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Gross morphology and cnidae of *edwardsiella* anemones and larvae (Anthozoa, Edwardsiidae) from the Swedish West Coast

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Abstract

The American ctenophore *Mnemiopsis leidyi*, recently found in Skagerrak off the Swedish west coast, carried parasitic anemone larvae identified as *Edwardsiella* sp. In the Western Atlantic Ocean, *M. leidyi* are commonly infected by larvae of American *Edwardsiella lineata* anemones. It was questioned whether the parasitic larvae came across the Atlantic with the invasive ctenophore, or whether they belonged to the native *E. carnea* anemones. However, recent molecular investigations identified *E. carnea* and *E. lineata* as different species and the Swedish larvae from *M. leidyi* as *E. carnea*. Present morphological investigation supports the distinction of *E. carnea* anemones from *E. lineata* by a changeable rounded to flattened physa without rugae and by white pigmentation on basal outer tentacles. One Swedish larva developed into a juvenile with this characteristic column differentiation, identifying our larva as *E. carnea* and confirming its ability to produce parasitic larvae. Cnidome descriptions of *E. carnea* anemones and larvae reveal three similar cnidae in *E. carnea* and *E. lineata* anemones and larvae, and cnidae specific to either anemone or larval stages. Cnidome characteristics support our parasitic larvae as an *Edwardsiella* member, but further comparable studies of *E. lineata* cnidomes are warranted for evaluating their taxonomic significance.

KEYWORDS

cnidocysts, *Milne-Edwardsia carnea*, *Mnemiopsis leidyi*, nematocysts, parasite

1 | INTRODUCTION

The American ctenophore *Mnemiopsis leidyi* Agassiz, 1856 was recorded in Skagerrak off the Swedish west coast in 2006 (Hansson, 2006). During 2007–2009, *M. leidyi* invaded the Swedish waters in huge numbers and endoparasitic anemone larvae were attached to its stomach or pharynx (Selander et al. 2009). Up to 40% of the ctenophores were reported infected by *Edwardsiella* sp. larvae. In one *Mnemiopsis*, eleven parasites were found (Selander et al. 2009). After years with less abundance, *M. leidyi* infected by parasitic larvae re-appeared in 2014. Since then, infected individuals of *M. leidyi* have appeared regularly in the Gullmar Fjord on the Swedish west coast (personal observations).

Parasitic larvae of *Edwardsiella lineata* (Verrill, 1873) are common in *M. leidyi*, along the North American east coast (Bumann & Puls, 1996; Reitzel et al. 2007). The larvae feed directly from stomodeum or pharynx of the host, thus affecting its growth (Daly, 2002b; Reitzel et al. 2009). *Edwardsiella lineata* anemones, closely resembling *E. carnea* (Gosse, 1856) native to Skagerrak (Carlgren, 1921, 1940), have not been reported from Swedish waters.

Observations of parasitized *M. leidyi* in Sweden raise several questions. Are *E. carnea* larvae able to parasitize *Mnemiopsis*? Have American *E. lineata* larvae drifted with the Gulf Stream with *M. leidyi* and established a population in Sweden (Selander et al. 2009)? Is it possible that *E. lineata* larvae are re-introduced with *M. leidyi* every year? However, a single report of *Edwardsiella sp*. larva, living in the ctenophore *Bolina* (syn. *Bolinopsis*) (Stephenson, 1935), is suggested to represent *E. carnea* (Selander et al. 2009).

Edwardsiella carnea and *E. lineata* are inconspicuous, small sea anemones, usually hard to sample (Daly, 2002a). Besides the studies of Carlgren (1921, 1940) and Carlgren and Stephenson (1928), there are no morphological or cnida descriptions of *E. carnea*, and the two species are regarded almost morphologically indistinguishable. Carlgren (1921, pp. 62, 64) referred the cnidae in *E. carnea* as capitulum nematocysts, scapus nematocyst, tentacle nematocysts and actinopharynx nematocysts, after following the column differentiation and structures of the anemone. Carlgren (1940, p. 25, fig. V6) referred these cnidae as microbasic b-mastigophors, microbasic p-mastigophors, basitrichs and spirocysts (Table S1).

Selander et al. (2009) hence tried to solve the identity of the parasitic larvae combining general morphological studies with phylogenetic analysis of 18S rRNA sequences. However, these data, compared with sequences from various *Edwardsia* and *Edwardsiella* species published in GenBank, gave no conclusive result. Dnyansagar et al. (2018) showed that transcriptomes of *E. carnea* and *Edwardsiella* parasites from Sweden were more than 99% identical, whereas transcriptomes of *E. carnea*, compared with published transcriptome data set of *E. lineata* anemone (Stefanik et al. 2014), were ~97% identical. Consequently, their results indicated that *E. carnea* and *E. lineata* anemones are genetically very similar, but distinct species and that the Swedish parasite belongs to *E. carnea* anemones and that *E. carnea* can produce parasitic larvae, infecting *M. leidyi*.

With the intension to (a) confirm that the Swedish anemone is *E. carnea* and not *E. lineata*, and morphologically characterize *E. carnea*, (b) manifest that *E. carnea* and *E. lineata* are two different species and (c) identify the Swedish parasitic larvae of *Edwardsiella*, the gross morphology and cnidomes of *E. carnea* are here described in detail. Carlgren (1940) described the cnidae of *E. carnea* anemone only by hand drawings; thus, this will be the first micrographic documentation of the anemone cnidome and the first description at all of the cnidome of the Swedish parasitic larva. Such a detailed description of *E. carnea* anemones and larvae can be helpful for monitoring a possible introduction of *E. lineata* in Swedish waters and confirm that *E. carnea* is capable of producing parasitic larvae.

2 | MATERIALS AND METHODS

Edwardsiella carnea anemones, collected by dredging in Skagerrak, off the Swedish west coast (58°21´N, 11°06´E; 58°21´N, 11°07´E), August 2012, depth 40 m, were found at the base of the soft coral *Alcyonium digitatum* (Figure 1a,b). Anemones kept in seawater were fed with cultured *Artemia* nauplii at Sven Lovén Centre for Marine Infrastructure, Kristineberg, Fiskebäckskil. Some of the collected anemones (Dnyansagar et al. 2018) were used in their transcriptome analyses of *E.* carnea, and some were used in present study.

The ctenophore *Mnemiopsis leidyi*, infected by endoparasitic larvae, were collected with plankton nets in the Gullmar Fjord on the Swedish west coast (58°21´N, 11°24´E), October to November 2014. Larvae from *M. leidyi* (Figure 2a) were kept free-swimming in saline water in a refrigerator (4°C), without food one to two weeks, until examined. Some of the collected parasitic larvae (Dnyansagar et al. 2018) were used in their transcriptome analyses of *Edwardsiella* larvae. Based on the results of Dnyansagar et al., showing the Swedish parasites in *M. leidyi* to be *E. carnea* larvae, our investigated parasitic *Edwardsiella* larvae are here referred as *Edwardsiella carnea*.

2.1 | Anemone morphology and cnidae

Morphological investigation was performed on only two extending *E. carnea* anemones, due to scarcity of anemones (Figure 1a,b, Table 1). Table S2 gives anemone and tube dimensions. Cnida measurements were made from squashed preparations of the two extending anemones, of one contracted anemone, of anemone tissues inside tubes and of tubes (Table S3). We failed to do a thorough cnida investigation from tissues inside column. The small contracted column was too tough to be successfully dissected longitudinally. Instead, column was cut into an apical part, including capitulum with actinopharynx, and one proximal part, including scapus with mesenterial filaments and physa. When possible, 20 undischarged capsules, from different anemone tissues and tubes, were measured for each cnidocyst type.

2.2 | Larval morphology and cnidae

From *M. leidyi*, 23 living *Edwardsiella* larvae were investigated (Figure 2). Free-swimming larvae, larvae under a cover slip, and gently or firmly squashed larvae were examined. Larvae referred small were ~1 mm or smaller; large larvae were ~2 mm or longer. Eosin added to the last six studied larvae before squashing made shaft, spine and tubule pattern clearer, facilitating identification of less conspicuous cnidae. When possible, 20 undischarged capsules were measured

