

## Prevalence and molecular characterisation of Acanthocephala in pinnipedia of the North and Baltic Seas

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### ABSTRACT

Harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) are final hosts of acanthocephalans in the German North and Baltic Seas. Parasitic infections in seals can cause pathological changes, which may result in deteriorated health of the host. Common gastrointestinal parasites of harbour and grey seals are acanthocephalans and a number of 275 of 2460 (11.2%) investigated seals from 1996 to 2013 were infected with *Corynosoma* spp. (Acanthocephala, Polymorphidae). The prevalence showed a wave-like pattern: it increased from 1.2% and 0.4% in 1996 and 1997, respectively, to 23.9% during the second phocine distemper epizootic in 2002 and decreased to 6.2% in 2004. In 2005, prevalence peaked again with 25.0% followed by a decrease to 9.3% in 2009 and an increase to 38.5% in 2012. Statistical analysis revealed that harbour seals originating from the North Sea showed a higher prevalence than grey seals, whereas no significant difference between grey and harbour seals from the Baltic Sea was observed. Furthermore, juvenile pinnipedia from the North Sea were significantly less infected with *Corynosoma* spp. than seals older than seven months. Molecular species identification as well as phylogenetic relationship analysis among the detected *Corynosoma* species were achieved by sequencing and comparisons of the ribosomal ITS1-5.8S-ITS2-complex and cytochrome-c-oxidase I gene. Molecular analysis resulted in a newly arranged distribution of Acanthocephala in the North Sea as in contrast to previous studies, *C. strumosum* could not be confirmed as predominant species. Instead, *C. magdaleni* and a *C. magdaleni* isolate (isolate Pv1NS) with an atypical number of longitudinal rows of hooks at the proboscis were detected. Furthermore, morphological and molecular analyses indicate the possible finding of a cryptic species (*Candidatus Corynosoma nortmeri* sp. nov.).

### 1. Introduction

Indigenous seals of the North and the Baltic Seas are harbour seals, *Phoca vitulina* (Linnaeus, 1758), grey seals, *Halichoerus grypus* (Fabricius, 1791) and ringed seals, *Phoca hispida* (Schreber, 1775). After the first PDV epizootic in 1988/89, a stranding network along the coast of the German federal state Schleswig-Holstein and an extensive assessment of health status of seals, including parasitic infections, was established (Siebert et al., 2007). Parasites can cause pathological changes, affecting the health of seals (Bergeron et al., 1997; Lehnert et al., 2007). The class of Acanthocephala (Kohleuter, 1771), also known as thorny headed worms, represent intestinal parasites associated with focal eosinophilic, granulomatous inflammation and enteritis as well as gastric ulcers and even perforation of the intestinal wall (Bergman and Olsson, 1985; Lehnert et al., 2007; Siebert et al., 2007). The genus *Corynosoma* (Lühe, 1904) as a member of the family

Polymorphidae (Meyer, 1931) is with 38 species the most speciose acanthocephalan genus (Golvan, 1994). The trunk of *Corynosoma* is described as small to medium sized, covered with a characteristically distribution of trunk spines. The proboscis is armed with 14–28 longitudinal rows of hooks. The life cycle is heteroxenous, containing usually an amphipod or isopod as first intermediate host, fishes as second intermediate hosts and waterfowl, sea birds or marine mammals as final hosts (Petrochenko, 1971; Crompton and Nickol, 1985; Nickol et al., 2002; García-Varela et al., 2005).

Examinations after the PDV epizootic in 1988/89 revealed that 70.2%–94.5% of the investigated harbour seals were infected with acanthocephalans, namely *Corynosoma strumosum* (Rudolphi, 1802) (Borgsteede et al., 1991; Strauss et al., 1991). Further prevalence determined for the North Sea at the same time period was 32% in the Kattegat-Skagerrak (Lunneryd, 1992), whereas the infection rate of harbour seals in the North Sea between 1997 and 2000 was 23% with *C.*

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*strumosum* as predominant species, but also *C. semerme* (Forssell, 1904) was detected in one animal (Lehnert et al., 2007). Before the second PDV epizootic occurring between 1996 and 2002, 12% of examined seals were infected with *Corynosoma* spp., while the prevalence increased to 20% after the second PDV epizootic between 2002 and 2005 (Siebert et al., 2007). In contrast, 100% of investigated ringed seals of the Bothnian Bay in Finland, as part of the Baltic Sea, were infected with *C. semerme* between 1988 and 1999, while 58.0% of seals were infected with *C. strumosum* and 81.0% with *C. magdaleni* (Valtonen et al., 2004). For the same region, Nickol et al. (2002) reported a species-specific tropism in the intestine of grey seals, with *C. strumosum* and *C. magdaleni* preferring the small intestine while *C. semerme* was found primarily in the rectum.

So far, *Corynosoma* species were mainly differentiated by morphological characteristics, but subtle differences between species may result in less reliable data (Van Cleave, 1953; García-Varela et al., 2005). However, reliable species discrimination is crucial for the understanding parasite epidemiology and its impact on the health status of the host. Therefore, the usage of molecular markers is beneficial to discriminate between species with identical or very similar morphological characteristics. According to Hebert et al. (2003), the mitochondrial gene cytochrome c oxidase I (COI) can serve as reliable DNA marker gene to analyse the systematic relationships between closely related species. Consequently, this marker has been used in several studies to discriminate species in, including *Corynosoma* spp. and related acanthocephalan genera (García-Varela et al., 2009). In addition, Blouin (2002) described the internal transcribed spacer (ITS)1–5,8S rRNA-ITS2-complex (ITS-complex) as an excellent tool for DNA diagnostics to distinguish between species. The ITS-complex has already been used successfully to differentiate acanthocephalans including *Corynosoma* spp. in harbour and grey seals (García-Varela et al., 2005).

The aim of the presented study was to compare the occurrence and distribution of acanthocephalans in harbour and grey seals of the German North and Baltic Sea between 1996 and 2012. Furthermore, acanthocephalan specimens of the seal carcasses were analysed using the ITS1-complex and COI as molecular markers to investigate their phylogenetic relationship.

## 2. Material and methods

### 2.1. Sample origin

The carcasses of harbour seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*) and ringed seals (*Phoca hispida*) originating from the North and Baltic Sea coasts of the German federal state Schleswig-Holstein were examined as part of the regional monitoring program (Siebert et al., 2007). Additionally, 17 seals from the Polish Baltic Sea were included in the analysis. Included animals were either found dead or mercy killed by officially appointed seal rangers and stored at –20 °C until further investigated. Necropsies were performed as described by Siebert et al. (2007). Specimens of acanthocephalans were collected during necropsy from the digestive tract of examined seals, washed with 0.9% NaCl solution and preserved in 70% ethanol for morphological characterisation and molecular differentiation. Results of the parasitological examination, combined with data comprising sex, age, sampling location, sampling date as well as body length and weight were noted. For this study, a dataset of 2460 seals examined between 1996 and 2013 were used for the epidemiological analyses on acanthocephalan infections. As data were not always completely available, sample sizes vary for below described investigations. Parts of the dataset were already analysed for different pathological aspects and published by Lehnert et al. (2007) and Siebert et al. (2007).

Age group classification of seals was determined by sampling date, body length and weight, as well as the navel and canine development in young seals (Mansfield and Fisher, 1960). Age group 1 included animals between 0 and 6 months, age group 2 those between 7 and 18 months

and age group 3 seals older than 18 months.

### 2.2. Surveillance of *Acanthocephala* infections in seals

The prevalence of intestinal acanthocephalan infection in the necropsied seals was compared between years 1996 and 2012. Furthermore, prevalence differences between harbour and grey seals from the North and Baltic Sea as well as between age groups were analysed.

### 2.3. Morphological characterisation of acanthocephalans

Morphological species discrimination of adult acanthocephalans collected from the intestine of necropsied seals was conducted according to criteria described by Delyamure (1955), Petrochenko (1971) and Nickol et al. (2002) using a stereomicroscope (Olympus SZH10 Research Stereo, Olympus, Japan).

Furthermore, 50 morphologically assigned acanthocephalans (35 specimens of *C. strumosum*, 10 of *C. semerme* and 5 of *C. magdaleni*) were characterised in detail by measuring the length and width of the body, fore- and hindtrunk and proboscis as well as determination of the trunk spine coverage and, if possible, the number of the proboscis' longitudinal hooks. Specimens were photographed with a digital camera (Olympus SC30 camera, Olympus, Japan) attached to a stereomicroscope (Olympus SZH10 Research Stereo, Olympus, Japan) or light microscope (Olympus Cx41, Olympus, Japan). Measurements were conducted with CellSens Entry software (version 1.5, Olympus, Japan).

