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ORIGINAL ARTICLE

Naupliar development of Facetotecta (Crustacea: Thecostraca) and the nature of the first nauplius instar in the Crustacea - pro et contra the Hexanauplia concept

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Abstract

From a number of partial moult series obtained by laboratory culture of plankton-caught specimens, we described all naupliar instars of the facetotectan species, *Hansenocaris itoi*. It reveals seven naupliar instars, instead of the five that were previously supposed for the Facetotecta. This number of naupliar instars is unique not only for Facetotecta, but also for Thecostraca and Hexanauplia as well. We studied the external morphology of each naupliar instar in detail with light microscopy and SEM. Nauplius 1 is entirely non-feeding and differs from subsequent instars in having a smooth, unsculptured cuticle and undeveloped armament of the labrum and the limbs. The subsequent naupliar development is characterized by an increase in size and in the number of cuticular plates and by the appearance of different armament of the limbs. We compare nauplius 1 across all Thecostraca and discuss its nature in relation to the larval development of Crustacea. We also discuss the presence of seven naupliar instars in Facetotecta and the concept of Hexanauplia.

Keywords Y-larvae · Naupliar instars · Larval development · Morphology · Ultrastructure · SEM

Introduction

The enigmatic Facetotecta (or y-larvae) represents the last significant group of Crustacea for which the adult forms are still unknown (Grygier 1996; Kolbasov and Høeg 2003; Glenner et al. 2008; Høeg et al. 2014). Their naupliar and cypridiform larvae have been found in the marine plankton worldwide. Facetotectan nauplii were first described in detail more than 100 years ago by Hansen (1899), who originally illustrated five different naupliar types of y-larvae from West Indian, equatorial Atlantic waters and from the Bay of Kiel in the Baltic. Subsequently y-larvae were reported from almost all oceans in the world (Kolbasov and Høeg 2003; Belmonte 2005; Ponomarenko 2006; Swathi and Mohan 2019). A post-naupliar instar or 'y-cyprid' resembling other thecostracan cypridiform larvae was first described by Bresciani (1965).

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Treatment with the crustacean moulting hormone 20-hydroxy ecdysone has recently been shown to induce y-cyprids to moult into a unique minute, slug-like stage, called the ypsigon (Glenner et al. 2008). The morphology of both the y-cyprid and the ypsigon suggest that unknown adult stages are advanced endoparasites in still to be identified hosts (Glenner et al. 2008; Pérez-Losada et al. 2009). Thus, the incompletely known life cycle of Facetotecta includes free-swimming naupliar stages, a cypridiform larva specialized for attachment and an ypsigon with an unknown role (Høeg et al. 2014; Pérez-Losada et al. 2009). The y-nauplii can be planktotrophic (feeding) or lecithotrophic (non-feeding), but the y-cyprid is always non-feeding, as is also the case for cypridiform larvae in other Thecostraca. At least thirteen naupliar morphotypes are known to date, but only some of these have been correlated with y-cyprids.

The naupliar body consists of a cephalic anterior part, covered by the dorsal head shield, and a posterior hindbody. The y-cyprid has a univalved carapace that only partially covers the larval body, six pairs of natatory thoracopods, a segmented thorax and an abdomen with furcal rami and several pore openings. The dorsal side of the naupliar head shield, the 'trunk', the carapace, and the telson of the y-cyprid have a surface pattern of reticulated cuticular ridges, which together form a series of interconnected plates.

Grygier (1985) erected the higher-level taxon Facetotecta to accommodate y-larvae and placed it inside the monophyletic Thecostraca. Facetotecta is considered as one of the three subclasses of the class Thecostraca also including Cirripedia and Ascothoracida (Grygier 1987; Høeg and Kolbasov 2002; Pérez-Losada et al. 2002, 2009; Chan et al. in-press). Itô (1985) proposed the new genus *Hansenocaris* for three new species, described on the basis of their respective y-cyprids. Currently, Facetotecta encompass seven species, established on the basis of y-cyprid morphology and assigned to a single genus, *Hansenocaris* (Itô 1990; Kolbasov et al. 2007). Additional five species of *Hansenocaris* were described on the basis of naupliar stages (Belmonte 2005; Swathi and Mohan 2019), but they remain dubious, because they were not established on the basis of y-cyprid morphology.

It has been tentatively stated that the larval development of Facetotecta consists of five naupliar instars and a single y-cypris stage (Itô 1990; Kolbasov and Høeg 2003). All naupliar instars of *Hansenocaris furcifera* Itô, 1989 and *Hansenocaris itoi* Kolbasov and Høeg, 2003 have setiform maxillulary rudiments and are therefore formally metanauplii. Two different nomenclatures for the ornamentation (dorsal plates) of the naupliar head shield has been established (Schram 1972; Itô 1987). Schram's system was based only on what he considered the first (really second) instar, while Itô's system includes a convention for plate divisions in later instars. We attempted to slightly modify and combine these two systems of plate terminology (using mostly Itô's), but the terminology breaks down after a few moults. Although we tried to trace the fate of cuticular plates through the moults, we cannot reliably follow the plates on the naupliar head shield through the entire larval development (especially in later instars).

Basic aspects of larval development, longevity and general biology are virtually unexplored in crustacean y-larvae. The worldwide presence but unknown biodiversity of Facetotecta calls for future more detailed work such as studies of the development of single-species and assessments of the facetotectan diversity based on both morphological and molecular data. Larval characters have significantly advanced our understanding of crustacean evolution and phylogeny, and this again calls for a wider study of the facetotectan larvae (Walossek 1993; Martin et al. 2014b; Olesen 2009; Høeg et al. 2003). While Oakley et al. (2013) specifically used the presence of six naupliar instars to morphologically characterize the new taxon 'Hexanauplia' (Copepoda + Thecostraca) tentatively identified by molecular markers, other molecular data are conflicting with this assessment. As such, both Malacostraca and Copepoda have been postulated to be the sister-group to Thecostraca (Pérez-Losada et al. 2009; Schwentner et al. 2018; Lozano-Fernandez et al. 2019).

Here, we describe the morphology of all naupliar instars of *H. itoi* in the White Sea (Russia) with special attention to those that were missed in earlier accounts (Kolbasov and Høeg 2003). These nauplii were referred to type IV in Hansen's (1899) classification (Kolbasov and Høeg 2003). For the first time, we systematically employ scanning electron microscopy on a crustacean y-larva throughout its larval development and hereby reveal the true first instar of a planktotrophic y-larva. We discuss our results in relation to naupliar development in other crustacean taxa, in particular focusing on the first naupliar instar and the presence of seven naupliar instars in facetotectan life cycle.

Material and methods

The main material was obtained in 2018 and 2019 from medio April to medio July, and included 163 specimens of different naupliar instars collected off the White Sea Biological Station (66°34'N, 33°08′E). In the course of this study we also used material containing about 330 specimens of nauplii collected in June–July 1998–1999 from the same locality and partially studied with SEM (Kolbasov and Høeg 2003). All larvae were captured with a 72 μm mesh net with 40 cm mouth opening at a depth of 0–40 m. In general we followed the methodology developed previously (Kolbasov and Høeg 2003). Some field-collected larvae were preserved directly, while others were reared to establish the larval sequence. Although the nauplii are planktotrophic, we did not attempt to feed them and no single larva was cultured from instar 1 to the y-cyprid. Instead, following the method outlined by Itô (1990), we cultured the individual larvae, including instar 1 and all subsequent instars onwards until they moulted and thereafter preserved and examined both the empty exuviae of the previous instars and the resulting new larvae. In this way we could assemble the whole larval series based on a combination of field-collected and cultured instars. Both live larvae and their shed exuviae were preserved in glutaraldehyde or in 4% formalin. To avoid a temperature stress of larvae, we sorted plankton samples using ice packs. The cultures were maintained at 0–9 °C in a cold room or a refrigerator. Since no individual larva was followed through its entire development, we cannot give the developmental time with any confidence. Due to presence of a significant thermocline in the White Sea and due to the fact that the larvae occur throughout a large depth range, we also cannot give the seawater temperature in their natural environment.

To establish the number and sequence of naupliar instars, all moult stages or their shed exuviae were examined using differential interference contrast (Nomarsky) optics with an Olympus BX51 light microscope. We used our previous description of naupliar instars of *H. itoi* as a basis for reconstructing the complete sequence of naupliar stages (Kolbasov and Høeg 2003). Five or more specimens of each instar were examined in SEM. The specimens were postfixed in 2% OsO4 for 2 h, dehydrated in acetone and critically-point dried with CO_2. Dried specimens were sputter-coated with an alloy of platinum–palladium and examined in a JEOL JSM-6380LA scanning electron microscope operating at voltages of 15–20 kV at the University of Moscow. The resulting photographs were touched up using CorelDraw X3 Graphics Suite.

Results

Subclass Facetotecta Grygier, 1985.

Genus *Hansenocaris* Itô, 1985.

Hansenocaris itoi Kolbasov and Høeg, 2003.

(Figs. 1–12, Supplementary videos 1–2)

Description of naupliar instars

All instars (except the non-feeding nauplius 1) are planktotrophic, semitransparent with a black nauplius eye and a brownish gut coloured by food particles (see Supplementary video files). They swim slowly forward in different directions, often upside down and bobbing up and down. The first instar is an orthonauplius, while all later stages are metanauplii.

The study of the larval behaviour faced several challenges related to differences in light and temperature between the laboratory setup and the seawater from where the material was collected. Both the increased temperature and the light of the microscope seemed to decrease the larval activity, and the study of natural behaviour was further hampered by the negative phototaxy of the larvae. Nevertheless, we attempted to examine the general movement behaviour of all collected larvae but succeeded only with a few of them (see Supplementary video files).

We use the following terminology for the nomenclature of cuticular plates:

B – brim plates; C – crescentic plates; E – elongated plates; F – frontal plates; G – gabled plates; H – hindbody plates; I – intercalary plates; M – marginal plates; O – occipital plates; P – polygonal plates; S – superlateral plates; W – window plate; number of plates division indicated by apostrophes (’).

Nauplius 1 (Figs. 1, 2, Supplementary video 1).

This instar has not been recognized previously (see Kolbasov and Høeg 2003), probably due to its short duration before moulting into nauplius 2, but it may also be that earlier collecting attempts were too late (June–July). We found 16 specimens of this instar and most of them began to moult into the next stage when collected (or soon after collecting) (Fig. 1a, d). Nauplii 1 first appear in the White Sea plankton in the second half of April, when the basin of the sea off the White Sea Biological Station is partially ice-free and water temperature is about −2-0 °C. The earliest findings were April 25 (2018) and April 23 (2019), with maximum occurrence around 30 April. They ceased to be found in the plankton after May 3 (see Table 1).

Body drop-shaped, about 245–260 μm long and 145–165 μm wide (means of 5 preserved specimens); body indistinctly subdivided into a wide anterior part corresponding to the head shield and a narrower posterior corresponding to the hindbody; dorsal part of hindbody without papilla/knob of dorsocaudal organ (Figs. 1a and 2a). Anterior end rounded, anteriormost part, corresponding to cuticular plates F3, F4, M1 and B1 of head shield, bent downwards (Figs. 1a, c and 2a). Posteriolateral ends of head shield rounded; ventral side of hindbody bulbous or inflated (Fig. 1b, c). Cuticle of body smooth, lacking cuticular ridges; head shield with 3 pairs of dorsal setae corresponding to those on plates P2, C1 and I3 of nauplius 2 (Figs. 1a, e and 3a, b). Posteriormost end tapering to short dorsocaudal spine with rare denticles; ventral side with pair of narrow, conical furcal spines armed with denticles (Figs. 1a, f and 2a, e). Posterior part of faciotrunk (hindbody) with 5 ventral transverse rows or combs of denticles corresponding to ventral cuticular ridges of hindbody in subsequent instars, length of these rows and density of denticles increase in posterior direction (Fig. 2e). New wrinkled cuticle with ridges of next instar visible in gap of shedding cuticle (Fig. 1a, d).

