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Novel morphological and molecular data for *Corynosoma hannae* Zdzitowiecki, 1984 (Acanthocephala: Polymorphidae) from teleosts, fish-eating birds and pinnipeds from New Zealand

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ABSTRACT

The polymorphid acanthocephalan, *Corynosoma hannae* Zdzitowiecki, 1984 is characterised on the basis of newly collected material from a New Zealand sea lion, *Phocarctos hookeri* (Gray), and long-nosed fur seal, *Arctophoca forsteri* (Lesson) (definitive hosts), and from Stewart Island shags, *Leucocarbo chalconotus* (Gray), spotted shags, *Phalacrocorax punctatus* (Sparrman) and yellow-eyed penguins, *Megadyptes antipodes* (Hombron & Jacquinot) (non-definitive hosts) from New Zealand. Specimens are described in detail and scanning electron micrographs for *C. hannae* are provided. Additionally, cystacanths of *C. hannae* are reported and described for the first time from the body cavity and mesenteries of New Zealand brill, *Colistium guntheri* (Hutton) and from New Zealand sole, *Peltorhamphus novaezeelandiae* Günther from Kaka Point, Otago in New Zealand. Partial sequence data for the mitochondrial cytochrome *c* oxidase 1 gene (*cox1*) for adults, immature specimens and cystacanths of *C. hannae* were obtained. Phylogenetic analyses of the newly-generated sequences and for available *cox1* sequences of *Corynosoma* spp. revealed a close relationship between *C. hannae* and *C. australe* Johnston, 1937, both species infecting pinnipeds in the Southern Hemisphere. However, a morphological comparison of the species suggests that *C. hannae* mostly closely resembles *C. evae* Zdzitowiecki, 1984 and *C. semerme* (Forssell, 1904), the latter of which occurs in pinnipeds in the Northern Hemisphere.

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1. Introduction

Species of the polymorphid genus *Corynosoma* Lühe, 1904 are cosmopolitan parasites of pinnipeds, and to a lesser extent, of cetaceans, marine birds and terrestrial mammals [1]. Their life cycle is complex and involves crustaceans (i.e. amphipods) as intermediate hosts [2,3] and many include teleosts as paratenic hosts [4–6]. Species of *Corynosoma* use trophic webs to spread their cystacanths (infective stage for the final host) between obligatory hosts. During their transmission, cystacanths of *Corynosoma* can also infect other top predators of the marine realm (i.e. cartilaginous fish, fish-eating birds or non-pinniped marine mammals (e.g. [7–10]), which may act as a dead end in their life cycle [11]). In these non-definitive hosts, acanthocephalans usually attain adult morphology without reaching sexual maturity [10].

The main morphological characters defining species of *Corynosoma* are a pipe-shaped body, an inflated fore-trunk forming a spiny disc, a hind-trunk bearing somatic spines on its ventral surface, and the presence or absence of genital pines surrounding the genital pore, more usually present in males [1,12]. Traditional taxonomical studies on these acanthocephalans have been based almost exclusively on morphological characters (e.g. [13–17]). However, studies combining morphological and molecular data are clearly necessary to facilitate the diagnoses of problematic taxa and to provide reliable tools for cryptic species delimitation.

In New Zealand reports of species of *Corynosoma* are scarce. To the best of our knowledge, the first record of these acanthocephalans dates back to 1958 when adult worms of four taxa, i.e. *Corynosoma australe* Johnston, 1937, *Corynosoma bullosum* (von Linstow, 1892), *Corynosoma semerme* (Forssell, 1904), and *Corynosoma* sp., collected from pinnipeds were described by Johnston and Edmonds [18]. Later, Grabda and Ślósarczyk [19] reported cystacanths of *C. semerme* in two fish paratenic hosts, namely blue grenadier, *Macruronus novaezelandiae*

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(Hector) and pink cusk-eel, *Genypterus blacodes* (Forster) from the South Island of New Zealand and the Auckland and Campbell Islands. Shiel et al. [20] reported specimens of *Corynosoma hannae* Zdzitowiecki, 1984 in the long-nosed fur seal, *Arctophoca forsteri* (Lesson) (as *Arctocephalus forsteri*) and yellow-eyed penguin, *Megadyptes antipodes* (Hombron & Jacquinot) from St Clair Beach, Dunedin, and the Otago Peninsula, respectively. More recently, García-Varela et al. [21] provided molecular data apparently for *C. australe* from New Zealand sea lion, *Phocarctos hookeri* (Gray) from Enderby Island.

In the present study, we use morphological and genetic data to characterise mature and immature adults of *C. hannae* from pinnipeds and fish-eating birds from New Zealand. We provide a detailed morphological description of the specimens including, for the first time, scanning electron micrographs of morphological traits useful for species identification. Additionally, cystacanths of *C. hannae* were found in the body cavity and mesenteries from flatfish from Otago, New Zealand. Cystacanths of this species are reported and characterised morphologically for the first time. We also obtained sequence data for the mitochondrial cytochrome *c* oxidase 1 gene (*cox1*) for adults, immature specimens and cystacanths of *C. hannae*. Newly generated sequences for *C. hannae* were then analysed with the available sequences of all congeneric species of *Corynosoma* and other polymorphids from GenBank. The resulting phylogenetic analyses suggest that these cystacanths, immature specimens and adults from teleosts, fish-eating birds and pinnipeds are conspecific and distinct from other available species of *Corynosoma*.

2. Materials and methods

2.1. Sample collection

Adult acanthocephalans were collected from the intestine of one *Phocarctos hookeri* found dead on Sandy Bay beach, Enderby Island, Auckland Islands (50°30′S, 166°16′W). Immature acanthocephalans were collected from the small and large intestines of four Stewart Island shags, *Leucocarbo chalconotus* (Gray), three spotted shags, *Phalacrocorax punctatus* (Sparrman), eight yellow-eyed penguins, *M. antipodes*, found dead at Otago Harbour, South Island (45°47′S, 170°38′W), and from one juvenile *A. forsteri* found dead at St Clair Beach, Dunedin (45°54′S, 170°30′W). Adult and immature acanthocephalans were washed in saline and fixed in either 70% or 100% ethanol or 5% formalin. Encysted cystacanths were collected from the body cavity and mesenteries of one New Zealand brill, *Colistium guntheri* (Hutton) and 28 New Zealand sole, *Peltorhamphus novaezeelandiae* Günther caught off Kaka Point, Otago (46°23′S, 169°47′W). Cystacanths were excysted and left in tap water in the fridge for 24 h until proboscides were everted, and then fixed in 70% or in 100% ethanol. Nomenclature and classification of marine mammals follows Wilson and Reeder [22] and Jackson and Groves [23], whereas for cormorants and shags we follow Kennedy and Spencer [24].

2.2. Morphological description

Immature specimens from *L. chalconotus* (n = 31) and adult acanthocephalans from *Phocarctos hookeri* (n = 8) were punctured with a fine needle and stained with iron acetocarmine or Mayer's paracarmine, washed in distilled water, dehydrated in ethanol, cleared in clove oil or methyl salicylate and mounted in Canada balsam. Cystacanths from *P. novaezeelandiae* (n = 9) and immature specimens from *M. antipodes* (n = 81) and *A. forsteri* (n = 2) were cleared in beechwood creosote and examined as temporary wet mounts. Only cystacanths from *P. novaezeelandiae* and immature specimens from *L. chalconotus* were used for the morphological description because this material was in larger quantity and of a better quality than the specimens from *C. guntheri* and *P. punctatus*. Measurements were taken as follows: from drawings made with the aid of a drawing tube or using an eyepiece micrometer (specimens from *A. forsteri,* and *M. antipodes*), or using the Leica Application Suite microscope software or with the Olympus digital camera for photographs and ImageJ (Wayne Rasband, NIH, USA). They are in micrometres unless otherwise stated and are presented as the mean ± standard deviation (where >30 measurements), (range and number of measurements where this varies from the total number of specimens measured). Fully mature eggs were measured from drawings of eggs in situ in the body cavity of female worms.