### 2.4. Molecular and phylogenetic analyses of acanthocephalan specimens

Genomic and mitochondrial DNA of the 50 worms characterised in detail was extracted individually using QIAamp DNA Micro Kits (Qiagen, Germany) according to the manufacturer's instructions. Isolated DNA was amplified by PCR using the GeneAmp® RNA PCR Core Kit (Applied Biosystems, USA). Amplification of the ribosomal internal transcribed spacer (ITS)-complex (ITS1-5.8S rRNA-ITS2) was carried out in a 50 µl reaction mixture containing 5 µl 10x PCR buffer II, 4 µl dNTP mix (1 mM each), 4 µl MgCl<sub>2</sub> (5 mM), each 5 µl primer FWcor2 and REVcor2 (0.2 pmol) (García-Varela et al., 2005; Luton et al., 1992), 0.25 µl AmpliTaq DNA Polymerase (0.025 U/µl), 10 µl template DNA and 16.75 µl DEPC-treated water. PCR cycling parameters comprised an initial denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min and subsequent final elongation at 72 °C for 5 min.

Parts of the mitochondrially encoded cytochrome c oxidase I (COI) DNA were amplified using 5 µl 10x Taq PCR buffer II (1 mM), 2.25 µl dNTP mix (0.9 mM each), each 5 µl primer Cox1FW and Cox1Rev (0.2 pmol) (Folmer et al., 1994; García-Varela et al., 2009), 0.25 µl PerfectTaq DNA Polymerase (5 PRIME GmbH, Germany) (0.025 U/µl), 5 µl template DNA and 27.5 µl DEPC treated water. Cycling conditions comprised an initial denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 1 min, 40 °C for 1 min, 72 °C for 1 min and subsequent final elongation at 72 °C for 5 min.

Obtained amplification products were sequenced with the Sanger method (SeqLab, Germany and Eurofins MWG Biotech, Germany). If sequencing of PCR products was not successful, PCR products were ligated into 4-TOPO™ vector and inserted in chemically competent TOP10 *E. coli* cells (OneShot™ TOP10) using the TOPO™ TA Cloning™ Kit (Life Technologies Inc., USA) and purified plasmids (NucleoSpin™ Plasmid DNA Purification Kit, Macherey-Nagel, Germany) were used for Sanger sequencing. Alignment and analysis of obtained sequences were carried out using BioEdit, version 7.2.5 (Hall, 1999). For species identification, obtained sequences were compared to published ITS (Table 1) and COI sequences (Table 2) of Acanthocephala. Primer sequences were removed from the sequences before submission to GenBank (accession nos. MF001277-MF001280 and MF078642-

**Table 1**  
Acanthocephala species, origin and GenBank accession numbers used for species discrimination based on the ITS1-5.8S rRNA-ITS2-region.

Species	Sample origin		GenBank acc. no.	Reference
	Host	Origin		
<i>Asplanchna sieboldi</i>	n.d.	n.d.	AF416411	Not published
<i>Bolbosoma cf. capitatum</i>	<i>Homo sapiens</i>	Kyoto, Japan	AB706182	Arizono et al., 2012
<i>B. nipponicum</i>	<i>Balaenoptera acutorostrata</i>	North Pacific Ocean	AB706183	Arizono et al., 2012
<i>Brachionus patulus</i>	n.d.	n.d.	AF416412	Not published
<i>Corynosoma australe</i>	<i>Otaria byronia</i>	n.d.	AF286307	García-Varela et al., 2005
<i>C. bullosum</i>	<i>Mirounga leonina</i>	n.d.	AF286308	García-Varela et al., 2005
<i>C. cetaceum</i>	<i>Pontoporia blainvillei</i>	n.d.	AF286310	García-Varela et al., 2005
<i>C. caspicum</i>	<i>Phoca caspica</i>	n.d.	AF286309	García-Varela et al., 2005
<i>C. enhydri</i>	<i>Enhydra lutris</i>	n.d.	AF286311	García-Varela et al., 2005
<i>C. hamanni</i>	<i>Leptonychotes weddelli</i>	n.d.	AF286312	García-Varela et al., 2005
<i>C. magdaleni</i>	<i>Phoca hispida</i>	n.d.	AY532065	García-Varela et al., 2005
<i>C. magdaleni</i> isolate Pv1NS	<i>Phoca vitulina</i>	North Sea	MF078643	this study
Cand. <i>C. nortmeri</i> sp. nov.	<i>Phoca vitulina</i>	North Sea	MF001280	this study
<i>C. semerme</i>	<i>Halichoerus grypus</i>	Baltic Sea	MF001279	this study
<i>C. strumosum</i>	<i>Phoca hispida</i>	n.d.	AF286313	García-Varela et al., 2005
<i>C. validum</i>	<i>Odobenus rosmarus</i>	n.d.	AF286314	García-Varela et al., 2005
<i>C. villosum</i>	<i>Eumetopias jubatus</i>	n.d.	AF286315	García-Varela et al., 2005
<i>Macracanthorhynchus ingens</i>	n.d.	n.d.	AF416414	Not published
<i>Moniliformis moniliformis</i>	n.d.	n.d.	AF416415	Not published
<i>Neoechinorhynchus golvani</i>	<i>Dormitator maculatus</i>	Mexico	FJ968109	Martínez-Aquino et al., 2009
<i>Octospiniferoides</i> sp.	<i>Petenia splendida</i>	n.d.	FJ388978	Not published
<i>Oligacanthorhynchus tortuosa</i>	n.d.	n.d.	AF416417	Not published
<i>Oncicola</i> sp.	n.d.	n.d.	AF416416	Not published
<i>Polymorphus</i> sp.	<i>Anas platyrhynchos</i>	n.d.	AF461421	García-Varela et al., 2005
<i>P. altmani</i>	<i>Enhydra lutris</i>	n.d.	AY532066	García-Varela et al., 2005
<i>P. brevis</i>	n.d.	n.d.	AF286306	Not published
<i>P. minutus</i>	<i>Gammarus pulex</i>	n.d.	AY532067	García-Varela et al., 2005
<i>Pomphorhynchus tereticollis</i>	<i>Platichthys flesus</i>	Stralsund, Baltic Sea	JF706705	Špakulová et al., 2011
<i>Tenuiproboscis</i> sp.	n.d.	n.d.	JF694277	Not published

n.d.: no data available.

MF078643).

Phylogenetic analyses were conducted using MEGA5 software (Tamura et al., 2011) with 1000 bootstrap replicates to test for reliability. For nucleotide alignment of the ITS-complex, a phylogenetic tree was constructed using maximum likelihood (ML) method, based on the Kimura 2-parameter model (Kimura, 1980). Pairwise distance analyses were carried out using the Maximum Composite Likelihood model

(Tamura et al., 2011). To eliminate silent mutations from the COI, nucleotide sequences were translated into amino acid sequences by using the ExPASy translate tool provided by SIB Swiss Institute of Bioinformatics for invertebrate mitochondrial genes. Phylogenetic analysis of the COI sequences was conducted using the maximum likelihood (ML) method, based on the Jones-Taylor-Thornton (JTT) model (Jones et al., 1992). Calculations of the pairwise distances were

**Table 2**  
Acanthocephala species, origin and GenBank accession numbers used for species discrimination based on partial COI sequences.