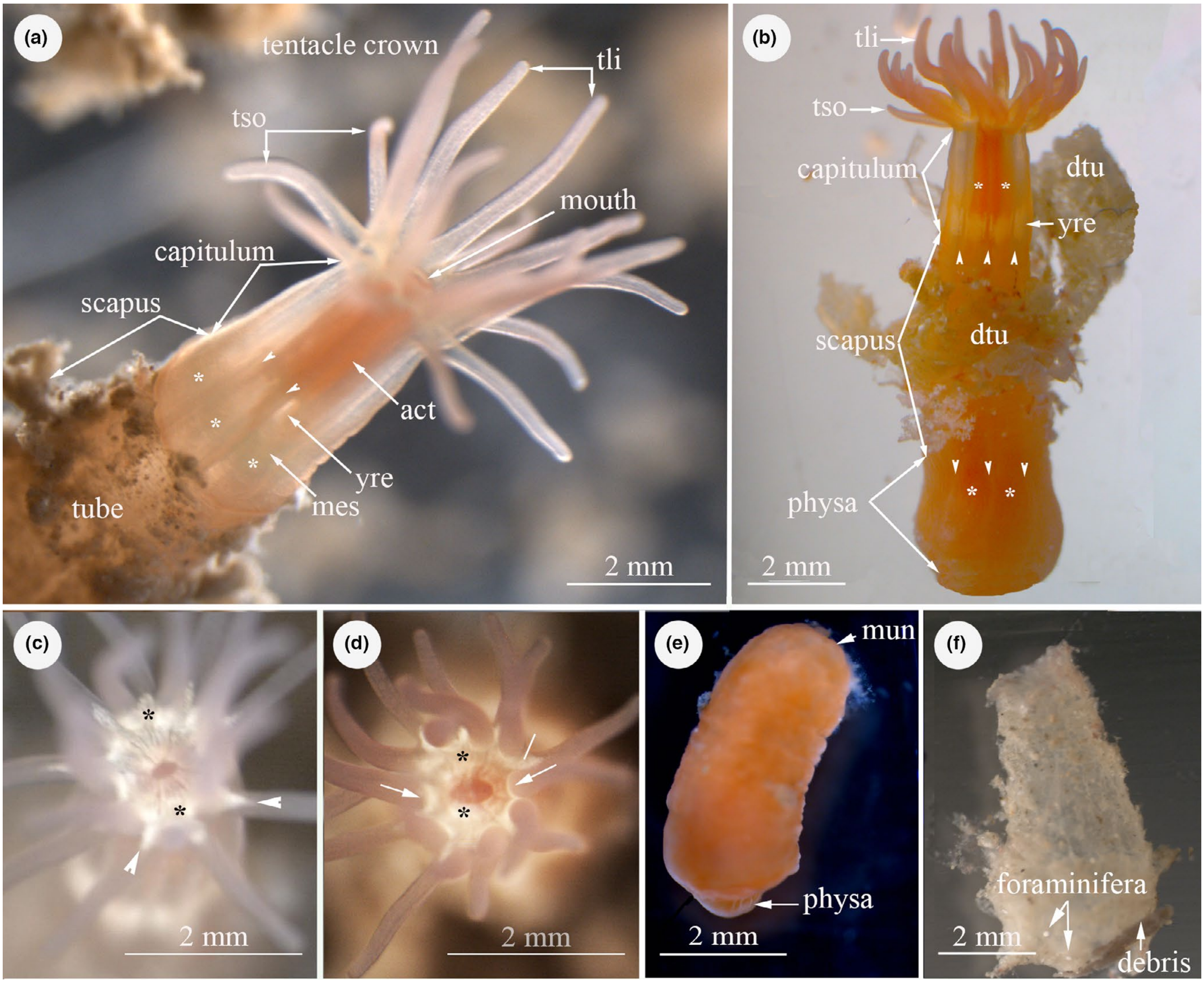

FIGURE 1 *Edwardsiella carnea.* (a) Light-coloured anemone extended from tube. (b) Reddish anemone extended from damaged tube. (a,b). Tentacle crown with outer and inner tentacles, mesenterial compartments (*) and mesenterial insertions/mesenteries (arrowheads) on capitulum, scapus and (in b) rounded physa. (c,d) Oral disc white with mouth, mesenterial compartments (*) and mesenterial insertions (red lines) and whitish coloured tentacle bases. (c) White coloured arrowheads at outer tentacle bases (arrowheads). (d) No white coloured arrowhead at inner tentacle base (arrows). Note white line (line) between inner tentacle bases, connecting white arrow on outer tentacle bases. (e) Contracted anemone without tubule, covered with mucus. Capitulum with yellow annulus completely contracted inside column, physa partly contracted. (f) Empty tubule attached with foraminifera and debris. Note broader width basally to fit broad physa. Abbreviation: *act*, actinopharynx; *arrows*; point at outer tentacle base with no arrowhead; *arrowheads,* point at inner tentacle base with white arrowhead or at mesenterial insertion; *dtu,* damaged tube; *line*, pointing at white line between inner tentacle bases, connecting white arrowhead on outer tentacle bases; *mes*, mesenterial filament, position; *tli*, tentacles, long, inner cycle; *tso,* tentacles, short, outer cycle; *yre*, yellow rectangles; *, indicates mesenterial compartment

from each cnida type, from at least five larvae; thereafter, some cnidae were measured from each larva (Table S4). As many as possible of the rare cnidae were measured.

2.3 | Microscopes and identifications

Examination was made with a Leitz DMRBE stereomicroscope and with a Leica MZI6A light microscope (LM) equipped with interference-contrast optics, 100x/1.30 Pl, fluotar objective both connected to the digital photograph equipment, Leica application suite, version 3.8 (LAS V3.8). Cnida measurements were made on photographs in Photoshop. Capsules measured in different foci showed slight width differences. Accuracy was generally ± 0.5 µm, and ± 0.2 µm for smallest cnidae.

The cnida nomenclature and classification system of Weill (1934) with modifications by Carlgren (1940, 1945), Mariscal (1974), Watson & Woods (1988) and Östman (2000) were used. Cnidae were placed in tiny-, small-, short-, medium-,

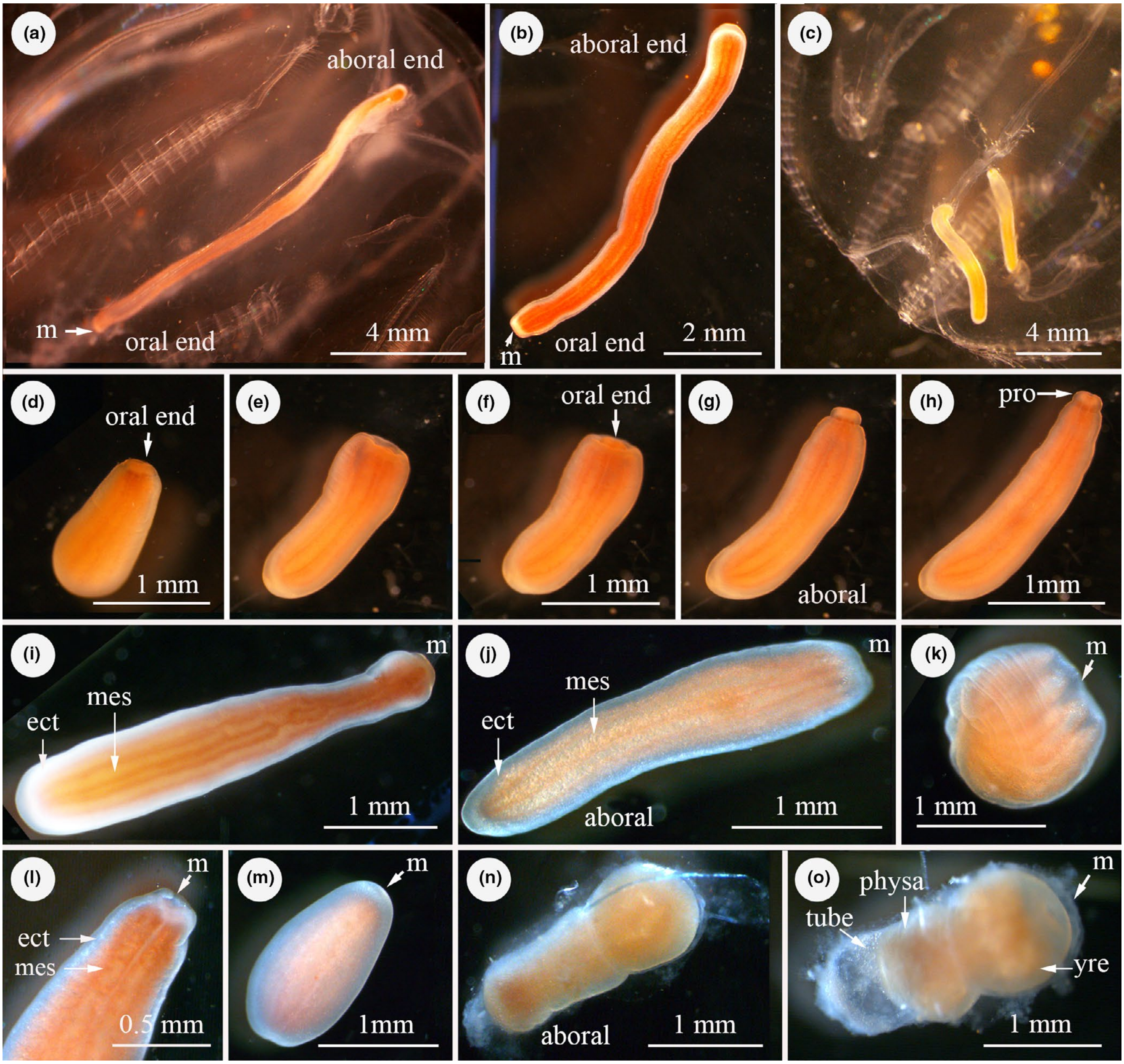

FIGURE 2 *Edwardsiella carnea* larvae. (a,b) Same long larva. (a). In *Mnemiopsis leidyi*. (b) Free-swimming slightly contracted. (c). Larvae in *M. leidyi*. (d-h). Contracted larva with apical end inside body extends, finally showing oral protrusion (pro); same magnification. (i-l). Note whitish ectoderm (ect) and mesenterial compartment (mes). (i) Extended larva with head-shaped oral end. (j,k) Same larva. (j) Extended. (k). Contracted. (l). Extended larva; note mouth (m). (m) Post-parasitic planula-shaped larva; note mouth (m). (n,o) Same larva metamorphosing to juvenile. (n) Free-swimming for 8 days; note three different column regions; bulb-shaped oral end conspicuous. (o) Free-swimming for 9 days; note mouth (m), yellow rectangles on bulb-shaped oral end, contracted aboral end (physa) and transparent tube with small structures, probably cnidoblasts and fibroblasts. Abbreviations: *ect,* ectoderm*; m,* mouth; *mes*, mesenterial compartment; *pro*, protrusion; *yre*, yellow rectangles

narrow-, long-, broad- and large-size groups (Tables S3–S6). Used cnida terminology are as follows: *basitrich*, well-developed spines only at tubule base; *b-mastigophore*, shaft gradually tapering into distal tubule, generally armed with prominent spines; *cnidae/cnidocysts*, include nematocysts and spirocysts; *distal tubule*, tubule distal to shaft; *holotrich/holotrichous/hoplotelic*, spines along tubule*; homotrich/homotrichous*, spines of same size and shape; *isorhiza*, same diameter throughout tubule; *microbasic*, shaft shorter than 1.5x capsule length; *p-mastigophore*, shaft tapers abruptly into distal tubule, prominent v-notch at base of inverted shaft; *shaft*, basally enlarged tubule, generally due its prominent spines.

