P. gracilis* is not yet distinctly set off from the head shield. The ventral surface also has an irregular arrangement of denticles similar to instar 2 (Fig. 7B). The furcal spines insert by a kind of "articulation" situated in a distinct depression at the posteriormost end of the hind body (Fig. 7H). The furcal spines are flanked by a pair of much smaller denticles on each side of the body (Fig. 7I).

***P. sulcata*.** The flotation collar, when retained, has a surface pattern of slightly wavy lines (Fig. 8B). The frontolateral horns and labrum have not changed. Both this and the succeeding instar have denticle-like processes on each side of the furcal spines. The ornamentation on the ventral surface of the hind body consists of a few irregularly distributed denticles and slightly pronounced, posteromedially orientated furrows.

### 3.2.5. Instar 5 (Figs. 9–10)

***P. gracilis*.** The head shield, both here and in *P. sulcata*, is dome-shaped in anterior view and somewhat spindle-shaped in lateral view, tapering posteriorly (Fig. 9A). Its overall shape heralds the lateral down-folding of the cypris shield (or "carapace") exactly as observed during in the development of *Briarosaccus tenellus* (WALOSSEK et al. 1996). Although no flotation collar was observed in any of the instar 5 larvae examined with SEM, all specimens of both species had a

distinct collar attachment ridge. The dorsal surface of the shield resembles that found in the preceding stages (Fig. 9B). The antennule has a thicker and even more distended second segment than in instar 4 (Fig. 9F), but the antenna and mandible have not changed (Fig. 9E). Compared to instar 4, both the frontolateral horns (Fig. 9C) and the hind body project more ventrally and the latter has a narrower shape. LM reveals a pronounced ornamentation on the hind body, already weakly perceptible in instar 4, which indicates the thoracic limbs of the cypris developing under the cuticle. The ornamentation and appearance of the furcal spines resemble instar 4, but in some specimens we noticed three spines on each side of the furcal spines rather than the two found in the preceding stage (Fig. 9D).

***P. sulcata*.** This stage is relatively large and with a noticeably arched head shield. Although all examined larvae lacked flotation collars, the "collar attachment ridge" was well pronounced, demonstrating the existence of the collar in this stage (Fig. 10A,D). The frontolateral horns are long. The furcal rami (Fig. 10C) are thin and considerably longer than in this instar of *P. gracilis*. The hind body seems to retain the ornamentation of the preceding stage (Fig. 10A).

### 3.3.6. Cypris (Fig. 11)

***P. gracilis*.** Our samples for SEM study contained only female cyprids. The head shield ("carapace") carries only a moderate number of setae. The shield also carries five pairs of lattice organs (LO1–5) of the pore-field type characteristic of rhizocephalans and thoraciicans (JENSEN et al. 1994a, b). LO1 and 3–5 have the normal straight shape, but the second pair of lattice organs (LO2) stands out in being bent into a distinct crescent shape (Fig. 11B). On each anteroventral rim of the shield the frontolateral horn glands open with two separate exit pores (Figs. 11A, C).

The third antennular segment has a short and broad shape with only a moderately dense carpet of cuticular villi on the attachment disc. The posterior perimeter of the disc is bordered by a distinctly raised cuticular skirt from which projects a long and thin cuticular structure, probably a sensillum (Figs. 11D, E: s). Slightly anterior to this raised skirt, and inside the attachment disc

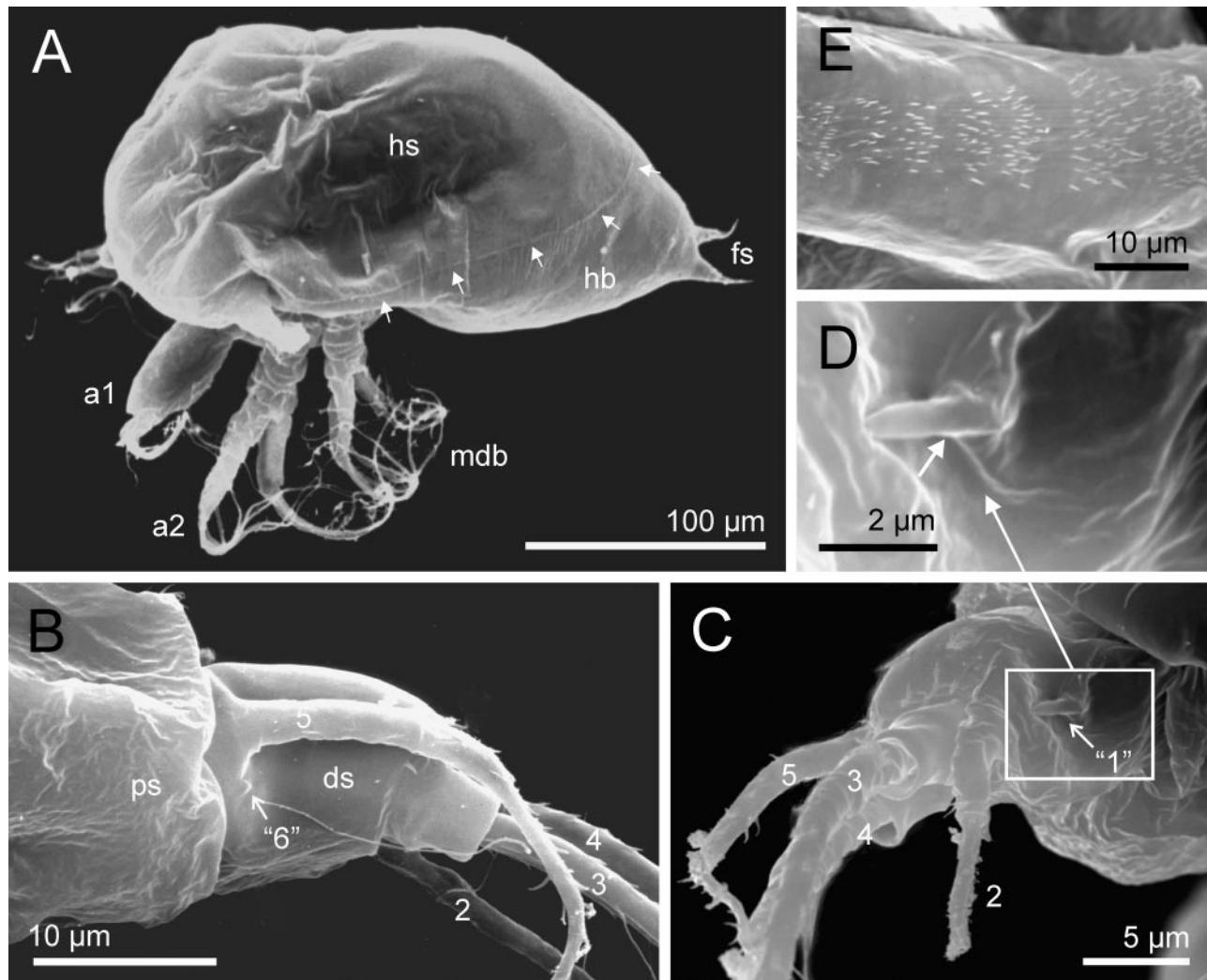
**Fig. 4.** *Peltogasterella sulcata*, nauplius 2. **A.** Oblique ventral view of whole nauplius, the wrinkled cuticle is an artifact. **B.** Paired frontal filaments (ff) (detail of A). **C.** Labrum with two minute, distal papillae (arrows). **D.** Antennule; anterolateral view of distal (third) segment. **E.** Antennule, spine (arrow) putatively from rudimentary seta "6" near base of seta 5. **F.** Antenna, enditic seta (arrow) distally on basipod. **G.** Mandible. **H.** Tip of frontolateral horn, with suture (su), subterminal seta (se), and crown of cuticular fringes (cf) around terminal opening; sphere trapped within fringes could be a secretion product or an artifact. **I.** Furcal spines. 1–5 = antennular setae, a1 = antennule; a2 = antenna; ba = basis; cf = cuticular fringes, ex = exopod, en = endopod, fh = frontolateral horn; fs = furcal spine; se = seta, su = suture.

proper, inserts the postaxial sensillum (Fig. 11D: *pas*). Two distinct grooves originate at each antero-lateral corner of the disc and merge into a median groove that proceeds posteriorly for some distance. Posterolateral to the confluence of these grooves an elongated cone shaped structure with a distinct terminal pore projects (Fig. 11D: *as*).

The absence of a large aesthetasc on the third segment confirmed that the cypris larvae we studied in SEM were all female (WALKER 1985; GLENNER et al. 1989). The terminal setae on the fourth segment are comprised of three long, apically pored sensilla (Fig. 11F: *ts1–3*) and one very short, sac-shaped sensillum (Fig. 11F: *ts4*).

The frontal filaments insert at the base of the antennules and have an elongate, tapering distal part distinctly set off from a swollen basal part (Fig. 11G).

