R e s e a r c h   a r t i c l e

Morphological observations on three Baltic species of *Corynosoma* Lühe, 1905 (Acanthocephala, Polymorphidae)

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Abstract. Necropsies of Baltic grey (*Halichoerus grypus*) and ringed seals (*Pusa hispida*) presented a rare opportunity to study their acanthocephalan fauna. Both species hosted adults of three species of the genus *Corynosoma* Lühe, 1904, namely *C. magdaleni* Montreuil, 1958, *C. semerme* (Forsell, 1904) Lühe 1911 and *C. strumosum* (Rudolphi, 1802) Lühe 1904. A comparative morphological analysis of these three species of *Corynosoma*, combining both light and scanning electron microscopy, was performed for the first time. Sexual dimorphism in the size and shape of the trunk was observed in both *C. magdaleni* and *C. semerme*, but there was insufficient material to investigate this phenomenon in *C. strumosum*. Genital spines were not observed in any of the female acanthocephalans. Three possible explanations for the presence of genital spines in some females, but not others are (i) cryptic speciation, (ii) phenotypic variation and (iii) loss by extraction or shearing when the copulatory cap is released. Copulatory caps were observed on female *C. semerme*. The size and morphology showed considerable variability and all caps were strongly autofluoresecent.

Keywords. Baltic Sea, grey seals, ringed seals, SEM, thorny-headed worms.

Introduction

The Baltic Sea area, one of the largest areas of brackish water in the world, is characterised by a strong salinity gradient from marine waters at the Swedish west coast, to brackish water in the Baltic Proper, and nearly freshwater in the Bothnian Bay and Gulf of Finland. In the latter regions, populations of grey seals, *Halichoerus grypus* (Fabricius, 1791) and ringed seals, *Pusa hispida* (Schreber, 1775)

coexist. Baltic seal species are often heavily infested with a variety of metazoan parasites, including the seal louse *Echinophthirius horridus* (von Olfers, 1816) (Arthropoda: Echinophthiriidae), the heartworm *Acanthocheilonema spirocauda* (Leidy, 1858) (Nematoda: Onchocercidae), the lungworm *Otostrongylus circumlitus* (Railliet, 1899) (Nematoda: Crenosomatidae) and thorny-headed worms of the genus *Corynosoma* Lühe, 1904 (Acanthocephala: Polymorphidae) (see Leidenberger *et al*. 2007; Leidenberger & Boström 2008, 2009).

Seals of the Baltic Sea are the definitive host of three species of *Corynosoma*, namely *C. magdaleni* Montreuil, 1958, *C. semerme* (Forssell, 1904) Lühe 1911 and *C. strumosum* (Rudolphi, 1802) Lühe 1904. The species of *Corynosoma* in the Baltic Sea are not morphologically well characterized. Most studies of these acanthocephalans addressed ecological questions (e.g., Sinisalo *et al*. 2003, 2004; Valtonen *et al*. 2004). Nickol *et al*. (2002) provided morphological descriptions of *C. magdaleni* and *C. strumosum* from the Gulf of Bothnia and these included detailed line drawings but no micrographs from light microscopy (LM) or scanning electron microscopy (SEM). Recently, Waindok *et al*. (2018) used DNA sequence data to characterize species of *Corynosoma* from harbour seals and grey seals of the German North and Baltic Seas. However, their morphological observations were restricted to a limited number of morphometric and meristic measurements.

In the present study, we used LM and SEM to make observations on the morphology of *Corynosoma* spp. found in grey and ringed seals in the Baltic Sea coast of Sweden.

Material and methods

Thorny-headed worms were collected during seal necropsies at the Swedish Museum of Natural History (SMNH) between 2007 and 2008 (Table 1). The seals were caught through hunting in Baltic Sea regions along the coast of Sweden at this time (Leidenberger & Bäcklin 2008). A total of ten grey seals, *H. grypus* of both genders, and ranging in age 0–21 years (determined by growth layer groups after Hewer 1964), were collected from the following Swedish provinces: Skärplinge and Östhammar/Upplands Province, Hudiksvall and Stocka/Gävleborgs Province, and Luleå/Norrbottens province (Fig. 1). Three ringed seals, *P. hispida*, were collected from Tisteröarna/Norrbottens Province (Table 1, Fig. 1). The seals were frozen after capture for storage, and then thawed prior to necropsy. All seals were infected by *Corynosoma* spp. (prevalence: 100%). One grey seal individual had a mixed infection of all three species, and four individuals had a mixed infection with two species. In ringed seals, only one individual had a mixed infection with two species. In grey seals, the intensity varied between 27 and 4387 parasites (mean: 1338) and in ringed seals between 149 and 616 parasites (mean: 348). The parasites were removed from the intestines (ileum, cecum and colon), then fixed and stored in 70% ethanol for microscopic examination.

Ideally, acanthocephalans should be collected live and then relaxed in tap water to evert the proboscis, before fixation (Van Cleave 1953). However, in the present study the acanthocephalans were killed before collection, when they were frozen inside their seal hosts, and so could not be relaxed prior to preservation (Leidenberger & Bäcklin 2008). Consequently, most specimens had a partially or fully introverted proboscis. Even in the few specimens with a fully everted proboscis, hooks were sometimes broken or obscured by host tissue debris. The three species of *Corynosoma* identified in the seal specimens from the Swedish Baltic Sea, *C. magdaleni*, *C. semerme* and *C. strumosum*, were discriminated primarily on basis of patterns of trunk spination. Morphometric and meristic data for the three taxa are listed in Table 2. Of the almost 1000 specimens of *C. semerme* examined, only 30 (14 females and 16 males) were in a suitable condition for morphological study, i.e., most conventional morphological characters were visible in each of these specimens (Table 2). *C. magdaleni* and *C. strumosum* were collected in smaller numbers (10 and 2 respectively), and it was not possible to find a single specimen of either species with a fully everted proboscis. Ten specimens (6 females and 4 males) of *C. magdaleni* and two

Table 1. Material of *Corynosoma magdaleni*, *C. semerme* and *C. strumosum* studied from seals in the Baltic Sea and studied by SEM and/or LM. Abbreviation: yrs = years.

Species	Host(s)	Host gender (age in yrs)	Locality	Habitat	Material	Number of specimens
C. magdaleni	grey seals	♂ (9)	Stocka/Gävleborgs province, Bothnian Sea	ileum	SMNH-162973	4 ♀♀, 1 ♂
		♂ (12)		colon	SMNH-162973	1 ♂
		♀ (0)	Skärplinge/Uppsala province, Bothnian Sea	colon	SMNH-162973	1 ♂
		♀ (2)	Luelå/Norrbottens province, Bothnian Bay	ileum	SMNH-162973	1 ♀
		♀ (21)		colon	SMNH-162973	1 ♂
	ringed seal	♀ (12)	Tistersöarna, Luleå/ Norrbottens province, Bothnian Bay	ileum	SMNH-162973	1 ♀
	grey seal	(unknown)	Åland, Bothnian Sea (Nickol *et al.* 2002)	(unknown)	HWML- 39480-1141-2 HWML- 39480-1041-3	1 ♀ 1 ♂
C. semerme	grey seals	♂ (12)	Stocka/Gävleborgs province, Bothnian Sea	colon	SMNH-162974 SMNH-162975	14 ♀♀ 10 ♂♂
		♂ (19)	Hudiksvall/Gävleborgs province, Bothnian Sea	colon	SMNH-162976	6 ♂♂
C. strumosum	grey seals	♂ (9)	Stocka/Gävleborgs province, Bothnian Sea	colon	SMNH-162977	1 ♀
		♂ (21)	Östhammar/Uppsala provinces, Ålands hav	ileum	SMNH-162978	1 ♂
		(unknown)	Åland, Bothnian Sea (Nickol *et al.* 2002)	(unknown)	HWML-39481-1158-2 HWML-39481-1142-4	1 ♀ 1 ♂

specimens (one of each gender) of *C. strumosum* were selected for morphological study on the basis that most taxonomic characters were visible in these worms.