TABLE 1 Morphological characteristics including cnidae for *Edwardsiella carnea* and *Edwardsiella lineata* anemones (Östman and Møller; Daly 2002b; Reitzel et al. 2009)

	E. carnea anemone Östman and Møller	*E. lineata* anemone Daly 2002b; Reitzel et al. 2009
Oral disc with tentacle crown	Oral disc whitish, connected with white lines to outer tentacles. Outer tentacle bases with white-pigmented arrowheads (Figure 1c). 17–18 tentacles (Figure 1a,c,d).	Oral disc whitish. White opaque flecks and opaque longitudinal marks on each tentacle. 18–36 tentacles (Daly 2002b, pp. 869, 871).
Column characteristics	Column length 1.5–2.5 cm (Goose 1856; Carlgren 1921). Broadest width <0.5 cm (Table S2). Differentiated in capitulum, scapus, physa (Figure 1a,b). Inner fibre net surrounding column (Figure S2h).	Column length 10–30 mm, diameter 2–5 mm (Daly 2002b, p. 871). Differentiated in capitulum, scapus, aboral end (Daly 2002b, p. 871; Reitzel et al. 2009). Inner fibre net not recorded.
Introvert-able Capitulum	Capitulum long, including actinopharynx and annulus, border to scapus (Figure 1a; Figure S1g,h).	Capitulum short, including actinopharynx, border to scapus. Annulus not present in capitulum, but in scapus (Daly 2002b; Reitzel 2009, Figure 1b).
Aboral column end region	Contractible rounded to flattened basal physa without rugae (Figure 1b; Figure S1i,j).	Long, gradually tapering basal end with rugae (Daly 2002b).
Cnidome	Broad microbasic b-mastigophores; narrow microbasic b-mastigophores; microbasic p-mastigophores; thin, long basitrich; spirocysts (Figure 3, Table S3).	Broad microbasic b-mastigophores; basitrichs (= narrow microbasic b-mastigophores); microbasic p-mastigophores; thin long basitrich; spirocysts (Daly 2002b, p. 874).

3 | RESULTS

3.1 | Morphology of *Edwardsiella carnea* anemones

One light-coloured, nearly transparent anemone, partly inside its tube, was studied when retracted and ejecting (Figure 1a; Figure S1). One reddish, less transparent anemone, due to its damaged tube, showed the whole scapus and physa (Figure 1b). Following Carlgren (1921, pp. 62, 63), extended anemones were differentiated into oral disc with mouth, tentacle crown attached to apical column including narrow capitular region, followed by capitulum, scapus and aboral physa region (Figure 1b,c).

Oral disc and tentacle crown (Figure 1c,d). Oral disc whitish. Eight intermesenterial compartments, macrocoels and mesenteries/septa, characteristic for Edwardsiid anemones, visible on oral disc (Figure 1c,d). Delicate, filiform tentacles in cycles; inner tentacles slightly longer than outer ones (Figure 1a; Figure S1h). Hollow tentacle coel visible at tentacle base of partly translucent anemone. A white-pigmented "arrowhead-shaped" region present at each outer tentacle base (Figure 1c), but not at inner tentacle base (Figure 1d). Between inner tentacle bases, a whitish line connects oral disc with the "arrowhead" region on each outer tentacle.

Column (Figure 1a,b), cylindrical, broadened gradually towards basal rounded physa. Proximal scapus and physa hide inside a mucous fibre tube/periderm. Disturbed anemone quickly and completely retracts into its tube (Figure S1a).

Capitulum (introvert) (Figure 1a), equivalent to scapulus (Carlgren & Stephenson, 1928; Carlgren, 1940), broadened slightly towards scapus. Mesenterial insertions and intermesenterial compartments referred as capitular ridges (Carlgren, 1921, p. 62), equivalent to scapulus ridges (Carlgren & Stephenson, 1928, p. 11), visible on extended column (Figure 1a). Actinopharynx reddish, visible through capitulum wall on partly transparent anemone, but invisible on reddish anemone. At basal capitulum, eight rectangular blotches, one on each intermesenterial compartment form the whitish to yellow annulus (Figure 1a). Like Carlgren (1921, p. 62), annulus forms external border between capitulum and scapus. Capitulum is invisible in fully retracted anemones. Intermesenterial compartments and opening into retracted capitulum are visible in the tube opening (Figure S1a). Early extending anemones (Figure S1b-f) are gradually showing the yellow annulus rectangles, as mesenterial capitulum compartments became larger, and the tentacle tips appear. On extended capitulum, annulus rectangles are first broader than long, and later slightly longer than broad (Figure S1f,g).

Scapus, basal to annulus, partly hidden inside tube, longest column part, nearly cylindrical, broadened gradually basally (Figure 1a,b). Mesenterial insertions and compartments visible, mesenterial filaments hardly visible.

Physa or *aboral region* (Figure 1b), broad, completely hidden inside tube; seen through tube wall when reddish (Figure S2a). Mesenterial compartments and mesenterial insertions visible (Figure 1b); border between physa and scapus not always clear on extended physa. Physa changeable during extension and contraction, from round to somewhat flattened Figure S1i,j).

*Tube, r*eaching halfway up extended column, built by tissues and coarse fibres transverse-oriented to tubal long axis, partly transparent (Figure 1f, Figure S2a,c). Tube basally broadened to fit the broad, rounded physa, vaguely visible through the partly transparent tube (Figure S2a). Tube presumably attached to substrates via mucus. Single and composed skeletal elements (sclerites) of *Alcyonium digitatum* scattered among tube fibres (Figure S2d-f,i,j); foraminifera, debris (Figure 1f) and sponge spicules may also attach. Separate tubes attached to each other (Figure S2g). Tube corresponds to scapus cuticula (Carlgren, 1921, p. 63) and to rough periderm (Daly, 2002b, p. 871). Daly gives no picture of the tube.

Contracted anemone, without tube, elongate oval with rounded ends (Figure 1e). The tough column wall gave resistance to cutting. A net of fine fibres, regularly arranged in right angles to each other, and to length/width of column axis, formed a tight pattern, basally mirroring mesenterial compartments (Figure S2h).

3.2 | Morphology of *Edwardsiella carnea* larvae

The reddish, parasitic larvae were attached with anterior mouth to pharynx or stomach of *M. leidyi* (Figure 2a-c). Like anemones, larvae were cylindrical, with visible mesenterial compartments, but with no differentiated body regions, except for retractable protrusion or vaguely head-shaped anterior end with mouth invagination (Figure 2d-j; Table 2). Larvae were swimming with posterior end forwards, with cilia constantly beating, spinning around the oral–aboral axis. Free-swimming larvae, shorter and thicker than when attached inside *M. leidyi* (Figure 2a,b), could contract to less than half their size when inside *Mnemiopsis*. Some larvae were repeatedly contracting and stretching (Figure 2d-l). Their apical body part, retracting into the anterior body and then ejected (Figure 2d-h), might mirror the introvertible capitulum of the anemone. After free swimming for 12 days, one larva became planula-shaped with mouth invagination (Figure 2m), similar to the post-parasitic planula stage of Reitzel et al. (2009, figure 3E). One larva, after 8 days swimming, became a juvenile planktonic polyp with three differentiated body regions (Figure 2n). The bulb-shaped anterior, with apical mouth and opaque-yellow patches, will likely develop to oral region with tentacles and capitulum with yellow annulus of adult anemones (Figure 1a). The cylinder-shaped mid-region will become scapus, and the slightly bulb-shaped basal region will be like physa. Same larva, one day older, had around its middle and posterior regions a thin, transparent tube, in which small structures, probably fibroblasts and cnidoblasts, were faintly visible (Figure 2o). The transparent larval tube will likely develop to the fibrous anemone tube, including tissue with b-mastigophores and fibroblasts (Figures 1f, 3a,b; Figure S2b,c).

Along larvae, ectoderm, and endoderm/endodermal intermesenterial compartments, bordered by insertions (mesenteries), were visible (Figure S3a,b). Posterior ectoderm broader than anterior ectoderm with pointing tips towards mesenteries (Figure S3b,c). Lateral ectoderm varied from broad, heavily ciliated to thin ectoderm with few cilia (Figure S3f-i). Ectodermal thickness changed during larval contraction and relaxation. Well-developed pharynx and mesenteries present in large larvae (Figure S3e). On each larval side, a round to elongate vacuole with beating cilia constantly moved small particles, probably food (Figure S3f). Larvae are ciliary suspension feeders (Reitzel et al. 2006, p. 832). Different sized lipid droplets were scattered in endoderm (Figure S3c,d,h,i). Lipid droplets few in small larvae increased in number and size with increasing larval size and were generally large and abundant in large larvae.

3.3 | Cnidae of *Edwardsiella carnea* anemones

Anemone cnidome consisted of broad microbasic b-mastigophores; narrow microbasic b-mastigophores; microbasic p-mastigophores; and thin, long basitrichs and

TABLE 2 Morphological characteristics for *Edwardsiella carnea* and *Edwardsiella lineata* larvae, planulae and juveniles (Östman and Møller (Ö, M); Reitzel et al. 2009)

Parasite stage		Post-parasitic planula		Juvenile/adult stage	
E. carnea	*E. lineata*	*E. carnea*	*E. lineata*	*E. carnea*	*E. lineata*
((Ö, M); Reitzel et al. 2009)		((Ö, M); Reitzel et al. 2009)		((Ö, M); Reitzel et al. 2009)	
Long, slender, vermiform. Body not differentiated into regions, with mouth, pharynx, mesenteries and mesenterial compartments (Figure 2; Figure S3b,e,f (Ö, M)); (Reitzel et al. 2009).		Stout, oval to cone-shaped, with mouth. Body not differentiated into regions, with external faintly visible mesenterial compartments (Figure 3m(Ö, M)); (Reitzel et al. 2009).		Body differentiated into anterior region (capitulum), mid-region (scapus) and aboral end, in *E. carnea* developed to a contractible rounded to flattened physa (Figure 2o,n (Ö, M)) In *E. lineata* aboral, tapering end, physa not mentioned (Reitzel et al. 2009).	