Species	Sample origin		GenBank acc. no.	Reference
	Host	Origin		
<i>Bolbosoma</i> sp.	<i>Callorhinus ursinus</i>	St. Paul Island, Alaska, USA	JX442190	García-Varela et al., 2013
<i>Corynosoma australe</i>	<i>Phocarctos hookeri</i>	New Zealand	JX442191	García-Varela et al., 2013
<i>C. enhydri</i>	<i>Enhydra lutris</i>	Monterey Bay, California, USA	DQ089719	García-Varela et al., 2013
<i>C. magdaleni</i>	<i>Phoca hispida saimensis</i>	Lake Saimaa, Finland	EF467872	García-Varela et al., 2011
<i>C. magdaleni</i> isolate Pv1NS	<i>Phoca vitulina</i>	North Sea	MF078642	this study
Cand. <i>C. nortmeri</i> sp. nov.	<i>Phoca vitulina</i>	North Sea	MF001278	this study
<i>C. obtuscens</i>	<i>Callorhinus ursinus</i>	St. Paul Island, Alaska, USA	JX442192	García-Varela et al., 2013
<i>C. semerme</i>	<i>Halichoerus grypus</i>	Baltic Sea	MF001277	this study
<i>C. strumosum</i>	<i>Phoca vitulina</i>	Monterey Bay, California, USA	EF467870	García-Varela et al., 2011
<i>C. validum</i>	<i>Callorhinus ursinus</i>	St. Paul Island, Alaska, USA	JX442193	García-Varela et al., 2013
<i>Ibhirhynchus dimorpha</i>	<i>Eudocimus albus</i>	Veracruz, Mexico	GQ981438	García-Varela et al., 2013
<i>Macracanthorhynchus ingens</i>	n.d.	n.d.	AF416997	Not published
<i>Mediorhynchus gallinarum</i>	n.d.	Indonesia	KC261352	Not published
<i>Moniliformis moniliformis</i>	n.d.	n.d.	AF416998	Not published
<i>Oligacanthorhynchus tortuosa</i>	n.d.	n.d.	AF416999	Not published
<i>Oncicola</i> sp.	n.d.	n.d.	AF417000	Not published
<i>Polymorphus brevis</i>	<i>Zoogoneticus purhepechus</i>	Central Mexico	KC549497	Alcántar-Escalera et al., 2013
<i>P. obtusus</i>	<i>Aythya affinis</i>	Baja California Sur, Mexico	JX442195	García-Varela et al., 2013
<i>P. trochus</i>	<i>Fulica americana</i>	Sinaloa, Mexico	JX442196	García-Varela et al., 2013
<i>Pomphorhynchus tereticollis</i>	<i>Gammarus pulex</i>	Chevigny, France	AY423353	Perrot-Minnot 2004
<i>Pseudocorynosoma</i> sp.	<i>Oxyura jamaicensis</i>	Durango, Mexico	JX442198	García-Varela et al., 2013
<i>Tenuiproboscis</i> sp.	<i>Lutjanus argentimaculatus</i>	n.d.	JF694276	Not published

n.d.: no data available.

achieved using the JTT matrix-based model.

### 2.5. Statistical analyses

The  $\chi^2$ -test was performed to test for significant differences between the infection rates over years and between the three age groups. Holm–Bonferroni method was used to control the familywise error rate.

One-way ANOVA and Kruskal-Wallis-test were used to test for statistically significant differences of morphological measurements between the identified groups. Statistical calculations were conducted with SigmaStat™ (version 3.11, Systat Software, Germany). In all analyses,  $P \leq 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Surveillance of Acanthocephala infections in seals

Throughout the study period from 1996 to mid-2013, a dataset including 2293 harbour seals (2120 from the North Sea, 54 from the Baltic Sea and 119 with unknown origin), 161 grey seals (67 from the North Sea, 76 from the Baltic Sea and 18 with unknown origin) and 6 ringed seals (5 from the Baltic Sea and 1 with unknown origin) was analysed for intestinal Acanthocephala infections. In total, 11.2% (275/2460) of investigated seals were infected. Since numbers of examined ringed seals were low ( $n = 6$ ; one infected animal), data were excluded from subsequent statistical analyses.

#### 3.1.1. Annual prevalence

At the beginning of the study period in 1996/1997, the annual prevalence of thorny headed worms in seals was 1.2% (2/170) and 0.4% (1/224). As of 1998, the infection rate increased in three waves up to a maximum of 38.5% (47/121) in 2012. An overview of the annual prevalences and statistically significant differences between 1996 and 2012 is provided in Fig. 1.

#### 3.1.2. Harbour and grey seals in North- and Baltic Seas

Overall, 10.5% (223/2120) harbour seals originating from the North Sea carried an infection with *Corynosoma* spp, whereas harbour

**Table 3**  
Prevalence of acanthocephalan infections in different age groups of harbour seals (*Ph. vitulina*) and grey seals (*H. grypus*) from the North and Baltic Seas.

	Age group 1	Age group 2	Age group 3
	positives/total (%)	positives/total (%)	positives/total (%)
<b>North Sea</b>			
<i>Ph. vitulina</i>	101/1460 (6.9) <sup>a</sup>	90/389 (23.1) <sup>a,d</sup>	42/313 (13.4) <sup>a,b</sup>
<i>H. grypus</i>	1/46 (2.2) <sup>d</sup>	9/15 (60.0%) <sup>a,b,c,d</sup>	5/15 (33.3) <sup>a,d</sup>
<b>Baltic Sea</b>			
<i>Ph. vitulina</i>	2/19 (10.5)	0/20 (0.0) <sup>c</sup>	2/11 (18.2)
<i>H. grypus</i>	13/42 (31.0) <sup>b,d</sup>	2/6 (33.3)	11/24 (45.8) <sup>a,b,c,d</sup>

<sup>a</sup> *Ph. vitulina* age group 1 from the North Sea is significantly less infected ( $P = 0.0001$ ).

<sup>b</sup> *Ph. vitulina* age group 3 from the North Sea is significantly less infected ( $P \leq 0.0315$ ).

<sup>c</sup> *Ph. vitulina* age group 2 from the Baltic Sea is significantly less infected ( $P = 0.0005$ ).

<sup>d</sup> *H. grypus* age group 1 from the North Sea is significantly less infected ( $P = 0.0009$ ).

seals from the Baltic Sea were significantly less frequently infected (7.4%; 4/54;  $P \leq 0.001$ ). In contrast, no statistically significant differences between grey seals from the North- (20.9%; 14/67) and Baltic Seas (34.2%; 26/76) were found. The infection rate of grey seals from the North- and Baltic Seas was significantly higher compared to harbour seals from the North Sea ( $P = 0.013$  and  $P \leq 0.001$ , respectively), whereas no significant differences between grey and harbour seals from the Baltic Sea were observed.

#### 3.1.3. Age class distribution

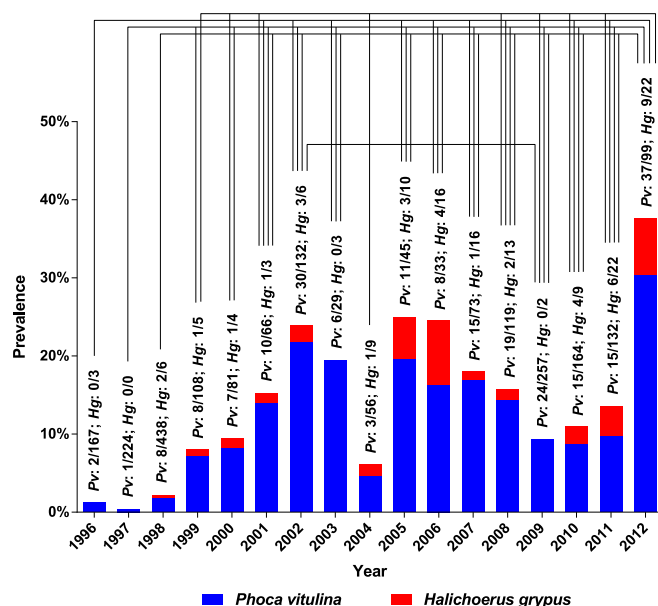
Detailed data on prevalences in different age groups of seals from the North and the Baltic Seas are shown in Table 3. Harbour seals of age group 1 originating from the North Sea were significantly less infected with *Corynosoma* spp. than harbour seals of age group 2 ( $P \leq 0.0001$ ) and age group 3 ( $P = 0.0001$ ) from the same habitat. In addition, grey seals belonging to age group 1 from the North Sea were also significantly less frequently infected than grey seals of age group 2 ( $P \leq 0.0001$ ) and age group 3 ( $P = 0.0004$ ) from the same habitat. Grey seals of age group 1 of the North Sea were significantly less frequently infected than grey seals of age group 1 of the Baltic Sea ( $P = 0.0002$ ); however, no statistically significant differences between grey seals of age group 2 and 3 between North Sea and Baltic Sea were determined. Furthermore, comparisons of harbour seals originating from the North Sea vs. Baltic Sea revealed no statistically significant differences between any of the age groups.