Voucher specimens are deposited in the invertebrate collection of the Department of Zoology, Swedish Museum of Natural History (SMNH), Stockholm, Sweden (Nos. SMNH-162973-162978, SMNH-172304-172310). Additionally, specimens of *C. magdaleni* and *C. strumosum*, collected by Nickol *et al.* (2002) from the Finish Baltic Sea, were borrowed from the University of Nebraska State Museum, Manter Laboratory of Parasitology (Nos. HWML 39480-39481).

For LM, 42 specimens stored in 70% ethanol were transferred to pure glycerine by a slow evaporation method (Seinhorst 1959) and mounted on slides in glycerine. Observations were made with a Leitz Laborlux S microscope and measurements were taken with an ocular micrometer. All measurements (Fig. 2, Table 2) are in micrometres, unless otherwise noted. The range is followed by the mean value in parentheses. Width measurements represent the maximum width. The fore-trunk does not include the proboscis or neck and the hind-trunk not the bursa in males. Eggs of gravid females were measured through the body wall. Measurements of the proboscis hooks represent the length of the blade and the width of the base of the blade, as defined by Wayland (2010).

Copulatory caps were imaged using a Leica SP5 confocal microscope. Serial optical sections were obtained at 2.4 μm intervals, using a plan-apochromat 10×/0.4 NA objective. A 405 nm laser was used for excitation, and autofluorescence was recorded in the range 415–514 nm using a photomultiplier tube. A maximum intensity projection was used to transform the serial optical sections into a 2D image. Image processing was performed in Fiji (Schindelin *et al.* 2012, 2015).
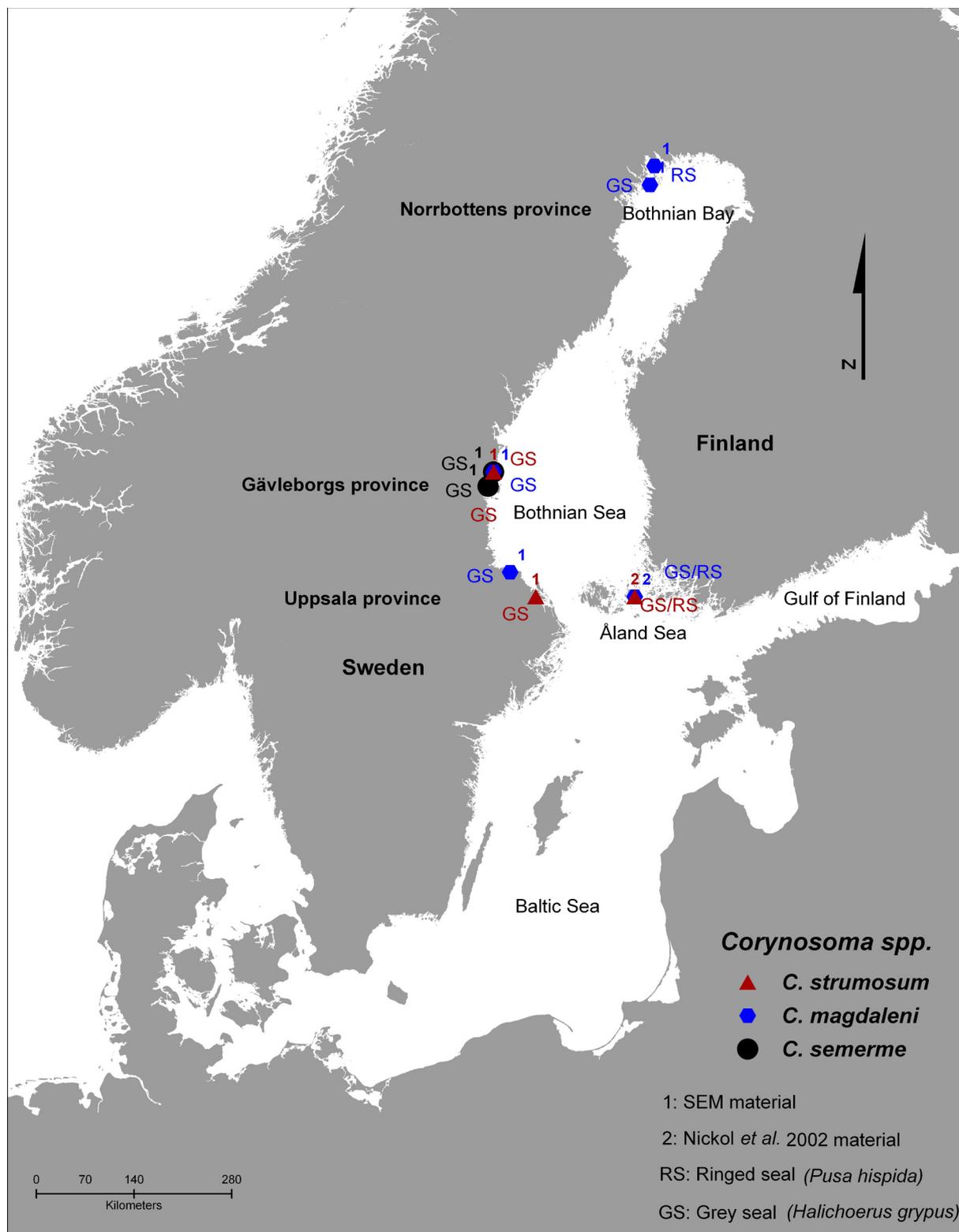
Fig. 1. Map of sampling localities.

Table 2. Morphometric characters in *Corynosoma magdaleni* Montreuil, 1958*, C. semerme* (Forsell, 1904) Lühe, 1911 and *C. strumosum* (Rudolphi, 1802) Lühe 1904 from grey seals, *Halichoerus grypus*, in the Baltic Sea presented as mean and (range). All measurements in micrometre (µm) unless otherwise noted. n = number of specimens measured is smaller than number of specimens available. * = proboscis not fully everted.