Four adult females from *Phocarctos hookeri* and 3 immatures (1 male and 2 females) from *L. chalconotus* were also studied by scanning electron microscopy (SEM). Adult females were dehydrated through an ethanol series, critical point dried, sputter-coated with gold and examined with a Hitachi Stereoscan Model S-2469N scanning electron microscope, operating at 15 kV at Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Immature worms were viewed with a JEOL 6700F field emission scanning electron microscope (JEOL Ltd., Tokyo, Japan) at the Otago Centre for Electron Microscopy (OCEM, University of Otago, New Zealand).

Voucher specimens are deposited in the Museum of New Zealand, Te Papa Tongarewa (MNZ), Wellington, New Zealand; the Otago Museum (OMD), Dunedin, New Zealand; and the Colección Nacional de Helmintos (CNHE), Instituto de Biología, UNAM, Ciudad de México, México. Additionally, hologenophores, i.e. anterior parts of a worm used for morphological studies, while the posterior part was used in molecular analyses (see [25]), are deposited in the OMD.

2.3. Molecular data

Total genomic DNA was isolated from three adult specimens ex *Phocarctos hookeri*, from the posterior part of one immature worm ex *Phalacrocorax punctatus*, five immature worms ex *L. chalconotus*, two cystacanths ex *C. guntheri* and one cystacanth ex *P. novaezeelandiae*. The anterior parts of cystacanths and immature worms were used for the morphological description (hologenophores). Additionally, genomic DNA was also isolated from one adult specimen of *C. australe* from a South American sea lion, *Otaria flavescens* Shaw from Northern Patagonia, Argentina. Adult acanthocephalans were placed individually in tubes and digested overnight at 56 °C in a solution containing 10 mM Tris-HCl (pH = 7.6), 20 mM NaCl, 100 mM Na_2 EDTA (pH = 8.0), 1% Sarkosyl and 0.1 mg/ml proteinase K. After digestion, DNA was isolated from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio, USA) according to the manufacturer's instructions. Partial fragments of the mitochondrial cytochrome *c* oxidase 1 gene (*cox1*) were amplified using the same forward primer #507 (5′-AGT TCT AAT CAT AA(R) GAT AT(Y) GG-3′ [26]) and reverse primer HC02198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ [27]) as those used for Polymerase chain reaction (PCR). PCR amplifications were carried out in 25 μl reactions, containing 1 μl of each primer (10 μM), 2.5 μl of 10× PCR buffer (Promega, Madison, Wisconsin, USA), 1.5 μl $MgCl_2$ (25 mM), 0.5 μl of dNTP mixture (10 mM), 0.125 μl of Platinum® Taq DNA polymerase (1 U/μl) (Invitrogen Corporation, São Paulo, Brazil) and 2 μl of diluted template DNA. PCR conditions included a first step of denaturation at 94 °C for 5 min, followed by 35 cycles (denaturation at 94 °C for 1 min, annealing at 40 °C for 1 min, and extension at 72 °C for 1 min), followed by a post-amplification incubation at 72 °C for 5 min. Positive PCR products were cleaned and sequenced with an ABI 3730 capillary DNA sequencer. Contiguous sequences were assembled and base-calling differences resolved using Codoncode Aligner version 5.0.2 (Codoncode Corporation, Dedham, Massachusetts, USA).

Genomic DNA was extracted from cystacanths and immature worm tissue in 200 μl of a 5% suspension of Chelex® in deionised water and containing 0.1 mg/ml proteinase K followed by incubation at 56 °C for 5 h, boiling at 90 °C for 8 min, and centrifugation at 14,000*g* for 10 min. Partial fragments of the *cox1* gene were amplified with the same forward and reverse primers as in adult specimens. PCR

amplifications were performed in 25 μl reactions containing 2.5 μl of extraction supernatant, 1× PCR buffer (16 mM (NH4)2SO4, 67 mM Tris–HCl at pH 8.8), 2 mM MgCl2, 200 μM of each dNTP, 0.5 mM each primer, and 0.7 units BIOTAQ™ DNA polymerase (Bioline Ltd.). Thermocycling conditions for the *cox1* fragment were as follows: initial denaturation at 95 °C for 2 min followed by 40 cycles with denaturation at 94 °C for 40 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s; with a final extension step at 72 °C for 5 min. PCR amplicons were purified prior to sequencing using ExoSap PCR pre-sequencing purification kit (GE Healthcare, Auckland, New Zealand). Amplicons were cycle-sequenced using PCR primers, employing BigDye® Terminator v3.1 Ready Reaction Cycle Sequencing Kit, alcohol-precipitated and run on an ABI 3730XL Analyser (Applied Biosystems, Foster City, CA, USA). All newly-generated sequences were submitted to GenBank (see Table 1 for accession numbers).

2.4. Phylogenetic analyses

Newly-generated sequences were aligned together with other sequences for species of *Corynosoma* (5 species) [21,28] retrieved from GenBank (Table 1) using ClustalW [29] with default parameters, implemented in MEGA v5 [30]. The alignment was trimmed to match the shortest sequence prior to phylogenetic analyses resulting in 573 sites. Sequences for species belonging to genera *Andracantha* Schmidt, 1975 (1 species) [31], *Bolbosoma* Porta, 1908 (2 species) [21], *Hexaglandula* Petrochenko, 1950 (1 species) [32], *Profilicollis* Meyer, 1931 (1 species) [21], *Polymorphus* Lühe, 1911 (2 species) [28,33] and *Pseudocorynosoma* Aznar, Pérez-Ponce de León & Raga, 2006 (2 species) [31], were used as outgroups in the phylogenetic analyses (Table 1). The best-fit nucleotide substitution model was selected using the program jModelTest 0.1.1. [34] and applying the Akaike Information Criterion (AIC); this was the TPM1uf model with estimates of invariant sites and gamma distributed among-site rate variation (TPM1uf + I + G). Phylogenetic trees were reconstructed by maximum likelihood (ML) and Bayesian inference (BI) analyses. For ML analyses, the program RA × ML v7.0.4 [35] was used. A GTRGAMMAI substitution model was used for ML analyses, and 10,000 bootstrap replicates were run to assess nodal support. The BI tree was constructed using MrBayes 3.1.2 [36], with two runs and four chains (one cold, three heated) per run. The Metropolis-coupled Markov chain Monte Carlo (MCMCMC) were run for 10^6 generations, sampled every 10^3 generation, and the first 1250 samples (25%) were discarded as 'burn-in'. The outputs of MrBayes were examined with Tracer v1.4 [37] to check for convergence of different parameters, determine the approximate number of generations at which log-likelihood values stabilised, identify the effective sample size (EES > 200) for each parameter, and the estimated magnitude of model parameters in individual and combined runs. Topological convergence in the two independent MCMC runs was checked with the compare plot in AWTY [38]. The initial 25% of MCMCs was verified to include all the generations before stationarity was archived. Posterior probability values were

Table 1
Taxa included in the phylogenetic analyses with data on life-cycle stage, host, locality and GenBank accession number. Abbreviations: A, adult; C, cystacanth; I, immature specimens.

