JIPK. Volume 13 No 1. April 2021
Sinta 2 (Decree No: 10/E/KPT/2018)
e-ISSN:2528-0759; p-ISSN:2085-5842
DOI=10.20473/jipk.v13i1.24486
Available online at https://e-journal.unair.ac.id/JIPK/index

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)

Short Communication

Morphological Identification of *Bolbosoma turbinella* (Acanthocephala) in *Balaenoptera borealis* (Sei Whale) from Straits of Malacca, Malaysia

Mohd Ihwan Zakariah[1], Mohd Tamimi Ali Ahmad[2], Mohd Shafiq Razak[1], Norainy Mohd Husin[1], Wahidah Wahab[1], Rabi-Atun Adawiah Abdullah[1], Nor Asma Husna Yusoff[1], Surzanne Agos[1], and Marina Hassan[1]

[1]Higher Institutional Centres of Excellence, Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, 21030. Malaysia.
[2]Turtle and Marine Ecosystem Centre (TUMEC), No. 1, Rantau Abang, Dungun, Terengganu, Terengganu, 23000. Malaysia.

OPEN ACCESS

ARTICLE INFO

Received: March 1, 2021
Accepted: March 24, 2021
Published: March 31, 2021

*) Corresponding author:
E-mail: ihwanz@umt.edu.my and marina@umt.edu.my

Keywords:
Sei Whale
Balaenoptera borealis
Morphological study
Bolbosoma turbinella

Abstract

The study of ectoparasite and endoparasites of marine mammals are not habitually done because some species are endangered and protected by law. A stranded Sei Whale, *Balaenoptera borealis* (Anderson, 1878) from the East Coast of Peninsular Malaysia was examined for endoparasites. The objective of this study is to identify the species of acanthocephalan in the intestine of the Sei Whale found in the straits of Malacca. A total of ten parasite specimens were collected from the fresh intestine, and were fixed in 10% buffered formalin for further histological procedures. The morphological features of this parasite viewed under Scanning Electron Microscope (SEM) are referred to as the proboscis armature and variations in the spination of the area between the anterior and posterior cephalic bulb. Genomic DNA extracted by using QIAGEN DNeasy Blood & Tissue Kit DNA and sequenced with First Base Sequencer showed that this species belonged to *Bolbosoma turbinella*. This was the first record of a sei whale carrying the endoparasites *Bolbosoma turbinella*, in Malaysian waters.

Cite this as: Zakariah, M. I., Ahmad, M. T. I., Razak, M, S., Husin, N. M., Wahab, W., Abdullah, R. A. A., Yusoff, N. A. A., Agos, S., & Hassan, M. (2021). Morphological Identification of *Bolbosoma turbinella* (Acanthocephala) in *Balaenoptera borealis* (Sei Whale) from Straits of Malacca, Malaysia. *Jurnal Ilmiah Perikanan dan Kelautan*, 13(1):73–80. http://doi.org/10.20473/jipk.v13i1.24486

1. Introduction

The Sei Whale, *Balaenoptera borealis* is from the family of Balaenopteridae; commonly inhabits temperate to subpolar oceans and thought to migrate in a long-distance journey from north to south (Norina *et al.,* 2017). Researchers also believe this Sei Whale does not remain in a single place for more than a year (Jafferson *et al.,* 2015). They mainly feed on small schooling fish, but in some areas, their diets consist extensively of zooplankton or shrimp-like crustaceans (krill). The study of ectoparasite and endoparasites of marine mammals are not habitually done because some species are endangered and protected by law (Pinto *et al.,* 2004). A few studies have been reported previously regarding the parasites found in whales (Measures, 1992; Omar and Margolis, 1998; Pinto *et al.,* 2004). There are multiple species of parasites found in infected whales, with different infection sites including intestines, kidney, liver, pancreas, stomach, uterus, and skin (Hermosilla *et al.,* 2015).