Labrum bulbous, potato-shaped, without apical papilla, ridges and pores (Fig. 2a, b). Two small hillocks of wrinkled cuticle with apical pore representing putative rudiments of frontal filaments located on fold anteriorly to labrum (Fig. 2b, c). Antennules (Figs. 1b, c and 2a, b, d) vaguely

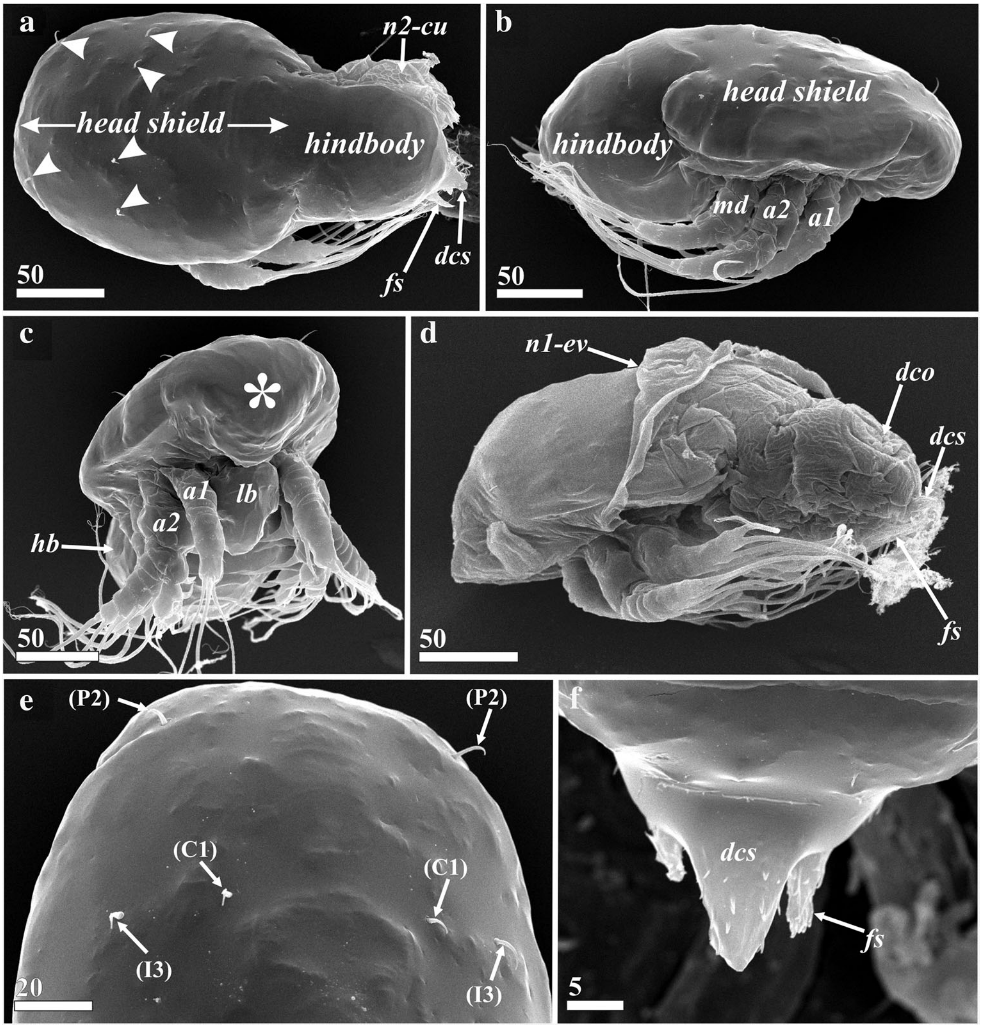

Fig. 1 *H. itoi*, nauplius 1, (SEM) **a** Dorsal side, general view, 3 pairs of setae indicated by arrowheads, nauplius began to moult and cuticle of subsequent nauplius 2 observed in right lateral side of hindbody. **b** Lateral side, general view. **c** Nauplius anterioventrally, bent anteriormost part (plates F3, F4, M1 and B1) of subsequent nauplius 2 indicated by asterisk. **d** Moulting nauplius 1, moulting cuticle preserved at anterior half, posterior half reveals compressed structure of subsequent nauplius 2, lateral view. **e** Anterior half of dorsal head shield showing 3 pairs of setae corresponding to setae on plates P2, C1 and I3 of subsequent nauplius 2. **f** Posteriormost end with dorsocaudal spine and furcal spines. Abbreviations: *a1* - antennule; *a2* - antenna; *dco* - papilla of dorsocaudal organ; *dcs* - dorsocaudal spine; *fs* - furcal spines; *hb* - hindbody; *lb* - labrum; *md* - mandible; *n1-ev* - shedding cuticle (exuvium) of nauplius 1; *n2-cu* - cuticle of nauplius 2. Scale bars in μm

subdivided into 5–6 annulations (segments?); 4 short proximal annuli without setae; terminal portion (segment) longest, may consist of two segments, with one short proximal, one short middle and three long apical setae (Fig. 2a, d). Antennae and mandibles biramous, distinctly segmented (Fig. 2a, d, e). Antennal and mandibular exopods twice longer than endopods, with 6 and 5 short segments respectively, apical segment tiny and hardly discernible from preceding segment, basal segment without seta, second segment with shorter seta, other segments with one long, distal inward seta; all setae serrated with small denticles in distal half and long, thin apical setules (Fig. 2d, e). Both antennal and mandibular endopods two-segmented, their proximal segments with prominent, inward curved spine and shorter distal seta, distal segments with two apical, long setae (Fig. 2a, d, e). Both limbs with two-segmented protopods, coxa and basis with prominent, curved spine on inner margin, without setae and denticles (Fig. 1d, e). All limbs with circular patches of small denticles around distal margin of segments. Nauplius 1 lacks setiform vestiges of maxillules and represents the orthonauplius (Fig. 2e).

Nauplius 2 (Figs. 3, 4, Supplementary video 1).

A full description of this instar follows here, since the previous treatment by Kolbasov and Høeg (2003) was based on a single specimen and an exuvium fouled with bacteria. Kolbasov and Høeg (2003) termed this instar ‘nauplius 1’ (real nauplius 2). This instar begins to occur in the White Sea plankton, together with nauplius 1, during the second half of April. Their first occurrence were on 25 April 2018 and 23

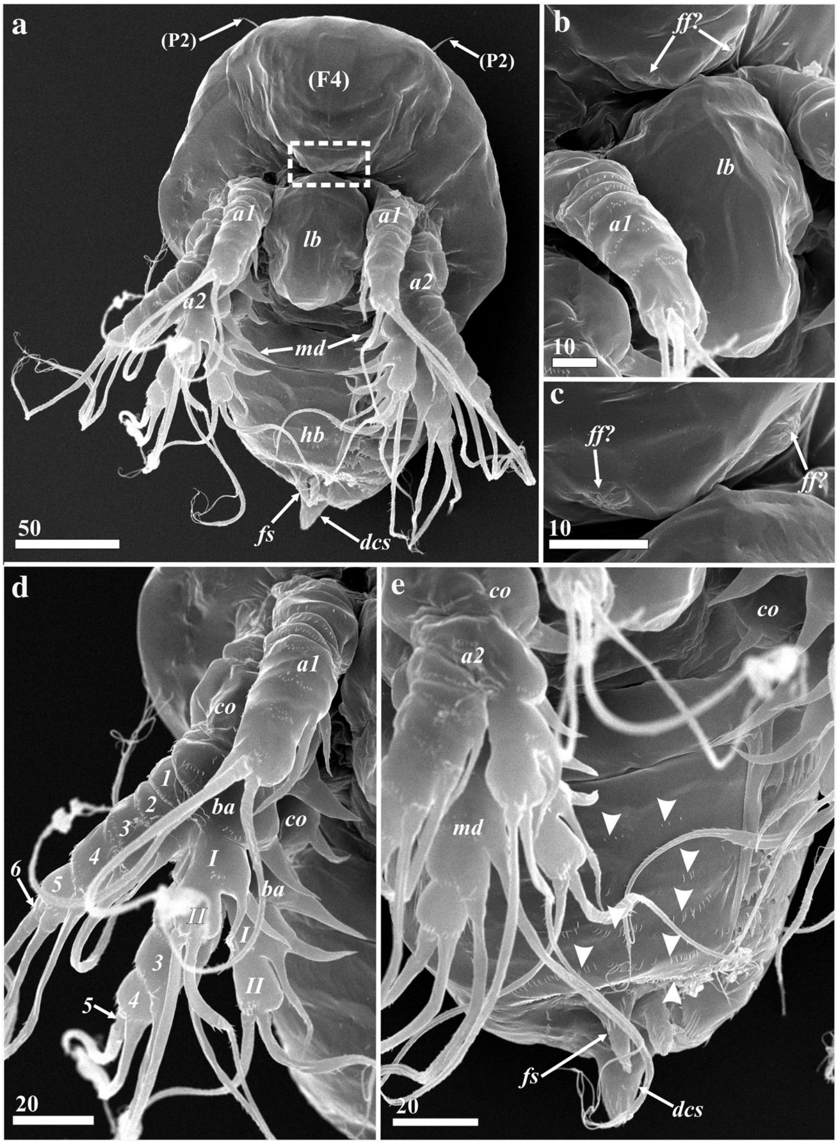

Fig. 2 *H. itoi*, nauplius 1, ventral side (SEM) **a** General view (cuticular folder with putative rudiments of frontal filaments from 'C' in rectangle area). **b** Labrum. **c** Putative rudiments of frontal filaments. **d** Right naupliar limbs, segments of exopods numbered in Arabic, segments of endopods numbered in Roman. **e** Posterior part, 5 transverse cuticular rows or combs of denticles corresponding to ventral cuticular ridges of hindbody in subsequent instars indicated by arrowheads. Abbreviations: *a1* - antennules; *a2* - antennae; *ba* - basis; *co* - coxa; *dcs* - dorsocaudal spine; *ff?* - putative rudiments of frontal filaments; *fs* - furcal spines; *hb* - hindbody; *lb* - labrum; *md* - mandible. Scale bars in μm

April 2019, with maximum frequency 28 April - 5 May. They cease to be found after May 10–15 (see Table 1).

Young (just after hatching from nauplius 1) and mature individuals differ in form and size (Fig. 3a, b). Young specimens of nauplius 2 (Fig. 3a) resemble those of nauplius 1 because cuticular plates of dorsal head shield (especially at anteriormost end) and hindbody (Fig. 1c, d) stay wrinkled and compressed for a while (about a day) and straighten out in mature forms (Fig. 3b). This change of form suggests an initially rather soft cuticle that only gradually becomes sclerotized. Young specimens with rounded anterior end, because anteriormost end bends downward (Fig. 3a); 290–300 μm long and 175–185 μm wide.

Mature specimens with slightly elongated, trapezoidal anterior end, 370–415 μm long and 210–220 μm wide, with dorsal head shield 270–290 μm long and triangular hindbody 115–120 μm long (Fig. 3b). Cuticle of dorsal side with pattern of fine reticulation (Fig. 3); narrow stripes of thin cuticle demarcate boundary of moult between head shield and faciotrunk (Figs. 3b, c and 4d, '*bm*'). Cuticular ridges divide dorsal head shield into 57 polygonal plates arranged in symmetrical pattern (Fig. 3b). Axial row consists of 14 plates (Fig. 3b): 4 unpaired frontal plates (F1–4), single semicircular window plate (W), then 2 pairs of occipital plates split by delicate median ridge (O1', O2') and 5 unpaired occipital plates (O3–7). Window plate situated above naupliar eye. Dorsolateral surface with 43 plates (Fig. 3b, c): 2 pairs of crescentic plates (C1–2), 2 pairs of elongated plates (E1–2), 3 pairs of intercalary plates (I1–3), 6 pairs of polygonal plates (P1–6), 6 pairs of marginal plates (M1–6) where long, undivided M2–3 or M3–4 representing two plates, one pair of superlateral plates (S), unpaired anteriormost brim plate (B1) and one pair of long, lateral brim plates (B2). Head shield with 4 pairs of simple pores without seta: one on I1, E2 and two on B2 (Figs. 3c, d,