Of the six pairs of thoracopods, only the first pair could be studied in detail. Both the exopod and the endopod have two segments (Figs. 11H, I). As in all cirripedes, a rather short and distinctly serrated seta inserts distally on the first exopod segment. The second exopod segment carries three long, natatory setae at or near its apical end (Fig. 11I). The lateral one has a single row of spines on its lateral face. The first endopod segment carries one long, natatory seta mediodistally. The second endopod segment carries three long, natatory setae distally. Two of these bifurcate several times soon after their origin



**Fig. 5.** *Peltogasterella gracilis*, nauplius 3. **A.** Lateral view; collar accidentally lost, revealing collar attachment ridge (arrows). **B.** Apex of antennule, lateral view. **C.** Apex of antennule, oblique lateral view. **D.** Detail of C, remnant of disappearing setae 1, this seta always totally absent in nauplius 4. **E.** Spines on ventral side of hind body, pattern identical to that described for *Briarosaccus tenellus* by WALOSSEK et al. (1996). 1–“6” = antennular setae; a1 = antennule; a2 = antenna; ds = distal antennular segment; fs = furcal spine; hs = head shield; hb = hind body; mdb = mandible; ps = penultimate antennular segment.

and this impedes attempts to count the number of setae unless their insertions can be clearly observed. We could not, therefore, accurately describe the setation of the more posterior thoracopods, but can state with confidence that they have at least as many as the first thoracopod. This whole setation pattern differs only in minor details from the one described by GLENNER & HØEG (1995) in *Balanus amphitrite* Darwin, 1854.

## 4. DISCUSSION

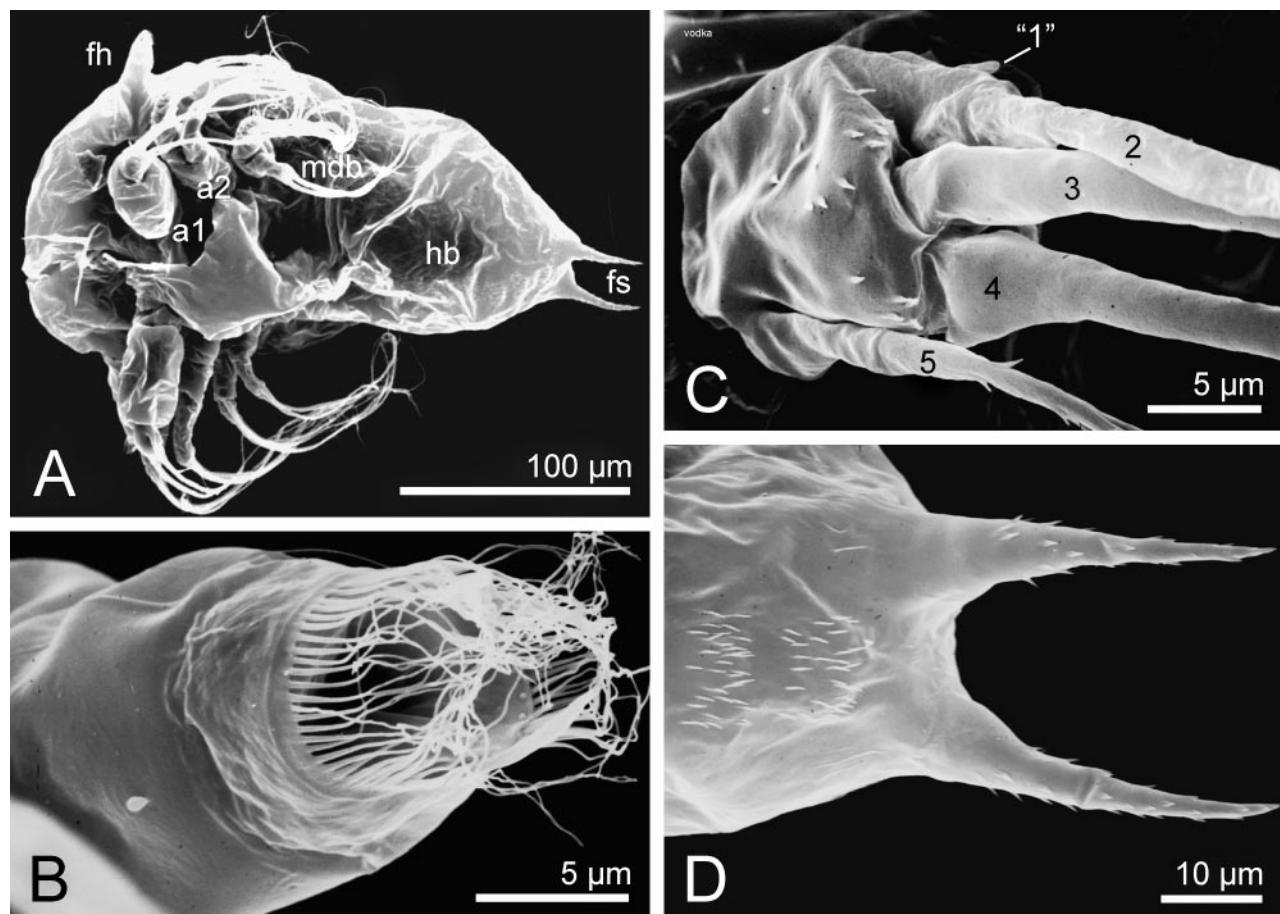
### 4.1. Discrimination between naupliar instars

**Instar 1** differs markedly from the subsequent ones in both species of *Peltogasterella*. It has a smaller size and an ovoid body shape; immediately after hatching the frontolateral horns are closely applied to the body and not articulated, and they lack a crown of thin long fringes around the terminal opening; the furca is but

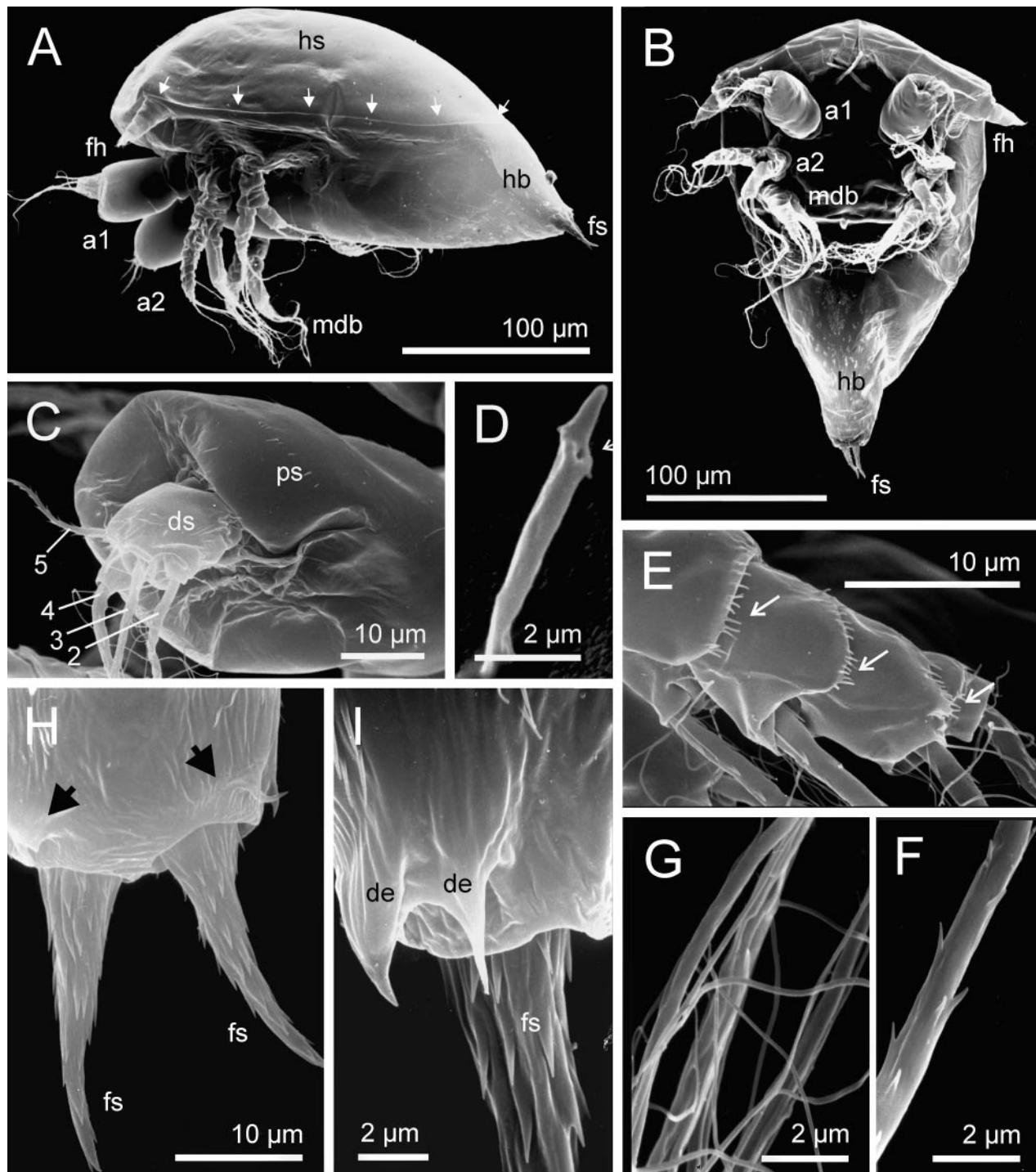
weakly developed. Most characteristically, instar 1 lacks a flotation collar, and the complete absence of a collar attachment ridge testifies that this is not due to accidental loss in the material studied (Tab. 1).