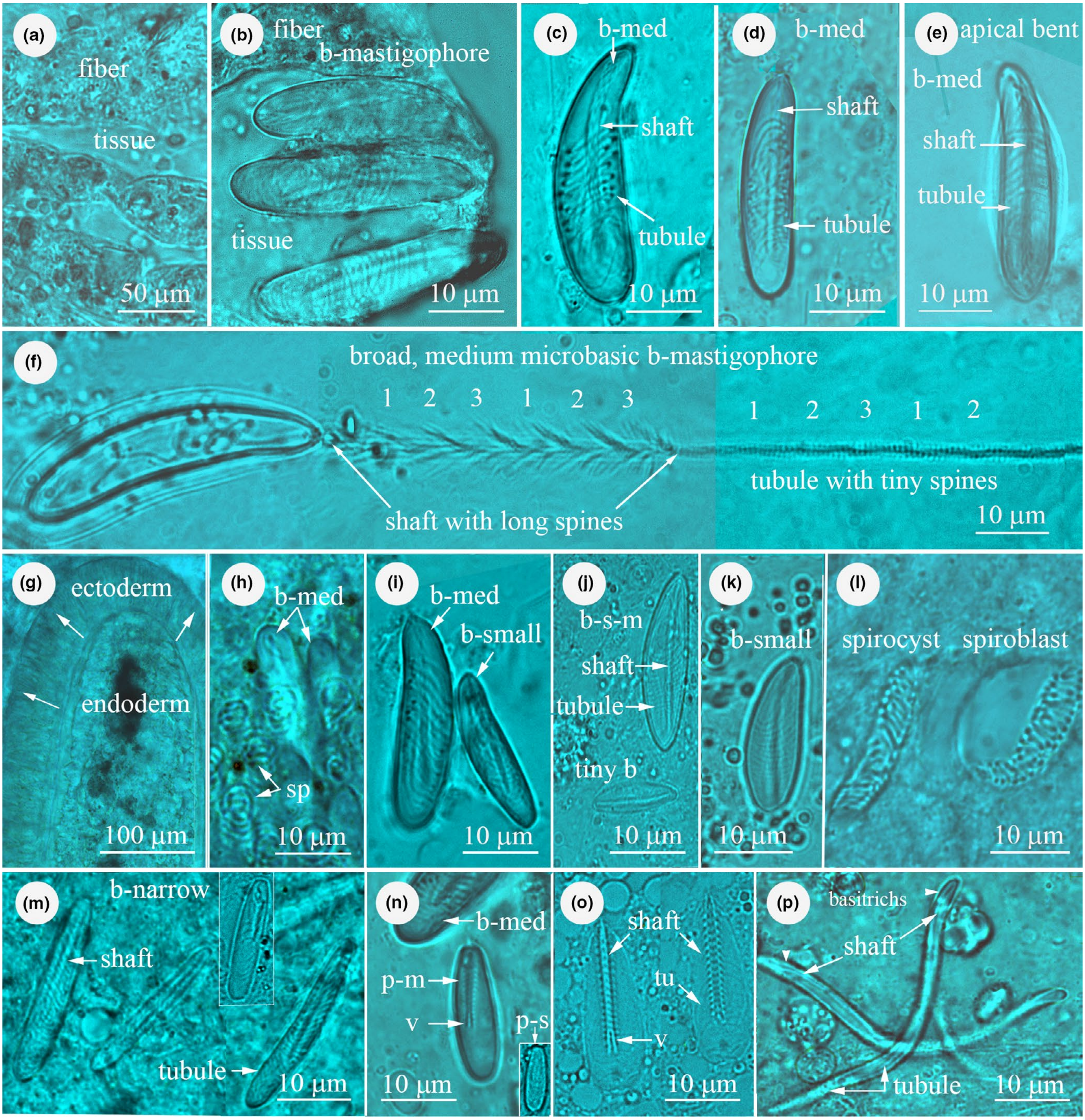

FIGURE 3 *Edwardsiella carnea* anemone, cnidae. (a-f) Tube part and broad, medium microbasic b-mastigophores from tube and column. (a) Tube fibres and tissue. (b) b-mastigophores in tissue between fibres. (c-f). From column. (b-e). From different views and foci, note change in capsule shape and width. (c) Lateral view; bow-formed shape. (d) Dorsal view; nearly straight shaft and capsule sides. (e) Ventral view; note bent apical capsule end. (f) Broad, medium microbasic b-mastigophore, partly discharged; inverted tubule inside capsule and in everted tubule. Spines in three helices (1, 2, 3), long spines on shaft, tiny spines on tubule. (g) Tentacle tip; ectoderm with parallel-oriented cnidae (arrows) and endoderm. (h-l) Tentacle cnidae. (h) Broad, medium b-mastigophores (b-med) and spirocysts (sp) in endoderm. (i,j,k) Tiny, small and medium b-mastigophores for size comparisons. (k) Immature; note long shaft. (l) Spirocyst and spiroblast. (m-p) Actinopharynx and mesenterial nematocysts. (m). Narrow, medium b-mastigophores; note shaft and tubule pattern. Inset: Narrow, small b-mastigophore. (n,o) Medium p-mastigophores; note v-shaped notch (*v*) at shaft end and tubule pattern. (n) Broad, medium b-mastigophore for size comparisons. Inset: Small p-mastigophores. (p) Thin, long basitrichs, note shaft, tubule, densely coiled at capsule end and anterior spatula-shaped capsule. Abbreviations: *arrow*, cnida orientation; *arrowheads*, point at anterior spatula-shaped capsule; *b-med*, broad, medium b-mastigophore; *b-narrow*, narrow, medium, b-mastigophores; *b-s-m*, broad, small-to-medium b-mastigophore; *p-m*, p-mastigophore, medium; *p-s*, p-mastigophore, small; *v*, v-notch; *tu*, tubule; *1, 2, 3*, three helical spine rows. All mastigophores microbasic [Colour figure can be viewed at wileyonlinelibrary.com]

spirocysts (Figure 3; Figure S4a-c). Detailed cnida variations, dimensions, size groups and abundance given in Table S3.

1. Broad microbasic b-mastigophores. Medium b-mastigophores dominated in tissue between tube fibres (Figure 3a,b) and in column (Figure 3c-f), in tentacles richly abundant surrounded by spirocysts (Figure 3g-i). Small b-mastigophores less common, abundant in tentacle bases (Figure 3i-k), tiny b-mastigophores few in column and in actinopharynx (Figure 3j).

C*apsule* of broad, medium b-mastigophores in lateral view, slightly bow-formed, often apically prominent bent (Figure 3c). In dorsal/ventral view (Figure 3d), capsules looked straight, slightly narrower (ca 1–2 μm) than in lateral view. In ventral view, apical bending faintly visible (Figure 3e). Small and tiny b-mastigophores less bow-formed, with no prominent apical bending (Figure 3i-k).

Inverted shaft rod-shaped, not reaching capsule end, in lateral view slightly bow-formed, following capsule shape, the upside-down, v-shaped spine pattern dimly visible (Figure 3c). Shaft in dorsal/ventral view looked straight (Figure 3d). Immature capsules, slightly broader than mature ones; immature, loosely twisted shaft reached capsule end (Figure 3k). Transition between shaft and distal tubule distinct. *Inverted distal tubule* in slightly oblique, regular coils, around and basal to shaft, filling medium-sized capsules, except for 1/4 to 1/5 of apical capsule (Figure 3c,i). Tubules nearly filled small and tiny capsules (Figure 3i,j).

Everted shaft (Figure 3f) microbasic (slightly longer than capsule), armed with 6–7 rows of homotrichous, 3–5 μm long spines in triple helices. Towards shaft end, spines decreased in length and shaft tubule tapered towards distal tubule. Entire d*istal tubule* with tiny spines in triple helices. No discharged tiny and small b-mastigophores observed.

2. Narrow microbasic b-mastigophores (Figure 3m). *Capsule* narrow, elongate, slightly bent, tapering apically, with blunt ends. Rod-shaped *inverted shaft* ~ ¾ capsule length; transition to thin *inverted distal tubule* distinct. Dense, regular, slightly oblique tubule coils filled capsule around shaft, irregular coils basal to shaft. *Everted shaft* microbasic (15–20 μm). No clear observation of discharged shaft and distal tubule.

3. Microbasic p-mastigophores (Figure 3n,o; Figure S4a). C*apsule* elongate, drop-shaped. *Inverted shaft* rod-shaped, pointed apically, with prominent v-shaped notch at end. Mature shaft at clear distance from capsule end. Clear upside-down, v-shaped spine pattern on loosely twisted shaft "late in development" (Figure 3o). *Distal tubule* emerging v-shaped notch, regularly coiled around shaft, in remaining capsule irregularly coiled. In small and tiny p-mastigophores, internal characteristics unclear (Figure 3n, insert). Discharged capsules not observed.

4. Thin, long basitrichs (Figure 3p; Figure S4b,c), only inside column. C*apsule* differently bent, varied in length, of constant width, except, slightly broader, spatula-shaped, apical end. *Inverted shaft* rod-shaped, pointed apically (Figure S4c). *Distal tubule* loosely coiled basal to shaft, densely coiled at capsule end (Figure S4b). Partly *everted shaft* narrow, without visible spine pattern (Figure S4c).

5. Spirocysts (Figure 3h,l) dominant in tentacles. *Capsule* elongate, basally rounded or narrow-pointed. *Inverted tubule* in distinct, regular coils, vertical to slightly oblique to capsule axis; sometimes some irregular coils. *Discharged tubule* spineless.