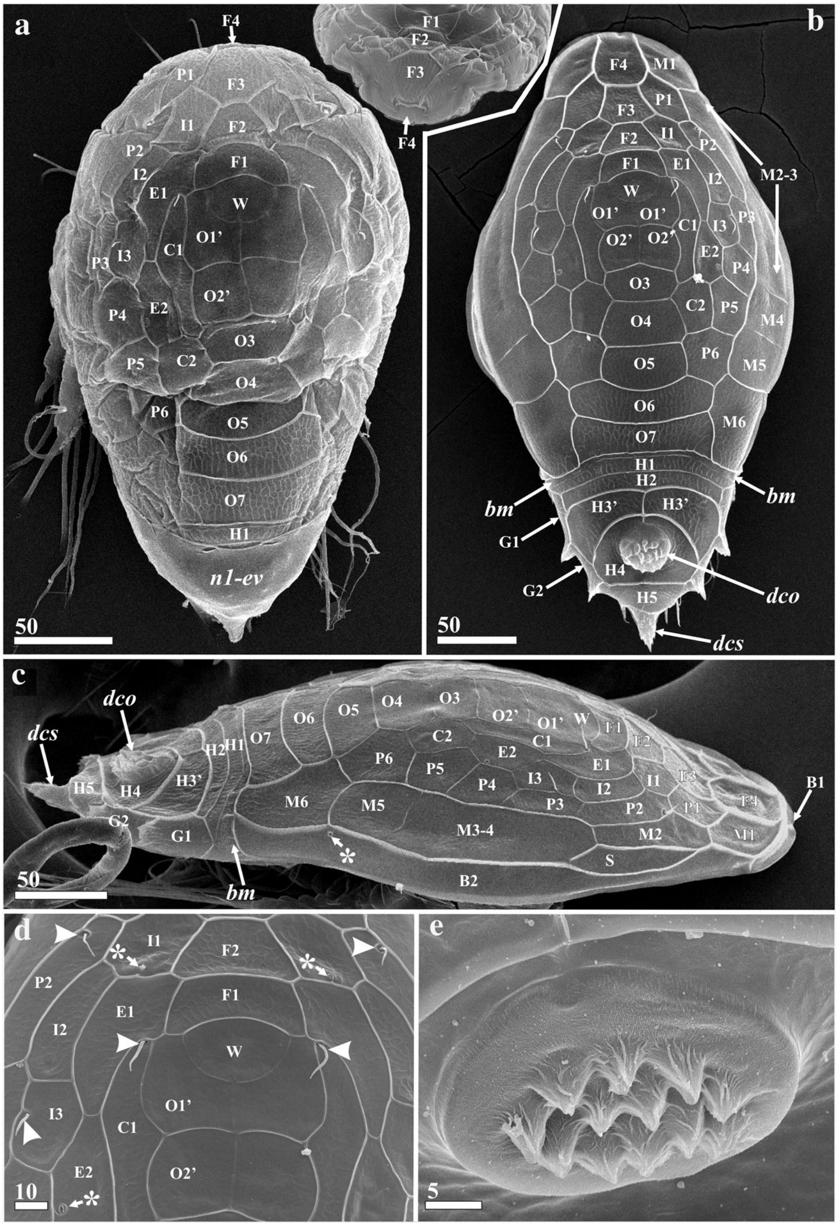

Fig. 3 *H. itoi*, nauplius 2, dorsal side (SEM) **a** Just moulted, young nauplius with remnants of cuticle of nauplius 1 at posterior end, cuticular plates compressed, anterior end round; bent anteriormost end in upper right. **b** Mature nauplius with stretched cuticular plates, anterior end elongated. **c** Mature nauplius, dorsolateral view, pore without setae indicated by asterisks. **d** Central area of head shield around 'window plate' (W), setae in pores indicated by arrowheads, pores without setae indicated by asterisks. **E** Papilla of dorsocaudal organ. Abbreviations: *bm* - boundary of moult between head shield and faciotrunk; *dco* - papilla of dorsocaudal organ; *dcs* - dorsocaudal spine; *n1-ev* - shedding cuticle (exuvium) of nauplius 1. Scale bars in µm

4d, indicated by asterisk) and 3 pairs of pores with seta inside on P2, C1 and I3 (Fig. 3c, d, indicated by arrowheads).

Dorsal and lateral sides of hindbody with 10 cuticular plates (Fig. 3b, c). Dorsal side with 6 hindbody plates: anteriorly 2 long and narrow, unpaired H1 and H2, then pair of bent H3 in middle, circular H4 around papilla of dorsocaudal organ and triangular posterior H5 terminating with conspicuous dorsocaudal spine with fine denticles and paired lateral pores at base (Figs. 3b, c and 4a, f). Lateral sides have 2 pairs of gabled plates G1 and G2, their posterior parts forming two sharp, lateral protrusions of hindbody. Papilla or knob of dorsocaudal organ prominent and round, with 9–13 sharp cuticular protrusions (Fig. 3b, e).

Anterior part of ventral side divided on several symmetrical plates by feeble cuticular ridges (Fig. 4d). Pair of papillae with terminal pores, probable rudiments of frontal filaments, projects from cuticular fold anteriorly to labrum (Fig. 4b, '*ff?*'). Prominent labrum bulbous, with round basal part with two lateral pores and narrower oval distal part with several indistinct symmetrical cuticular ridges, unpaired subterminal pore and apical papilla with tuft of cuticular villi (Fig. 4a, b, e). Antennules (Fig. 4a, c, e) consist of two portions: basal portion with 3–4 indistinct annulations, lacks setae; terminal elongated portion or segment, with one short proximal seta and one short seta at middle on inner margin and two long and one short apical setae, two circular rows of denticles (ctenes) in middle area and 2–3 feeble apical ctenes (Fig. 4a, c, e). Antennal and mandibular exopods with 6 and 5 short segments respectively, apical segment tiny and hardly discernible from penultimate segment, circular row of rare, small

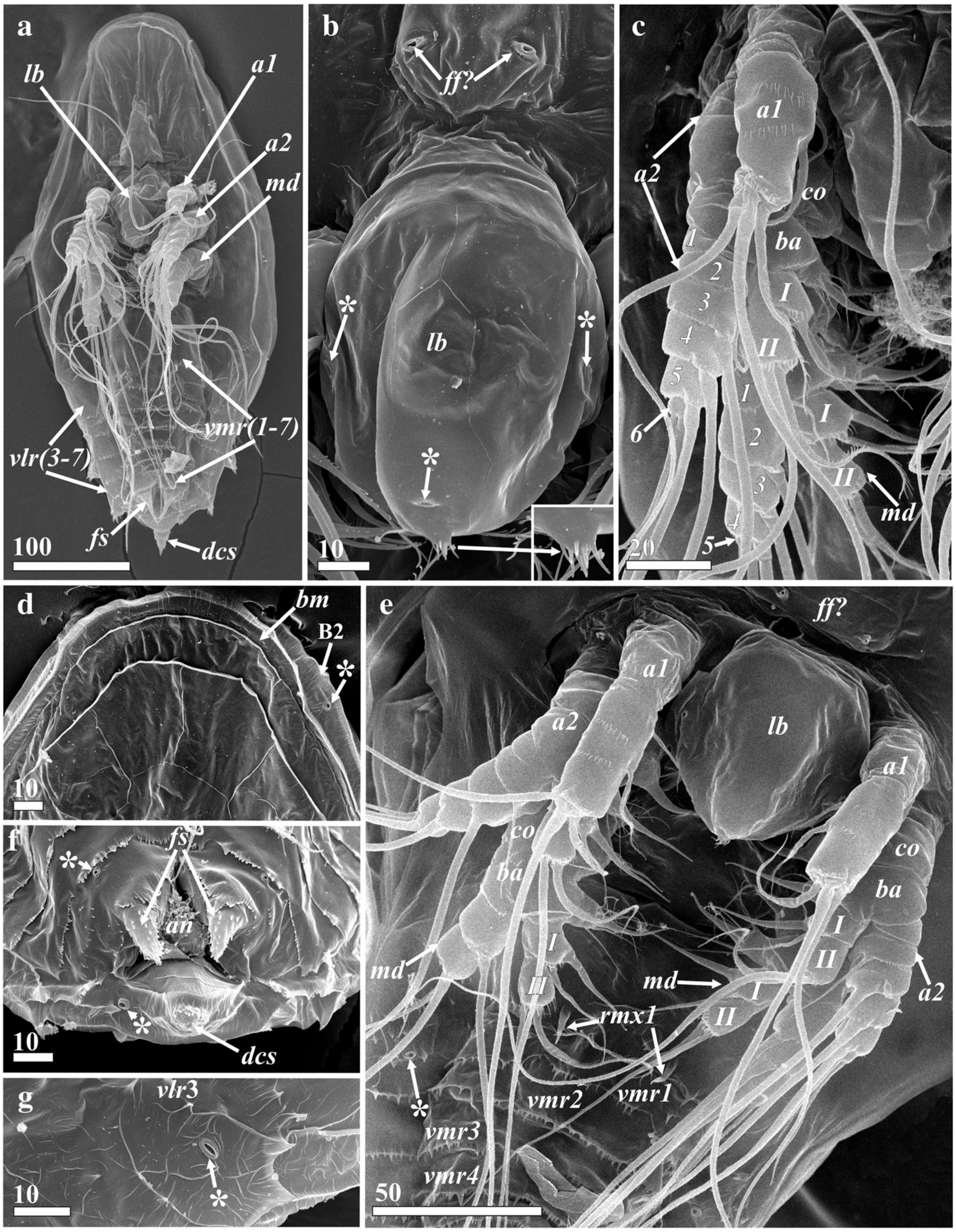

Fig. 4 *H. itoi*, nauplius 2, ventral side (SEM) **a** General view. **b** Labrum, terminal papilla of labrum in lower right angle in rectangular area (pores of labrum indicated by asterisks). **c** Right naupliar limbs, segments of exopods numbered in Arabic, segments of endopods numbered in Roman. **d** Anteriormost end (pore indicated by asterisk). **e** Central area with labrum and naupliar limbs (pore indicated by asterisk, segments of endopods numbered in Roman). **f** Posteriormost end, posterioventral view (pores indicated be asterisks). **g** Pore on ventrlolateral cuticular ridge (*vlr3*) in posterior part (indicated by asterisk). Abbreviations: *a1* - antennules; *a2* - antennae; *an* - anus; *ba* - basis; *bm* - boundary of moult of faciotrunk; *co* - coxa; *dcs* - dorsocaudal spine; *ff?* - putative rudiments of frontal filaments; *fs* - furcal spines; *lb* - labrum; *md* - mandible; *rmx1* - rudiments of maxillules; *vlr* – ventrolateral cuticular ridges; *vmr* – ventromedial cuticular ridges. Scale bars in µm

denticles around distal margin of segments, basal segment of antenna without seta and with short seta in mandible, apical segment with two long setae, other segments with one mediodistal, inward seta, shorter in segment 2 of antenna and longer in remaining ones (Fig. 4c, e). Antennal and mandibular endopods two-segmented, their proximal segments with prominent, inward curved spine and shorter distal seta at base of spine, distal segments with two apical, long setae, distal ends of segments with long denticles (Fig. 4c, e). Inner margin of coxa of antenna with long, curved, massive spine without setae and denticles; inner margin of coxa of mandible carries seta with setules in proximal part, this seta shorter and thinner in comparison with coxal spine of antenna (Fig. 4c, e). Inner margin of basis of antenna and mandible with long, slightly curved, massive spine with corolla of thin, proximal setae (Fig. 4c, e). Pair of short and stout setae representing rudiments or vestiges of maxillules inserted after naupliar limbs (Fig. 4e, '*rmx1*').

Ventral area of facoitrunk or hindbody posteriorly to vestiges of maxillules ornamented by 7 prominent unpaired ventromedial ridges and 7 paired ventrolateral ridges (4a, e, f, '*vmr1–7*' and '*vlr1–7*'), these ridges armed with minute spines and may represent borders of future trunk somites. Paired pores inserted mediolaterally in *vmr1* (Fig. 4e), laterally in *vlr3* (Fig. 4g) and at base of furcal spines (Fig. 4f). Paired conical furcal spines

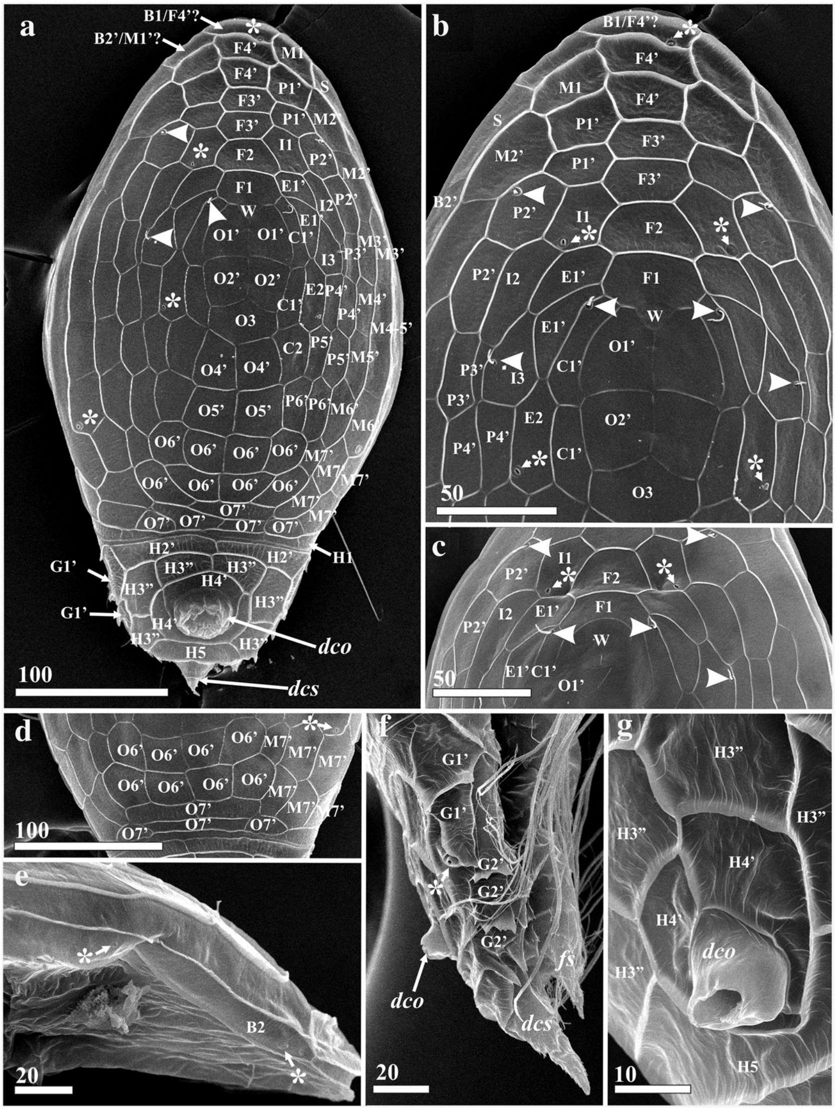

Fig. 5 *H. itoi*, nauplius 3, dorsal and lateral sides (SEM) **a** General view, dorsal side. **b** Anterior half of head shield, dorsal view (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). **c** Central area of head shield around 'window plate' (W), dorsal view (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). **d** Posterior part of head shield, dorsal view. **e** Anterior part, lateral view (pores without setae indicated by asterisks). **f** Posterior part, lateral view (pore without setae indicated by asterisks). **g** Area around papilla of dorsocaudal organ, dorsal side. Abbreviations: *dco* - papilla of dorsocaudal organ; *dcs* - dorsocaudal spine; *fs* - furcal spines. Scale bars in μm

covered with denticles terminate hindbody and surround triangular anus (Fig. 4f).

