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Organisms, Diversity & Evolution 6 (2006) 83–97
ORGANISMS DIVERSITY & EVOLUTION
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A new species of Cycliophora from the mouthparts of the American lobster, *Homarus americanus* (Nephropidae, Decapoda)

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Received 9 November 2004; accepted 19 May 2005

Abstract

The present study describes a new species of Cycliophora with the aid of light- and electron microscopy. The animals live attached or free-living on the mouth appendages of the American lobster, *Homarus americanus*. Individuals occur in dense clusters of up to several thousand individuals. The new species is named *Symbion americanus* sp. nov. according to the name of its host; it can be distinguished from the previously described species *Symbion pandora* by the presence of a posterior pair of retractable tubular appendages or toes in the Prometheus larva. Morphological variation among cycliophorans on *H. americanus* collected in different localities seems to be high. In several sexual populations of *S. americanus*, older feeding individuals with a female typically have 5–13 rings of cuticular scars and a thicker cuticle. Moreover, attached Prometheus larvae frequently contain three males, chordoid cysts possess a distal appendix, and chordoid larval morphology varies among localities. These differences in morphology might indicate the existence of cryptic species. The presence of toes in the Prometheus larva could support a cycliophoran relationship with rotiferan taxa, although additional ultrastructural studies are needed. Considering that the genus *Homarus* is at least 60 My years old, and with regard to its history of speciation, it seems possible that the two *Symbion* species separated during the Pleistocene.

Keywords: *Symbion americanus* sp. nov.; *Homarus americanus*; Complex life cycle; Dwarf male; Symbiont-host; Animal phylogeny

Introduction

Cycliophorans are acoelomate metazoans of microscopic size. They were described first from the mouth appendages of the Norway lobster, *Nephrops norvegicus* Linnaeus, 1758, where they live attached as commensals (Funch and Kristensen 1995; Funch 1996; Funch and Kristensen 1997; Kristensen and Funch 2002). Cycliophoran feeding individuals are attached to the mouthparts of the lobster and obtain food by active filter feeding (Riisgard et al. 2000). The taxon Cycliophora is regarded as a distinct metazoan phylum so far containing a single described species: *Symbion pandora* Funch and Kristensen, 1995. The phylogenetic position of Cycliophora is still unclear (Kristensen 2002; Sørensen and Kristensen 2004; Funch et al. 2005); analyses suggest relations either to gnathiferan taxa (Winnepenninckx et al. 1998; Giribet et al. 2000; Peterson and Eernisse 2001; Zrzavỳ et al. 2001; Giribet et al. 2004) or to Entoprocta (Zrzavỳ et al. 1998; Sørensen et al. 2000; Obst 2003).

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doi:10.1016/j.ode.2005.05.001

The life cycle of cycliophorans is very complex, with six different stages that arise throughout alternate cycles of asexual and sexual reproduction. The feeding individual is so named because it is the only stage in the life cycle with a functional digestive system. Asexual reproduction predominates at the beginning of the life cycle when young feeding individuals reproduce by means of the Pandora larva that develops inside their brood chamber. Upon liberation, the Pandora larva settles nearby and develops into a new feeding individual. Later in the life cycle the feeding individuals develop sexual stages in their brood chamber: a Prometheus larva (Funch and Kristensen 1999; Obst and Funch 2003) or a female. The Prometheus larva (male larva) settles on the trunk of a feeding individual and develops usually one to two males inside, but we have also observed three males inside an attached Prometheus larva of *S. pandora* (pers. obs. 1994–2002). Upon fertilization the female moves away from the medial areas of the mouth and encysts. Inside the cyst the embryo grows and engulfs the tissue of the mother animal. The embryo develops into the chordoid larva, resting inside the chordoid cyst. Once liberated the chordoid larva is able to swim to a new host.

Cycliophorans occur also on the mouthparts of the European lobster, *Homarus gammarus* Linnaeus, 1758, as well as on the American lobster, *Homarus americanus* H. Milne-Edwards, 1837 (see Funch and Kristensen 1997; Nedvěd 2004). Only few morphological differences in any of the stages could be detected (pers. obs. 1990–2004) between cycliophorans from *N. norvegicus* and *H. gammarus*; therefore *S. pandora* might live on both lobsters in European waters. However, cycliophorans from *H. americanus* show differences in both morphology and life history. The American lobster inhabits the coastal and oceanic waters of the Atlantic Ocean along the east coasts of Canada and the United States from Labrador to North Carolina (Hughes and Matthiessen 1962; Cobb 1971; Lawton and Lavalli 1995). The animals have been found from the intertidal zone down to a depth of 720 m (McRae 1960). Major coastal aggregations of the American lobster are found in the Gulf of Maine and the coastal waters of New Brunswick and Nova Scotia, Canada.

Methods

Live observations

Living commensals from samples 1, 3, 4 and 6 (see Table 1) were studied on dissected mouthparts of *H. americanus* in seawater, using LEICA and ZEISS stereo microscopes at 8–50 × magnification. Free cycliophoran stages were caught with an Irwin loop or a fine Pasteur pipette, mounted on microslides in seawater, and photographed in various light microscopes.

Whole-mount preparations

Mouthparts from sample 1 (Table 1) were briefly dipped in 7% magnesium chloride, then fixed in 4% formaldehyde (from paraformaldehyde) buffered with 0.1 M phosphate-buffered saline (PBS). Mouthparts from samples 2 and 3 were fixed in 2% formaldehyde buffered with Borax. For light microscopy (LM), preparations of the fixed mouthparts were washed several times in distilled water before further manipulation. Attached individuals were gently shaved off their host's mouthparts. Non-attached stages were isolated by filtering the fixative of the mouthparts through a filter with 32 μm mesh size. Single individuals were isolated with an Irwin loop under a ZEISS stereo microscope with 8–50 × magnification, placed on a microslide, and covered with a coverslip. Afterwards the specimens were dehydrated in a graded glycerol series (5%, 25%, 50%, 80%, 100%). The coverslip was sealed with Glyceel®. Photos were taken with a ColorView II digital camera mounted on an Olympus BX60 light microscope with DIC optics. All stages were drawn with the aid of a camera lucida from whole-mounted preparations.

Electron microscopy

For scanning electron microscopy (SEM), lobster mouthparts with Cycliophora from samples 1 and 2 (Table 1) were fixed in 4% formaldehyde unbuffered or buffered with 0.1 M PBS. Some specimens were briefly dipped in 7% magnesium chloride before fixation. The mouthparts were washed in distilled water. Small pieces of lobster integument with attached individuals and isolated stages were placed in cages closed with 10 μm mesh net lids to allow for the exchange of solutions without losing the specimens. The specimens were postfixed with 1% OsO_4 (1 h, 23 °C). Material from sample 4 was fixed directly in 1% OsO_4 (1 h, 23 °C) and rinsed in distilled water. All fixed and rinsed specimens were slowly dehydrated with ethanol or acetone, critical point dried using carbon dioxide, mounted on aluminium stubs with sticky carbon pads, sputter-coated with gold, and examined with a CamScan MaXim 2040 EnVac SEM (University of Aarhus), a JEOL field emission SEM (University of Copenhagen), or an FEI Quanta 200 SEM (Harvard University), using a secondary electron detector in all cases.

For transmission electron microscopy (TEM), specimens from samples 1 and 5 were briefly dipped in 7% magnesium chloride and then fixed in 4% paraformaldehyde buffered with 0.1 M PBS and postfixed in Dalton's chrome-osmium fixation (Dalton 1955),

Table 1. Overview of samples, their treatment and storage

Sample no.	Locality	Global position	Date	Method	Specimens	Storage	Accession no.	Figures
Type material								
1	Birch Harbor, Maine	44°23′N, 68°02′W	8 April 2002	LM	Live	—	—	7A
				LM	Holotype, fixed	wm in ZMUC	CYC 231 ZMUC	1, 2
					Paratypes, fixed	wm in ZMUC	CYC 232–266 ZMUC	3D
					Paratypes, fixed	wm in MCZ	MCZ 61718–61722	8A
				SEM		—	—	9A
				TEM	Paratypes, fixed	Grids in ZMUC	Grid box no. M02.01.1	—
Additional material								
2	Domariscotta, Maine	44°01′N, 69°31′W	September 1996	LM	Fixed	wm in ZMUC	CYC 267–291 ZMUC	3C, 3E, 3F, 4–6, 7C–E, 8B, 8D
				SEM	Fixed	—	—	9B–E
3	Maine	—	May 2001	LM	Live/fixed	wm in ZMUC	CYC 292–293 ZMUC	3A, 3B
4	Cape Cod Bay near Sandwich, Massachusetts	41°46′N, 70°28′W	October 2003	LM	Live	—	—	7B, 8C
				SEM	Fixed	—	—	10A–E
5	Birch Harbor, Maine	44°23′N, 68°02′W	Unknown	TEM	Fixed	Grids in ZMUC	Grid box nos. 99.990–99.992	11A–B
6	Maine	—	August 2004	LM	Live/fixed	wm in ZMUC	CYC 294–314 ZMUC	—

All specimens were collected in the USA. MCZ = Museum of Comparative Zoology, Harvard University, USA; wm = whole mount; ZMUC = Zoological Museum, University of Copenhagen, Denmark.