**Instars 2 and 3** are very similar to each other, but in the latter the antennular seta 1 is reduced to a mere rudiment. Instar 3 is also somewhat larger, with longer and more slender furcal rami and with a somewhat more swollen middle antennular segment, which is especially apparent in *P. sulcata*. Both species have a similar ornamentation of the ventral surface of the hind body. In stage 2 this ornamentation has an irregular denticulation while in stage 3 it has a distinct longitudinal band of denticles. For sexual differences in instar 3, see below.

**Instar 4** has a dorsally arched body, tapering towards the posterior end. The head has become somewhat weakly set off from the posteriorly projecting hind body just as in *Briarosaccus tenellus*. Compared to



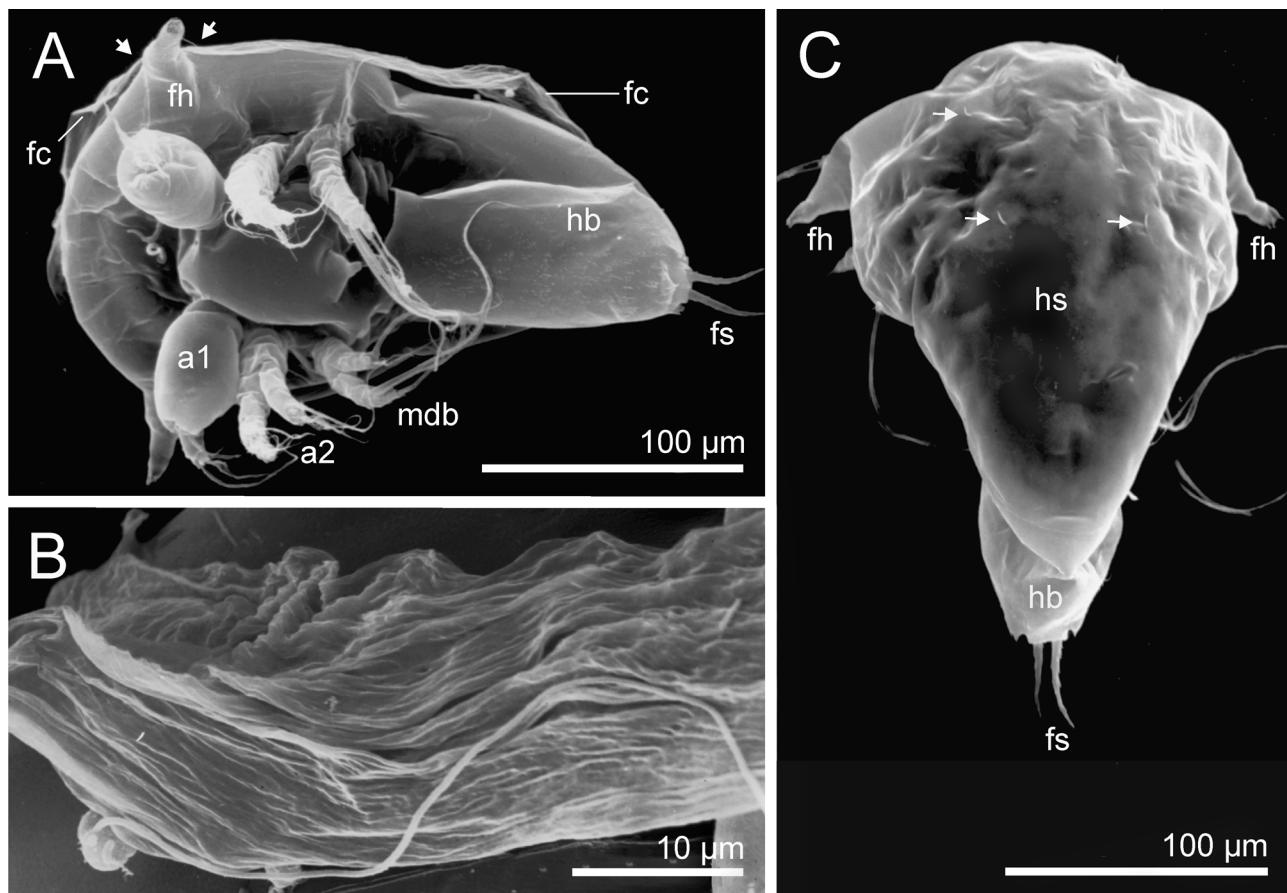
**Fig. 6.** *Peltogasterella sulcata*, nauplius 3. **A.** Oblique ventral view. **B.** Tip of frontolateral horn; gland pore encircled by cuticular fringes. **C.** Antennule, distal segment, oblique apical view showing disappearing seta "1". **D.** Hind body, ventral view, with denticles and furcal rami. 1–5 = antennular setae, a1 = antennule, a2 = antenna, fh = frontolateral horn; fs = furcal spine; hb = hind body, mdb = mandible.



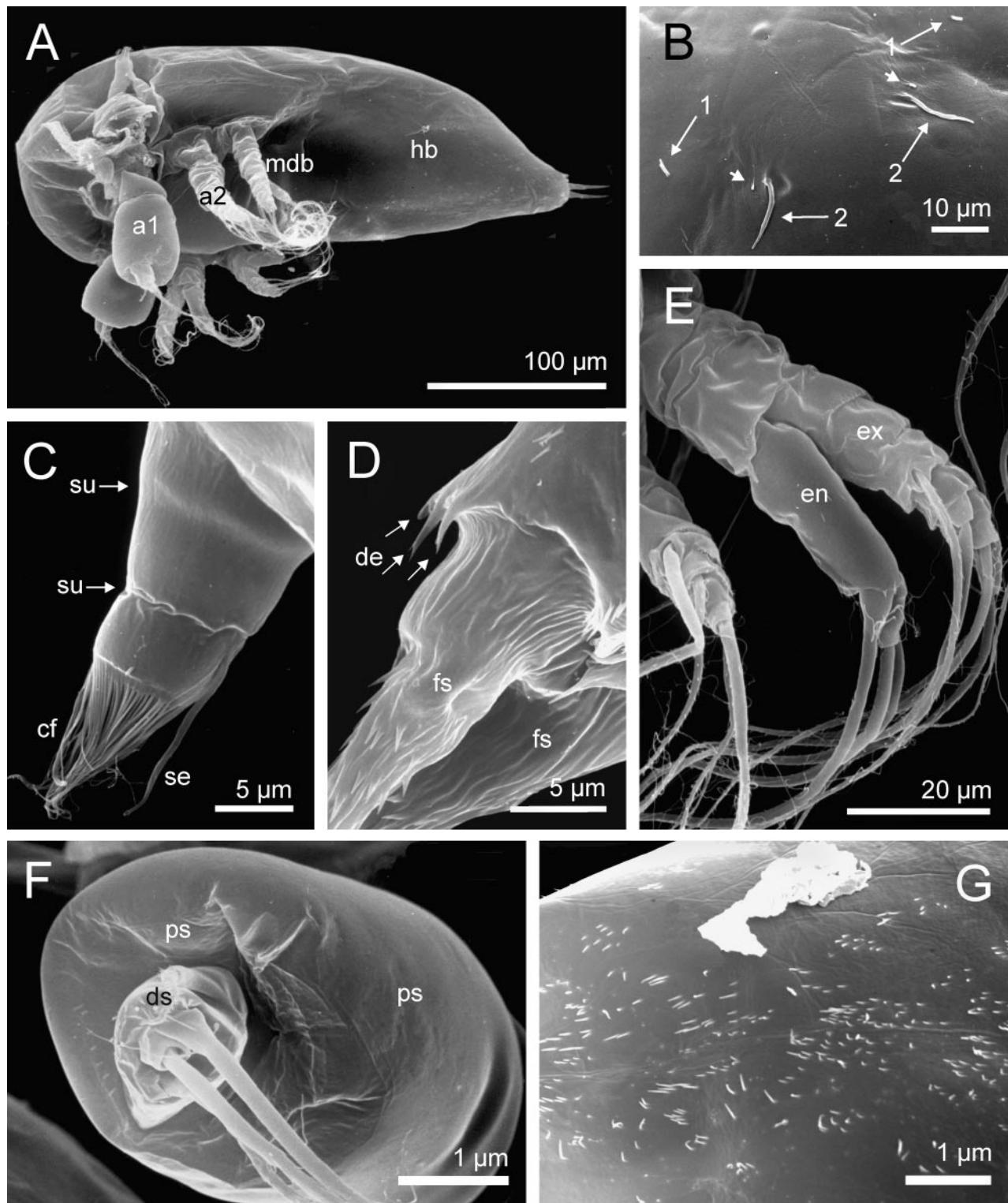
**Fig. 7.** *Peltogasterella gracilis*, nauplius 4. **A.** Lateral view; collar accidentally lost, revealing collar attachment ridge (arrowheads). **B.** Ventral view; note developing hind body here and in A. **C.** Antennule; apical view, setae on distal segment (ds) and its insertion on penultimate segment (ps); seta 5 distinctly setulated; note how penultimate segment has expanded, heralding the attachment disc of the cypris; **D.** Seta from second pair on head shield, precursor of second pair of lattice organs in cypris, note the terminal pore (arrow). **E.** Mandibular exopod with denticles on distal part of annuli; natatory setae deeply socketed. **F.** Seta 4 on antennule. **G.** Antenna; distal plumose setae. **H.** Furcal spines, dorsal view, deeply socketed in hind body and flanked proximally by denticles (arrowheads). **I.** Pair of short denticles (arrowheaded in H) at base of heavily denticulated furcal spine. 2–5 = antennulary setae, a1 = antennule, a2 = antenna, de = dencicle, ds = distal antennulary segment, fh = frontolateral horn, fs = furcal spine, hb = hind body, hs = head shield, mdb = mandible, ps = penultimate antennulary segment.