Nauplius 3 (Figs. 5, 6, Supplementary video 1).

This instar has not been found previously (see Kolbasov and Høeg 2003). Nauplii 3 occur in the White Sea plankton throughout May, with maximum occurrence May 11–20 (see Table 1).

The dorsal surface of nauplius 3 has a larger number of cuticular plates compared to the previous instar (Fig. 5a). These plates may have arisen by subdivision of plates of nauplius 2 or some of them may originate de novo. Homologies may be established between nauplii 2 and 3 for axial plates and for those plates having pores and setae, while the origin of numerous lateral plates is suggested putatively to be derivatives of existing plates of nauplius 2. The plates of the hindbody are less in number in comparison with those of the head shield and their homology may be traced more or less reliably.

Body ellipsoid, with rounded anterior end, 450–480 μm long and 220–240 μm wide, with dorsal head shield 330–350 μm long and triangular hindbody 120–130 μm long (Fig. 5b). Head shield carries at least 105 dorsal polygonal cuticular plates arranged in symmetrical pattern (Fig. 5a-d). Axial row consists of 29–30 plates (Fig. 5a-d): 7 unpaired frontal plates (F), single semicircular window plate (W) and 22 occipital plates, where only O3 unpaired, while O1, O2, O4 and O5 paired, but O6 divided in 6 plates and O7 - in 4–5 plates. Dorsolateral faces with 75–76 plates (Fig. 5a-d): 3 pairs of crescentic plates (C), 3 pairs of elongated plates (E), 3 pairs of intercalary plates (I), 12 pairs of polygonal plates (P), 14 pairs of marginal plates (M), one pair of superlateral plates (S) and 2 pairs of brim plates (B). Intercalary (I1–3), occipital (O1', O2', O3), frontal (F1, F2), crescentic (C2), elongated

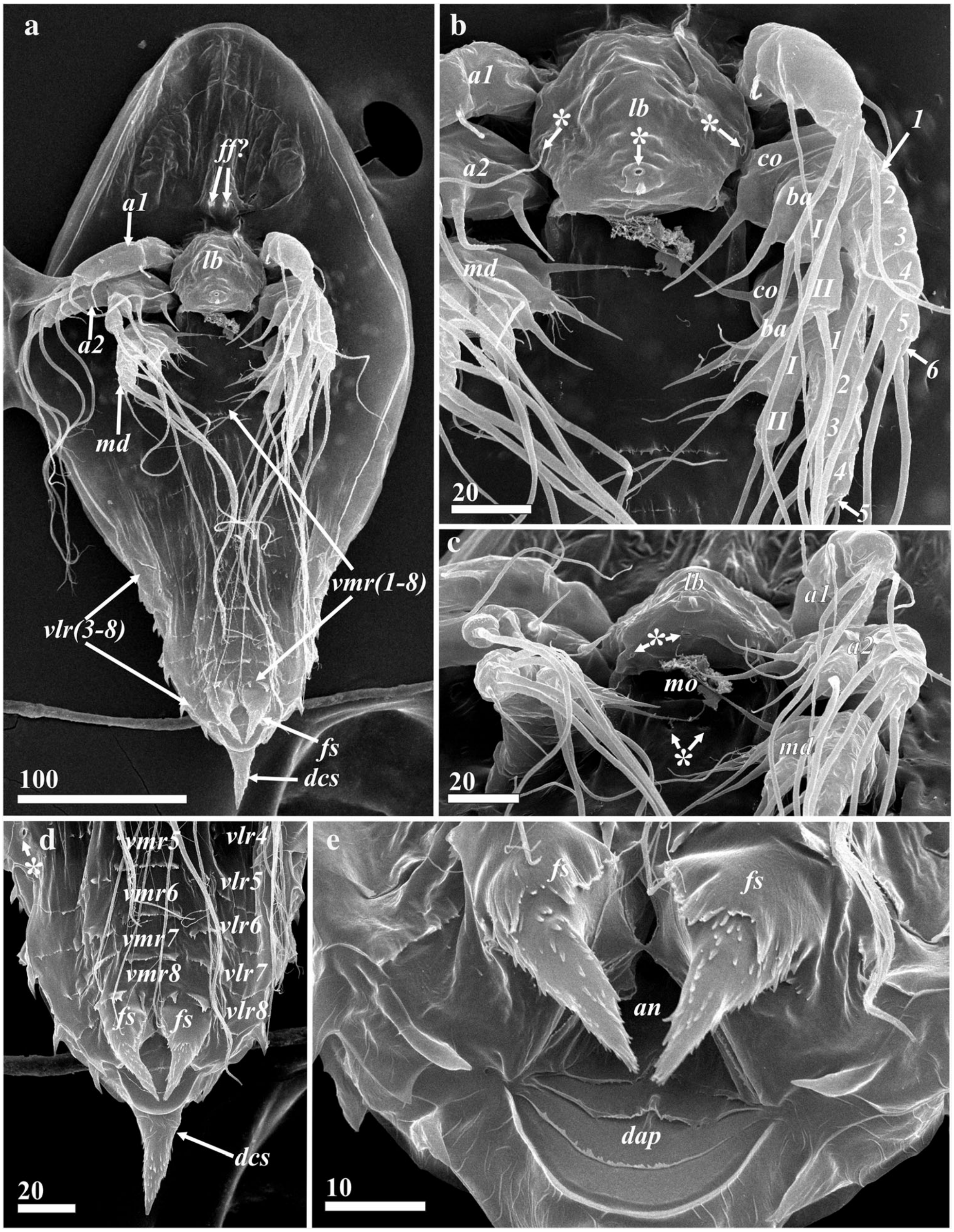

Fig. 6 *H. itoi*, nauplius 3, ventral side (SEM) **a** General view. **b** Central area with labrum and naupliar legs (pores indicated by asterisk, segments of exopods numbered in Arabic, segments of endopods numbered in Roman). **c** Area around slit-like mouth opening (pores indicated by asterisks). **d** Posterior part (pore indicated by asterisk). **e** Anal region. Abbreviations: *a1* - antennules; *a2* - antennae; *an* - anus; *ba* - basis; *co* - coxa; *dap* - dorsal anal plate; *dcs* - dorsocaudal spine; *ff?* - putative rudiments of frontal filaments; *fs* - furcal spines; *lb* - labrum; *md* - mandible; *mo* - mouth opening; *vlr* – ventrolateral cuticular ridges; *vmr* – ventromedial cuticular ridges. Scale bars in μm

(E2), marginal (M1) and superlateral (S) plates stay undivided after nauplius 2 moulting. Head shield carries 5 pairs of simple pores without seta: one on I1, E2, M6 and two on B2 (Fig. 5a-e, indicated by asterisk) and 3 pairs of pores with seta inside on P2', C1' and I3 (Fig. 5b, c, indicated by arrowheads). Several specimens had additional unpaired, asymmetrical simple pore on anteriormost plate (Fig. 5b).

Dorsal and lateral sides of hindbody with 22 cuticular plates (Fig. 3b, c). Dorsal side carries 12 hindbody plates: anteriorly one long and narrow H1 and paired elongated H2', then 3 pairs of bent H3" in middle, two circular H4' around papilla of dorsocaudal organ and unpaired posterior H5 terminating with conspicuous dorsocaudal spine with fine denticles and paired lateral pores at base (Fig. 5a, g). Lateral sides have 2 pairs of gabled plates G1' with pore on posterior pair, and 3 pairs of G2' with sharp posterior parts (Fig. 5f). Only H1 and H5 plates stay undivided after nauplius 2 moulting. Papilla or knob of dorsocaudal organ prominent, volcano-shaped (Fig. 5a, f, g).

Ventral side (Fig. 6) has similar morphology to that in preceding nauplius 2. Almost vertical position of labrum in examined specimen allows for examining the mouth cavity (Fig. 6c). Additionally to two lateral and one subterminal pores on upper side (Fig. 6b), labrum has two lateral pores and one pore beneath apical papilla on lower side (Fig. 6c). Mouth opening slit-like, with two pores on ventral surface in front of entrance (Fig. 6c). Although naupliar limbs have almost the same structure as in nauplius 2, they differ in several

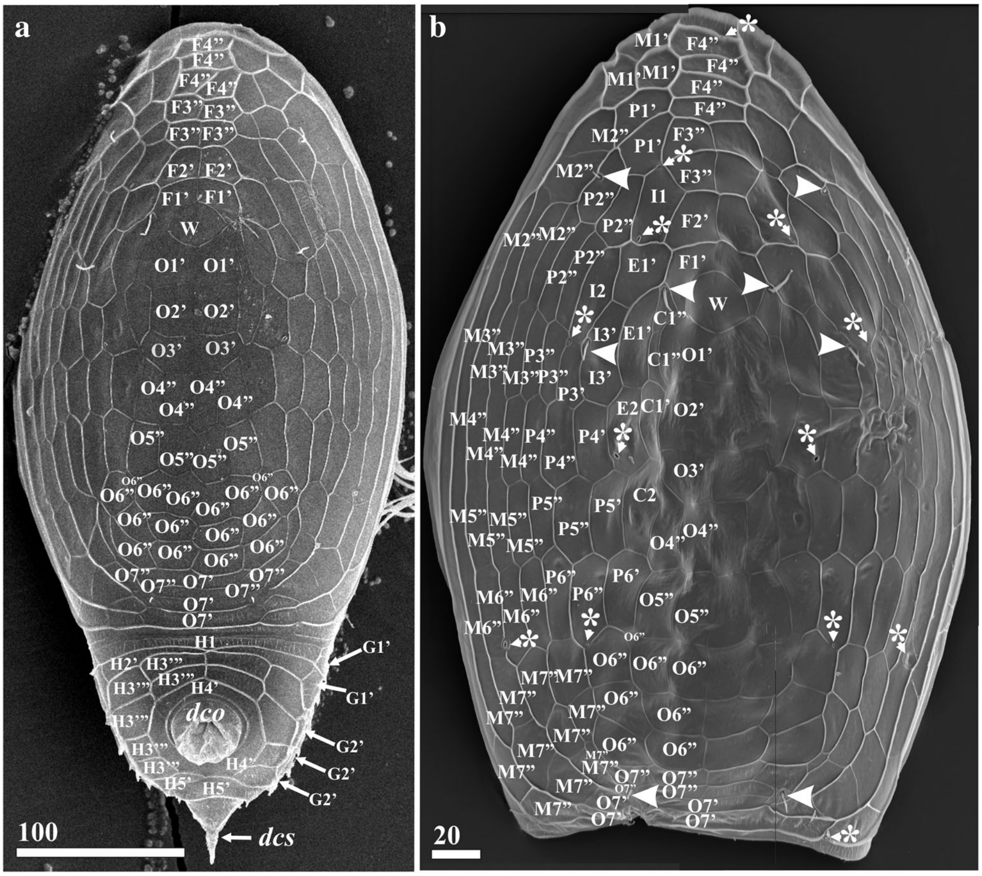

Fig. 7 *H. itoi*, nauplius 4, dorsal side (SEM). **a** General view. **b** Head shield (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). Abbreviations: *dco* - papilla of dorsocaudal organ; *dcs* - dorsocaudal spine. Scale bars in μm

characters of armament: terminal segment of antennule with one newly formed seta on the middle of the outer margin; mandibular coxa with curved, massive spine without setules, instead of seta with setules in nauplius 2; curved, massive spine of antennal basis without corolla of thin, proximal setae (Fig. 6b, c).