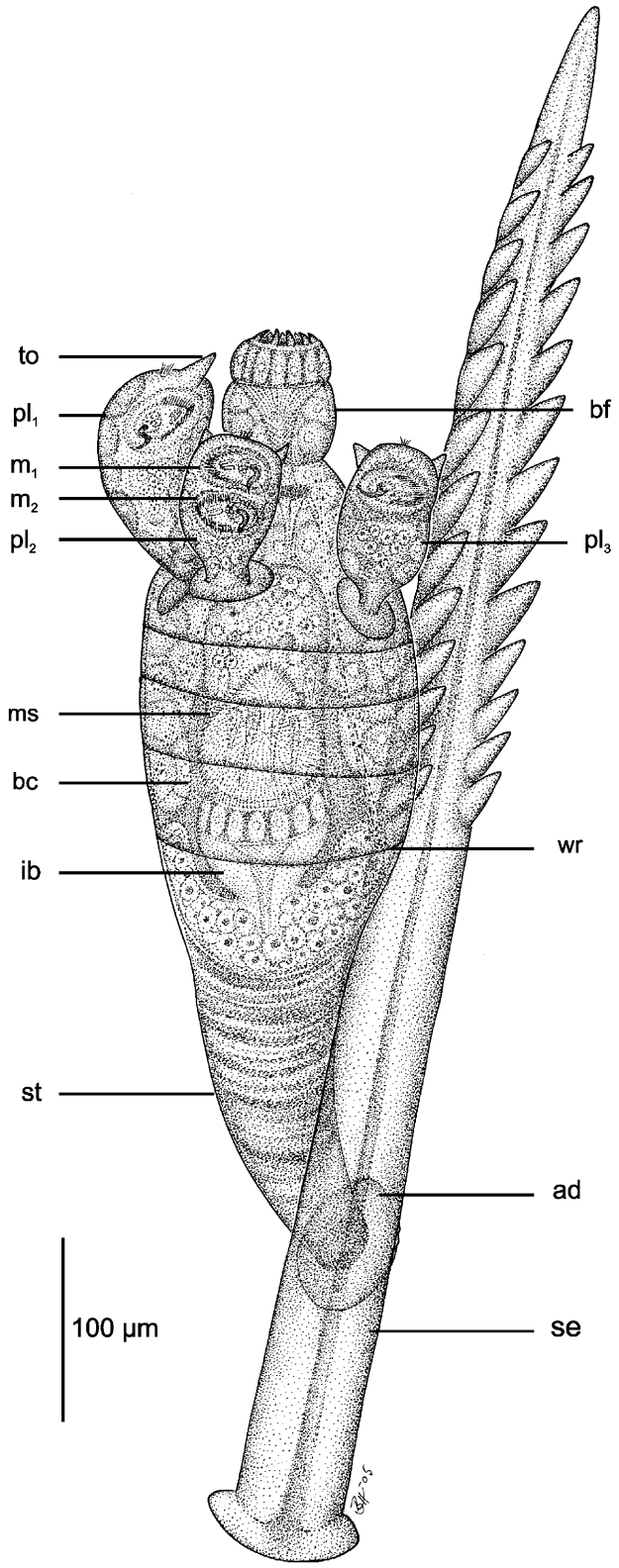

Fig. 1. *Symbion americanus* sp. nov., habitus of holotype with three (of five) attached paratypical Prometheus larvae ($pl_{1–3}$). Abbreviations: ad = adhesive disc; bc = brood chamber; bf = buccal funnel; ib = internal bud; $m_{1, 2}$ = male 1, 2; ms = motile stage; se = host seta; st = stalk; to = toe; wr = wrinkle.

dehydrated in an acetone/ethanol series, transferred to propylene oxide, and subsequently embedded in TAAB 812®. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963). Sections were examined and micrographs were obtained with a Phillips MORGAGNI 268 TEM.

Taxonomic section

-Phylum Cycliophora Funch and Kristensen, 1995
-Class Eucycliophora Funch and Kristensen, 1995
-Order Symbiida Funch and Kristensen, 1995
-Family Symbiidae Funch and Kristensen, 1995
-Genus *Symbion* Funch and Kristensen, 1995 (Type species: *Symbion pandora* Funch and Kristensen, 1995)

Symbion americanus sp. nov. (Figs. 1, 2)

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Etymology

The species epithet has been chosen in accordance with that of the host, *H. americanus*. Its ending matches the masculine gender (Funch and Kristensen 1995) of the genus name.

Type material

Holotype: feeding individual (whole mount, CYC 231 ZMUC) from sample 1 at Birch Harbor, Maine, USA, 44°23′N, 68°02′W, 8 April 2002. Paratypes (whole mounts, CYC 232–266 ZMUC and MCZ 61718–61722; TEM sections, grid box no. M02.01.1): all life stages except for free females and free chordoid larvae, found on mouthparts of ten *H. americanus* collected as sample 1. See also Table 1.

Additional material

All life cycle stages, collected in the USA. Sample 2: near Domariscotta, Maine, September 1996; Sample 3: observations in Boston on lobsters from Maine, May 2001; Sample 4: Cape Cod Bay near Sandwich, Massachusetts, October 2003; Sample 5: Birch Harbor, Maine (type locality), date unknown; Sample 6: observations in Stockholm, Sweden, on lobsters from Maine, August 2004. See also Table 1.

Diagnosis

Sessile feeding individuals of *Symbion americanus* sp. nov. on mouth appendages of *H. americanus* (Decapoda, Nephropidae). Feeding individual with buccal funnel, trunk, stalk, and adhesive disc (Figs. 1, 2). Free or attached Prometheus larva with paired toes at posterior end (Figs. 1, 3D, 7A); attached Prometheus

Fig. 2. *Symbion americanus* sp. nov., light-microscope (DIC) image of holotype with Prometheus larvae.

larva usually with two dwarf males inside (Fig. 1). Chordoid cysts often aggregated on exopodites and lateral areas of the mouthparts of *H. americanus*.

Descriptions

Holotype. Feeding individual (Figs. 1, 2) with closed buccal funnel and six transverse wrinkles (not scars) in the trunk, attached to a host seta with a slightly lobed, non-striated adhesive disc. Collected from a sexual population on the mouth appendages of *H. americanus*. Mounted in glycerol. Measurements: feeding individual length = 486 µm; maximum width = 134 µm; trunk length = 288 µm; acellular stalk length = 139 µm; closed buccal funnel length × width = 59 × 51 µm. Brood chamber of feeding individual with immature stage, probably a young Pandora larva or a Prometheus larva. Anterior end of internal stage fully developed, with glandular and ciliary organs; posterior end undifferentiated, without egg or buccal funnel; stage length = 120–140 µm. Basal trunk of the feeding individual with internal bud consisting of undifferentiated cells and fully developed buccal funnel; internal bud maximum length × width = 104 × 81 µm, buccal funnel width = 61 µm.

Paratypes – attached Prometheus larvae. Five Prometheus larvae on trunk of holotype. Three individuals attached to distal part of trunk, very close to buccal funnel (Fig. 1; $pl_{1\text{-}3}$). Measurements: pl_1 length = 125 µm, pl_2 length = 99 µm, pl_3 length = 101 µm, pl_1 toe length = 14 µm (Fig. 1; to). Two Prometheus larvae situated underneath holotype, attached to middle part of trunk, hardly visible (not drawn in Fig. 1). Two buds are inside pl_1, two males are inside pl_2 (Fig. 1; $m_{1,\,2}$), one fully developed male and one bud are inside pl_3.

Paratypes – others. Feeding individuals on average 376 µm long (Table 2), carry up to 13 shallow, ring-like wrinkles in cuticle of trunk (Fig. 1; wr). When closed, buccal funnel (Fig. 1; bf) has average diameter of 54 µm.

Pandora larva on average 187 µm long (Table 2), with a ventral ciliated field extending 54 µm in length and covered with locomotory cilia of about 12 µm length. Internal buccal funnel always present.

Prometheus larva (Figs. 3D, 7A) on average 125 µm long (Table 2), with a ventral ciliated field extending on average 43 µm in length, bearing locomotory cilia of 7 µm length. Anterior sensory organs consist of ventralia and frontalia (Fig. 7A; ve, fr) that are about 30 µm long. Larva with a posterior pair of tubular appendages or toes situated lateroventrally (Figs. 3D, 7A; to); the latter 19–24 µm long. LM observations on live Prometheus larvae show that toes are retractable (Fig. 7A). LM observations on whole mounts reveal several large muscle attachments ventrally at bases of toes, and tiny cuticular attachment fibres that may reach as far as tips of toes. Other live observations suggest presence of glands in the toes (Fig. 3D; gl?). It could not be ascertained whether gland ducts extend into tips of toes. TEM observations of posterior bodies of attached Prometheus larvae from type material reveal presence of mucus granules with a stippled appearance, but specimens too degraded to obtain more information on gland organization. Examination with SEM did not reveal any pores in the toe cuticle. However, a pore was observed nearly midventrally on each toe by LM in one specimen (whole mount CYC 232 ZMUC). Whether or not gland cells are associated with these pores remains to be documented. Free-swimming Prometheus larva lacks any penial structure, but

two internal buds (Fig. 3D; ib) were frequently observed that subsequently developed into dwarf males (Fig. 1; $m_{1,\ 2}$).