| Sex	*Corynosoma magdaleni* ♀	*Corynosoma magdaleni* ♂	*Corynosoma semerme* ♀	*Corynosoma semerme* ♂	*Corynosoma strumosum* ♀	*Corynosoma strumosum* ♂
Number of specimens	6	4	14	16	1	1
Body length (mm)	4.36 (3.67–5.11)	3.61 (2.53–4.39)	2.50 (1.83–3.13)	2.32 (1.91–2.73)	4.90	5.67
Fore-trunk length (mm)	0.86 (0.74–0.95)	0.92 (0.73–1.20)	1.11 (0.77–1.49)	1.00 (0.78–1.46)	0.91	1.43
Fore-trunk width (mm)	0.58 (0.50–0.68)	0.49 (0.40–0.58)	0.95 (0.64–1.21)	0.89 (0.72–1.04)	0.58	0.99
Hind-trunk length (mm)	3.50 (2.86–4.37)	2.69 (1.81–3.19)	1.39 (0.63–1.79)	1.32 (0.85–1.53)	3.99	4.24
Hind-trunk width (mm)	0.35 (0.30–0.41)	0.34 (0.26–0.46)	0.68 (0.39–0.86)	0.63 (0.51–0.79)	0.35	0.6
Trunk spines length (fore-trunk)	30 (20–40)	32 (30–40)	30 (10–50)	34 (20–50)	20	30
Neck length	165 (150–190) (n = 3)	113 (n = 2)	175 (90–280) (n = 10)	168 (140–230) (n = 13)	–	160
Neck width	221 (210–240) (n = 3)	153 (110–190) (n = 2)	314 (240–410) (n = 10)	262 (230–300) (n = 13)	–	350
Proboscis length	[306 (250–360)]* (n = 2)	[219 (190–250)]*	393 (280–510) (n = 7)	472 (360–610) (n = 7)	[140]*	[270]*
Proboscis width	176 (140–200)	144 (140–150)	242 (180–290)	226 (190–260) (n = 14)	140	190
Proboscis receptacle length	490 (n = 1)	666 (640–690) (n = 2)	941 (690–1230) (n = 8)	776 (630–1250) (n = 10)	–	790
No. of longitudinal rows of hooks	18 (16–20)	17 (16–20)	23 (20–25)	23 (22–24)	20	18
No of hooks in each row	10 (8–11)	8 (8–10)	11 (10–12)	12 (10–14)	10	10
Subdivision of hooks in each row	3 (2–3) 3 (2–4) gap 4 (3–4)		5 (4–6) 3 (2–3) 4 (4–5)		4 2 4	
Hook length, anterior series	30 (n = 2)	38 (30–50) (n = 2)	39 (20–50) (n = 9)	41 (10–60) (n = 15)	20	30
Hook length, middle series	47 (20–50)	44 (40–50)	59 (30–80)	60 (30–80) (n = 15)	30	60
Hook length, posterior series	29 (20–40) (n = 5)	24 (20–30) (n = 2)	28 (10–40) (n = 12)	27 (10–40) (n = 13)	20	30
Genital spines length	–	17 (n = 3)	–	43 (30–50)	–	20
Testes length	–	–	–	283 (150–400) (n = 13)	–	430
Testes width	–	–	–	223 (140–330) (n = 13)	–	190
Cement glands length	–	88 (n = 1)	–	165 (90–230) (n = 12)	–	150
Cement glands width	–	50 (n = 1)	–	164 (100–240) (n = 12)	–	170
Saefftigen's pouch length	–	566 (240–890) (n = 2)	–	214 (140–290) (n = 12)	–	480
Saefftigen's pouch width	–	81 (60–100) (n = 2)	–	126 (80–210) (n = 12)	–	70
Bursa length	–	258 (160–390) (n = 3)	–	385 (290–510) (n = 3)	–	190
Bursa width	–	271 (160–440) (n = 3)	–	440 (350–490) (n = 3)	–	220
Egg length	57 (50–60) (n=5)	–	91 (40–120) (n = 10)	–	–	–
Egg width	17 (n=5)	–	27 (10–40) (n = 10)	–	–	–
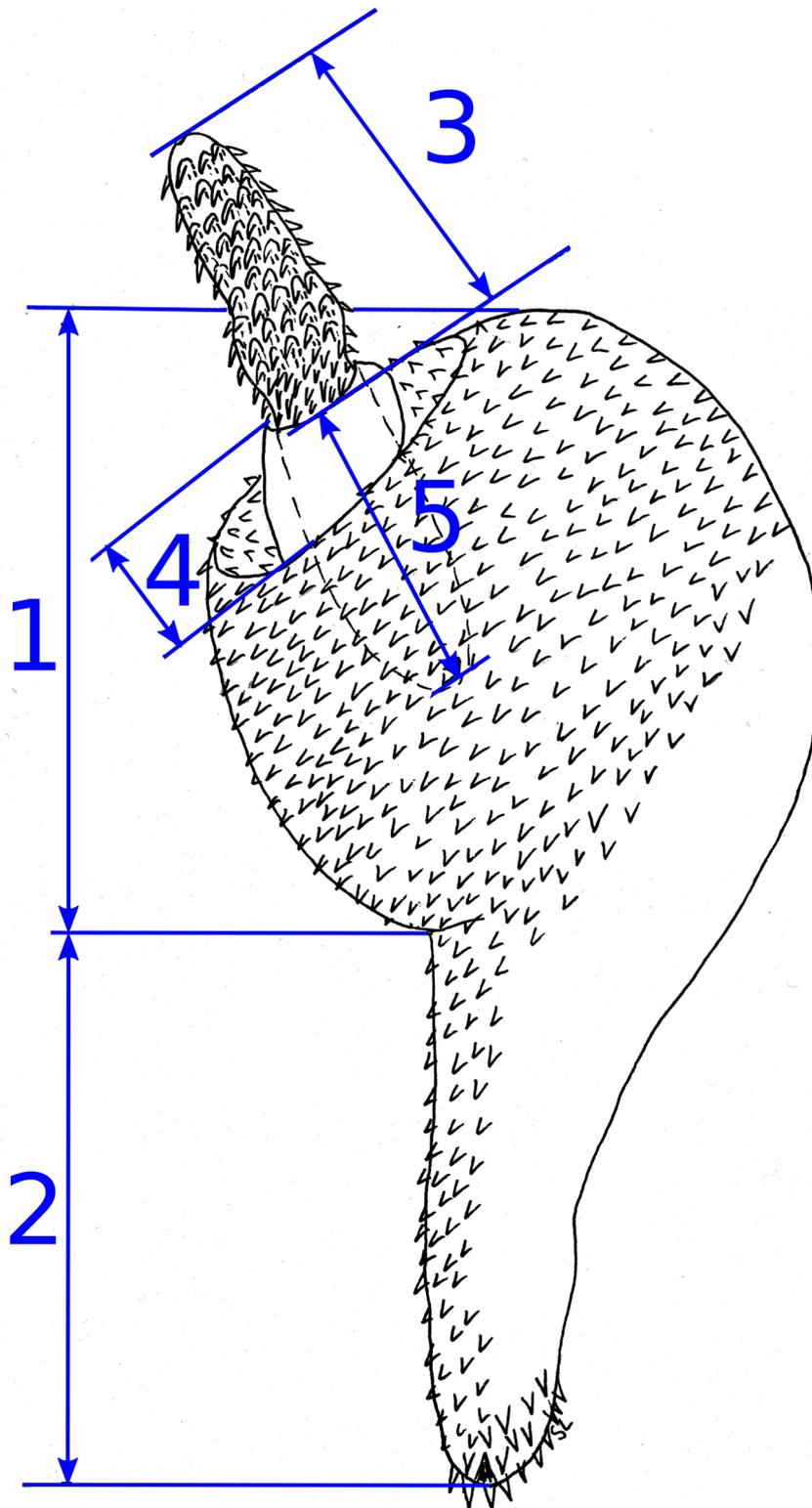
Fig. 2. Schematic diagram of praesoma and trunk of a male of *Corynosoma semerme* illustrating the length measurements recorded from all three species of *Coryonosma*; internal organs except proboscis receptacle excluded. 1 = fore-trunk. 2 = hind-trunk. 3 = proboscis. 4 = neck. 5 = proboscis receptacle.

For SEM, 41 specimens fixed in 70% ethanol were dehydrated through an ethanol series. Specimens were critical point dried in liquid CO_2, mounted on stubs, gold-plated under vacuum to a thickness of 20 nm in an Agar High Resolution Sputter Coater Model 20, and examined in a Hitachi S-4300 SEM at an accelerating voltage of 10 kV and photographed by digital imaging software. Figure plates were prepared using FigureJ (Mutterer & Zinck 2013).

Institutional abbreviations

HWML = University of Nebraska State Museum, Manter Laboratory of Parasitology, Lincoln, USA
SMNH = Swedish Museum of Natural History, Stockholm, Sweden

Results

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Phylum Acanthocephala Rudolphi, 1802
Class Palaeacanthocephala Meyer, 1931
Order Polymorphida Petrochenko, 1956
Family Polymorphidae Meyer, 1931

Genus *Corynosoma* Lühe, 1904

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The morphology of all three species conformed to the generic diagnosis of *Corynosoma* according to Lühe (1904, 1911), Van Cleave (1953), Arai (1989) and Aznar *et al*. (2006). The genus is characterized by a small body size, comprising a clubbed fore-trunk and a cylindrical hind-trunk. Trunk spines on the anterior and ventral surface of the fore-trunk. Neck trapezoid, short and spineless; can be retracted into fore-trunk. Proboscis subcylindrical, enlarged midway along its length or just posterior to the midpoint. Proboscis hooks arranged in longitudinal rows. Posterior 3–5 hooks in a longitudinal row without roots; all other hooks with simple, unbranched roots. Largest hooks occur on broadest part of proboscis. Proboscis receptacle double-walled. Lemnisci shorter than proboscis receptacle, broad and leaf-like. Gonopore terminal or slightly subterminal in both genders. Male reproductive system either confined to the hind-trunk or extending into posterior part of fore-trunk. Paired testes elongate-oval, partially overlapping. Six pyriform cement glands. Eggs fusiform, with relatively short polar prolongations of the thickened shell membrane, filling body cavity of gravid females. Gonopore of female sometimes covered by a copulatory cap.