Acanthocephalan is an endoparasites, which are typically found in the internal organ particularly the stomach and intestines of their host body. Their life cycle is heterogeneous, in which they usually need one to two intermediate hosts including crustaceans and fishes, in order to complete their life cycle (Hermosilla *et al.,* 2015). Common acanthocephalans belong to the general *Bolbosoma* and *Corynosoma*, where genus *Bolbosoma* are mainly found in whales whilst *Corynosoma* in pinnipeds (Golvan, 1959). Common *Bolbosoma* sp. infected-whales come from the species of *B. brevicolle*, *B. capitatum*, and *B. physeteris* (Baylis, 1932; Gubanor, 1951; Delyamure, 1955). The whale can be infected by these Acanthocephala parasites either through the ingestion of *Bolbosoma*-infected krill or by feeding the second intermediate host which is fishes (Hermosilla *et al.,* 2015). A previous study has reported the presence of *B. balaenae* encapsulated in cephalothorax of large whales in the Northeast Atlantic Ocean (Gregori *et al.,* 2012). Other than whale as their host, *Bolbosoma* sp. also has been found hosting in the black scabbard fish, oceanic horse mackerel and in common dolphins (Costa *et al.,* 2000). The high presence of *Bolbosoma* sp. parasites embedded in submucosa may lead to complete luminal obliteration and haemorrhage, which eventually caused death; as reported by Diaz-Delgado *et al.* (2018), on the death of adult female stranded cetaceans in the Canary Island circa 2006-2012.

In current study, the whale was found dead in Sungai Sarang Buaya, Semerah, Batu Pahat, Johore, Malaysia after being stuck in the mud. The fresh sample of the intestine was collected in-situ and the worms extracted from fresh intestines were subsequently fixed in 10% buffered formalin for further species identification, as detailed described in the materials and methods part. To the best of our knowledge, this is the first study that reported the presence of *B. turbinella* hosting Sei Whale, found in Malacca Straits, Malaysia. Findings are important to control the spread of these parasites into other aquatic areas and the possibility to infect other aquatic animals.

2. Materials and Methods

2.1 Sample collection

The dead Sei Whale (*B. borealis*) was examined, with an estimated length: 11.7 m and weight: 10 tonnes. A total of ten worms are taken freshly from the dead whale's intestine organ, and fixed in 10% buffered formalin for histopathological purposes. The analysed samples were viewed under Portable Scanning Electron Microscope (SEM) purposely to study their morphological characteristics. The morphology characteristic of bulb, neck, proboscis, and hook are the important keys for the identification of the parasites as compared to the previous study. Standard operational procedures for fish diagnosis were applied in this study to acquire better analysis results. The standard procedures for diagnostic are referred to as the standard method by the Department of Agriculture and Water Resources, Australian Government. Methods for DNA applications for the parasitological study were performed as described in Bass *et al.,* (2015).

2.2 DNA Extraction

Firstly, the genomic DNA was extracted by using QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, USA). The cytochrome c oxidase subunit one mitochondrial gene of the parasite was amplified by Primer CO1 Forward (5'-GTT CCA CAA ATC ATA AGG ATA TTG G-3'), and Reverse (5'-TAC ACC TCA GGG TGA CCA AAA AAC CA-3'). The PCR conditions of CO1 rDNA were as follows: each 25 ml reaction contained 2.5ul of 10X EasyTaq® buffer solution, 1.0ul of deoxynucleotide triphosphate dNTP max 10 mmol/L, 2.3ul of total DNA (20ng/ul), 0.6ul of each primer (25umol/L), 3.0ul of EasyTaq® DNA Polymerase; 5U/ul, Invitrogen (Sigma-Aldrich, USA), and bi-distilled water to complete the final volume.

The PCR consisted of the following temperature cycles: initial denaturation for 2 minutes at 94°C, followed by 35 cycles of 30 s at 94°C for denaturation, 30 s at 50°C for hybridization, and 60 s at 72°C for an extension, with a final extension of 10 minutes. The quality of extracted DNA and PCR products was assessed using electrophoresis on 1.2% agarose gel in TBE buffer and

Table 1. Morphology measurement of *Bolbosoma turbinella* from the previous study

Morphology	Measurement (mm)	
	Bolbosoma turbinella (Measures, 1992)	*Bolbosoma* (This Study)
Total Length	19.9	22.0
Bulb Width	2.5	1.2
Bulb Length	1.2	0.4
Neck Length	3.25	2.5
Neck Width	7.25	4.0
Proboscis Length	7.99	2.0
Proboscis width	6.80	3.0
Proboscis Receptacle Length	1.5	0.5
Proboscis Receptacle Width	6.23	3.0
No. of Longitudinal Row of Hooks on Proboscis	2.0	9
No. of Hooks Per Row on Proboscis	06-07	10-13
Hook Length	-	0.4

Table 2. Maximum likelihood fits of nucleotide substitution models of this study.

Model	Parameters	BIC	AICc	InL
HKY	13	3.392.647	3.310.923	-1.642.416

Table 3. Estimates of evolutionary divergence between sequences of this study.