Dwarf males inside an attached Prometheus larva are oriented with dorsal side towards rear of larva and posterior end towards trunk of feeding individual. Free

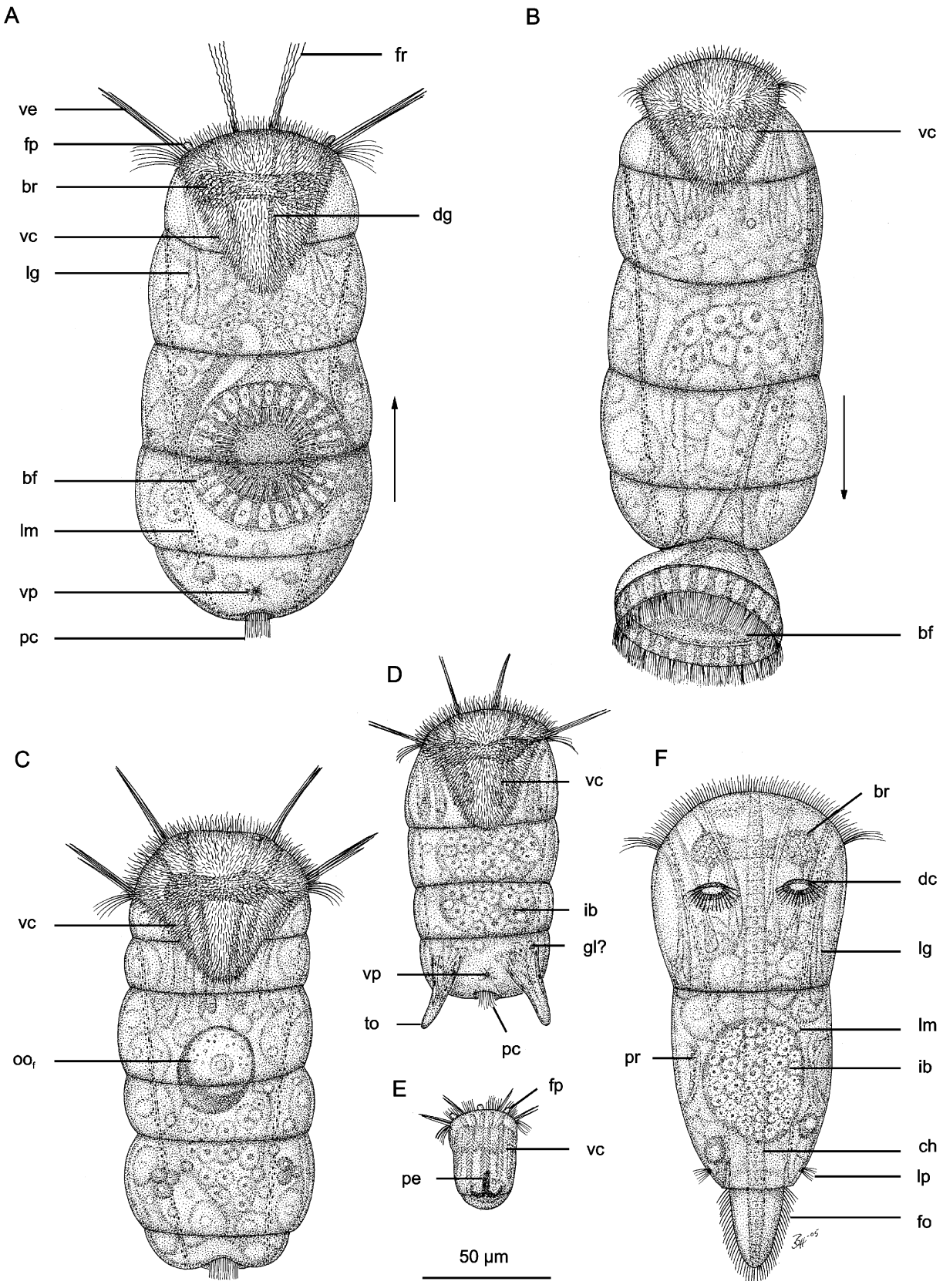

Table 2. Morphometrical comparison between *S. pandora* and the type materials of *Symbion americanus* sp. nov.

		S.pandora	*S.americanus*				
		Mean (μm)	Mean (μm)	Stdev (μm)	*n*	Min (μm)	Max (μm)
Feeding	Length	347	376	78	111	226	570
individual	Width	113	111	14	67	85	142
Pandora larva	Length	160	187	19	3	165	203
	Width	69	81	12	3	70	94
Prometheus larva	Length	110	125	6	6	119	137
	Width	45	62	12	6	50	80
Male	Length	33	57	7	2	52	63
	Width	21	40	12	2	31	49
Chordoid cyst	Length	145	157	15	27	123	186
	Width	103	80	9	27	51	96

Values for *S. pandora* are taken from the literature (Funch 1996; Funch and Kristensen 1997; Obst and Funch 2003); *n* = number of measured specimens; Min = lowest value; Max = highest value; Stdev = standard deviation.

dwarf males on average 57 μm long (Table 2). Ventral and frontal ciliated fields of male 27 μm long each, locomotory cilia on average 5 μm in length. Anterior sensory organs in the male, lateralia and frontalia, 20 μm long; frontal palps 5 μm long. Ventral side of male with a tubular falciform penis about 10 μm long and tapered towards tip.

We did not observe free-swimming females or chordoid larvae in the type material, although the intermediate stage, the chordoid cyst, was present (Fig. 8A). Cysts are on average 157 μm long (Table 2); they do not possess the distal unpaired appendix that is a typical feature in chordoid cysts from specimens collected in sample 2 (see below).

All ten lobsters in sample 1 were infested with populations of up to several thousand cyclophorans. Feeding individuals typically aggregated on the medial rims of all mouthparts in dense clusters (Fig. 9A). We counted up to 35 feeding individuals attached to a single host seta. If present, chordoid cysts often aggregate on the exopodites and lateral areas of the mouthparts.

Non-type feeding individuals. Cuticle of feeding individuals from sample 5 consists of thick procuticle and thin (40–60 nm), multilayered epicuticle with superficial polygonal sculpture; diameter ranging from 0.5 to 0.8 μm. Cuticle in buccal funnel relatively thin (0.5–1.3 μm), becomes thicker (1.5–3.2 μm) in upper trunk (Fig. 11A, B; cu), reaches up to 4.5 μm in lower trunk.

Fixed material from sample 2 and live material from sample 6 contain two types of feeding stages. Many presumably old feeding stages overgrown by cyanobacteria, with thick cuticle, a female in the brood chamber, and a trunk marked with deep, ring-like scars (Figs. 4, 5; sc). These overlapping pieces of thick cuticle appear different from wrinkles known from *S. pandora*. Acellular stalk (Figs. 4, 5; st) with distinct striation; adhesive disc (Figs. 4, 5, 6; ad) slightly lobed. However, younger feeding individuals that are without a brood chamber or with a Pandora larva inside lack these deep, ring-like cuticular scars and look very similar to feeding individuals from the type material (Figs. 1, 2).

TEM observations on feeding individuals from sample 5 show mass of nervous tissue, diameter approx. 7–8 μm (Fig. 11A; br), probably constituting cerebral ganglion at base of the buccal funnel. Nerve cells in buccal funnel contain one type of loosely distributed osmiophilic granules < 100 nm in diameter. In the trunk, two cells apparently of neural origin situated near rectum (Fig. 11B; nc?). Cell diameter 1.5–2.0 μm, cells filled with small osmiophilic vesicles 0.2 μm in diameter.

Non-type Pandora larvae. Observations on live Pandora larvae from samples 3 and 4 revealed presence of

Fig. 3. *Symbion americanus* sp. nov., free-living stages; A–E: ventral, F: dorsal view; A, B from sample 3, C, E, F from sample 2, D from type material. (A) young Pandora larva; whole mount CYC 292 ZMUC. (B) Pandora larva 22 h after escape from feeding stage; whole mount CYC 293 ZMUC. (C) female; whole mount CYC 272 ZMUC. (D) Prometheus larva; whole mount CYC 243 ZMUC. (E) dwarf male; whole mount CYC 268 ZMUC. (F) chordoid larva; whole mount CYC 276 ZMUC. Arrows indicate swimming direction. Abbreviations: bf = buccal funnel; br = brain; ch = chordoid organ; dc = dorsal ciliated organ; dg = dorsal glands; fo = foot; fp = frontal palps; fr = frontalia; gl? = glands?; ib = internal bud; lg = lateral glands; lm = longitudinal muscle; lp = lateral ciliated pit; oo_f = (presumably fertilized) oocyte; pc = posterior ciliated tuft; pe = penis; pr = protonephridium; to = toe; vc = ventral ciliated field; ve = ventralia; vp = ventral pore.