Species	Life-cycle stage	Host	Locality	GenBank accession no. (*cox1*)	Source
Genus *Andracantha* Schmidt, 1975					
A. gravida (Alegret, 1941)	A	*Nannopterum auritus* (Lasson)	Yucatan (Mexico)	EU267822	[31]
Genus *Corynosoma* Lühe, 1904					
C. australe Johnston, 1937	A	*Otaria flavescens* Shaw	Northern Patagonia, Chubut (Argentina)	KX957714	Present study
	A	*Phocarctos hookeri* (Gray)	Enderby Island (New Zealand)	JX442191	[21]
C. enhydri Morozov, 1940	A	*Enhydra lutris* (L.)	Monterey Bay, California (USA)	DQ089719	[21]
C. hannae Zdzitowiecki, 1984	C	*Colistium guntheri* (Hutton)	Kaka Point, Otago, South Island (New Zealand)	KX957724-KX957725	Present study
	C	*Peltorhamphus novaezeelandiae* Günther	Kaka Point, Otago, South Island (New Zealand)	KX957726	Present study
	I	*Leucocarbo chalconotus* (Gray)	Otago Harbour, South Island (New Zealand)	KX957718-KX957721, KX957723	Present study
	I	*Phalacrocorax punctatus* (Sparrman)	Otago Harbour, South Island (New Zealand)	KX957722	Present study
	A	*P. hookeri*	Enderby Island (New Zealand)	KX957715-KX957717	Present study
C. obtuscens Lincicome, 1943	A	*Callorhinus ursinus* L.	St. Paul Island, Alaska (USA)	JX442192	[21]
C. magdaleni Montreuil, 1958	A	*Phoca hispida saimensis* (Nordquist)	Lake Saimaa (Finland)	EF467872	[28]
C. strumosum (Rudolphi, 1802)	A	*Phoca hispida botnica* Gmelin	Baltic Sea (Finland)	EF467871	[28]
C. validum Van Cleave, 1953	A	*C. ursinus*	St. Paul Island, Alaska (USA)	JX442193	[21]
Genus *Bolbosoma* Porta, 1908					
B. turbinella (Diesing, 1851)	A	*Eschrichtius robustus* Lilljeborg	Monterey Bay, California (USA)	JX442189	[21]
Bolbosoma sp.	A	*C. ursinus*	St. Paul Island, Alaska (USA)	JX442190	[21]
Genus *Hexaglandula* Petrochenko, 1950					
H. corynosoma (Travassos, 1915)	A	*Nyctanassa violacea* (L.)	Nayarit (Mexico)	EU189488	[32]
Genus *Profilicollis* Meyer, 1931					
P. bullocki Mateo, Córdova & Guzmán, 1982	C	*Emerita analoga* Stimpson	Caleta Lenga (Chile)	JX442197	[21]
Genus *Polymorphus* Lühe, 1911					
P. brevis (Van Cleave, 1916)	A	*Nycticorax nycticorax* L.	Michoacan (Mexico)	DQ089717	[33]
P. minutus (Goeze, 1782)	C	*Gammarus pulex* (L.)	Dijon (France)	EF467865	[28]
Genus *Pseudocorynosoma* Aznar, Pérez-Ponce de León & Raga, 2006					
P. anatarium (Van Cleave, 1945)	A	*Bucephala albeola* L.	Durango (Mexico)	EU267821	[31]
P. constrictum (Van Cleave, 1918)	A	*Anas clypeata* L.	State of Mexico (Mexico)	EU267820	[31]

calculated obtained from the 50% majority rule consensus of sample trees after excluding the initial 25% as 'burn-in'. Trees were drawn using FigTree software version 1.3.1 [39]. Genetic distances (uncorrected p-distance) were calculated with MEGA v5.

3. Results

Description of adults of *Corynosoma hannae* Zdzitowiecki, 1984 from otariids, immature specimens from fish-eating birds and cystacanths from fish paratenic hosts (Figs. 1–4).

3.1. Description of adult (Figs. 1, 2, 3a–b)

General Polymorphidae, with characters of the genus *Corynosoma* [12,13]. Living specimens white. Females larger than males (Fig. 1a, c). Proboscis subcylindrical, widens markedly at posterior third (Figs. 1c, 2a), armed with 21–24 longitudinal rows with 11–13 hooks (Fig. 3a); males with 21 or 22 rows; females with 22 or 24 rows: hooks 1–7/8, prebasal, with simple roots, hooks 1–5/6 longer than roots, hooks 6,7/7,8 shorter than roots, hooks 8/9–11/13 small with basal discs (Fig. 2a, b, c); hook combinations (anterior/basal) of 7/4, 7/5, 7/6; 8/4 and 8/5. Measurements of hooks are presented in Table 2. Neck trapezoid. Trunk expanded anteriorly into disc (Figs. 1a, b, c, 3c); fore-trunk shorter, hind-trunk elongated posteriorly; spinose, single field, extending ventrally along 89–97% of the trunk in males (Fig. 1a, b), almost to genital pore in females (Fig. 1c). Aspinose areas on ventral and dorsal surfaces of disc (Figs. 1b, c, 3b). Genital spines surround genital pore in males (Figs. 1a, 2d). Proboscis receptacle double-walled; cephalic ganglion ellipsoidal, situated at mid-length of proboscis receptacle. Lemnisci broad flat, equal size, shorter than proboscis receptacle.

Male (measurements based on 4 mature specimens) Trunk 2.7 mm (2.6–2.9 mm) long by 992 (919–1055) wide at disc level; hind-trunk 549 (524–582) maximum width. Disc diameter 1167 (919–1267). Trunk spines 35 (32–38, n = 12) long by 8 (8–9, n = 12) wide; Proboscis 531 (495–559, n = 4) long by 217 (202 − 233) maximum width. Neck 86 (80–93) long by 282 (244–324) wide. Proboscis receptacle 763 (636–881) long by 211 (186–235) wide. Lemnisci 614 (563–652, n = 3) long by 315 (263–363, n = 3) wide (Fig. 1a). Testes symmetrical, ovoid, posterior to proboscis receptacle (Fig. 1a). Right testis 435 (398–469) long, by 312 (297–336) wide. Left testis 431 (394–462) long, by 341 (269–399) wide. Cement glands claviform, 6 in 3 pairs, 256 (169–401, n = 11) long, by 142 (107–183, n = 11) wide Saefftigen's pouch 503 (460–573) long. Genital spines 41 (33–49, n = 12) long, by 11 (9–13, n = 12) wide; 32 (28–40, n = 3) in number (Fig. 2d). Copulatory bursa in all specimens inverted. Genital pore terminal.

Female (measurements based on 4 gravid specimens) Trunk 3.0 mm (2.8–3.4 mm) long by 1335 (1208–1490) wide at disc level; hind-trunk 701 (639–800) maximum width. Disc 1552 (1357–1726) in diameter. Trunk spines 33 (29–35, n = 5) long by 10 (9–11, n = 12) wide (Fig. 2e). Proboscis 638 (607–669) long by 273 (262–290) maximum width. Neck 101 (84–117, n = 2) long by 390 (361–408) wide. Proboscis receptacle 807 (731–856, n = 3) long by 211 (167–246, n = 3) wide. Spines near genital pore 37 (30–41, n = 5) long, by 10 (8–11, n = 5) wide (Fig. 2f). Mature eggs fusiform, with polar prolongations of the middle membrane (Fig. 2g), 88 (86–94, n = 12) long by 26 (24–32, n = 12) wide. Genital pore slightly subterminal.

3.2. Description of immature specimens (Fig. 3c–f)

General Males and females equal in size. Proboscis, subcylindrical, widens markedly at posterior third (Fig. 3c, d), armed with 18–24 longitudinal rows with 11–13 hooks; males with 18 to 24 rows; females with 18 to 24 rows: hooks 1–7/8 large, prebasal hook largest, with simple roots, hooks 1–5/6 longer than roots, hooks 6,7/7,8 shorter than roots, hooks 8/9–11/13 small with basal discs; hook combinations (anterior/basal) of 7/4, 7/5, 8/4 and 8/5. Measurements of hooks are presented in Table 2. Trunk spinose, spreading posteriorly covering 98–100% of ventral trunk in males, reaching to but not surrounding genital pore in females (Fig. 3c). Aspinose areas on ventral and dorsal surfaces of disc and between disc and hind-trunk on ventral surface (Fig. 3c).