Ventral area of faciotrunk or hindbody posteriorly to vestiges of maxillules ornamented by 8 prominent unpaired ventromedial ridges and 8 paired ventrolateral ridges (6a, d, ‘*vmr1–8*’ and ‘*vlr1–8*’). Paired pores inserted laterally in *vlr4* (Fig. 6d). Paired slightly curved, conical furcal spines covered with denticles terminate hindbody and surround triangular anus with semicircular cuticular plate on dorsal/upper side (Fig. 6e).

Nauplius 4 (Fig. 7, Supplementary video 2).

This instar was previously described as ‘nauplius 2’ by Kolbasov and Høeg (2003). They gave a detailed account of both this and the following instars, whence we here offer only a short description for these stages. Nauplius 4 begins to occur in the White Sea plankton from the first half of May (first finding May 8) to the first decade of June, with maximum occurrence in the end of May (see Table 1).

Body ellipsoid, with rounded anterior end, 490–500 μm long and 230–250 μm wide, with dorsal head shield 350–370 μm long and triangular hindbody 130–140 μm long (Fig. 7). Head shield carries approximately 180 plates of different sizes (Fig. 7 herein and Fig. 2A in Kolbasov and Høeg 2003). Axial row includes about 60 plates (Fig. 7): 3–5 unpaired anterior frontal plates and 4–5 pairs of posterior frontal plates (F), single semicircular window plate (W) and about 46 occipital plates. There is some slight individual variability in the numbers and shapes of the more laterally situated plates (Fig. 7a, b). Intercalary (I1–2), occipital (O1’, O2’), crescentic (C2) and elongated (E2) plates stay undivided as in nauplius 2. Head shield carries at least 6 pairs (5 in nauplius 3) of simple pores without seta: on I1, I2, E2, P6”, M6” and M7” (Fig. 7, indicated by asterisk) and 4 pairs (3 in nauplius 3) of pores with seta inside on P2”, C1”, I3′ and O7” (Fig. 7b, indicated by arrowheads). Several specimens had additional unpaired, asymmetrical simple, small pores on anteriormost plate and in anterior part of left I1 plate (Fig. 7b).

Dorsal and lateral sides of hindbody divided into 30–35 cuticular plates (Fig. 7a), with 20–21 hindbody plates (H). Lateral sides have 5 pairs of gabled plates (G) with sharp posterior parts (Fig. 7a). Only H1 plate stays undivided as in nauplius 2.

Ventral side has similar morphology to that in preceding nauplius 3, except extra subterminal seta on inner margin of terminal segment of antennules; corolla of

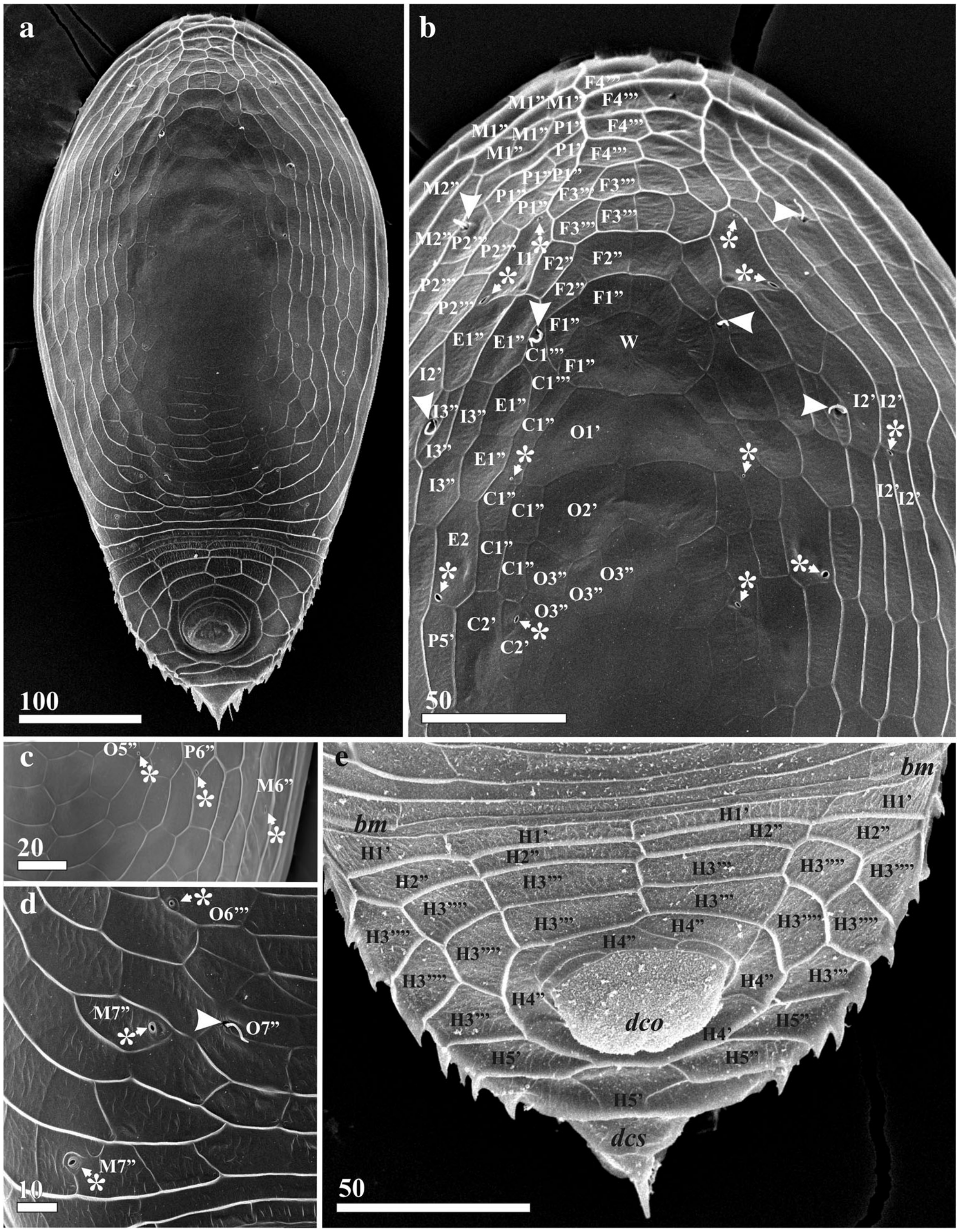

Fig. 8 *H. itoi*, nauplius 5, dorsal side (SEM) **a** General view. **b** Anterior half (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). **c** Middle-posterior part of head shield, right side (pores without setae indicated by asterisks). **d** Posterior part of head shield (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). **e** Posterior part of faciotrunk. Abbreviations: *bm* - boundary of moult of faciotrunk; *dco* - papilla of dorsocaudal organ; *dcs* - dorsocaudal spine. Scale bars in μm

thin, proximal setae at basis of massive spine of antenna; and 10 ventromedial and ventrolateral ridges.

Nauplius 5 (Figs. 8, 9, Supplementary video 2).

Previously described as 'nauplius 3' (see Kolbasov and Høeg 2003). It occurs in the White Sea plankton from the second half of May (first finding May 26) to the second half of June, with maximum occurrence in the beginning of June (see Table 1).

Body ellipsoid, with rounded anterior end, 540–590 μm long and 270–290 μm wide, with dorsal head shield 390–440 μm long and triangular hindbody 140–150 μm long (Fig. 8a). Head shield carries approximately 280 plates of different sizes (Fig. 8a-d herein and Fig. 3A in Kolbasov and Høeg 2003). There is some individual variability in the numbers and shapes of these plates. Borders between axial (occipital) plates in central area ephemeral, hardly discernible (Fig. 8a, b). Unlike instar 4, instar 5 has no unpaired frontal plates; anterior part with 28–30 frontal plates (F) (Fig. 8b). Only four plates: intercalary (I1), occipital (O1', O2') and elongated (E2) stay undivided as in nauplius 2. Head shield carries 12 pairs (6 in nauplius 3) of simple pores without seta: two pores on I1, one pore on I2', C1", C2', E2, O5", O6"', P6", M6" and on two plates M7" (Fig. 8b-d, indicated by asterisk) and 4 pairs (as in nauplius 4) of pores with seta inside (Fig. 8b, d, indicated by arrowheads).

Dorsal and lateral sides of hindbody divided into 42–44 cuticular plates (Fig. 8a, e herein and Fig. 3A in Kolbasov and Høeg 2003), with 30–32 hindbody plates (H). Lateral

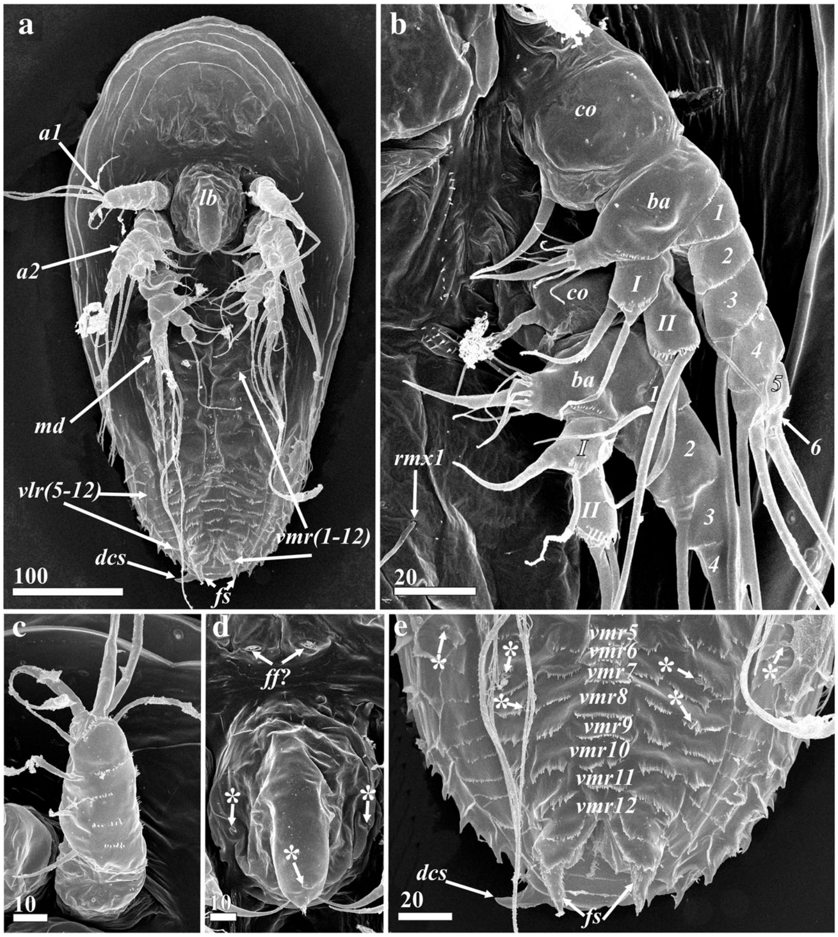

Fig. 9 *H. itoi*, nauplius 5, ventral side (SEM) **a** General view. **b** Left antenna and mandible (endopod segments numbered in Roman, exopod segments numbered in Arabic). **c** Right antennule. **d** Labrum (pores of labrum indicated by asterisks). **e** Posterior part (pores indicated by asterisks). Abbreviations: *a1* - antennules; *a2* - antennae; *ba* - basis; *co* - coxa; *dcs* - dorsocaudal spine; *ff?* - putative rudiments of frontal filaments; *fs* - furcal spines; *lb* - labrum; *md* - mandible; *rmx1* - rudiments of maxillules; *ob* – oban; *vlr* – ventrolateral cuticular ridges; *vmr* – ventromedial cuticular ridges. Scale bars in µm

sides have 6 pairs of gabled plates (G) with sharp posterior parts. All hindbody plates, including H1, are divided and paired.

Ventral side (Fig. 9) has similar morphology to that in preceding nauplius 4, except extra seta on inner margin of terminal segment of antennules (now 4 setae instead 3 in nauplius 4) and 12 ventromedial and ventrolateral ridges with 3 pairs of pores in *vlr 5, 7, 8* (Fig. 9a, c, e).

Nauplius 6 (Fig. 10, Supplementary video 2).

Previously described as 'nauplius 4' (see Kolbasov and Høeg 2003). Nauplius 6 begins to occur in the White Sea plankton from the beginning of June (first finding June 02) to the end of June, with maximum occurrence in the middle of June (see Table 1).

This instar differs from instar 5 mostly in the morphology of the hindbody and the dimensions of the body, but its existence is certain as we obtained it from plankton samples and by the moulting of nauplius 5 in our cultures in 1998–1999 (Kolbasov and Høeg 2003) and in 2018–2019.