**Tab. 1.** Comparison of the naupliar stages in *Briarosaccus tenellus* and two species of *Peltogasterella*. The ground plan stages (T1–6) are those present in the Thoracica and also assumed for the Thecostracan ancestor. T3 has been lost from the development of *Peltogasterella*. Note that the first instar never has a flotation collar and that antennular seta 1 (f) always disappears at the 2<sup>nd</sup> moult. \*) Original stage designation of Walossek et al. (1996) in parentheses. \*\*) inferred by comparison with other species. \*\*\*) a spine-like rudiment of h<sub>1</sub> seta retained in the nauplius 3 of *P. gracilis* and *P. sulcata*.

Ground plan	Stage	Features				
		<i>Briarosaccus tenellus</i> *	<i>Peltogasterella gracilis &amp; sulcata</i>	collar	f-seta	frontolateral horns “articulated” and with fringed terminal opening
T1	1 (missed **)	1		—	+	—
T2	2 (N0)	2		+	+	+
T3	3 (N1)	not present		+	—	+
T4	4 (N2)	3		+	— ***	+
T5	5 (N3)	4		+	—	+
T6	6 (N4)	5		+	—	+



**Fig. 8.** *Peltogasterella sulcata*, nauplius 4. **A.** Oblique ventral view of whole larva, this specimen has retained the flotation collar (fc) which clearly passes above the frontolateral horns (arrows). **B.** Detail of flotation collar, note absence of reticulated pattern; the semiparallel longitudinal wrinkles are a shrinkage artifact of dehydration. **C.** Dorsal view, note setae on head shield (arrows) and developing hind body (hb). a1 = antennule, a2 = antenna, ch = cardhu, mdb = mandible, fc = flotation collar, fh = frontolateral horn, fs = furcal spines, hb = hind body, hs = head shield.

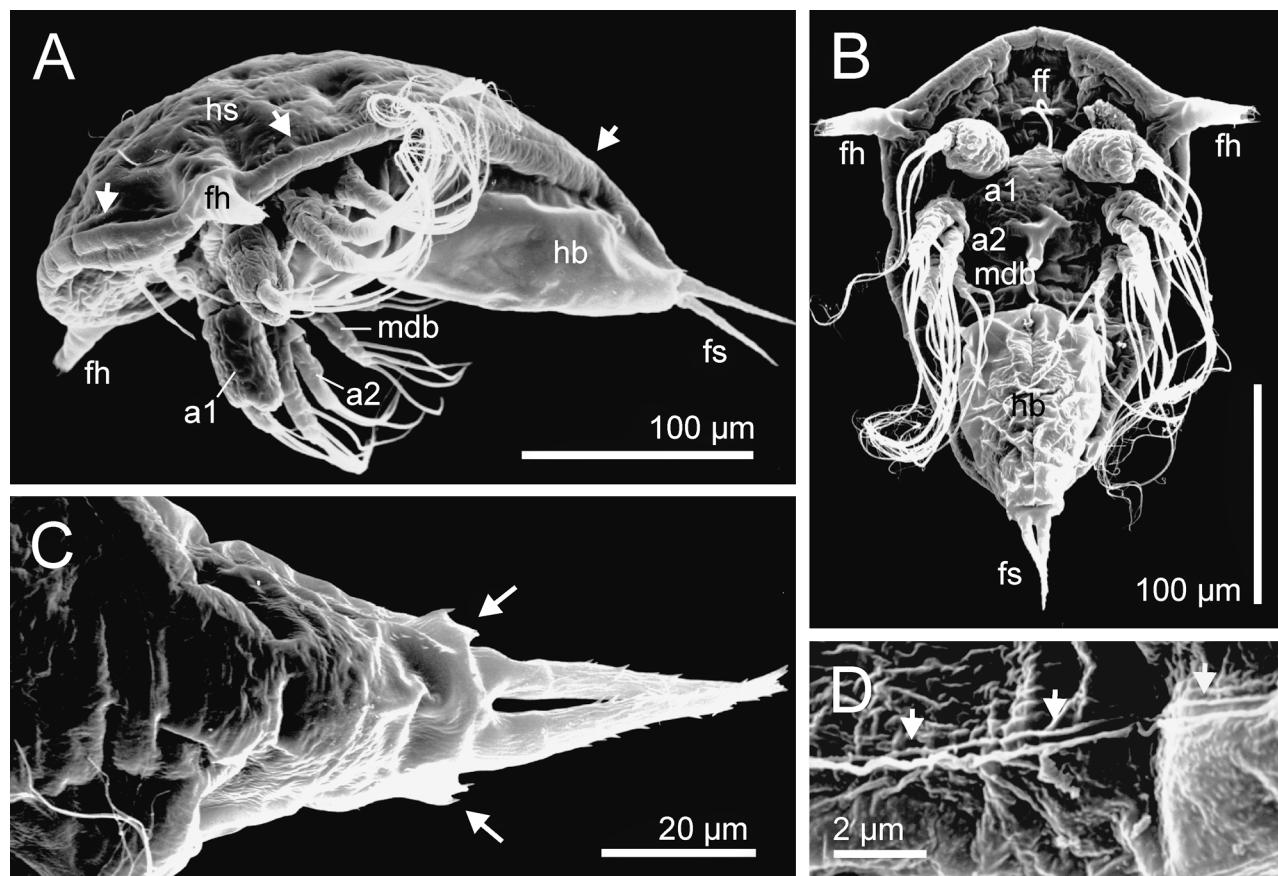


**Fig. 9.** *Peltogasterella gracilis*, nauplius 5. **A.** Ventrolateral view, collar accidentally lost. **B.** Seta pair 1 and 2 dorsally on head shield; these setae develop into lattice organs pair 1 and 2 in the cypris; note additional diminutive, but unpored, setae at base of seta 2 (arrowheads). **C.** Tip of frontolateral horn; encircled by proximal inconspicuous and distal prominent sutures (su); seta (se) inserted at distal suture, distal cuticular fringes (cf) encircles gland pore. **D.** Furcal spines (fs); three denticles (de) lateral to insertion in hind body. **E.** Antenna; oblique lateral view. **F.** Antennule; apical view, insertion of distal third segment on greatly expanded penultimate (second) segment. **G.** Ventral side of hind body. a1 = antennule; a2 = antenna; ds = distal antennular segment; en = endopodite; ex = exopodite; hb = hindbody; mdb = mandible; ps = penultimate antennular segment.