### 3.2. Morphological characterisation of acanthocephalans

Morphological discrimination of *Corynosoma* species was possible for 217 of the 275 infected seals, from the remaining 58 seals no specimens were preserved. The specimens of 191 seals (177 harbour seals from the North Sea, 11 grey seals from the North Sea and 3 harbour seals from the Baltic Sea) were morphological identified as *C. strumosum*. Interestingly, some specimens identified as *C. strumosum* showed an atypical number of 16 longitudinal hook rows as described by Amin et al. (2011). Specimens of 5 seals (4 grey seals and 1 ringed seal, all originating from the Baltic Sea) were identified as *C. magdaleni*. *C. semerme* was identified in 26 seals (1 harbour seal originated from the North Sea and 25 grey seals from the Baltic Sea). Coinfections of *C. semerme* and *C. strumosum* occurred in 1 harbour seal from the North Sea, whereas 4 grey seals from the Baltic Sea were coinfecting with *C. semerme* and *C. magdaleni*.

### 3.3. Molecular and phylogenetic analyses of acanthocephalan specimens

A total of 50 thorny headed worms were used for molecular species determination based on published ITS and COI sequences.



**Fig. 1.** Prevalence of acanthocephalan infections in harbour and grey seals from the German North and Baltic Seas between 1996 and 2012. Notations indicate the Acanthocephala positive and total number of examined *Phoca vitulina* (Pv) and *Halichoerus grypus* (Hg). Connecting lines indicate statistically significant differences between annual prevalences after Holm–Bonferroni correction ( $P \leq 0.001$ ).



### 3.3.1. ITS sequence analysis

The 10 sequenced acanthocephalans morphologically identified as *C. semerme* showed identical ITS sequences. However, as no genetic data of *C. semerme* were available, no species-specific percentage identity with sequences deposited in public databases could be determined. The 5 specimens morphologically identified as *C. magdaleni* showed 100% identity with the published ITS sequence of *C. magdaleni* (GenBank acc. no. AY532065). However, none of the 35 specimens morphologically determined as *C. strumosum* was confirmed by molecular species discrimination. Instead, 13 sequenced specimens showed 100% identity with the published ITS sequence of *C. magdaleni*. Further 19 specimens showed 99% identity to *C. magdaleni* due to one transversion (g.288G > C) and one transition (g.304G > A). These samples are referred to as *Corynosoma magdaleni* isolate Pv1NS in the following. The remaining 3 specimen contained 86 single nucleotide polymorphisms (SNPs) compared to the published *C. magdaleni* ITS sequence as well as 16 nucleotide insertions at six positions (g.611\_612insAAT; g.616\_617insACTGT; g.635\_636insA; g.649\_650insT; g.728\_729insGCGCT and g.766\_767insG) and 5 nucleotide deletions at three positions (g.522del, g.526\_529del and g.565del) compared to the published *C. magdaleni* ITS sequence. Compared to *C. strumosum*, as which the three specimens were determined morphologically, 102 SNPs as well as 17 nucleotide insertions at seven positions (g.611\_612insAAT; g.616\_617insACTGT; g.635\_636insA; g.649\_650insT; g.728\_729insGCGCT; g.766\_767insG and g.769A) and 5 nucleotide deletions at three positions (g.522del, g.527\_529del and g.565del) were detected. As these sequences were substantially different from any other known species, they are referred to as “Candidatus *Corynosoma nortmeri* sp. nov.” in the following.

An alignment of previously published ITS sequences of *C. strumosum* (GenBank acc. no. AF286313) and *C. magdaleni* (GenBank acc. no. AY532065) with selected nucleotide sequences obtained in the present study [*C. semerme* (GenBank acc. no. MF001279), *C. magdaleni* isolate Pv1NS (acc. no. MF078643) and Cand. *C. nortmeri* sp. nov. (acc. no. MF001280)] is provided in Fig. 2.

Phylogenetic analysis of the ITS-complex was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. This model yielded a single best tree with the highest log likelihood of  $-16,028.00$  (Fig. 3). Analysis involved 25 nucleotide sequences with a total of 636 positions in the final dataset. The genus *Corynosoma* forms a monophyletic group with *C. caspicum* (Golvan and Mokhayer, 1973), *C. magdaleni* and the obtained sequence of *C. magdaleni* isolate Pv1NS as sister species as well as *C. enhydri* (Morozov, 1940) and *C. strumosum*. By contrast, Cand. *C. nortmeri* sp. nov. and *C. semerme* showed less close relationships to these species.

The nucleotide distance based on the Maximum Composite Likelihood model between *C. magdaleni* and *C. magdaleni* isolate Pv1NS. was low (0.005) compared to the distance between *C. magdaleni* and *C. strumosum* with a value of 0.033. The class of Cand. *C. nortmeri* sp. nov. showed the closest relationship to *C. magdaleni* (0.204).

### 3.3.2. COI sequence analysis

The 10 sequenced acanthocephalans morphologically identified as *C. semerme* showed identical COI sequences. Again, no comparison with previously published *C. semerme* sequences was possible. 5 specimens morphologically identified as *C. magdaleni* showed 100% amino acid identity with the published sequence of *C. magdaleni* (GenBank acc. no. EF467872), confirming molecular ITS species determination. Again, none of the 35 specimens morphologically identified as *C. strumosum* matched perfectly to published COI amino acid sequences, but the 13 specimens identified as *C. magdaleni* on ITS level showed 100% identity with the published COI sequence of *C. magdaleni*. Similarly, COI sequences of the 19 specimens referred to as *C. magdaleni* isolate Pv1NS (see section above) were identical to those of *C. magdaleni*. The sequences of the three Cand. *C. nortmeri* sp. nov. specimens contained one amino acid substitution compared to sequences of *C. magdaleni*

(p.Ile12Val) as well as *C. strumosum* (p.Leu5Trp). An alignment showing *Corynosoma* COI sequence comparison is provided in Fig. 4.

Construction of the phylogenetic tree was conducted by using the Maximum Likelihood method based on the Jones-Taylor-Thornton (JTT) model. The tree with the highest log likelihood ( $-2479.70$ ) is shown in Fig. 5. In total, 19 amino acid sequences with a total of 201 positions in the final dataset were involved in the analysis. *Corynosoma* species grouped together as a monophyletic assemblage, even though the bootstrap values were moderate. The estimated evolutionary divergence between Cand. *C. nortmeri* sp. nov. and *C. strumosum* as well as *C. magdaleni* was each 0.005.

### 3.4. Morphological characteristics of *Corynosoma* spp. determined by molecular analysis

Analysed specimens were assigned to the *Corynosoma* species resulting of the molecular species discrimination. Determined morphological parameters are shown in Table 4. Due to the small sample size of Cand. *C. nortmeri* sp. nov. (n = 3), One-way ANOVA was conducted only between *C. magdaleni* and *C. magdaleni* isolate Pv1NS. The latter had a significant larger body size than *C. magdaleni* ( $P \leq 0.001$ ), associated with a significantly longer and wider fore- ( $P \leq 0.001$  and  $P \leq 0.001$ , respectively) and hindtrunk ( $P \leq 0.001$  and  $P \leq 0.001$ , respectively).

## 4. Discussion

An extensive assessment of health status of seals has been undertaken since the first phocine distemper virus (PDV) epizootic in 1988/89 (Siebert et al., 2007). Besides histological alterations and microbiological infections, special attention of the assessment has been given to reveal the parasitic burden of seals in German waters (Lehnert et al., 2007; Siebert et al., 2007). One frequently occurring class of parasites are thorny-headed worms, scientifically known as Acanthocephala. Within the health monitoring, Siebert et al. (2007) reported an acanthocephalan prevalence of 12% between 1996 and 2002 and 20% between 2002 and 2005 for seals originating from the coast of the German federal state Schleswig-Holstein. Lehnert et al. (2007) found 23% of harbour seals of the North Sea to be infected with *Corynosoma* spp between 1997 and 2000. To extend obtained data, this study focused on the investigation of annual prevalences over an extended period of time (1996–2012), taking host preferences and age dependent infection patterns of acanthocephalans in seals of the North and Baltic Seas into account. While the seal populations in the German Wadden Sea grew constantly (Galatius et al., 2016), the prevalence of acanthocephalan infections fluctuated during this period. In the beginning of the study, the annual prevalence of infected seals was low, but increased continuously until the second PDV epizootic in 2002, when 23.9% of the seals were infected. Afterwards, the Acanthocephala prevalence decreased to 6.2% in 2004. The PDV infection could have led to a decreased immune response, which may contribute to a predisposition for parasitic infections (Borgsteede et al., 1991), but as this was not confirmed during the second PDV epidemic in 2002, it is assumed that the PDV infection progresses too rapidly and severely to influence the parasitic burden (Siebert, 2003). In the following years, this wavy pattern continued by increasing again to high levels (25.0%) in 2005 decreasing to 9.3% in 2009 and reaching the overall highest prevalence level of 38.5% in 2012. Influential factors on marine parasite populations, which may contribute to the observed wave-like pattern of the annual Acanthocephalan prevalences, are the abundance of intermediate host like amphipods and fishes as well as abiotic factors such as the pH, temperature and phosphate level in the environment (Marcogliese and Cone, 1997; Poulin, 2006; Sinisalo, 2007). However, limited information is available on the intermediate hosts of *Corynosoma* spp. in the North Sea.