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Corynosoma magdaleni Montreuil, 1958
Figs 3–4, Table 2

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Diagnosis

Corynosoma magdaleni is characterised by a body 3.67–5.11 mm long in females and 2.53–4.39 mm long in males, a slender trapezoid neck structure, spines covering most of fore-trunk, spines covering more than half the length of ventral surface of hind-trunk, proboscis with 16–20 longitudinal rows of hooks in both genders with 8–11 hooks in each row in females and 8–10 hooks in each row in males.

Material examined (see Table 1 and Fig. 1)

FINLAND • 1 ♀; Åland, Bothnian Sea; HWML- 39480-1141-2 • 1 ♂; Åland, Bothnian Sea; HWML-39480-1041-3.

SWEDEN • 4 ♀♀, 2 ♂♂; Stocka/Gävleborgs Province, Bothnian Sea; SMNH-162973 • 1 ♂; Skärplinge/Uppsala Province, Bothnian Sea; SMNH-162973 • 1 ♀, 1 ♂; Luelå/Norrbottens Province, Bothnian Bay; SMNH-162973 • 1 ♀; Tistersöarna, Luleå/Norrbottens Province, Bothnian Bay; SMNH-162973.

Corynosoma semerme (Forsell, 1904) Lühe, 1911
Figs 5–6, Table 2

Diagnosis

Corynosoma semerme is characterised by a body 1.83–3.13 mm long in females and 1.91–2.73 mm long in males, a robust trapezoid neck structure, spines covering most of fore-trunk, spines covering entire ventral surface of hind-trunk, proboscis with 22–25 longitudinal rows of hooks with 10–12 hooks in each row in females and 22–24 longitudinal rows of hooks with 10–14 hooks in each row.

Material examined (see Table 1 and Fig. 1)

SWEDEN • 14 ♀♀; Stocka/Gävleborgs Province, Bothnian Sea; SMNH-162974 • 10 ♂♂; same data as preceding; SMNH-162975 • 6 ♂♂; Hudiksvall/Gävleborgs Province, Bothnian Sea; SMNH-162976.

Corynosoma strumosum (Rudolphi, 1802) Lühe, 1904
Figs 7–8, Table 2

Diagnosis

Corynosoma strumosum is characterised by a body 4.90 mm long in females and 5.67 mm long in males, a robust trapezoid neck structure, spines covering most of fore-trunk, spines covering anterior third
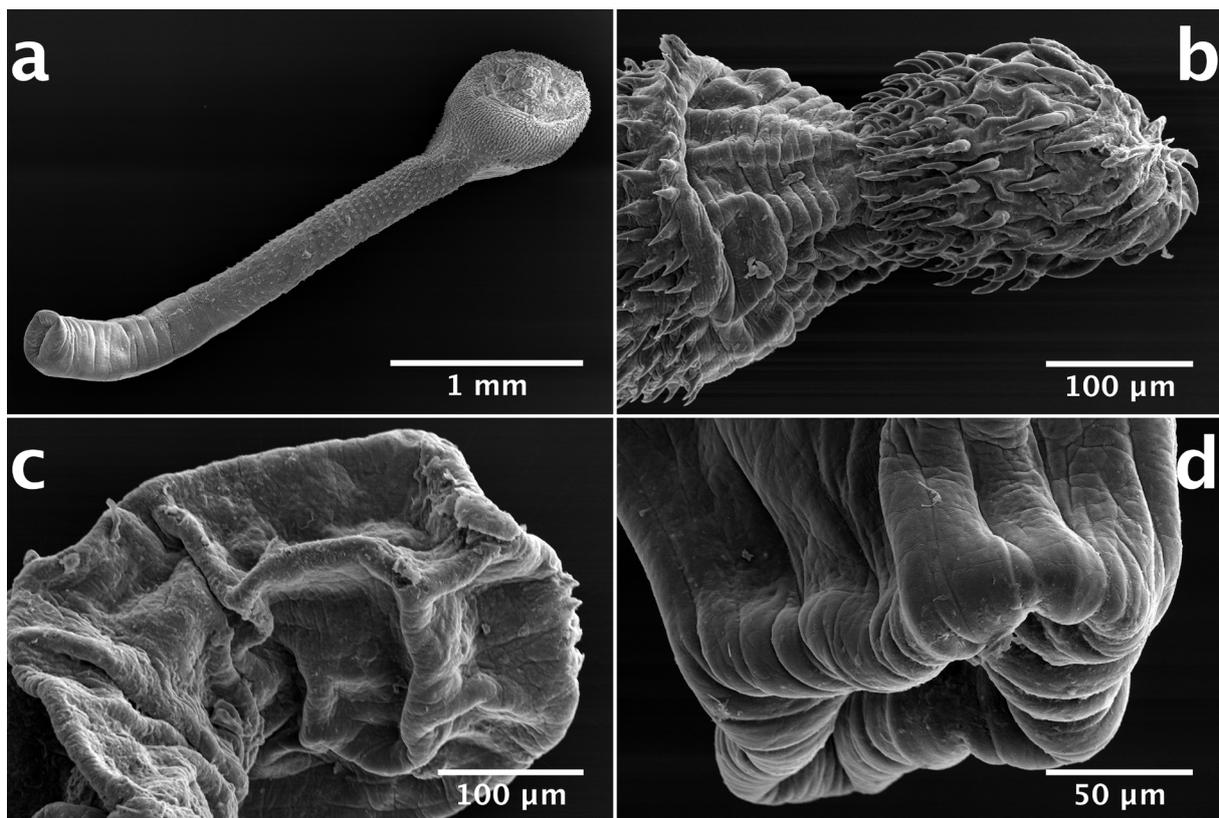