Body ellipsoid, with rounded anterior end, 600–645 µm long and 300–320 µm wide, with dorsal head shield 450–470 µm long and triangular hindbody 150–170 µm long (Fig. 10a, d). Head shield carries approximately 290 plates of different sizes (Fig. 10a-c herein and Fig. 4 in Kolbasov and Høeg 2003). There is some individual variability in the numbers and shapes of these plates, even on left and right sides of one specimen (Fig. 10b) that breaks the symmetrical pattern of cuticular plates. As in nauplius 5, borders between occipital plates in central area ephemeral, hardly discernible (Fig. 10a-c). Anterior part with numerous small, irregular plates, 30–34 of them may represent frontal plates (F) (Fig. 10a, b). Only occipital (O1', O2') and elongated (E2) plates stay undivided as in nauplius 2. Normally intercalary I1 plate also stays undivided, but in one specimen it was divided into 2 or 3 plates on the left side, while undivided in right (Fig. 10b). Head shield carries 14 pairs of simple pores of different size without seta (Fig. 10b, c, indicated by asterisk; some pores are absent on images) and 4 pairs (as in nauplii 4, 5) of pores with seta inside (Fig. 10b, c, indicated by arrowheads).

Dorsal and lateral sides of hindbody divided into 50–52 cuticular plates (Fig. 10a, d herein and Fig. 4 in Kolbasov and Høeg 2003), with 34–36 hindbody plates (H), where 4–

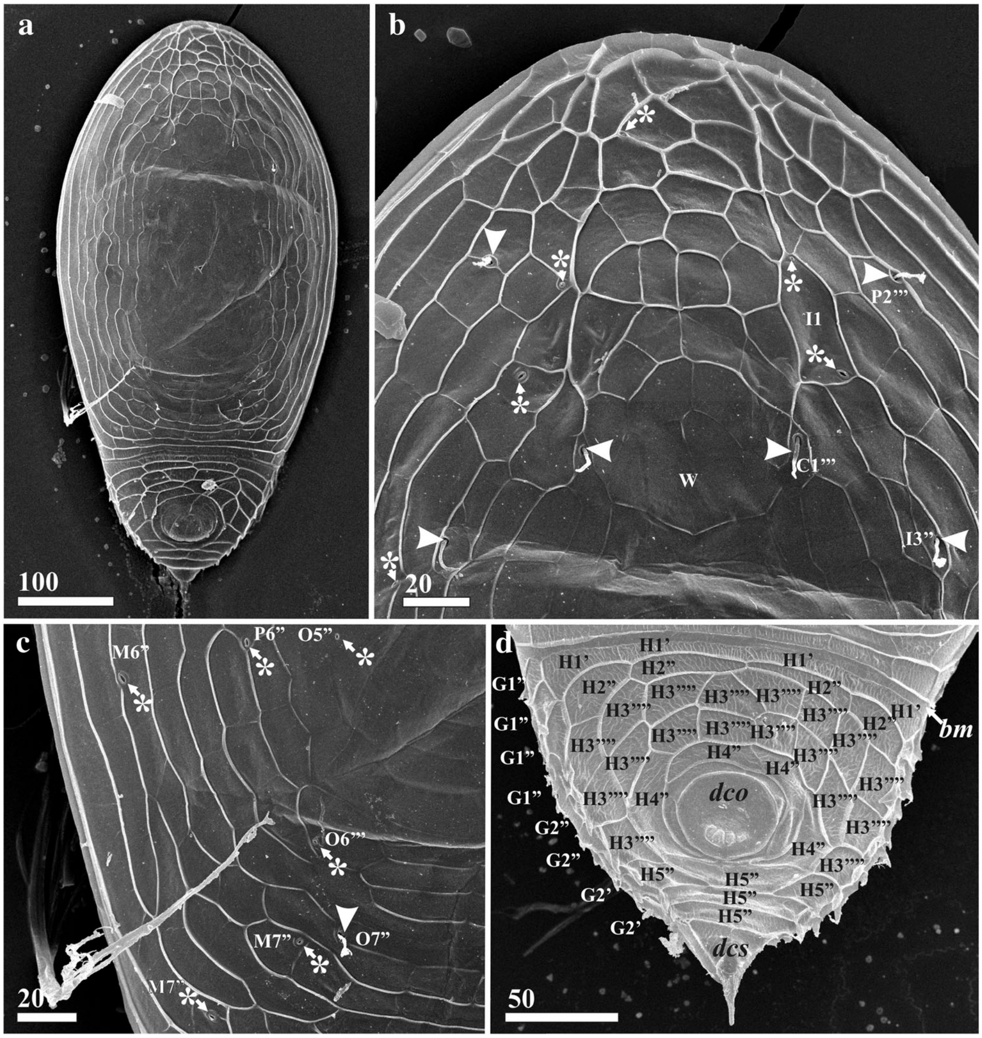

Fig. 10 *H. itoi*, nauplius 6, dorsal side (SEM) **a** General view. **b** Anterior part of head shield (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). **c** Posterior part of head shield, left side (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). **d** Posterior part of faciotrunk. Abbreviations: *bm* - boundary of moult of faciotrunk; *dco* - papilla of dorsocaudal organ; *dcs* - dorsocaudal spine. Scale bars in μm

5 central H3’’’ plates of nauplius 5 seem to be split into a total of 8–9 smaller H3’’ “plates. Lateral sides have 8 pairs of gabled plates (G) with sharp posterior parts.

The structure of the ventral side and of the appendages is very similar to that of instar 5.

Nauplius 7 (Figs. 11, 12, Supplementary video 2).

This last naupliar instar is larger than all preceding ones, and it can easily be distinguished from them by the distinct compound eyes of the y-cyprid forming inside the nauplius. The active beating of the cyprid thoracopods inside nauplius 7 eventually releases the y-cyprid via a rupture between the head shield and the ventral faciotrunk in the anterior part (Figs. 11b and 12a). Although this naupliar instar was also described in details earlier as ‘nauplius 5’ (see Kolbasov and Høeg 2003), we prefer to provide a supplementary description of nauplius 7 because several new details of its morphology of corresponding stages of other species of y-larvae were added recently (see Grygier et al. 2019). Nauplius 7 first occurs in the White Sea plankton from the end of June (first finding June 23) and was found until the second half of July (last finding July 9), with maximum occurrence in the beginning of July (see Table 1).

Body drop-shaped, with rounded anterior end and elongated, narrower posteriorly hindbody (Fig. 11a), 670–700 μm long and 290–310 μm wide, with dorsal head shield 490–495 μm long and sharp triangular hindbody 160–210 μm long. Head shield consists of 290–315 dorsal polygonal cuticular plates of different sizes (Fig. 11a-c herein and Fig. 5A, B in Kolbasov and Høeg 2003). Borders between occipital plates in central area ephemeral, hardly discernible, marginal plates have no transverse borders/ridges and look as 4 longitudinal ribbons (Fig. 11a-c and Fig. 5A, B in Kolbasov and Høeg 2003). Frontal and some occipital plates form rather asymmetrical patterns of distribution. Anterior part with numerous small, irregular plates, 32–40 of them may represent frontal plates (F) (Fig. 11a, b herein and Fig. 5A, B in Kolbasov and Høeg 2003). As in nauplius 6, two occipital (O1’, O2’), one intercalary (I1) and one elongated (E2) plates stay undivided as in nauplius 2. Head shield carries 15–17

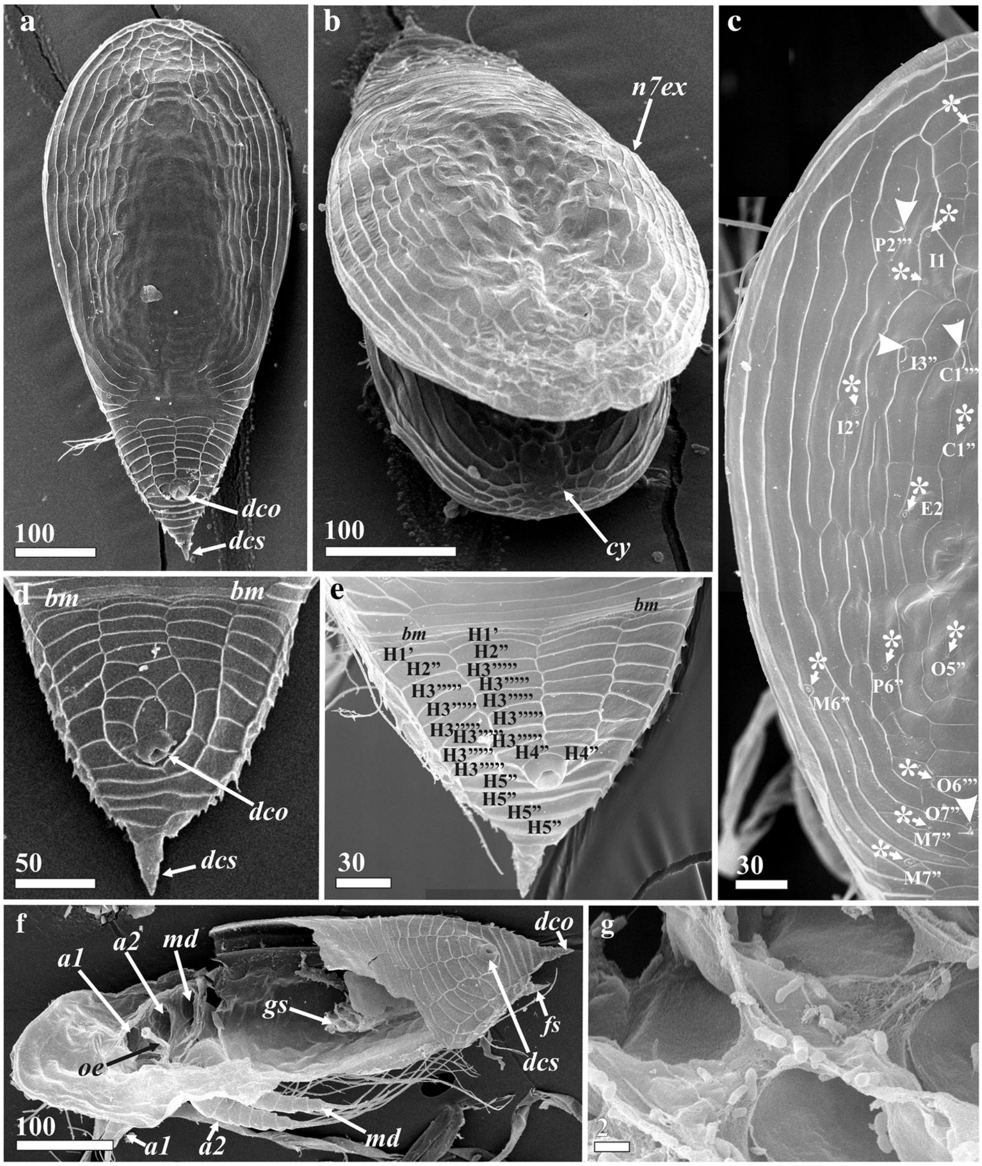

Fig. 11 *H. itoi*, nauplius 7, dorsal side (SEM) **a** General view. **b** Moulting into y-cypris stage. **c** Head shield, left side (setae in pores indicated by arrowheads, pores without setae indicated by asterisks). **d**, **e** Posterior part of faciotrunk. **f** Dorsolateral view showing inner side of exuvium with 'ghost sling' of y-cypris (head shield removed). **g** Surface of 'ghost sling' showing delicate reticulated curicular membrane. Abbreviations: *a1* - antennules; *a2* - antennae; *cy* - y-cypris larva; *dco* - papilla of dorsocaudal organ; *dcs* - dorsocaudal spine; *fs* - furcal spines; *gs* - 'ghost sling' of y-cypris; *md* - mandible; *n7ex* - exuvium of nauplius 7; *oe* - cuticle of oesophagus. Scale bars in μm

pairs of simple pores of different size without seta, including those on brim plates (Fig. 11 c, indicated by asterisk; some pores are absent on image) and 4 pairs (as in nauplii 4–6) of pores with seta inside (Fig. 11 c, indicated by arrowheads).

The elongated hindbody (Fig. 11a, d, e) differs strongly from that in all previous instars. Heralding the condition in the ensuing y-cypris (see Kolbasov and Høeg 2003), in anterior half it carries four longitudinal rows of narrow, brick-shaped, rectangular plates. Normally rear part with several (4) single narrow plates. In a few individuals central and posterior plates may be irregular (Fig. 11d herein, and Fig. 5A, in Kolbasov and Høeg 2003). Dorsal surface has 36–40 hindbody plates (H), lateral sides with 10 pairs of gabled plates (G) with sharp posterior ends (Figs. 11d, e and 12c). External part of knob of dorsocaudal organ papilliform, smaller in comparison with nauplii 2–6 (Fig. 11d, e). Dorsocaudal spine long, with small sharp denticles (Figs. 11d, e and 12d).