It might be assumed that acanthocephalan prevalences in harbour

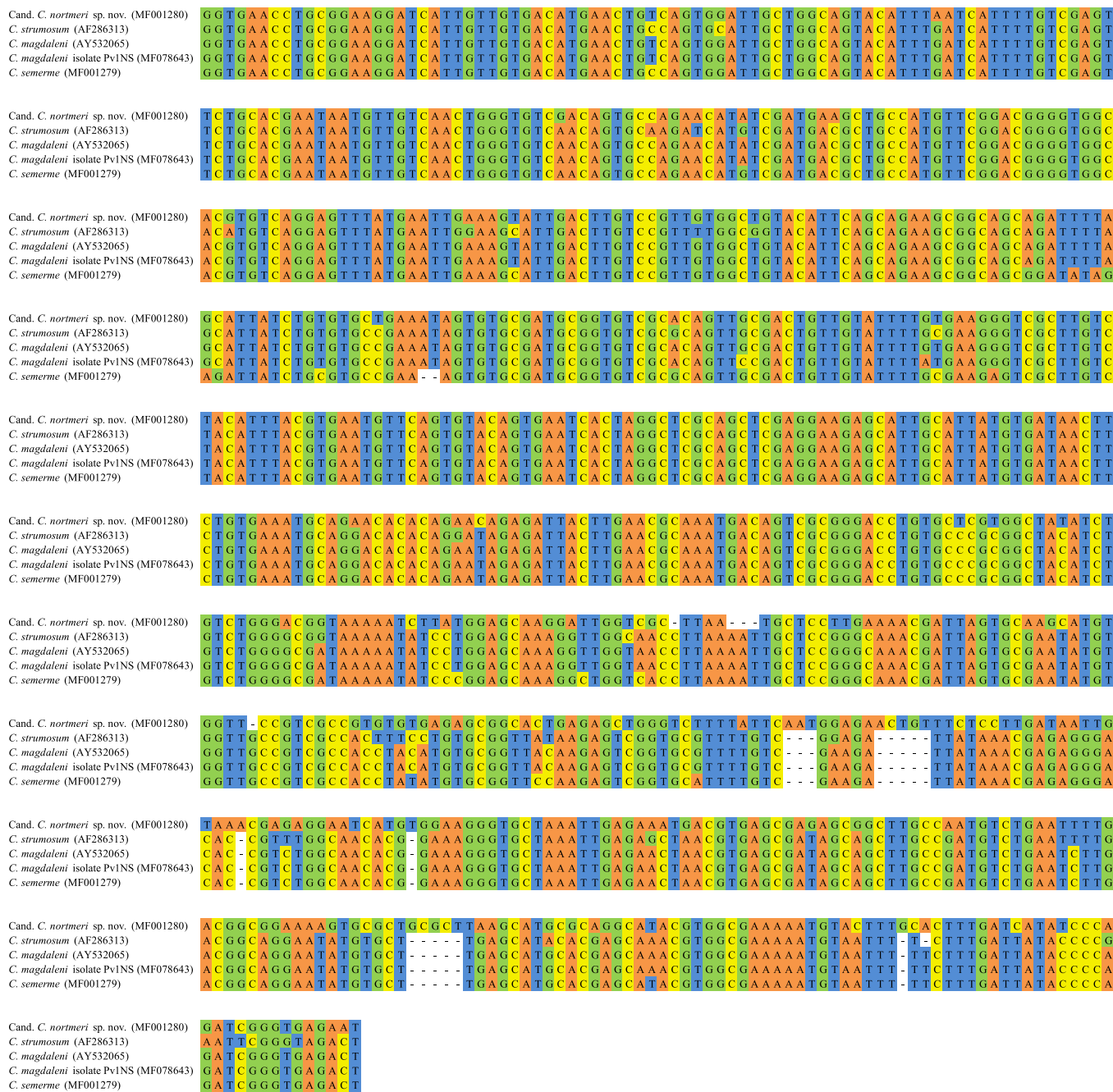


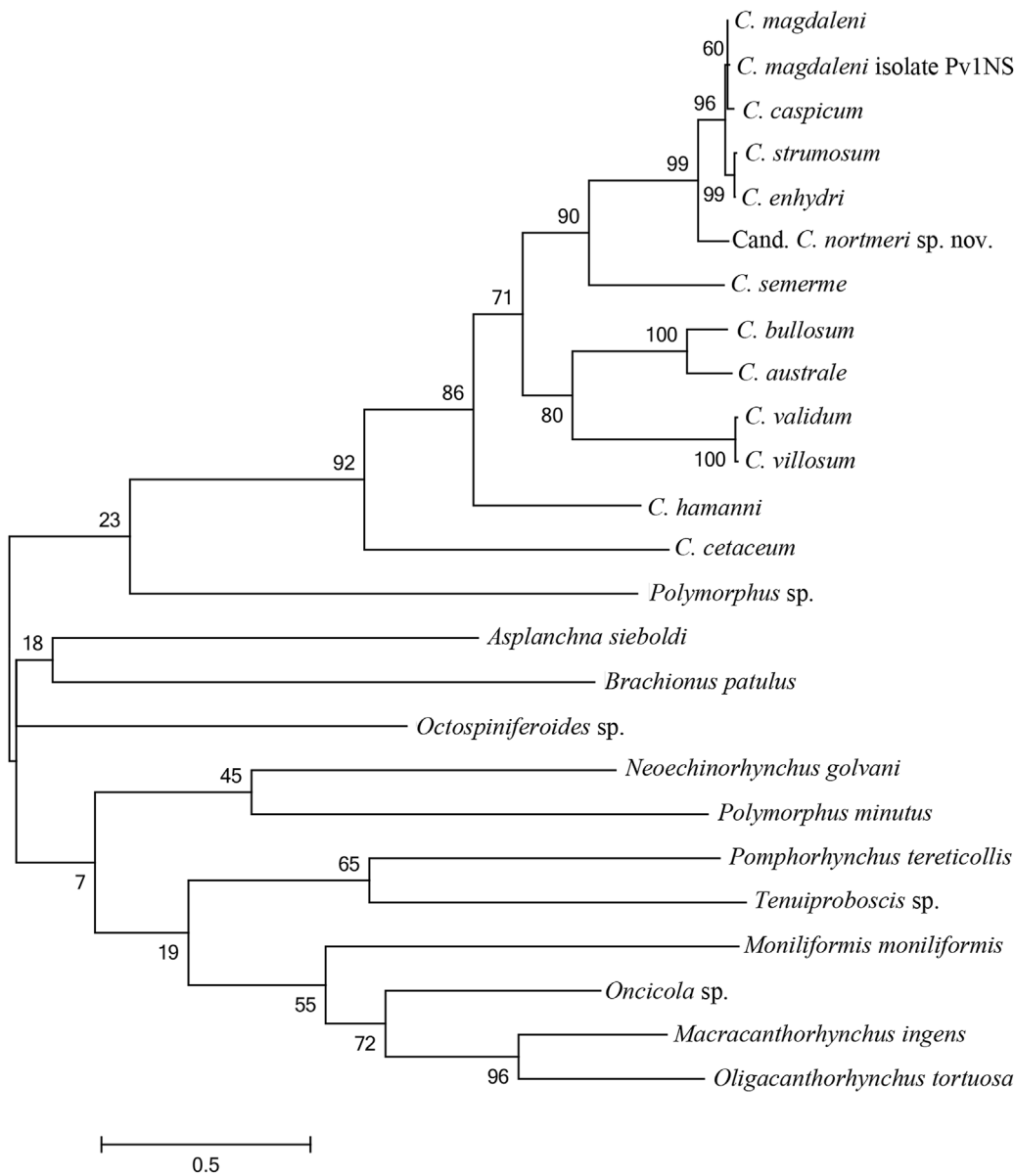
Fig. 2. Nucleotide alignment of the ribosomal ITS1-5.8S-ITS2-complex of *Corynosoma* species including obtained ITS (GenBank acc. nos. MF001279, MF001280 and MF078643) and previously published sequences (GenBank acc. nos. AY532065 and AF286313).

and grey seals, as trophically transmitted pathogens, are almost identical due to their overlapping prey spectrum (King, 1983). However, in the presented study, grey seals of the North Sea were more frequently infected (20.9%; 14/67) than harbour seals in the same habitat (10.5%; 223/2120). However, in the Baltic Sea no statistically significant differences between both seal species were determined, but rather low sample sizes from the Baltic Seas (54 harbour seals; 7.4% positives vs. 76 grey seals, 34.2% positives) may have hampered statistical significance.