Fig. 3. SEM micrographs of *Corynosma magdaleni* from grey seals, *Halichoerus grypus,* in the Swedish Baltic Sea. **a**. ♀, entire worm, ventro-lateral view, from the Bothnian Bay, Norrbotten, Luleå. Ventral spination extends over more than half the length of the hind-trunk. **b**. Proboscis of a ♀ showing the spineless neck and the arrangement of the hooks. **c**. Posterior end of a ♂ showing the extended copulatory bursa. **d**. Posterior end of a ♀ in which the gonopore has been retracted into the gential vestibule.
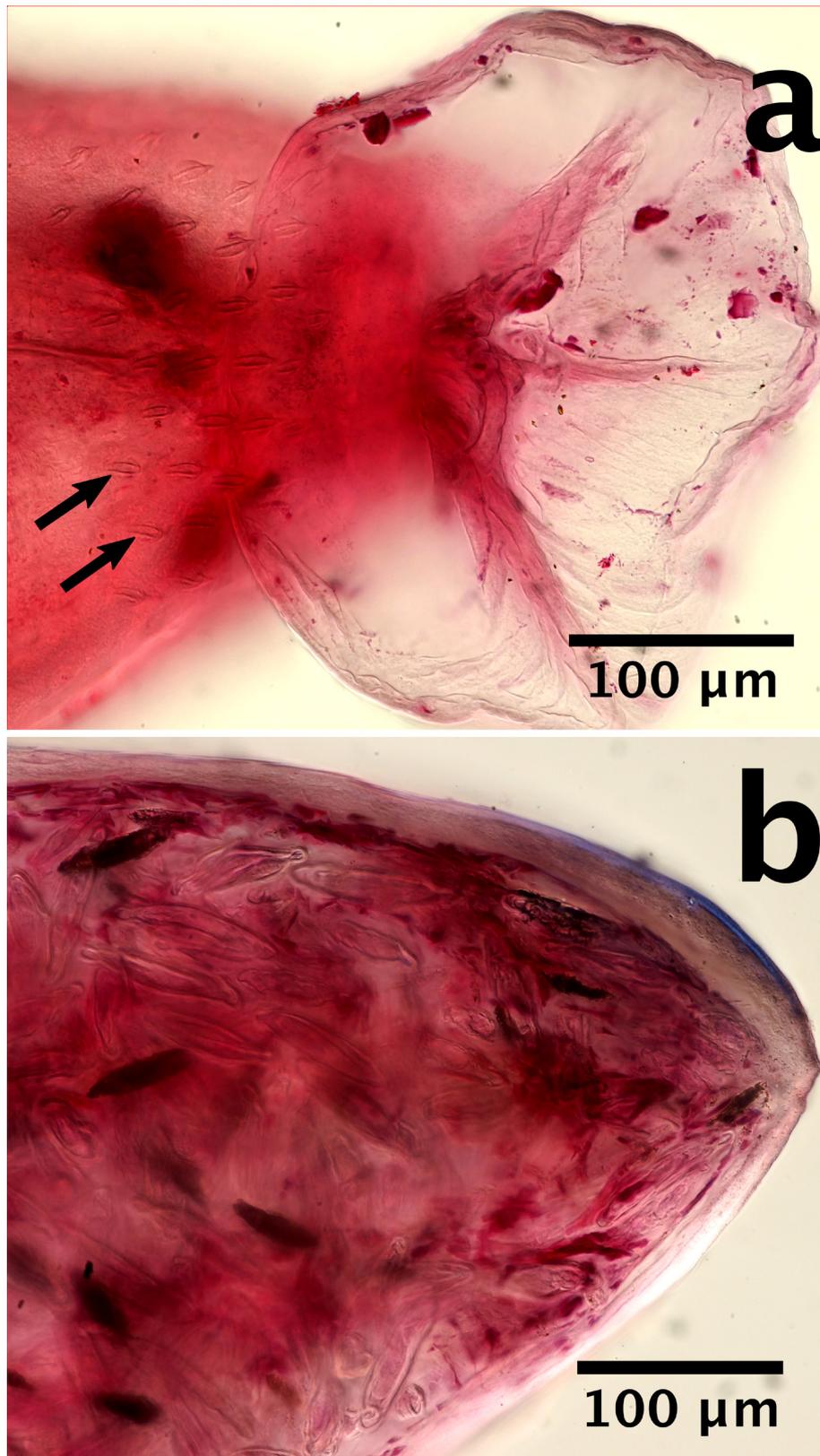
Fig. 4. LM micrographs showing posterior end of *Corynosoma magdaleni* from a grey seal, *Halichoerus grypus,* from the archipelago of Åland (Finland). **a**. ♂ showing everted bursa and genital spine (arrows) (HWML 39480–1041-3). **b**. ♀ (HWML 39480–1141-2).
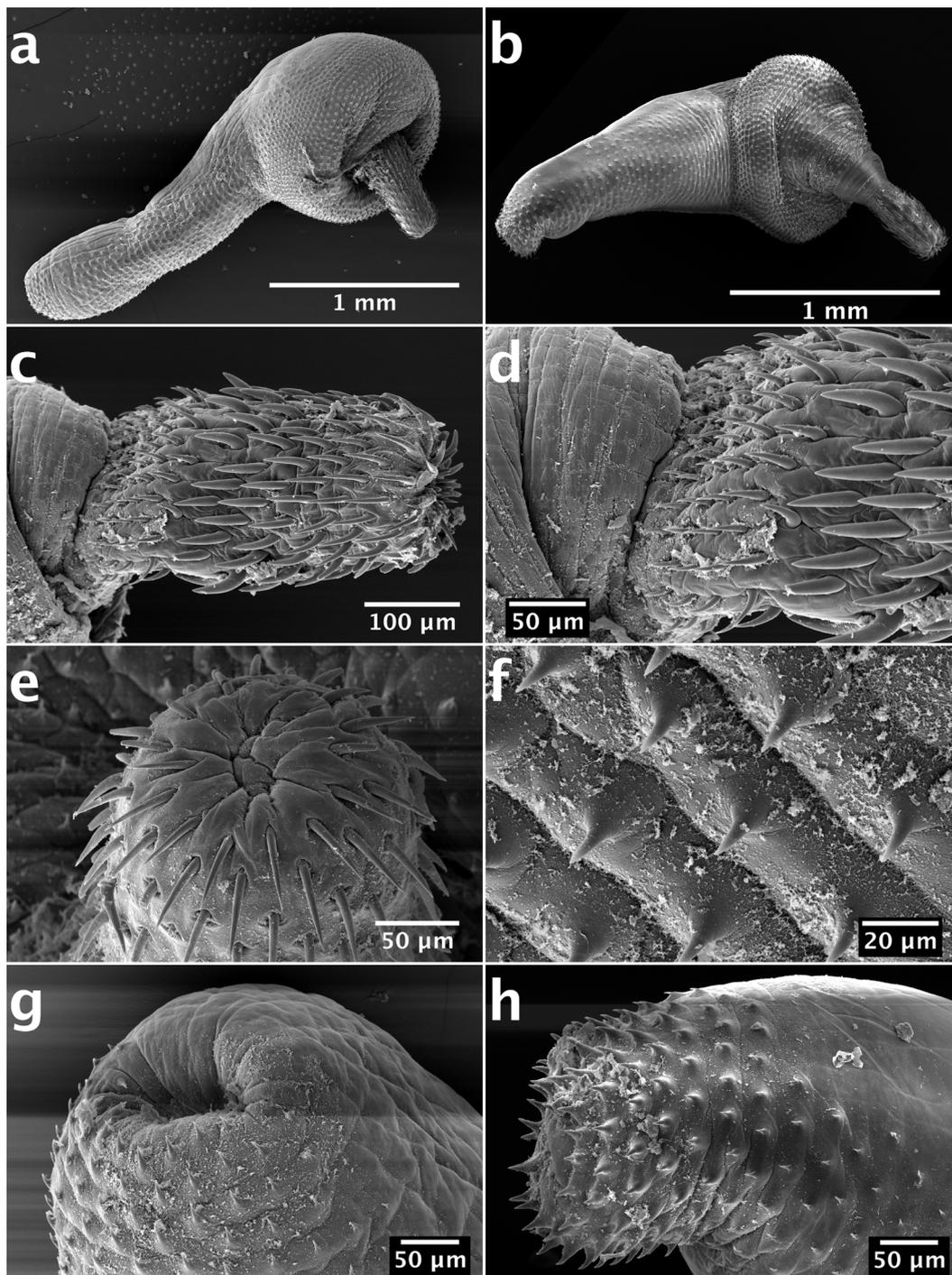
Fig. 5. SEM micrographs of *Corynosoma semerme* from grey seals, *Halichoerus grypus,* in the Swedish Baltic Sea. **a**. ♀, entire worm, ventro-lateral view, from the Bothnian Sea, Hälsingland, Stocka. Spines cover the entire ventral surface of the hind-trunk. **b**. ♂, whole worm, ventral view, from the Bothnian Sea, Hälsningland, Stocka. Ventral spination of the entire hind-trunk and numerous genital spines. **c**. Proboscis of a ♀, ventral view, showing the arrangement of the hooks. **d**. Base of proboscis of a ♀, lateral view, showing the spineless neck and the posterior and middle series of hooks. **e**. Anterior end of proboscis of a ♀ showing the bare tip. **f**. Trunk spines of the fore-trunk of a ♂, ventral view. **g**. Posterior end of a ♀ showing the trunk-spines that extend over the ventral surface as far as the gonopore. **h**. Posterior end of a ♂ with numerous genital spines.

of ventral surface of the hind-trunk, proboscis with 20 longitudinal rows of hooks in females and 18 longitudinal rows of hooks in males with 10 hooks in each row in both genders.

Material examined (see Table 1 and Fig. 1)

FINLAND • 1 ♀; Åland, Bothnian Sea; HWML- 39481-1158-2 • 1 ♂; Åland, Bothnian Sea; HWML-39481-1142-4.

