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The Complete Larval Development of the Pedunculated Barnacle *Capitulum mitella* (Crustacea: Cirripedia) Using a Standardized Terminology

Ryusuke Kado[1,2,*], Niklas Dreyer[3,4], Jørgen Olesen[4], Dieter Waloszek[5], and Jens T. Høeg[6]

[1]*School of Marine Biosciences, Kitasato University, 1-15-1 Kitasato, Minami-ku, Sagamihara-shi, Kanagawa, 252-0373 Japan. *Correspondence: E-mail: rukado@kitasato-u.ac.jp (Kado)*

[2]*Present affiliation: Marine Ecology Research Institute, St. Luke's Tower 34F, 8-1, Akashicho, Chuo-ku, Tokyo, 104-0044 Japan*

[3]*Centre de recherche du CHU Sainte-Justine, 3175 Chem. de la Côte-Sainte-Catherine, Montréal, QC H3T 1C5, Canada. E-mail: niklas.dreyer.hsj@ssss.gouv.qc.ca (Dreyer)*

[4]*Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark. E-mail : jolesen@snm.ku.dk (Olesen)*

[5]*Department of Geology, Lund University, Sölvegatan 12, 223 62 Lund, Sweden. E-mail: dieter@waloszek-d.de (Waloszek)*

[6]*Marine Biology Section, Department of Biology, University of Copenhagen, Universitetsparken 4, DK-2100 Copenhagen, Denmark. E-mail: jthoeg@bio.ku.dk (Hoeg)*

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We describe the complete larval development of the pedunculated barnacle *Capitulum mitella*, using both light microscopy and scanning electron microscopy. This includes all six naupliar instars and the final cypris stage. Many previous accounts on the development of barnacle larvae suffer from a shortage in detail and habitually use terminologies that are either inconsistent or hard to compare with those used for other crustaceans. We therefore propose and use a new completely standardized terminology to enable comparison both within barnacles and with larvae of other crustaceans. Rather than a stage-by-stage description, our account follows changes in specific features during larval development. The morphological, ecological and phylogenetic significance of these characters is discussed. Special attention is paid to the feeding apparatus and how it may have undergone adaptive evolution in response to changes in the availability of food items through geologic time. *C. mitella* is universally agreed to be placed lower in the barnacle phylogeny than acorn barnacles (Balanomorpha), and fossil forms very similar to this species can be traced back to the Upper Jurassic. This makes *C. mitella* central to understanding the large-scale evolution within barnacles.

Keywords: *Capitulum mitella*; adaptive evolution; feeding apparatus; larval morphology; standardized terminology

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BACKGROUND

Juveniles and adults are often very different both structurally and functionally. This raises important questions about how and why such life cycles evolved (Nielsen 2012). Both dispersal and the ability of larvae and adults to grow in different habitats have been forwarded as explanations hereof (Strathmann 1993; Carrier et al. 2018). Cirripedia (barnacles) is an excellent taxon for studying the evolution and ecological role of larvae. They all have a pelagic larval phase, while the adults are permanently sessile and displaying an amazing variation in habitat, lifestyles and morphology. Adult cirripedes, or barnacles, occur from semi-terrestrial habitats to the deep sea and have lifestyles ranging from hard bottom suspension feeders over a wide variety of epibiotic forms to some of the most specialized parasites found in all Metazoa (Anderson 1994; Høeg and Møller 2006, Chan and Høeg 2015; Chan et al. 2021). The biology of barnacle larvae remains widely accepted as one of the major drivers of their immense evolutionary success and biodiversity (Høeg and Møller 2006; Martin et al. 2014; Yu et al. 2020; Dreyer et al. 2022). Most species commence development with a series of naupliar instars, but the larval phase always terminates with the very different cypris larva that is uniquely adapted to locating and irreversibly attaching at the final settlement site and initiate metamorphosis into a juvenile (Anderson 1994; Høeg and Møller 2006; Yu et al. 2020; Dreyer et al. 2022). The hatching larva, the nauplius, swims and potentially feeds using its three pairs of appendages (antennules, antennae and mandibles), but the torpedo-shaped cyprid propels itself through the water with six pairs of thoracopods while also being capable of bipedal walking on a substrate using a pair of highly specialized, four-segmented antennules (Lagersson and Høeg 2002; Høeg and Møller 2006).

Across barnacles, the developmental patterns vary considerably in terms of larval size, duration of the pelagic phase and the nauplii being feeding or non-feeding (Ewers-Saucedo and Pappalardo 2019).

Feeding (planktotrophic) nauplii is found among most species of Balanomorpha (acorn barnacles) and Lepadoidea and enables the larvae to both grow and attain long-distance dispersal. Among species with non-feeding (lecithotrophic) larvae, the large group of parasitic Rhizocephala (Høeg 1995) have the smallest nauplii in all Cirripedia, enabling the production of very large brood sizes. Conversely, many deep-sea barnacles have lecithotrophic nauplii that are very large, containing copious nutrient resources that enable dispersal for weeks or months over vast areas with few food particles other than marine snow in the inhospitable sea bottom that offers no option for settlement. The cost for this scheme is reduced brood sizes (Mortensen and Høeg 2006; Yorisue et al. 2013). Finally, many barnacles possess an abbreviated development, where larvae hatch as cyprids (Høeg 1995; Høeg and Møller 2006; Mortensen and Høeg 2006 2013; Dreyer et al. 2020). This may be an adaptation that ensures that the larva remains within a suitable but confined habitat. The final stage, the cyprid, has a rather stereotyped general structure throughout all cirripedes, but SEM studies reveal interesting differences in the organs used for substrate location and attachment (Glenner et al. 1989; Chan et al. 2017; Dreyer et al.2022; Yap et al.2022, 2023). In addition, it is remarkable that this settlement stage varies in size from a mere 70 µm in some rhizocephalans to more than 1mm in some species of *Lepas* Linnaeus, 1758.

It follows that barnacles (Cirripedia or even Thecostraca in general) are excellent models for studying how larval structure, development and biology evolved within a taxon with a very wide range of adult life forms. Due to its importance in biofouling, there is a host of studies on the settlement biology of the cyprid. In contrast, truly detailed studies on cirripede larval development

and their adaptations during the pelagic are relatively few (Bassindale 1936; Grygier 1994; Jones and Crisp 1954; Kado 1982, Kado and Hirano 1994; Lang 1979; Moyse 1987; Norris and Crisp 1953; Walker and Rainbow 1976; Walley 1969; Walossek et al. 1996). Most accounts on nauplii are wanting in detail and habitually use terminologies that are either inconsistent or hard to compare with those used for other crustaceans, such as copepods.

In this study we have used both light microscopy (LM) and scanning electron microscopy (SEM) to examine the larval development of *Capitulum mitella* (Linnaeus, 1758), a pedunculated species with planktotrophic naupli that is common in intertidal rocky habitats in East Asia (Fig. 1). Pedunculated barnacles are universally agreed to be more plesiomorphic than acorn barnacles (Balanomorpha). Fossil forms similar to *C. mitella* and species of the closely related genus *Pollicipes* Leach, 1817 can be traced back to the Mesozoic and are central to understanding the large-scale evolution of barnacles (Gale 2018; Chan et al. 2021). This makes *C. mitella* an obvious choice for a detailed study of cirripede larval development.

Previous studies on larval development in *C. mitella* are few and somewhat lacking in detail (Yasugi 1937; Lee et al. 2000). Here, we describe how all externally visible larval features change during development. We use a new, completely standardized terminology to enable comparison both within barnacles and with larvae of other crustaceans. The main goal is to provide a platform based on external morphology, facilitating comparative studies of larval development across the Crustacea in a phylogenetic context (Martin et al. 2014; Lozano-Fernandez et al. 2019; Bernot et al. 2023).

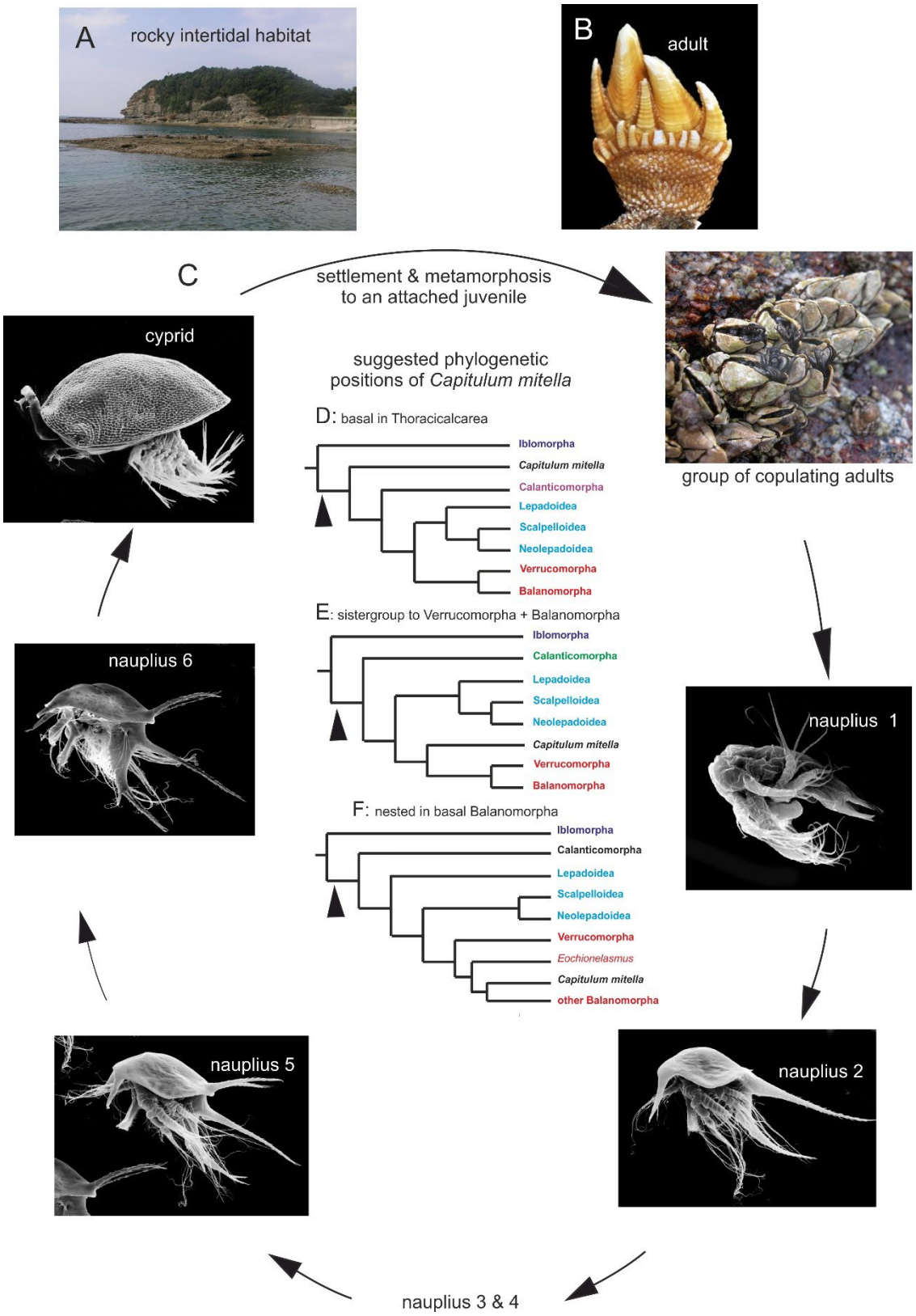

Fig. 1. Habitat, general biology, life cycle and phylogenetic position of *Capitulum mitella*. A: Typical rocky intertidal habitat in Japan. B: Adult specimen. C: The life cycle, where adults release larvae that develop through six nauplius instars into the terminal cyprid, followed by cypris settlement and metamorphosis into an attached juvenile (nauplius instars 3 and 4 and metamorphosis not depicted). D–F: Different phylogenetic positions suggested for *C. mitella*. D: Basalmost in the Thoracicalcarea = taxa above the arrow. E: Sistergroup to Balanomorpha + Verrucomorpha (= "sessilian barnacles"). F: Nested within basal Balanomorpha. Genera *Pollicipes* and *Lithotrya*, closely related to *Capitulum*, omitted for clarity (see details in Chan et al. 2021).

MATERIALS AND METHODS

The length of the reproductive season of *Capitulum mitella* was assessed by performing monthly samples in the summer of 1979. This was followed by monthly sampling of brooding specimens with embryos from May 1980 to January 1981, which provided the source of larvae for culture. Sampling took place on rocky shores at Jogashima, Kanagawa Pref., Japan. Mass culture of larvae followed the technique of Kado (1982). For a detailed survivability assessment under controlled laboratory conditions and diverse feeding regimes, ca. 61 nauplii were cultured individually in 4.5 ml of filtered seawater with algae in an incubator at 23°C exposed to continuous light (3500–4000 lux). Different lots of larvae were fed with either of three species of algae: The diatom *Skeletonema costatum* (Grev.) Cleve grown in medium SWII (Iwasaki 1961); the dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller and the Haptophyceae *Pavlova lutheri* (Droop) J.C. Green, with the latter two grown in f/2 medium (Guillared and Ryther 1962). We changed the seawater and the algae every day after checking for the presence of exuviae. Algae were added to reach a concentration of 2-3 × 10^5 cells/mL for *S. costatum* or 3-6 × 10^4 cells/mL for *P. minimum* and 5–10 × 10^5 cells/mL for *P. lutheri.*

Larvae for light microscopy (LM) study were first examined live, subsequently fixed in 5% neutralized seawater formalin and finally mounted and cleared in diluted glycerol. Exuviae from individual cultures and from a mass culture were also fixed. Larval appendages were dissected under a dissecting microscope and mounted in glycerol. Larvae for scanning electron microscope (SEM) were postfixed with OsO4, dehydrated in alcohol and acetone, critical point dried in CO_2 and examined using a JEOL JSM-840 SEM at the Natural History Museum of Denmark.

Size measurements were carried out under LM with an ocular micrometer for structures larger than 100 μm using 20 to 40 individuals of each larval stage from mass culture. SEM photographs were used for measuring smaller structures, but we are aware that length measurements from SEM micrographs are not always accurate. Measurements were: shield width (the greatest width of the head shield, excluding frontolateral horns); total body length, from the anterior margin of the shield to the tip of the dorsocaudal spine for nauplii; shield length, from the anterior to the posterior margin of the head shield in nauplii (carapace for cyprids) excluding the posterior shield spines; height (for cyprids), the maximum distance between the dorsal and ventral margins of the carapace. Drawings were made from LM preparations with a camera lucida or from SEM pictures. Terminology of spines on the hind body is based on Norris and Crisp (1953) but modified by us as explained below. Setal terminology principally follows Lang (1979) and Branscomb and Vedder (1982) but was modified by using the terminology of Watling (1989), Lavalli and Factor (1992) and Garm (2004). Setation formulae follow the system of Newman (1965). Formulae for the setae and teeth on the antennal gnathobase, here called the naupliar process as in Martin et al. (2014), are based on Kado and Hirano (1994). We provide details below on the principles of terminology and the problem inherent in comparing and homologizing setae and spines both between instars and between species.

RESULTS

GENERAL BIOLOGY OF *CAPITULUM MITELLA*

Life cycle and reproduction

General development: The development comprises six naupliar stages (N1-N6), henceforward called instars, and a final cyprid stage (Figs. 1, 2). In field collections, specimens brooding unhatched embryos in the mantle cavity were only present during the summer months (June to August).

Larval development and survival under the three different food regimes are given in table 1. Nauplii failed to develop into cyprids on a diet of *Skeletonema costatum*. Fed on *Pavlova lutheri*, survival to cyprids was poor, while fed on *Prorocentrum minimum* we observed a very high ratio of survival (91.8%). Development time (at 23°C) was slowest when fed on *S. costatum*, and all larvae had perished before they reached N6. Fed on *P. minimum*, the mean developmental time to the cyprid was significantly faster (13.7 days) than when fed on *P. lutheri* (18.9 days). The time spent in N6 was like that of the preceding instars, except for N1, which moults into N2 within a few hours after release. The duration of the cyprid is variable, as it terminates either at settlement or when this larva dies from lack of energy resources (Lucas et al. 1979; Høeg and Ritchie 1987).

Table 1. Survival and intermolt periods of naupliar instars of *Capitulum mitella* under three different algal diets and segregated rearing conditions at 23°C

Initial no. of nauplii reared*	Survival no. and intermolt period (days) Mean S.D. (Min.-Max.)				
	N2	N3	N4	N5	N6
61	61 2.1 0.6 (1.5–2.6)	42 2.6 0.9 (1.0–5.0)	18 4.0 1.0 (2.0–5.9)	7 5.4 (3.0–9.0)	0
59	53 2.6 0.4 (2.5–3.5)	39 2.0 0.4 (1.0–2.0)	23 2.5 0.9 (1.0–4.8)	14 4.7 1.3 (2.9–7.2)	6 8.2 1.8 (6.1–11.2)
61	60 1.7 0.2 (1.6–2.6)	60 1.9 0.6 (1.0–3.1)	59 2.2 1.1 (1.0–5.0)	58 2.7 1.1 (2.0–8.1)	56 5.3 1.4 (3.0–11.9)

Initial no. of nauplii reared*	Duration (days) from N2 to N6 Mean S.D (Min.-Max.)	Survival rate through naupliar stage	No. of cyprids metamorphosed	Algal diet
	N2-N6	(%)		
61	0	0	0	*Skeletonema costatum*
59	18.9 2.3 (15.5–22.6)	10.2	6	*Pavlova lutheri*
61	13.7 3.1 (11.0–27.9)	91.8	56	*Prorocentrum minimum*

*: N2 nauplii were used for rearing experiments due to very short intermolt period (less than a few hours) of N1.

Larval Terminology od Features

For the structures in the nauplii, our terminology is provided below and in figure 3. It relies principally on Walossek (1993), Anderson (1993), Walossek et al. (1996), Chan et al. (2014), and especially on Martin et al. (2014). For the final larval instar, we use "cyprid/cyprids" as a noun and "cypris" as an adjective, the reason being that "cypris" has no plural form. For this instar we strictly follow the terminology of Høeg et al. (2004) and Bielecki et al. (2009).

Orientation: For motile structures (appendages and labrum) the terminology of orientation needs clarification. For the labrum, we use "anterior" and "posterior" side, referring to the situation when the labrum is directed ventrally, as in figure 9: N6. For the appendages, traditional cirripede literature uses preaxial for the lateral or outer side and postaxial for the median or inner side. These terms are not used in the otherwise rather similar larvae of copepods or elsewhere in crustacean literature. We, therefore, use outer (lateral) for preaxial and inner (median) for postaxial as shown in figures 19, 22 and 24. For the antennular setae in the cyprid, where the appendage axis is twisted relative to the nauplius, the terms "postaxial seta 2" and "postaxial seta 3" are so well engrained, that we continue using those names (see: Walossek et al. 1996; Bielecki et al. 2009).

Appendages – general structure: Excepting antennules and the caudal appendages, the remaining ones are biramous, consisting of a stem carrying two branches, an *exopod* and an *endopod* (Figs 3, 20, 21, 22, 25, 26). The stem is two-segmented, consisting of a *coxa* and a *basis*.
Appendage segments and annuli: The morphology and terminology of crustacean larval appendages, including those of nauplii, have been extensively treated previously (Walossek 1993; Dahms 2000; Boxshall 2004; Boxshall and Jaume 2009; Martin et al. 2014). The components of the cypris antennule have always been called segments in the specialized literature on this important appendage. For this reason, we use "segment" both for the divisions of this appendage, for the endopods of the antenna and mandible, and for endo- and exopods of the thoracopods. We use "annulus" for narrow, ring-like divisions of the mandibular and antennal exopods, although they are not always complete rings, as already shown for branchiopod nauplii (Walossek 1993; Maruzzo et al. 2009). We also acknowledge that a clear structural distinction between appendage segments (articles) and annuli does not always exist, as shown for branchiopod nauplii (Olesen and Grygier 2004).

Numbering of appendage components: During some moults, appendage segments seem to "split" as from N3 to N4 in the antennal endopod. Also, an annulus can be added, as basally in the antennal exopod during the same moult. We have highlighted such events in the text to facilitate comparison and numbering of the segments and annuli.

Abdomen (ab): A small component posterior to the thorax and only manifested in the cyprid.

Antennule (a1) (or 1st antenna): the uniramous, anteriormost appendages on the head.

Antenna (a2) (or 2nd antenna): the biramous, second pair of head appendages.

Anus (an): a concavity clearly fringed with folded cuticle and situated between the dorso-caudal spine and the posterio-ventral part of the hind body.

Arthrodial membrane (am): a thin and flexible cuticle connecting body and appendage partitions.

Atrium oris (ao): A shallow depression in the ventral cuticle in which the mouth is located

(Moore an McCormic 1969; Walossek 1993).

Basis (ba): Distal segment of the stem (protopod) of the antenna, mandible and postmandibular appendages.

Carapace: The greatly enlarged and laterally downfolded outgrowth of the maxillary segment of the cyprid, where it encloses the entire larval body in a spacious cavity (Figs. 1, 2). The cavity enclosed by the cypris carapace is called the mantle cavity because it is ontogenetically continuous with the mantle cavity, or brood chamber, in the adult barnacle (Høeg et al. 2004)., see *head shield* (Fig. 2).

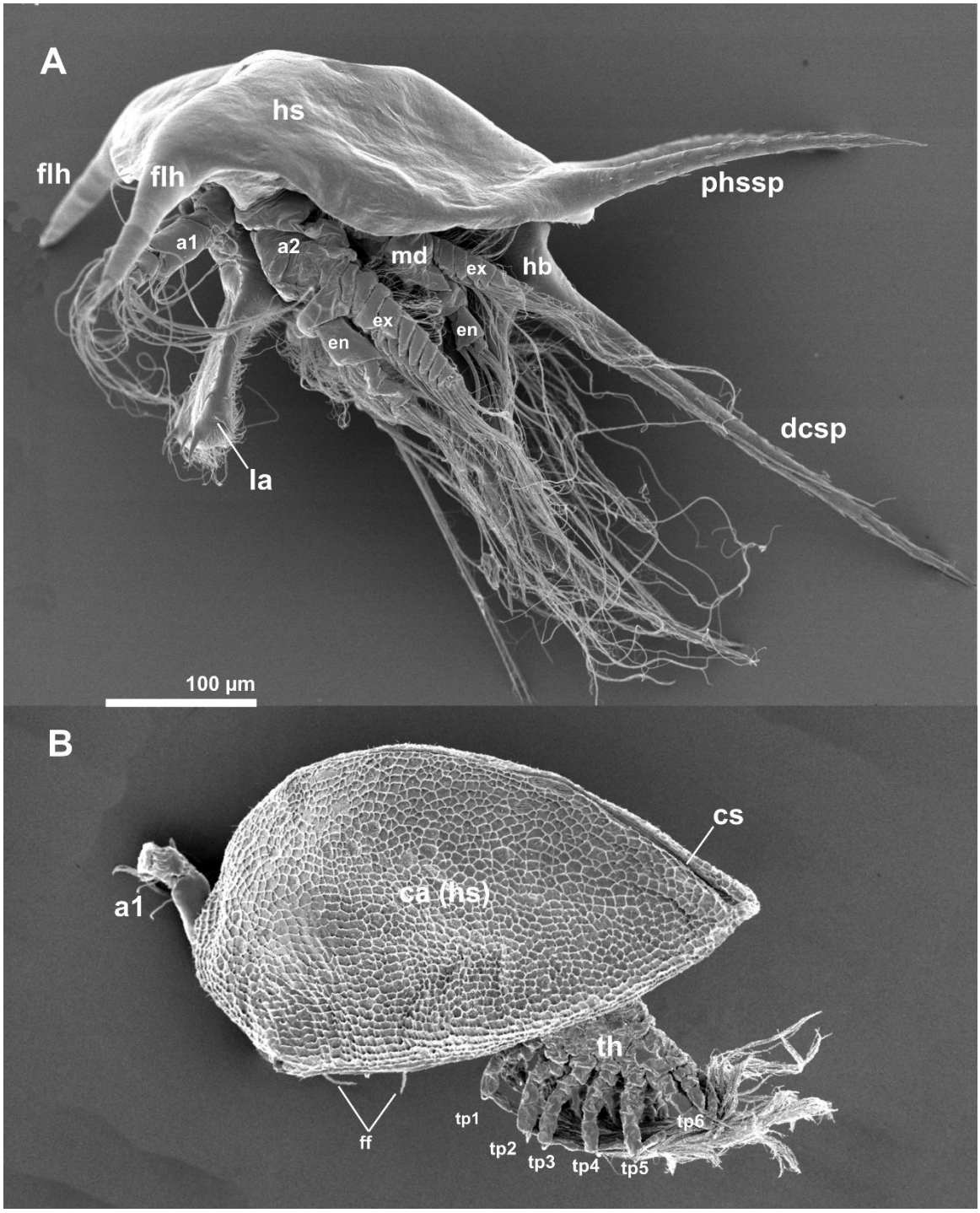

Fig. 2. Nauplius and Cyprid. A: The subterminal naupliar instar (N5). B: The cyprid that follows nauplius 6. The nauplius has a pair of antennules, antennae and mandibles for swimming and feeding and a head shield covering the dorsal part of the body, with no segmentation yet in the hind

body. The cyprid has a head shield (carapace) enclosing the entire body. Its antennules are used for bidepal walking on the substratum before settlement and six pairs of thoracopods used for rapid swimming. At the last larval moult the cyprid is formed beneath the cuticle of nauplius instar VI, including the limbs. Legends: *a1*, antennule; *a2*, antenna; *ca*, carapace; *cs*, carapace slit; *dcsp*, dorsocaudal spine; *en*, endopod; *ex*, exopod; *ff*, frontal filament; *flh*, frontolateral horn; *hb*, hind body; *hs*, head shield; *la*, labrum; *md*, mandible; *phssp*, posterior head shield spine; *th*, thorax; *tp1-6*, thoracopod 1-6.

Caudal appendages (cap): a pair of rod-shaped structures articulated to the posteriormost end of the cypris body, also called "furcal rami" (Walossek 1993; Walossek et al. 1996). In *C. mitella*, they are attached on a distinct telson (Fig. 29D). These appendages contain muscles, are motile and sweep over the substratum in a sensory matter during surface exploration.

Coxa (cx): the proximal segment in the stem of the antenna, mandible and postmandibular appendages.

Coxal process (cxpr): see naupliar process

Dorsal sensilla (ds): sensory setae along or near the midline of the head shield.

Dorsocaudal spine (dcsp): a process extending from the postero-dorsal part of the hind body (Figs. 2–4).

Endite (ed): See naupliar process.

Endopod (en): In a biramous appendage, the ramus closest to the body midline.

Exopod (ex): In a biramous appendage, the ramus extending furthest from the midline

Feeding chamber: Called setose region by Groom (1894), it is a complex space confined anteriorly by the labrum, laterally by setae on the endopods of antennae and mandibles and posteriorly by the hind body (Fig. 27). The atrium oris (see above) is located in the "roof" of this chamber, *i.e.*, on the ventral side of the body.

Frontal filaments (ff): a pair of slender sensory papillae; in nauplii situated between the anterior margin of the head shield and the base of the labrum; in the cyprid basally attached to one of the compound eyes (Figs. 2, 3). They are putatively sensory structures containing branching paraciliary extensions (Walker 1974; Høeg et al. 2004; Høeg and Møller 2006).

Frontolateral horns (flh): a pair of laterally or latero-ventrally projecting processes on the anterior part of the head shield (Figs 2, 3). A distal opening on the tip is the exit for two unicellular glands. The tip also carries innervated setae. The cyprid has no horns, but the glands are retained and open with pores on each side of the carapace near the anterior end (Walker 1974; Høeg et al. 2004; Høeg and Møller 2006; Semmler et al. 2009).

Furcal spines (fsp): A pair of spines diverging as a "fork" posterior-most on the naupliar hind body (Figs. 3, 4, 7, 8, 9, 16). They may be primordia of the articulated caudal appendages present only in the cyprid (see also Walossek 1993).