Posterior part of faciotrunk contains a unique internal supporting structure for y-cypris trunk and thoracopods (Fig. 11f, g, '*gs*') recently described as 'a ghostly support sling for cypris y' (Grygier et al. 2019). This delicate, fibrous structure of unknown nature consists of external and internal layers and representing paired deep pockets enveloping the faciotrunk with developing thoracopods of y-cypris (Fig. 11g), while anterior part of the y-cyprid seems to have no external enveloping structure excepting the exuvium of nauplius 7 (Fig. 11b, f).

Ventral side similar to that in nauplii 4–5 (Fig. 12), but putative rudiments of frontal filaments (*ff?*) more elongated (Fig. 12f), apical papilla of labrum with dense, fine denticles,

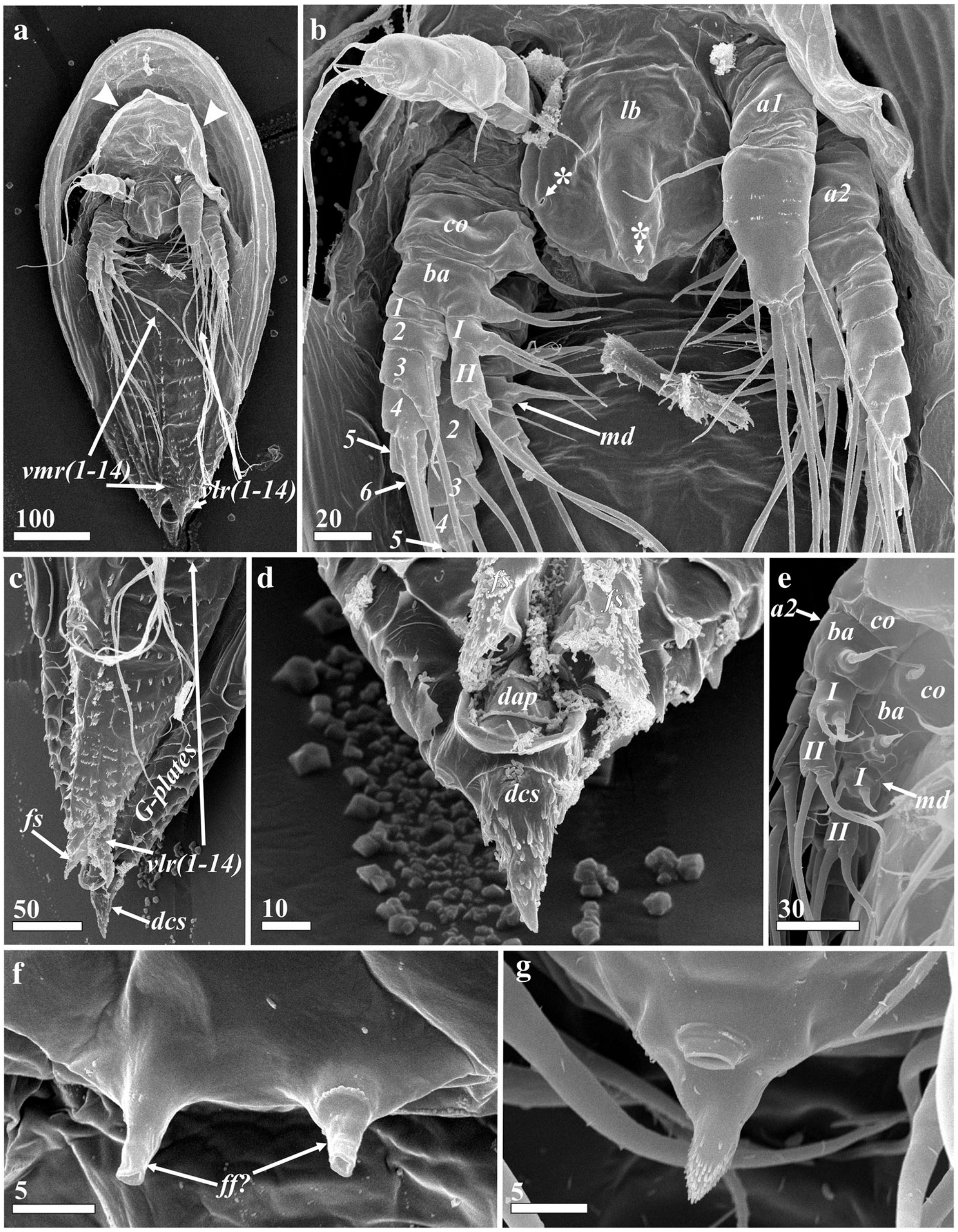

Fig. 12 *H. itoi*, nauplius 7, ventral side (SEM) **a** General view, exuvium (rupture of naupliar cuticle for y-cypris release indicated by arrowheads). **b** Central part with legs and labrum (endopod segments numbered in Roman, exopod segments numbered in Arabic, pores of labrum indicated by asterisks). **c** Posterior part. **d** Posteriormost end. **e** Armament of antenna and mandible. **f** Putative rudiments of frontal filaments. **g** Tip of labrum. Abbreviations: *a1* - antennules; *a2* - antennae; *ba* - basis; *co* - coxa; *dap* - dorsal anal plate; *dcs* - dorsocaudal spine; *ff?* - putative rudiments of frontal filaments; *fs* - furcal spines; *lb* - labrum; *md* - mandible; *vlr* – ventrolateral cuticular ridges; *vmr* – ventromedial cuticular ridges. Scale bars in µm

but without tuft of cuticular villi (Fig. 12g). Contrary to previous instars, antennules of nauplius 7 seem to consist of 4 (or even 5) distinct segments (Fig. 12b). In comparison with nauplii 5 and 6, corolla of thin, proximal setae presents only at basis of massive spine of mandible, but absents in antenna (Fig. 12b, e). Posterior half of faciotrunk with 14–15 ventromedial and ventrolateral ridges (Fig. 12c).

Comparison of naupliar instars

In Table 1 we summarize the main differences between naupliar instars of *H. itoi.* It lists the principal morphological features that distinguish the seven nauplii and provides the period of time where these are present in the plankton.

Discussion

For the first time it is shown that the naupliar phase of a planktotrophic Facetotecta can comprise a total of seven naupliar instars, starting with a non-feeding nauplius 1 (the presumed hatchling). This raises questions concerning 1) the number of nauplar instars in the ground pattern of the Thecostraca 2) the significance and phylogenetic implications of this number when comparing to development in other

Table 1 Main differences between naupliar instars of *H. itoi*

Instar	Size, length and width (μm)	Body shape and characters	Head shield	A1	A2	Mdb	Hindbody	Period of occurrence
N1	245–260, 145–165	drop-shaped; rounded anterior end; no vestiges of mx1	no distinct head shield, no cuticular ridges,	with 5 setae	gnathobases without corolla of proximal setae	gnathobases without corolla of proximal setae	ventral part inflated, no cuticular ridges, no dorsocaudal knob	April 23 – May 3
N2	370–415, 210–220	sharpen, trapezoid anterior end	with 57 cuticular plates	with 5 setae	gnathobase of basis with corolla of proximal setae	gnathobase of basis with corolla of proximal setae	dorsal side with 10 cuticular plates	April–May 15
N3	450–480, 220–240	ellipsoid, with rounded anterior end	with ~105 cuticular plates	with 6 setae	gnathobase of basis without corolla of proximal setae	gnathobase of basis with corolla of proximal setae	dorsal side with 22 cuticular plates	May 01–May 30
N4	490–500, 230–250	ellipsoid, with rounded anterior end	with ~180 cuticular plates	with 7 setae	gnathobase of basis with corolla of proximal setae	gnathobase of basis with corolla of proximal setae	dorsal side with 30–35 cuticular plates	May 08 – June 10
N5	540–590, 270–290	ellipsoid, with rounded anterior end	with ~280 cuticular plates	with 8 setae	gnathobase of basis with corolla of proximal setae	gnathobase of basis with corolla of proximal setae	dorsal side with 42–44 cuticular plates	May 26–June 20
N6	600–645, 300–320	ellipsoid, with rounded anterior end	with ~290 cuticular plates; asymmetrical, irregular frontal plates	with 8 setae	gnathobase of basis with corolla of proximal setae	gnathobase of basis with corolla of proximal setae	dorsal side with 50–52 cuticular plates	June 02–June 30
N7	670–700, 290–310	drop-shaped, with rounded anterior end; compound eyes of y-cypris	with 290–315 cuticular plates	with 8 setae	gnathobase of basis without corolla of proximal setae	gnathobase of basis with corolla of proximal setae	elongated, narrower posteriorly; dorsal side with 46–50 cuticular plates	June 23–July 09

crustaceans, and 3) the role of these many instars in a larval ecological context.

Nauplius 1 in Facetotecta and other Thecostraca (Figs. 1, 2, 13, Supplementary video 1)

The larval development of *Hansenocaris itoi* was previously argued to comprise only five naupliar instars (Kolbasov and Høeg 2003), but the failure to detect the total number was, at least in part, due to lack of sampling in spring (April–May). The presence of a non-feeding nauplius 1 is intriguing since all later nauplii (2–7) are feeding (planktotrophic, Supplementary videos 1, 2). In the following we compare the non-feeding instar 1 with the presumed equivalent first instar nauplii of other thecostracans (Fig. 13) and briefly also with other Crustacea. Based on this, we then discuss whether our nauplius 1 is the real/true first instar for *H. itoi.*

In *H. itoi* we identified several clear differences in the external morphology between nauplius 1 and later nauplii. The later six instars are clearly planktotrophic in having limbs with long setae serving for both feeding and locomotion, and well-developed gnathobases of both antennae 2 and the mandibles with functional setae and setules associated with feeding. In addition, their intestine contains food particles, and the functional labrum has secretory pores and an apical papilla. None of these feeding-related structures are present in nauplius 1.