SWEDEN • 1 ♀; Stocka/Gävleborgs Province, Bothnian Sea; SMNH-162977 • 1 ♂; Östhammar/Uppsala Province, Ålands hav; SMNH-162978.
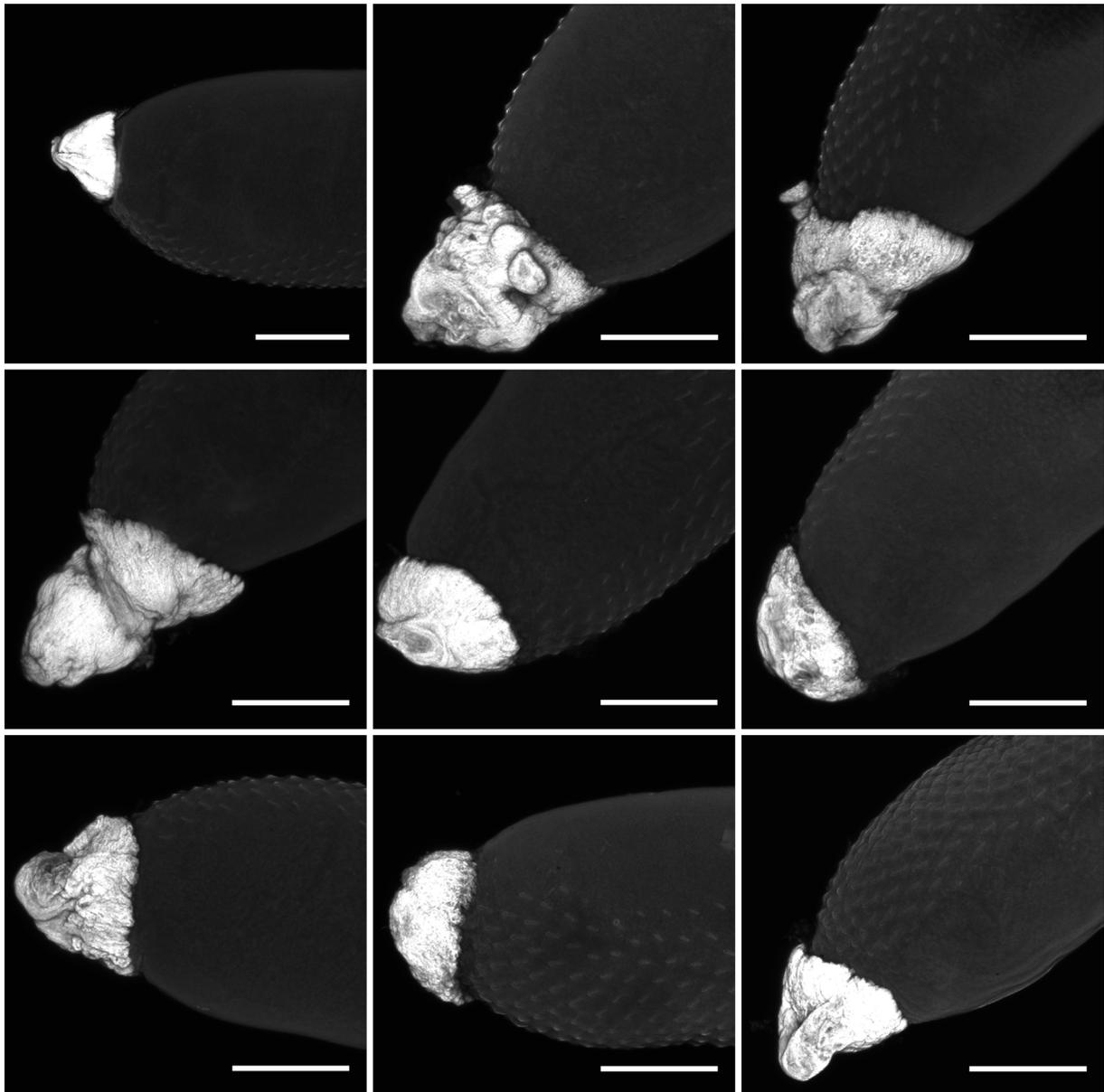

Fig. 6. Variation in the morphology and size of copulatory caps found on ♀ *Corynosoma semerme* from a ringed seal, *Pusa hispida,* in the Swedish Baltic Sea. Images are maximum intensity projections of confocal image stacks. The copulatory caps show strong autofluorescence when excited with a 405 nm laser. All scalebars represent 200 μm.

Comparative analysis of conventional morphological characters

In all three species, spines cover most of the fore-trunk, but are only found on the ventral surface of the hind-trunk. The extent of spine coverage of the ventral surface of the hind-trunk shows interspecific variation and is the most useful character for discriminating the three species. In *C. magdaleni*, spines cover more than half the length of the hind-trunk, and the density of spines decreases from anterior to posterior (Fig. 3a). *Corynosoma semerme* exhibits spines over the entire ventral surface of the hind-trunk (Fig. 5a–b). Spines cover only the anterior third of the ventral surface of the hind-trunk in *C. strumosum* (Fig. 7a–b).

Corynosoma semerme can be distinguished from the other two taxa on the basis of having more longitudinal rows of hooks (22–25 vs 16–20 in *C. magdaleni* and 18–20 in *C. strumosum*). In *C. magdaleni*, longitudinal rows of hooks are punctuated by a gap between the middle series of 2–4 large, robust hooks and the proximal series of 3–4 short, slender hooks (Fig. 3b); this gap was not observed in the other two taxa. The trapezoid neck structure is robust in *C. semerme* (Fig. 5c–d) and *C. strumosum* (Fig. 7c), but more slender in *C. magdaleni* (Fig. 3b).

Genital spines were observed surrounding the gonopore of males in all three taxa (e.g., Fig. 5h). These genital spines are larger than the trunk spines (Table 2). Genital spines were not observed in any of the female acanthocephalans. However, the gonopore was retracted into the genital vestibule in many
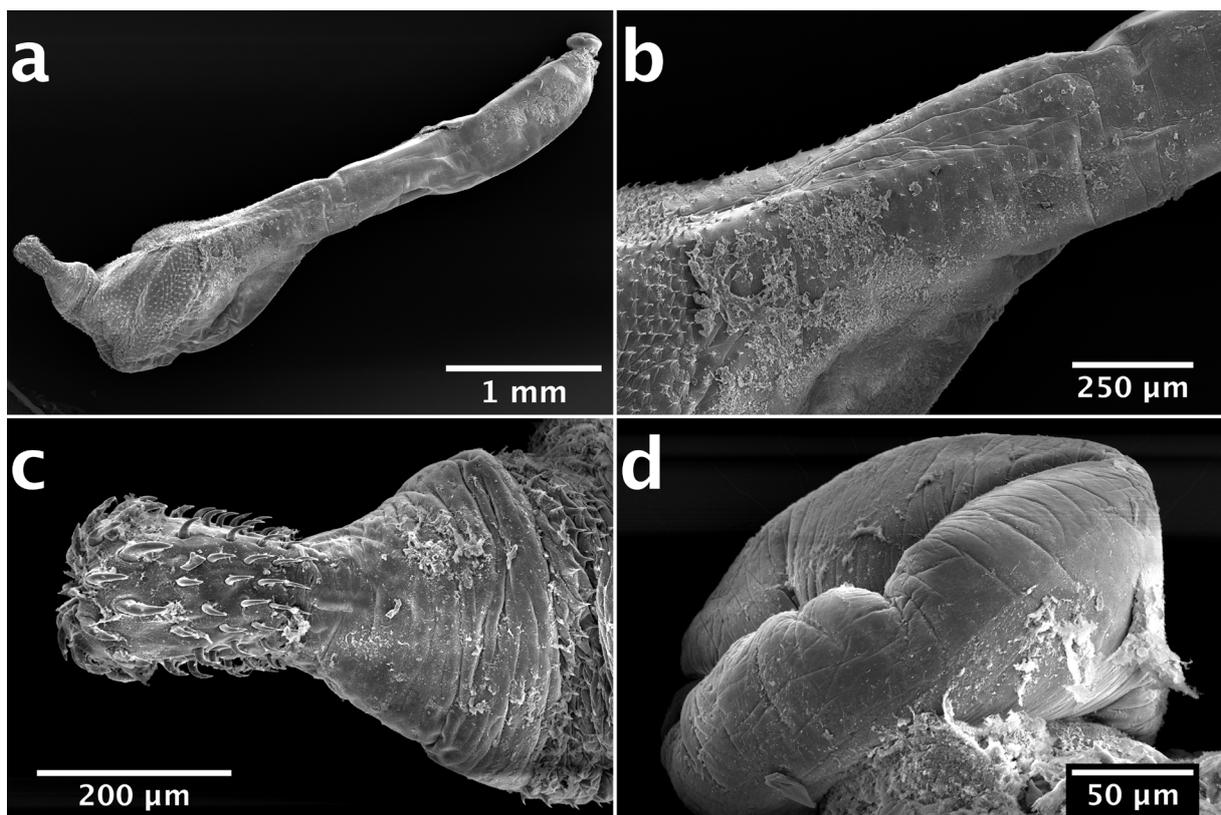