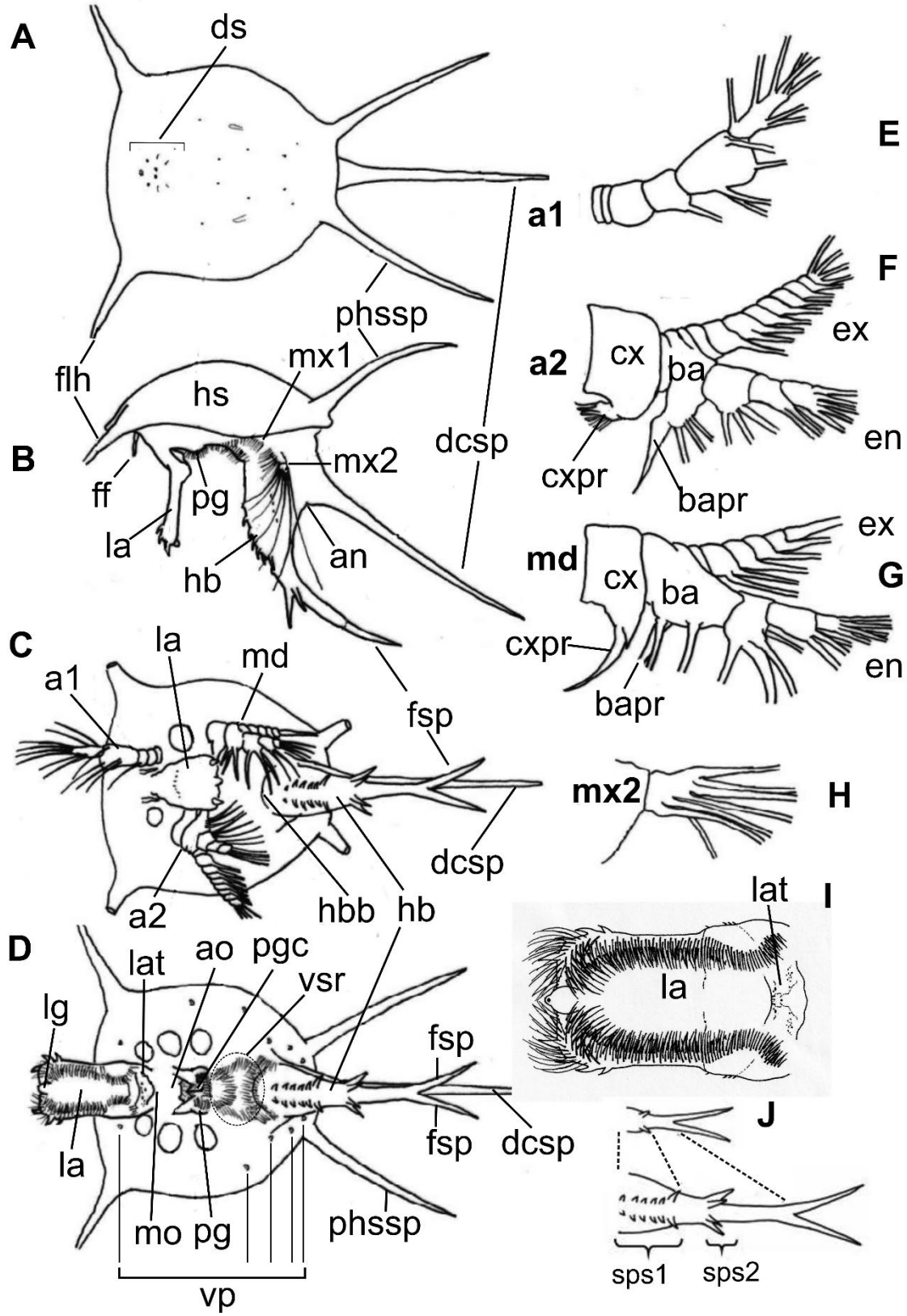

Fig. 3. Terminology of larval features. Various views and details of nauplius instar 6 of *Capitulum mitella*. A: Dorsal view. B: Lateral view. C: Ventral view with right side a1 and mdb and left side a2 removed. D: Ventral view with labrum artificially tilted anteriorly and all appendages removed to visualize the oral area. E–H: Drawings of the appendages (antennule, antenna, mandible and diminutive maxilla. I: Detail of D; posterior side of labrum ("inner" when tilted anteriorly). J: Ventral views of hind body and spinulated area in comparison with instar 2. Legends: *a1*: antennule, *a2*: antenna, an: anus, *ao*: atrium oris, *ba*: basipod; *bapr*: basal process, *cx*: coxa, *cxpr*: coxal process, *dcsp*: dorsocaudal spine, *ds*: dorsal sensila, *en*: endopod, *ex*: exopod, *ff*: frontal filament,

flh: frontolateral horn, *fsp*: frucal spine, *hb*: hind body, *hbb*: hind body boundary, *hs*: head shield, *la*: labrum, *lat*: labral tectum, *lg*: labral grand, *md*: mandible, *mo*: mouth, *mx1*: maxillule, *mx2*: maxilla, *pg*: paragnath, *pgc*: paragnath channel, *phssc*: posterior head shleld spine, *sps1*: hind body spine series 1, *sps2*: hind body spine series 2, *vp*: ventral pores, *vsr*: ventral setose region.

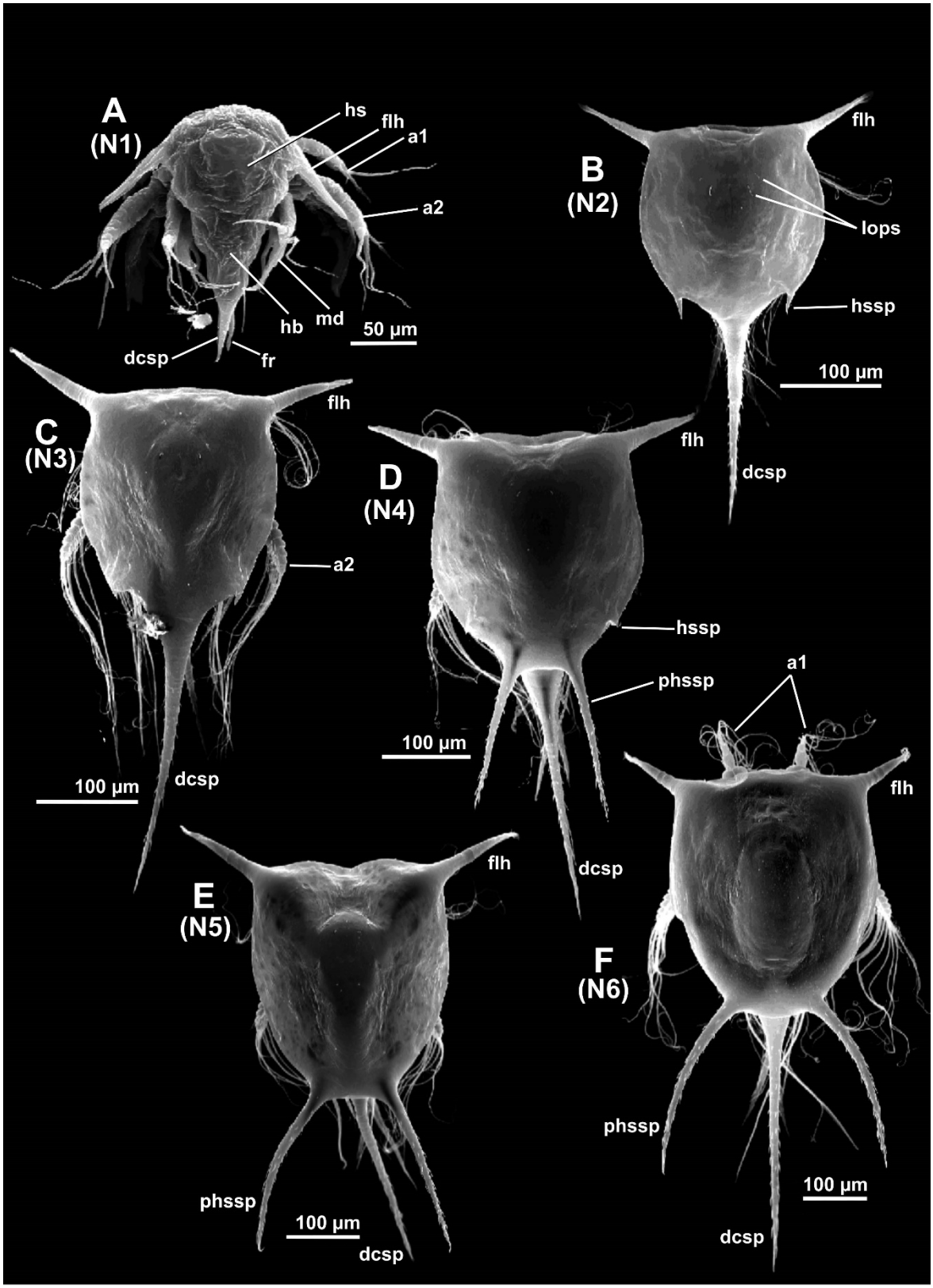

Fig. 4. Dorsal views of naupliar instars of *Capitulum mitella*. A: N1, B: N2, C: N3, D: N4, E: N5; F: N6. Legends: *a1*, antennule; *a2*, antenna; *dcsp*, dorsocaudal spine; *flh*, frontolateral horn; *fsp*, furcal spine; *hb*, hind body; *hssp*, head shield spine; *md*, mandible; *phssp*, posterior head shield

spine.

Head shield (hs): The head shield is an outgrowth of the antennal segment, which covers the dorsal part of head region (antennular, antennal, and mandibular segments).

Headshield spines (hssp, phssp): extend laterally from the naupliar head shield. They comprise smaller headshield spines (*hssp*) and much longer posterior head-shield spines *(phssp)* (Fig. 4): The cypris carapace carries no such spines.

Hind body (hb): The hind body is the post-cephalic part of the body, which grows progressively larger during the naupliar phase. It represents the incipient thorax, abdomen and telson, but segmentation or thoracic appendages are not externally manifested until the cyprid.

Hindbody boundary (hhb): is a groove separating the cephalic region and the hind body (Fig. 17D).

Hinge line (hl): A more or less distinct mid-dorsal cleft in the cypris carapace that facilitates the opening and closing of the valves (Fig. 28D).

Labral gland (lg): a gland in the labrum exiting through a single pore on its distal rim (Fig. 13H).

Labrum (la): a postero-ventrally protruding lobe, which overhangs the atrium oris and forms the anterior margin of the oral opening. We name the two surfaces “anterior” and “posterior” based on the position of the labrum shown in figure 9: N6. The labrum consists of a proximal part (lapp) and a distal part (ladp), the two being angled slightly relative to each other (Fig. 9). The labrum contains two pairs of lateral muscles (Semmler et al. 2009).

Labral tectum (lat): The proximal, posterior side part of the labrum; with three to seven gland pores (Figs. 3I, 13I).

Lattice organs (lo): These are chemosensory structures situated dorsally on the carapace in all thecostracan cyprids (El'fimov 1986; Jensen et al. 1994a; Høeg et al. 1998; Kolbasov and Høeg 2002 2007). In Cirripedia, they usually occur as five pairs of elongated structures, organized into two anteriorly (lo1 and lo2) and three posteriorly situated pairs (lo3-5) (Figs. 28A, D, E). The two anterior pairs occur in nauplii as two pairs of setae called lattice organ precursor setae (lops), anteriorly on the head shield (Rybakov et al. 2003).

Mandible (md): the third pair of head appendages, with biramous morphology and very reminiscent of the antenna. See above.

Maxillule (mx1) and maxilla (mx2): The maxillule (first maxilla) and the maxilla (second maxilla) are the appendages following the mandible in the crustacean head. Below we argue why we consider two pairs of setal regions in the nauplii, developing into distinct humps, as being the maxillule (mx1) and maxilla (mx2).

Naupliar process: A prominent, medially directed process situated on either the coxa or basis of the antenna (*a2*) and mandible (*md*). The specific morphology of the often complex armature of setae and spines depends on the instar. Such processes have also been called *endite*, *arthrite*, or *gnathobase* in other crustacean nauplii. Martin et al. (2014) discussed this in detail and recommended the term “naupliar process” if situated on the coxa but did not discuss similar structures on the basis. We follow Martin et al. (2014) in part by extending their term to cover such structures on both coxa (*coxal process, cxpr*) and basis (*basal process, bapr*). When necessary, we include the name of the limb in question in the abbreviation (thus: *a2cxpr*, *a2bapr, mdcxpr, mdbapr*).

Paragnaths (pg): a pair of lobes or humps located on either side of the midline between the bases of mandibles and forming postero-lateral boundaries of the atrium oris. They are not considered as appendages but derived from the general ventral cuticle (Walossek 1993; Wolff & Scholtz 2006).

Paragnath channel (pgc): The narrow channel in the midline between the paragnaths.

Posterior shield spine(s) (phssp): see head shield spines.

Setae and spines: For such structures, bewildering classifications and names exist. Here, we follow the system of Garm (2004), which in part builds on Watling (1989). Garm considers the setae of crustaceans to be structurally well-defined, having a basal articulation and a lumen extending throughout. Spine (or spinule) is a term for almost any cuticular processes that are not setae, and they can be of various sizes and shapes, including themselves carrying secondary spines. We emphasize that it is not always clear in LM or SEM whether a structure is a seta or a spine. Setae can carry various types of processes. If basally articulated, such side branches are called setules. If setules occur in two opposing rows, it is *plumose* (P seta); if such setules are long and closely spaced, the seta is *feathery plumose* (FP seta). Small, unarticulated setal ornaments are *spinules*, and if there are one or several rows of these, the setae are *serrulate*. Some of the *C. mitella* setae carry two rows of rather long and robust side branches. We here refer to these as *combed setae* (Fig. 26, Table 2), but were unable to accurately determine whether the side branches are setules (articulated side branches) or spinules (unarticulated).

Setal regions 1 & 2: Paired regions situated ventrally behind the mandibles and assumed to represent primordia of the maxillule and maxilla (Figs. 17-A and -B). See these and Discussion.

Setation formulas. These have traditionally been used in descriptions of cirriped naupliar development, especially for the antennules. Such formulas consist of a series of characters describing the seta: S = simple, P = plumose, F = feathered plumose, C = combed seta; starting from the outer ("preaxial") side up to the terminal-most seta and proceeding inwards along the inner ("postaxial") side. See table 2.

Setation numbering on appendages: To facilitate our account, we have numbered the setae on the antennule, antenna, and mandible in the line drawings (Figs. 19, 22, 26). Numbering starts basally on the outer (or lateral) side and proceeds apically and onwards towards the base on the inner (or medial) side. When new setae appear at a moult, they are given a higher number than the highest in the preceding instar. Numbering is as follows: Setae on the antennule (a#): on the antennal or mandibular basis (b#); on the antennal or mandibular exopod (x#); on the antennal or mandibular endopod (n#). In the text, setal types are also, when appropriate, designated as simple (S), plumose (P), feathered plumose (F) and combed (C), and this is summarized in Table 2. When several setae sit on a single segment, our numbering may not always precisely correspond ontogenetically to a previous instar, as this issue can only be solved when looking at setae in formation during a moult. We emphasize that our numbering system is not intended for general use, but only to facilitate the interpretation of our figures of *C. mitella* nauplii.

Telson (te): only present in the cyprid (Fig. 29) as a short component behind the short abdomen, and carrying the caudal appendages, often also called furcal rami (Walossek 1993).

Ventral pore (vp): pores, likely from glands, opening ventrally on the peripheral surface of the head shield (Fig. 7).

Ventral setose region (vsr): space (forming a feeding chamber) fenced by series of setae on the ventral surface between the paragnaths and the hind body (*pf*, *mf*, and *lf* in Figs. 7 and 17).

Table 2. Setation formulae for the naupliar instars of Capitulum mitella. Colons and semicolons indicate different segment and unclear segmentation; separation of the seta within the same segment marked by "."

Instar	Antennule			
	Segment number			
	IV	III	II	I
N6	(P)(P)PPSS.SP:	(S)P.SP:	S:	
N5	(S)(P)PPSS.SP:	(S)S.P:	S:	
N4	(S)(P)PPSS.SP:	P:	S:	
N3	(S)PPS:	SP.P:	S:	
N2	SPSS:	SP.P:	S:	
N1	(Sr)SrSrSrSr;	Sr:	Sr:	

Instar	Antenna												
	Exopod									Endopod			Basis	Coxa
	Segment number									Segment number				
	IX	VII I	VII	VI	V	IV	III	II	I	III	II	I		
N6	4P:	P:	P:	P:	P:	P:	PP:	P:		PPSPP:	SSS:	SFFF:	SPFH:	NP
N5	3P:	P:	P:	P:	P:	P:	PP:	P:		PPSPP:	SSS:	SFFF:	SPFH:	NP
N4	2P:	P:	P:	P:	P:	P:	PP:			PPSPS:	SSS:	FSF:	SPFH:	NP
N3	2P:	P:	P:	P:	P:	P:	P:			PPsP:	SS:	FF:	SSFH:	NP
N2		SP:	P:	P:	P:	P:	P:			PPS:	SS:	FF:	SFH:	NP
N1		Sr:	Sr:	Sr:	Sr:	Sr:	Sr:			SrSrSr;	SrSr;	SrSr;	SrS:	NP

Instar	Mandible										
	Exopod						Endopod			Basis	Coxa
	Segment number						Segment number				
	VI	V	IV	III	II	I	III	II	I		
N6	P:	P:	P:	P:	PP:		SSSSS:	SSPP:	SPCP:	PPP:	NP
N5	P:	P:	P:	P:	PP:		SSSSS:	SSPP：	SPCP:	PPP:	NP
N4	P:	P:	P:	P:	P:		SSSSS:	SPP:	SPCP:	PPP:	NP
N3	P:	P:	P:	P:	S:		SSS:	SP:	PCP:	PPP:	NP
N2	P:	P:	P:	P:	S：		SSS:	SP:	PCP:	PP:	NP
N1	S:	S:	S:	S:			SrSr;	SrSr;	SrSr;	SrS:	NP

C: combed seta; F: F-plumose seta; H: hispid seta; NP: naupliar coxal process; P: plumose seta; s: small simple seta, sometimes primordial; S: simple seta; Sr: serrulate seta; Parenthesis indicate that the seta is sited apically on the segment.

Larval Morphogenesis

In this section, we follow individual larval features through all six naupliar instars (N1 - VI) and the final cyprid. The metamorphic moult to the cyprid involves significant structural and functional changes, but many features can be traced back to the naupliar phase. For the cyprid, Rao and Lin (2013) already gave a detailed and complete account based on SEM, including a very informative table on the antennular setae. We can add little new for this instar, but for comparison with the nauplius phase, we also show important cyprid features in the figures.

Naupliar head shield (Figs. 2, 4, 6–9)

The head shield and the entire larval body undergo drastic changes in shape three times during development: from N1 to N2, from N3 to N4, and finally from N6 to the cyprid, where a carapace replaces the shield. As naupliar development progresses, the shield becomes progressively larger and increasingly overhangs the body both laterally and posteriorly. In N1 (Figs. 4A, 6A, and 7-N1), the shield has a simple pear-shaped form with posteriorly directed frontolateral horns. N2 and N3 have almost similarly shaped shields, but now the frontolateral horns are projecting antero-laterally. In N1 to N3, the shield is only indistinctly separated from the hind body on the dorsal side, but ventrally there is a clear border behind the mandibular region, except in N1. In N4 to N6, the enlarging hind body is separated from the cephalic region dorsally by being located underneath the posteriorly projecting head shield (Fig. 8: hatched arrow).

Head shield spines (Figs. 4, 6, 8, 10)

A single pair of small *head shield spines* is present in N2-N3 (Fig. 4: *hssp*). In N4, there are two pairs of spines. A small, more anteriorly situated pair (*hssp*) and a much longer pair of *posterior head shield spines* (*phssp*) flanking the dorso-caudal spine (Figs. 4, 6, 8). On the ventral side, near the shield margin, there are in N4-N6 also a small setae or spines, except between the two posterior shield spines. The number of these spines increases with instar (N4: 8-10, N5: 16-20, N6: up to 30-36) and some of them have terminal pores.

Cypris carapace (Figs. 2, 28)

In the cypris, the head shield is replaced by a *carapace* that has an elongated shape in both dorsal and lateral views and can enclose the entire body in its large mantle cavity (Fig. 28). In *C. mitella,* there is a mid-dorsal groove or "hinge" (Fig. 28). Posteriorly, the carapace is divided into two halves by a dorsal cleft. The carapace has a honeycomb-like surface structure but lacks conspicuous ornaments such as spines and frontolateral horns. The pores of the frontolateral horn glands are retained (Fig. 28B). Dorsally, the lattice organs are situated in two groups (Fig. 28D, E). Both the thorax with the natatory thoracopods and the antennules can be extended ventrally from the mantle cavity such as happens when the larva is either swimming or walking on the substratum (Fig. 28). At rest, both antennules and thorax are usually completely retracted, and the same is true in most fixed specimens. In this condition, a ventral view shows only the narrow opening between the contracted valves of the carapace (Fig. 28C).

Dorsal sensory setae and pores (Fig. 11)

Lattice organs: Throughout all naupliar instars, the head shield carries two pairs of setae, which are precursors of the first two pairs of lattice organs (*lo1*, *lo2*) in the cyprid (Jensen et al. 1994a; Rybakov et al. 2003). These *lattice organ precursor setae* (*lops*) are situated near the midline in the anterior half of the naupliar head shield (Figs. 4B, 11D, E). In N1, the precursor setae are separated by 45–50 μm; in later instars, the lo1 precursor setae are separated by 55–65 μm,

while only 30–45 µm separates the lo2 precursor pair. The lo1 precursor setae are 4 µm long, with a basal annulation and a narrow side branch extending at the top of the annulated part. The lo2 precursors are 8 µm long with small spinules on their setal shaft. In later instars, both pairs of lattice organ precursors change to simple setae. The posterior pairs (*lo3-5*) have no external precursors in the nauplii but appear in the cyprid just in front of the cleft in the carapace.

Other sensilla and pores: In the naupliar instars, there is a single pore in the midline between the first pair of lattice organ precursor setae (*lops*) setae and the anterior shield margin (Fig. 11C–E, large arrow). In the cyprid, this anterior midline pore remains positionally associated with the anterior lattice organs. In later naupliar instars, additional pores develop anteriorly between the lo1-precursors, with one present in N5 and three (two newly added) in N6.

In addition to the lattice organ precursors, the shield also carries several additional pairs of small setae from N3 onwards (Fig. 5). These are all furnished with apical pores, ca. 2 µm long and extend from a small pore or depression located on the side of a hillock-like papilla, measuring 1 µm in basal diameter and height. In N3-N6, the first such pair of setae is sited between the anterior midline pore and the first pair of lo-precursor setae. An additional two pairs (in N3), or four pairs (in N4–N6), are situated in the middle to posterior shield area (Fig. 5). In the cyprid, there are many small sensory setae widely distributed on the surface of the carapace (see below).

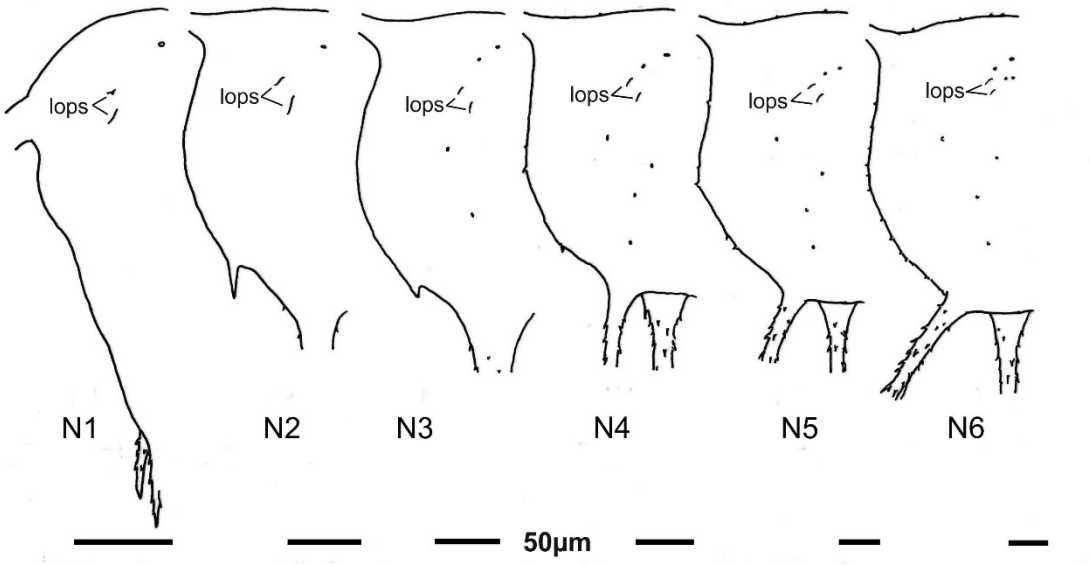

Fig. 5. Drawings of dorsal views of head shield (left side only) of the six naupliar instars of *Capitulum mitella,* showing the (left side) lattice organ precursor setae (*lops*) and other setae and pores.

Frontolateral horns (Fig. 12)

The newly hatched N1 nauplius has postero-laterally directed frontolateral horns. Distally, the horn lacks a true opening but terminates in a concavity, above which a small, spine-like process extends. Presumably, the two gland cells terminating at the tip of each horn are not yet active in N1. From N2 the horns are open-ended, ventro-laterally directed and increase in size through the instars. N2 has tubular-shaped horns. The tip has clefts in both the dorsal and in the ventral margins, and a row of perforations lies along the ventral one. In N3 to N6 the horns terminate as three small

prongs, with the two lateral ones carrying many thin cuticular villi rolled inward. (Høeg and Møller 2006; Chan et al. 2014)

Frontal filaments (Figs. 3, 6)

From N3, the nauplii carry a pair of frontal filaments on the ventral surface close to the anterior margin of the head shield and the labrum. A filament consists of a proximal, thicker part and a distal, thinner and flexible part, which projects antero-ventrally, with no articulation existing between the two parts. This shape remains the same through all naupliar instars, but the length increases from ca. 40 μm in N2 to ca. 60 μm in N6. In the cyprid, the filaments are located in the anterior part of the mantle cavity and attached at the base of the compound eyes. Since the eyes can be operated by muscles, such movements also affect the filaments. Being much longer than in nauplii, the frontal filaments often extend out of the mantle cavity when the cyprid explores a surface (Høeg 1985; Høeg et al. 2004; Chan et al. 2014).

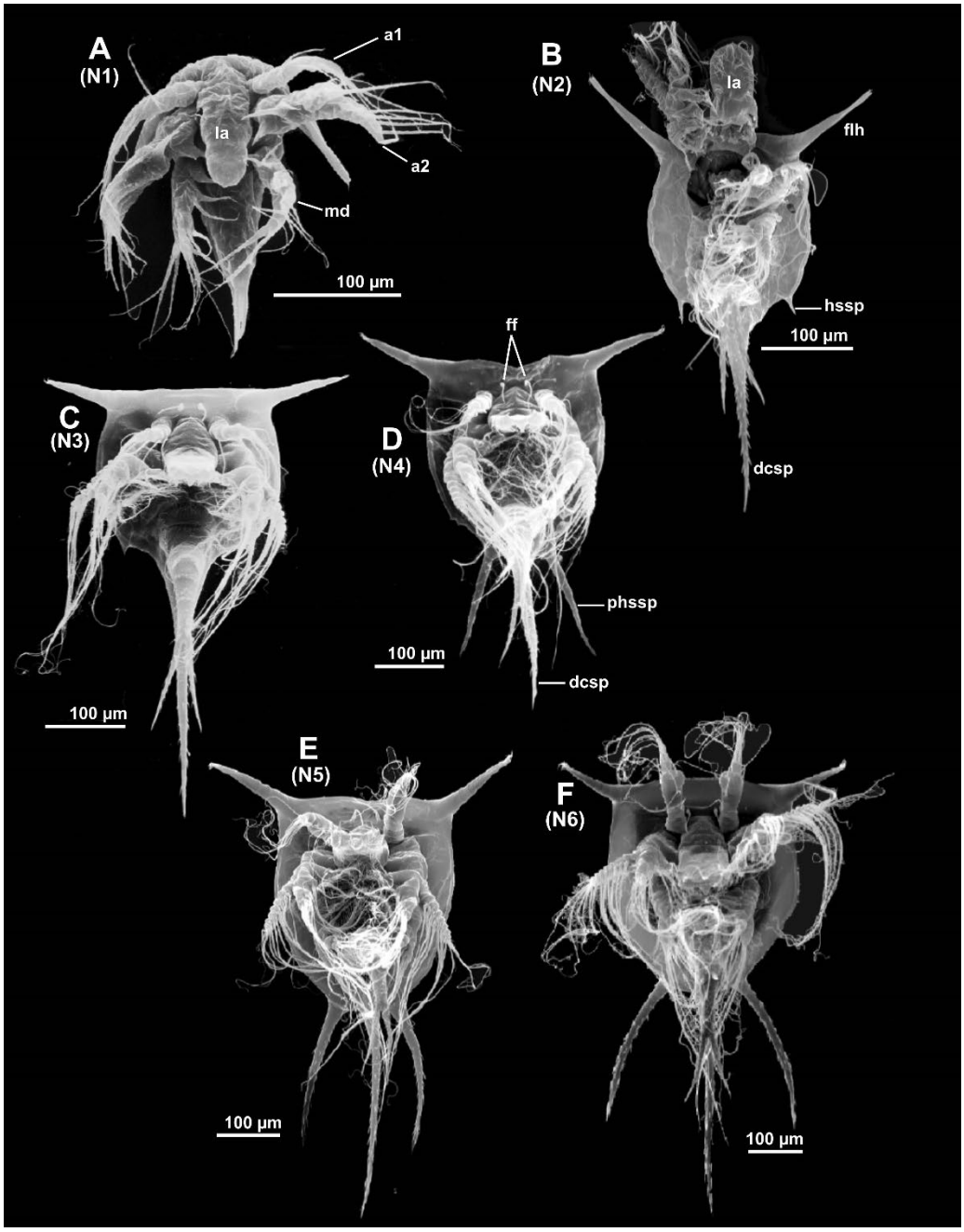

Fig. 6. Ventral views of naupliar instars of *Capitulum mitella.* A: N1, B: N2 with labrum tilted artificially anteriorly; right a1and a2 somewhat damaged in preparation. C: N3, D: N4, E: N5; F: N6. Legends: *a1*, antennule; *a2*, antenna; *dcsp*, dorsocaudal spine; *ff*, frontal filament; *flh*, frontolateral horn; *hssp*, head shield spine; *la*, labrum; *md*, mandible; *phssp*, posterior head shield spine.