3.4 | Cnidae of *Edwardsiella carnea* larvae from Sweden

Larval cnidome consists of seven different cnida types, whereof three types were present in anemones. Detailed cnida variations, dimensions, size groups, abundance and development are given in Figures 4, 5 and 6; Table 2,3; Figure S4d-k and Table S4. Not all cnida types present in each larva, especially not in small larvae, with few cnidae. Only ~30 cnidae and some developing cnidoblasts were observed in one small larva. Cnidae generally not identified in anterior ectoderm of small larvae, whereas in posterior ectoderm, cnidae were abundant (Figure S5a). Cnidae increased in number with increasing larval size, in large larvae richly abundant, except for in anterior ectoderm. A thin, probably starved 2.2 mm long larva with few lipid droplets had few cnidae. Cnidae mostly oriented with apical end pointing out from ectoderm or towards ectodermal rim (Figure S5b-e), except for broad, large isorhizas, often oriented with long capsule axis parallel to rim (Figure S5f-h)

1. Broad, medium, microbasic b-mastigophores (Figure 4a-g) richly abundant, dominated in ectoderm of large larvae, in small larvae rare, mostly present in posterior ectoderm. *Capsule* in lateral view, broad, slightly bow-formed (Figure 4a-c); in dorsal/ventral view, straight, slightly narrower (~1–2 μm) (Figure 4e). Immature capsules often broad and bent (Figure 4g).

Inverted shaft rod-shaped, slightly bow-formed, following capsule shape (Figure 4a-c). In dorsal/ventral view, the centrally placed shaft, straight or nearly so (Figure 4e). Mature shaft not reaching capsule end (Figure 4a). Less twisted shaft, slightly undulating, close to capsule end, might be immature "late in development" (Figure 4b-d). Shaft in focus showed the upside-down, v-shaped spine rows of varied number (10 in Figure 4b, 9 in Figure 4c, 8 in Figure 4d. Transition between shaft and *distal tubule* clear (Figure 4a). *Inverted distal tubule* filled capsule with slightly oblique, regular coils around and basal to shaft, except for 1/5 - 1/6 of apical capsule (Figure 4a).

Everted shaft microbasic with 6–10 rows, homotrichous, 3–5 μm long spines in triple helices (Figure 4f); 8 spine rows seemed most common. Spines decreased in length towards

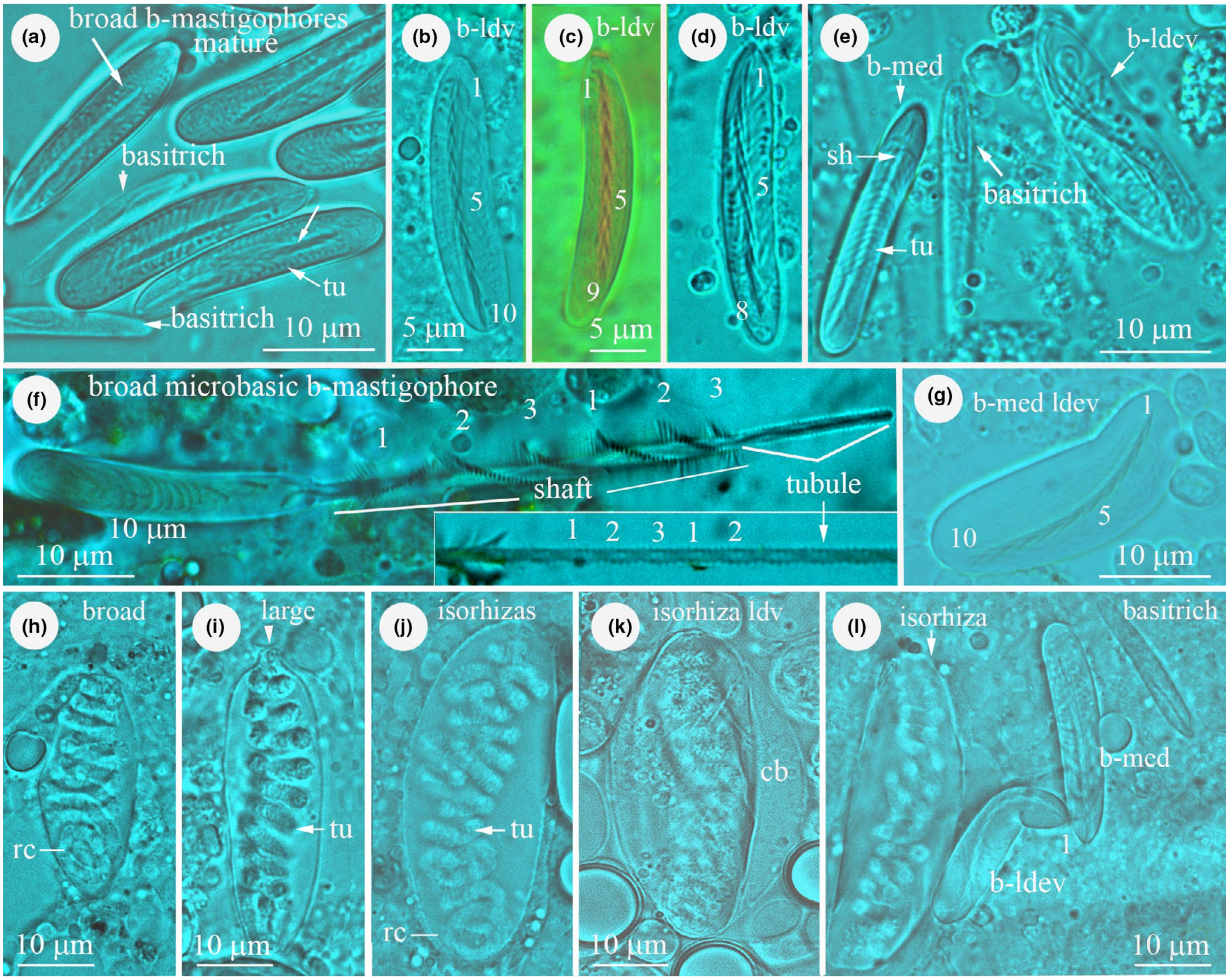

FIGURE 4 *Edwardsiella carnea* larva, cnidae. (a-g). Broad, medium microbasic b-mastigophores. Note differences in capsule shape, size and shaft pattern. (a-e,c,g) Same magnification. (a). Mature b-mastigophores. Narrow basitrichs for size comparisons. (b to d) Capsules in lateral view, shafts "late in development". Spine pattern with ten spine rows in (b) (1, 5, 10), nine in (c) (1, 5, 9) and eight in (d) (1, 5, 8). (c) Eosin coloured shaft. (d, e) Broad b-mastigophores "late in development" (b-ldev). Note long, developing shaft with loose spine helices, making narrower angles to long capsule axis than on mature, shorter shafts in (a). (e) Broad b-mastigophore (b-med) in dorsal/ventral view, broad b-mastigophore "late in development" (b-ldev) with undulating shaft, and narrow basitrich for size comparison. (f) Eosin coloured, partly discharged b-mastigophore. Shafts with large spines in triple helices (1, 2, 3). Distal tubule with small spines. Note inverted tubule inside everted tubule. Inset: Tubule with small spines in triple helices (1, 2, 3). Magnification as in (f). (g) Broad b-mastigophores "late in development" (b-med ldev). Capsule markedly broader than mature b-mastigophore in (a). (h-l) Same magnification. Broad, large isorhizas. Note size and shape differences, last rounded tubule coil (rc) in (h, j) and protruding apical capsule end (arrowhead) in (i). (h) Smallest capsule. (j) Broadest capsule. (k) Cnidoblast (cb), "late in development", surrounding capsule with tubule. (l) Broad b-mastigophores and basitrich for size comparison to large isorhiza. Abbreviations: *arrowhead*, points at protruding apical capsule tip; *b-*, *b-med*, broad, medium b-mastigophore; *cb*, cnidoblast; *ldev*, late development; *rc*, round, last tubule coil; *sh*, shaft; *tu*, tubule; *1–10*, spines rows; *1, 2, 3*, triple spine helices [Colour figure can be viewed at wileyonlinelibrary.com]

shaft end, which gradually tapered towards *distal tubule*. Tiny spines in triple helices along tubule (350–600 µm long) (Figure 4f, inset)

2. *Broad, large isorhizas* (Figure 4h-l) significantly larger than other cnidae, evenly scattered in large larvae, except in anterior and posterior ectoderm, where it is absent. Long capsule axis often parallel-oriented to ectodermal margin (Figure S5f). C*apsule* varied in size and shape, from broad oval to broad elongate with nearly straight sides; broadest part close to mid-capsule or slightly more apically (Figure 4h-j; Figure S4d,e). Anterior capsule often broader than narrow posterior end; small protruding tip apically.