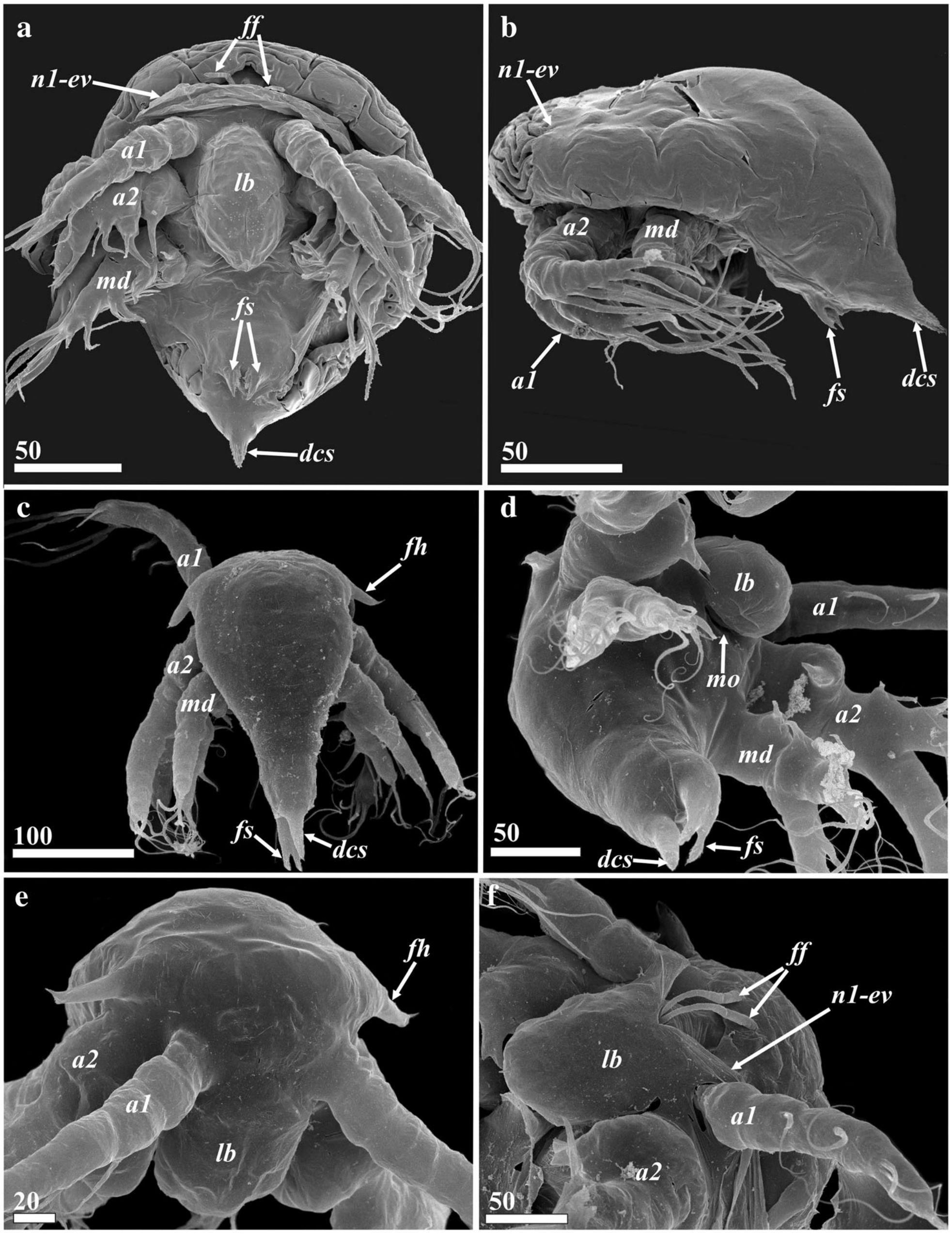

Fig. 13 Nauplii 1 of Ascothoracida (*Sessilogoga captiva* Kolbasov et al. 2020 from Taiwan) - **a**, **b** and Cirripedia (*Balanus balanus* (Linnaeus 1758) from the White Sea) - **c**-**f**; note absence of frontal filaments and vestiges of maxillules; smooth cuticle without developed sculpture and long setae; smooth labrum without pores and setae; limbs without distinct segmentation; half-formed feeding armament of limbs bearing shorter swimming setae and gnathobases; and short furcal and dorsocaudal spines **a** Ventral side of moulting nauplius 1, shedding cuticle (*n1-ev*) peeled away to reveal frontal filaments (*ff*) of second instar. **b** Lateral side, anterior cuticle of dorsal shield (*n1-ev*) peeled away to reveal wrinkled cuticle of second instar. **c** Dorsal side, note frontolateral horns pressed to the side. **d** Ventrolateral view from posterior end, note undeveloped gnathobases and small, slit-shaped mouth (*mo*). **e** Anterior side, note absence of frontal filaments. **f** Anterioventral view, shedding cuticle (*n1-ev*) peeled away to reveal frontal filaments (*ff*) of second instar. Abbreviations: *a1* - antennule; *a2* - antenna; *dcs* - dorsocaudal spine; *ff* - frontal filaments; *fh* - frontolateral horns; *fs* - furcal spines; *lb* - labrum; *md* - mandible; *mo* - mouth; *n1-ev* - shedding cuticle (exuvium) of nauplius 1. Scale bars in µm

All instars, except the first, also have the dorsal side superficially organized into a complex pattern of polygonal cuticular plates diagnostic for Facetotecta. This 'infantile' appearance of nauplius 1 dovetails with its transitory and non-feeding role in development. Nauplii 1 and 2 were found in the plankton simultaneously and in culture most of the nauplius 1 specimens moulted into nauplius 2 within one day after collecting. This again suggests a very short duration of the first instar. A similar short instar 1 duration is recorded from rhizocephalan nauplii (Rybakov et al. 2002).

A non-feeding instar 1 followed by feeding nauplii is known elsewhere, both in Thecostraca (Fig. 13) and in other Crustacea, and fits well into a broader pattern of crustacean naupliar development. In Branchiopoda (e.g., Anostraca and Spinicaudata), the first 1–3 nauplii are commonly swollen with yolk and non-feeding with weakly developed mouthparts (Olesen 2014; Olesen and Grygier 2014). In Copepoda lecithotrophy evolved convergently several times, but nauplii of most taxa are planktotrophic (Huys 2014), while malacostracan nauplii (in euphausiaceans and dendrobranchiate shrimps) are universally lecithotrophic (Scholtz 2000; Martin et al. 2014a; Akther et al. 2015).

A comparison of early nauplii within Thecostraca is complicated by most studies relying on sampling and only few being based on laboratory culture (Grygier 1987, 1992, 1993, 1995; Boxshall and Böttger-Schnack 1988; Itô 1986, 1987). The only way to document the true nauplius 1 is to directly observe hatching or spawning from an adult. This has never been observed in Facetotecta, but a number of times in the parasitic Ascothoracida (Grygier 1987, 1990a, b; Itô and Grygier 1990, Kolbasov et al. 2020) and in the Cirripedia (Anderson 1965, 1994; Kaufmann 1965; Lewis 1975; Rybakov et al. 2003; Nogata and Matsumura 2006; Semmler et al. 2008). In all studied Ascothoracida, nauplius 1 is a non-feeding orthonauplius followed by feeding metanauplii, except in the fully lecithotrophic *Baccalaureus falsiramus* Itô and Grygier, 1990). Nauplii 1 in *H. itoi* and the ascothoracidans *B. falsiramus* and *S. captiva* are similar in being of short duration and in having only rudimentary feeding structures, a labrum without pores, no distinct head shield, and a smooth external cuticle without pores and setae (in contrast to the sculptured cuticle of the following instar). There is also an interesting similarity in the nauplius 1–2 moulting between Facetotecta and Ascothoracida (Itô and Grygier 1990; Kolbasov et al. 2020). It proceeds without any definite fissure lines and the exuvium of nauplius 1 is crumpled and often torn into pieces. This differs from moulting between all subsequent instars, where special fissure lines or 'moult boundaries' are seen between the head shield and the faciotrunk (Figs. 3b, c and 4d). In this later phase of the development, the dorsal head shield normally splits from the faciotrunk during the moulting process (Fig. 7b). A moult line indicating such a split is also clearly seen encircling the body of nauplii in the kentrogonid rhizocephalans (Rybakov et al. 2002).

In Cirripedia, nauplius 1 is a short-lived, non-feeding orthonauplius (Fig. 13c-f) with many similarities to both the Ascothoracida and Facetotecta in having a simple morphological outline of limbs, labrum and body, and spine armament compared with later stages (Costlow and Bookhout 1958; Lewis 1975; Lang 1979; Yan 2003; Poltarukha and Korn 2008; Chan et al. 2014). One of the most extreme cases of such highly reduced nauplius 1 is seen in species of *Octolasmis* (Yap et al. 2015). Taken together, it thus seems that the ancestral state for Thecostraca is to have a structurally simple and non-feeding orthonauplius in the first larval instar followed by feeding metanauplii.

Thecostraca and the concept of Hexanauplia

Within Crustacea, the number of naupliar instars has been used both as a primary phylognetic argument or to characterize taxa indentified by other means such as molecular data. Many copepods and cirripedes have a development comprising six naupliar instars (e.g. Huys 2014; Chan et al. 2014) and this is also considered basal for the Ascothoracida (Itô and Grygier 1990). Transcriptomic data has provided some support for a Copepoda-Thecostraca clade, named 'Hexanauplia' under the assumption that six naupliar instars is the ground pattern for the entire Thecostraca (Oakley et al. 2013; Lozano-Fernandez et al. 2019). This ground pattern number of instars is now complicated both by our observation of more than six naupliar instars in Facetotecta and there is furthermore not non-universal phylogenetic support for 'Hexanauplia', since some analyses return Thecostraca and Malacostraca as sister taxa (see Schwentner et al. 2017; Lozano-Fernandez et al. 2019).

Before this study five naupliar instars were considered as an apomorphy for Facetotecta compared to the ground pattern of six instars in Cirripedia (Itô 1990; Kolbasov and Høeg 2003). The seven instars presently found in *H. itoi* could either be a plesiomorphy or an apomorphic condition, perhaps associated with an exceptionally long larval life for this species, here estimated to last about three months. If seven naupliar instars represent the ground pattern for Thecostraca, the term 'Hexanauplia' would be etymologically imprecise even if the clade persists based on molecular markers. Within Thecostraca the trend seems always to be a reduction in number, and normally, this occurs by completely loss of instars although they can be present and internally brooded in the females (Anderson 1994; Chan et al. 2014; Dreyer et al. 2020; Grygier 1984; Walossek et al. 1996; Rybakov et al. 2002). The climax of such reduction is seen in species that omit nauplii altogether and hatch as cypridiform larvae, such as in many rhizocephalans and in scalpellid thoracicans (Høeg et al. 2014; Chan et al. 2014).

For now, we consider it most likely that the seven naupliar instars in *Hansenocaris itoi* represent the ground pattern for Facetotecta. Since Facetotecta currently is recovered as the sistergroup to the remaining Thecostraca (Pérez-Losada et al. 2009), this also impacts on the number of naupliar instars hypothesised for the ground pattern of all Thecostraca.

Lozano-Fernandez et al. (2019) viewed the presence of a 'naupliar phase' in certain crustaceans as a result of limb suppression during the early larval development followed by a jump in morphological appearance into a juvenile stage/phase (e.g., cyprid or copepodite). But Lozano-Fernandez et al. (2019) pointed out that this is essentially similar to what is seen in some malacostracans that sport a naupliar phase in their early development. Dendrobranchiate shrimps usually pass through a naupliar phase with five to six stages with postmandibular limbs present only as limb buds followed by an abrupt shift into a postnaupliar phase with more active appendages (protozoea) (e.g. Chio and Hong 2001; Martin et al. 2014a), similarly to the conditions in copepods and thecostracans. Therefore, the presence of a 'naupliar' phase (as a result of suppressed limbs development) may well be a Multicrustacea synapomorphy (Copepoda + (Thecostraca + Malacostraca)), a taxon established by Regier et al. (2010), rather than a Hexanauplia synapomorphy sensu Oakley et al. (2013).

The 'ghostly' support sling in *Hansenocaris itoi*

A 'ghostly support sling for cypris y' was recently found in the hindbody of the last naupliar instar of lecithotrophic facetotectan nauplii by Grygier et al. (2019). The presence of a similar "ghost sling" in the planktotrophic nauplius 7 of *H. itoi* suggests that this structure is universally present in Facetotecta, as was proposed by Grygier et al. (2019). The composition of the "ghost sling" is still unknown but it may in fact be a thin-walled cocoon enveloping the entire future cypridiform larva. If cuticular in nature, the facetotectan 'ghost sling' may represent the remnants of an instar previously intercalated between nauplius 7 and the free swimming cypridiform larva, such as what is actually present in dendrogastrid ascothoracidans (Kolbasov et al. 2008). Certainly, the drastic metamorphic change from nauplius to cypridiform larva seen in all Thecostraca must have evolved by condensation into a single moult of a more gradual (anamorphic) ontogenetic pattern with instars that are now lost (Walossek 1993). A 'ghost' has not been reported in any other studies on nauplius-cypris metamorphosis (Kaufmann 1965; Walley 1969; Molares et al. 1994; Collis and Walker 1994; Korn et al. 2000), as it is hard to detect and may simply have been overlooked.

Larval developmental times in Thecostraca

In the White Sea, the larval development of acorn barnacle species, including cypris settlement and metamorphosis into a sessile juvenile, lasts about 30–40 days long and is completed by the end of May (Kolbasov own data). Opposed to this, we recorded y-larvae in the White Sea plankton over a total span of almost 3 months, and although direct evidence is lacking we predict that *H. itoi* has a relatively long larval life. There seems to be no clear pattern in Thecostraca concerning the relative duration of planktotrophic compared to lecithotrophic larval development. The developmental time also depends on the ambient temperature, whence it is necessarily longer in the deep sea and other cold habitats such the White Sea (Walossek et al. 1996; Yorisue et al. 2012). Thecostracan nauplii generally serve the purpose of either feeding or dispersal or both (Høeg and Møller 2006; Martin et al. 2014b). In Thecostraca, most examples of a very long naupliar phase in the plankton seem to concern deep sea species inhabiting rare and patchily distributed substrata, and this is probably an adaptation to ensure long distance dispersal over the large area of inhospitable sea bottom (Buhl-Mortensen and Høeg 2006; Yorisue et al. 2012). In the ascothoracidan *Baccalaureus falsiramus* the larval development with six naupliar instars and a final cypridoid stage takes about one month (Itô and Grygier 1990). A short developmental time of one week or less is found in many species of rhizocephalan barnacles. All these examples also show that there is no clear correlation between larval developmental time and whether the adult is a parasite or not.

Summary and outlook

We have shown that facetotectan larval development can be of exceedingly long duration and comprise seven naupliar instars, one in excess of what has been found elsewhere in Thecostraca. Even in planktotrophic forms, y-nauplii are characterized by the reduction of segmentation and setation of natatory limbs in comparison with other thecostracan nauplii in Ascothoracida and Cirripedia (see Grygier 1987), which potentially is a synapomorphy of Facetotecta. The first naupliar instar of Facetotecta differs from those in Ascothoracida and Cirripedia by the presence of frontal filaments, if we consider two papilliform projections inserted anteriorly to the labrum as putative rudiments of these structures. Such filaments are absent in both ascothoracidan and cirripede nauplii 1, although present in subsequent larval instars, including the cypridoid stage (Walker 1974; Høeg et al. 2009). The presence of seven instars raises questions about the naupliar development in general within Crustacea, but a clear evolutionary pattern cannot yet be established. Given the worldwide distribution of Facetotecta, future studies should in detail investigate larval development in single species while also using morphological and molecular means to document their apparent, but poorly known species diversity. This again, may facilitate the ultimate goal of identifying their suspected hosts and thus "closing the life cycle".

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Compliance with ethical standards

Ethics statement All applicable international, national, and/or institutional guidelines for animal testing, animal care, and use of animals were followed by the authors.

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