Ventral pores (Figs. 7, 10)

From N3, the head shield has an increasing number of small pores located along its ventral margin. Each pore is situated on a tubular or papillary structure (Fig. 10). Their position, remaining almost invariable through the instars, is shown in figure 7, where the number also indicates their order of appearance during development.

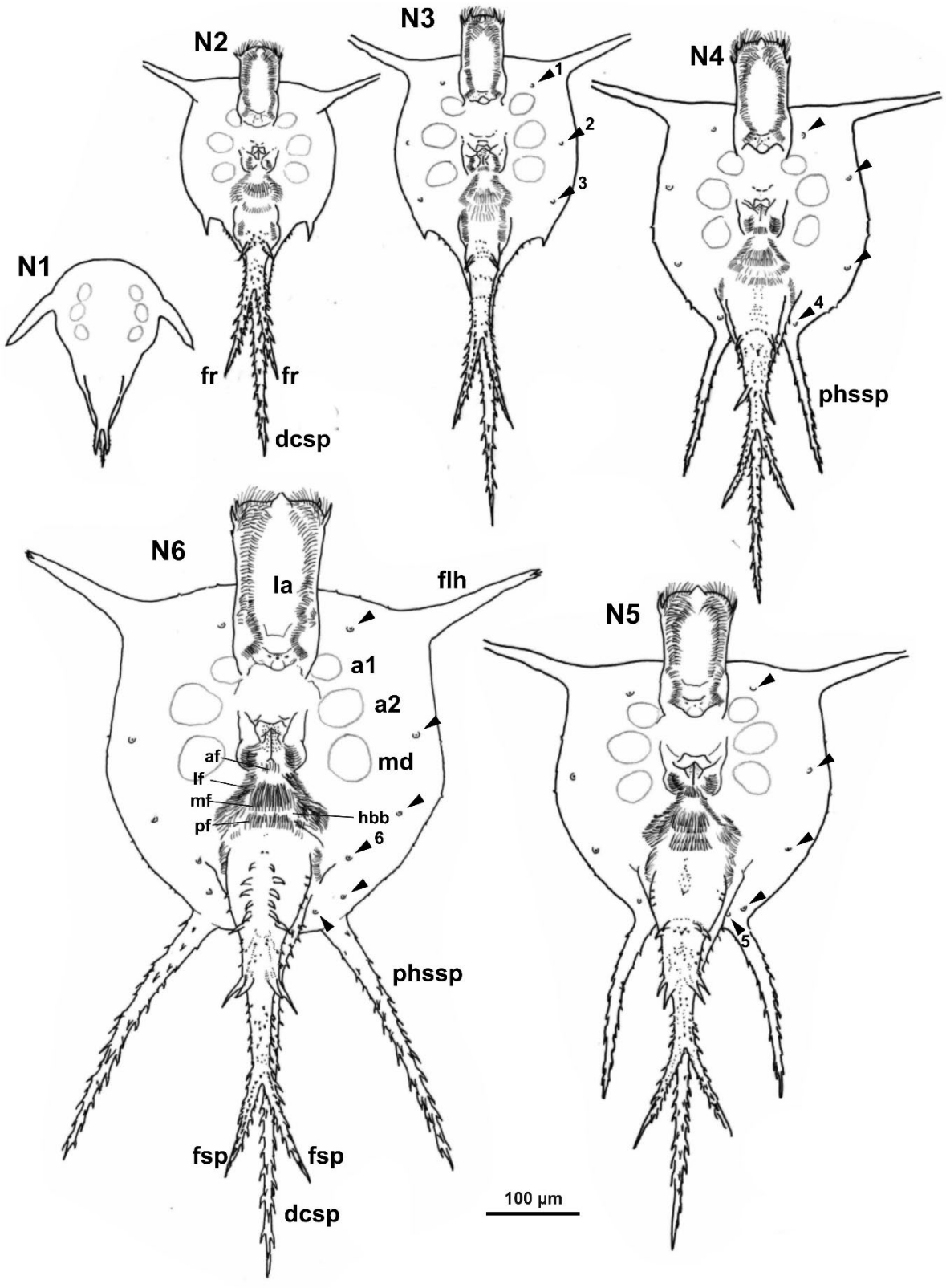

Fig. 7. Drawings of ventral views of the six naupliar instars of *Capitulum mitella*. All with an anteriorly tilted labrum and the appendages removed. Arrowheads point to the ventral pores; pores 1–3 appear together in N3, pore 4 in N4, pore 5 in N5 and pore 6 in N6. Ventral fences of setae are indicated as anterior fence (*af*), middle fence (*mf*), posteior fence (*pf*) and lateral fence (*lf*). Legends: *a1*, antennule; *a2*, antenna; *dcsp*, dorsocaudal spine; *fsp*, frucal spine; *hbb*, hind body bounday; *phssp*, posterior head shield spine.

Labrum (Figs 13, 14, 15)

The rectangularly shaped labrum extends ventrally from the body. Our microscopic observations on live nauplii showed that it can swing forwards and backwards to some extent. We could not fully document its mobility, but its approximately anteriormost stance is shown in figures 3B and 8C, D. It tilts backwards to some extent (Fig. 8B) but never to fully covers the feeding chamber. On its posterior surface, the proximal part, facing the atrium oris, is furnished with gland openings and small spines (Fig. 13I) that increase in number through the instars (Fig. 15). The more distal part of the labrum has a much thickened margin, fringed with many slender and posteriorly directed setae. This forms a central, deep concavity running through the posterior surface of the labrum facing the feeding chamber (Fig. 13). The labrum and its fringing setae serve to confine the feeding chamber anteriorly (Figs. 20, 27). The labral structure becomes increasingly complex through the instars. In N1, it is rather simple, the anterior surface of the distal part having short, fine setae, while few setae and no special structures being present on the posterior surface. The opening of the labral gland appears in N2 and lies on a distinct protrusion at the distal end, where there are also several fine setae and stout spines (Fig. 13H). From N2, its relative size and inner muscular swelling increase with each moult, as does the number and size of the marginal setae. There are two spine pairs on the distal margin in N2 and N3 but four spine pairs in N4 to N6 (Figs. 14, 15). There is also an increase in the number of small spines on the anterior surface (Fig. 15). In the cyprid, the labrum is a simple hump of unsclerotized cuticle without any setae or spines.

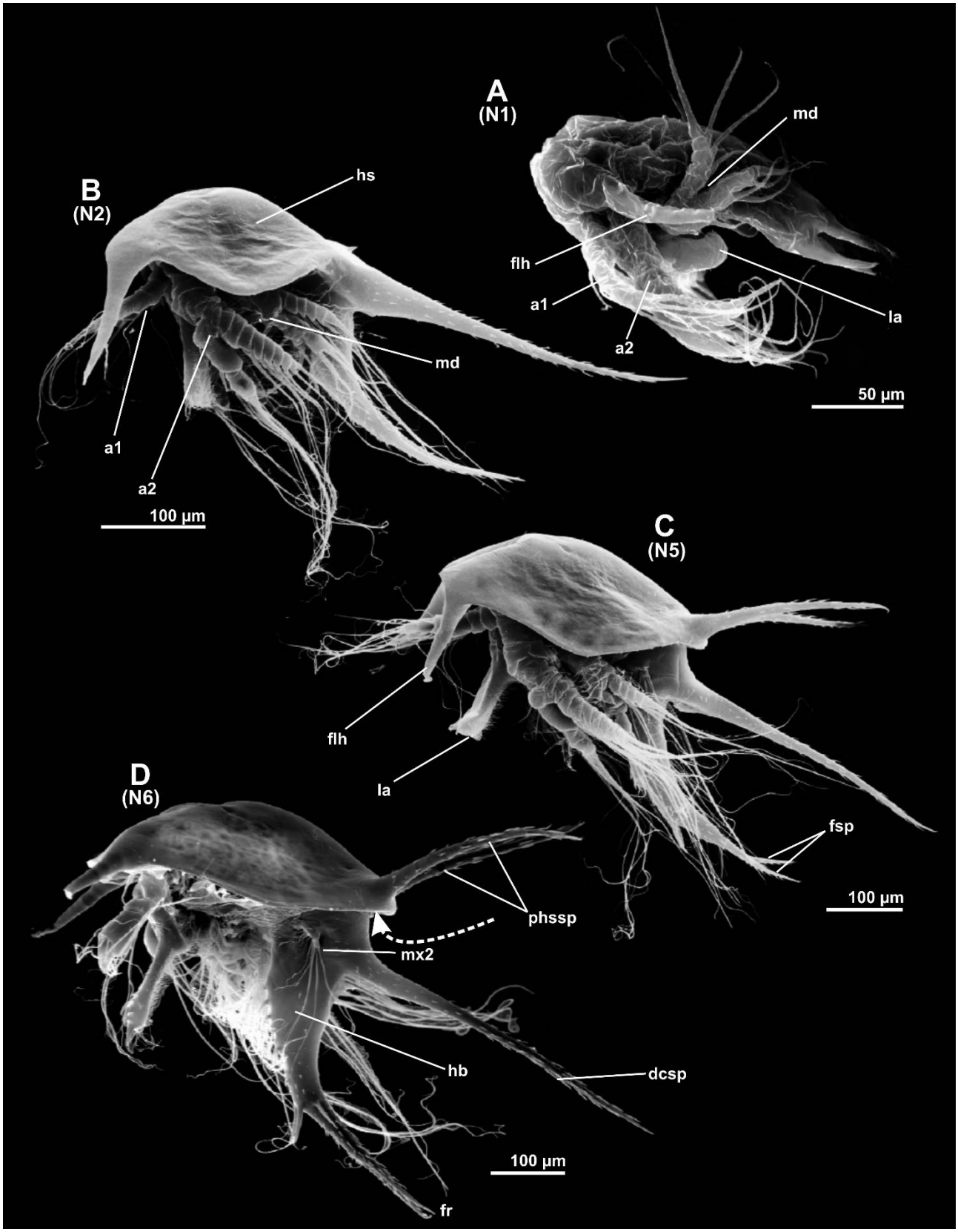

Fig. 8. Left lateral views of naupliar instars of *Capitulum mitella*. A: N1, B: N2, C: N5, D: N6. Dotted arrow indicates clear separation of head shield from the hind body.

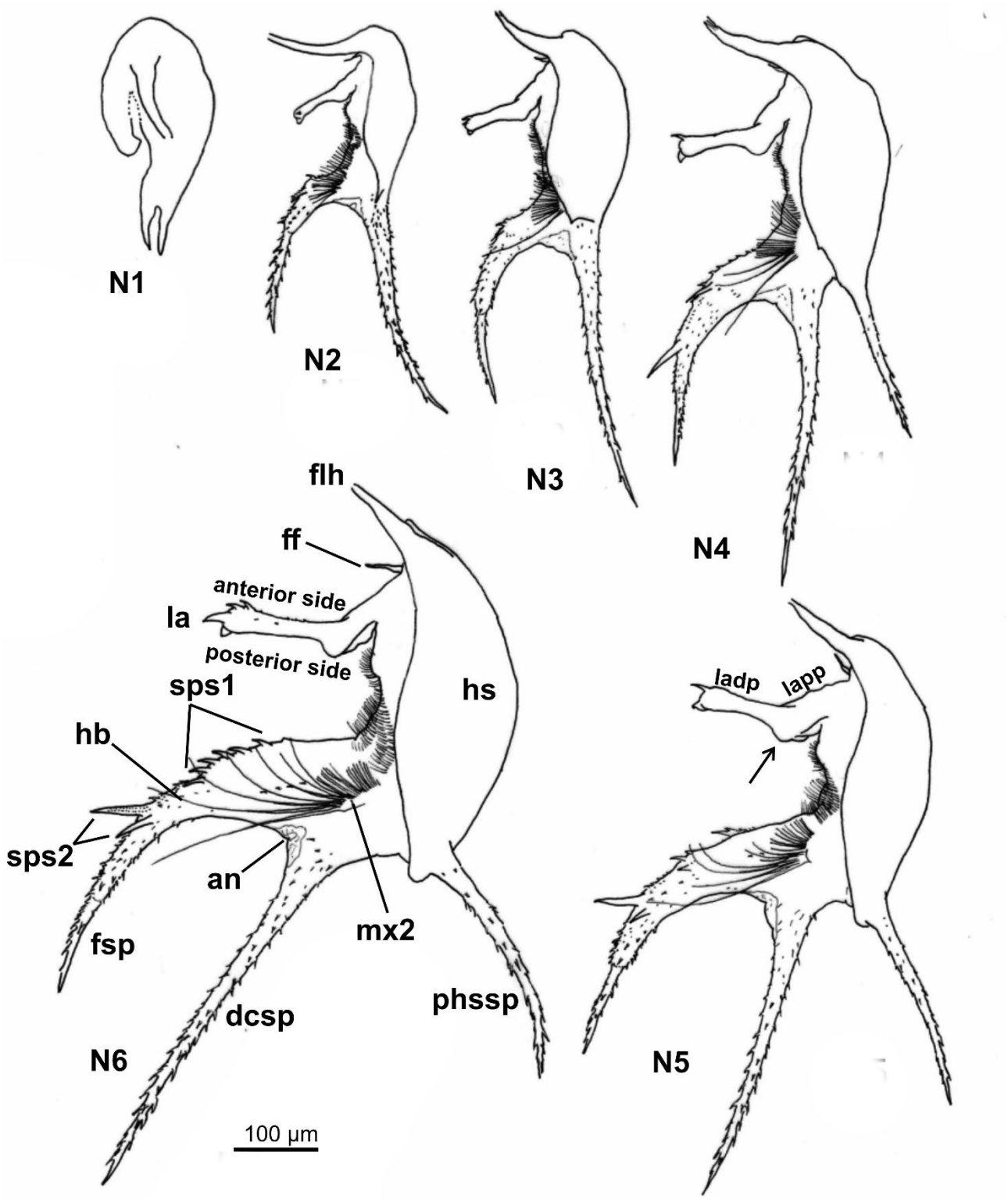

Fig. 9. Drawings of left lateral views of the six naupliar instars of *Capitulum mitella*. The angle between the proximal part of the labrum (*lapp*) and the distal part (*ladp*) is indicated by an arrow. Two series of spines on the hind body (*sps1*, *sps2*); the anterior one consisting of six pairs and a minute pair (see Fig. 23F). Legends: *an*, anus; *dcsp*, dorsocaudal spine; *fsp*, furcal spine; *hb*, hind body; *hs*, head shield; *la*, labrum; *phssp*, posterior shield spine; *sps1*, series 1 hind body spine; *sps2*, series 2 hind body spine.

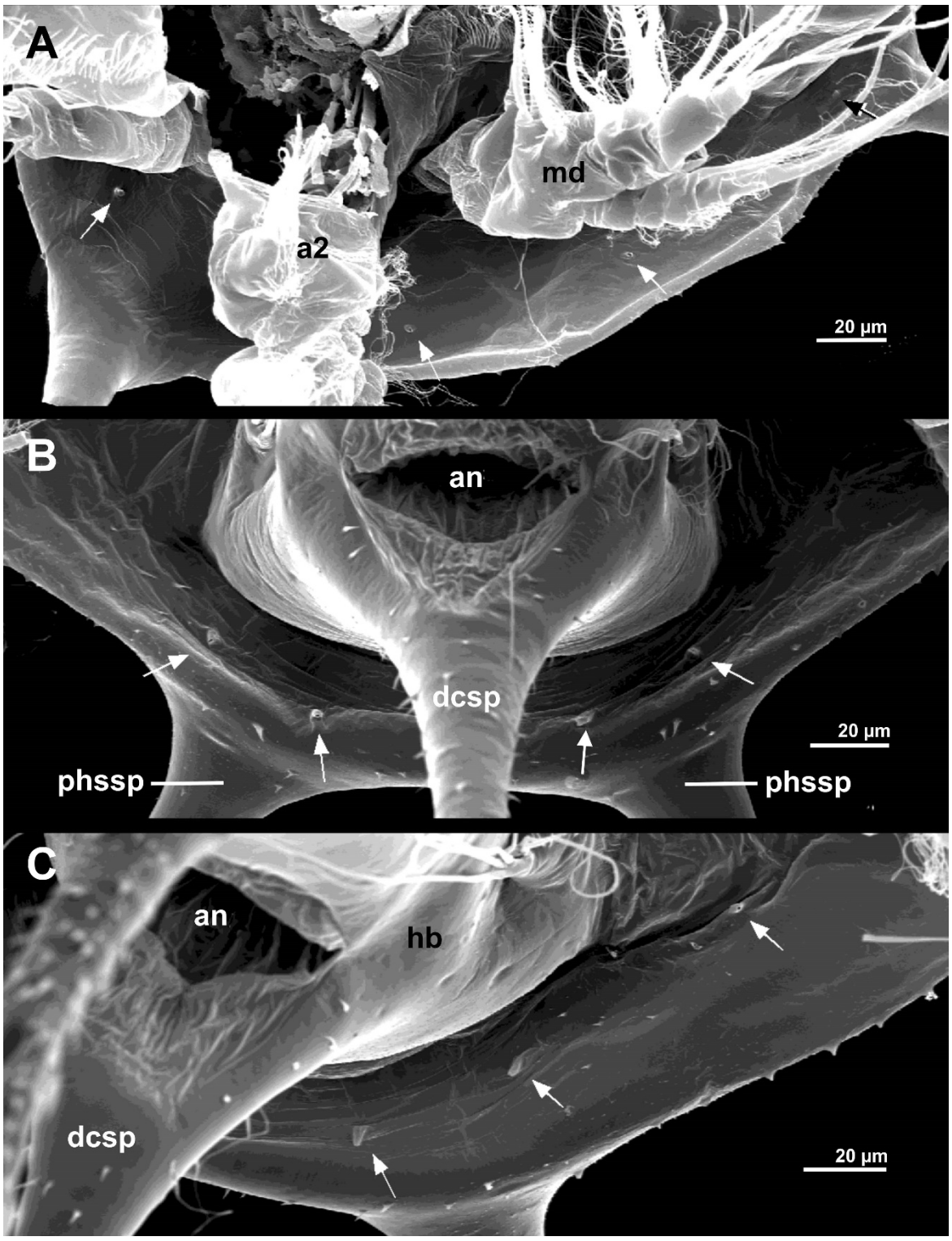

Fig. 10. Ventral views, showing details of the naupliar instars of *Capitulum mitella*. A: N4, anterior end is left, showing details of right body side. B: N5, anterior end is up, showing details in the area near the anus. C: N6, oblique of the area near the anus. Note in B and C how the anus (*an*) is situated just ventral to the base of the dorsocaudal spine (*dcsp*). Arrows indicate ventral pores. Legends: *a2*, antenna; *hb*, hind body; *md*, mandible; *phssp*, posterior head shield spine.

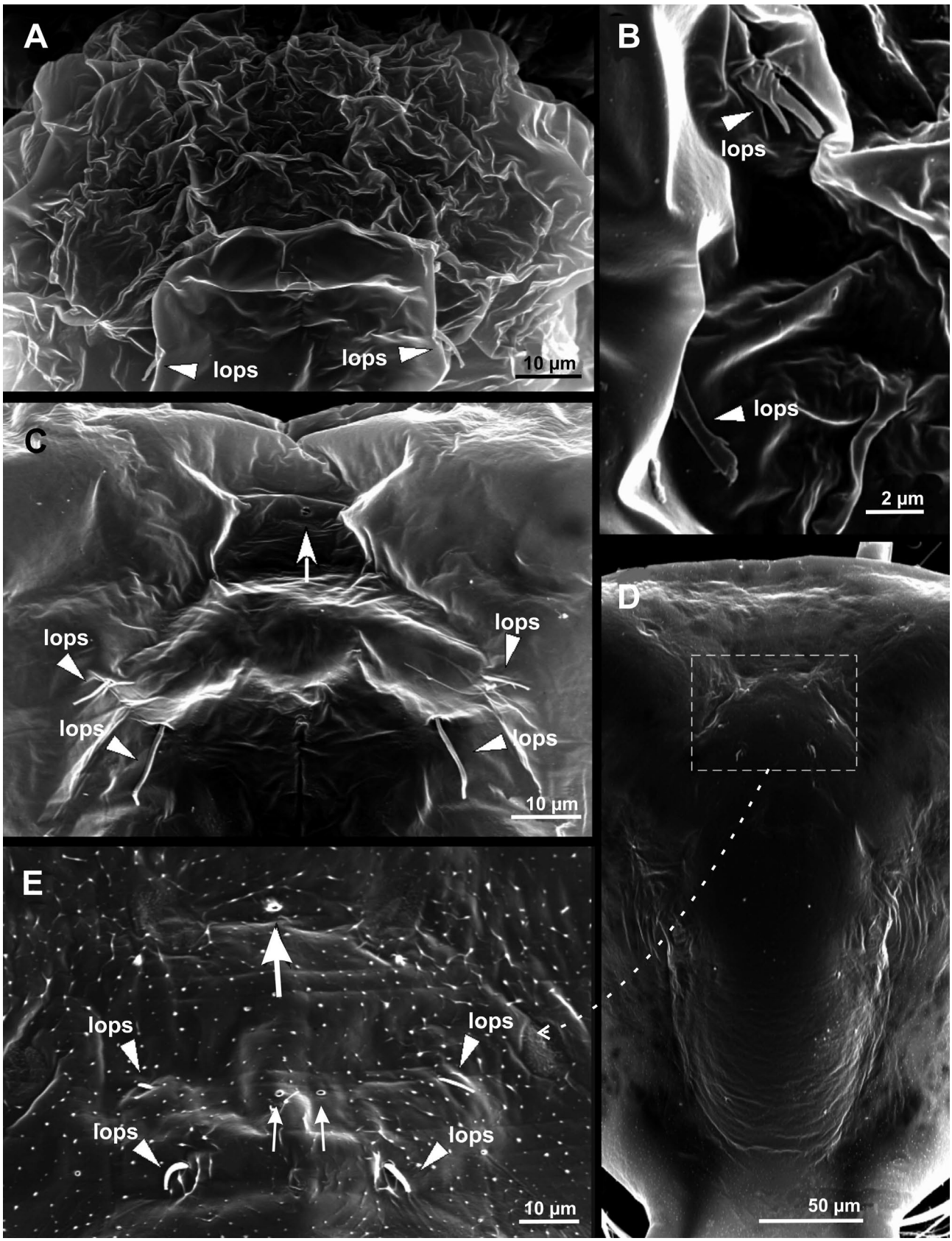

Fig. 11. Dorsal sensilla in naupliar instars of *Capitulum mitella.* A: N1 (anterior pair of lattice organ precursor setae (*lops*) not in view), B: high magnification of the right side lattice organ precursor setae in A. C: N2, D: N6, E: high magnification of the rectangle part in D. Large arrow point to the anterior midline pore, small arrows to other pores. Arrowheads point to small seta.

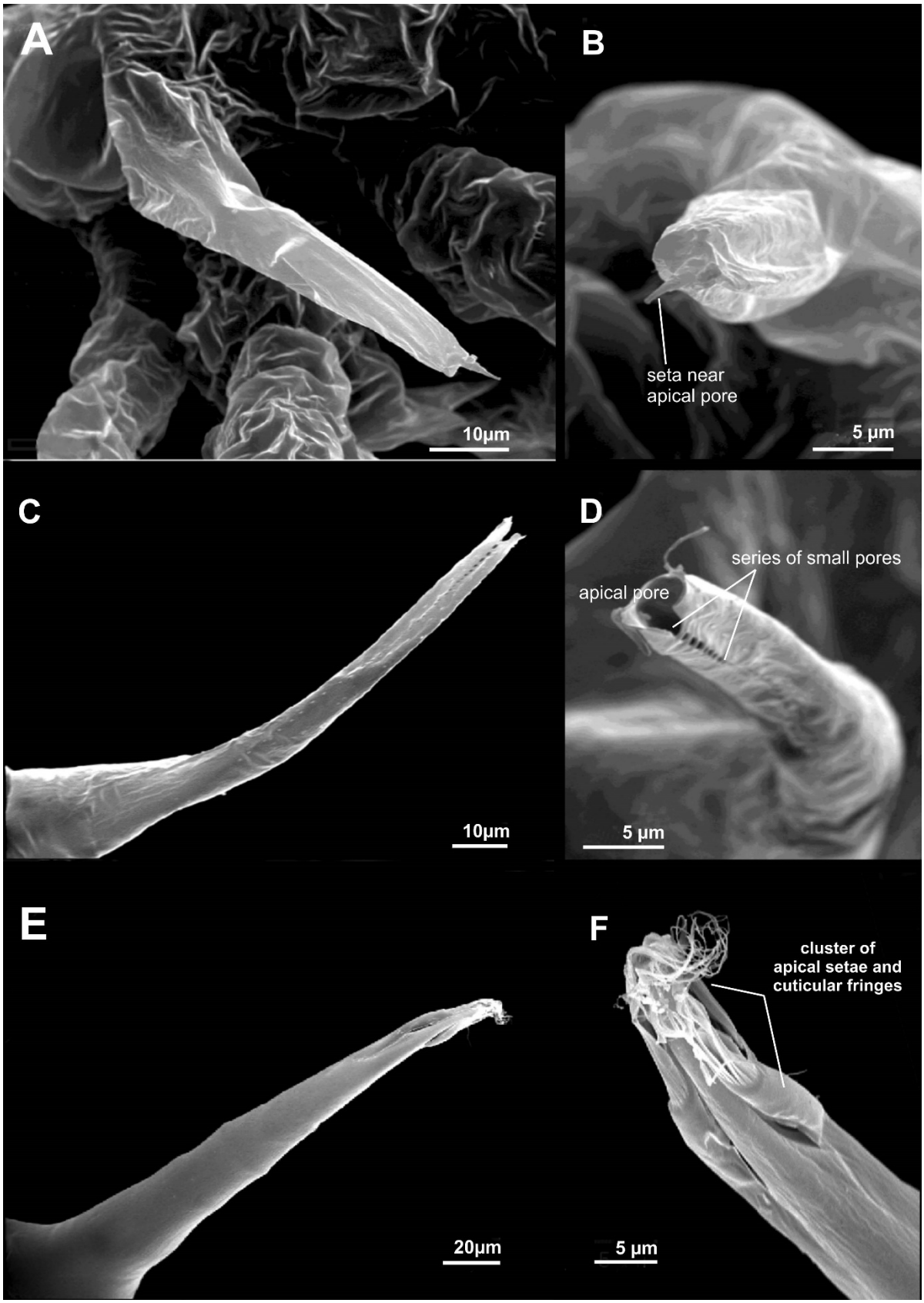

Fig. 12. Frontolateral horns of naupliar instars of *Capitulum mitella*, showing how the apical end changes through the instars. A: N1. Dorso-lateral view of left frontolateral horn. B: N1. Top view of frontolateral horn. C: N2. Ventral view of left frontolateral horn. D: Oblique apical view of frontolateral horn, showing gland pore and series of small pores. E: N6. Ventral view of left frontolateral horn. F: N6. Oblique apical view of frontolateral horn; note the several setae or cuticular fringes.