Inverted tubule broad, in horizontal, somewhat irregular, slightly oblique, loose coils. All coils not reaching capsule wall. Last coil in a round circle close to capsule end (Figure 4h,j). Inverted tubule tightly twisted, stained red by eosin, clearly visible also in partly everted tubule and in

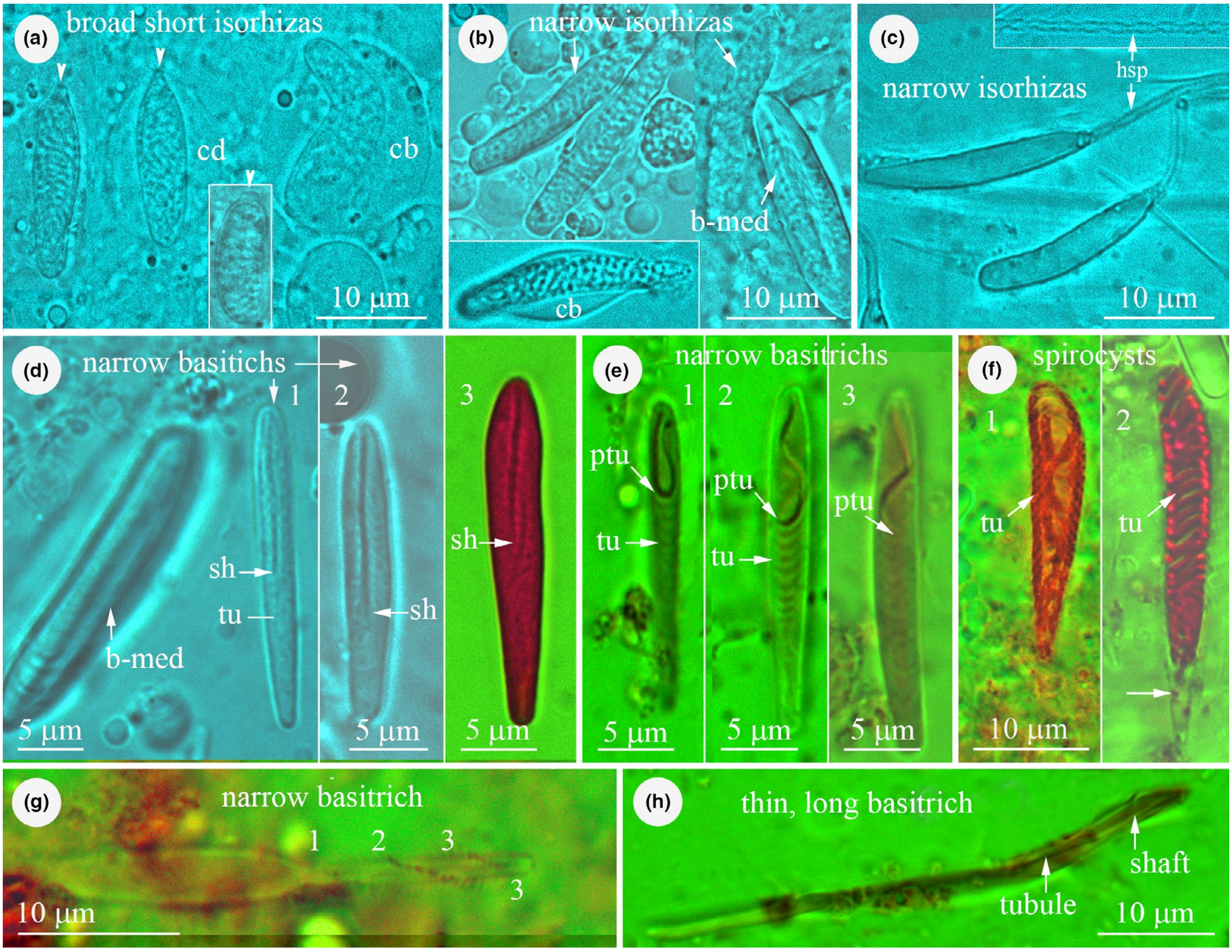

FIGURE 5 *Edwardsiella carnea* larva, cnidae. (a) Broad, short isorhizas. Note large size variation, protruding apical tip (arrowhead), irregular, dense tubule coils and isorhizas "late in development", surrounded by cnidoblast (cb). Inset: Small, probably mature isorhiza. (b, c) Narrow isorhizas. (b) Undischarged isorhizas with irregular, dense tubule coils. Broad, medium b-mastigophore (b-med) for size comparison. Inset: Note cnidoblast (cb). (c) Discharged, narrow isorhizas with helical spine pattern (hsp) or spineless tubule. Note narrow apical capsule. Inset: Clear helical spine pattern. (a-c) same magnification. (d, e) Narrow basitrichs. Note size differences. (d) Basitrichs (1–3) with slightly undulating shaft (sh) of different length, distal tubule coils filling whole capsule. Broad b-mastigophore (b-med) for size comparison. (e) Basitrichs (1–3) with an apical, prominent tubule coil (ptu), which size might decrease as basitrichs are maturing; remaining coils (tu) basal to prominent coil. (d3-e1-3). Eosin coloured. (f) Eosin coloured spirocysts with irregular tubule coils (tu) in central capsule (f1) and regular tubule coils and narrow-pointed capsule end (f2). (g) Partly discharged, narrow basitrich with three rows of small spines (1, 2, 3) on shaft. (h) Thin, long basitrich with shaft in spatula-shaped anterior capsule and part of visible tubule in remaining capsule. Abbreviations; *arrowhead*, points at apical protruding tip*; b-med*, broad, medium b-mastigophore; *cb*, cnidoblast; *ldv*, late development; *hsp*, helical spine pattern; *ptu*, prominent tubule coil; *sh*, shaft; *tu*, tubule; *1, 2, 3*, three spine rows

surrounding cnidoblasts (Figure S4d,i,k). *Everted tubule* isodiametric, spineless (Figure S4f,i,j). One not fully discharged tubule ~500 µm

3. Broad, short isorhizas (Figure 5a) found in eight larvae, whereof three in small larvae; mostly present at ectodermal rim. *Capsule* varied in shape and size; small protruding tip at narrow apical end, basal capsule broader. *Inverted tubule* in irregular dense coils, completely filling capsule. *Everted tubule* isodiametric, spineless

4. Narrow isorhizas (Figure 5b,c) in small larvae, most abundant and dominant in posterior ectoderm. In large larvae present throughout ectoderm, but far less abundant than broad b-mastigophores. *Capsule* narrow, straight or slightly bow-formed, slightly pointed anterior and rounded posterior. *Inverted tubule* in dense, oblique, irregular coils completely filling capsule. *Everted tubule* (170–220 µm) isodiametric with tiny, sometimes barely visible spines, forming triple helices (Figure 5c, inset). Some tubules seemed spineless (Figure