Fig. 7. SEM micrographs of *Corynosoma strumosum* from grey seals, *Halichoerus grypus*, in the Swedish Baltic Sea. **a**. ♂, whole worm, ventro-lateral view, from the Bothnian Sea, Uppland, Östhammar. **b**. Ventral spination on the hind-trunk of a ♂ restricted to one-third of its length only. **c**. Proboscis of a ♂, lateral view, showing the spineless neck and the arrangement of the hooks. **d**. Posterior end of a ♂ showing the extended copulatory bursa.

females (Fig. 3d), which would obscure genital spines if present. In female *C. semerme*, the trunk spines extend as far as the gonopore (Fig. 5g), but do not differentiate into distinctive genital spines or surround the genital pore as they do in the males (Fig. 5h). Therefore, we consider genital spines to be absent in *C. semerme* of the Baltic Sea.

Egg size can aid the discrimination of gravid females of *C. magdaleni* from those of *C. semerme*, the former species having shorter eggs (50–60 vs 40–120 µm). The only female specimen of *C. strumosum* found in this study was not gravid and so we were unable to measure the eggs of this species.

Copulatory caps

Copulatory caps were observed covering the gonopore of some females of *C. semerme* (Fig. 6). These copulatory caps showed considerable variation in size and all were strongly autofluorescent when illuminated with light of a wavelength of 405 nm. Copulatory caps were not observed on male *C. semerme* or on any specimens of the other two species examined.

Study of the material from Finnish Baltic Sea (Nickol *et al.* 2002)

Nickol *et al.* (2002) stated that their "descriptions of *C. magdaleni* and *C. strumosum* are based on 80 specimens that were fully extended or were extended enough to show most of the anatomical features". Surprisingly, very little of their material was preserved and deposited as voucher specimens: four slides, each with one male and one female of each species. The material was of limited use for our morphological studies. One male of *C. magdaleni* (HWML 39480-1041-3) had a fully everted proboscis which facilitated the measurement of hook morphometrics in two complete longitudinal rows of hooks (one on
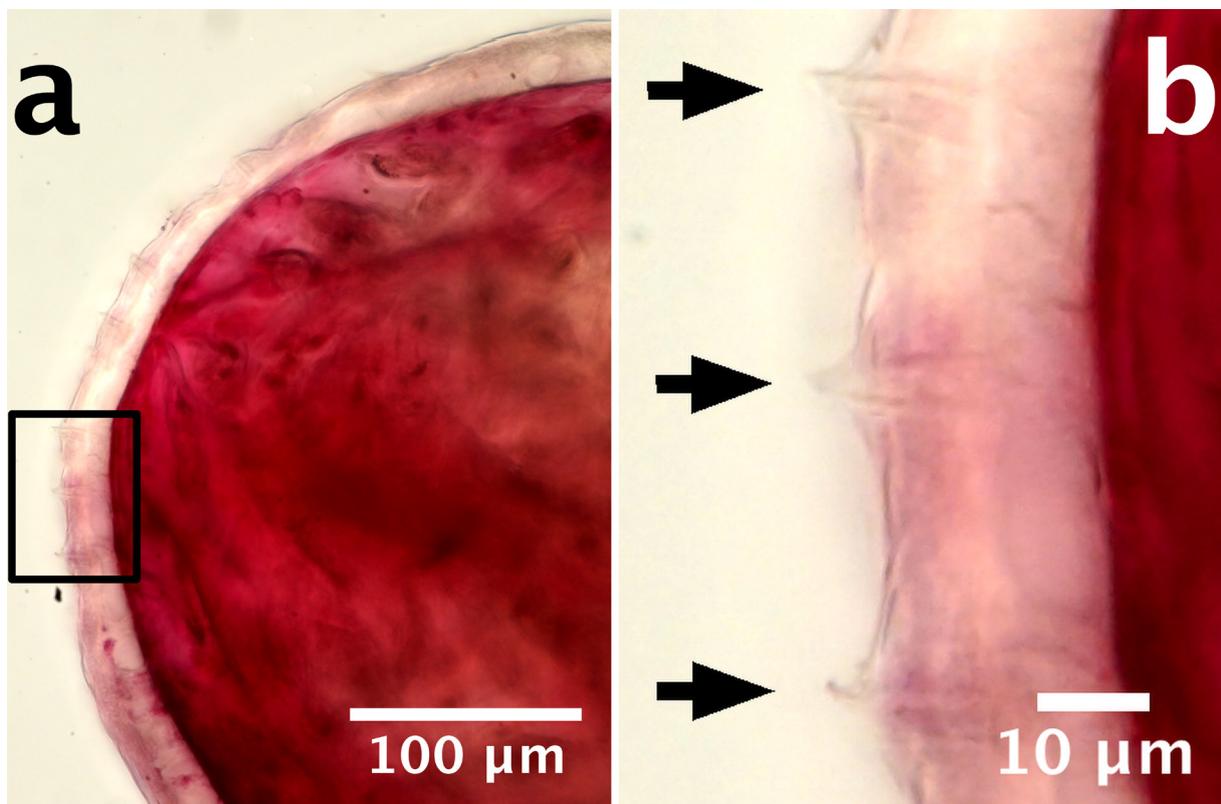

Fig. 8. LM micrographs of genital spines of a ♀ *Corynosoma strumosum* (HWML 39481–1158-2) from a grey seal, *Halichoerus grypus,* from the archipelago of Åland (Finland). **a**. Posterior of trunk. **b**. Enlargement of rectangular region in subfigure a.

the dorsal surface or the proboscis and the other on the ventral). The partially inverted proboscides of the other three specimens obscured the anterior hooks, preventing analysis of hook patterns. It is important to note that the material of Nickol *et al*. (2002) was "stained with Mayer´s carmalum, dehydraded in ethanol, cleared in xylene and mounted in Canada balsam", which differed from our preparation method (see Material and methods). Therefore comparisons of morphometric data between the two studies should be treated with caution.

Genital spines were clearly visible in the male *C. magdaleni* (HWML 39480-1041-3) (Fig. 4a), which also agrees with the description by Montreuil (1958). In the female (HWML 39480-1141-2), genital spines were not observed (Fig. 4b), which agrees with the description by Montreuil (1958) ("small number of spines (2 to 10) in less than the half of the females") and with the description by Nickol *et al*. (2002) ("absent in most females", but contrary to their fig. 2). We observed a few genital spines in the *C. strumosum* female (HWML 39481-1158-2) (Fig. 8), which concurs with the descriptions by Van Cleave (1953) and Nickol *et al*. (2002). Genital spines were not visible on the male *C. strumosum* (HWML 39481-1142-4), but the genital pore was introverted into the genital vestibule, possibly obscuring any spines that might be present.