			1	2	3	4	5	6
1	3428032 A2	*Bolbosoma* (this study)		0.01	0.01	0.01	0.02	0.02
2	KU314823.1	*Bolbosoma tubinella*	0.03		0.00	0.01	0.02	0.02
3	KU314823.1	*Bolbosoma tubinella*	0.03	0.01		0.01	0.02	0.02
4	JX442189.1	*Bolbosoma tubinella*	0.12	0.09			0.02	0.02
5	KF156891.1	*Bolbosoma caenoforme*	0.20	0.17	0.17	0.16		0.00
6	JX442190.1	*Bolbosoma* sp.	0.20	0.17	0.17	0.16	0.00	

observed under UV light. Fragments were purified and sequenced by using First Base Sequencer (Axil Scientific Pte Ltd., Singapore).

3. Results and Discussion

This is the first report of *Bolbosoma* sp. found in the intestine of whales stranded in Malaysia. The previous study had reported the death of this Sei Whale was due to decompression sickness, lost its body balancing which eventually stranded in mud and died. However, in this study, infection of these parasites contributed to the factor of host death.

A total of ten samples of parasites were analysed for morphological study and genetic study that proved the result of the sequence was *B. turbinella*. The previous report of *B. turbinella* was described in blue whales from North American waters, and it is the first publication from the eastern coast of North America (Measures, 1992). As the earlier identification of *Bolbosoma* sp. is difficult because of the unavailability of the sample, it may be useful to mention the genus of *Bolbosoma* was reported in whales from North American waters. However, this sample from Malaysia, the DNA sequence indeed belongs to *B. turbinella* (Figure 3).