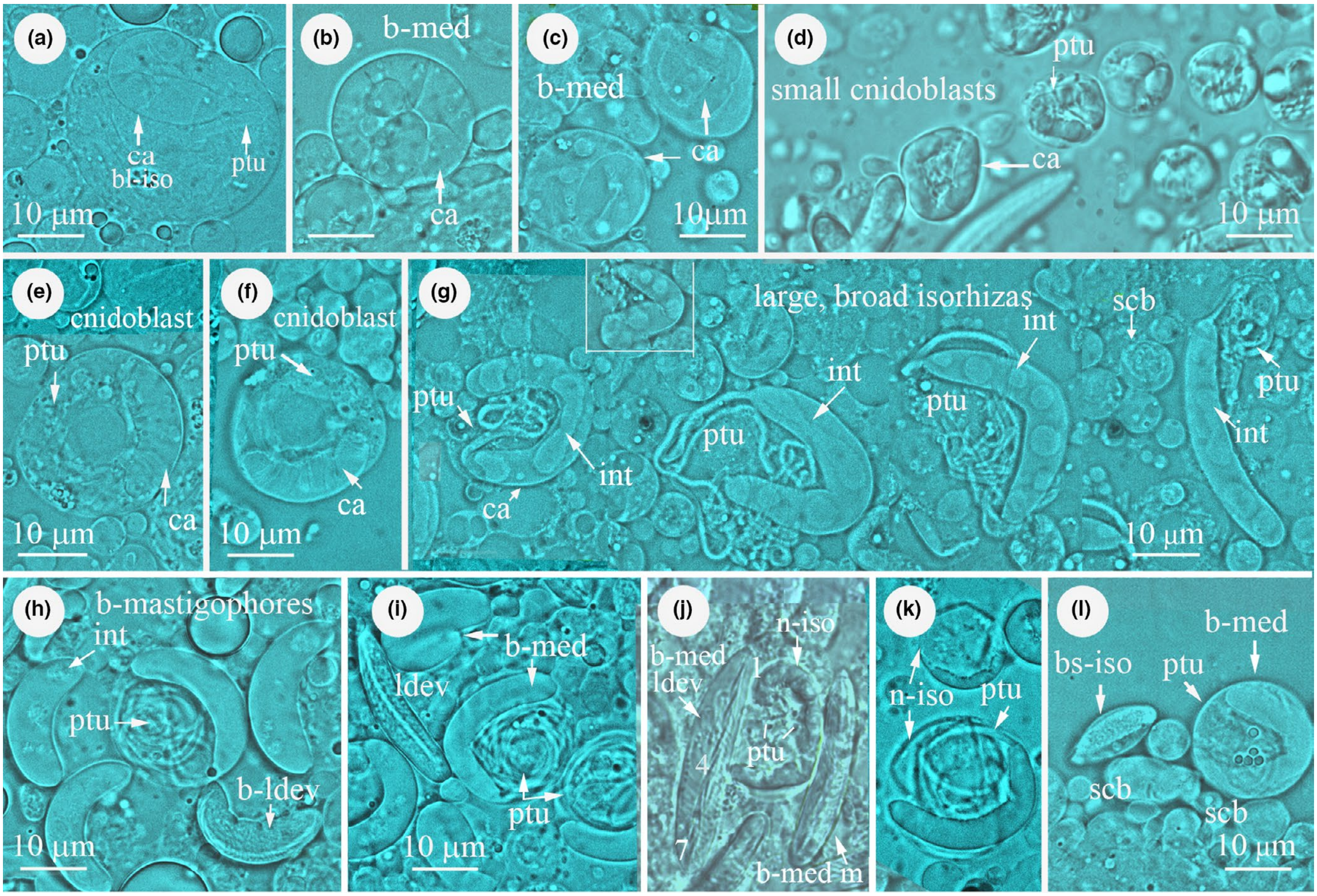

FIGURE 6 *Edwardsiella carnea* larva. Cnida development. (a) Large cnidoblast, probably broad, large isorhiza with capsule (ca) and pre-inverted tubule (ptu). (b,c) Cnidoblasts, probably broad, medium b-mastigophores with developing capsule; no pre-inverted tubule. (d). Small cnidoblasts (scb) "early in development". Capsules bent twice or once, some with pre-inverted folded tubules (ptu). (e,f) Cnidoblasts "early in development", probably broad, medium b-mastigophores with pre-inverted tubule (ptu) in fragments. (g) Probably broad, large isorhizas "early in developments", without cnidoblast, with pre-inverted tubule (ptu) and round to elongate internal capsular structures (int) of different sizes. From left to right: capsules bent twice, once and nearly straight. Inset: Probably broad, medium b-mastigophore with internal structures. (h,i) Broad, medium b-mastigophores with or without pre-inverted tubule (ptu) and small, rounded internal capsular structures. Note capsules "late in development" (ldev), slightly bent or almost straight, with long inverted shaft. (j) Immature, broad b-mastigophore "late in development" (b-med ldev) showing seven spine rows (1, 4, 7) on loosely twisted shaft, making narrow angle to capsule axis; and probably a narrow isorhiza (n-iso) "early in development", with bent capsule and pre-inverted tubule (ptu) in fragments.(k) Probably small and medium narrow isorhizas (n-iso), with pre-inverted tubule. (l) Cnidoblast with broad, short isorhiza capsule (bs-iso); broad, medium b-mastigophore (b-med) for size comparisons. Abbreviations: *bl-iso*, broad, large isorhiza; *b*, *b-med,* broad, medium b-mastigophore; *bs-iso*, broad, short isorhiza; *ca,* capsule; *int,* small rounded to elongate internal structures; *ldev*, late development; *n-iso*, narrow isorhiza; *ptu*, pre-inverted tubule; *scb*, small cnidoblast. All pictures same magnification. All mastigophores microbasic [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Cnidae present in *Edwardsiella carnea* and *Edwardsiella lineata* larvae (Östman and Møller, Table S4; Reitzel et al. 2009)

	Parasitic larvae, *E. carnea* (Östman and Møller, Figures 4 and 5)	**Parasitic larvae, *E. lineata* (Reitzel et al. 2009, Figure 6)**
Cnidome	Broad microbasic b-mastigophores*, broad large isorhizas; broad short isorhizas; narrow small and medium isorhizas; narrow basitrichs with rod-liked, thin shaft; narrow basitrichs with apical tubule coil; thin long basitrich*, spirocysts*	Microbasic b-mastigophores*, holotrichous (= broad large isorhizas); not mentioned: broad short isorhizas; narrow isorhizas, narrow basitrichs with rod-liked, thin shaft; not mentioned: narrow basitrich with apical tubule coil, (thin long basitrich found in one juvenile)*; spirocysts*

*Nematocysts present also in adult anemones.

S4j). Isorhizas "late in development" partly surrounded by a cnidoblast (Figure 5b, inset)

5. Narrow basitrichs (Figure 5d,e) abundant in large, stained larvae; not identified in small, unstained larvae, here probably overlooked. *Capsule* narrow, elongate, slightly broader apically. Two shaft/tubule pattern distinguished. One basitrich subtype with thin, rod-like or slightly undulating *shaft*, running towards mid-capsule or longer (Figure 5d). *Inverted tubule* almost filling remaining capsule with irregular, slightly oblique coils. Other basitrichs formed an apical, conspicuous tubule coil of different length (Figure 5e), thereafter, tubule filled distal capsule with irregular, slightly oblique, dense coils. Only few, unclear *everted shafts* and *distal tubules* noticed. Tiny spines in triple helices formed 3–4 coils on shaft (Figure 5g). Small dots on tubule, interpreted as spines. Different basitrich subtypes separately measured (Table S4).

6. Thin, long basitrichs (Figure 5h) identified in eight, mostly eosin-stained larvae. Twenty basitrichs recorded from one stained larva, none in unstained, small larvae; here certainly overlooked. C*apsule* differently bent, varied in length, but with constant width, except slightly broader, spatula-shaped apical end. *Inverted shaft* rod-shaped or slightly undulating; transition to *distal tubule* vague. *Inverted tubule* in large, undulating, loose coils apically; dense, small coils basally. No discharged capsules observed.

7. Spirocysts (Figure 5f) most abundant in large larvae, rare in small. Capsule narrow, elongate, broadest apically; basal end broad, rounded or narrow-pointed. Inverted tubule in regular coils, vertical to slightly oblique to capsule axis. Sometimes, irregular tubule coils in capsule centre. Generally, no coils in narrow capsule end (Figure S4g). Everted tubule broad, spineless, stained red by eosin, sometimes with a drop of internal substance at tubule tip (Figure S4h).

3.5 | Cnida development in larvae

Cnidoblasts of different developmental stages (Figure 6), referred as "early" or "late in development" appeared in proliferation zones in large larvae (>2–3 mm). Large cnidoblasts with largest capsules match broad, large isorhizas (Figure 6a), second largest cnidoblasts with broad capsules, might match broad, medium b-mastigophores (Figure 6b,c), cnidoblast with narrow small or narrow medium capsules might match narrow isorhizas (Figure 6j,k), and small cnidoblasts matched the smallest cnidae (Figure 6d). In certain areas, different cnida types often dominated. Broad, large isorhiza dominated in one area (Figure 6g), broad b-mastigophore cnidoblasts (Figure 6h,i) and small cnidoblasts in other areas (Figure 6d). All developing cnida types were not always found in each larva. Only few developing broad, short isorhizas identified (Figure 6l).

Capsule inside cnidoblast during "early development" is first unstructured, later successively formed (Figure 6a-c). Pre-inverted tubule appeared, first in fragments (Figure 6d,j) and then irregularly folded, surrounded by the bent capsule (Figure 6g-i,k). Maturing capsules, with internal matrix of different size and shape, decreased its bendings from three, two and one (Figure 6g).

Immature cnidae with tubule and shaft completely inverted in a bent, often unusual broad capsule, are referred "late in development" (Figure 6h,i). Immature *broad b-mastigophores* were commonly identified in bent, generally broader capsules than mature capsules. On their, helically, loosely twisted shaft, thus longer than mature shaft and "late in development", their conspicuous spine rows formed narrow angles to long capsule axis (Figures 4d; 6j). Spine pattern on mature, densely twisted, short shaft often dimly visible (Figure 4a).

Immature *broad, large isorhizas*, "late in development", surrounded by a cnidoblast, showed the tightly, twisted tubule inside the slightly bow-formed to nearly straight capsule (Figure 4k). Capsules of *broad, short* and *narrow isorhizas, narrow basitrichs* and *spirocysts*, "late in development", often slightly bent, surrounded by a cnidoblast (Figure 5a,b, inset). Immature broad, short isorhizas sometimes markedly larger than mature ones. Cnidoblast in later stages only partly covered the capsule (Figure S4g,k). The cnidoblast of *thin, long basitrich* appeared in parts.

In general, immature capsules were slightly bent and broader than mature capsules; immature, loosely, helically twisted shafts, slightly longer, with clearer spine pattern than mature, densely twisted shafts.

4 | DISCUSSION

4.1 | Morphology: *Edwardsiella carnea* and *Edwardsiella lineata* anemones

The morphology of present *E. carnea* anemone is close to descriptions of Gosse (1856) and Carlgren (1921), and similar to *E. lineata* but with a few distinctions (Table 1). The aboral *E. carnea* physa, without rugae, and changeable from round to flat, when retracted into itself (Figure 1b; Figure S1i,j), is the main obvious feature separating *E. carnea* from *E. lineata*, with long, tapering aboral end provided with rugae (Daly, 2002b). Daly did not mention physa. Moreover, the long introvert-able *E. carnea* capitulum is not identical to the shorter *E. lineata* capitulum. In *E. lineata*, the border between capitulum and scapus is close proximal to actinopharynx (Reitzel et al. 2009, Figure 1B). Since annulus is not, as in *E. carnea* included in capitulum (Figure 1a), *E. lineata* annulus seemed thus not to retract into column.

Additional characteristics separating *E. carnea* and *E. lineata* anemones are the pigmentation on oral disc and tentacles (Figure 1c,d; Table 1). The low tentacle number of our *E. carnea* specimens compared with those studied by (Gosse 1856, Gosse 1960) and to *E. lineata* (Daly, 2002b, 869) (Table 1, Table S2) may be because our anemones were not fully grown. The fine *fibre net*, surrounding the *E. carnea* column (Figure S2h), might be precursor to the coarse tube. Neither Carlgren nor Daly mentioned this fibre net.

The polygonal column pattern, formed by conspicuous capitulum and scapus ridges (intermesenterial compartments), and the conspicuous mesenterial insertions and compartments on physa of *E. carnea* (Gosse, 1856, plate 9, Figures 1-4; Fautin, 2007), are certainly fixation artefacts, making ridges and insertions prominent, due to contractions. Live *E. carnea* is cylindrical, capitulum and scapus ridges, mesenterial insertions and compartments on physa are visible, but not conspicuous (Figure 1a,b). In everting anemones, capitulum ridges grew distinct (Figure S1c-f).

4.2 | Larval morphology

Edwardsiella carnea larvae, like anemones, are cylindrical, with pharynx and for Edwardsiids characteristic mesenteries and mesenterial compartments; however, the larval body is not differentiated into regions (Figure 2a-j; Figure S3a,b,e,f; Table 2). Reitzel et al. (2007, p. 1392, Figure 1B; Reitzel et al. 2009, p. 102, Figures 2B, 3C,E) recorded in *Edwardsiella lineata* following life cycle stages: parasite stage, post-parasitic planula, juvenile and adult polyp (the anemone). Our collected *E. carnea* larva, like in Selander et al. (2009, pp. 2005, 2006, Figure 3), is similar to the parasite stage.

A free-swimming larva metamorphosed to a juvenile planktonic polyp with three visible body regions (Figure 2n,o), which likely were equivalent to the anemone capitulum, scapus and physa (Figure 1b). The aboral juvenile body end, the physa, contracted and flattened as the anemone physa (Figure S1i,j), a feature confirming the affiliation of our parasitic larva to *E. carnea*. Another larval behaviour is the repeatedly retracting and ejecting of the apical larval body part (Figure 2d-h), a pattern, which might reflect the retracting and ejecting of the anemone capitulum.