Male (measurements of 18 specimens) Trunk 2.0 mm (1.9–2.5 mm) long by 841 (533–1135) maximum width at disc level. Trunk spines

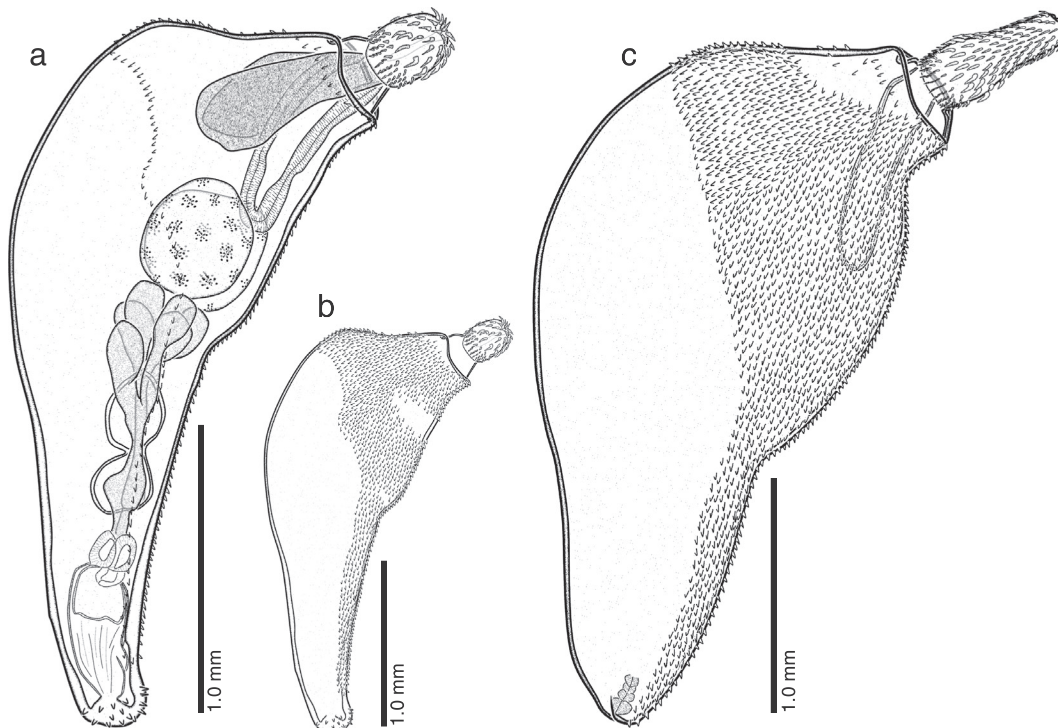

Fig. 1. *Corynosoma hannae* from *Phocarctos hookeri*: (a) adult male, whole worm, lateral view; (b) adult male showing complete trunk armature, lateral view; (c) adult female, whole worm, lateral view.

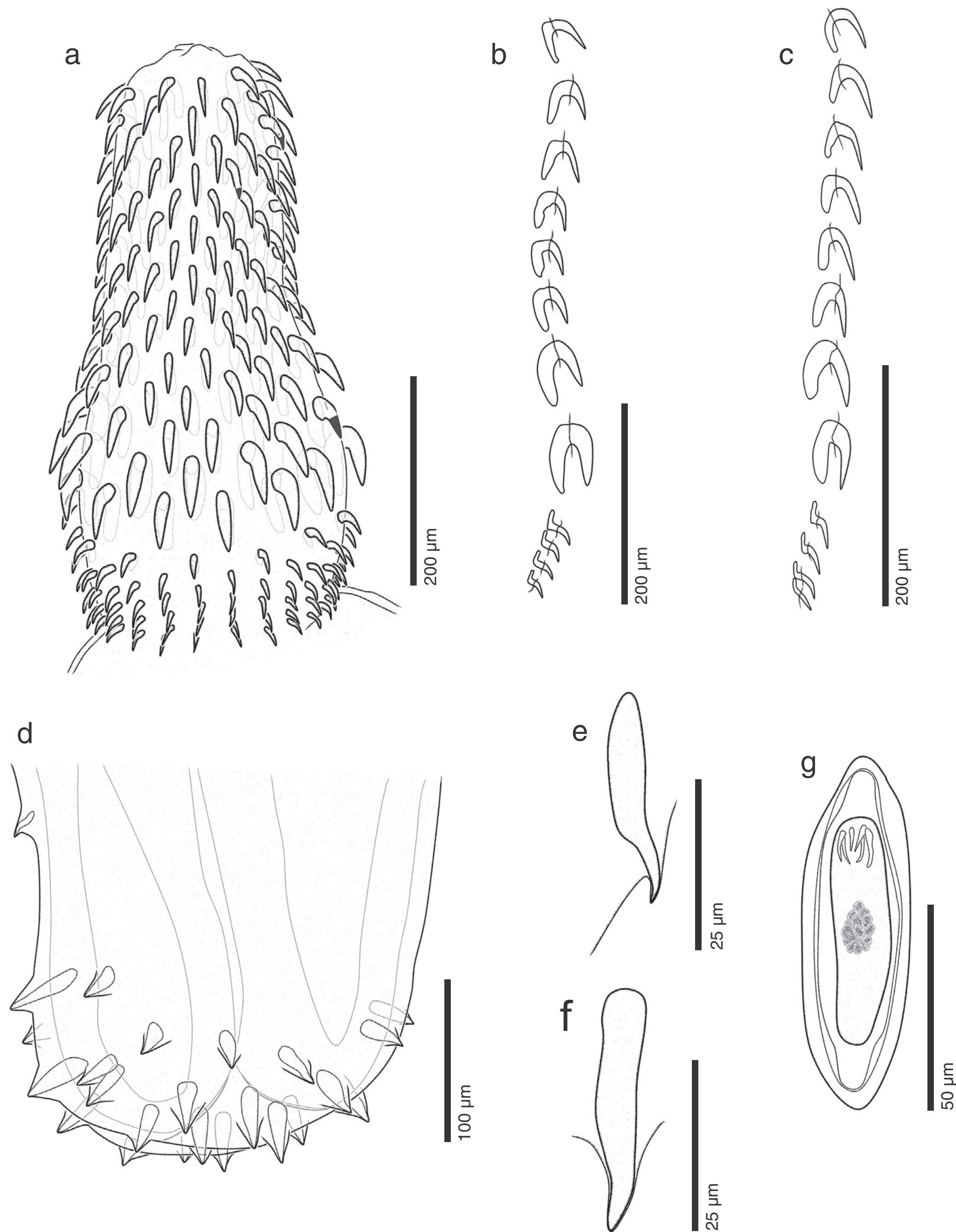

Fig. 2. *Corynosoma hannae* from *Phocarctos hookeri*: (a) female proboscis armature, lateral view. Missing large hooks have been reconstructed with a black shadowed area; (b) row of hooks of female, lateral view; (c) row of hooks of male, lateral view; (d) posterior end of male showing genital spines, lateral view; (e) somatic spine of female, lateral view; (f) spine near genital pore of female, lateral view; (g) Egg.

40 ± 3 (29–45, n = 64) long by 6 ± 1 (5–7, n = 64) wide. Proboscis 524 (425–591, n = 14) long by 245 (180–292, n = 17) maximum width. Neck 142 (110–180, n = 16) long by 324 (242–413, n = 16) wide. Proboscis receptacle 830 (605–1070) long by 219 (153–310) wide. Lemnisci 582 (493–740) long by 264 (253–284, n = 3) wide. Testes 185 ± 16 (165–220, n = 23) long, by 147 ± 29 (95–187, n = 23) wide. Saefftigen's pouch 364 ± 56 (293–441, n = 5) long. Genital spines 45 ± 4 (38–54, n = 64) long, by 10 ± 1 (8–12, n = 64) wide; c.40 in number (Fig. 3e).

Female (measurements of 13 specimens) Trunk 2.1 mm (1.6–2.6 mm) long by 994 (743–1210) maximum width at disc level. Trunk spines 40 ± 4 (29–45, n = 51) long by 6 ± 1 (5–7, n = 64) wide. Proboscis 611 (460–692) long by 285 (221–334) maximum width. Neck 141 (112–176, n = 9) long by 361 ± 58 (285–460, n = 9) wide. Proboscis receptacle 991 ± 122 (802–1286) long by 233 (156–302) wide. Lemnisci 517 (443–626) long. Genital spines absent (Fig. 3f).

3.3. Description of cystacanths (Fig. 4a–b)

General Males and females equal in size. Proboscis, subcylindrical, widens markedly at posterior third (Fig. 4a, b), armed with 18 longitudinal rows with 12–13 hooks; hook combinations (anterior/basal) of 7/5 and 8/5. Measurements of hooks are presented in Table 2. Trunk spinose, spreading posteriorly covering 98–100% of ventral trunk in males (Fig. 4b), reaching to but not surrounding genital pore in females.