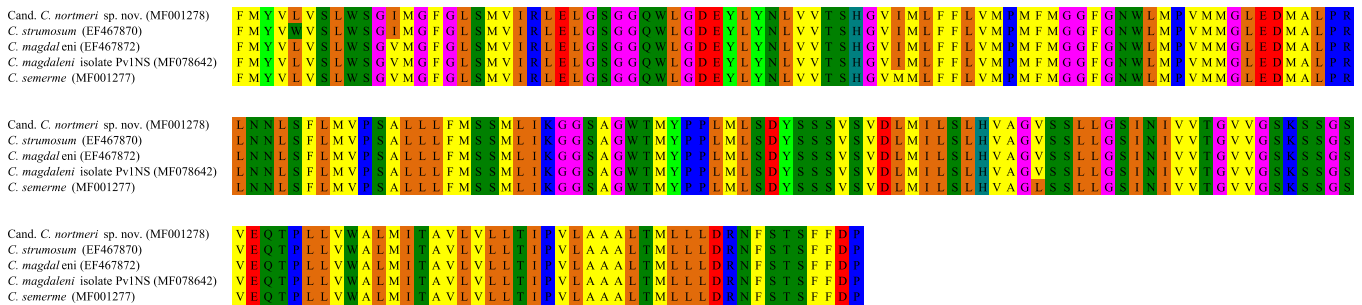
Parasitic infections with pulmonary nematodes, heartworms and seal lice in harbour seals seem to be age-dependent (Claussen et al., 1991; Lehnert et al., 2007, 2016; Ulrich et al., 2016), probably due to development of protective immunity and/or a shift in the prey spectrum during adolescence (Lehnert et al., 2007, 2016; Lundström et al., 2010). The latter might also be assumed for acanthocephalan

infections, as studies during the first PDV epidemic revealed that young seals were less frequently infected with *Corynosoma* spp. than subadult and adult seals, which did not show differences between their age classes (Borgsteede et al., 1991; Strauss et al., 1991). Similar results were observed in the presented study. However, the juvenile age group 1 includes data of a number of seals which died during their weaning period and were consequently not exposed to intermediate hosts. Nevertheless, subadult seals from the North Sea are more often infected with thorny-headed worms than adults.

Regarding *Corynosoma* species identification, the intraspecific variance and the interspecific conformance of morphological characteristics between *C. strumosum* and *C. magdaleni* is a barrier to reliably distinguishing them morphologically. Therefore, molecular species differentiation using the ITS1-5,8S rRNA-ITS2-complex as well as COI was additionally conducted for selected individuals. To our knowledge,



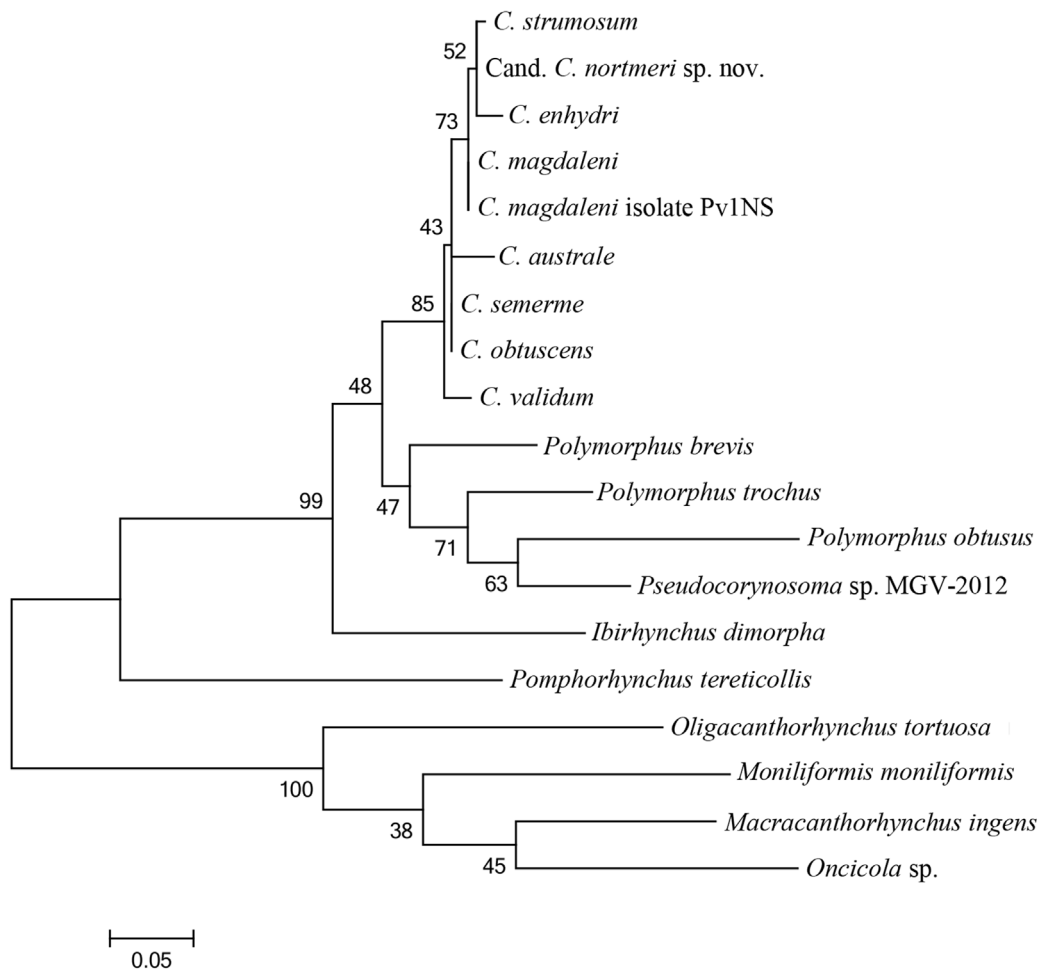
**Fig. 3.** Phylogenetic analysis of the ribosomal ITS1-5.8S-ITS2-complex using the Maximum Likelihood method based on the Kimura 2-parameter model. The log likelihood is -16,028.00. The percentage of trees based on 1000 bootstrap replicates in which the associated taxa clustered together is shown next to the branches. GenBank accession numbers of analysed nucleotide sequences are listed in Table 1.



**Fig. 4.** Amino acid alignment of the partial mitochondrial COI sequence of *Corynosoma* species including obtained (GenBank acc. nos. MF001277, MF001278 and MF078642) and previously published sequences (GenBank acc. nos. EF467872 and EF467870).

this study provides the first genetic sequences of *C. semerme* and their phylogenetic classification. All sequenced worms morphologically identified as *C. semerme* resulted in identical sequences. In terms of the ITS-complex, *C. semerme* did not show any closer relationship to another known species of the genus *Corynosoma*. In contrast, the translated amino acid sequence of the COI was identical to a published sequence of *C. obtuscens*. This species is widely distributed along North and South

America with a broad range of definitive hosts (Lincicome, 1943; Tantaletán et al., 2005). Moreover, terrestrial animals like the Andean fox, *Pseudalopex culpaeus* (Molina, 1782), are described as hosts (Tantaletán et al., 2007). *C. obtuscens* and *C. semerme* are morphologically similar and can be differentiated only by subtle differences in the spination of males and the number of longitudinal hook rows at the proboscis (Neiland, 1962). Unfortunately, no ITS-sequence of *C.*



**Fig. 5.** Maximum likelihood tree based on COI sequences using the Jones-Taylor-Thornton (JTT) model. The log likelihood is -2479.70. The percentage of trees based on 1000 bootstrap replicates in which the associated taxa clustered together is shown next to the branches. GenBank accession numbers of analysed amino acid sequences are listed in Table 2.