4.3 | Cnidae in *E. carnea* and *E. lineata* anemones

The cnidome of present *E. carnea* anemones closely conforms the cnida descriptions of Carlgren (1921, p. 64; Carlgren 1940, p. 25 (Table S1), except for the probably overlooked thin, long basitrich and tiny b-mastigophores. The overall broader size ranges of present *E. carnea* cnidae (Table S3), compared with cnida range recorded by Carlgren (1921, 1940) (Table S1), is certainly due to our numerous measurements.

Broad, medium microbasic b-mastigophores from column of *E. carnea* (Figure 3c, Table S3), correspond to capitulum and scapus nematocysts of Carlgren (1921) and microbasic b-mastigophors of Carlgren (1940) (Table S1). Like Carlgren (1940) broad, medium b-mastigophores were the most numerous and largest nematocysts in *E. carnea* anemones, had bow-formed capsule, 5 to 6 prominent spines rows on discharged shaft, and tiny, hoplotelic spines (spined throughout distal tubule) (Figure 3c,f). Their presence from tube tissues is only our observations (Table S3).

Our broad, small and medium microbasic tentacle b-mastigophores (Figure 3i-k) corresponded to tentacle nematocysts of two size classes (Carlgren, 1921) and tentacle b-mastigophors (Carlgren, 1940). Contrary to our findings, Carlgren (1921) reported very numerous small tentacle nematocysts and few larger ones. Perhaps Carlgren (1921) investigated tentacle bases, where small b-mastigophores dominate.

Microbasic b-mastigophores are recorded from tentacles, column and actinopharynx of *E. lineata* (Daly, 2002b, table 1). The differently named nematocysts, microbasic b-mastigophores, scapus basitrichous and basitrichs from column and tentacles of *E. lineata* (Reitzel et al. 2009, p. 105, figure 4B), might all include *broad microbasic b-mastigophores*. Basitrich in Edwardsiids is the old term for broad, microbasic b-mastigophore. Carlgren (1940) remarked "all wide basitrichs could be microbasic b-mastigophores and perhaps also hoplotelic" (spined throughout tubule). Microbasic b-mastigophores (Daly 2002b, table 1) of *E. lineata* might, due to their narrow width (1.6–2.6 μm), include both broad microbasic b-mastigophores (Figure 3c), and narrow b-mastigophores (Figure 3m).

Narrow microbasic b-mastigophores (Figure 3m) are certainly equivalent to actinopharynx nematocysts (Carlgren, 1921, p. 64, spirocysts might been included herein), to basitrichs from actinopharynx and filaments (Carlgren, 1940, p. 25) (Table S1) and partly to basitrich (C) and microbasic b-mastigophores from actinopharynx (Daly, 2002b, table 1).

Microbasic p-mastigophores (Figure 3n,o) correspond to actinopharynx nematocysts (Carlgren, 1921, p. 62), to microbasic p-mastigophors from actinopharynx and filament (Carlgren, 1940, p. 25) (Table S1) and to microbasic p-mastigophores of *E. lineata* (Daly, 2002b, table 1). The largest length/width measurements of *E. lineata* p-mastigophores might include broad, medium microbasic b-mastigophores (Tables S5 and S6).

Thin, long basitrichs (Figure 3p) and *spirocysts* (Figure 3l) matched *E. lineata* basitrich (F) and spirocysts, respectively (Daly, 2002b, figure 3B,F, table 1). Spirocysts, the most abundant tentacle cnidae, are less important for species identification. Their capsule shape and regular, coiled tubule pattern are closely similar within anthozoan spirocysts.

Although Daly (2002b, figure 3, table 1) have incorrectly named some of her cnida pictures, not shown any correct picture of narrow, microbasic b-mastigophores (basitrich C) and of p-mastigophore, and partly mixed up their measurements (see Tables S5 and S6), our opinion is that the cnidomes of *E. carnea* and *E. lineata* anemones are closely similar. Both consist of broad, microbasic b-mastigophores (Figure 3c); narrow, microbasic b-mastigophores (Figure 3m, partly equivalent to basitrich (C) of *E. lineata*); microbasic p-mastigophores (Figure 3o); and thin, long basitrichs (Figure 3p, equivalent to basitrich F of *E. lineata*) and spirocysts.

4.4 | Cnida comparisons between *Edwardsiella larvae*

Of the eight different cnida types in present *E. carnea* larvae, Reitzel et al. (2009) recorded four for *E. lineata* larvae (Tables S4 and S6).

Broad, microbasic b-mastigophores, common in *E. carnea* larvae (Figure 4a), were close in size and shape to larval *E. lineata* microbasic b-mastigophores, pictured in dorsal/ventral view (Reitzel et al. 2009, fig. 6D, table 1); however, their smallest width (2.25 μm for *E. lineata*) might include narrow basitrichs (Table S4).

Broad, large isorhizas (Figure 4h,l) matched developing holotrichs surrounded by cnidoblasts and mature holotrichs of Reitzel et al. (2009, figs 5E, 6A, table 1). However, our large isorhizas are spineless (Figure S4f), not holotrichous (spined throughout tubule). Note *E. lineata* holotrich is oriented with last tubule coil upwards. Present large capsule variation (Figure 4h-l; Figure S4d,e) may be due to large number (163) examined isorhizas.

Narrow basitrich, subtype with thin, vaguely, undulating shaft (Figure 5d), might correspond to *E. lineata* basitrichs; given largest width (6.14 μm, Reitzel et al. 2009, fig. 6E, table 1) might, however, include broad b-mastigophores (Table S4). Our basitrich subtype with proximal tubule loop (Figure 5e), not noted in *E. lineata* larvae.

Broad, short and narrow isorhizas (Figure 5a-c) not noted in *E. lineata* larvae (Table S6). *Thin, long basitrichs* (Figure 5h) only identified in one juvenile (Reitzel et al. 2009, fig. 6C, table 1). *Spirocysts* (Figure 5f) abundant in the two *Edwardsiella* larvae.

Certainly misled by the p-mastigophore picture from *E. lineata* anemone, representing one b-mastigophore in dorsal/ventral view (Daly, 2002b, figure 3D), (Reitzel et al. 2009, table 1) recorded *p-mastigophores* in larvae. No picture was given for *E. lineata* larvae with the characteristic p-mastigophore v-notch at shaft end; if present, the larval *E. lineata* cnidome will differ from that of *E. carnea* larvae.

4.5 | Identification and problems of larval cnidae

Cnidae during "early development" within cnidoblasts are primarily identified by their capsules. Except for the large, broad *isorhizas*, nematocysts are not easily recognized (Figure 6a-f). Cnidae "late in development," with inverted shaft and tubule, are more easily identified. Immature *broad microbasic b-mastigophores* are distinctly recognized by their broad, bow-shaped capsule, and long, loosely twisted shaft with clear v-shaped spine pattern (Figure 6h-j).

Mature, discharged, *broad microbasic b-mastigophores* (Figure 4f) are recognized by prominent spines in helical rows on short shaft, and small spines in clear helical pattern on narrow, distal tubule. The broad, spineless tubules from large isorhizas are distinct from narrow isorhiza tubules (Figure 5c; Figure S4f,j).

Narrow basitrichs, commonly identified in stained large larvae (Figure 5d,e), might been overlooked when unstained. Two basitrich subtypes may exist. However, basitrichs with a prominent apical coil (Figure 5e) might be early developing basitrichs with undulating shaft (Figure 5d). The three helical coils of small spines, dimly visible on the discharged shaft (Figure 5g), but slightly larger than the small dots on the distal tubule, may characterize these basitrichs. *Thin, long basitrichs*, mostly identified from stained larvae (Figure 5h), might been overlooked when unstained.

Spirocysts with some irregular tubule coils (Figure 5f), unstained and partly covered by tissues, can be mistaken for *narrow isorhizas* (Figure 5b), as their capsule size are overlapping (Table S4). However, the isorhiza capsule is narrow apically and broader basally (Figure 5c). The spirocyst capsule is broadest apically, and has narrow-round or narrow-pointed base, seldom with tubule inside (Figure 5f, Figure S4g).

5 | CONCLUSIONS

1. In agreement with Dnyansagar et al. (2018), *Edwardsiella carnea* collected in Sweden and American *Edwardsiella lineata* are regarded as two distinct species (Table 1). Primarily, the anemones are distinguishable by the rounded to flattened physa without rugae in *E. carnea* (Figure 1b; Figure S1i,j) versus the tapering, aboral column with rugae in *E. lineata* (Daly, 2002b) in addition to their different pigmentation on oral disc and tentacles (Figure 1c,d; Daly, 2002b).

2. The fine fibre net around *E. carnea* column (Figure S2h) is not mentioned for *E. lineata* and may be a distinctive feature of *E. carnea*.
3. The present detailed morphological descriptions will facilitate the monitoring of a possible introduction of *E. lineata* to Skagerrak.
4. Studies of the development of the Swedish parasitic larva into a juvenile, with aboral rounded to flattened, contractible physa (Figure 2o), identify the larva as *E. carnea*. The larval cnidae place the larva as a member of *Edwardsiella*.
5. Microbasic b-mastigophores, thin, long basitrichs and spirocysts, are recorded in both anemones and larvae. Narrow microbasic b-mastigophores and microbasic p-mastigophores are specific for anemones. Three isorhiza types and the two narrow basitrich subtypes are specific larval cnidae, whereof (Reitzel et al. 2009, table 3) only reported holotrichs (our broad, large isorhizas) and basitrichs (our narrow basitrichs with undulating shaft) in *E. lineata* larvae (Table 6).
6. Cnida data from *E. lineata* (Daly, 2002b; Reitzel et al. 2009*)* are insufficient for distinguishing this species from *E. carnea*.
7. Knowledge of the detailed cnida variations, including their developmental stages, can be essential for species identification.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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