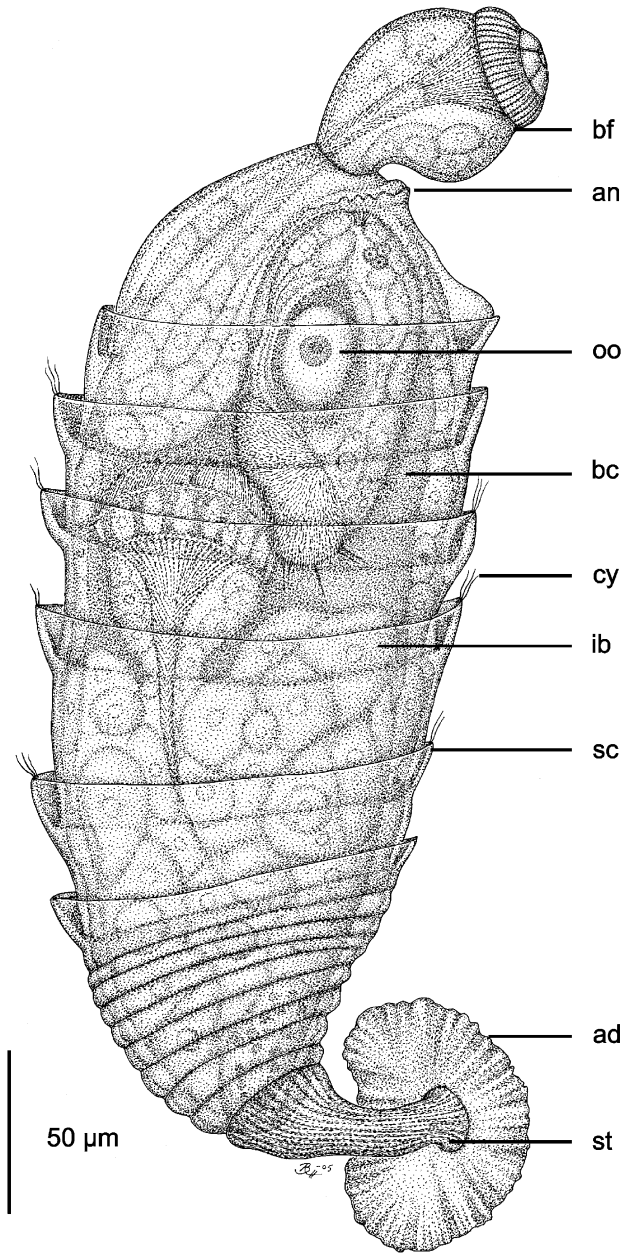

Fig. 4. *Symbion americanus* sp. nov., feeding stage with young female inside; from sample 2; whole mount CYC 272 ZMUC. Abbreviations: ad = adhesive disc; an = anus; bc = brood chamber; bf = buccal funnel; cy = cyanobacteria; ib = internal bud; oo = oocyte; sc = transversal scar; st = stalk.

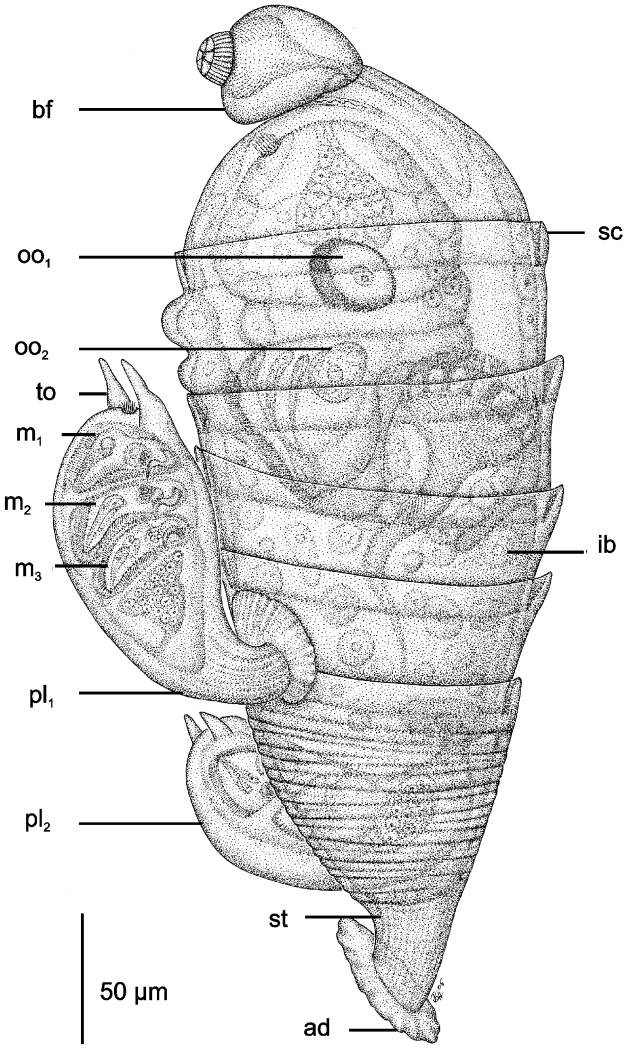

Fig. 5. *Symbion americanus* sp. nov., feeding stage with two attached Prometheus larvae ($pl_{1,2}$), each with three dwarf males inside ($m_{1–3}$); from sample 2, whole mount CYC 272 ZMUC. The feeding stage shows five transverse scars (sc) or 'moulting rings', and has a fully developed female with two oocytes inside ($oo_{1,2}$); a new buccal funnel with an immature gut arises from the internal bud (ib). Abbreviations: ad = adhesive disc; bf = buccal funnel; st = stalk; to = toe.

paired ventralia (Fig. 7B; ve) about 25 µm long, retractile frontalia (Fig. 3A; fr), and a pair of frontal palps situated medial to the ventralia (Fig. 3A; fp). More than 100 free Pandora larvae observed for about 20–24 h after removal of lobster mouth parts exhibited some moving with buccal funnel protruding from posterior part (see Fig. 3B).

In material from sample 2, two pores present at posterior end of Pandora larva. A small pore (3–7 µm) with ciliary protrusions and presumed sensory function situated ventrally (Fig. 9E; vp). A larger pore with a ciliary tuft situated at posterior end (Fig. 9B, E; pc), perhaps serving as opening for buccal funnel.

Non-type Prometheus larvae. Material from sample 2 includes a Prometheus larva with anterior glands in front of the cerebral ganglion, two pairs of lateral glands (45 µm long), and three pairs of dorsal glands (69 µm long), the latter having their outlets in front of the ventral ciliated field. Frontal palps, similar to those of the Pandora larva, have also been observed. Same material reveals two posterior pores, similar to Pandora larva. A small ventral pore (Fig. 9C; vp) may have

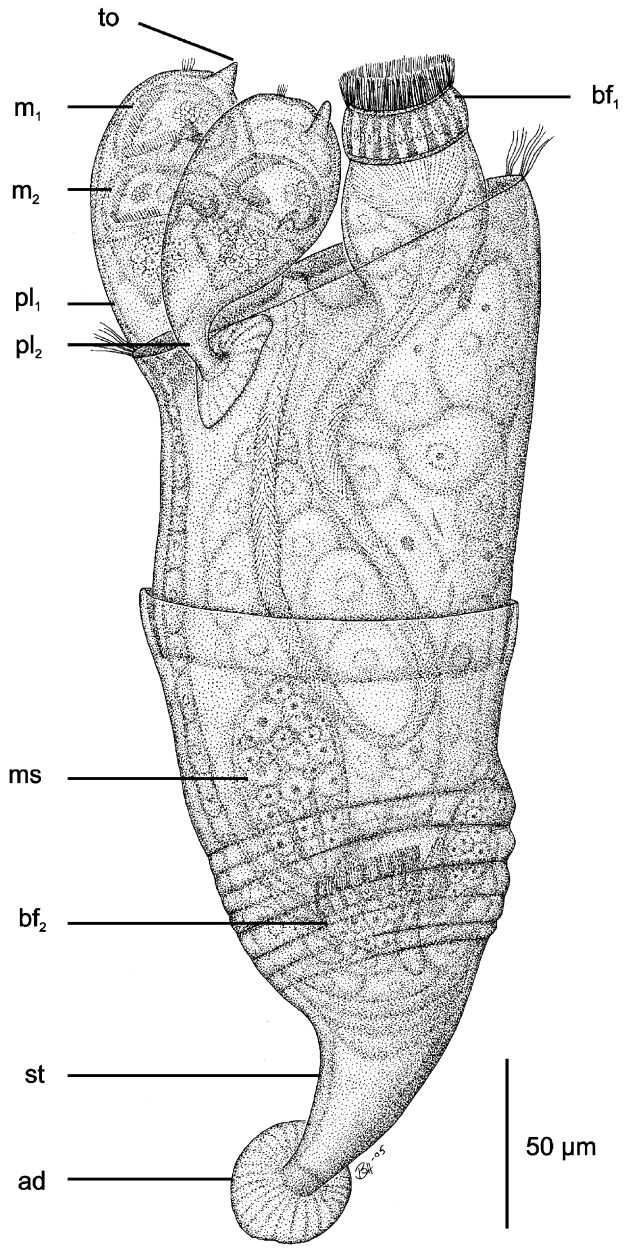

Fig. 6. *Symbion americanus* sp. nov., feeding stage with a new buccal funnel (bf_1); from sample 2, whole mount CYC 272 ZMUC. A free stage, possibly a female, has just left the feeding individual and broken the cuticle in a sharp line forming a scar or 'moulting ring'. Two Prometheus larvae ($pl_{1,2}$), each with two dwarf males inside ($m_{1,2}$), are attached close to the break in the cuticle. Inside the feeding stage a new buccal funnel (bf_2) and a motile stage (ms) are in early development. Abbreviations: ad = adhesive disc; st = stalk; to = toe.

sensory function, a larger posterior ciliated tuft (Fig. 9C; pc) may serve as exit for males. Furthermore, feeding individuals from this locality frequently carry attached Prometheus larvae containing three internal buds and/or males (Fig. 5; m_{1-3}).

Non-type males. Free males were observed in material from samples 2 and 4. One measured specimen with unpaired testis of 10 µm width and 7 µm length (Fig. 7E; te), densely packed with 25–30 spermatozoa.

Non-type females. We only observed two free females in the population from sample 2. Both individuals 164 µm long, with ventral ciliated field on average 56 µm in length, and with locomotory cilia about 11 µm in length. Each female with a single egg or zygote inside (Fig. 3C; oo_f), with average diameter of 25 µm and a large nucleus of about 5 µm. Females inside old feeding individuals seem to contain more than one oocyte (Fig. 5; $oo_{1,2}$), a feature never observed in *S. pandora*. Nearly all old feeding individuals from sample 2 contain a female in the brood chamber.