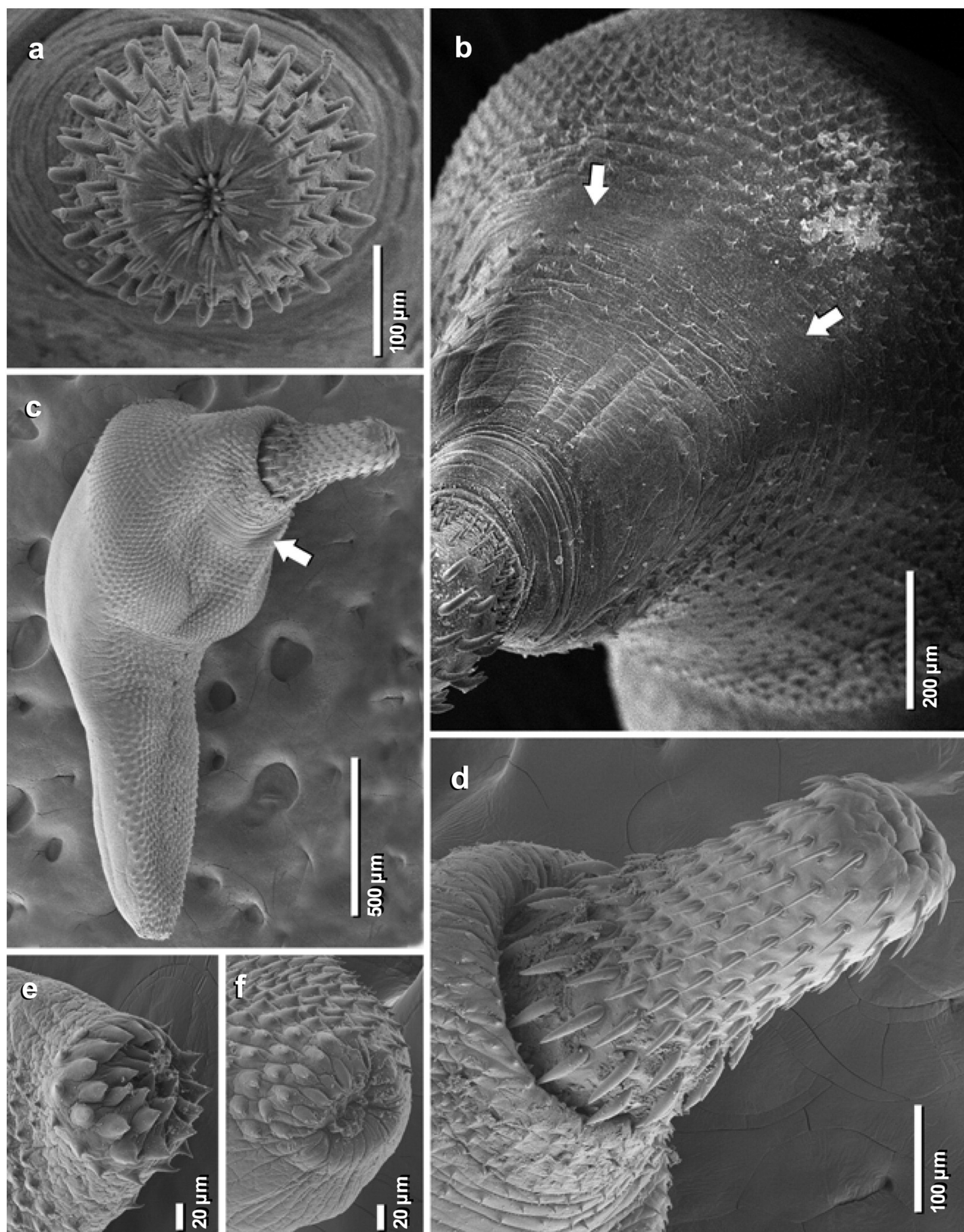

Fig. 3. Scanning electron micrographs of *Corynosoma hannae* from *Phocarctos hookeri*: (a) gravid female proboscis, apical view; (b) gravid female disc, dorsal view. Scanning electron micrographs of *C. hannae* from *Leucocarbo chalconotus*: (c) immature female, whole worm, ventro-lateral view; (d) immature female proboscis, lateral view; (e) genital spines of immature male, basal view; (f) spines near genital pore of immature female, basal view. Arrows points to areas lacking spines on the ventral and dorsal surface of the disc.

Aspinose areas on ventral and dorsal surfaces of disc and between disc and hind-trunk on ventral surface (Fig. 4a, b).

Male (measurements of 5 specimens) Trunk 2.0 mm (1.7–2.1 mm) long by 838 (713–950) maximum width at disc level. Trunk spines 35 ± 3 (30–40, n = 18) long by 6 ± 1 (5–7, n = 18) wide. Proboscis 590 (546–624) long by 267 (233–285) maximum width. Neck 142 (116–200) long by 302 (277–340) wide. Proboscis receptacle 805 (715–904) long by 171 (152–187) wide. Lemnisci 609 (515–752) long by 340 (252–423) wide. Genital spines 42 (34–47, n = 18) long, by 9 (8–11, n = 18) wide.

Female (measurements of 4 specimens) Trunk 2.3 mm (2.2–2.5 mm) long by 1093 (994–1261) maximum width at disc level. Trunk spines 34 ± 3 (30–39, n = 14) long by 6 ± 1 (5–7, n = 14) wide. Proboscis 671 (630–745) long by 310 (287–346) maximum width. Neck 155 (120–182) long by 385 (342–464) wide. Proboscis receptacle 957 (838–1020) long by 224 (212–236) wide. Lemnisci 722 (643–819) long by 411 (360–518) wide. Genital spines absent.

3.4. Taxonomic summary

Type host leopard seal, *Hydrurga leptonyx* (de Blainville) (Carnivora: Phocidae).

Type-locality King George Island, South Shetlands, Antarctic.

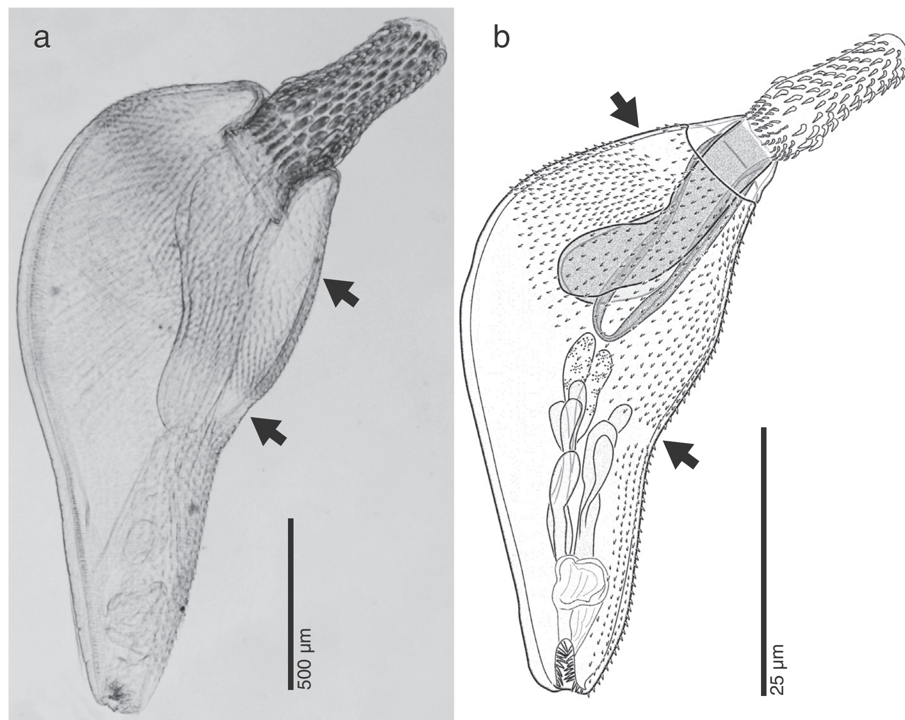

Fig. 4. *Corynosoma hannae* from *Peltorhamphus novaezeelandiae*: (a) photomicrograph of an excysted male cystacanth; (b) line drawing of a male cystacanth. Arrows point to areas lacking spines.