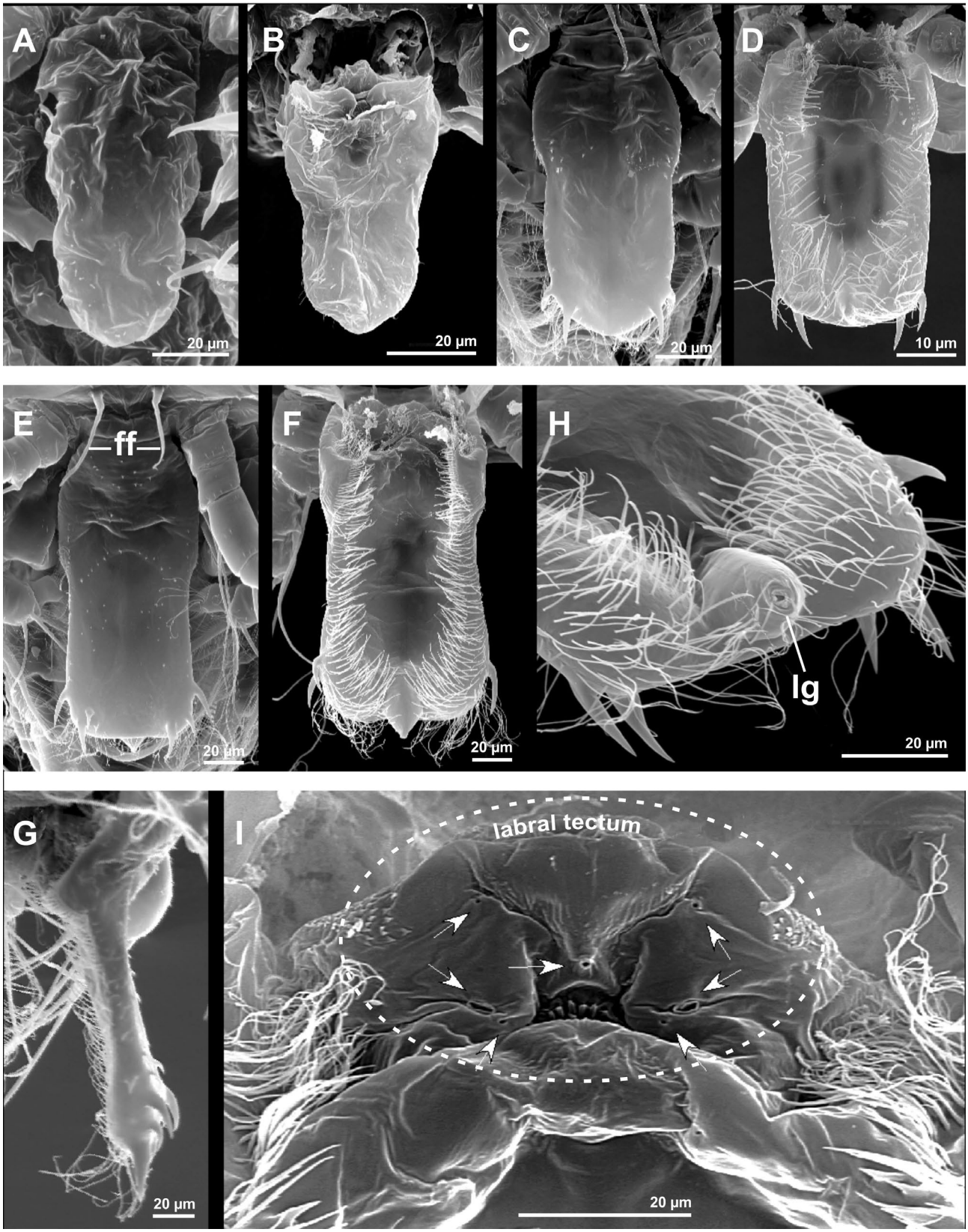

Fig. 13. Labrum of naupliar stages of *Capitulum mitella.* Anterior (outer) and posterior (inner) views; see also Figs 14 and 15. A: N1; anterior (outer view). B: N1; posterior (inner view). C: N2; Anterior (outer) view. D: N3; posterior (inner view) showing labrum fringed by setae. E: N4; anterior (outer view). F: N6; posterior (inner) view with labrum fringed by dense fence setae. G: N6; lateral view. H: N5; oblique posterior view of distal; showing pore of labral gland (*lg*), setae and spines, I: N5; posterior view of proximal labral part (*lat*). Arrows indicate gland openings; labral tectum indicated by hatched lines.

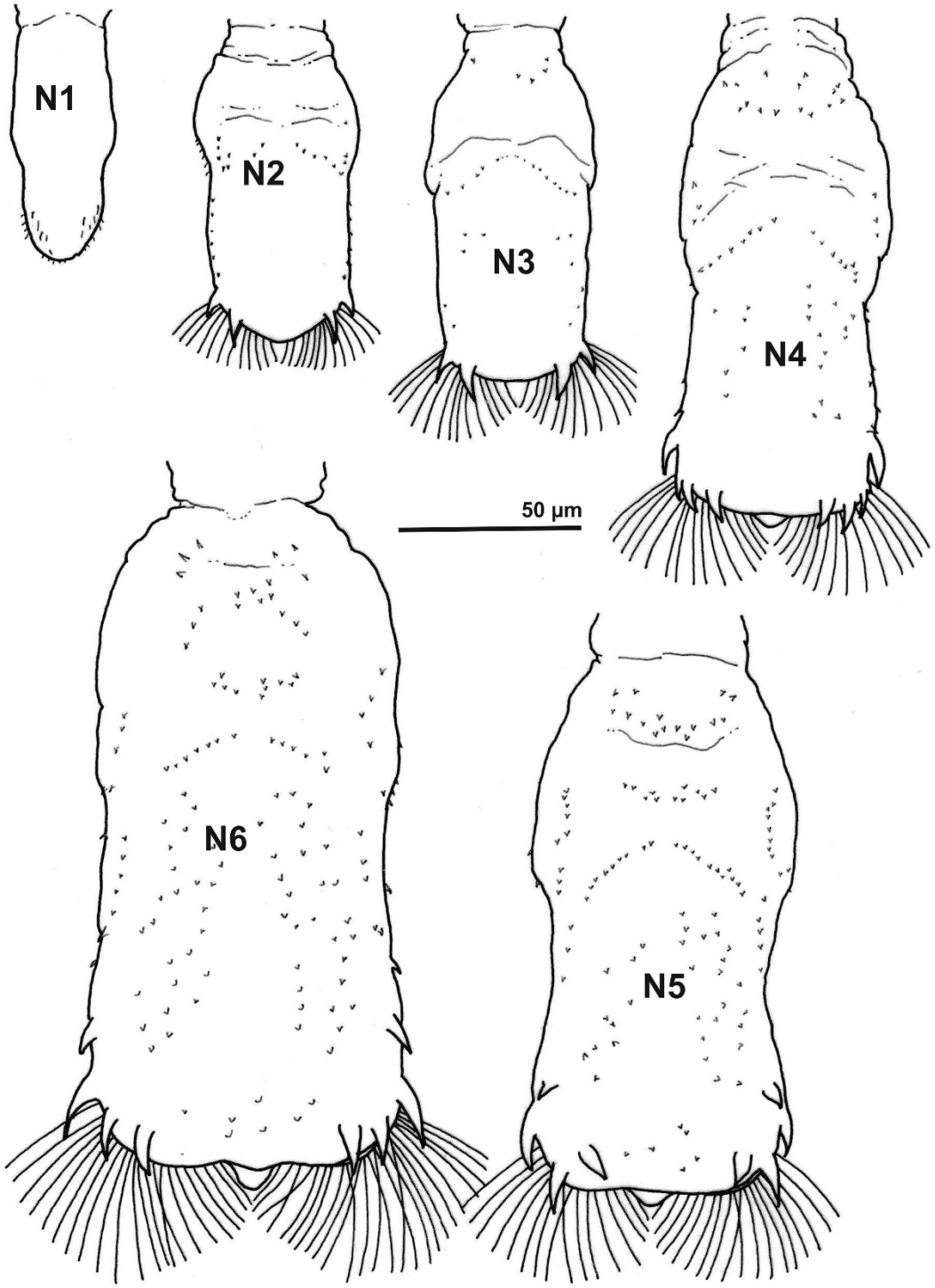

Fig. 14. Drawings of anterior (outer) views of labrum of naupliar instars of *Capitulum mitella.* Distal end is down. Note increasing numbers of lateral and distal spines.

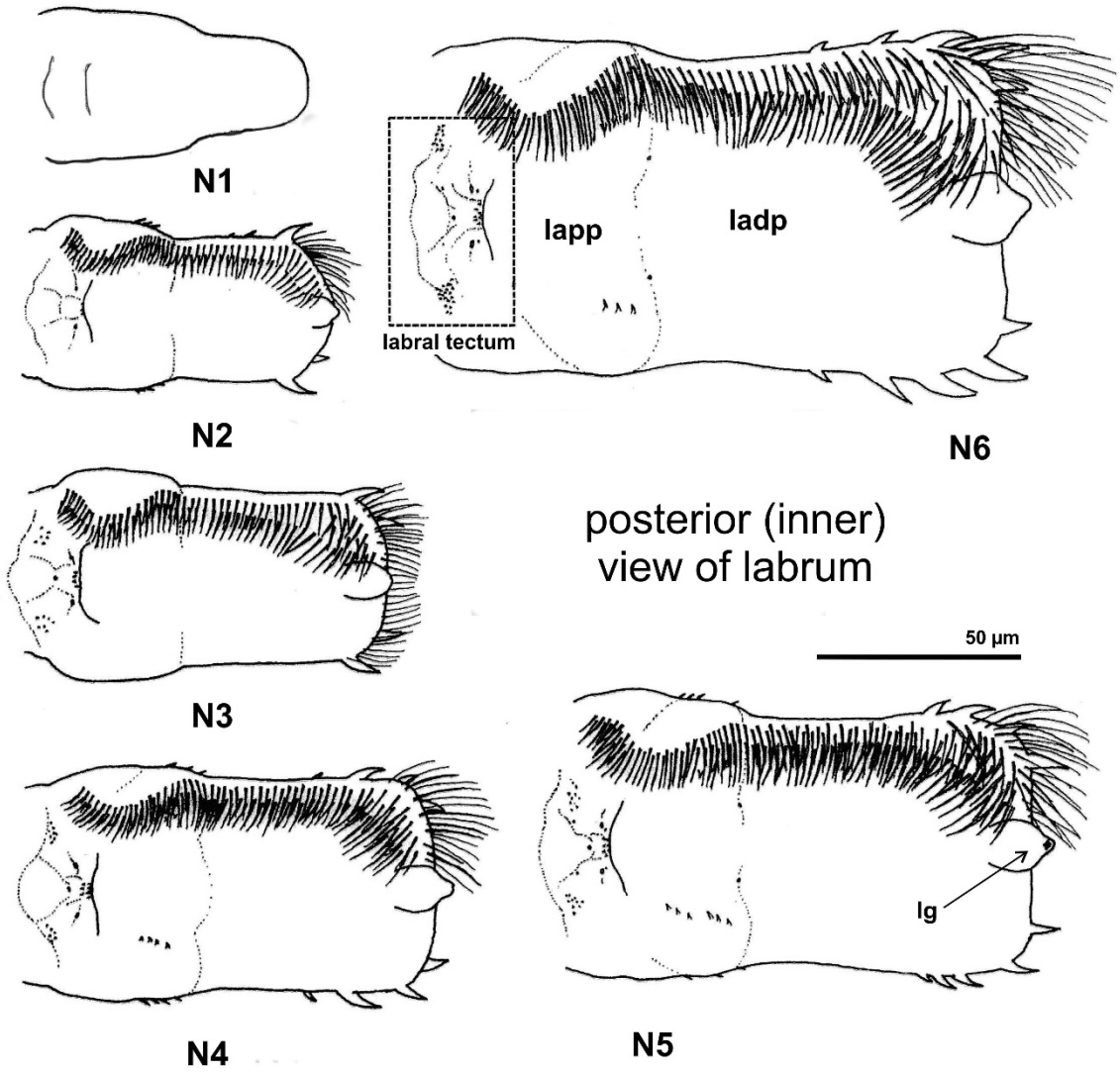

Fig. 15. Drawings of posterior (inner) views of labrum of naupliar instars of *Capitulum mitella.* The fringing (lateral) setae on the labrum are omitted on the body left side (here lower edge) to better visualize the denticles. In N6, the area covering the mouth (labral tectum) also shown in rectangular broken line. Opening of the labral gland on a protuberance at the distal end (arrowed in N5). Legends: *ladp*, distal part of labram; *lapp*, proximal part of labrum.

Oral region and feeding chamber (Fig. 27)

The oral region consists of the mouth, atrium oris, and paragnaths, none of which are present in the non-feeding N1. The mouth opens in N2 as a transverse, elongated slit between the antennules and just behind the basalmost part of the labrum. What we here choose to call “atrium oris” is a depression located immediately posterior to the mouth. It is a V-shaped pit traversed by a narrow groove called the paragnath channel (Fig. 27B, D). Anteriorly this channel passes into the mouth. The sides of the atrium oris lack setae and spines. The hump-shaped paragnaths form the posterior confinement of the atrium oris, carrying a row of inwardly directing setae along their lateral rims. The atrium oris and paragnaths lie in the dorsal roof of the feeding chamber, which is the complex space where captured food items are trapped before final ingestion. When antennae and mandibles are in their most median stance, the naupliar processes of these appendages meet in

the midline just behind the mouth (Fig. 27C, E). In this stance, the chamber is also a functionally closed space, confined anteriorly by the labrum, laterally by the endopodal setae of antenna and mandibles and posteriorly by the hind body, when the latter is tilted forward during feeding, as we observed on live nauplii (Figs. 20, 27). We used light microscopy to examine both healthy nauplii and those that died during culture. The healthy specimens always had a very clean feeding chamber. In contrast, the ones perishing during culture had a feeding chamber containing algae sticking to setae and spines.

Post-oral region

Behind the mouth, the ventral surface carries some setal fences (Figs. 7, 17D, F). An *anterior fence* runs transversely immediately behind the paragnaths (Fig. 7: *af*). A median fence and a posterior fence closely border the hind body boundary (Fig. 17D, F). In addition, there is a pair of *lateral fences*, running on each side as arcs from the paragnaths to the hind body boundary (Fig. 17F: *lf*). The setae of the transverse fences point antero-ventrally, and those of the lateral fences antero-inwardly. Both the setation of fences and the oral region, in general, remain unchanged after N2.

Hind body (Figs. 16, 17)

N1 has a small hind body with no setae but carrying a dorso-caudal spine and a pair of furcal spines, both furnished with spinules. The anus is not yet open (Fig. 16C), and ventrally there is no clear border between the head region and hind body. From N2 both the dorso-caudal spine and the furcal spines are much longer, also relative to body size, and heavily armed with spinules. Ventrally, the distinct hind body boundary separates the head region and hind body, fenced by rows of setae (Fig. 17: *hbb*). A further marker is the primordial maxillules and maxillae, since they are situated on the head. In N5 and N6 the hind body is located beneath the posterior part of the overhanging head shield. In N2, the hind body is mid-ventrally armed by a pair of serrate spines (Fig. 16E: arrows) and some simple spines. In later instars, such as N6, the ventral armature consists of six pairs of very distinct spines, suggesting the future six thoracic segments in the cyprid and a minute pair anterior to these (Fig. 7). The development of these spines is shown in table 3. The anus appears in N2 as a slit beneath the basis of the dorsocaudal spine. It is bordered by less sclerotized and folded cuticle dorsally and ventrally, indicating some flexibility of the opening (Figs. 10, 16, 17).

Table 3. Nauplii of Capitulum mitella. Distribution of ventral spines on the hind body, numbers of setae on maxilla and numbers of setae and teeth at antennal naupliar process. Spine arragement on hind body and arrangement of setae on the antennal naupliar process ("gnathobase") is based on Kado and Kim (1996)

Instar	Ventral spines on the hind-body	
	Series 1	Series 2
N1	None	None
N2	1 pair	None
N3	1 pair	None
N4	1 to 2 median + 1 pair	1 pair
N5	1 to 2 median + 1 pair	2 pairs
N6	6 pairs + rudimantary pair anteriorly	2 pairs

Instar	Numbers of setae	Numbers of setae and spines at A2 coxal process			
	on maxilla (distal segment)	Series 1	Series 2	Series 3	spines
N1	None	None	None	None	None
N2	None	9	7-9	None	2
N3	1	many	6-7	2-4	2
N4	3	many	7	2-3	2
N5	7	many	7-8	2-4	2
N6	7	many	8-10	0-3	3

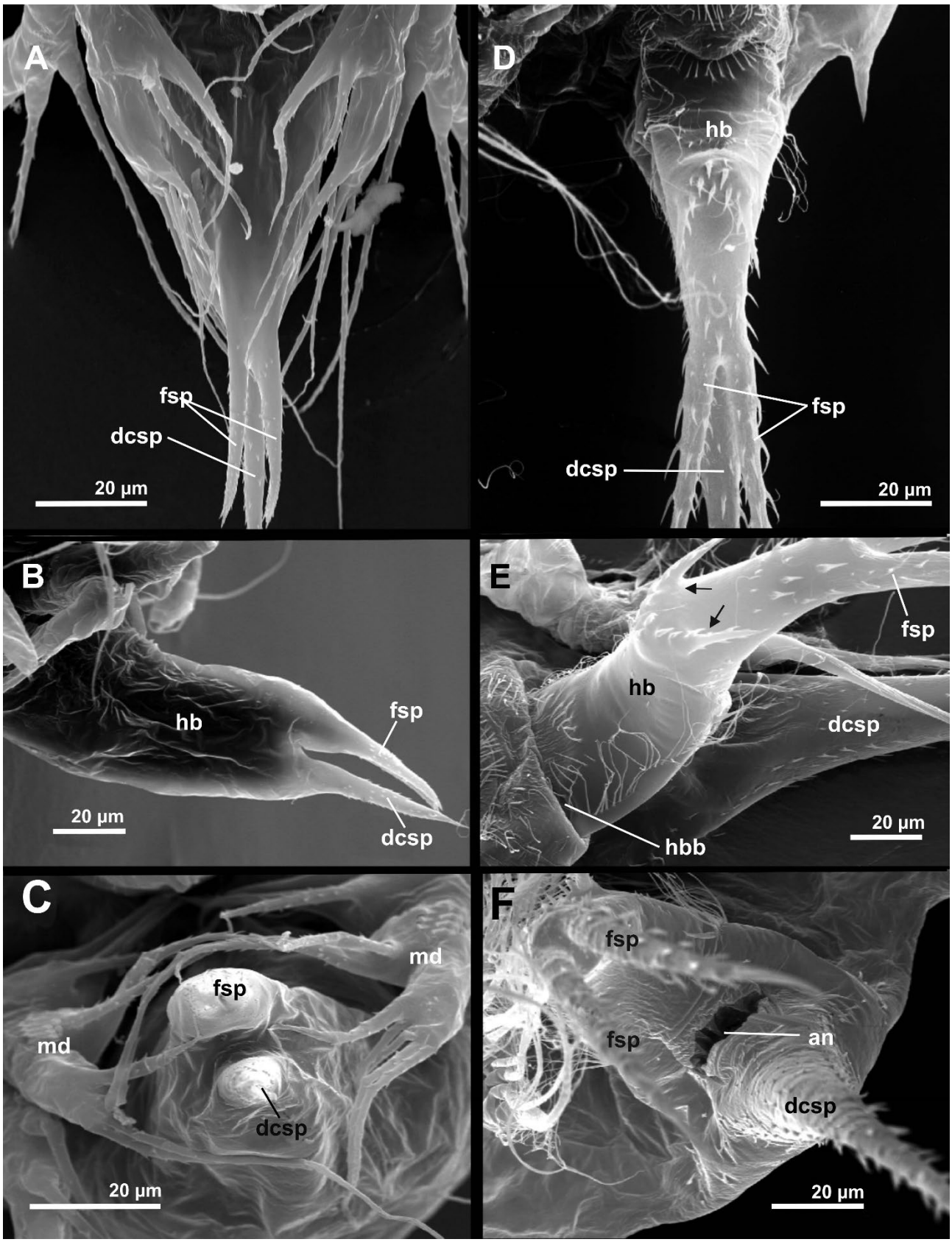

Fig. 16. Hindbody in naupliar instars of *Capitulum mitella*. A, D: ventral views. B, E: oblique lateral views. C, F: posterior views. In N1 (A, B) no spines on the ventral side and no clear demarcation of the head-hindbody border. D and E (N2) shows a clear head-hindbody boundary (*hbb*) and conspicuous spines on hindbody, caudal spines and dorso-caudal spine. In C (N1) and F (N2) the caudal spines (*csp*) and dorsocaudal spine (*dcsp*) are seen almost end on. Legends: *an*, anus; *fsp*, furcal spine; *hb*, hind body; *md*, mandible.

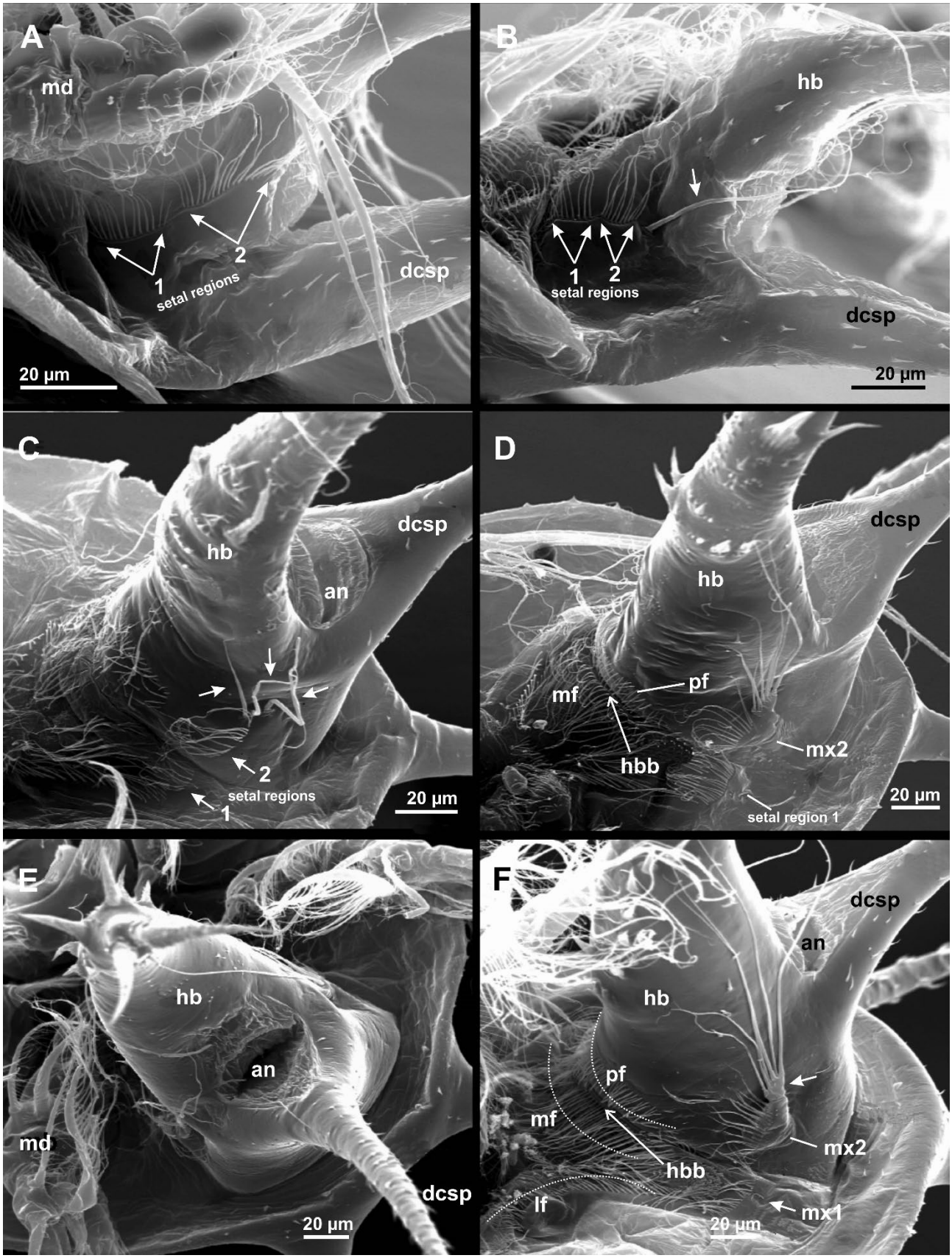

Fig. 17. Hind body in naupliar instars of *Capitulum mitella,* showing hindbody and setal regions (1, 2) that develop into the primordial maxillule (*mx1*) and maxilla (*mx2*). A: N2, lateral view, ventral side is up. B: N3, lateral view, ventral side is up. C: N4, ventro-lateral view. Three setae appeared posteriorly in the setal region 2. D: N5, ventro-lateral view, showing the transverse middle (*mf*) and posterior (*pf*) setal fences. Setal regions 1 and 2 have now matured into a primordial

maxillule (*mx1*) and maxilla (*mx2*). E: N6, posterior view. F: N6, ventro-lateral view. Maxillule (*mx1*) and Maxilla (*mx2*) even more appendage-like with an articulated distal element (arrow). The dotted lines trace the median (*mf*), posterior (*pf*) and lateral (*lf*) setal fences. Legends: *an*, anus; *dcsp*, dorsocaudal spine; *hb*, hind body; *hbb*, hind body boundry.

Antennule (Figs. 18, 19)

Naupliar antennule: The development and setation follow from figures 18, 19 and table 2. The antennule initially (N1) carries eight setae and ends up with 13 setae (N6). The setae can be simple, plumose or serrulate, but serrulate setae occur only in N1. The simple numbers in parentheses below refer to setal numbers in figure 19. We describe the ontogeny closely because some antennular seta become morphologically diverse sensilla in the cyprid (Nott and Foster 1969).

Segment1 (as1): lacks setae in all instars and articulates to the body by means of a "double annulation" of arthrodial membrane.

Segment 2 (as2): bears one distally situated, inner seta (1) in all instars and similarly so in the cyprid, where it is called postaxial seta 2 (*ps2*).

Segment 3 (as3): bears one inner seta in N1 (2) but ends up (N6) with four setae, one outer (12) and three inner (2, 11, 13). In N1, the articulation between the third and the fourth segment is indistinct. In N2 and N3, the third segment carries three inner setae, two distal (3, 4) and one medial (2), with setae 3 and 4 having shifted their location proximally relative to the distal-most setae (5-8). Interestingly, the same two setae (3, 4) seemingly jump to the fourth segment from N3 to N4, leaving the third segment with seta (2) only, but in N5 it carries three setae (2, 11, 12) since a new distal postaxial seta (11) and a new outer seta (12) have appeared; seta (12) being sited very close to the joint to segment 4 and directed ventro-laterally. In N5 and especially in N6, the third antennular segment has a distinct distal swelling, caused by the development of the cypris attachment organ within.

Segment4 (as4): initially (N1) bears six setae (3–8), and it ends up (N6) with eight setae; two outer (9, 10), four apical (5–8) and two inner (3, 4). The seta (8), slightly away from the outside in N1, shifts to an apical position in N2, which therefore has four apical ones (5–8). In N3, segment 4 acquires an outer seta (9) and yet another one (10) is added in N4. Furthermore, the two inner setae (3, 4), sited on segment 3 until N3, have in N4 shifted position to segment 4. Accordingly, the apical segment 4 carries eight setae in N4 and this stays unchanged in N5 and N6. The setation formula of the four apical setae is PPSS. The cyprid has nine setae on segment 4, but with radically changed morphologies and their relation to the naupliar ones is discussed below.

Cypris antennule: The cypris antennule consists of four segments just as in the naupliar stages. This is invariable among cirripedes, but the shape, biomechanics and function of the appendage change drastically between the N6 and the cypris (Nott and Foster 1969; Walley 1969; Walossek et al. 1996). Many details on the *C. mitella* antennules were given in Moyse et al. (1995), Al-Yahya et al. (2016) and particularly in Rao and Lin (2013), so here we only summarize the gross morphology relevant to make comparisons with N6. Segment 1, not easily visible by SEM, is a compound structure, consisting of two articulating sclerites being devoid of setae. Segment 2 has a long, cylindrical shape with a single seta (*ps2*), derived from the similarly sited seta (*1*) in the nauplius. Segment 3 is short and nearly symmetrically bell-shaped. It terminates in the villus-covered attachment disc, while the small, cylindrical segment 4 is spatially displaced laterally. The

attachment disc is densely populated with cuticular villi and surrounded by a distinct velum consisting of narrow cuticular flaps. Segment 3 carries a single seta (*ps3*) outside the attachment disc and several setae on the disc itself or around its periphery. As in all other Thoracica, segment 4 carries four subterminal and five terminal setae, which differ very little in structure within that taxon. Their relation to the naupliar setae is discussed below.