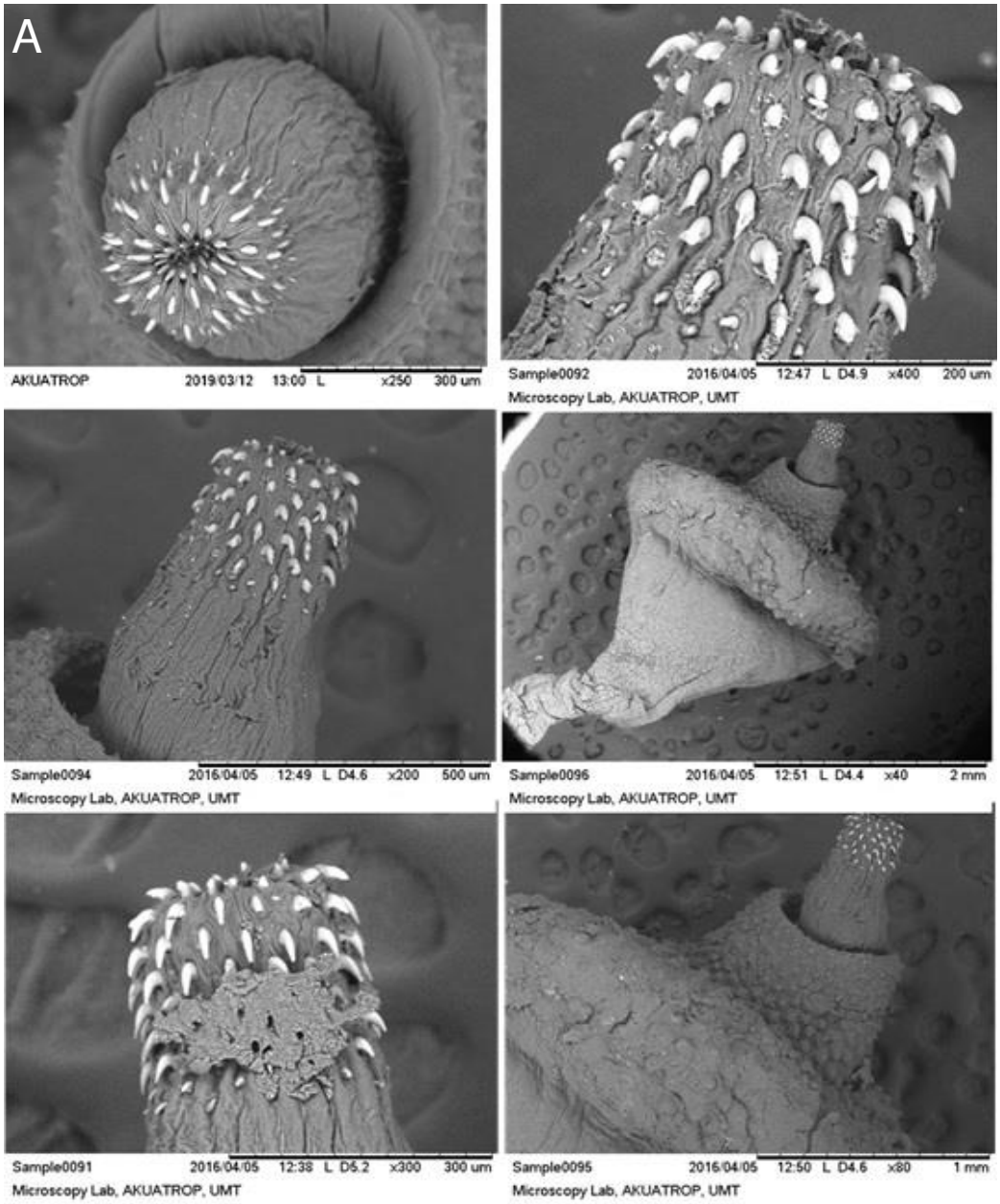

Figure 1. Portable Scanning Electron Micrograph (Hitachi TM-1000) of the anterior portion of an individual *Bolbosoma turbinella* from Whale small intestine (D and F). The pictures showed the typical proportions of presomal parts, distribution of cuticular spines of the two cephalic fields, and some posterior extension of anterior bulbar spines ventrally (A, B, C and E).

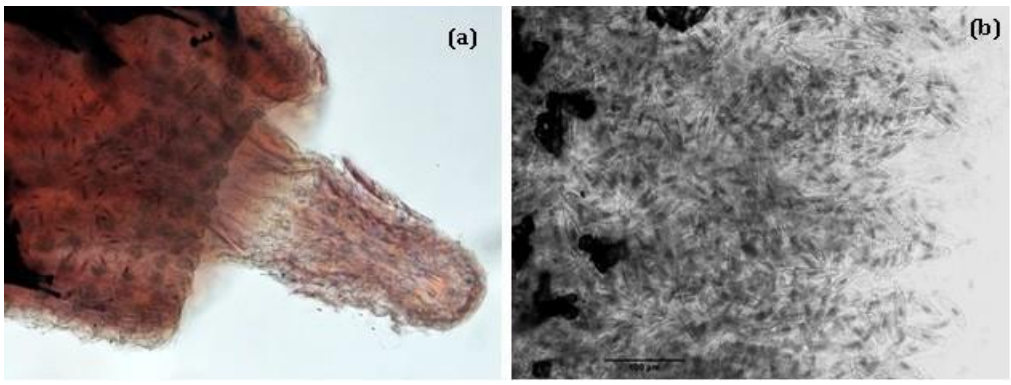

Figure 2. Sample female collected showed (a) the structure of proboscis of *Bolbosoma turbinella* stained with carmine and (b) parasite eggs

The result was supported with the previous research where they found *B. turbinella* in the Sei Whale (*B. borealis*) from the Pacific Ocean of British Columbia (Margolis and Pike, 1955). In this study, the morphological structures of the examined parasites showed the typical proportions of presomal parts, distribution of cuticular spines of the two cephalic fields, and some posterior extension of anterior bulbar spines ventrally (Figure 1). Factually, *Bolbosoma* sp. infection has little relevance on host health such as local reactions produced by the proboscis of the parasites (Vongpakorn *et al.,* 2012). According to the previous report, this acanthocephalan was identified as *Bolbosoma* sp. The morphology of the worms had a bulb that could divide into three parts: anterior, posterior, and intermediate ringbulb, in the proboscis containing mostly 14 longitudinal rows of hooks, and each row contains 8-9 hooks respectively (Yamaguti, 1963). This parasite had been reported possibly zoonotic and potentially infected humans. The symptom shown is acute abdominal pain (Tada, 1983). Surprisingly, these samples of parasites are in the matured stage and carrying an egg (Figure 2). The lifecycle of these parasites is still not completely reported before.

In term of host specificity, *B. turbinella* is not restricted only to blue whales. It also had been reported previously from several Mysticetes; Blue Whale, Sei Whale, Fin Whale, Humpback Whale, and North Atlantic Right Whale and one Odontocete; the North Atlantic Bottlenose Whale which is the host type according to Diesing (1851). The distribution of *B. turbinella* in whales includes the Atlantic and Pacific Oceans in the northern and southern hemispheres (Measures, 1992). Ionita *et al.* (2008) also reported that this *Bolbosoma* sp. also found in the intestine of seals. The possibilities of these parasites to infect humans are also significantly positive because they are categorized as zoonotic parasites (Shuko *et al.*, 2019).

In term of DNA study, the sequence submitted for sequencing showed a positive result of *B. turbinella*. This result of the size of the parasites did not show a significant difference from a previous study by Measures (1992) (Table 1). The morphology character of bulb, neck, proboscis, and hook of the parasite shows the most likely similarity to the previous study report (Figure 1). The DNA study which then supports the confirmation of this study result using molecular phylogenetic analysis by maximum likelihood method (Figure 3).