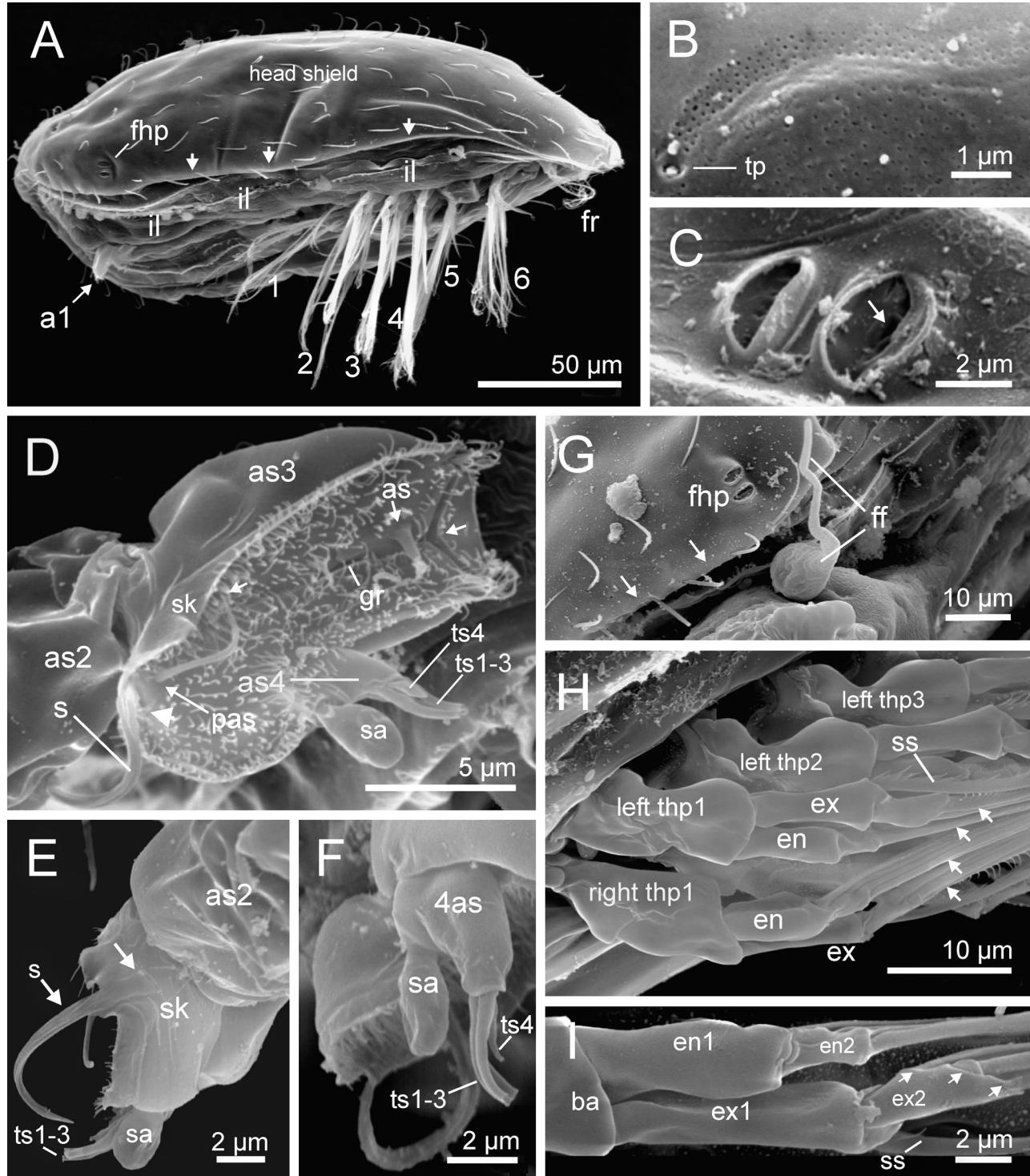
instar 3, the furcal rami insert in depressions in the hind body and are flanked by spine-like processes.

**Instar 5** resembles instar 4, but in *P. gracilis* it differs by the now rather pronounced ornamentation of the hind body reflecting the thoracic segments of the cypris. This pattern is useful in discriminating between the naupliar instars of *P. gracilis*: it is not pronounced in instar 2; instar 3 possess developed transverse grooves, which in instar 4 become oriented at 45° to the longitudinal body axis. Finally, instar 5 has very pronounced furrows, especially near the end of this stage when the naupliar cuticle envelopes the almost completed cypris. *P. sulcata* has but a weak ventral ornamentation, which cannot be used to separate the larval stages. In both *P. gracilis* and *P. sulcata* SEM observations show that in instars 4 and 5 the ventral surface of the hind body has an irregular denticleation and a median longitudinal groove (Fig. 12).

The lecithotrophic development explains the extremely reduced labrum and why the appendages lack true gnathobases and have significantly fewer setae than in planktotrophic nauplii. The only enditic remnant is the single, short seta on the antennal basipod. Unlike the Thoracica, the attenuated limb setation of rhizocephalan nauplii remains almost unchanged during development and affords little help in separating the instars. The change from five to four antennular setae by the reduction of seta 1 to a mere denticle provides a notable exception and allows a clear separation of instars 2 and 3 in *Peltogasterella*. Beginning with instar 3, the middle region of the antennule becomes increasingly swollen. This trend heralds the development of the attachment disc (third segment) in the cypris antennule and is similar to events in other cirripedes (NOTT & FOSTER 1969; WALOSSEK et al. 1996).



**Fig. 10.** *Peltogasterella sulcata*, nauplius 5. **A.** Lateral view of whole body, flotation collar accidentally lost (see D) revealing the non-homologous and slightly swollen rim of the head shield (arrowheads); note the very swollen penultimate segment of antennule (a1); hind body well developed. **B.** Ventral view. **C.** Dorsal view of furcal rami, flanked by lateral pairs of denticles (arrows). **D.** Collar attachment ridge; two closely adjacent rims of cuticle (arrowheads) marks where flotation collar broke free from naupliar head shield. a1 = antennule, a2 = antenna, ff = frontal filaments, fh = frontolateral horn, fs = furcal spine, hb = hind body, hs = head shield, mdb = mandible, sc = scapa.



**Fig. 11.** *Peltogasterella gracilis*, female cypris. **A.** Whole cypris, ventrolateral view, sparsely setose head shield folded down as carapace on each side of body; ventral rim of shield indicated by arrows, cuticle continues as soft inner lamella (il) lining mantle cavity; antennules (a1) almost retracted but the six thoracopods (1–6) and furcal rami (fr) extend posteriorly as during swimming, note how inner lamellae from each side meet in the midline and closely adjoin the extended appendages. Frontolateral horn pores (fhp) correspond to the terminal pores of frontolateral horns in nauplii. **B.** Crescent shaped second pair of lattice organs (LO2), note the terminal pore (tp); remaining lattice organs (LO1 & LO3–5) are all perfectly straight. **C.** Paired pores of the two frontolateral horn gland cells exiting near ventral rim of head shield (see A and G), gland opening (arrow) sur-

#### 4.2. Sex and species in *Peltogasterella* nauplii

Male larvae are larger than female larvae in all rhizocephalans of the suborder Kentrogonida (which includes the genus *Peltogasterella*) (HØEG & LÜTZEN 1995). These differences are significant in *P. gracilis* as well (Tab. 2) and fertilized male and female eggs already show significant differences in size (for *P. sulcata*, we studied only male larvae). Some other rhizocephalan species have a considerable overlap in the size ranges, especially in the naupliar stages. Male and female cyprids always differ by clear cut morphological traits in the antennules (WALKER 1985; 1999; GLENNER et al. 1989), while sexual differences other than size were, until now, unknown among the nauplii. We have demonstrated at least one such difference for instar 3 larvae of *P. gracilis*. Females of this instar have a midventral longitudinal groove and fine transverse grooves on the ventral side of the hind body; such grooves are absent in males (Fig. 12). These ornaments are only weakly pronounced in both sexes of *P. sulcata*. Identifying sexual differences early in ontogeny could be advantageous in species such as

**Tab. 2.** Length of eggs and naupliar instars in male and female *Peltogasterella gracilis*.

Stage	Length (μm)	
	Male	Female
Egg	245.0 ± 5.3	185.0 ± 8.5
1	263.0 ± 7.7	217.1 ± 14.0
2	290.1 ± 7.9	245.3 ± 10.3
3	308.2 ± 9.4	264.8 ± 10.3
4	359.4 ± 22.0	300.2 ± 10.5
5	377.6 ± 7.6	309.9 ± 7.8
cypis	305.3 ± 2.2	250.9 ± 4.1

*Sacculina carcinii*, which are under serious consideration for use as biological control agents (parasitic castigators) to fight immigrant crabs (Papers in THRESHER 1997).

*P. gracilis* and *P. sulcata* larvae are very similar in both size and morphological features. From our data it appears that, *P. sulcata* larvae are larger than those of *P. gracilis*. However, we have good grounds to believe that the two complete series of *P. sulcata* nauplii studied here represent exclusively male larvae, because the stage 4 nauplii from another, incomplete, series were significantly smaller and probably represented females. In all stages, the larvae of *P. sulcata* have significantly longer furcal rami and slightly longer frontolateral horns than those of *P. gracilis*. In instar 1 the terminal openings of the frontolateral horns are fringed with finger-like processes in *P. gracilis* but are absolutely smooth in *P. sulcata*. As mentioned above, the ventral ornamentation of the trunk provides an important feature for separating the instars of *P. gracilis*, but it is only weakly pronounced in *P. sulcata*, and therefore not useful for separating the instars of that species.