Sexual dimorphism and interspecific variation in trunk morphology

Both *C. magdaleni* and *C. semerme* exhibited subtle sexual dimorphism in trunk shape. In *C. magdaleni*, trunk length, hind-trunk length and fore-trunk width were typically larger in gravid females than in males (Table 2). In *C. semerme*, fore-trunk length and width showed the most evidence of sexual dimorphism. Males were typically smaller than gravid females. *Corynosoma magdaleni* and *C. semerme* appear to differ in trunk shape. *Corynosoma magdaleni* has a longer, but narrower fore-trunk and hind-trunk. These differences can be observed in both females and males (Table 2). The trunk shape of *C. strumosum* is similar to that of *C. magdaleni*.

Discussion

Conventional morphological characters

Overall, our morphological measurements of the three species of *Corynosoma* agree with those found in the literature. For example, in the original description of *C. magdaleni*, Montreuil (1958) noted that the females were slightly larger than the males, and this sexual dimorphism in size was observed in the present study. Montreuil (1958) also noted that the neck exhibited greater extension in females than in males, a characteristic also observed in *C. magdaleni* and *C. semerme* in our study. The specimens of *C. semerme* males (2.60±0.32 mm, n = 173) and females (2.93±0.39 mm, n = 172) found by Helle & Valtonen (1981) were somewhat larger than those encountered in the present study. Helle & Valtonen (1981) reported that the total body length of *C. strumosum* from ringed seals of the Bothnian Bay was 4.71 ±0.68 mm for males (n = 373) and 5.34 ±0.77 mm for females (n = 390). Popov & Fortunato (1987), Nickol *et al*. (2002) and Kaimoto *et al*. (2018) also reported that females of *C. strumosum* were larger than males. However, in our material, that consists only of one individual per gender, the male was larger than the female (Table 2). While most studies in Europe (except Lühe 1911), including the present one, describe slight differences between the body size of the genders, sexual dimorphism is rarely reported in the North American literature (Van Cleave 1953). Waindok *et al*. (2018) and Nickol *et al*. (2002) did not separate the measurements for males and females.

Populations of *C. semerme* from the coast of Sweden and the Gulf of Finland appear to differ in the number of longitudinal rows of hooks, the former displaying 20–25 (present study) and the latter 26 (Forssell 1904). However, the number of hooks observed in each row seems very similar: 10–14 (this study) vs 12–14 (Forssell 1904). Lühe (1911) described the German material with 24–26 longitudinal rows and 13 hooks in each row, while Van Cleave (1953) described the North American material with

22–24 rows of 12–13 hooks each. For *C. magdaleni*, the examined proboscis armature of Swedish worms differed slightly from the Canadian material described by Montreuil (1958). The Swedish material has fewer longitudinal rows and hooks in each row than the Canadian material (rows: 16–20 vs 17–23; hooks: 8–11 vs 9–11) (see also Table 2), but agrees with the description by Waindok *et al*. (2018) who found 16–19 rows of hooks for *C. magdaleni*. For *C. strumosum*, Rudolphi (1802) described a short and cylindrical proboscis with 16 and Lühe (1911) with 18 longitudinal rows of hooks for material collected in Germany. Our Swedish material showed 18–20 rows of hooks with 10 hooks each (Table 2). This agrees with the German studies mentioned above, while the North American material exhibited up to 11 hooks in each row (Van Cleave 1953). The extensive study by Popov & Fortunato (1987) showed for *C. strumosum* material from ringed seals variations between 15 and 21 longitudinal rows with 8–12 hooks in each row.

Overall, cross-study comparisons suggest that the number of longitudinal rows and number of hooks in each row show intraspecific variation related to geographical region and definitive host. However, in the absence of molecular data we cannot be certain that this apparent intraspecific variation is not in fact interspecific variation among cryptic species (Väinölä *et al*. 1994; Steinauer *et al*. 2007; Waindok *et al*. 2018). Intraspecific morphological variation related to host has been observed in *Corynosoma hannae* Zdzitowiecki, 1984 (see Hernández-Orts *et al*. 2017).

The genital spines may be obscured if the tip of the tail is retracted into the genital vestibule (Van Cleave 1945). Males have numerous genital spines around the tail (e.g., Figs 2, 5b, 5h), which vary in size between the species (see Table 2) and clearly differ from the trunk spines. We could, however, not observe any genital spination in females in the Swedish material (e.g., Fig. 5g), but was seen in the prepared Finnish female of *C. strumosum* analysed by Nickol *et al*. (2002) (Fig. 8). Forssell (1904, 1905) and Lühe (1911) did not describe genital spines in Finnish and German females of *C. semerme* and *C. strumosum*. By contrast, some genital spines in females were described for the North American material (e.g., Montreuil 1958 for *C. magdaleni*). Van Cleave (1953) did observe the same pattern for females of *C. strumosum* ("often introverted into the genital vestibule and not observable from the surface"). He suggested that the few spines occasionally found in females may be lost with the copulatory cap. This could be one explanation why some authors did not observe and describe them.

Another possibility to explain the differences observed, may be morphological divergence between the Northern European and North American material. Our own observations disagree with the description of *C. strumosum* by Nickol *et al*. (2002) ("genital spines long in both sexes"). The study by Nickol *et al*. (2002) did not separate the measurements for males and females, and combined the Finnish results with those from Van Cleave (1953) and Montreuil (1958), both studies from North American material. It would have been more helpful for us to compare the Swedish results with a pure Finnish data set which is missing. The morphological description by Nickol *et al*. (2002) should be handled with caution or avoided, because they combined specimens from two different geographical areas (North America and the Baltic Sea). Popov & Fortunato (1987) did not observe genital spines in 80 female individuals of *C. strumosum* collected from the Chaun Bay in the Eastern Siberian Sea, although genital spines were present in all other collected samples (n = 500). Amin *et al*. (2011: fig. 4) presented one SEM picture of female genital spines for an acanthocephalan identified as *C. strumosum* from the Caspian seal, *Pusa caspica* Gmelin, 1788, from the Caspian Sea. However, specimens of *Corynosoma* from Caspian seals are likely to be *Corynosoma caspicum* (Golvan & Mokhayer, 1973) (see García-Varela *et al*. 2005).

Trunk shape

Corynosoma magdaleni and *C. strumosum* share a similar trunk shape, which is longer and more slender than that of *C. semerme*. The phenotypic divergence of *C. semerme* from the other two species reflects the phylogenetic relationships of the three taxa; molecular genetic data have revealed that *C. magdaleni*

and *C. strumosum* have a closer affinity to each other, than to *C. semerme* (Waindock *et al*. 2018; Lisitsyna *et al*. 2019).

In the majority of acanthocephalans, the proboscis is the primary organ of attachment to the intestinal wall of the definitive host; and, therefore, adaptive radiation is often expressed in divergence of proboscis morphology. However, in *Corynosoma* both the trunk and proboscis anchor the acanthocephalan to the wall of the intestine (Aznar *et al*. 1999). We might speculate that adaptation to different regions of the digestive tract might be accompanied by changes in trunk shape and spination. Spine coverage may be linked to trunk shape (Hernández-Orts *et al*. 2012), but further analyses are needed.

Copulatory caps

Copulatory caps were only observed on females of *C. semerme*. The copulatory caps are formed from a substance produced by the cement gland secretions of male worms (Dezfuli *et al*. 1999) and are thought to prevent further inseminations and thus sperm competition (Crompton 1985). The considerable variation in the size of the copulatory caps may be random; or, alternatively, might represent differences between males in the allocation of resources to prevent sperm competition. The copulatory caps were strongly autofluorescent when illuminated with a 405 nm laser. Dezfuli *et al*. (1998) demonstrated that the cement glands of *Pomphorhynchus laevis* (Zoega *in* Müller, 1776) produce a protein with a molecular weight of 23 kDa. However, little else is known about the composition of acanthocephalan copulatory caps.

Future work

Tandem morphological and molecular analyses are required to investigate the diversity of species of *Corynosoma* and to identify the best phenotypic characters for discriminating the species found. Given the difficulties of retrieving high quality specimens from dead definitive hosts, alternative sampling methods should be investigated, such as rectoscopy or intestinal biopsy of anaesthetized seals.

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