Other definitive hosts New Zealand sea lion, *Phocarctos hookeri* (Gray), long-nosed fur seal, *Arctophoca forsteri* (Lesson) (Carnivora: Otariidae).

Other localities for definitive hosts Balleny Islands, D'Urville Sea, Antarctica; Enderby Island, Auckland Islands (50°30′S, 166°16′W) and St Clair Beach, Dunedin, South Island, (45°54′S, 170°30′W), New Zealand.

Site in definitive hosts Large intestine (type host).

Non-definitive hosts Stewart Island shag, *Leucocarbo chalconotus* (Gray), spotted shag, *Phalacrocorax punctatus* (Sparrman) (Pelecaniformes: Phalacrocoracidae); yellow-eyed penguin, *Megadyptes antipodes* (Hombron & Jacquinot) (Sphenisciformes: Spheniscidae).

Localities for non-definitive hosts Otago Harbour, South Island (45°54′ S, 170°30′W), New Zealand.

Site in non-definitive hosts Small and large intestines.

Paratenic host New Zealand brill, *Colistium guntheri* (Hutton), New Zealand sole, *Peltorhamphus novaezeelandiae* Günther (Pleuronectiformes: Pleuronectidae).

Locality for paratenic host Kaka Point, Otago (46°23′S, 169°47′W), New Zealand.

Site in paratenic hosts Body cavity and mesenteries.

Infection parameters in paratenic hosts Ex *C. guntheri*, 56 cystacanths in one fish; ex *P. novaezeelandiae*, prevalence 100% ($n = 28$), mean intensity/abundance = 33 per fish.

Voucher specimens Ex *Phocarctos hookeri*, 4 specimens (CNHE 9940); ex *M. antipodes,* 6 specimens (MNZ ZW 1489–94); ex *L. chalconotus*, 15 specimens (OMD IV58500).

Hologenophores Ex *L. chalconotus*, 3 specimens (IV64671–3); ex *Phalacrocorax punctatus*, 1 specimen (IV64676); ex *C. guntheri*, 1 specimen (IV64675); ex *P. novaezeelandiae*, 1 specimen (IV64674).

Representative sequences C. hannae ex *Phocarctos hookeri* GenBank KX957715-KX957717 (*cox1*); ex *L. chalconotus* GenBank KX957718-KX957721, KX957723 (*cox1*); ex *Phalacrocorax punctatus* GenBank KX957722 (*cox1*); ex *C. guntheri* Genbank KX957724-KX957725 (*cox1*); ex *P. novaezeelandiae* Genbank KX957726 (*cox1*).

References Zdzitowiecki [12,13]; Stryukov [40]; Shiel et al. [20]; Present study.

3.5. Taxonomic remarks

Corynosoma hannae was described by Zdzitowiecki [13] from 3 specimens (1 male, 1 gravid female and 1 female without mature embryophores) collected from the large intestine of the leopard seal, *Hydrurga leptonyx* (de Blainville) from Antarctica. Interestingly, Zdzitowiecki [13] designated the gravid female as the holotype which is not usual taxonomic practice. Later, *C. hannae* was reported by Stryukov [40] from the intestine of *H. leptonyx* from the Balleny Islands, D'Urville Sea, Antarctica, and by Shiel et al. [20] from *A. forsteri* and *M. antipodes* from New Zealand. The specimens from fish-eating birds, fur seal and sea lion from New Zealand in this study exhibit all the morphological features of *C. hannae* (i.e. body shape; proboscis with well pronounced dilation at posterior third; hooks arranged in c.22 rows of 12–13 hooks per row, including 7–8 apical hooks and 4–6 basal hooks per row; somatic spines spreading almost to posterior body end; absence of genital spines in females). Our mature specimens from a sea lion were slightly smaller than the type specimens as were the immature specimens from the fur seal. Intraspecific morphological and biometrical variability induced by different otariid host species, has been observed for species of *Corynosoma* ([41]; J.S. Hernández-Orts, unpubl. data). Therefore we consider that all the material from New Zealand belongs to *C. hannae*.

The ranges of measurements for the specimens of *C. hannae* from fish-eating birds are also smaller than those from leopard seals, as was expected since they were all immature (Table 3). Measurements of the hook length and root length of immature and adult specimens from New Zealand are similar to the values for adults reported by Zdzitowiecki [13] (Table 2).

The specimens from New Zealand provided the lower and higher limits of the range for the number of rows of hooks (18–24) for *C. hannae*. For those from fish-eating birds, the most usual number of

Table 2
Comparative data for the proboscis hooks of *Corynosoma hannae* from paratenic fish hosts, non-definitive hosts and definitive hosts. Measurements in micrometres.

Host	*Peltorhamphus novaezeelandiae* Günther[a]						*Leucocarbo chalconotus* (gray)[b]					
Locality	Otago, New Zealand						Otago, New Zealand					
Life-cycle stage	Cystacanth						Immature					
Source	Present study						Present study					
	Male			Female			Male			Female		
	n	Hook	Root	n	Hook	Root	n	Hook	Root	n	Hook	Root
Apical	5	47–61	41–52	1	58	52	6	44–53	36–42	6	38–57	40–51
Subapical I	5	50–55	38–54	3	50–59	51–56	8	38–57	35–54	12	44–62	39–50
Subapical II	5	40–58	37–51	4	51–58	44–60	12	34–56	33–44	12	45–63	40–54
Subapical III	5	43–58	35–47	4	54–55	45–60	13	38–52	35–46	12	41–56	40–48
Subapical IV	5	35–58	34–48	4	45–52	44–55	14	42–50	37–48	13	41–54	40–48
Subapical V	5	41–57	37–49	4	44–57	43–57	14	41–51	42–53	13	38–53	42–52
Subapical VI	5	42–60	41–62	4	50–58	51–67	15	49–60	50–56	13	53–65	51–62
Subapical VII	5	61–78	65–104	4	60–84	70–83	16	60–69	62–72	13	64–84	71–89
Basal I	5	35–50	–	4	32–50	–	14	28–38	–	12	29–42	–
Basal II	5	31–36	–	4	31–40	–	14	26–36	–	12	29–37	–
Basal III	5	30–34	–	4	30–40	–	14	23–32	–	12	25–38	–
Basal IV	5	26–32	–	4	27–37	–	14	19–31	–	11	23–33	–
Basal V	5	23–31	–	4	25–37	–	10	16–29	–	10	24–28	–
Basal VI		–	–		–	–	–	–	–	–	–	–

Host	*Phocarctos hookeri* (Gray)[c]						*Hydrurga leptonyx* (de Blainville)[c]		
Locality	Enderby Island, New Zealand						King George Island, Antarctic		
Life-cycle stage	Adult						Adult		
Source	Present study						Zdzitowiecki [13][d]		
	Male			Female			Male		
	n	Hook	Root	n	Hook	Root	n	Hook	Root
Apical	5	52–56	39–50	8	46–70	46–59	1	56	47
Subapical I	5	46–52	37–44	10	44–59	44–54	1	52	44
Subapical II	5	41–52	37–41	10	41–56	37–52	1	51	43
Subapical III	5	37–46	31–41	10	37–52	33–50	1	51	46
Subapical IV	5	39–48	37–41	10	35–50	39–56	1	53	48
Subapical V	5	44–48	31–57	10	43–56	44–56	1	54	52
Subapical VI	5	54–69	48–61	9	50–69	44–78	1	60	23
Subapical VII	5	50–67	54–63	5	65–81	67–78	1	75	29
Basal I	12	28–37	19–30	10	28–44	17–41	1	32	36
Basal II	11	22–30	17–22	10	24–33	19–24	1	29	24
Basal III	10	20–28	13–20	10	22–30	15–22	1	30	17
Basal IV	10	19–26	13–17	59	22–30	13–19	1	29	17
Basal V	5	17–24	13–15	5	22–24	13–17	1	28	13
Basal VI	–	–	–	1	22	13	–	–	–

[a] Fish paratenic host.
[b] Non-definitive host.
[c] Definitive host.
[d] Measurements from type-material.

rows was 18–20 (73% of the sample for specimens collected from *L. chalconotus*); whereas in seals, the most usual number of rows was 22 (62.5% in the New Zealand sea lion, and 50% in the long-nosed fur seal).