#### 4.3. Comparison with other rhizocephalan nauplii

##### 4.3.1. Time of development

Species of *Peltogasterella*, *Sacculina*, and *Lernaeodiscus* Müller, 1862 have larvae of approximately the same size and they can all accomplish larval development into cyprids within about 5 days at 15–20 °C. By contrast, the much larger larvae of the cold-water *Briarosaccus* require about a month to reach the cypris stage. However, HØEG & LÜTZEN (1995) reported that larvae of Swedish populations of *Peltogaster paguri* Rathke, 1842 can survive and develop into cyprids even at temperatures in the 5–10 range °C.

rounded by an outer cuticular rim. **D.** Antennulary segments 3 and 4, oblique ventral view; attachment disc of segment 3 with moderately dense carpet of cuticular villi; seta-like structure (s) inserts (arrowhead) at skirt (sk) lining the posterior perimeter disc; the postaxial sense organ (pas) inserts slightly proximally on disc proper; cone-shaped, and terminally pored axial sense organ (as) situated just behind confluence (arrow) of three distinct grooves (gr) in disc, the cement gland probably exits within these grooves; antennulary segment 4 (as4) inserts laterally on as3 and carries a short aesthetasc (sa) subterminally, and three apically pored setae (ts1–3) and a fourth, smaller seta (ts4) terminally. **E.** Posterior view of attachment disc showing how seta-like structure (s) inserts outside and near basis of expanded skirt (sk). **F.** Antennulary segment 4; short, subterminal aesthetasc (sa) and the four terminal setae (ts 1–4); the subterminal aesthetascs are short and extending only slightly beyond the base of the terminal setae. **G.** Frontolateral horn pores (fhp) near ventral rim (arrows) of left side head shield; frontal filament with swollen base and thin projection extends from mantle cavity. **H.** Anteroventral view of anterior pairs of thoracopods (thp); anterior is left; note stout, conspicuously serrated seta (ss) distally on first exopodal (ex) segment; longer, natatory setae on endo- (en) and exopods indicated by white arrows. **I.** Thoracopod 1, shorter endopod (en, top) and longer exopod (ex, bottom); note on exopod how serrated seta (ss) originates from first segment (ex1), while natatory setae (arrowheads) insert along oblique terminal edge of second segment (ex2). ba = basis

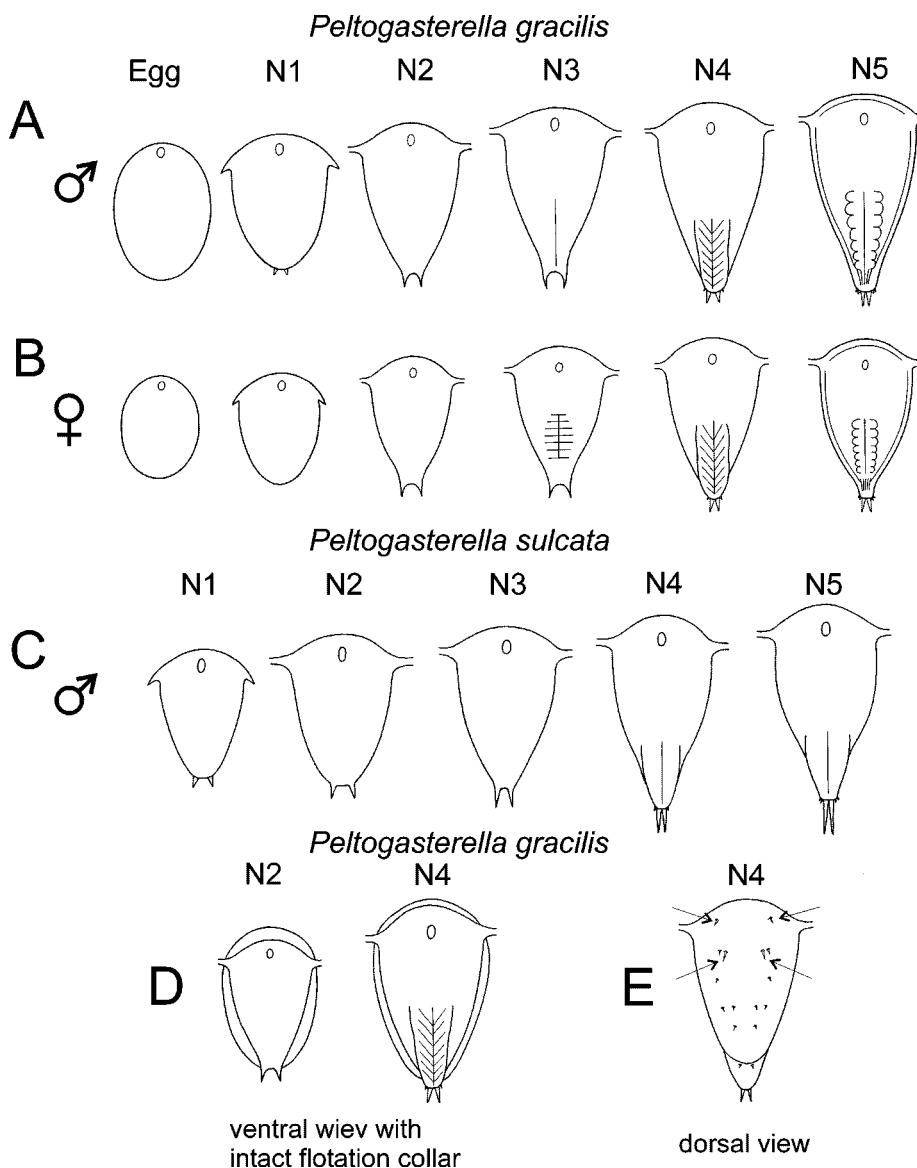
#### 4.3.2. Flotation collar

The flotation collar in *Peltogasterella* nauplii is small and with an almost smooth or only faintly wavy surface pattern. It therefore differs from the much larger collar present in nauplii of both *Peltogaster* Rathke, 1842 and *Briarosaccus*, where the surface has a very conspicuous reticulated pattern (WALOSSEK et al. 1996). A detailed comparative account of the collar will be provided in a forthcoming paper (HØEG & RYBAKOV, in prep.). Early papers on *Peltogasterella* nauplii failed to mention or illustrate the collar (ICHIKAWA & YANAGIMACHI 1957; YANAGIMACHI 1961a, b), but it is very often lost during specimen han-

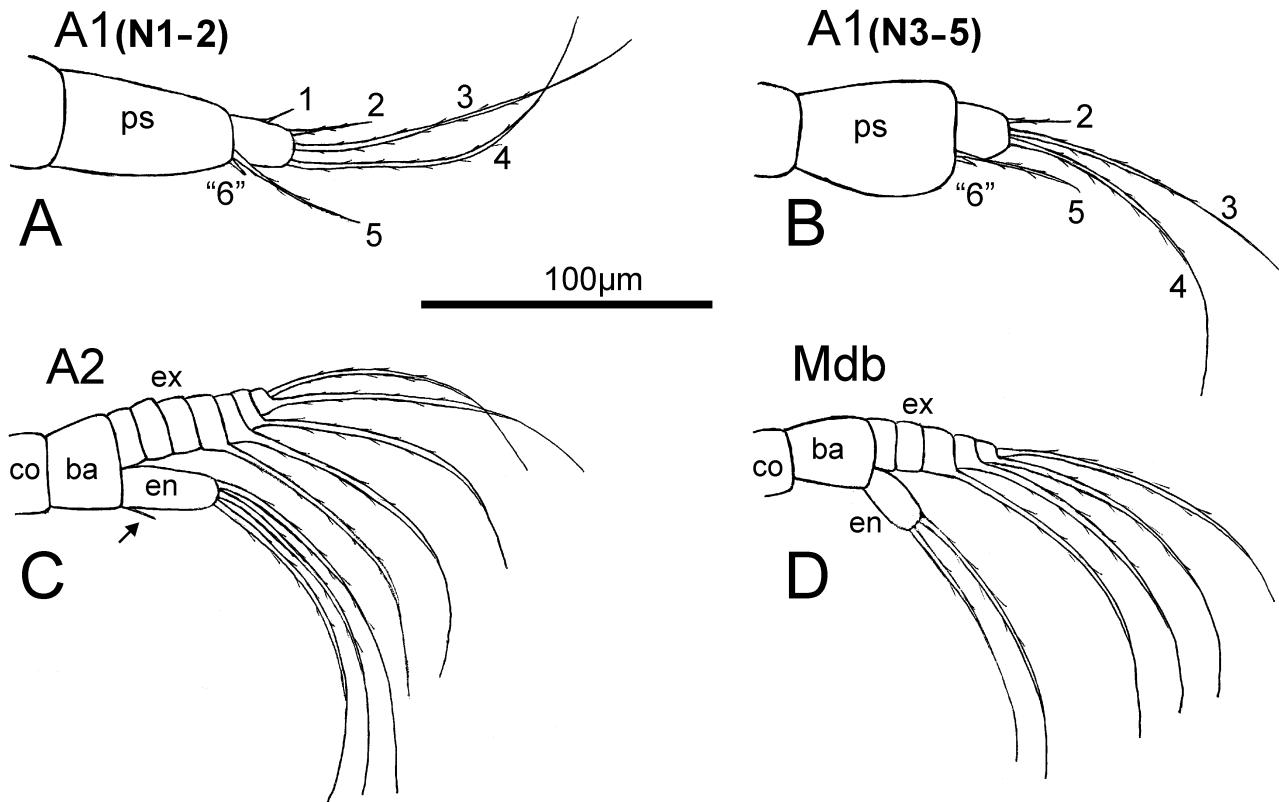
dling, leaving only the “attachment ridge”, and we can testify to the difficulty of seeing it with LM, even when it is specifically searched for. When retained, the flotation collar is small and can only be seen with difficulty using the light microscope, most easily when viewed head on.