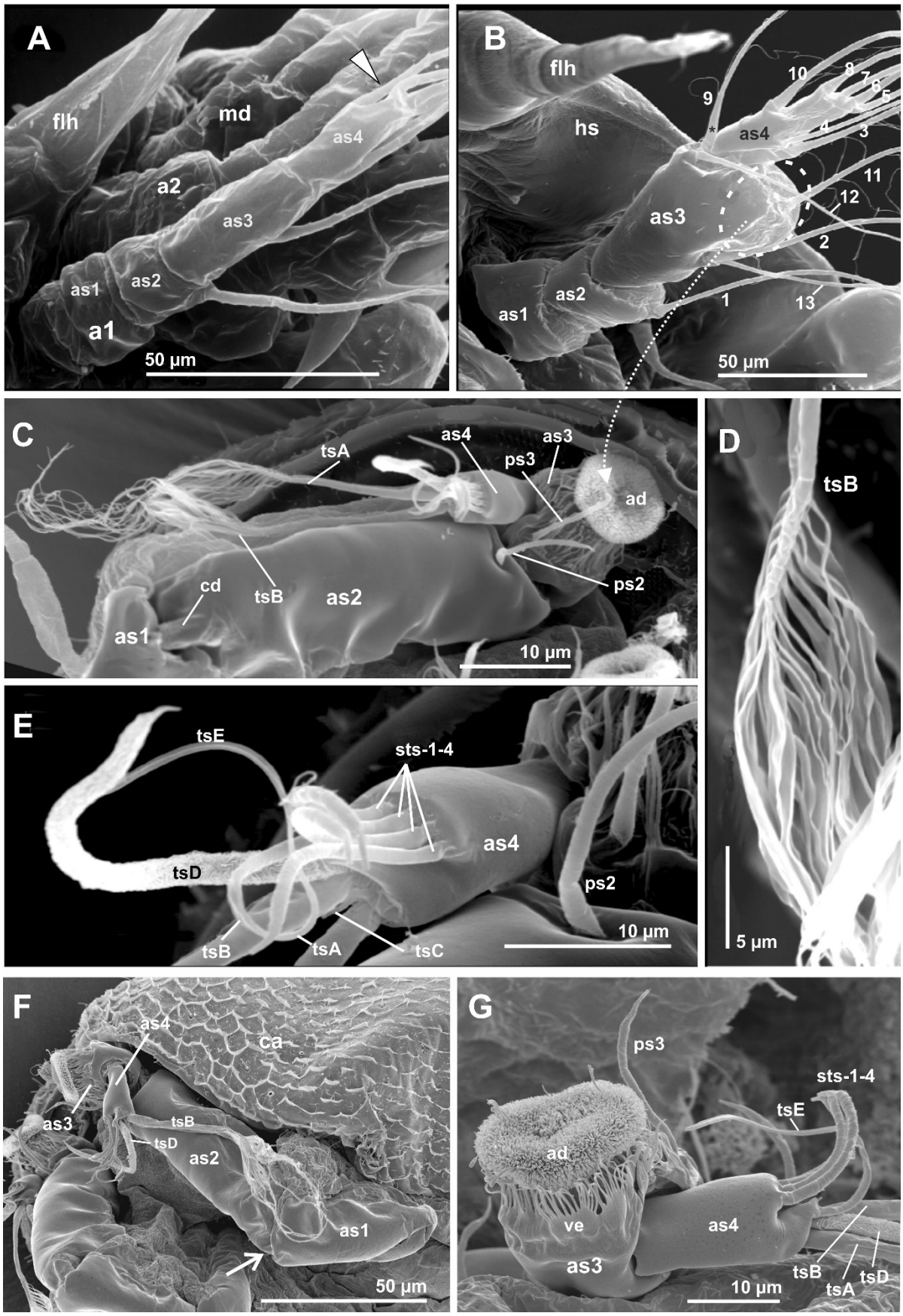

Fig. 18. Antennule of nauplii and cyprid of *Capitulum mitella*. A: N1. Anterior view; no seta on segment 1; segments 2 and 3 each carry a single seta; on segment 4 one seta (arrowhead) is located slightly more proximally than the other three. B: N6. Anterior view; eight setae on the terminal segment 4, numbered as in Fig. 19; segment 3 is distally expanded, heralding the attachment disc in

the cyprid (hatched oval). C: Cyprid; distal three segments in ventral view; a large condyle (*cd*) in the joint between segment 1 and segment 2. D: Plumose terminal seta B (*tsB*) on segment 4; *tsA* is similar. E: Close-up of segment 4 with four subterminal setae (*sts-1-4*) and five terminal setae (*tsA-E*). F. Lateral view of partially extended left antennule, exposing the distal sclerite of segment 1 and segments 2-4; arrow points to the ventral joint between segments 1 and 2; carapace is heavily sculptured. G: Close-up view of segments 3 (*as3*) and 4 (*as4*). Legends: *a1*, anntenule; *a2*, antennule; *ad*, attachment disc; *ps1*, proximal seta 1; *ps2*, proximal seta 2; *ps3*, proximal seta 3; *ve*: velum.

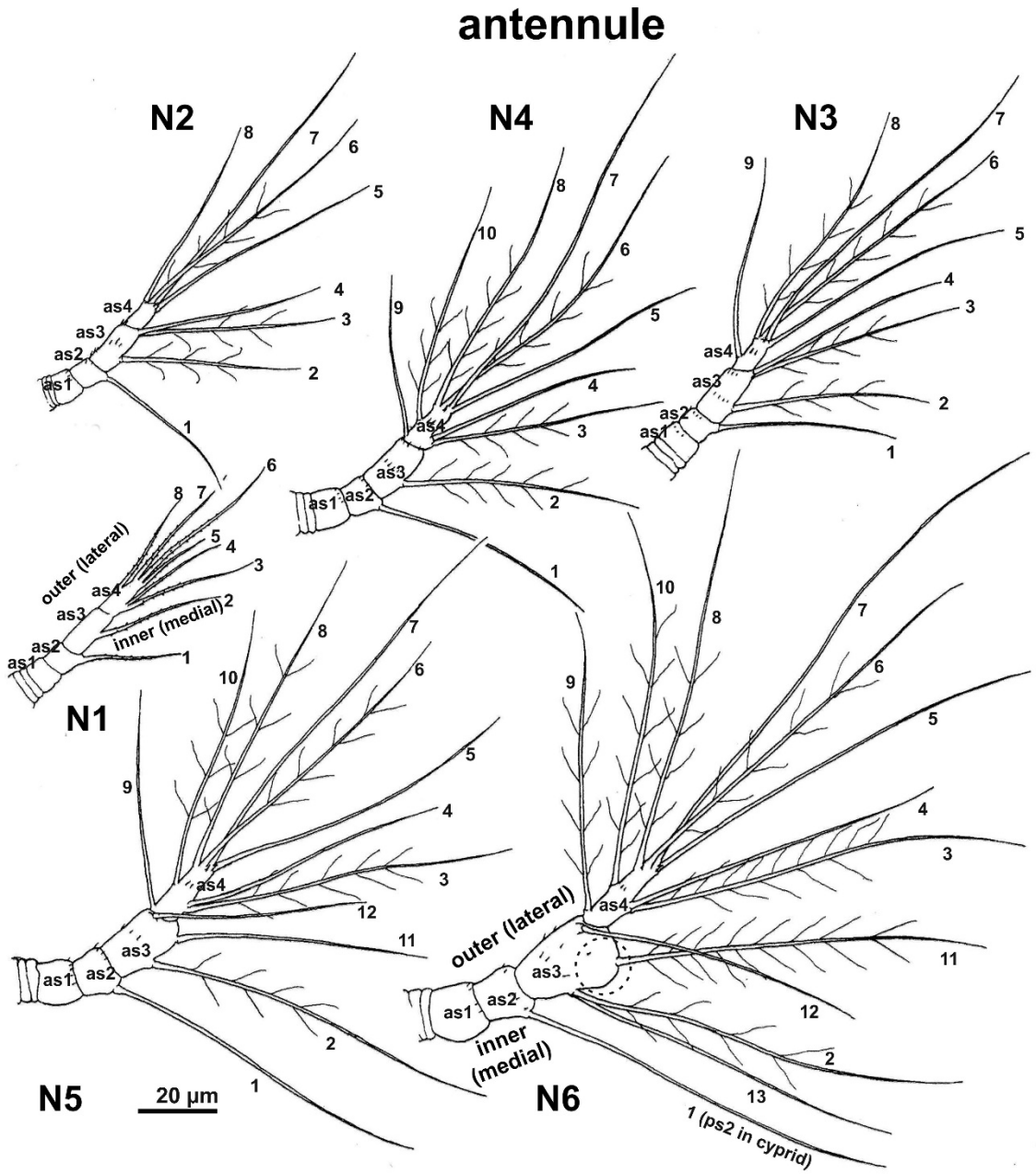

Fig. 19. Drawings of antennules of the six naupliar instars of *Capitulum mitella*. The setae have been numbered according to first their location from inner (medial) to outer (lateral) side and then by order of appearance through the six instars. Setae are drawn according to whether they are simple or plumose. Note that setae 3 and 4 shift position relative to segments during development. In N6 the ellipsis on segment 3 indicates the approximate location of the attachment disc in the cyprid. New seta during development: (N3: 9), (N4: 10, 11, 12); (N6: 13). Legends: *as1-4*, antennular segment 1-4.

Antenna (Figs. 20–22)

The antenna is biramous, consisting of a coxa, a basis, a segmented endopod, and an annulated exopod. In the cyprid, there is no trace of the antenna. This dovetails with this appendage being completely absent from settled juvenile and adult barnacles. The antenna can carry four types of setae: simple, serrulate, plumose, and feathered plumose (F-plumose). It is rather simple in N1 since plumose setae are present only from N2. In N2, the morphology has not changed drastically, but the setation gradually increases in number and complexity, and an endopod segment and an exopod annulus are added from N4. The complexly shaped naupliar process on the antennal coxa is described separately below. The basis carries a stout and inwardly extending naupliar process (Fig. 22: *bapr*) furnished with numerous fine setae and one (N1), two (N2), or three (N3-N6) setae on the inner side. One of these (*b1*) is F-plumose from N2, while another stout and simple seta becomes plumose from N4 (*b2*).

From N2, F-plumose setae (*n1*, *n2* and from N4 n9) are present on the basis and on endopod segment 1, where they extend inward, covering the feeding chamber behind the mouth when the antennae are in the inwardly folded position. More distally sited endopod setae are either simple or plumose, while all exopodal ones are plumose from N3. In all these plumose setae, the setules are rather widely spaced, and there are none on the distal part of the shaft.

At the N3-N4 moult, the distal (2^{nd}) endopod segment splits into two. This results in the setae on the original apical segment being distributed onto segments 2 and 3 in N4. The two originally (N3) subapical setae become located on segment 2 (*n3*, *n4*), while the original four (N3) apical setae and two newly added ones (N4) are located on segment 3 (*n5-8*, *n10-11*). In N4 and N5, one simple seta on endopod segment 1 points laterally (*n12*), opposed to the three post-axially pointing F-plumose ones.

The exopod ends up having nine divisions, here called annuli although the separations between them are incomplete, especially in the younger instars. It carries an increasing number of setae, all of which are plumose from N3 and ends up with a total of 12 setae in N6. Very fine setae line the outer edge of the exopod, being only 30 μm in N2 but increasing to a length of 60 μm in N6.

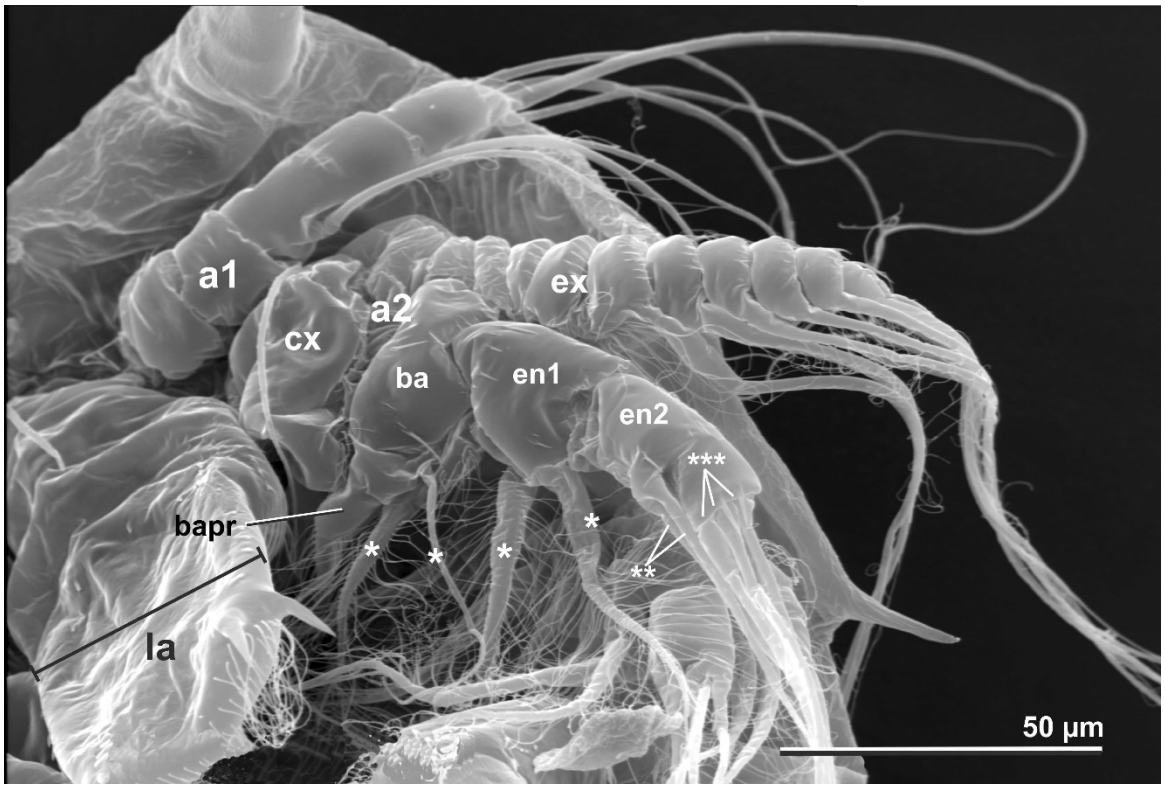

Fig. 20. N2. Oblique left anterior view of labrum, antennule and antenna. The antennal coxal naupliar process is inserted beneath and hidden behind the labrum: the antennal basal naupliar process (*bapr*) is only partially hidden. Asterisks show basal and endopodal setae. Legends: *a1*, antennule; *a2*, antenna; *ba*, basis; *cx*, coxa; *en1-2*, endopdal segment 1-2; *ex*, exopod; *la*: labrum.

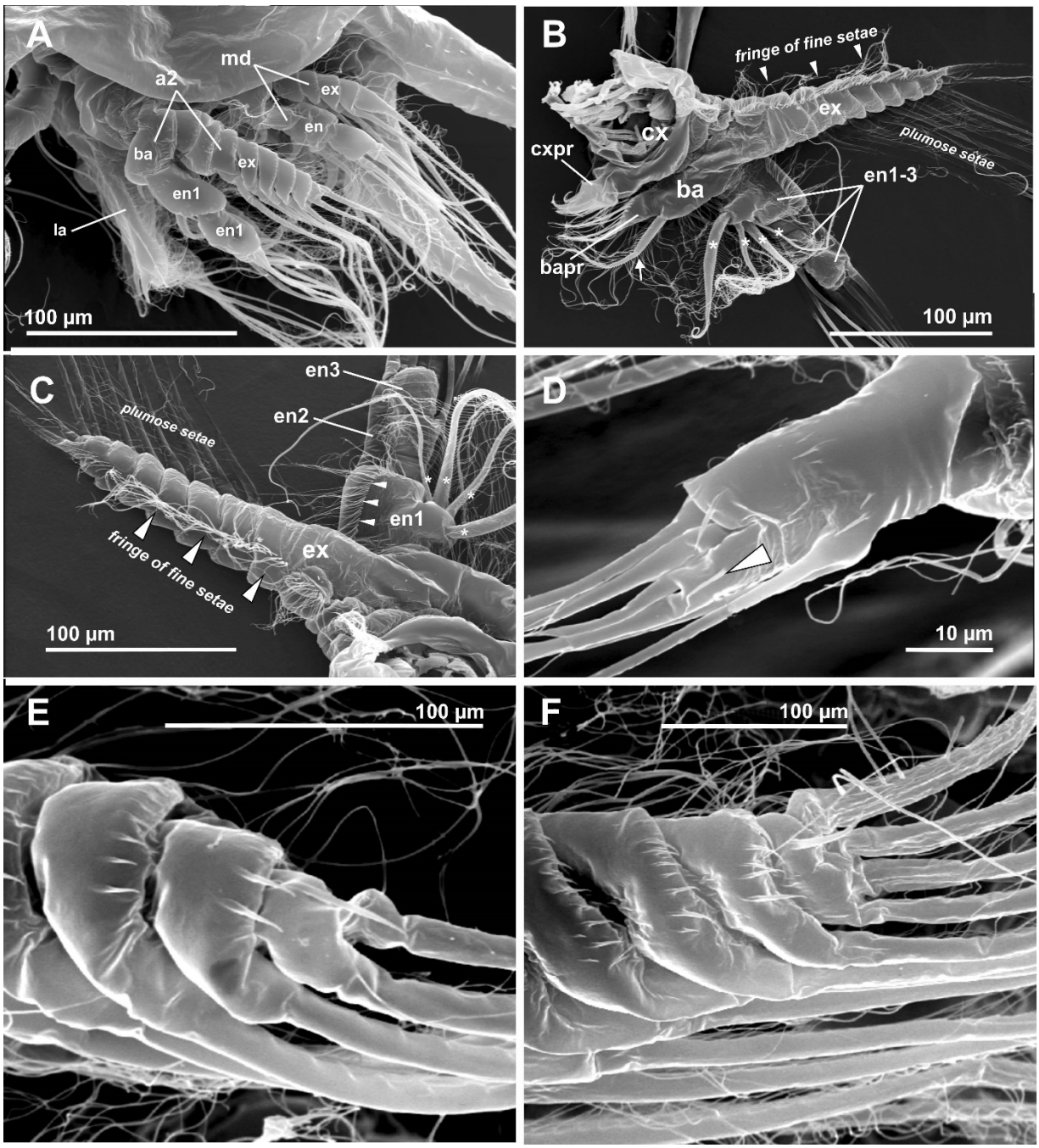

Fig. 21. N3. Antenna of nauplii of *Capitulum mitella*. A: Left lateral view of of labrum, antenna and mandible. Both appendages in a stance where they "guard" the ventral feeding chamber behind the labrum. B: N5. Right antenna dissected loose and shown in oblique dorsal view. Note naupliar process on coxa (*cxpr*), naupliar process on basis (*bapr*) with a F-plumose seta (arrow), and four prominent F-plumose setae on endopod segment 1(asterisks). C: N5. Exopod, showing fringe of thin hairs along the outer side and plumose setae (vaque in this picture) along the inner side. D: N3; distal segment of antennal endopodite; the two ventral-most of the four distal setae are fused in their basal part (arrowhead). E: N2, end of antennal exopodite, anterior view; two setae on the terminal annulus. F: N6, distal end of antennal exopodite, anterior view, with four setae on the last annulus. Legends: *a1*, antennule; *a2*, antenna; *ba*, basis; *cx*, coxa; *en1-2*, endopdal segment 1-2; *ex*, exopod; *la*: labrum.

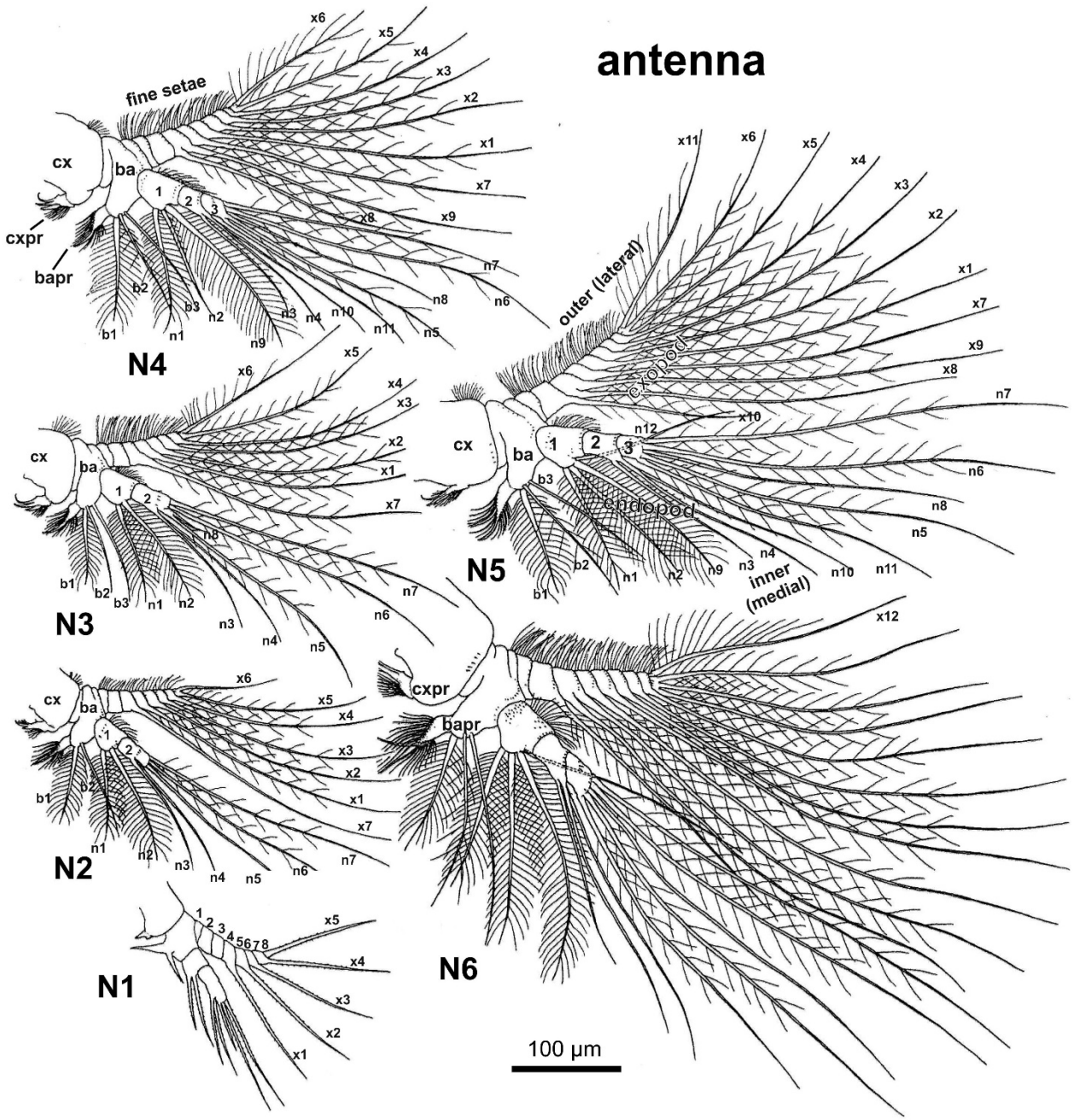

Fig. 22. Drawings of the antenna in the naupliar instars of *Capitulum mitella*. For N3 and N4 the endopodal segment numbers are indicated. The fine hairs on the exopod are present in all instars from N2. New setae appearing during development: (N2: *x6*), (N3: *n8*, *x7*), (N4: *x8*, *9*, *n9*, *n10*, *n11*), (N5: *n12*, *x10*, *x11*), (N6: *x12*). Legends: *b1-3*, numbers of setae on basis; *ba*, basis; *bapr*, basal naupliar process; *cx*, coxa; *cxpr*, coxal naupliar process; *n1-12*, setal numbers on endopod; *x1-12*, setal numbers on exopod.

Antennal naupliar process (Figs. 23, 24)

The process is located on the inner side of the antennal coxa and consists of a basal part and stout, inwardly curving spine-like processes on the distal margin. When the antennae are in the medially folded position, the processes on both coxa and basis from either side meet in the midline (Fig. 27). The coxal naupliar process changes from N1 to N2 and from N2 to N3, but afterwards its now rather complex morphology stays almost the same. In N2, it carries a bifurcated spine (Fig. 24:

bsp) consisting of an inner (*isp*) and an outer spine (*osp*). The inner spine is armed with several teeth-like spinules on the inner margin and several (< 10) simple setae on the posteriorly facing side (Fig. 23). The coxal naupliar process is also fringed with many simple setae on its distal half (fringe setae: *fs*) and has two (N2) or three (N3-N6) serrulate setae (*ss*) on the posterior surface. In addition, there are two small spines (*ssp*) on the innermost side (Figs 23, 24). In N3, the bifurcated spine has been completely cleaved into two separate spines. In addition, the N3 naupliar process carries one curved and biserrate spine (*bssp*) medial to the inner spine. There are now also 20–30 simple setae fringing the outer side. The inner spine has several teeth-like spinules on the inward margin, a sharp tooth-like spine on its outward margin (Fig. 23D: arrow) and a row of several (< 10) simple setae. When both processes meet in the midline, the tips of the curved biserrate spines (*bssp*) will fit into the left and right sockets in the anterior edge of the atrium oris depression (Fig. 27E).

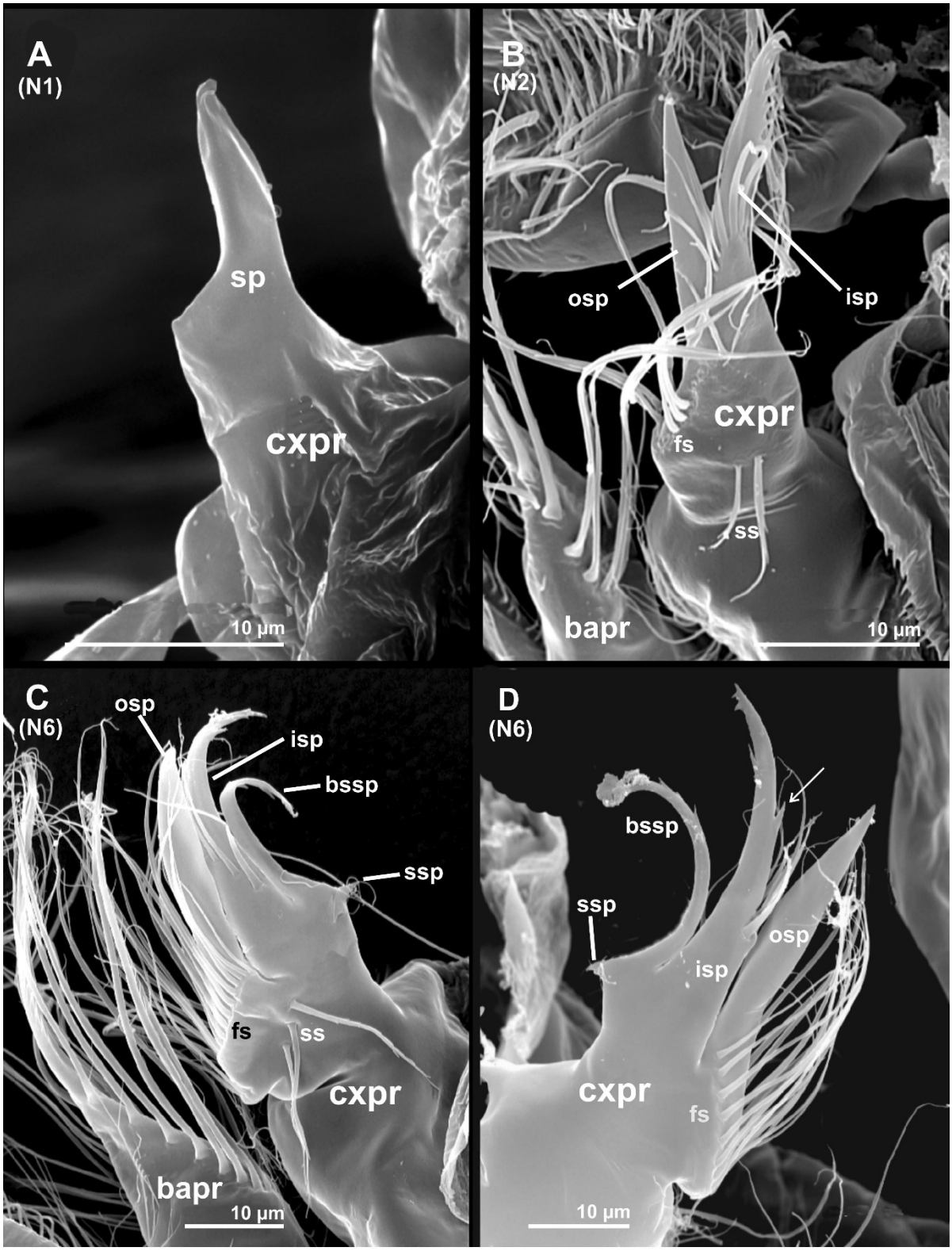

Fig. 23. Antennal coxal naupliar process in nauplii of of *Capitulum mitella.* A: N1, dorsal (inner) view. B: N2, dorsal (inner view). C: N6, dorsal inner view. D: N6, ventral (outer) view. Legends: *bapr*, basal naupliar process; *cxpr*, coxal naupliar process; *bssp*, bi-serrulate spine; *fs*, fringing setae; *isp*, inner spine; *osp*, outer spine; *sp*, spine; *ss*, serrulate setae.