**Table 4**  
Morphological characteristics of *Corynosoma* spp. specimens determined by molecular analysis. Sizes are indicated as mean values with minimum and maximum values in brackets.

	<i>C. semerme</i> (n = 10)	<i>C. magdaleni</i> (n = 18)	<i>C. magdaleni</i> isolate Pv1NS (n = 19)	<i>Cand. C. nortmeri</i> sp. nov. (n = 3)
body-length	2966 μm (2769–3329 μm)	5431 μm (3988–6597 μm)	7545 μm (6374–8314 μm) <sup>a</sup>	5956 μm (4690–7837 μm)
fore trunk				
length	1393 μm (1235–1583 μm)	2064 μm (1119–2700 μm)	3000 μm (2286–3621 μm) <sup>a</sup>	2409 μm (1844–3248 μm)
width	1113 μm (836–1226 μm)	1341 μm (863–1790 μm)	2009 μm (1577–2324 μm) <sup>a</sup>	1693 μm (1231–2100 μm)
hind trunk				
length	1575 μm (1462–1804 μm)	3273 μm (2714–4522 μm)	4532 μm (3424–5280 μm) <sup>a</sup>	3546 μm (2851–4577 μm)
width	528 μm (444–598 μm)	627 μm (470–748 μm)	880 μm (660–1079 μm) <sup>a</sup>	781 μm (695–951 μm)
proboscis				
length	500 μm (418–571 μm)	629 μm (592–661 μm)	812 μm (610–1047 μm)	767 μm (707–883 μm)
width	262 μm (232–331 μm)	350 μm (270–600 μm)	367 μm (300–541 μm)	518 μm (365–773 μm)
proboscis hook rows (no.)	n.d.	18–19	16–18	16
trunk spine coverage	100%	50.3%	45.6%	51.8%

n.d.: not determined.

<sup>a</sup> Statistically significant differences (P ≤ .05) between *C. magdaleni* and *C. magdaleni* isolate Pv1NS.

*obtusens* has been published so far, so that it remains unknown whether this gene region is a suitable marker for reliable differentiation of these two species.

Samples of *C. magdaleni* isolate Pv1NS showed the same COI amino acid sequence as *C. magdaleni*, but two polymorphisms in the ITS-complex were detected. Furthermore, there were remarkable differences in morphological characteristics. According to the emended descriptions of Nickol et al. (2002), the body of *C. magdaleni* is 3.6–5.3 (mean 4.7) mm long. Individuals of this study determined as *C. magdaleni* by molecular analysis exhibited a slightly longer body length of 4.0–6.3 (mean 5.3) mm, whereas *C. magdaleni* isolate Pv1NS was with 6.4–8.3 (mean 7.6) mm significantly longer than those of *C. magdaleni*. In addition, proboscises of *C. magdaleni* isolate Pv1NS were armed with 16–18 longitudinal hook rows while *C. magdaleni* is commonly

described with 17–23 rows (18–19 in the presented study, cf. Table 4). From obtained results it remains questionable whether *C. magdaleni* isolate Pv1NS represents a variant occurring simultaneously with *C. magdaleni* in the same habitat or a different species. Future investigations using further genetic markers such as the small (SSU) and large (LSU) subunit of nuclear ribosomal DNA (García-Varela et al., 2011) are needed to answer this question. However, if proven to be a variant only, the morphological description of *C. magdaleni* needs extension by parameters of the presented study, in particular body length and the proboscis armature.

According to DeSalle et al. (2005), boundaries of a taxon are determined by geographical, morphological, ecological, reproductive and behavioural information. Consequently, morphologically cryptic species can only be detected at the DNA sequence level. The ITS-region of



Cand. *C. nortmeri* sp. nov. revealed considerable nucleotide substitutions, insertions and deletions compared to other published *Corynosoma* ITS-sequences. The phylogenetic analysis resulted in an apparent evolutionary distance between Cand. *C. nortmeri* sp. nov. and *C. magdaleni*, the closest related species. Additionally, on COI amino acid level, Cand. *C. nortmeri* sp. nov. differed from other *Corynosoma* species and showed a substitution of Leucine to Tryptophan (p.Leu5Trp) compared to *C. strumosum* as well as Isoleucine to Valine (p.Ile12Val) compared to *C. magdaleni* and *C. semerme*. Furthermore, all three sequenced Cand. *C. nortmeri* sp. nov. individuals had only 16 longitudinal hook rows at their proboscis. According to Petrochenko (1971), only *C. hadweni* as well as *C. magdaleni* isolate Pv1NS described in this study features similar morphological characteristics. These species, however, are larger than Cand. *C. nortmeri* sp. nov., particularly in the minimum size (*C. hadweni* 6.0–8.3 mm and *C. magdaleni* isolate Pv1NS 6.4–8.3 mm vs. Cand. *C. nortmeri* sp. nov. 4.7–7.8 mm) and *C. hadweni* was only described once, when discovered in Alaska in 1953 (Van Cleave, 1953). Even though only three Cand. *C. nortmeri* sp. nov. individuals were available, results indicate the potential finding of a cryptic species. Nevertheless, further studies including on an appropriate sample size are crucial to confirm this potential new species. When these data are provided, we propose the name *Corynosoma nortmeri* sp. nov., named after Nortmer, a late Middle High German notation for the North Sea.

The high interspecific conformance of *Corynosoma* spp. complicates discrimination between some species and Van Cleave (1953) criticised the high rate of incorrectly determined samples as *C. strumosum*. Specimens of *Corynosoma* spp. with 16–18 longitudinal hook rows and remarkable alterations in body size and shape originated from the Caspian Sea were determined as *C. strumosum* (Amin et al., 2011), even though *C. strumosum* is described with 18 longitudinal hook rows only (Nickol et al., 2002). Previous studies on the acanthocephalan distribution in seals of the North Sea during the first PDV epizootic and later found *C. strumosum* as predominant species. Based on the molecular analysis in this study, *C. magdaleni* and the here described *C. magdaleni* isolate Pv1NS instead of *C. strumosum* turned out as predominant acanthocephalan species. Therefore, the complicated species determination by morphological characteristics within the genus *Corynosoma* should be complemented by molecular species discrimination as valid species discrimination is crucial for the understanding parasite epidemiology and the assessment of the parasitic impact on the health status of the host.

## 5. Conclusion

In this first study with an elaborated epidemiological analysis of Acanthocephala infections in Seals from the North and Baltic Seas, the prevalence of *Corynosoma* spp. showed a wave-like pattern over the study period of 1996–2012, with annual prevalences ranging between 0.4% and 38.5%. For the first time, molecular species differentiation of acanthocephalan species in the North and Baltic Sea was performed, resulting in an altered distribution of acanthocephalans in German parts of the North and the Baltic Sea compared to previous morphology-based studies: None of the examined parasites was identified as *C. strumosum*, but rather *C. magdaleni* was found in seals from both, the North and Baltic Seas. The combination of morphology and molecular analysis indicate the potential finding of a cryptic species, for which as the name Candidatus *Corynosoma nortmeri* sp. nov. is proposed.