Non-type chordoid cysts. Morphology of chordoid cysts from sample 2 (Fig. 8B) differs remarkably from type material in sample 1 (Fig. 8A). Cysts ($n = 13$) larger: average length 220 µm, average width 95 µm (compare to Table 2). Each cyst from this locality possesses a conspicuous, distal, unpaired appendix (Figs. 8B, 9D; ap) with an average length of 26 µm. Appendix generally devoid of tissue; no pore observed with LM or SEM. However, all observations on sample 2 from fixed material; chordoid tissue sometimes difficult to observe in the cysts.

Non-type chordoid larvae. Morphology of chordoid larva also varies among localities. Chordoid larvae from sample 2 ($n = 13$) wedge-shaped and continuously tapered towards posterior end; average length 184 µm, maximum width 76 µm (Figs. 3F, 8D). Note that shape and length of body depend on contraction of musculature; contracted longitudinal muscles can result in more ovoid body shape. Unicellular lateral glands of these chordoid larvae (Figs. 3F, 8D; lg) have total length of 100–110 µm, start at posterior end of ventral ciliated field, and pass underneath buds towards anterior end. Gland duct is narrower (3–5 µm) in first two-thirds than in last one-third (duct width 6–9 µm). Outlets situated laterally, close to beginning of anterior ciliated bands. Terminal gland nucleus diameter 5 µm. Undifferentiated cells that form internal buds are present in posterior body. Buds either unpaired (Fig. 3F; ib) or paired (Fig. 8D; ib). If paired, buds slim and long, on average 77 µm long, 17 µm wide.

Chordoid larvae from sample 4 with oval-shaped body with a distinct foot (Figs. 8C, 10A, B). Paired internal buds round (Fig. 8C; ib), lateral glands shorter than in chordoid larvae from sample 2. Paired dorsal ciliated organs (Fig. 10A, E; dc) with diameter of 6–8 µm, and with bundle of cilia projecting from them. A pair of lateral ciliated pits, presumably of sensory function, located lateroventrally at junction between body and foot (Fig. 10B–D; lp). These organs 2 µm in diameter, consisting of fewer than 20 cilia about 4 µm in length and thicker at their bases. Lateral ciliated pits

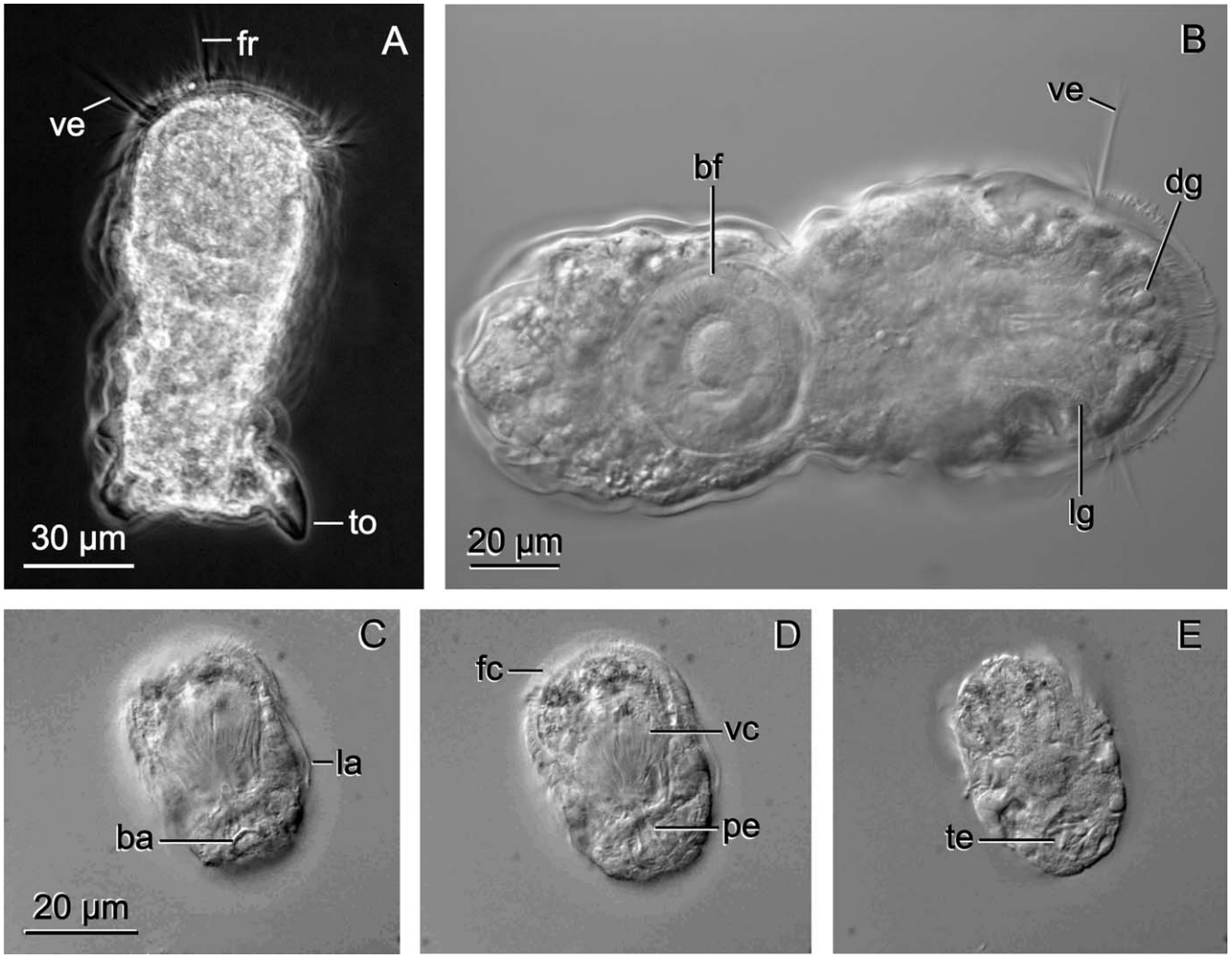

Fig. 7. *Symbion americanus* sp. nov., light-microscope images of motile life cycle stages. (A) dark-field view of a living Prometheus larva (from type material), with focus on the paired posterior toes (to), the frontalia (fr), and ventralia (ve); toes can be retracted individually. (B) DIC view of a living Pandora larva (from sample 4), showing long ventralia (ve), unicellular lateral (lg) and dorsal glands (dg), and a fully developed buccal funnel (bf) inside the posterior body. (C–E) DIC optical sections from ventral to dorsal of a fixed dwarf male (from sample 2, whole mount CYC 268 ZMUC); C with focus on penis base (ba) and lateralia (la), D with focus on frontal ciliated field (fc), penis (pe) and ventral ciliated field (vc), E with focus on testis (te).

also seen by LM in chordoid larvae from sample 2 (Fig. 3F).

Discussion

Morphological variation

There seems to be a high degree of morphological variation among the cycliophoran populations sampled from *H. americanus*. Considerable variation also occurs among cycliophorans from the European lobster *H. gammarus*; e.g., feeding individuals collected at the Danish island Anholt in 1991 are between 353 and 916 µm long (Funch 1992). Cycliophorans collected in sample 2 of the present study partly differ from the remaining material, although the Prometheus larva possesses the typical toes. In this population chordoid cysts show a typical distal appendix (Figs. 8B, 9D; ap), and chordoid larvae are wedge-shaped (Fig. 8D) with long and slim internal buds and lateral glands (Fig. 8D; ib, lg). Furthermore, in sample 2 we frequently observed three males inside attached Prometheus larvae (Fig. 5; m_{1-3}), and old feeding individuals appeared to have a slightly thicker cuticle marked with deep, ring-like scars. Such a high degree of morphological variation among cycliophorans on *H. americanus* may be due to the presence of cryptic species representing separate genetic lineages, as described for many other marine inverte-