Seven species of *Corynosoma* parasitizing pinnipeds have been reported from the Southern Hemisphere, namely *C. arctocephali* Zdzitowiecki, 1984, *C. australe*, *C. bullosum*, *C. evae* Zdzitowiecki, 1984, *C. hamanni* (Linstow, 1982), *C. pseudohamanni* Zdzitowiecki, 1984 and *C. gibsoni* Zdzitowiecki, 1984 [12]. *Corynosoma semerme* was also reported from the sub-Antarctic Campbell and Auckland Islands in New Zealand [18]. However, this species is currently considered a widespread acanthocephalan in the Northern Hemisphere (see below). Compared with the other species from the Southern Hemisphere, *C. hannae* most closely resembles *C. evae* morphometrically, each with similar proboscis and body shape, and having a proboscis armature of 20–24 longitudinal rows of hooks. However, *C. evae* possesses 8–10 large hooks and 3–4 small basal hooks, whereas *C. hannae* has 7–8 large hooks and 4–5 small basal hooks. In addition *C. hannae* differs from *C. evae* by the extent of somatic spines in adult specimens (100% of trunk length vs. 69% for females; and 89–97% of trunk length vs. 61% for males, see Fig. 4a and b in Zdzitowiecki [13]).

Corynosoma hannae is clearly distinguishable from *C. australe* by the shape of the proboscis (approximately cylindrical with a swelling at the base vs. cylindrical) and by the numbers of hooks in each rows (7–8 large hooks and 4–5 small basal hooks vs. 9–11 large hooks and 2–4 small basal hooks) [9,13]. *Corynosoma hannae* differs from *C. arctocephali* and *C. bullosum* in having a considerably shorter trunk length (2.8–5.1 vs. 6.6–8.4 and 12.0–17.8, respectively for females; and 2.6–3.6 vs. 4.1–6.6 and 8.0–11.7, respectively for males), and by the extent of somatic spines in adult specimens (100% of trunk length vs. 58–69%5 and 28–38, respectively for females; and 89–97% of trunk length vs. 55–60% and 33–54%, respectively for males) [13,42]. Additionally, *C. arctocephali* possesses 19–22 and *C. bullosum* 16–18, usually 16, rows of hooks compared with 18–24 usually 22 in *C. hannae*. *Corynosoma hamanni* can be differentiated from *C. hannae* in having a longer trunk (5.2–6.4 vs. 2.8–5.1 for females; and 5.2–7.1 vs. 2.6–3.6 for males), by the number of hooks per row (usually 14–15 vs. 11–13)

Table 3
Comparative data of males and females of *Corynosoma hannae* from different hosts. Trunk in millimetres, other measurements in micrometres.

Reference	Present study[a]	Present study[a]	Present study[a]	Present study	Zdzitowiecki [13][b]	Stryukov [40][c]
Hosts	*Megadyptes antipodes* (Hombron & Jacquinot)	*Arctophoca forsteri* (Lesson)	*Leucocarbo chalconotus* (Gray)	*Phocarctos hookeri* (Gray)	*Hydrurga leptonyx* (de Blainville)	*H. leptonyx*
Locality	Otago Harbour, New Zealand	St Clair Beach, Dunedin, Otago	Otago, New Zealand	Enderby Island, New Zealand	South Shetlands, Antarctic	Balleny Islands, Antarctica
Life-cycle stage	Immature	Immature	Immature	Adult	Adult	Adult
General						
No. of rows of hooks	22–24	22–24	18–24	21–24	22	–
No. hook per row	12–13	12–13	11–13	11–13	12–13	11–13
No. apical hooks per row	7–8	7–8	7–8	7–8	7–8	7–8
No. basal hooks per row	4–5	4–5	4–5	4–6	4–6	4–5
Largest hook	69–72	66–73	69–84	69–81	75	–
Female						
Trunk	1.7–2.2 × 0.4–1.1	2.9	1.6–2.6 × 0.8–1.2	2.8–3.4 × 1.2–1.5	5.1 × 1.6	3.5 × 1.6
Proboscis	670 × 268	724 × 245	460–692 × 221–334	607–669 × 262–290	684 × 334	702 × 328
Neck	201 × 235	–	112–176 × 285–460	84–117 × 361–406	150 long	–
Trunk spines % ventral cover	–	99%	98–100%	99–100%	99%	99%
Trunk spines	–	–	29–46 × 5–6	29–35 × 9–11	52 × 11	43 × 8
Proboscis receptacle	737–1275 × 221–272	1190	802–1286 × 156–274	737–856 × 167–246	1410 × 310	1162 × 359
Lemnisci	600–700	–	443–626	–	1000 × 600	720 × 750
Egg size	–	–	–	86–94 × 24–32	105–130 × 42–53	112 × 39
Male						
Trunk	1.5–2.2 × 0.7–1.1	2.4	1.9–2.5 × 0.5–1.1	2.6–2.9 × 0.9–1.1	3.6 × 1.3	3.6 × 1.5
Proboscis	503–670 × 228–268	582 × 221	425–591 × 180–292	495–559 × 202–233	585 × 296	628 × 341
Neck	134 × 335	–	111–180 × 242–413	80–93 × 244–324	190 long	–
Trunk spines % ventral cover	–	–	98–100%	89–97%	95%	100%
No. of genital spines	–	–	40	28–40	40	–
Trunk spines	–	–	28–45 × 5–7	32–38 × 8–9	42 × 10	34 × 8
Genital spines	–		38–54 × 8–11	33–49 × 9–13	46 × 16	39 × 9
Proboscis receptacle	623–1090 × 161–460	804 × 221	605–1070 × 153–310	636–881 × 186–235	950 × 240	–
Lemnisci	600	630	493–740 × 253–284	563–652 × 263–363[d]	940 × 600	–
Testes	132–348 × 106–307	335–369 × 226–275	165–220 × 95–187	394–469 × 269–399	520–590 × 400	482–496 × 345–369
Säfftigen's pouch	–	–	293–441	460–573	890 × 240	394

[a] Immature specimens.
[b] Measurements from type-material.
[c] All measurements calculated from the published figures.
[d] Lemnisci not fully extended.

and the distribution of the genital armature which spreads dorsally in both sexes (see Fig. 1a and b in Zdzitowiecki [14]). Morphological differences between *C. pseudohamanni* and *C. hannae* include males larger than females (vs. females larger than males), a slightly longer trunk length in males (3.5–4.9 vs. 2.6–3.6) and a longer proboscis (804–1001 vs. 607–702 for females, 799–929 vs. 495–628 for males). *Corynosoma hannae* differs from *C. gibsoni* by the shorter minimum trunk length in females (2.8 vs. 4.6), the extent of somatic spines in females (extending almost to genital pore vs. about 3/4 of trunk length, see Fig. 1a in Zdzitowiecki [43]) and the smaller egg length (86–130 vs. 155–188).

Considering the species of *Corynosoma* from the Northern Hemisphere, *C. hannae* mostly closely resembles *C. semerme*, both with slight sexual dimorphism, a proboscis with well pronounced dilation at posterior third, similar body length, number of rows of hooks, number of hooks per row, somatic armature extending almost to genital pore and egg size (see Supplementary Table S1). According to Zdzitowiecki [13], *C. semerme* differs from *C. hannae* occurring in *H. leptonyx* by having smaller body size, proboscis, hooks and eggs. However our specimens, from *Phocarctos hookeri*, have similar morphometrics to those of *C. semerme*. Rather, *C. hannae* differs from *C. semerme* by the smaller maximum number of rows of hooks (24 vs. 26), the smaller maximum number of apical hooks per row (8 vs. 9) and the presence of genital spines. Genital spines are found only in males of *C. hannae* but in both males and females of *C. semerme*.