#### 4.3.3. Appendages

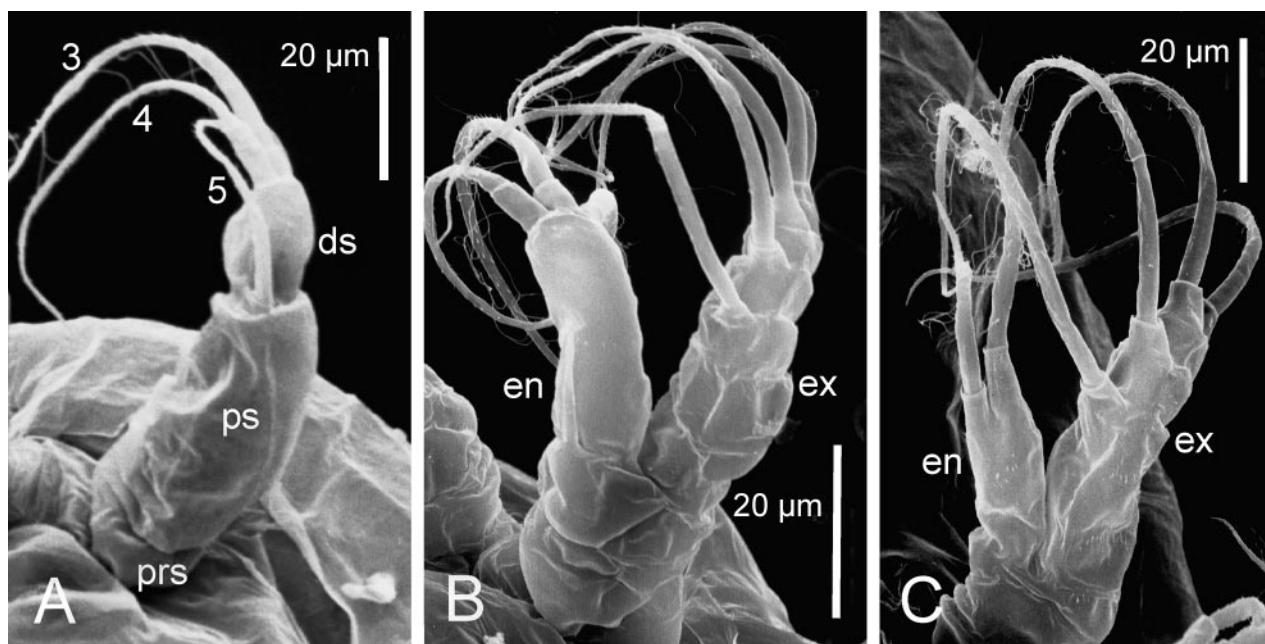
All naupliar stages carry a short seta on the antennal basipod close to the insertion of the endopod. The small size of this seta seems to characterize nauplii of *Peltogasterella*. Nauplii of *Briarosaccus* and *Peltogaster* carry a homologous, but significantly longer,



**Fig. 12.** Body outlines of naupliar instars of *Peltogasterella gracilis* and *P. sulcata*; only females of *P. sulcata* were cultured. A–C. Ventral views with the flotation collar removed from instars 2–5 (naturally absent in N1). D. Ventral views including outline of flotation collar. E. Dorsal view with setation, arrows indicate the setal precursors of lattice organ pairs 1 and 2.



**Fig. 13.** Naupliar appendages of *Peltogasterella gracilis*. Drawn using light microscopy. **A.** Antennule (A1) in nauplius 1–2. **B.** Antennule (A1) in nauplius 4; seta 1 has disappeared; seta 6 always remains rudimentary; note how the penultimate segment has expanded, heralding the attachment organ in the cyprid. **C.** Antenna (A2), arrow indicates the single enditic spine. **D.** Mandible (Mdb). Co = coxa, ba = basis, en = endopod, ex = exopod, ps = penultimate antennulary segment.



**Fig. 14.** Naupliar appendages from instar 1 of *Peltogasterella gracilis* (ventral view, right side). SEM. Cp. to Fig. 13. **A.** Antennule. **B.** Antenna. **C.** Mandible. En = endopod, ex = exopod; ds = distal antennulary segment, prs = proximal antennulary segment, ps = penultimate antennulary segment, 3,4,5 = antennulary setae.

seta (HAWKES et al. 1985; WALOSSEK et al. 1996), whereas the antennal basipod is unarmed in nauplii of *Sacculina* (WALKER 1988; COLLIS & WALKER 1994). We consider this enditic seta as the only rudiment of the original feeding armature, which should have been present in the planktotrophic ancestor. Accordingly, the absence of this seta in *Sacculina* represents an apomorphic condition and provides another larval character of phylogenetic value.

#### 4.3.4. Frontolateral horns

The nauplii of all examined species of Rhizocephala, including *Peltogasterella*, *Peltogaster*, *Sacculina*, and *Briarosaccus*, have a crown of fringes around the terminal openings of the horns from the second naupliar instar on. The presence of only one thick subterminal seta at the distal part of the frontolateral horns is another characteristic of nauplii in *Peltogasterella*. In *Briarosaccus* (WALOSSEK et al. 1996) and *Peltogaster* (unpublished) the frontolateral horns carry two setae, while the horns of *Sacculina* nauplii appear to lack setae (COLLIS & WALKER 1994).

### 4.4. Sex and morphology in cyprids

#### 4.4.1. Antennules

GLENNER et al. (1989) studied only male *P. sulcata* cyprids, while we had only females of *P. gracilis* available in our samples used for SEM. The third antennular segment of male *P. sulcata* cyprids differs from that of *P. gracilis* in having a very elongated shape and in lacking the raised cuticular skirt around the posterior border of the attachment disc. We consider this as a difference between male and female cyprids in both species of *Peltogasterella* rather than a difference between the cyprids of the two species. We base this on the micrographs in YANAGIMACHI (1961a), which show a similar difference in shape of the third antennular segment (elongated vs. short) between male and female *P. gracilis* cyprids. Incidentally, these photos also clearly show the well known third segmental aesthetasc in the male cyprid. Our observations agree with that of WALKER (1999), from *Heterosaccus lunatus* Phillips, 1978 that male and female cyprids in the Rhizocephala can differ not only in the armament of antennular sensilla but also in the shape of the attachment organ itself. It is possible that these differences relate to the fact that females settle directly on the host animal while males settle only on virginal female parasites (HØEG & LÜTZEN 1995).

Except for the 3rd segmental aesthetasc, the antennule of female *P. gracilis* cyprids carry the same structures as illustrated in male *P. sulcata* cyprids by GLENNER et al. (1989). These authors did not explicitly describe the

seta-like structure extending from the posteriormost part of the skirt, but it appears in their Figs. 23 and 25. A seta originating posteriorly on the skirt has not been reported for other rhizocephalan cyprids or from cirripedes in general, and could therefore represent a structure unique to *Peltogasterella* (WALKER 1985; GLENNER et al. 1989; MOYSE et al. 1995). It obviously cannot be the postaxial sense organ, which clearly originates close to the posterior skirt, but inside the attachment disc (Fig. 11D, pas). GLENNER et al. (1989) mistook the postaxial sense organ for the axial sense organ, an error corrected by MOYSE et al. (1995). The true axial sense organ is normally hidden in the carpet of cuticular villi and can rarely be seen with SEM. Both the female *P. gracilis* cyprids studied here and the male cyprids of *P. sulcata* and *Peltogaster paguri* (GLENNER et al. 1989: figs. 21, 26) have an almost identical conical process anteriorly on the attachment disc. Since it occurs in both sexes it cannot be the so-called spinous process which is found in males only (HØEG 1987; GLENNER et al. 1989). Following MOYSE et al. (1995) we suggest that it is the true axial organ, but its proper characterization in the above discussed genera must await an analysis using TEM.

#### 4.4.2. Lattice organs

In *Peltogasterella gracilis*, *P. sulcata*, *Peltogaster paguri*, and *Briarosaccus tenellus* (Peltogastridae), and in *Lernaeodiscus* sp. aff. *porcellanae* Müller, 1862 (Lernaeodiscidae), the second pair of lattice organs deviates from the remaining four pairs in having a distinct crescent shape (JENSEN et al. 1994b; WALOSSEK et al. 1996). This characteristic feature (Fig. 11B) is a putative synapomorphy for these two families. It is also the second pair of lattice organs that deviates in another rhizocephalan family, the Clistosaccidae, where they are very elongate and hairpin-shaped. In other Rhizocephala, and in the Thoracica and Acrothoracica all five pairs of lattice organs are straight and similar to the posterior ones (JENSEN et al. 1994a, b).