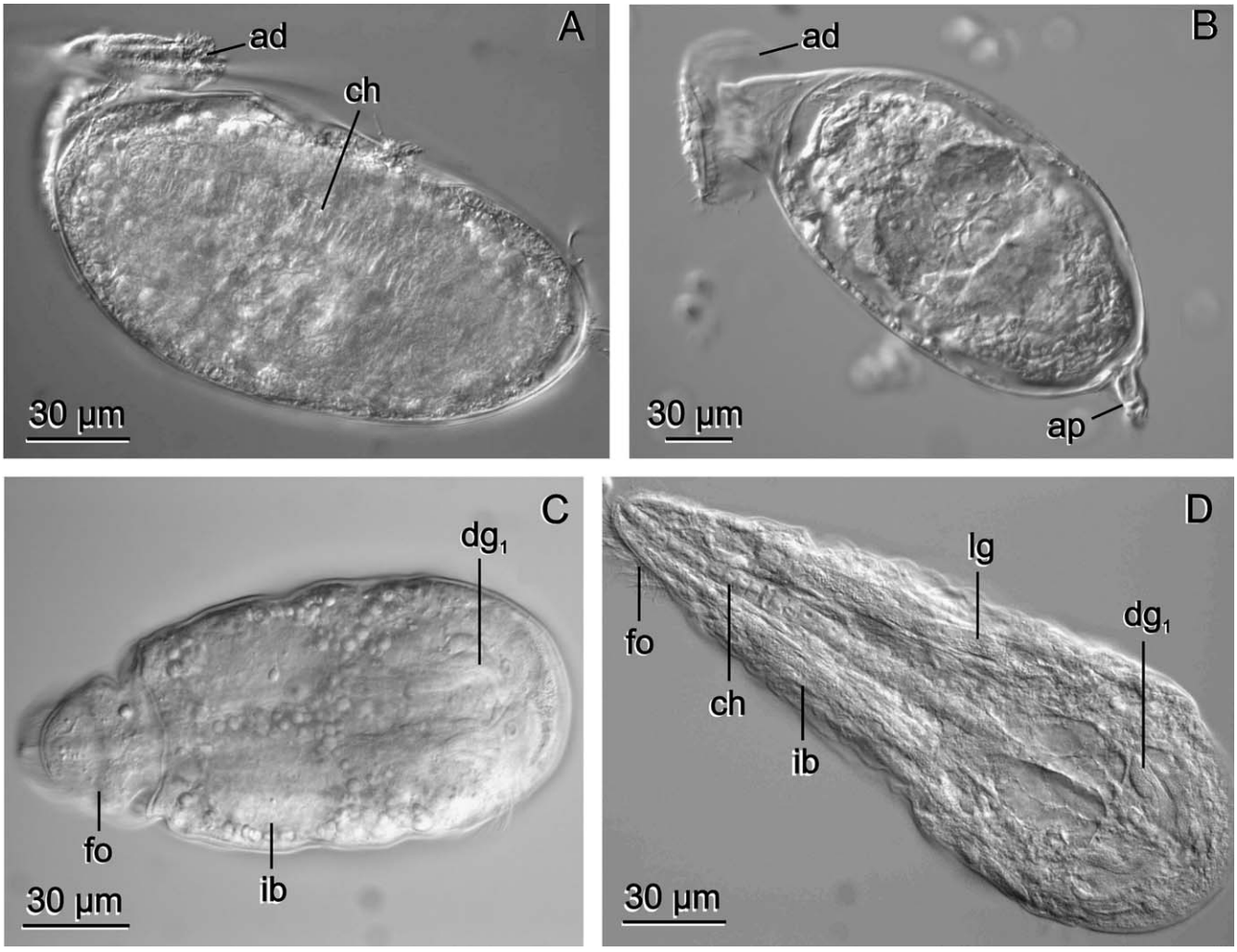

Fig. 8. *Symbion americanus* sp. nov., light-microscope (DIC) images of sexually produced stages from different localities. (A) fixed chordoid cyst; from type material, whole mount MCZ 61721. (B) fixed chordoid cyst; from sample 2, whole mount CYC 282 ZMUC. (C) living chordoid larva; from sample 4. (D) fixed chordoid larva; from sample 2, whole mount CYC 286 ZMUC. Abbreviations: ad = adhesive disc; ap = appendix; ch = chordoid organ; dg_1 = anterior dorsal glands; fo = foot; ib = internal bud; lg = lateral glands.

brates with a wide distribution, such as chaetognaths (Peijnenburg et al. 2004) or bryozoans (Thorpe et al. 1978). A study by Obst et al. (2005) actually indicates a high genetic diversity among cycliophorans on American lobsters. However, this diversity is also found among individuals from the same locality. Therefore, the existence of a single species on the American lobster cannot be ruled out.

We still lack information on parts of the life cycle; e.g., two types of feeding individuals possibly exist: one asexual type with thin cuticle forming the Pandora larva, and one sexual type with thicker cuticle and ring-like scars that usually develops a female. The presence of two types of sessile feeding individuals has been indicated previously by Funch and Kristensen (1997). The deep, ring-like scars (Figs. 4, 5; sc) in the cuticle of the old feeding individuals from samples 2 and 6 have not been observed before, and might originate from a different process than the wrinkles in *S. pandora*. Funch and Kristensen (1997) suggested that the wrinkles of the feeding individuals of *S. pandora* arise from repeated replacements of the buccal funnel, while free stages are released through the anus (cloaca) independent of these replacements. We observed several Pandora larvae of *S. americanus* sp. nov. escaping from living feeding stages through the anus, leaving no deep scars in the cuticle (Figs. 1, 2). The deeper scars observed in this study (Figs. 4–6), however, could arise if the replacement of the buccal funnel also involved a discarding of the distal part of the trunk. This more dramatic event would allow an internal stage, e.g. a female, to escape from the brood chamber. Unfortunately, we never observed the release of females in living *S. americanus* sp. nov., but have seen a single female fixed in the process of leaving a feeding

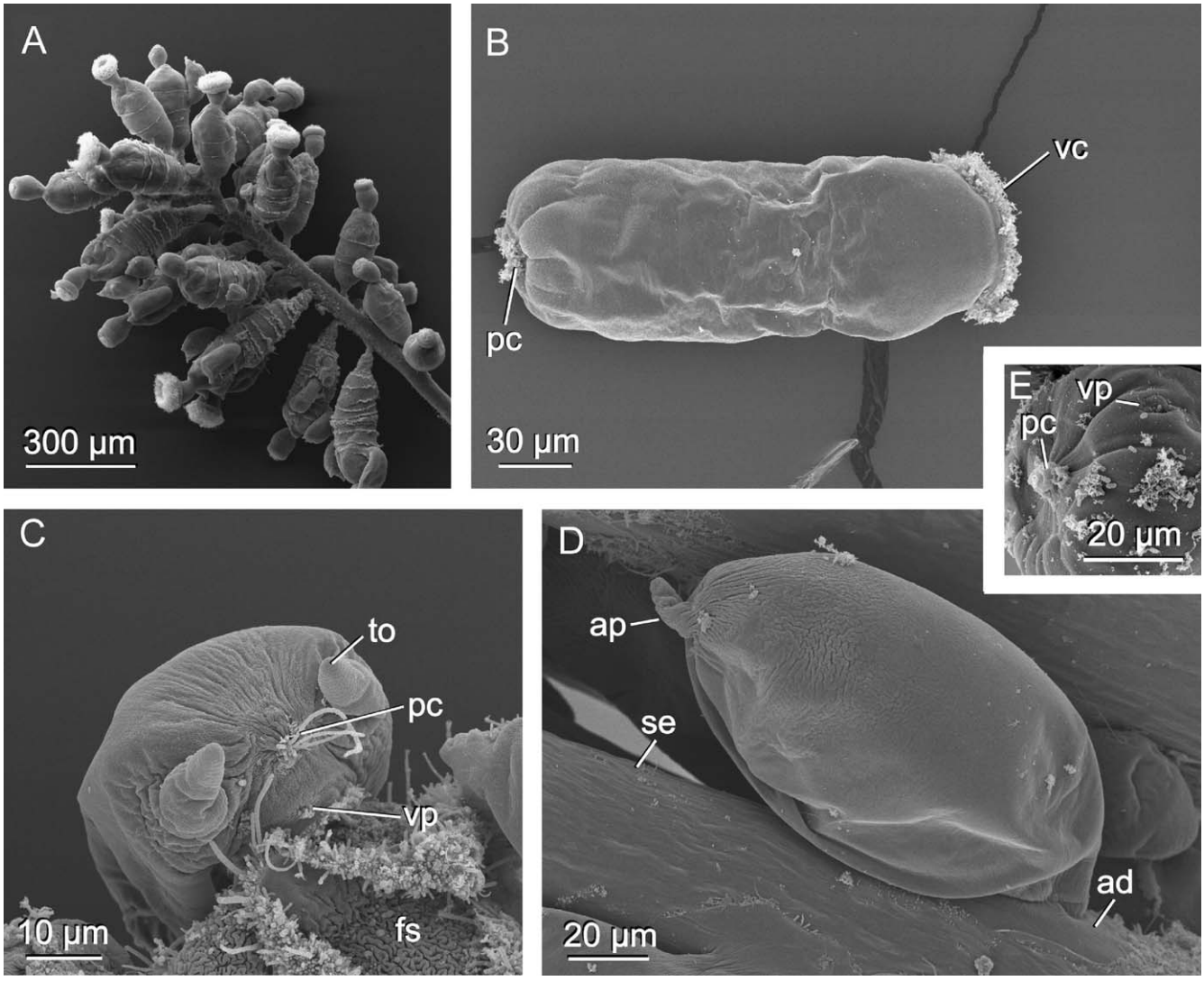

Fig. 9. *Symbion americanus* sp. nov., SEM micrographs of different life cycle stages; A from type material, B–E from sample 2. (A) feeding individuals with attached Prometheus larvae on a host seta. (B) Pandora larva, dorsal view. (C) attached Prometheus larva on trunk of a feeding stage (fs). (D) chordoid cyst attached to a host seta (se). (E) rear part of Pandora larva. Abbreviations: ad = adhesive disc; ap = appendix; pc = posterior ciliated tuft; to = toe; vc = ventral ciliated field; vp = ventral pore.

stage with a detached distal trunk and buccal funnel. Further studies of living material are needed to clarify whether the characteristic scars are connected to the release of internal stages.

Regardless of the variation among the cycliophoran populations on American lobsters, *Symbion americanus* sp. nov. can be distinguished clearly from *S. pandora* by the key diagnostic character: the presence of toes in the Prometheus larva. The populations of feeding individuals and chordoid cysts on *H. americanus* are usually denser than those of *S. pandora* on *N. norvegicus*, and often consist of up to several thousand individuals. Also, the rate of infestation with cycliophorans is higher among the investigated adult *H. americanus* compared to *N. norvegicus* (Obst and Funch 2005).