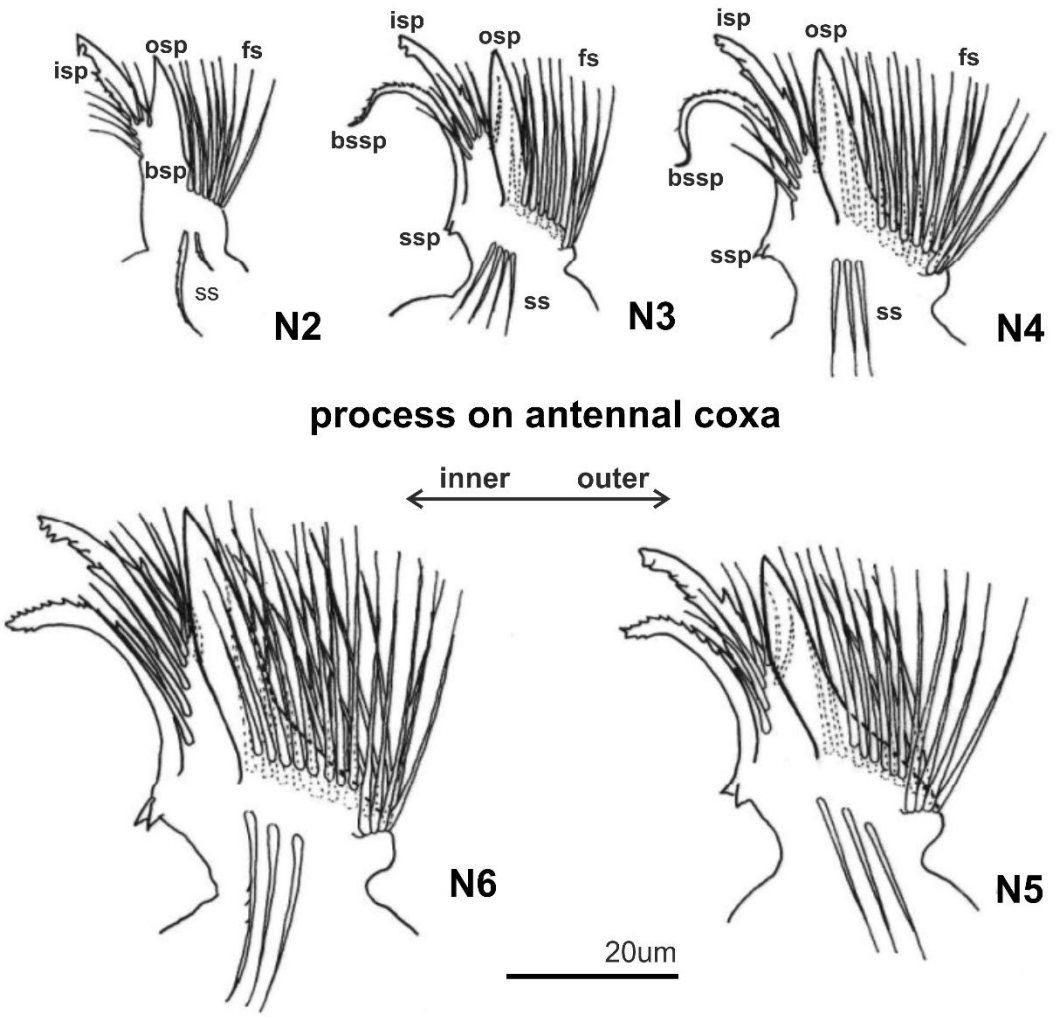

Fig. 24. Drawings of antennal coxal naupliar process in N2 to N6 instar nauplii of *Capitulum mitella*. All are inner views. Legends: *bsp*, bifurcated spine; *bssp*, bi-serrulate spine; *fs*; fringing setae; *bapr*, basal naupliar process; *cxpr*, coxal process; *isp*, inner spine; *osp*, outer spine; *ss*; serrulate setae; *ssp*, small spines.

Mandible (Figs. 25, 26)

The mandible is biramous, consisting of coxa, basis, a segmented endopod and an annulated exopod. It does not change drastically throughout the naupliar instars. Four types of setae appear through development: simple setae, serrulate setae, plumose setae and combed seta. Unlike the antenna, there are never any F-plumose seta on the mandible. In N1, all setae are serrulate, and the long spine or seta on the coxa also has a serrulate structure. From N2, the coxal spines have developed into a true naupliar process (Fig. 26; *cxpr*), curving inwardly and furnished with a row of simple setae. On the posterior side of the coxa next to the naupliar process sits a small, stout spine (Fig. 26: *ssp*). In N1, the basis carries two inwardly curving setae, which become plumose in N2 (Fig. 26: *b1*, *b2*). From N3 to N6, the basis carries three such plumose setae (*b1-b3*). In N1, the endopod segmentation is unclear (Fig. 25), but there are two inner setae and five setae at or near the distal end, all slightly serrulate. From N2, the endopod is three-segmented and remains so through N6 (Fig. 25). In N2 and N3, endopod segment 1 carries three setae (*n1*, *n2*, *n8*); n2 and n3 are setulated, but the robust and medially curving n1 seta is combed (in Table 2: C). In N4, a simple and more apically-sited seta is added (*n10*). Thereafter, setation remains constant on segment 1, but the

three setae (*n1*, *n2*, *n8*) diverge somewhat in both length and robustness. The second segment bears two setae in N2, three setae in N3 and N4, and four setae in N5 and N6 (two simple: *n3*, *n4*, and two plumose: *n9*, *n13*). Apically, the third endopod segment bears three simple setae in N2 and N3 (*n5*, *n6*, *n7*) and five simple ones in N4-N6 (*n5*, *n6*, *n7*, *n11*, *n12*). The exopod consists of six annuli throughout the naupliar instars if we count in the distinct basal swelling of the distal-most seta. The first annulus is never setulated. In N1 there is a serrulate setae on each of the distal four annuli. From N2 to N4 each annulus, except the first, carries at least one seta. The second annulus has a simple seta N2 and N3 (*x5*), but from N4 all the exopodal setae are setulated. In N5 and N6 there are six plumose setae on the exopod, two on the second annulus (*x5*, *x6*) and one on each of the more distal annuli. Many fine setae (ca. 20 µm long in N2 to 40 µm long in N6) line the cuticular joints of the exopod.

In the cyprid, the rudimentary mandible is located just behind the labrum, followed by the maxillule and maxilla. The mandible still consists of coxa, basis and degenerated endo- and exopod (Fig. 28G). The basis and the rami do not develop in adult mandibles. In the cyprid, both mandibles and other oral features lack setae and spines, and being made up of soft, unsclerotized cuticle, they appear wrinkled in SEM.

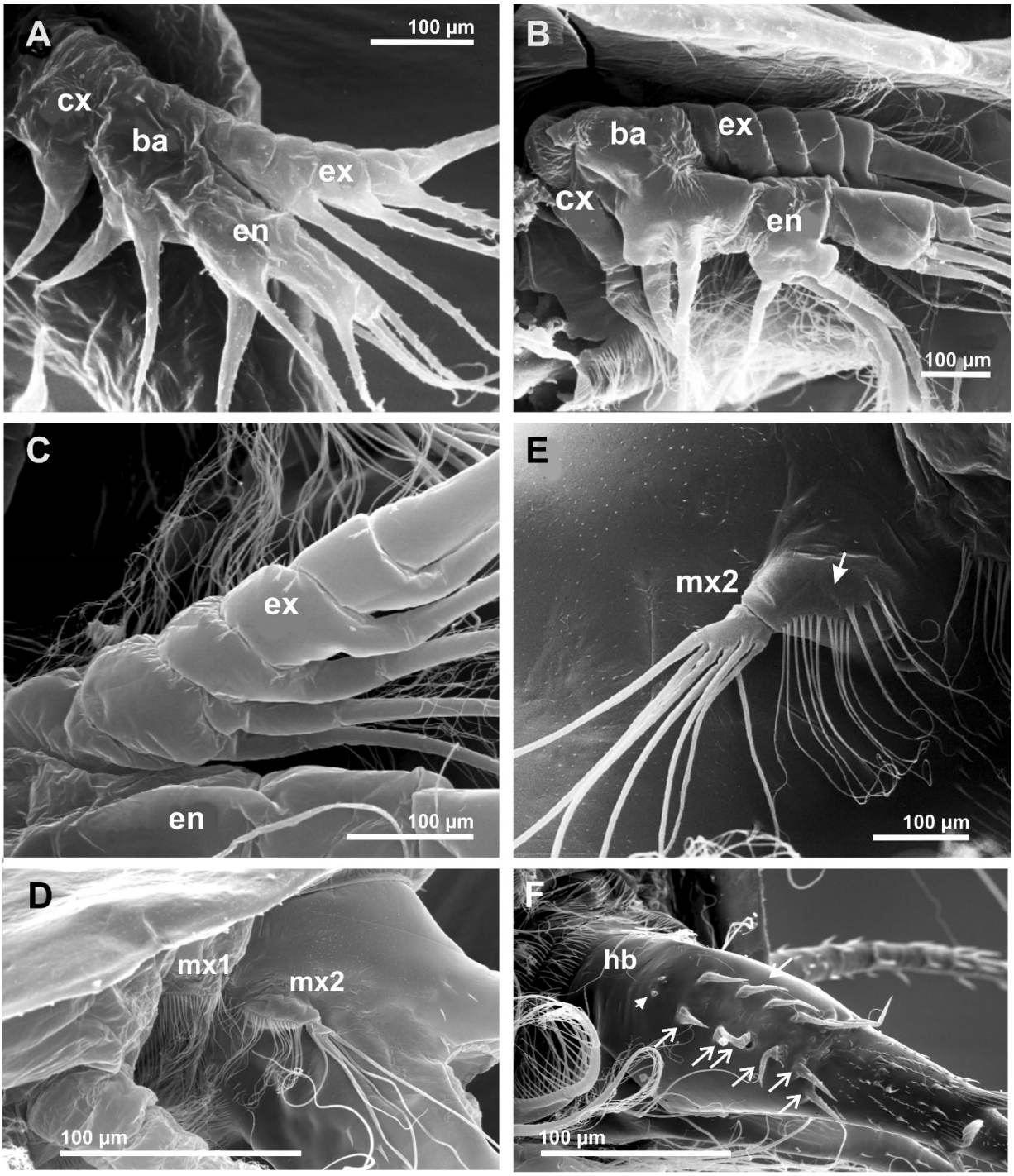

Fig. 25. Mandible, maxilla and spines on the hind body of *Capitulum mitella*. A: N1, mandible, anterior view. B: N6, mandible, anterior view. C: N6, mandibular exopod, anterior view. D: N6. Maxillule (*mx1*) in primordial form) and maxilla (*mx2*) close to the base of the hindbody. E. N6. Right maxilla, lateral view, articulated basal protusion (arrow) with a row of ca. 16 setae and articulated (arrow) distal segment with seven longer setae. F. N6. Six large pairs of spines on ventral side of hind-body (arrows) and anteriorly to these a pair of minute spines (arrowhead). Legends: *ba*, basis; *cx*, coxa; *en*, endopod; *ex*, exopod; *hb*, hind body.

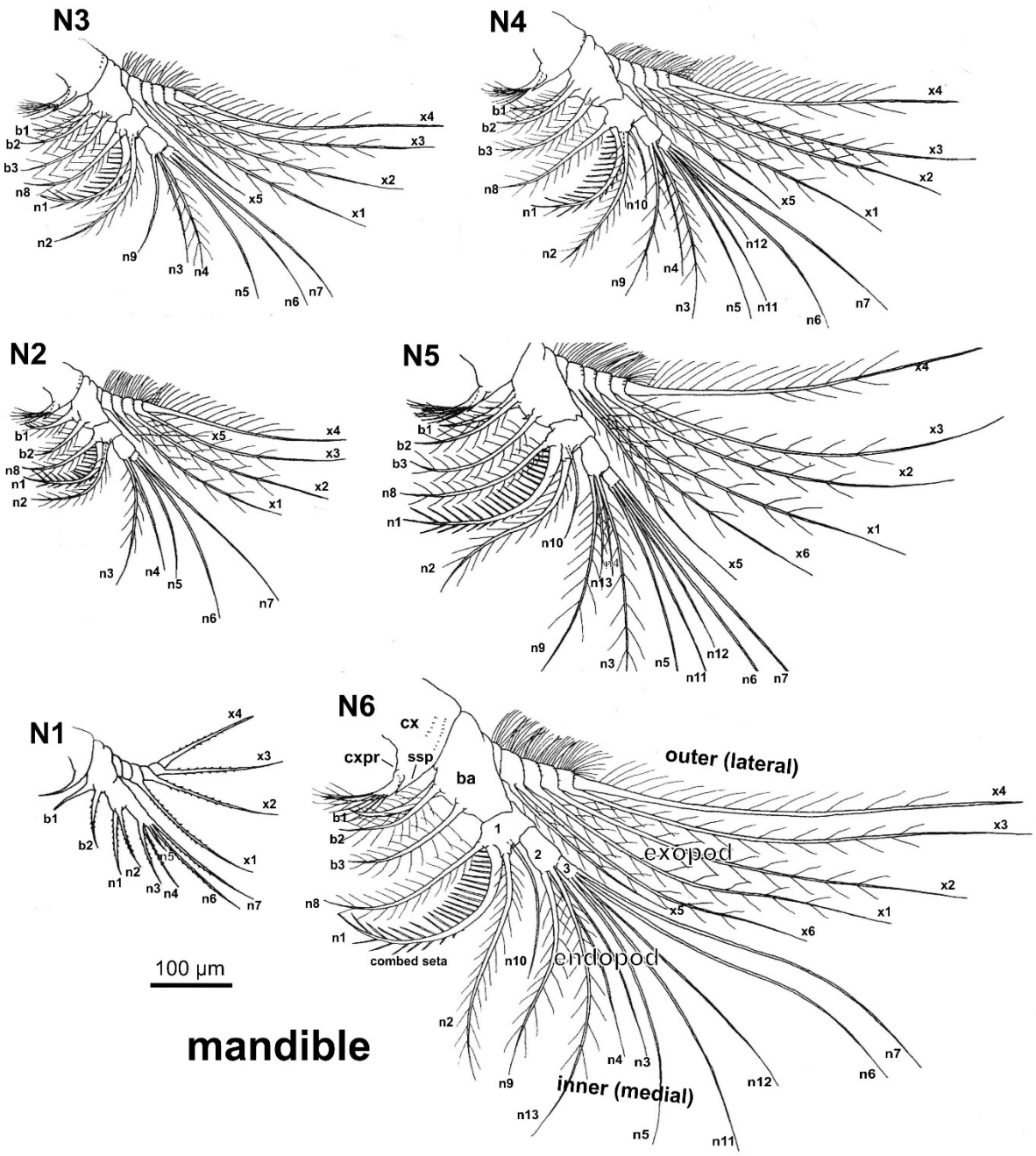

Fig. 26. Drawings of the mandible in the naupliar stages of *Capitulum mitella*. Legends: *b1-b3*, setal numbers on basis; *ba*, basis; *cx*, coxa; *cxpr*, coxal naupliar process; *n1-12*, setal numbers on endopod; *ssp*, small spine; *x1-6*, setal numbers on exopod.

Maxillule and maxilla (mx1, mx2) (Fig. 17, 25E & D)

Two pairs of setal regions (Fig. 17A, B, C) are located on each side of the body, near the base of the hind body. During development, these regions grow in size and complexity, and the setae come to sit on distinct protuberances. In the discussion below, we argue that we consider them as primordia of maxillule (*mx1*) and maxilla (*mx2*) and the figures are labelled accordingly (Figs. 17, 25). In N1 and N2 both setal regions form a row of fine and simple, ca. 15-20 µm long setae, but yet without any limb shape (Fig. 17A, B). In N3, a single thick and longer seta is added at the posterior end of setal region 2, and in N4, there are three such longer setae in the same position (Fig. 17B, C: arrows). Through N4 to N6, the setae of both regions come to sit on distinct hump-like elevations in

the cuticle. From N5, setal region 2 has developed into a mx2 anlagen, consisting of a basal part, which is articulated to a distal part (segment), carrying seven simple setae. The basal part of the rudimentary mx2 still carries the original row of fine setae (Figs. 17F, 25E, D). The more anteriorly situated setal region 1 also becomes hump-like in N5 and is considered a first maxilla (mx1) in N6. In the cyprid, these appendages are rudimentary, unsclerotized and located immediately behind the mandibles, with the maxillae (*mx2*) located more medially between the maxillules and with their distal parts meeting in the midline (Fig. 28F).

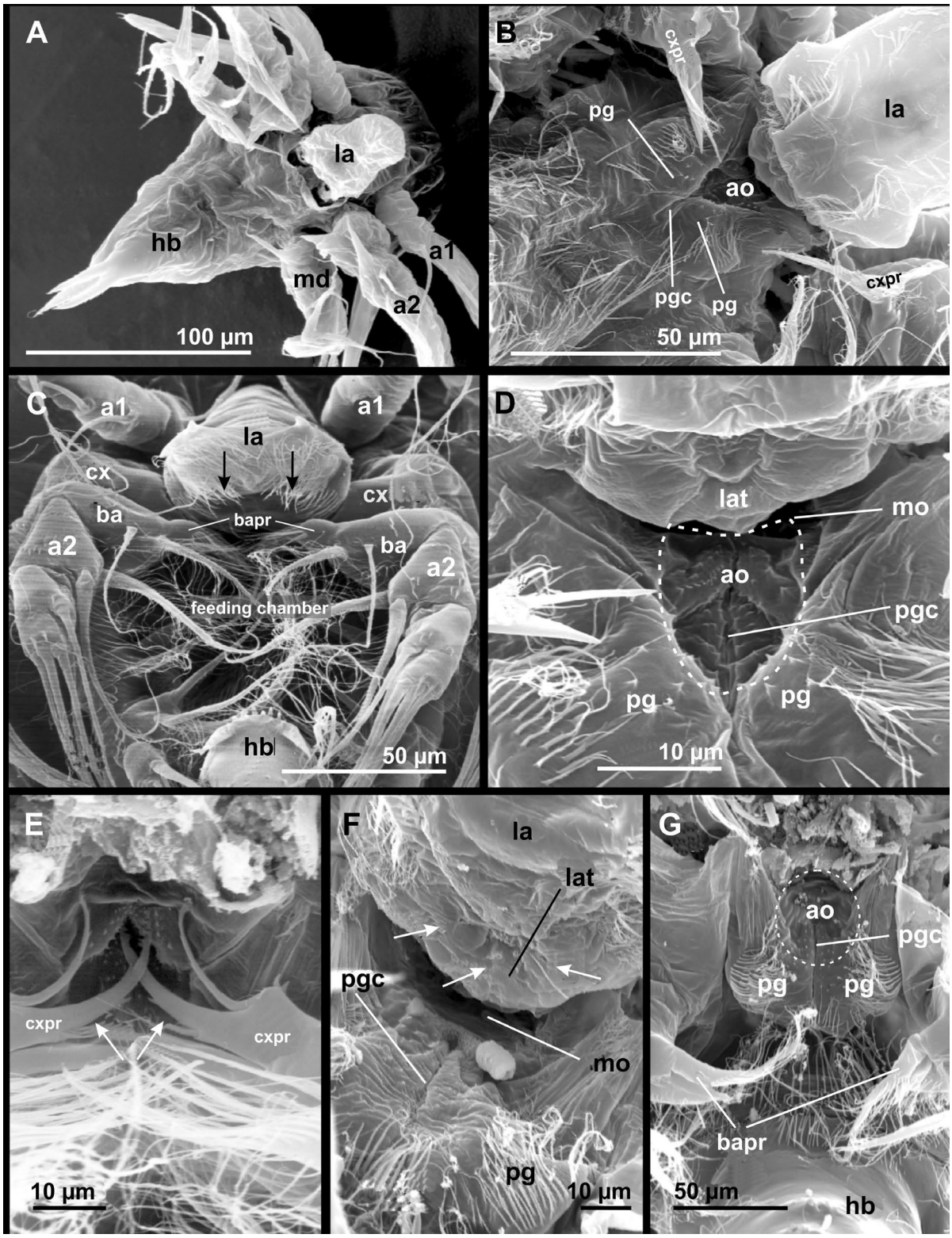

Fig. 27. Oral and ventral setose region of the nauplii of *Capitulum mitella*. A: N1 ventral view

with labrum anteriorly tilted; B: N2, ventral view with antennal coxal process. C: N2, midventral view anterior end is up, antennules and mandibles are in their apposed positions; the vertical arrows indicate where naupliar processes of antenna and mandible meet in the midline beneath the labrum. D: N2, oral region flanked by the paragnaths (*pg*). Dotted circle is atrium oris depression. E: N4, labrum removed, exposing antennal coxal processes meeting in the midline; arrows indicate inner spines; compare to D, where the processes are in their medial-most position, leaving the atrium oris open. F: N4, oral region, showing paragnath setae; arrows indicate gland pore openings on labral tectum. G: Nauplius with labrum and antennae removed, revealing the mandibular coxal processes meeting in the midline. Legends: *a1*, antennule; *a2*, antenna; *ao*, atrium oris; *ba*, basis; *bapr*, basal naupliar process; *cx*, coxa; *cxpr*, coxal naupliar process; *hb*, hind body; *la*, labrum; *lat*, labral tectum; *md*, mandible; *mo*, mouth; *pgc*, paragnath channel.

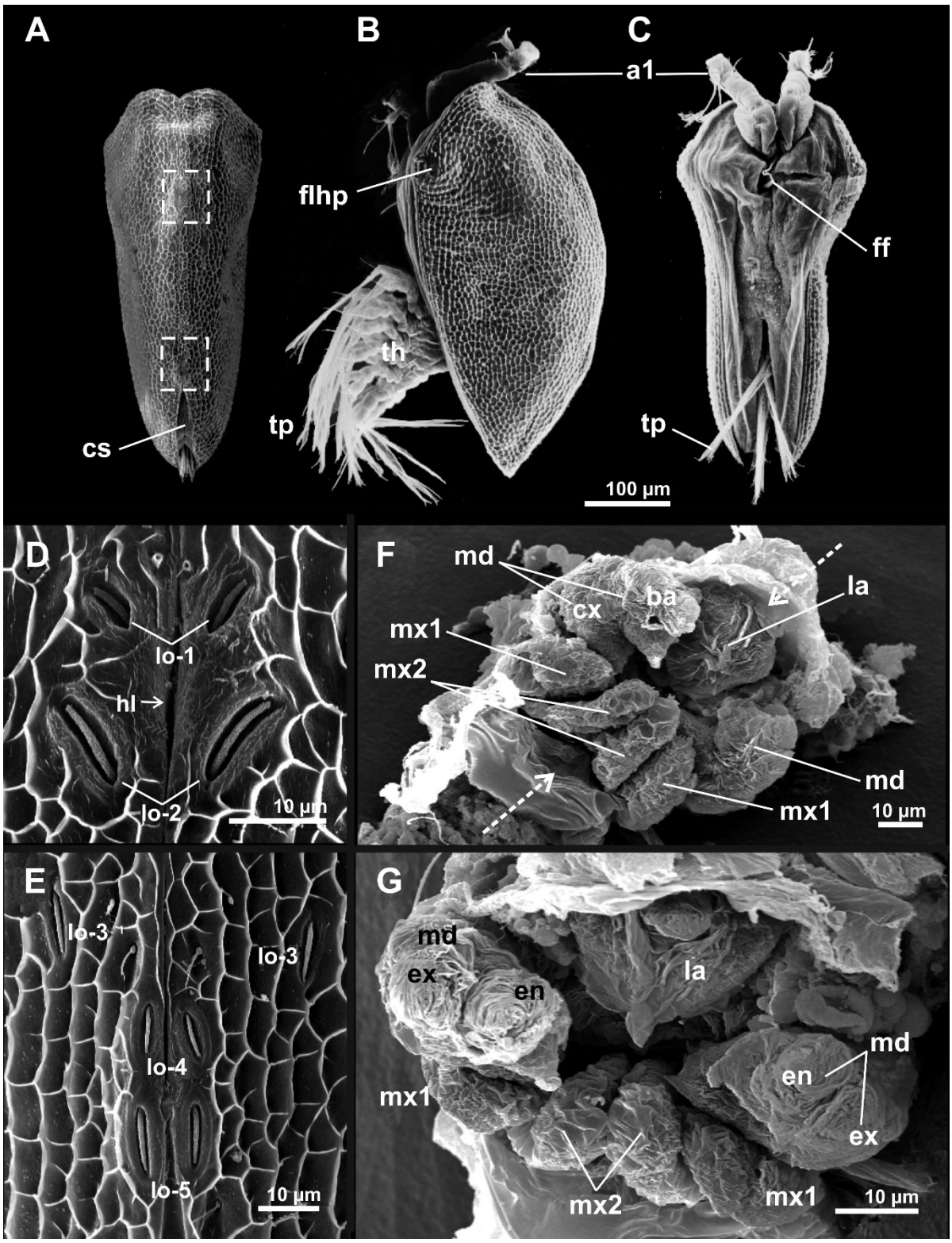

Fig. 28. Cyprid of *Capitulum mitella.* A–C: Dorsal (A), left lateral (B) and ventral (C) views of whole cyprid. D: Anterior rectangle in (A) showing the anterior two pairs of the lattice organ (*lo1*, *lo2*), which originate from dorsal sensilla on the naupliar head shield. E: Posterior rectangle in (A), showing the the posterior three pairs of the lattice organs (*lo3-5*); *lo3* lying more laterally than *lo4* and *5*. F. Ventral view of cyprid with antennules and thoracopods removed exposing diminutive mouth parts; these comprising, labrum, mandibles, maxillules and maxillae. Body midline indicated by two hatched arrows. G: Magnified ventral view of diminutive, mouth parts; almost apical views of mandibles with exopod and endopod sitting on top of the (largely hidden) mandibular basis. Legends: *a1*, antennule; *ba*, basis of mandible; *cs*, carapace slit; *cx*, coxa of mandible; *en*, endopod of mandible; *ex*, exopod of mandible; *ff*, frontal filament; *flhp*, frontolateral horn pore; *la*: labrum;

la1-5, lattice organ pairs 1-5; *md* mandible; *mx1*, maxillule; *mx2*; maxilla; *th*, thorax; *tp*, thoracopod.

Thorax and thoracopods

In the naupliar instars, there is no segmentation of the hind body and no external manifestation of appendages, but the future partition of the thorax is indicated by the transverse row of spines appearing on the ventral surface of the hind body (Figs. 17F, 25F). The thorax segmentation and the six pairs of natatory thoracopods appear abruptly at the moult between the N6 and the cyprid. The structure and setation of the biramous thoracopods were fully clarified by Rao and Lin (2013), while our SEM photos did not reveal all the details, especially in the endopods. Both the endopod and exopod are two-segmented. The basis is somewhat elongated, whereas what we designate as the coxa (Fig. 29B, E) is a much shorter, ring-shaped segment. Proximally to the basal joint of the "coxa", there are several sclerotized elements whose nature is unclear (Fig. 29: asterisks), but they are probably important in the natatory biomechanics.