### 4.5. Antennular setae in cirripede nauplii

GRYGIER (1994) analysed the antennular structure in larvae of the Ascothoracida, Facetotecta, and Thoracica (forming the monophylum Thecostraca) and advanced a hypothesis on the homologies of the setae. He provided good evidence that in the groundplan of the Thoracica the antennule of first-stage nauplii carried one subapical seta on the preaxial side (the so-called f-seta) and three apically-situated setae, a condition exemplified in all nauplii of the Lepadidae. Since a similar pattern is observed in the nauplii of Ascothoracida and Facetotecta, Grygier considers this pattern as plesiomorphic for the Thecostraca. GRYGIER (1994),

however, did not discuss the nauplii of the Rhizocephala. Our observations and those of COLLIS & WALKER (1994) and WALOSSEK et al. (1996) agree that the antennule of first instar rhizocephalan nauplii have a setation quite similar to the thoracican and thecostracan groundplan, with one preaxial seta (1) and three apical setae (2–4). We therefore conclude that seta 1 of the rhizocephalan naupliar antennule is homologous to the f-seta of lepidid nauplii. This implies that the rhizocephalan pattern exemplifies the thecostracan groundplan in accordance with its position as the sister group to the Thoracica (SPEARS et al. 1994; HØEG 1995). We recommend the designation of the f-seta as a “subapical seta” rather than the “preaxial seta”, to distinguish it from the true preaxial seta of the Thoracica (the  $h_1$ -seta in Grygier’s terminology). The latter does not appear until the thoracican instar 3 and has no homology with the f-seta. We also emphasize that in the Facetotecta, the “f-seta” *sensu* GRYGIER (1994) appears in instar 2. This delayed appearance indicates a homology to the cirripede  $h_1$ -seta rather than to the true f-seta. The facetotectan nauplii seem to lose the true f-seta in stage 1. If so, this event suggests a homology between the facetotectan instar 2 and the ascothoracidan and thoracican instar 3. In the Rhizocephala, seta 5 on the antennule seems to be homologous to the thoracican d-seta. The short dent at the basis of seta 5 in *Peltogasterella* is isolated from the latter and may therefore represent a rudiment of the e-seta, which in thoracicans inserts only slightly apically of the d-seta. WALOSSEK et al. (1996) believed that seta 1 in *Briarosaccus tenellus* (the f-seta) inserts apically on the penultimate rather than on the apical segment. We illustrate (Fig. 1F) how but the perfect angle of photography may give that impression. The true position of seta 1 (on the apical segment) is also evident on our unpublished SEM photos of *B. tenellus*.

#### 4.6. Previous observation on *Peltogasterella*

The species of *Peltogasterella* have five naupliar instars, although they have a development lasting only about 5 days. Except for WALOSSEK et al. (1996), all previous studies on rhizocephalan nauplii have found only four instars, and it seems that they have missed not the first but either the second or third instar. Instar 1 differs to an extent where it can hardly be overlooked even if only a few are present in the samples. Instar 2 appears several minutes after the hatching and moults into instar 3 within the first 12 h and might be overlooked. Moreover, instars 2 and 3 have an almost identical gross morphology and could not easily be distinguished under a light microscope. This probably explains why previous studies on *Peltogasterella* reported the true third instar immediately after the first

(ICHIKAWA & YANAGIMACHI 1957; YANAGIMACHI 1961b). Instar 3 could be overlooked as well, because fast developing Rhizocephala pass through instars 2 and 3 together in less than 24 h. With a daily sampling scheme, the second sample may accordingly contain mostly or only instar 4. We recommend that future studies on rhizocephalan development take samples with shorter intervals, of no more than 12 hours.

Both ICHIKAWA & YANAGIMACHI (1957) and YANAGIMACHI (1961b) presented photo series of larval development in *Peltogasterella gracilis*. From measuring these larvae we conclude that YANAGIMACHI (1961b: fig. 1) showed the following instars of a male generation (authors designations in brackets): instar 1 [1 hour after hatching]; instar 3 [1 day after hatching], lacking the “segmentation” in the abdominal region; instar 4 [2 days after hatching]; and instar 5 [4 days after hatching]. ICHIKAWA & YANAGIMACHI (1957: fig. 4) depicted four naupliar instars of a female generation: instar 1 [immediately after hatching], instar 3 [1 day after hatching] and with “segmentation” in the abdominal region; instar 4 [2 days after hatching], and instar 5 [3 days after hatching]. Instar 2 was missed in both cases. The drawings “1 day after hatching” in both these papers demonstrate the sexual dimorphism of the nauplii.

#### 4.7. Larval instars in the Rhizocephala

From a comparison of our data with the study of WALOSSEK et al. (1996) we suggest that *Briarosaccus tenellus* likely has a development with 6 naupliar instars rather than 5 reported (Tab. 1). Six instars is also the number found in most other Cirripedia and it is the ground pattern of the Thecostraca in general.

Until recently it was believed that the lecithotrophic Rhizocephala had an abbreviated development with only 4 naupliar instars (HAWKES et al. 1985; WALKER 1988; COLLIS & WALKER 1994), and this was also the number previously reported for *P. gracilis* (ICHIKAWA & YANAGIMACHI 1957; YANAGIMACHI 1961a,b). More recently, WALOSSEK et al. (1996) found 5 naupliar stages in *Briarosaccus tenellus*. They believed that the surplus stage represented the hatching instar, previously unnoticed due to its extremely short life time, and they accordingly designated it as “N0” (a ‘zero’ stage). We have re-examined both the drawings and the original photos of the five naupliar instars of *Briarosaccus tenellus*. From this we conclude that WALOSSEK et al. (1996) missed the first instar, while their stage “N0” is actually the second of a total of six instars. Thus, their “N0” of *B. tenellus* is not homologous to the first instar described for *Peltogasterella gracilis* (YANAGIMACHI 1961a), *Sacculina carcinii* (WALKER 1988; COLLIS & WALKER 1994), or *Briarosaccus callosus* Boschma,

1930 (HAWKES et al. 1985). The *B. tenellus* "N0" has a flotation collar, laterally directed and "articulated" frontolateral horns with terminal openings fringed with long setae, and relatively long furcal rami (see WALOSSEK et al. 1996: figs. 5a, b, e). In the development of *P. gracilis*, *B. callosus*, and *S. carcinii* these features do not appear until instar 2 (*S. carcinii* nauplii never develop any collar). Instars 2 and 3 are very similar in rhizocephalan development and differ only by the reduction of the subapical antennular seta (1) to a mere rudiment in stage 3. In all examined Rhizocephala this represents the only change in appendage setation during the entire development, and it always occurs after the second moult (YANAGIMACHI 1961a; HAWKES et al. 1985; WALKER 1988), not after the first, as originally claimed for *B. tenellus* by WALOSSEK et al. (1996).

From these comparisons we conclude that WALOSSEK et al. (1996) missed the true first instar. The development of *B. tenellus* actually comprises a total of 6 instars, thus having the most plesiomorphic development so far known from the Rhizocephala. Tab. 1 summarizes the number and homologies of naupliar instars in the Rhizocephala and Thecostraca.

Development of *Peltogasterella* includes only five stages and it appears that the third instar 3 (T3) of the groundpattern (Tab. 1) has been suppressed. In support of this the "N2" instar in *B. tenellus* and instar 3 in *Peltogasterella* have, among other common characters, a similar and very characteristic ornamentation of the ventral surface of the hind body, where the spines form a longitudinal band (see Fig. 13 and WALOSSEK et al. 1996: fig. 25c).

## 5. CONCLUSIONS

As previously shown for cyprids (GLENNER et al. 1989; HØEG & RYBAKOV 1996; WALKER 1999), this paper demonstrates how detailed studies of naupliar morphology can provide important information for rhizocephalan systematics and phylogeny, and in characters that can be directly compared to those of free-living cirripedes. Examples include the flotation collar, lattice organs and their precursors, frontolateral horns, appendage setation, and development of the hind body. A comparative analysis of numbers and morphologies of naupliar stages within the Rhizocephala will require a broader analysis of species from all three kentrogonid families (the species of the suborder Akentrogonida all hatch as cyprids). Such studies should cultivate small numbers of larvae in each container, with only brief intervals between sampling of larvae, and employ both SEM and light microscopy to describe the stages. Together with molecular datasets, these ultrastructural-

level larval traits will result in a more phylogenetically based taxonomy than the traditional one, which is almost exclusively based on limited characters of the adult parasites (HØEG & LÜTZEN 1996; ØKSNEBJERG 2000)

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