Phylogeny and speciation

The presence of a pair of posterior extensions, here called toes, in the Prometheus larva of *Symbion americanus* sp. nov. is a new character not observed in *S. pandora*. Comparable posterior extensions are present in other taxa, e.g. the toes in *Notommata copeus* (Monogononta, Rotifera) (Clément and Wurdak 1991), the spurs of *Philodina roseola* (Bdelloidea, Rotifera) (Dickson and Mercer 1966), or the furca of most chaetonotid gastrotrichs (Ruppert 1991) and the larva of *Nanaloricus mysticus* (Loricifera) (Kristensen 1991). The gland granules with stippled ultrastructure in the toes of the Prometheus larva are similar to those reported from the chordoid larva of *S. pandora* (Funch 1996). Such granules have been described from various

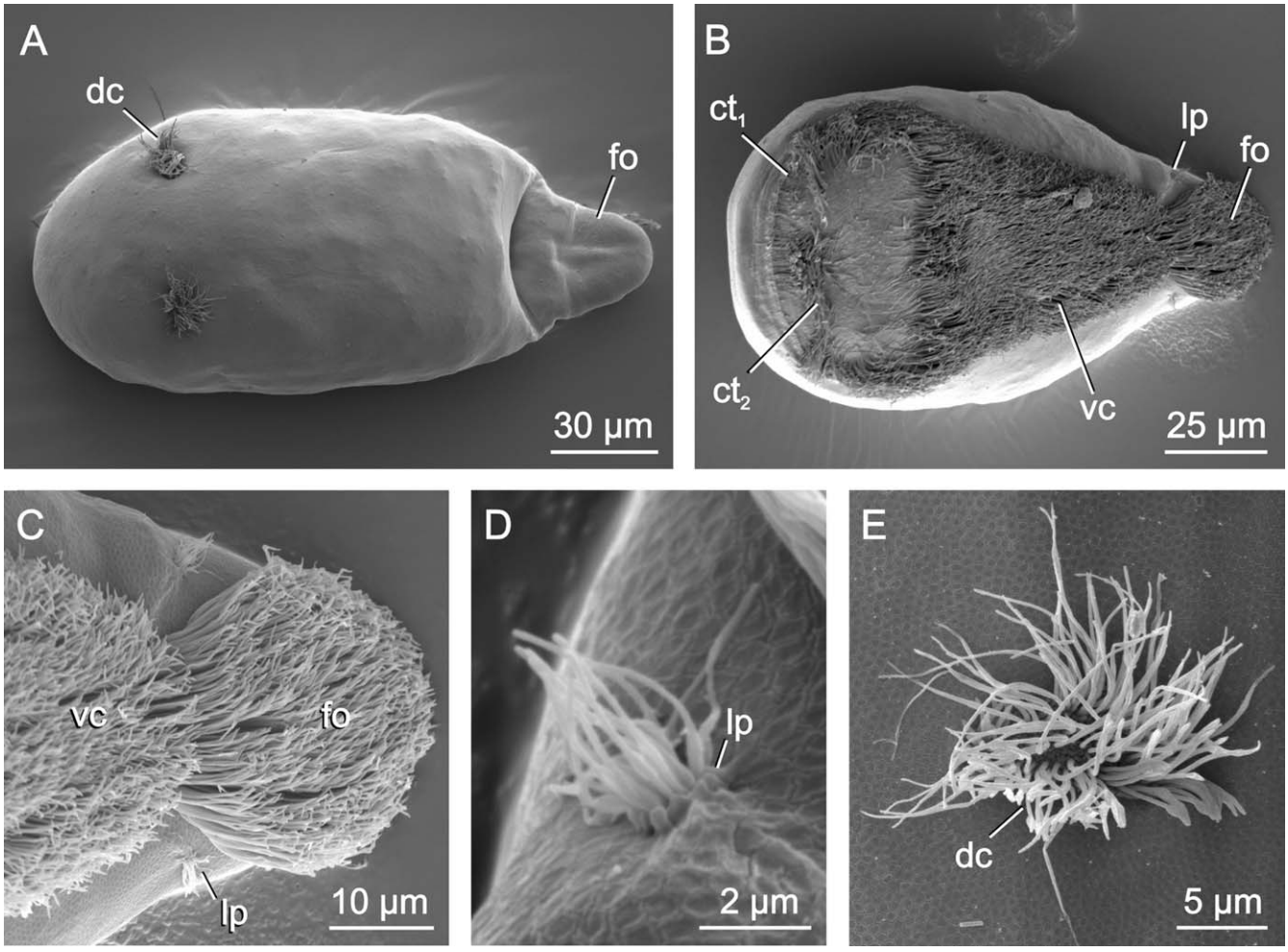

Fig. 10. *Symbion americanus* sp. nov., SEM micrographs of chordoid larvae; from sample 4. (A) dorsal view. (B) ventral view; showing the four ciliated areas: the two anterior ciliated bands ($ct_{1,\ 2}$), the ventral ciliated field (vc), and the ciliated foot (fo). (C) ventral view of junction between body and foot (fo). (D) lateroventral view of lateral ciliated pit (lp). (E) dorsal view of dorsal ciliated organ (dc). Abbreviations: dc = dorsal ciliated organ; fo = foot; lp = lateral ciliated pit; vc = ventral ciliated field.

taxa, e.g. from the rotifer *P. roseola* (Dickson and Mercer 1966), the kinorhynch *Pycnophyes greenlandicus* (Kristensen and Higgins 1991), and from the larva of the bryozoan *Bowerbankia gracilis* (Reed 1988). Future ultrastructural studies of the toes of the free Prometheus larva are needed before any homology statements can be postulated.

The cycliophoran species found to date live on the three closely related lobsters of the family Nephropidae (see Tshudy and Babcock 1997), on both sides of the North Atlantic. In Europe *S. pandora* lives on *N. norvegicus*, and another possible species is found on *Homarus gammarus*, while in North America *S. americanus* sp. nov. lives on *H. americanus*. Hedgecock et al. (1977) showed a relatively small genetic divergence between *H. gammarus* and *H. americanus* and suggested a recent speciation. This could imply that the above-described morphological differences between *S. pandora* and *S. americanus* sp. nov. arose after the hosts separated. Estimates for divergence of the two hosts range from 82,000 to 2 million years ago and suggest a Pleistocene separation due to glaciation. During that period, temperate habitats along the Atlantic margins were compressed, and today *Homarus* still is absent from coastal waters around Greenland and Iceland. Lobster populations from America and Europe, which were once connected through the North Atlantic, may have been separated ever since.

Additional cycliophoran species may occur on clawed lobsters other than the known hosts, *Homarus* and *Nephrops*. The genus *Homarus* is at least 60 My years old, based on fossil records dating back to Cretaceous (see Tshudy and Sorhannus 2003). The sister genus of *Homarus* is the monotypic genus *Homarinus*, which contains the rare Cape lobster *H. capensis* (Herbst, 1792) that is known only from a few localities in South Africa (Kornfield et al. 1994). This species has not been investigated for the occurrence of Cycliophora.

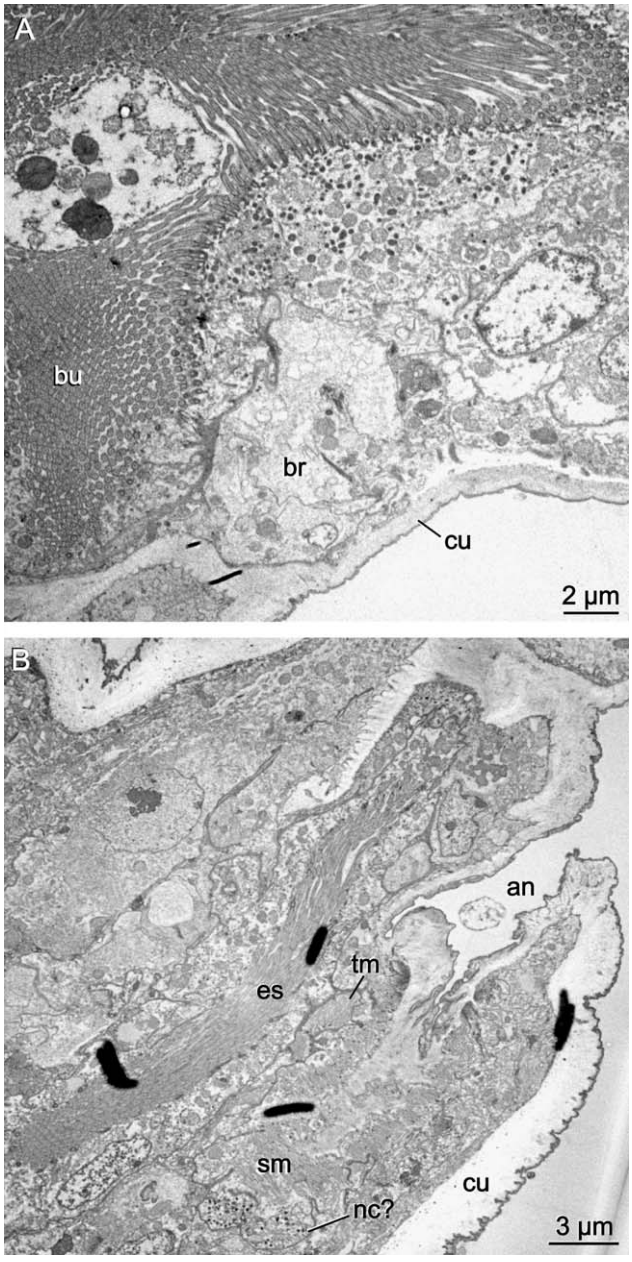

Fig. 11. *Symbion americanus* sp. nov., TEM micrographs of longitudinal sections around neck region of a feeding individual; from sample 5. (A) anal side of basal buccal funnel with nervous tissue probably constituting the brain (br). (B) distal part of trunk; two cells with osmiophilic vesicles (nc?) are located near a large sphincter muscle (sm) that surrounds the rectum; between anus (an) and esophagus (es) a long trunk muscle (tm) inserts in the body wall. Abbreviations: bu = buccal cavity; cu = cuticle.