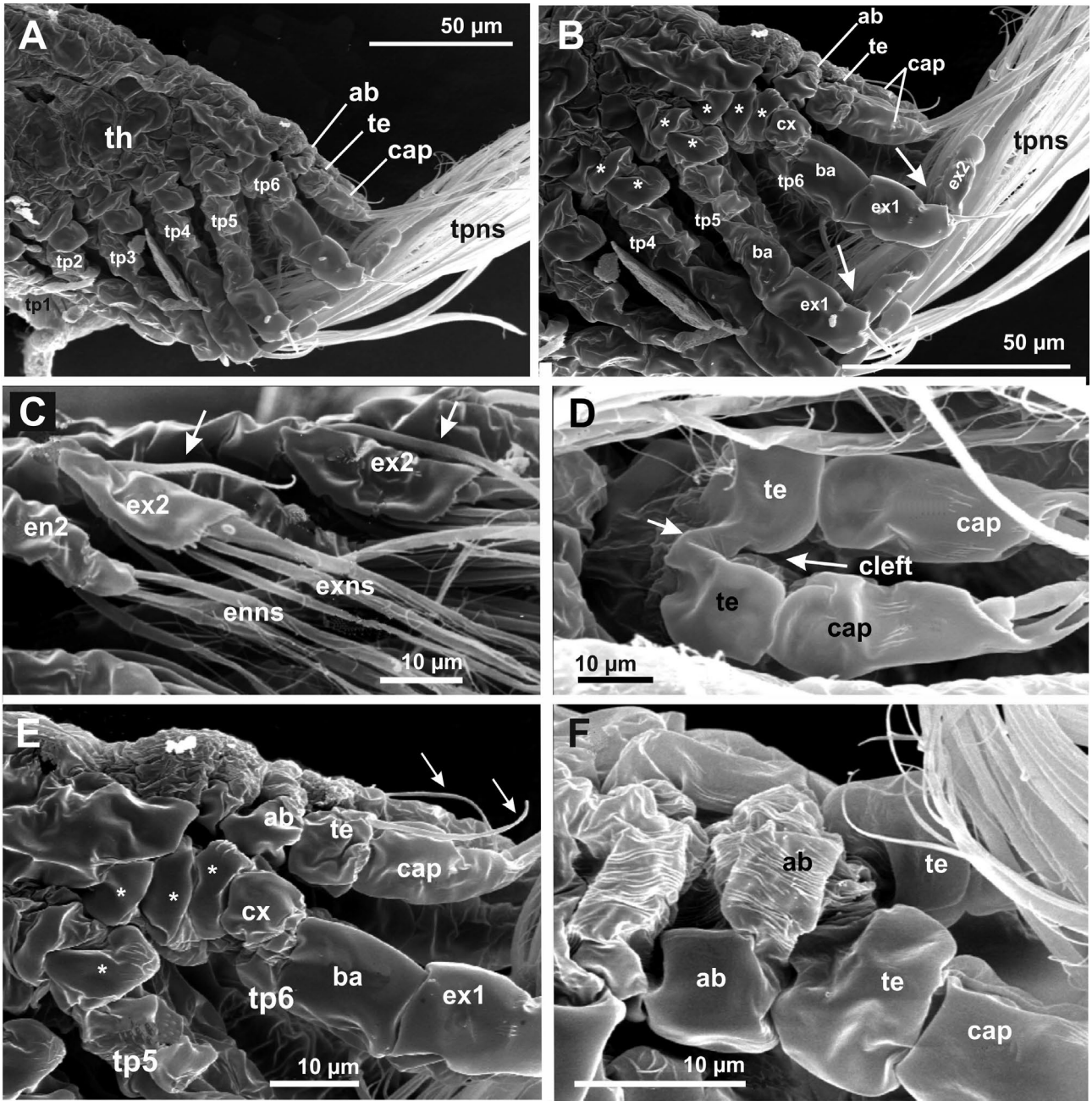

Fig. 29. *Capitulum mitella* cyprids with carapace removed. A: Overview of thorax (*th*) and left side thoracopods 1–6 (anterior thoracopod damaged). Posteriormost the small abdomen and telson with caudal appendages. B: Detail of A showing posterior thoracopods, abdomen and telson. Note the distinct flexure between exopod segments 1 and 2, as likely happens during the recovery stroke. Numerous sclerites at the base of the limbs. C: Oblique close up of thoracopods showing natatory setae sited apically on endopod and exopod segments 2. Arrow points to short and stout seta apically on exopod segment 1. D: Ventral view of showing the deeply cleaved telson (long arrow) and the narrow bridge (short arrow) between the two telson lobes. One-segmented caudal appendages attached to telson lobes. E. Oblique view of posteriormost end of the cyprid showing coxa (*cx*) and basis (*ba*) of thoracopods. Multiple sclerites at base of limbs (asterisks). Arrows point to two short setae situated dorsally on telson. F. Close-up dorsal view of abdomen, telson and caudal appendages. Legends: *ab*, abdomen; *ba*, basis; *cap*, caudal appendage; *cx*, coxa; *en*, endopod; *enns*, endopod natatory setae; *ex*, exopod; *exns*, exopod natatory setae; *te*, telson; *th*, thorax; *tp*, thoracopod; *tpns*, thoracopod natatory setae.

Abdomen and telson (Fig. 29)

There is no external manifestation of an abdomen or telson in the nauplii. In the cyprid, the abdomen appears as a small, narrow element intercalated between the much wider thorax and telson. The telson is articulated to the abdomen and has the same width but is slightly longer. It has no anal opening, dovetailing with the cyprid not having a functional gut. The telson is deeply cleaved, and each lobe carries a single, rather short seta dorsally that does not extend beyond the single segment of the caudal appendage (Fig. 29B). A dorsal view gives the impression that the telson is two separate elements (Fig. 29F), but a perfect ventral view reveals the narrow, interconnected bridge (Fig. 29D: arrows). Each telson lobe carries an unsegmented caudal appendage with three posteriorly extending setae (Fig. 29D). During cypris exploratory walking, the telson and caudal appendages are often flexed downwards and even forwards, thus touching the substratum and acting as an additional leg for walking in addition to the antennules (Maruzzo et al. 2012).

DISCUSSION

Detailed knowledge of larval morphology is essential for understanding the biological and evolutionary significance of the various metazoan larval stages and types, including both the functions of individual specimens and their role in broader marine ecology. Larval traits also hold promise as phylogenetically important characters (*e.g.*, Walossek 1993 1995; Emlet and Ruppert 1994; Young et al. 2003; Nielsen 1994 2012; Martin et al. 2014; Tyler et al. 2018). Conversely, if a robust phylogeny is available, it can be used to trace the evolution of larval features within a taxon. For all this, it is important to use standardized terminology when comparing characters between taxa. For cirripedes a detailed description of the cyprid using standardized terminology has now been achieved (Høeg et al. 2004; Bieliecki et al. 2009), but this was not so for the nauplii, where many details were little known and several different terminologies were often employed. General morphology and biology of cirripede nauplii have, until now, been overwhelmingly studied in species of the Balanomorpha (acorn barnacles). Yet, this taxon is deeply nested within thoracican barnacles, and many of their features in both larvae and adults are likely to be highly derived. In contrast, a pedunculated barnacle with planktotrophic nauplii, such as *C. mitella*, is much better suited for understanding general aspects of larval development in cirripedes. It is also important that *C. mitella* belongs to the Pollicipedomorpha that emerged already in the middle of the Jurassic, while balanomorphans did not arise until the mid-Cretaceous (Kado 1982; Chan et al. 2021). Therefore, pollicipediomorphans take centre stage when trying to understand the general evolution of the thoracican barnacles (Chan et al. 2021; Gale 2018). We hope that our account using both light and scanning electron microscopy of all features in all larval stages of C. *mitella* will contribute to these aims.

Larval morphology and phylogeny

The many and often complex morphological features in cirripede larvae offer potentials for use in phylogenetic studies. Larval life-cycle traits have also begun to be utilised in genome-scale phylogenetic analyses, demonstrating great promise for elucidating the evolution of complex larval

characters (Bernot et al. 2022). In Cirripedia, the first attempt at using cladistic methodology based on larval features was made by Newman and Ross (2001). They analyzed thoracican phylogeny using a matrix of naupliar characters, but the result was unconvincing as it was mostly contradictory to existing taxonomy. Also, their analysis was probably biased by using a "hypothetical ancestor" rather than a proper outgroup. In their combined morpho-molecular analysis, Pérez-Losada et al. (2004) initially included the naupliar characters of Newman and Ross (2001), but ultimately chose to focus on adult features in their final analysis. The failure of the Newman and Ross (2001) matrix to yield phylogenetic resolution was undoubtedly due to lack of details in the character. In many older accounts, scoring of naupliar character was often rather superficial, such as failing to precisely describe the position of setae on appendage segments. This is in marked contrast to the situation for Copepoda, where Huys and Boxshall (1991) have brilliantly shown how appendage morphology offers much phylogenetic insight, but only if painstaking attention is paid to both limb segmentation and the precise shape and position of the setae. If, as in the present account, this is achieved for a broad sampling of cirripede larvae, such a database may yet contribute to assist phylogenetic studies in Cirripedia. For the Balanomorpha, Chan (2003) gave a notable example on how to conduct such studies.

Cypris larvae offer an additional and very extensive set of characters for potential use in phylogeny (Jensen et al. 1994a b; Høeg et al. 2004, 2009; Pérez-Losada et al. 2009; Al-Yahya et al. 2016; Chan et al. 2017). In a modern context, Grygier's (1994) account of the antennule in thecostracan larvae, including cirripedes, pointed that way. In parasitic barnacles (Rhizocephala), where adult characters are too specialized to be of much use, cyprid features have supported phylogenies derived from molecular data (Glenner et al. 2009). This suggests that cypris characters may also offer phylogenetic information for the remaining cirripedes, but until now, cyprids of too few species have been studied in sufficient detail. At present, it seems to be more productive to employ the emerging molecular phylogenies as a platform for analyzing the evolution of larval traits, lecithotrophic developmental schemes, modification of developmental modes and cypris settlement (*e.g.*, Ewers-Saucedo and Pappalardo 2019; Wong et al. 2018; Dreyer et al. 2022). In the following, we therefore discuss how principal larval characters emerge and change through development in *C. mitella*. We shall also compare these characters to what is seen in other cirripede larvae, but only briefly touch on their phylogenetic value.

Head shield and carapace

Although the head shield expands through the naupliar instars, the transition into the cypris carapace, which can enclose the entire body, is nevertheless a dramatic metamorphic event. Closure of the carapace valves downfolded on both lateral sides happens by contraction of a carapace adductor muscle (Walley 1969; Glenner and Høeg 1998). In some cirripedes, such as *C. mitella* and species of *Lepas* and *Pollicipes*, the carapace has a distinct dorsal hinge line that facilitates opening and closure of the valves, but often such a structure is lacking and movement relies solely on the elastic property of the cuticle (Høeg 1985, 1987; Glenner et al. 1989; Jensen et al. 1994a; Blomsterberg et al. 2004; Høeg et al. 2004).

The naupliar head shield comprises the shield proper, dorsal sensilla, the precursor setae of lattice organs, other setae, and shield spines. The precise shape of the naupliar head shield varies considerably across cirripedes. Yet, although larval morphology of more than 100 cirripede species

has been reported, only Wong et al. (2018), using computer morphometrics, have attempted to understand the functional significance of variation in this body aspect. These analyses suggested that size-related biomechanical or developmental constraints and feeding requirements are important in shaping the evolution of the naupliar body form.

Head shield spines

We demonstrated the presence of two distinct posterior shield spines in nauplii of *C. mitella* and this separates them from nauplii of the pollicipedomorphan species *Pollicipes polymerus* Sowerby, 1833 and *P. pollicipes* (Gmelin, 1971) (Lewis 1975; Al-Yahya 1991), where such spines are absent. Nauplii of the iblomorphan *Ibla cumingi* Darwin, 1851 have a pair of very small posterior shield spines (Høeg et al. 2009). In *Lepas*, there is a distinct pair of rather robust posterior shield spines, but also additional shorter pairs of spines more anteriorly along the shield margins (Moyse 1987). In addition, *Lepas anatifera* Linnaeus, 1758 nauplii sport a long and robust middorsal spine, a feature not reported from species outside this genus. Within Balanomorpha, the presence of paired, posteriorly situated shield spines seems to vary. They are present in some Chthmaloidea (Kado 1982; Molares et al. 1994), but within that taxon, their presence seems to vary, since they have been documented in *Octomeris sulcata* Nilsson-Cantell, 1932 and *O. angulosa* (Sowerby, 1825) but are absent in *Chamaesipho brunnea* Moore, 1944 (Barker 1976; Kado and Kim 1996). Many nauplii of coronuloids (*e.g.*, *Tetraclita* Schumacher, 1817) and balanoids have paired posterior shield spines (Costlow and Bookhout 1957; Kado 1982; Lang 1979; Walker et al. 1987; Chan 2003; Zardus and Hadfield 2004; Nogata and Matsumura 2006), and this may suggest that their presence has some systematic value, but the underlying (potentially) adaptive significance is unclear. Clearly, such spines, especially when long, affect the hydrodynamic properties of the larval body at low Reynolds numbers, but how this might relate to, *e.g.*, swimming is unclear (Wong et al. 2020a). It is also possible that structures such as shield spines and frontal horns are devices to impede predation (Morgan 1989), but Cowden et al. (1984) found no effect of presumed antipredator structural features in cirripede nauplii. The issue of antipredatory devices in small-sized planktonic crustaceans was recently well-reviewed by Bashevkin and Morgan (2020).

Head shield and carapace setae

Since all setae on the naupliar head shield have apical pores, they are likely to be chemosensory, and this has been verified for the lattice organs and their precursor setae (Høeg et al. 1998; Jensen et al. 1994a; Rybakov et al. 2003). Lattice organs are one of the few synapomorphies for the entire Thecostraca (Høeg et al. 2009), and our study is the first to document the presence of the lo1 and lo2 precursor setae in all naupliar instars. There is normally a distinct shift in external structure between the precursor setae in the nauplii and the equivalent lattice organs in the cyprid. In rhizocephalans and most thoracicans, the organs in the cyprid are elongated, flat areas in the cuticle, perforated by small pores or pits that facilitate diffusion of chemical substances into a sensory chamber in the cuticle that contains branching paracilia (Høeg et al. 1998). It is, therefore, noteworthy that the lattice organs in cyprids of *C. mitella* have an exceptional structure, resembling a reclined seta perforated by numerous small pores. This is probably indicative of their ontogenetic origin from setae in the nauplii and ultimately of their evolutionary origin as true setae also in the

cyprid. A similar lattice organ shape is present in cyprids of the closely related *P. pollicipes* and in the thecostracan taxon Ascothoracida. In the latter, lattice organs can also occur in adult males and generalized females that have a cypridoid habitus (Jensen et al. 1994a; Kolbasov et al. 2008).

In cirripede cyprids, the number and length of carapace setae vary considerably. They are mostly fairly short, but in some species, such as those of the rhizocephalan genus *Peltogaster* Rathke, 1842, the carapace carries numerous and rather long setae (Glenner et al. 1989). Cypris setation has not been extensively mapped, but significantly, Jensen et al. (1994b) showed how setation patterns in rhizocephalan cyprids offered crucial phylogenetic insights that were later verified by molecular data (Glenner et al. 2009, Høeg et al. 2019). This underscores that cypris features, traditionally only superficially addressed in taxonomically oriented studies on cirripede larval development, can offer crucial phylogenetic information, something that was also recently shown for y-cyprids (Facetotecta) (Glenner et al. 1989; Høeg et al. 2004; Blomsterberg et al. 2004; Olesen et al. 2022).

Dorsal head shield pores

The anteriomedian pore is almost certainly the exit for a gland that also occurs in the nauplii of *Semibalanus balanoides* (Linnaeus, 1767) (Kauri 1962; Walley 1969). Høeg (unpublished) has also observed it in rhizocephalan nauplii, indicating that it might be omnipresent in cirripedes. In the cyprid, the anterior midline pore and its associated gland remain positionally associated with the anterior lattice organs, but it is unknown whether there is a functional relation (Høeg et al. 1998). Most of the remaining pores on the head shield are likely to be exit pores for unicellular epidermal glands, as shown for *S. balanoides* (Walker and Lee 1976).

Ventral head shield pores

In the nauplii, we identified several pore pairs on the ventral side near the shield margin. There are very few other reports on such pores in cirripede nauplii, possibly due to a lack of use of SEM. In a very detailed SEM study on tetraclitid larval development, Chan (2003) found ventral pores in cyprids but not in nauplii. In the rhizocephalan *Briarosaccus tenellus* Boschma, 1970, Walossek et al. (1996) found three pairs of pores around the body near the attachment of the ‘floatation collar’-ridge that surrounds the naupliar body in this species, and their number remains constant throughout all instars. In N3, the ventral pores of *C. mitella* are similar to those of *B. tennellus* in number, position and shape, but in *C. mitella* the number increases to six pairs through larval development. Also, the pores in *B. tenellus* may function in inflating the flotation collar after ecdysis, whereas no such structure is present in *C. mitella*. Hence, any homology between these features is doubtful.

Frontal filaments

Frontal filaments are apparently present in larvae of all Thecostraca (Høeg et al. 2009). As is the case for lattice organs, they contain branching ciliary extensions (Walker 1973; Høeg 1985, 1987) and are almost certainly chemosensory structures. There is rarely any structural change through the naupliar instars (Walossek et al. 1996; Chan 2003; this study). Being usually transferred into the cyprid, they are here relatively longer and can, as in *C. mitella*, extend ventrally from the

mantle aperture (Fig. 2B).

Frontolateral horns

Possessing a pair of frontolateral horns in nauplii is a characteristic apomorphy for all Cirripedia (Høeg and Møller 2006; Høeg et al. 2009). Grygier (1990, 1993) suggested that the cirripede horns might be homologous with marginal processes found in some ascothoracidan nauplii, but a single pair of horns is definitely unique to cirripedes. Their omnipresence in all cirripede species testifies to a crucial adaptive value, but their function nevertheless remains obscure (Høeg and Møller 2006; Høeg et al. 2009). They are complex structures, consisting of the horn itself and two large, unicellular glands opening at the tip, which also carry small, innervated setae (Walker 1973; Høeg 1985 1987; Semmler et al. 2009). It adds to the discussion of function that both we, Walossek et al. (1996), and Chan (2003) found that the horn structure differs between naupliar instars. The cyprid lacks horns, but the two gland cells on each side are, as in *C. mitella* transferred into this larva, where they exit in distinct pores frontolaterally on the carapace (Høeg et al. 2004). Therefore, these glands must function in both of these biologically very different larval stages. These pores in cyprids may be homologous to the anterior pits of the carapace found in the Ascothoracida (Kolbasov and Newman 2018).

Antennules

The increase in the number of setae on the antennule during ontogeny is common among planktotrophic cirripede nauplii. Grygier (1994) is so far the only attempt to discuss all Thecostraca in terms of the homologies in setation and segmentation of the naupliar antennules. Here, we cannot add any information relevant to his scheme. Within Cirripedia, the lecithotrophic nauplii of the Rhizocephala have relatively large and swollen antennules, but their setation is very simple. It stays constant at four setae after the loss of the outer ("preaxial") one at the first naupliar moult (Walossek et al. 1996; Rybakov et al. 2003). Within Thoracica, there are only small differences in the general outline of the antennules (Kado and Kim 1996; Chan 2003; Semmler et al. 2009). In contrast, the setation (setation formula) of the antennules of thoracican nauplii exhibits clear differences in the distal segments. Traditionally, setation formulas (see Terminology) rather than detailed morphological descriptions have been used to compare barnacle naupliar antennules. The setation for *C. mitella* in NII-NVI (setation formula PPSS) is similar to that found in *Pollicipes polymerus* and *P. pollicipes* (Al-Yahya 1991; Bernot et al. 2022). Different setation patterns are found in the pachylasmatidae *Octomeris sulcata* (PSPS) and *O. angulosa* (PSSP) and in the chthamalidae *Chthamalus dalli* Pilsbry, 1916, *C. challenger* Hoek, 19883, *C. antennatus* Darwin, 1854 and *Chamaesipho tasmanica* Foster & Anderson, 1986 (Korn and Ovsyannikova 1979; Kado 1982; Egan and Anderson 1989; Kado and Kim 1996). Other variations are seen in balanoids and tetraclitids that usually have the setation formulas PSPP and SSPS. The phylogenetic information inherent in these variations is still far from clear, and we recommend a more detailed character description for this purpose.

This study revealed that both the separation of antennular segments and the exact position of some setae can be obscure. In N5 and N6 a seta (Fig. 19: *9*) is situated on the border of two segments. Another seta (Fig. 19: *12*) is not sited on the outer side but rather on the semi-outer side, and it faces

ventrally as seen in nauplii of some lepadids (Moyse, 1987). We need additional SEM studies to clarify the situation for these setae in the other pollicipedomorphan species (Lewis 1975; Kugele and Yule 1996; Al-Yahya 1991; Dineen 1987; Bernot et al. 2022). There are no plumo-denticulate setae present in the naupliar antennules of *C. mitella*, coronuloids and balanoids, but such setae are found in the Lepadidae and Poecilasmatidae (Moyse 1987), and their presence could therefore be a synapomorphy for Lepadomorpha.

Antenna

The antenna and mandible share many structural similarities, and both play a vital role in the propulsion and feeding of the larvae. We have thoroughly analyzed the developmental patterns of both these appendages in *C. mitella* larvae, but this has rarely been done broadly for cirripedes in general (Kado 1982, Kado and Hirano 1994, Kado and Kim 1996).

In *C. mitella*, the segmentation of the antennal exopod was incomplete for several distal segments. A similar situation exists in nauplii of the rhizocephalan *Briarosaccus tenellus* (Walossek et al. 1996) and the fossil *Bredocaris admirabilis* (Müller and Walossek, 1988), but how this can be compared to other crustaceans and to naupliar appendage evolution in general remains unclear. For the antennal exopod in *C. mitella*, segmentation and arrangement of setae through the six naupliar instars is virtually identical to that seen in many other cirripede species, including other pollicipedomorphans, lepadids, verrucids and chthamaloids. This is in contrast to an extensive variation in setal number of both antennal and mandibular exopods of many balanoid nauplii (Kado and Hirano 1994; Kado and Kim 1996). For the antennal endopods, the setal numbers vary in many pedunculated barnacles. Using both SEM and LM, we found 12 endopodal setae in the antennae of NVI nauplii of *C. mitella,* suggesting that the 15 endopodal setae reported by Lee et al. (2000) may be in error. There are 10 endopodal setae in *Octolasmis mülleri*, 16 in *Conchoderma autrium* (Linnaeus, 1767) and 18 in *Lepas anatifera* (Dalley 1984).

Kado (1982) examined the planktotrophic nauplii of 18 cirripede species, including *C. mitella*, two chthamaloid and 15 coronuloid and balanoid species. He pointed out that segmentation patterns of the antennal and mandibular exopods have similar morphologies and also noted that the number of setae for these species does not exceed five in N1, six in N2, seven in N3, nine in N4, eleven in N5, and 12 in N6. Both exopod and endopod bear proximal rows of fine setae or setules in many thoracican nauplii, while these are not present in the rhizocephalan naupliar stages. Furthermore, F-plumose setae on the basis and the first endopodal segment are common in planktotrophic nauplii of chthmaloids, verrucids, and lepadids (Moyse 1987), but again are not found in rhizocephalans. These differences probably relate to the lecithotrophy in rhizocephalan nauplii. F-plumose setae on the basis and endopod are also present in some ascothoracid nauplii, so we suggest their presence as a plesiomorphy within Thecostraca. On the antennal basis, the cuspidate naupliar process is armed with two rows of small setae and terminates in a long and narrowly curved tip. This structure resembles the condition in many lepadids and chthmaloids such as *Octomeris* (*Pseudoctomeris* Poltarukha, 1996 in the current classification) (Kado and Kim 1996). For *C. mitella*, Lee et al. (2000) referred to this spine as ‘hispid’. In coronuloids and balanoids, one of the two setae on the first endopodal segment resembles the F-plumose setae on this segment in *C. mitella*, having the same thickness and flattened shape with a little narrow base and longer setules, although fewer in number (Kado 1982). If confirmed by SEM, this coronuloid-balanoid feature is putatively an

ancestral trait in these barnacles. Grygier (1993) compared the number of setae on the antenna and mandible in nauplii of cirripedes to the situation in unidentified petrarcid Ascothoracida. The petrarcids have more segments and more setae on the antennal exopods and he considered this to be a plesiomorphic state. From N4, cirripede nauplii generally carry maximally two setae on the third segment of the antennal exopod, and this is most likely an apomorphic condition.

Mandible

Both in the *C. mitella* nauplius and in cirripeds in general, the mandibular endopod consists of three segments in N4, N5 and N6. In cirripeds, the second exopodal annulus frequently carries two setae, just as in *C. mitella*, but this is never so in nauplii of Facetotecta (Itô 1986; Olesen et al. 2022) and Ascothoracida (Grygier 1992, 1993). It could, therefore, be an apomorphy for Cirripedia. Grygier (1993) stated that from N4, the cirripede nauplius has two setae on the distal annulus of the exopod, but this has not been thoroughly studied and is not the case in *C. mitella*.

Naupliar processes

The naupliar processes on the antenna and mandible are undoubtedly crucial for the final manipulation and ingestion of the captured food items. It is, therefore, interesting that they vary structurally between thoracican cirripedes species since this could well reflect differences in diet, such as size and structure of the algal cells. However, the functional and the evolutionary implications of this have seldom been studied or discussed in detail (Kado 1982, Kado and Hirano 1994, Kado and Kim 1996).

Antennal coxal process: There are at least three types of such processes in planktotrophic cirripede nauplii: The pollicipedomorphan/chthmaloid-type, the coronuloid/balanoid-type and the lepadid type (Kado 2018). The coxal naupliar process on the antenna in *Verruca stroemii* resembles a pollicipoid/chthmaloid-type but requires further study. The lepadid-type process, found in nauplii of *Lepas*, is characterized by a long basal part bearing a row of many setae on the outer margin, two prominent spines projecting distally and a long, serrated spine on the inner margin (Moyse 1987). In the poecilasmatid *Octolasmis unguisiformis* Kobayashi & Kato, 2003, the coxal antennal process also has a long basal part but carries long and curved serrated spines on the inner margin and two series of setae on the outer margin, just as in the pollicipedomorphan/chthmaloid-type (Kado, unpublished information). If this type of process is common in other poecilasmatid nauplii, it could represent an intermediate between lepadid- and pollicipedomorphan/chthmaloid-types. The lepadid-type is closest to the one seen in the nauplii of petrarcids Ascothoracida, which carry one outer F-plumose seta on the proximal part of the base (Grygier 1993). Therefore, the lepadid-type naupliar process must be considered as plesiomorphic within the Cirripedia. Among the other apomorphic types, the coronuloid/balanoid-type naupliar process resembles that of *C. mitella*. This relates to the current uncertainty of whether pollicipedomorphans diverged basally in the Thoracicalcarea or close to the split between Verrucomorpha and Balanomorpha (see Fig. 1).

Mandibular coxal process: In *C. mitella*, the mandibular coxa bears a spine that later develops into a true naupliar process. This structure carries only small and simple setae, which has also been confirmed in lepadid and chthamaloid nauplii. The coxal process on mandibles in coronuloid and balanoid nauplii is armed with a row of setae (Walker et al. 1987) and is usually referred to as being

cuspidate.

Labrum morphology and evolution

The labrum in *C. mitella* is a complex structure that serves in capturing and ingesting food. It has a long, semi-rectangular shape, comprising a proximal part, a distal part, the distally exiting labral gland and a multitude of spines and setae on the inner surface (Figs 13, 15, 30). In planktotrophic cirripede nauplii, the labrum may be either unilobed as in *C. mitella* or have a trilobed shape, consisting of a small middle part and two lateral parts (Fig. 31). In species with lecithotrophic nauplii, such as Rhizocephala and Scalpellidae, the labrum is non-functional and hence reduced to a tiny process (Martin et al. 2014). A trilobed labrum is considered an apomorphy for a group comprising coronuloid (whale- and turtle barnacles) and balanoid Balanomorpha (Pérez-Losada et al. 2004; Høeg et al. 2009). It consists of a small middle lobe flanked by two larger lateral lobes. All remaining cirripeds with planktotrophic nauplii have the plesiomorphic state of a unilobed labrum (Moyse 1987; Lewis 1975; Al-Yahya 1991; Chan 2003) and this is also true for planktotrophic nauplii of the outgroup Ascothoracida, such as *Zibrowia* (Høeg et al. 2014).