As noted above, *C. semerme* was described by Johnston and Edmonds [18] from the intestine of *Phocarctos hookeri* (as *Otaria hookeri*). Their specimens are similar to the New Zealand specimens of *C. hannae* from *Phocarctos hookeri* in having a cylindrical proboscis with a swelling at the base (see Plate I, Fig. 1 in Johnston and Edmonds [18]), and a proboscis armature of 22–24 longitudinal rows of 7–8 large hooks and 4–6 small basal hooks vs. 21–24 rows of 7–8 large hooks and 4–6 small basal hooks (see Supplementary Table S1). However, *C. semerme* sensu Johnston and Edmonds clearly differs from *C. hannae* in having a smaller body size in females (2.0–2.4 vs. 2.8–3.4), a smaller minimum size for males (1.8 vs. 2.6), by the extent of somatic spines in adult males (82% of trunk length vs. 89–97%), and by the presence of genital spines surrounding the genital pore in males and females vs. genital spines only in males. Golvan [44] suggested that the acanthocephalans described as *C. semerme* by Johnston and Edmonds [18] could belong to a different species based on their geographical distribution and the extent of the somatic spines. From our study, it appears that the taxonomic characters differentiating *C. hannae* from *C. semerme* sensu Johnston and Edmonds although few, may be valid. Unfortunately the specimens described by Johnston and Mawson could not be found and so could not be re-examined. There are currently no DNA sequences reported

from *C. semerme* available for comparison. Further studies are needed to clarify the status of *C. semerme* and *C. semerme* sensu Johnston and Edmonds compared with the current concept of *C. hannae*.

3.6. Molecular results

A total of 13 partial *cox1* sequences was generated (12 for *C. hannae* and 1 for *C. australe*). Newly generated *cox1* sequences of *C. hannae* (including specimens from sea lions, shags and fish) were almost identical (intraspecific genetic divergence ranged between 0.0 and 2.8%). The sequence for *C. australe* of García-Varela et al. [21] (JX442191; isolate from New Zealand) exhibited a strong association with our sequences for *C. hannae* (Fig. 5; mean genetic divergence was 0.5 ± 0.01%). This result suggests that the sequence reported to be *C. australe* of García-Varela et al. [21] belongs to *C. hannae*. In comparison with other species of *Corynosoma*, *C. hannae* exhibited the lowest divergence level with the newly generated sequence of *C. australe* (13.9 ± 0.5%), and the highest divergence level with the sequence of *Corynosoma enhydri* Morozov, 1940 (16.8 ± 0.4%). The phylogenetic analyses inferred with ML and BI methods yielded the same topologies. Both trees placed the specimens identified as *C. hannae* in a clade receiving strong bootstrap support and Bayesian posterior probability values (Fig. 5). The phylogenetic tree obtained in the present study suggests that *C. hannae* is a sister taxon to *C. australe*, both species infecting pinnipeds in the Southern Hemisphere.

4. Discussion

The complete life cycle of *C. hannae* is still unknown; however results from the present study suggest that teleosts act as paratenic hosts, fish-eating birds as non-definitive hosts and pinnipeds as definitive hosts. The early stages in the life cycle probably involve amphipods as intermediate hosts, as reported for other species of *Corynosoma* (e.g. [3,4]). Prior to this study, cystacanths of only *C. semerme* from *M. novaezelandiae* and *G. blacodes* were reported in New Zealand waters [19]. Following the brief description provided by Grabda and Ślósarczyk [19], it seems that these cystacanths could belong to *C. hannae* (i.e. a proboscis armed with 23–24 longitudinal rows with 12–13 hooks, with 7 prebasal hooks and 5 small hooks and genital spines surrounding the genital pore only in the male). Further studies are necessary to confirm the identity of these fish species as paratenic hosts for *C. hannae*. However, our study provides the first morphological and molecular characterisation of cystacanths of *C. hannae* from ray-finned fishes.

Aspinose areas in the ventral and dorsal armature of the disc or between the disc and the hind-trunk found in some specimens of *C. hannae* from New Zealand (Figs. 1b, 3b, c, 4), were observed independently of the developmental stage or sex of the worm, and were highly variable as to size and location. Interestingly, aspinose areas were not reported by Zdzitowiecki [13] and Stryukov [40] for *C. hannae* from the Antarctic. In other species of *Corynosoma*, aspinose areas in the trunk armature have only been reported in the hind-trunk of *C.*

Fig. 5. Bayesian inference (BI) tree derived from *cox1* rDNA sequences for polymorphid acanthocephalans with nodal support (posterior probabilities >0.50 shown only) followed by bootstrap values (>51% shown only) from Maximum likelihood (ML) analysis. The newly generated sequences are indicated in bold. Isolate labelled as *C. australe* by Garcia-Varela et al. [21] (GenBank acces. no. JX442191) is considered as conspecific with *C. hannae* in the present study (see molecular results). The scale bar indicates the expected number of substitutions per site.

cetaceum Johnston & Best, 1942 from small cetaceans by Aznar et al. [45], and by Sardella et al. [9] and Aznar et al. [46] in cystacanths of *C. cetaceum* from fish paratenic hosts. Distribution of trunk spination should be examined in other species of *Corynosoma* to detect any other instances of inhibition of spine growth, and to shed light on the way this morphological feature is produced.

The presence of 2 bare zones, forming 2 fields of trunk spines has been proposed as the only diagnostic morphological difference between *Andracantha* Schmidt, 1975 and *Corynosoma* [1,47]. Although some specimens of *C. hannae* possess an irregular bare ventral patch within a single field of trunk spines (Figs. 1b, 3c, 4a and b) these patches can be clearly distinguished from the aspinose area encircling the disc of species of *Andracantha* which divide the trunk spines into two fields (see Figs. 1, 2, 6, 7, 11 in [47]). The presence of aspinose areas in *C. hannae* is a highly variable character insufficient alone to distinguish our specimens from either other species of *Corynosoma* or other polymorphid genera.

A total of 12 partial *cox1* sequences were generated for *C. hannae* from New Zealand plus one sequence reported as *C. australe* from Patagonia, Argentina from a sea lion. Genetic divergence estimated among the 13 isolates was low, and ranged from 0.0 to 2.8%. This level of genetic divergence among isolates of *C. hannae* is similar to that found in other species of polymorphids. For example, the genetic divergence in *cox1* among isolates of *Hexaglandula corynosoma* (Travassos, 1915), *P. brevis,* and *Southwellina hispida* (Van Cleave, 1925), *Profilicollis botulus* (Van Cleave, 1916) and *Pseudocorynosoma constrictum* (Van Cleave, 1918) ranged from 0 to 3% [28,31,48,49]. The low levels of genetic divergence, in addition to the systematic position of the 13 isolates in the resulting phylogenetic trees, in combination with morphological data, clearly demonstrate that all the isolates belong to the same evolutionary lineage, i.e., they represent the species *C. hannae*.

Prior to our study, molecular data had never been obtained from cystacanths of *Corynosoma* spp. according to the GenBank dataset. In the present study, we have provided sequences for cystacanths of *C. hannae*, which allow us to link isolates of larvae from fish paratenic hosts and adults from the seal definitive hots, and thus partially elucidate the life-cycle of this acanthocephalan. Therefore, the use of cystacanths of *Corynosoma* in future molecular studies will provide valuable information about the diversity, evolution or host-parasite interactions of these acanthocephalans, and their usefulness as biological markers in population studies of marine fish [50]. Furthermore, fish paratenic hosts are usually easier to collect and examine than definitive hosts.

Finally, the present study raises new questions about the geographical distribution of species of *Corynosoma*. Previous taxonomical studies on *Corynosoma* suggest two main groups of species, one associated with Holarctic fauna and the other in fauna contiguous with the Antarctic [51, 52]. The geographical distribution of these groups was used as a biological trait to differentiate species (e.g. [44]), although morphological characters differ very little between them (e.g. [13]). However, recent integrative taxonomical studies, combining morphological and molecular data, in other groups of otariid parasites suggest that a single parasite species can be widely distributed between the Northern and Southern Hemispheres, infecting a wide range of pinniped species [53]. Thus, it is also possible that some species of *Corynosoma* could be distributed in both hemispheres. Future re-evaluation of several species of *Corynosoma* from different hosts and localities, using a combination of morphological and molecular data, is necessary to clarify the real distribution of these acanthocephalans.

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