Acknowledgements

We would like to thank Susanne Eriksson (Kristineberg) and Ole Tendal (Copenhagen) for collecting and sending lobster samples, Seth Tyler (Orono) and Gonzalo Giribet (Harvard) for providing laboratory space, SEM access, and embedded specimen for TEM, and Arvid Maunsbach (Aarhus) for access to TEM facilities. We appreciate the lobsters purchased by funds from the 'Stifterverband für die Deutsche Wissenschaft' during the first Euroscience Open Forum in Stockholm, Sweden, in August 2004. Furthermore, we would like to acknowledge technical support from Else Bomholt Rasmussen (Aarhus) during the TEM work, Birgitte Rubæk (Copenhagen) who contributed the drawings, and E. Davis Parker (Aarhus) for English language corrections. Financial support for this study was provided by the Danish Research Agency (contract grant nos. 21-02-0455 and 21-04-0047) and by the US National Science Foundation (contract grant no. 0334932).

References

Clément, P., Wurdak, E., 1991. Rotifera. In: Harrison, F.W., Woollacott, R.M. (Eds.), Microscopic Anatomy of Invertebrates. vol. 4. Aschelminthes. Wiley-Liss, New York, pp. 219–297.

Cobb, J.S., 1971. The shelter-related behaviour of the lobster *Homarus americanus*. Ecology 52, 108–115.

Dalton, A.J., 1955. A chrome-osmium fixative for electron microscopy. Anat. Rec. 121, 281.

Dickson, M.R., Mercer, E.H., 1966. Fine structure of the pedal gland of *Philodina roseola* (Rotifera). J. Microsc. 5, 81–90.

Funch, P., 1992. Beskrivelse af en ny klasse–Mesoprocta. Unpublished Master's thesis, University of Copenhagen.

Funch, P., 1996. The chordoid larva of *Symbion pandora* (Cycliophora) is a modified trochophore. J. Morphol. 230, 231–263.

Funch, P., Kristensen, R.M., 1995. Cycliophora is a new phylum with affinities to Entoprocta and Ectoprocta. Nature 378, 711–714.

Funch, P., Kristensen, R.M., 1997. Cycliophora. In: Harrison, F.W., Woollacott, R.M. (Eds.), Microscopic Anatomy of Invertebrates, vol. 13. Lophophorates, Entoprocta and Cycliophora. Wiley-Liss, New York, pp. 409–474.

Funch, P., Kristensen, R.M., 1999. Cycliophora. In: Knobil, E., Neill, J.D. (Eds.), Encyclopedia of Reproduction, vol. 1. Academic Press, New York, pp. 800–808.

Funch, P., Sørensen, M.V., Obst, M., 2005. On the phylogenetic position of Rotifera–have we come any further? Hydrobiologia 546, 1–18.

Giribet, G., Distel, D.L., Polz, M., Sterrer, W., Wheeler, W.C., 2000. Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cycliophora, Plathelminthes, and Chaetognatha: a combined approach of 18S rDNA sequences and morphology. Syst. Biol. 49, 539–562.

Giribet, G., Sørensen, M.V., Funch, P., Kristensen, R.M., Sterrer, W., 2004. Investigations into the phylogenetic position of Micrognathozoa using four molecular loci. Cladistics 20, 1–13.

Hedgecock, D., Nelson, K., Simons, J., Shleser, R., 1977. Genic similarity of American and European species of lobster *Homarus*. Biol. Bull. 152, 41–50.

Hughes, J.T., Matthiessen, G.C., 1962. Observations on the biology of the American lobster, *Homarus americanus*. Limnol. Oceanogr. 7, 414–421.

Kornfield, I., Williams, A.B., Steneck, R.S., 1994. Assignment of *Homarus capensis* (Herbst, 1792), the Cape lobster of South Africa, to the new genus *Homarinus* (Decapoda: Nephropidae). Fish. Bull. 93, 97–102.

Kristensen, R.M., 1991. Loricifera. In: Harrison, F.W., Woollacott, R.M. (Eds.), Microscopic Anatomy of Invertebrates. vol. 4. Aschelminthes. Wiley-Liss, New York, pp. 351–375.

Kristensen, R.M., 2002. An introduction to Loricifera, Cycliophora, and Micrognathozoa. Integr. Comp. Biol. 42, 641–651.

Kristensen, R.M., Funch, P., 2002. Cycliophora. In: Young, C.M., Sewell, M.A., Rice, M.E. (Eds.), Atlas of Marine Invertebrate Larvae. Academic Press, London, pp. 199–208.

Kristensen, R.M., Higgins, R.P., 1991. Kinorhyncha. In: Harrison, F.W., Woollacott, R.M. (Eds.), Microscopic Anatomy of Invertebrates. vol. 4. Aschelminthes. Wiley-Liss, New York, pp. 377–404.

Lawton, P., Lavalli, K.L., 1995. Postlarval, juvenile adolescent and adult ecology. In: Factor, J.R. (Ed.), Biology of the Lobster *Homarus americanus*. Academic Press, San Diego, pp. 47–81.

McRae, E.D., 1960. Lobster explorations on continental shelf and slope off northeast coast of the United States. Commer. Fish. Rev. 22, 1–7.

Nedvěd, O., 2004. Occurrence of the phylum Cycliophora in the Mediterranean. Mar. Ecol. Prog. Ser. 277, 297–299.

Obst, M., 2003. Cycliophoran relationships revisited. Cladistics 19, 159–160.

Obst, M., Funch, P., 2003. Dwarf male of *Symbion pandora* (Cycliophora). J. Morphol. 255, 261–278.

Obst, M., Funch, P., 2005. The microhabitat of *Symbion pandora* (Cycliophora) on the mouthparts of *Nephrops norvegicus* (Decapoda: Nephropidae). Mar. Biol. in press, doi:10.1007/s00227-005-0131-1.

Obst, M., Funch, P., Giribet, G., 2005. Hidden diversity and host specificity in cycliophorans: a phylogeographic analysis along the North Atlantic and Mediterranean. Sea. Mol. Ecol. 14, 4427–4440.

Peijnenburg, K.T.C.A., Breeuwer, J.A.J., Pierrot-Bults, A.C., Menken, S.B.J., 2004. Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. Evolution 58, 1472–1487.

Peterson, K.J., Eernisse, D.J., 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. Evol. Dev. 3, 170–205.

Reed, C.G., 1988. Organisation of the nervous system and sensory organs in the larva of the marine bryozoan *Bowerbankia gracilis* (Ctenostomata: Vesiculariidae): functional significance of the apical disc and pyriform organ. Acta Zool. 69, 177–194.

Reynolds, E.S., 1963. Use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17, 208–212.

Riisgard, H.U., Nielsen, C., Larsen, P.S., 2000. Downstream collecting in ciliary suspension feeders: the catch- up principle. Mar. Ecol. Prog. Ser. 207, 33–51.

Ruppert, E.E., 1991. Gastrotricha. In: Harrison, F.W., Woollacott, R.M. (Eds.), Microscopic Anatomy of Invertebrates, vol. 4. Aschelminthes. Wiley-Liss, New York, pp. 41–109.

Sørensen, M.V., Funch, P., Willerslev, E., Hansen, A.J., Olesen, J., 2000. On the phylogeny of the metazoa in the light of Cycliophora and Micrognathozoa. Zool. Anz. 239, 297–318.

Sørensen, M.V., Kristensen, R.M., 2004. Cycliophora. In: Schlager, N. (Ed.), Grzimek's Animal Life Encyclopedia, vol. 1. Lower Metazoans and Lesser Deuterostomes. Schlager Group Inc., Chicago, pp. 351–354.

Thorpe, J.P., Beardmore, J.A., Ryland, J.S., 1978. Genetic evidence for cryptic speciation in marine bryozoan *Alcyonidium gelatinosum*. Mar. Biol. 49, 27–32.

Tshudy, D., Babcock, L.E., 1997. Morphology-based phylogenetic analysis of the clawed lobsters (family Nephropidae and the new family Chilenophoberidae). J. Crustac. Biol. 17, 253–263.

Tshudy, D., Sorhannus, U., 2003. *Hoploparia*, the best-known fossil clawed lobster (family Nephropidae), is a 'wastebasket' genus. J. Crustac. Biol. 23, 700–711.

Winnepenninckx, B.M.H., Backeljau, T., Kristensen, R.M., 1998. Relations of the new phylum Cycliophora. Nature 393, 636–638.

Zrzavỳ, J., Mihulka, S., Kepka, P., Bezdék, A., Tietz, D., 1998. Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. Cladistics 14, 249–285.

Zrzavỳ, J., Hypša, V., Tietz, D.F., 2001. Myzostomida are not annelids: molecular and morphological support for a clade of animals with anterior sperm flagella. Cladistics 17, 170–198.