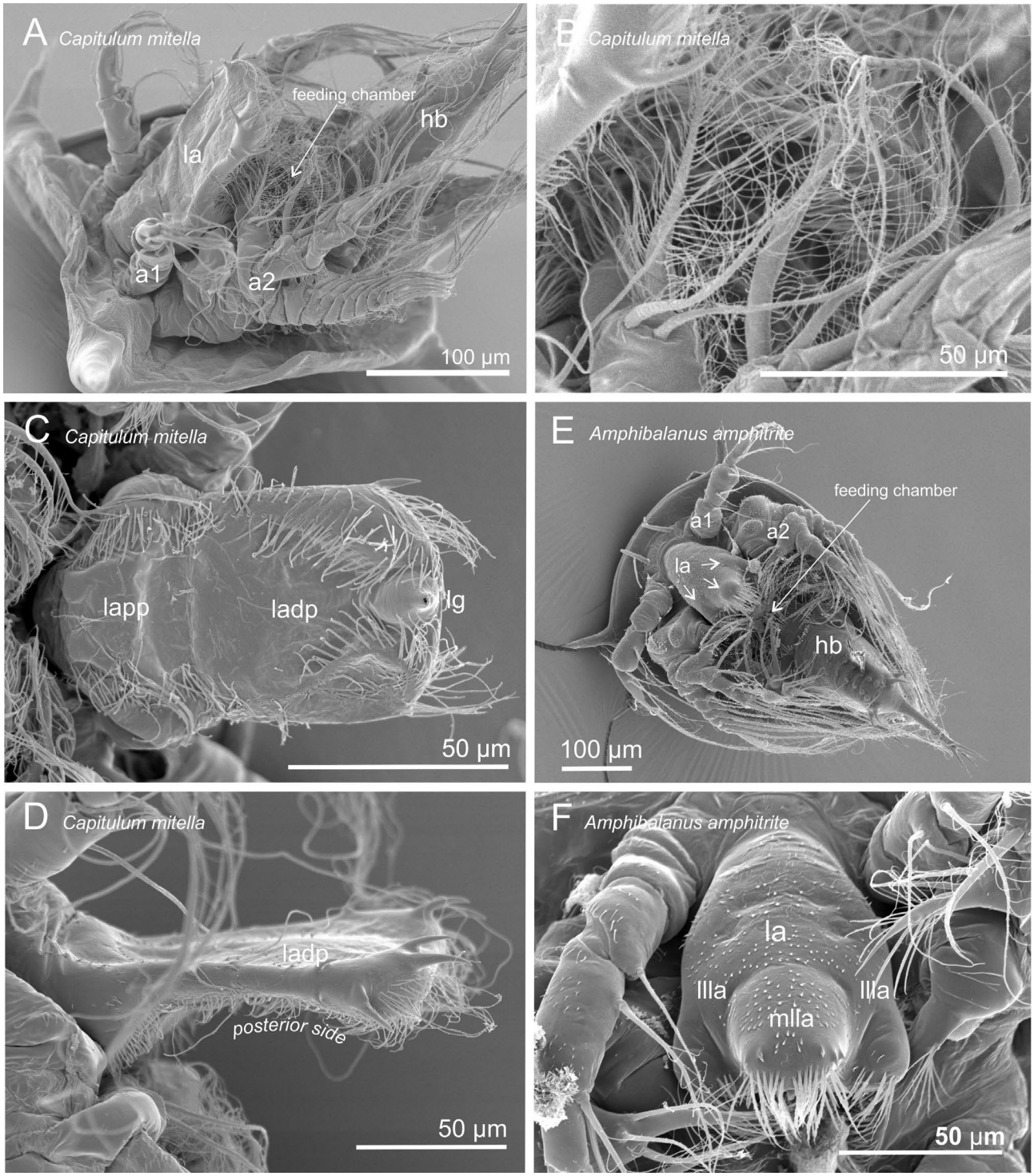

Fig. 30. Comparison of labrum and feeding chamber in the pedunculated barnacle *Capitulum mitella* (A–D) and the balananoid *Amphibalanus amphitrite* (E–F). A: Right lateral view of NV. The feeding chamber is guarded anteriorly by the long, unilobed labrum, laterally by setae on the antenna and mandible and posteriorly by the hind body that can be flexed anteriorly. B: Detail from A; F-plumose setae on the antenna; this being absent in balanomorphan nauplii. C: Inner (posterior) view of the unilobed labrum, showing the distal part and the proximal part; *lapp*, proximal part of labrum; *ladp*, distal part of labrum; *lg*, labral gland. D: Right lateral view of labrum of N6, showing the dense marginal setation and distal spines. E: Ventral view of N4. The labrum is short, and trilobed (arrows). Close up of anterior surface of labrum; *mlla*, labrum middle lobe; *llla*, labrum lateral lobe. Legends: *a1*, antennule; *a2*, antenna; *hb*, hind body; *la*, labrum.

From structural comparison we suggest that the trilobed labrum evolved from a unilobed shape by a reduction in size of the distal part to become the middle lobe of the trilobed form, with the lateral lobes corresponding to the former proximal part (Fig. 31). This interpretation gets support from details in the setation patterns. In the unilobed labrum, the posterior (inner) side of the proximal part is furnished with paired C-shaped rows of slender, inwardly directed setae and several spines (Figs 15, 31), and a similar arrangement is seen on the lateral lobes of the trilobed labrum (Egan and Anderson 1987, 1988). The middle part of a trilobed labrum is short and lacks inwardly directed setae on its posterior surface. Additionally, the middle part is in a lateral view angled slightly to the surface plane of the lateral lobes, a situation closely resembling the distal part of the unilobed labrum of *C. mitella*, where the distal part is similarly angled to the proximal lobe (Figs 2A, 3B, 9 NVI, Fig. 31).

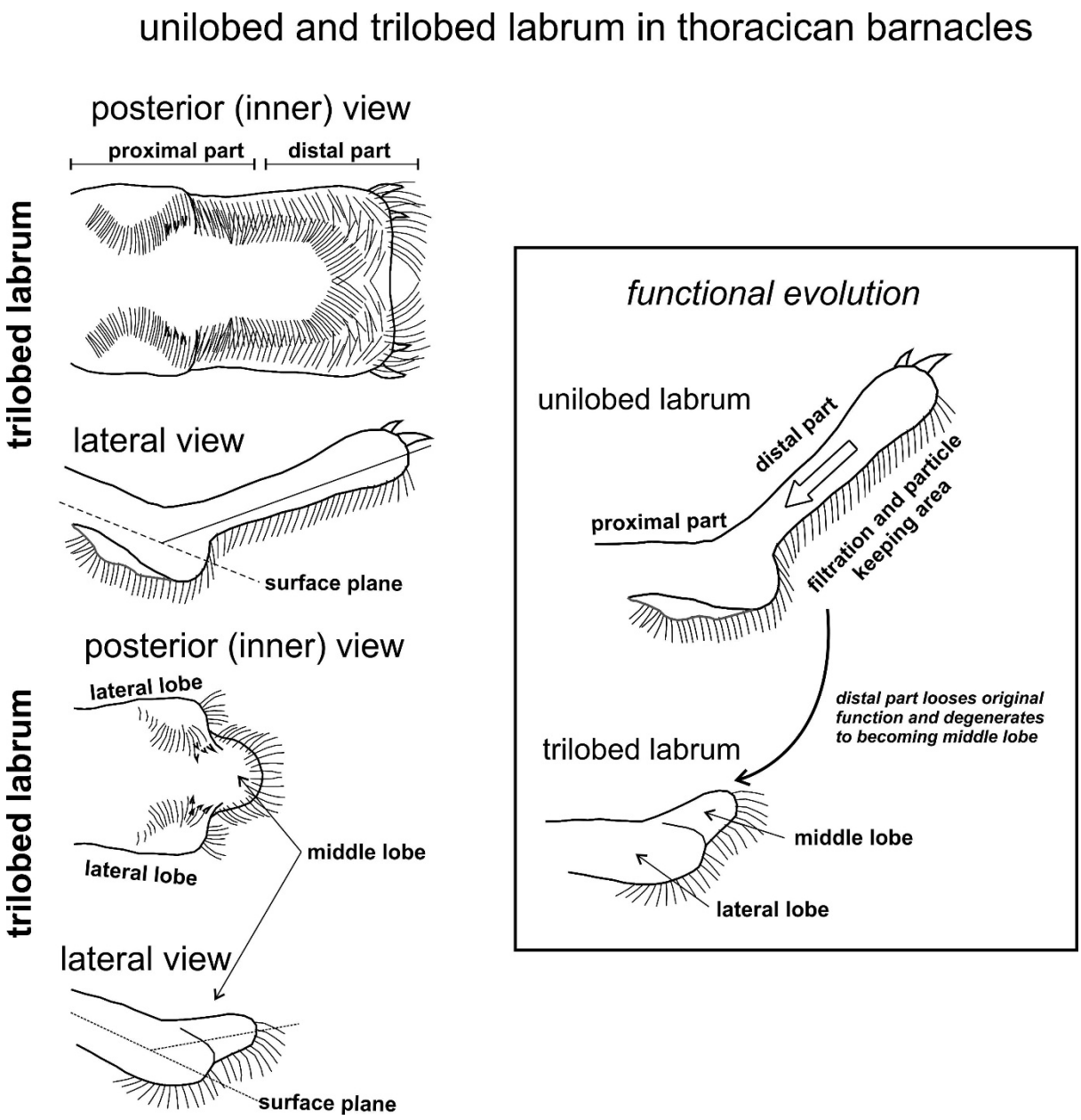

Fig. 31. Labrum evolution. Schematic representation of the unilobed labrum in *Capitulum mitella* and the trilobed labrum in balanoid barnacles. The distal part of the unilobed labrum is argued to have evolved into the small, middle lobe of the trilobed labrum, while the proximal part remained functional and were termed lateral lobes as they were located lateral to the middle lobe of the

trilobed labrum of the balanoid barnacles. Further explanation in text. Legends: *lapp*, proximal part of labrum; *ladp*, distal part of labrum.

Feeding chamber and paragnaths

The feeding chamber behind the mouth is confined anteriorly by the labrum, posteriorly by the hind-body when flexed anteriorly and laterally by the numerous and mostly plumose setae on antennae and mandibles, when these appendages are in their inner (median) stance. The density of setae bordering the feeding chamber is comparatively much greater in *C. mitella* and in the two abovementioned lepadid species than in *S. balanoides* and *B. improvisus* Darwin, 1854 (Semmler et al. 2009). Paragnaths are a basic part of the armature of crustacean nauplii, being paired, humplike protrusions of the mandibular sternite part of the sternum on each side of the atrium oris (Walossek 1993) and generally covered with fine setules, occurring irregularly distributed or in rows. These setules may support the sweeping of food over the surface and occurred already in the Cambrian Phosphatocopina. This character, therefore, belongs to the labrophoran level in arthropod evolution (Maas et al. 2003).

In *S. balanoides*, the anterior protrusions on the ventral surface just under the labrum might be equivalent to the paragnaths in *C. mitella*. In nauplii of *Lepas anatifera* and *L. pectinata* the paragnaths were not seen by Moyse (1987), but the reason may be that this otherwise fine study used light microscopy, only.

Historical changes in phytoplankton and adaptive evolution of feeding structures

In *C. mitella*, the non-feeding N1 have appendages with a rather simple structure and in particular they lack F-plumose (feathery plumose) setae, underlining the role of this type in food collection. All subsequent instars have F-plumose setae on the antenna and the same is true for nauplii of *Pollicipes pollicipes* (Al-Yahya 1991; Bernot et al. 2022). In balanomorphan nauplii, the antennae never carry F-plumose setae. However, in many coronuloid and balanoid barnacles there is one seta (broad, slightly flattened with longer fine setules) with a morphology similar to F-plumose seta on the first segment of the antennal endopod in *C. mitella* (Kado 1982, Kado and Hirano 1994). This may be the apomorphic state of the F-plumose seta. We suggest that both the loss of this setal type on the feeding apparatus and the change to a tri-lobed labrum in coronuloid-balanoid species occurred in association with a change in algal diet.

The Balanomorpha radiated early in the Palaeogene concomitant with a change in the composition of planktonic algae. In the Cretaceous the dominant forms were unicellular coccolithophorids, but after the KT-boundary, a number of factors combined to favour the radiation and rapid dominance of the large-sized diatom algae, a situation that has persisted until now (Benoiston et al. 2017). Diatoms habitually form chains consisting of 10 or more cells which render them an even larger food item. Therefore, the long and closely spaced setules found on F-plumose setae may no longer have been needed for the capture and retention of food items. Similarly, the change in labrum shape could also be associated with this new diet of diatoms. A shift in food preference between early and later barnacle lineages seems to have some experimental backup by results on feeding regimes (Moyse 1963; Kado 1982; Kado and Kim 1996). Although limited in scope, our feeding experiments also agree with *C. mitella* nauplii thriving better on non-diatom

algae. In this context, it would be highly interesting to compare naupliar feeding in selected species across the taxon Cirripedia Thoracica to elucidate how morphology and food preference have co-evolved. For comparison with outgroups, such a study should optimally also include species of Acrothoracica, Ascothoracida and Facetotecta with planktotrophic nauplii (Martin et al. 2014). The phylogeny of the entire Thecostraca is now becoming increasingly known (Chan et al. 2021) so there is every opportunity to study how the naupliar feeding apparatus, food capture and algal diet evolved within this monophyletic and extremely diverse taxon.

Feeding

Small-sized aquatic animals such as crustacean nauplii operate in a low Reynolds number regime, which fundamentally impacts their behaviour, including food collection. For adult copepods, this has now largely been clarified using advanced video techniques on animals under semi-natural conditions. (*e.g.*, Kiørboe et al. 2016, Tyrell et al. 2020). In contrast, very little is known about swimming and feeding in copepod or cirripede nauplii, as their much smaller size seriously impedes observation, even with the best available equipment (*e.g.*, Henriksen et al. 2007; Paffenhöfer & Lewis 1990). For cirripede nauplii, the feeding mechanism was originally studied for balanids (Lochhead, 1936, Norris and Crisp 1953, Rainbow and Walker 1976, Walker et al. 1987) and lepadids (Moyse 1984). Many statements in these older studies may well hold true, but they definitely need to be re-investigated under conditions that truly emulate their natural behaviour at low Reynolds numbers. Such studies must go in concert with detailed morphological studies of the structures involved, such as in Walker and Rainbow (1976) and here for *C. mitella*. Unfortunately, the feeding chamber in species of *Pollicipes*, close relatives to *C. mitella*, has not yet been investigated in detail, although the SEM analyses of Bernot et al. (2022) provide some detail. In a benchmark study, Walossek (1993) used SEM to describe the entire larval development of the upper Cambrian branchiopod *Rehbachiella* Müller, 1983. By comparison with the work of Barlow and Sleigh (1980) for *Artemia* Leach, 1819 and Fryer (1983) for *Branchinecta ferox* (H.Milne-Edwards, 1840), he suggested the motion of the three had appendages and the labrum in the *Rehbachiella* nauplius as follows: When the two antennae swung anteriorly, the mandible reached its posterior maximum and then started with its 'recovery stroke'. At this phase the labrum was raised passively to enhance the opening of the atrium oris. During the 'power stroke' the antennae met the mandible which then also swung backwards. This caused the labrum to be lowered again to cover the atrium oris. Lastly, the two antennae moved anteriorly again, being flexed far backwards, while the mandible still continued its backward-inward movement. On the other hand, the flexure of the naupliar appendages during the anterior swing ('recovery stroke') is not so much to reduce drag, but produces a lower pressure behind the limb, which sucks water and nutrient particles towards the body. Once the particles are close enough, they are trapped in the capture area, here in the postlabral feeding chamber. Wong et al (2020b) analyzed the swimming kinematics of N2 nauplii of *Tetraclitella japonica* by examining the locomotion of three appendages and tracing of microalgae (*Isochrysis galbana*) and microplastic beads around the body. They interpreted the swimming and feeding of the N2 nauplius as follows: *Tetraclita* nauplii swam by beating only the mandibles and antennae in a similar metachronal power stroke, but the antennules moved away from the other two appendage pairs at the mid power stroke. At the mid-recovery stroke, antennules and mandibles began to move away from each other during the recovery stroke. The relative fluxes showed that

fluid did not flow toward the nauplius' body during the power stroke, instead, fluid flowed toward the body only during the recovery stroke. *Tetraclita* nauplius drew particles with good accuracy toward its food capture region under the labrum at the end of the recovery stroke. Furthermore, the anti-phase beating of antennules might play a role in anchoring the moving body during the recovery stroke. Considering their speculation that the frontolateral horns and dorsocaudal spine of *Tetraclita* nauplii may also increase drag and enhance the anchoring effect, much longer frontolateral horns and dorsocaudal spine in addition to longer posterior head shield spines of *C. mitella* N6 nauplii may be morphologically much more effective in collecting food particles, albeit with a tradeoff against predation risk.

Whatever the method of capture, the collected items eventually end up in the semi-confined feeding chamber, whence they must be passed into the mouth opening. The role of the labrum in feeding is unclear, but it can likely be moved only slightly from the position shown in our figures. In nauplii of *Amphibalanus amphitrite*, Semmler et al. (2009) found no muscles extending from the labrum and into the body that could affect a shift in its position (angle) relative to the body. They did find two pairs of lateral muscles inside the labrum (Semmler et al. 2009), but their specific function is unknown. The coxal naupliar processes of antennae and mandibles may well act in finally conveying food items into the mouth. We observed that nauplii that perished during culturing had contaminated feeding chambers and this might indicate these specimens having had problems with feeding and ingestion, thus contributing to their demise. Unfortunately, Figs 20 and 27C show how the labrum covers most feeding structures from view, highlighting how difficult it will be to observe details of food manipulation and final ingestion under natural conditions.

Maxillule and maxilla

The presence of both a vestigial maxillule and a maxilla has never before been explicitly claimed for barnacle nauplii. In *C. mitella*, we argue that both these appendages are present, first as simple setal regions (1 and 2) that later develop into hump-like protuberances, although neither of them acquires a basal articulation to the body. The mx2 vestige becomes most appendage-like, having a complex setation and developing a distinct, articulated distal part. The mx1 anlage remains less complex. An apparently similar situation was reported by Olesen et al. (2024) for y-nauplii in facetotectans. The presence of both maxillule and maxilla in the cyprid, albeit in vestigial form, has also not been found before. In other cirripede nauplii, only a single pair of appendage anlage has been described and mostly considered as the maxillules. Grygier (1993) reported maxillules in NV and NVI metanauplii from petrarcid ascothoracidans. Olesen et al. (2024) found that the vestiges in facetotectans most likely represent maxillae, and that the identity maxillulary/maxillary anlagen need re-evaluation in all groups of Thecostraca based on new evidence, for example by confirming their nervous-system connections (see, *e.g.*, Kalke et al. 2020). In cirripede nauplii, setae first appear on these rudiments in N3, and their number then increases through development. Lepadids have three setae at N4 and four to five at N5-6. Pollicipedomorphans and chthmaloids have three setae in N4 and 6-7 in N5-N6 (Kado and Kim 1996; Al-Yayha 1991), while coronuloids and balanoids have 1-2 setae in N4 and 1-6 in N5-N6. A comparison of these setal numbers with our results from *C. mitella* suggests that these structures correspond to what we now consider as being the maxillae. Neither maxillules nor maxillae seem to function in the nauplii and the cyprid of *C. mitella*. In the cyprid, they and the mandibles are even more reduced, carrying no setae at all. In

contrast, the metamorphosed thoracican barnacle has a full set of functional mouthparts, including a mandible with palp, maxillule and maxilla (Høeg et al. 1994). Their presence in cyprids, albeit as mere vestiges or humps, shows that these mouth parts do not first disappear and then originate from "nothing" during cypris metamorphosis to the adult. In fact, their apparent absence in the cyprid caused the famous cirripedologist Krüger (1940) to speculate about the homology of the adult ones. In this context, it is also noteworthy that rhizocephalans, which lack appendages altogether in their adults, exhibit no trace of either maxillules or maxillae in their nauplii and cyprids (Walossek et al. 1996).

Hind body

The naupliar hind body carries the dorsocaudal spine, the anus, the caudal spines and some ventrally sited trunk spines. The six pairs of distinct spines on the hind body are indicative of the internal development of the six thoracic segments and their natatory appendages in the cyprid and are also found in y-cyprids (Facetotecta) (Grygier et al. 2019; Dreyer et al. 2023; Olesen et al. 2024). The minute spines anterior to these are of unknown homology. An alternate hypothesis to the one given below, is that these could represent the maxilla (*mx2*), in which case the hump developing from setal region 2 would be the emerging maxillule. But this would then leave the hump developing from setal region 1 and much resembling the setal region 2 hump unexplained. Although conspicuous, the dorso-caudal spine and the caudal spines in *C. mitella* are surpassed in length by those carried by lepadid and poecilasmatid nauplii (Moyse 1987; Al-Yahya 1991; Anderson 1994; Kado 2018; Dreyer et al. 2020, 2022). Balanomorphan nauplii have very short hind bodies with shorter spines and little variation within the group (Bassindale 1936; Walker et al. 1987; Semmler et al. 2009). Concerning ventrally situated spines on the hind-body, Norris and Crisp (1953) described from *Balanus perforatus* Bruguière, 1789 three different series of hind body spines. But considering the ontogeny of trunk spines of *C. mitella* nauplii (see Fig. 7, Table 3), they should rather be classified as two spine series rather than three. This developmental pattern of hind body spines is common among lepadomorph, pollicipedomorph and balanomorph nauplii and most likely represents an apomorphy of Cirripedia.

The cyprid

Details in the structure of *C. mitella* cyprids, including those of the head shield, the antennules, and the thoracopods, were described and discussed in detail previously (Jensen et al. 1994a; Moyse et al. 1995; Al-Yahya et al. 2016; Chan et al. 2017). In addition, Rao and Lin (2014) offered a very detailed SEM-based account of virtually all features of the *C. mitella* cyprid. Our results agree in all aspects with those of Rao and Lin (2014). The antennular segmentation and setation much resemble that described by Lagersson and Høeg (2002) for *Amphibalanus amphitrite* and Bielecki et al. (2009) for *Megabalanus rosa*. The setation of the natatory thoracopods is very similar to that described from *A. amphitrite* by Glenner and Høeg (1995). As in all cirripede cyprids, the caudal appendages consist of a single segment, but they have frequently (*e.g.*, Glenner and Høeg 1995) been described as 2-segmented. The reason is that the partially cleaved telson in some views masquerades as "a basal segment in two-segmented caudal appendages" (see Figs 29D, F; Kolbasov et al. 1999; Kolbasov and Høeg 2007).

The kinematics of swimming cyprids have only recently attracted attention (DiBacco et al. 2011; Maleschlijski et al. 2015; Lamont 2017; Lamont and Emlet 2018, 2021). For *C. mitella*, a detailed account of swimming in both nauplii and cyprids was provided by Wong et al. (2020). During swimming the joints between the segments of the thoracopodal rami (Fig. 29B: arrows) are oriented straight during the power stroke and flexed during the recovery stroke (Lamont and Emlet 2021). For the much slower antennular walking on the substratum a detailed account was given for *A. amphitrite* in Lagersson and Høeg (2002). Almost all cirripede cyprids have very similar antennular morphologies in terms of musculature and segmentation, whence the description for *A. amphitrite* is probably also valid for *C. mitella*, not least because both species settle in the same hard bottom intertidal habitat.

Nauplius-cypris metamorphosis

It is often overlooked that cirripede development includes two separate metamorphic events, both of which involve fundamental changes in the structure and function of the individual. The final metamorphosis of the settled cyprid to a juvenile has been closely studied, and events in *C. mitella*, as documented by Lin and Rao (2017), closely resemble those found in other thoracican species (Walley 1969; Glenner and Høeg 1993; Høeg et al. 2012; Maruzzo et al. 2012). The morphological change from nauplius to cypris is well documented (Walley 1969), but no attention has been given to how the larvae cope with this change while still swimming in the plankton. Cypris metamorphosis occurs in an immobile, attached organism, but the nauplius must, while still swimming, pass through a radical change in both morphology and mode of locomotion. All naupliar instars propel themselves using their antennules, antennae and mandibles. In contrast (Figs 1, 2), the cypris swims using the thoracopods, which are not at all externalized in the nauplius, and the naupliar antennule changes drastically into an appendage suited for bipedal walking during surface exploratory behaviour (Nott and Foster 1969; Lagersson and Høeg 2002). The change in head shield shape to a bivalved shape is likely due to contraction of the carapace adductor muscle present only in the cyprid. The thoracopods are probably ready to function as soon as the naupliar cuticle is shed. It is more problematic how the naupliar antenna and mandibles can function during swimming up to the very moment of ecdysis, when both sets are essentially reduced to “humps” or entirely absent in the resulting cyprid (Høeg 1985, 1987, this study). Furthermore, the musculature of the antennules of the new cypris is fine-tuned to bipedal walking, not swimming (Lagersson and Høeg 2002). Using video microscopy on live, metamorphosing nauplii, it may be possible to get insighty into some of these questions.

Evolution of nauplius and cyprid larvae

Development through naupliar instars must now be considered an apomorphy for all Eucrustacea (Martin et al. 2014). The nauplius (orthonauplius) is a “short head larva” bearing three pairs of appendages, and its ground pattern evolved already in the Upper Cambrian (Walossek 1993; Walossek and Müller 1997, 1998). many of those original features are clearly present also in cirripede nauplii such as *C. mitella*, even though cirripedes are otherwise a highly advanced taxon. These include: (1) a small head shield dorsal to the three pairs of limbs; (2) uniramous antennule (A1) and biramous antenna (A2) and mandible (Md); (3) A2 and Md are very similar in shape and

function for both locomotion and feeding; (4) The limb stems of A2 and Md consists of a coxa and a basis; (5) A flexible labrum with a labral gland and setae projects in front of the mouth; (6) A mouth opening recessed into an atrium oris; (7) A post-oral sternum resulting from a fusion of the antennal and mandibular sternites with characteristic sets of setae; (8) A pair of paragnath humps in the oral region; (9) Characteristic setation in the oral region and on appendages to serve both swimming and feeding; (10) The trunk terminates with an anus and caudal spines, a dorsocaudal spine and a pair of furcal spines (Walossek 1993).

A characteristic of all Thecostraca is an abrupt change in morphology and motion during the single moult from nauplius to the cypridoid instar (Walossek et al. 1996; Høeg and Møller 2006; Dreyer et al. 2023). Interestingly, a similar change occurs in the Cambrian "Orsten fossil" *Bredocaris admirabilis*, and may point to a thecostracan relation. (Walossek et al. 1996; Høeg et al. 2009). The origin of the cypris (or cypridoid) instar remains obscure, although it must have been driven by the need to successfully accomplish substrate location and attachment, which initiates the sessile juvenile and adult phases found in all Thecostraca (Dreyer et al. 2022). It is interesting that while most thecostracans have permanently sessile adults, those of synagogid Ascothoracida are at least in principle motile. Obviously, the change between the two larval types must originally have been more gradual. In this context, it is interesting that Watanabe et al. (2007) described the "precocious" development of the antennular attachment organ in a deep-sea barnacle. This could be a pointer to how this structure originally evolved. It is also interesting that adults in the most plesiomorphic species of Ascothoracida have a morphology that deviates little, if at all, from their settlement stage, here called an a-cyprid (Grygier 1996; Høeg et al. 2014; Dreyer et al. 2022). Furthermore, some Ascothoracida sport two succeeding a-cyprid instars, again suggesting remnants of an ancestral more gradual development (Grygier 1996; Kolbasov et al. 2008). Nagler et al. (2017) discussed the possible relations and evolution of the Thecostraca, but both the origin of the cyprid and the metamorphosis leading to the sessile lifestyle remain totally obscure. The Silurian *Ramphoverritor* has some claim to be placed early on the thecostracan lineage, but its morphology is too specialized to offer much information (Briggs et al. 2005; Høeg et al. 2009). For cirripedes proper, the Cambrian *Priscansermarinus*, previously considered as a pedunculated cirripede (Newman et al. 1969), is certainly not an arthropod at all (Pérez-Losada et al. 2009; Chan et al. 2021). For fossil information, this leaves only some late Paleozoic and Mesozoic pedunculated barnacles that were already fully fledged suspension feeders (Chan et al. 2021).

CONCLUSIONS

We have described the complete larval development of *Capitulum mitella*, covering all externally visible features. This is the first time this has been done in such detail for any thoracican barnacle, the only comparison being the account by Walossek et al. (1996) of a rhizocephalan barnacle. We trust that our study can serve to understand cirrripede larval development in general and serve as a comparison with larval development in other crustaceans. The phylogenetic position of *C. mitella* is somewhat uncertain, but adult barnacles comparable to this species and those of the closely related genus *Pollicipes* can be traced back to the Mesozoic, and we suggest that *Capitulum/Pollicipes*-like forms represent the plesiomorphic type of thoracican barnacles from above divergence of the Iblomorpha (Fig. 1: arrowhead). Moreover, *C. mitella* and *Pollicipes* spp.

are among the relatively few pedunculated barnacles that have planktotrophic nauplii, which certainly represents the plesiomorphic condition within cirripedes (Martin et al. 2014). It would be highly desirable if a study as detailed as ours were done on *Pollicipes pollicipes* and especially on a species of *Ibla*. Species of the Iblomorpha are with high confidence among the most plesiomorphic extant barnacles, but unfortunately their larval development is insufficiently described. We also call for detailed studies on naupliar food preference, food capture and ingestion including digestive enzymes in a wide selection of barnacle species, something that will challenge even the most advanced techniques available today.

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Authors' contributions: RK, JTH and DW contributed to study design; RK performed larval culture, collected the data, LM study, and illustrations; RK, JTH, ND and JO performed SEM study; RK, JTH and ND wrote the draft and revised the manuscript; JO and DW revised the manuscript.

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Availability of data and materials: The data presented in this study, including all underlying SEM images and drawings, are available electronically on request from RK and JTH.

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Ethics approval consent to participate: Agreed by all authors.

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