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## References

- Alcántar-Escalera, F., García-Varela, M., Vázquez-Domínguez, E., Pérez-Ponce de León, G., 2013. Using DNA barcoding to link cystacanths and adults of the acanthocephalan *Polymorphus brevis* in central Mexico. *Mol. Ecol. Res.* 13, 1116–1124.
- Amin, O., Heckmann, R., Halajian, A., El-Naggar, A., 2011. The morphology of a unique population of *Corynosoma strumosum* (Acanthocephala, Polymorphidae) from the Caspian seal, *Pusa caspica*, in the land-locked Caspian Sea using SEM, with special notes on histopathology. *Acta Parasitol.* 56, 438–445.
- Arizono, N., Kuramochi, T., Kagei, N., 2012. Molecular and histological identification of the acanthocephalan *Bolbosoma cf. capitatum* from the human small intestine. *Parasitol. Int.* 61, 715–718.
- Bergeron, E., Measures, L., Huot, J., 1997. Lungworm (*Otostrongylus circumlitus*) infections in ringed seals (*Phoca hispida*) from eastern Arctic Canada. *Can. J. Fish. Aquat. Sci.* 54, 2443–2448.
- Bergman, A., Olsson, M., 1985. Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome. *Finn. Game Res.* 44, 47–62.
- Blouin, M.S., 2002. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. *Int. J. Parasitol.* 32, 527–531.
- Borgsteede, F., Bus, H., Verplanke, J., van Burg, W., 1991. Endoparasitic helminths of the harbour seal, *Phoca vitulina*, in The Netherlands. *Neth. J. Sea Res.* 28, 247–250.
- Claussen, D., Strauss, V., Ising, S., Jäger, M., Schnieder, T., Stoye, M., 1991. The helminth fauna from the common seal (*Phoca vitulina vitulina*, Linné, 1758) of the Wadden sea in lower saxony. Part 2: Nematodes. *J. Vet. Med. B* 38, 649–656.
- Crompton, D.W.T., Nickol, B.B., 1985. *Biology of the Acanthocephala*. Cambridge University Press.
- Delyamure, S.L., 1955. *Helminthofauna of Marine Mammals (Ecology and Phylogeny)*. Gel' mintologicheskaya Laboratoria, Akademiya Nauk SSSR, Moskva. English translation: Israel Program for Scientific Translations, Jerusalem 1968.
- DeSalle, R., Egan, M.G., Siddall, M., 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 360, 1905–1916.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- Galatius, A., Brasseur, S., Czeck, R., Jensen, L.F., Jeß, A., Körber, P., Pund, R., Siebert, U., Teilmann, J., Klöpffer, S., 2016. Aerial Surveys of Harbour Seals in the Wadden Sea in 2016 – Population Still in Stagnation. Common Wadden Sea Secretariat, Wilhelmshaven, Germany 2016.
- García-Varela, M., Aznar, F.J., Pérez-Ponce de León, G., Piñero, D., Lacllette, J.P., 2005. Molecular phylogeny of *Corynosoma* Lühe, 1904 (Acanthocephala), based on 5.8 S and internal transcribed spacer sequences. *J. Parasitol.* 91, 345–352.
- García-Varela, M., Pérez-Ponce de León, G., Aznar, F.J., Nadler, S.A., 2009. Systematic position of *Pseudocorynosoma* and *Andracantha* (Acanthocephala, Polymorphidae) based on nuclear and mitochondrial gene sequences. *J. Parasitol.* 95, 178–185.
- García-Varela, M., de León, G.P.-P., Aznar, F.J., Nadler, S.A., 2011. Erection of *Ibirhynchus* gen. nov. (Acanthocephala: Polymorphidae), based on molecular and morphological data. *J. Parasitol.* 97, 97–105.
- García-Varela, M., de León, G.P.-P., Aznar, F.J., Nadler, S.A., 2013. Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences. *Mol. Phylogenet. Evol.* 68, 176–184.
- Golvan, Y., 1994. Nomenclature of the Acanthocephala. *Res. Rev. Parasitol.* 54, 135–205.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.
- Hebert, P.D., Cywinska, A., Ball, S.L., 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond., B, Biol. Sci.* 270, 313–321.
- Jones, D.T., Taylor, W.R., Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. *Bioinformatics* 8, 275–282.
- Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120.
- King, J., 1983. *Seals of the World*. British Museum (Natural History), London, pp. 240.
- Lehnert, K., Raga, J., Siebert, U., 2007. Parasites in harbour seals (*Phoca vitulina*) from the German Wadden sea between two phocine distemper virus epidemics. *Helgol. Mar. Res.* 61, 239–245.
- Lehnert, K., Schwanke, E., Hahn, K., Wohlsein, P., Siebert, U., 2016. Heartworm (*Acanthocheilonema spirocauda*) and seal louse (*Echinophthirus horridus*) infections in harbour seals (*Phoca vitulina*) from the North and Baltic Seas. *J. Sea Res.* 113, 65–72.
- Lincicome, D.R., 1943. Acanthocephala of the genus *Corynosoma* from the California sealion. *J. Parasitol.* 29, 102–106.
- Lundström, K., Hjerne, O., Lunneryd, S.-G., Karlsson, O., 2010. Understanding the diet composition of marine mammals: grey seals (*Halichoerus grypus*) in the Baltic Sea. *ICES J. Mar. Sci.* 67, 1230–1239.
- Lunneryd, S., 1992. *Dipetalonema spirocauda* (Leidy) (Nematoda) and *Corynosoma strumosum* (Rudolphi) (Acanthocephala) infection in harbour seal from the Kattegat-

- Skagerrak and the Baltic. *Sarsia* 76, 267–271.
- Luton, K., Walker, D., Blair, D., 1992. Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). *Mol. Biochem. Parasitol.* 56, 323–327.
- Mansfield, A., Fisher, H., 1960. Age determination in the harbour seal, *Phoca vitulina* L. *Nature* 186, 92–93.
- Marcogliese, D.J., Cone, D.K., 1997. Food webs: a plea for parasites. *Trends Ecol. Evol.* 12, 320–325.
- Martínez-Aquino, A., Reyna-Fabián, M.E., Rosas-Valdez, R., Razo-Mendivil, U., de León, G.P.-P., García-Varela, M., 2009. Detecting a complex of cryptic species within *Neoechinorhynchus golvani* (Acanthocephala: Neoechinorhynchidae) inferred from ITSs and LSU rDNA gene sequences. *J. Parasitol.* 95, 1040–1047.
- Neiland, K.A., 1962. Alaskan species of acanthocephalan genus *Corynosoma* Luehe, 1904. *J. Parasitol.* 48, 69–75.
- Nickol, B., Valtonen, E., Helle, E., 2002. *Corynosoma magdalenii* in grey seals from SW Finland Baltic Sea, with emended description of *C. magdalenii* and *C. strumosum*. *J. Parasitol.* 88, 1222–1229.
- Perrot-Minnot, M.-J., 2004. Larval morphology, genetic divergence, and contrasting levels of host manipulation between forms of *Pomphorhynchus laevis* (Acanthocephala). *Int. J. Parasitol.* 34, 45–54.
- Petrochenko, V., 1971. Acanthocephala of Domestic and Wild Animals. Volume II. Jerusalem, Israel Program for Scientific Translations. US Department of Agriculture & The National Science Foundation, pp. 487.
- Poulin, R., 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology* 132, 143–151.
- Siebert, U., 2003. Monitoring the Health Status of harbour seals: pathological investigations before and during the PDV-virus outbreak. *Wadden Sea Ecosys. Rep.* 17, 33–38.
- Siebert, U., Wohlsein, P., Lehnert, K., Baumgärtner, W., 2007. Pathological findings in harbour seals (*Phoca vitulina*): 1996–2005. *J. Comp. Pathol.* 137, 47–58.
- Sinialo, T., 2007. Diet and Foraging of Ringed Seals in Relation to Helminth Parasite Assemblages. University of Jyväskylä.
- Špakulová, M., Perrot-Minnot, M., Neuhaus, B., 2011. Resurrection of *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Acanthocephala: Pomphorhynchidae) based on new morphological and molecular data. *Helminthologia* 48, 268–277.
- Strauss, V., Claussen, D., Jäger, M., Ising, S., Schnieder, T., Stoye, M., 1991. The helminth fauna of the common seal (*Phoca vitulina vitulina*, Linné, 1758) from the Wadden sea in lower saxony Part 1: Trematodes, cestodes and acanthocephala. *J. Vet. Med. B* 38, 641–648.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Tantaleán, M., Mendoza, L., Riofrío, F., 2007. El zorro Andino, *Pseudalopes culpaeus*, un nuevo huésped para *Corynosoma obtuscens* (Acanthocephala) en el Perú. *Rev. Peru. Biol.* 14, 51–52.
- Tantaleán, M., Sánchez, L., Gómez, L., Huiza, A., 2005. Acanthocéfalos del Perú. *Rev. Peru. Biol.* 12, 83–92.
- Ulrich, S.A., Lehnert, K., Rubio-García, A., Sanchez-Contreras, G.J., Strube, C., Siebert, U., 2016. Lungworm seroprevalence in free-ranging harbour seals and molecular characterisation of marine mammal MSP. *Int. J. Parasitol. Parasites Wildl.* 5, 48–55.
- Valtonen, E., Helle, E., Poulin, R., 2004. Stability of *Corynosoma* populations with fluctuating population densities of the seal definitive host. *Parasitology* 129, 635–642.
- Van Cleave, H.J., 1953. A preliminary analysis of the acanthocephalan genus *Corynosoma* in mammals of North America. *J. Parasitol.* 39, 1–13.