The maximum likelihood fits of nucleotide substitution, Hasegawa-Kishino-Yano (HKY) models are suggested as the best model to apply this test (Table 2). Models with the lowest BIC scores (Bayesian Information Criterion) with a value of 3392.647 are considered to describe the substitution pattern the best (Table 2). The AICc value (Akaike Information Criterion, corrected), the Maximum Likelihood value (lnL), and the number of parameters (including branch lengths) are also presented for each model (Hasegawa *et al.,* 1985). Non-uniformity of evolutionary rates among sites may be modelled by using a discrete Gamma distribution (+G) with five rate categories, and by assuming that a certain fraction of sites is evolutionarily invariable (+I). Whenever applicable, estimates of the gamma shape parameter and/or the estimated fraction of invariant sites are shown. Evolutionary divergence between sequences is shown similarity with the range of divergence between 0.01 to 0.09 with the previous study (Table 3).

Assumed or estimated values of transition/transversion bias (R) are shown for each model as well. They are followed by nucleotide frequencies (f) and rates of base substitutions (r) for each nucleotide pair. Relative values of instantaneous r should be considered when evaluating them. The sum of r values is made equal to 1 for

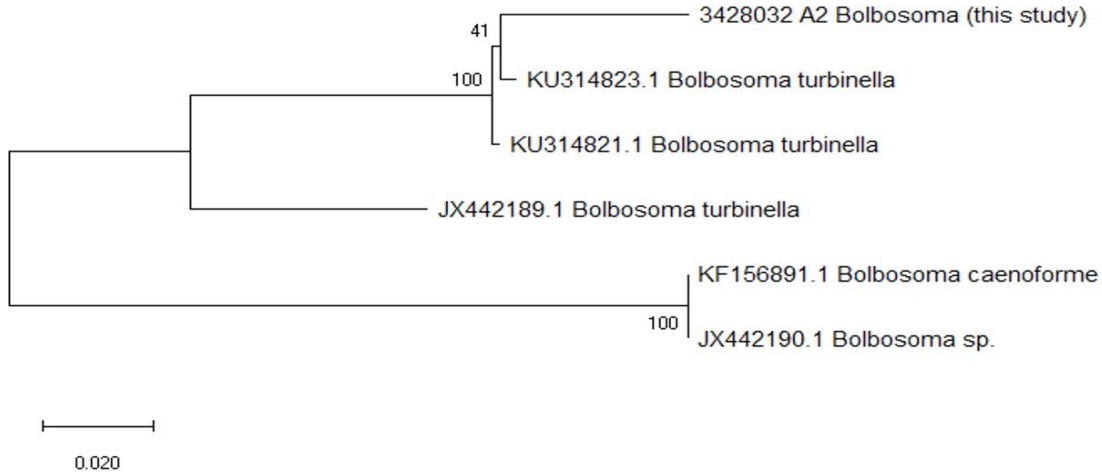

Figure 3. Molecular phylogenetic analysis by Maximum Likelihood method

each model for simplicity. Tree topology was automatically computed for estimating ML values. The results involved six nucleotide sequences. Codon positions in the sequences included were 1st+2nd+3rd+Noncoding. There was a total of 704 positions in the final dataset of sequences. Evolutionary analyses were conducted in MEGA X software (Kumar *et al.,* 2018).

The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa *et al.,* 1985). The tree with the highest log likelihood (-1641.71) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches with 100% similarity (Figure 3). Initial tree(s) for the heuristic search were obtained automatically by applying neighbor joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log-likelihood value.

The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved six nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There is a total of 704 positions in the final dataset. Molecular phylogenetic analysis showed that *Bolbosoma* sp. From this study is more likely related to *Bolbosoma turbinella* with 100% of similarity (Figure 3). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

The number of base differences per site from between sequences is shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates). The analysis involved six nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 704 positions in the final dataset. Evolutionary analyses were conducted in MEGA X software (Hasegawa *et al.,* 1985).

4. Conclusion

This is the first report of *Bolbosoma* sp. found in the intestine of Sie Whale stranded in Malaysia. Total of 10 samples of parasites were analysed, and DNA genetic study has been done, and positively proved that the result of sequence reading showed the species of *B. turbinella.*

Acknowledgement

I wish to express appreciation to the Malaysia Department of Fisheries (DoF), who notified the UMT parasitology team of the stranding cases. The hard work of DoF during the necropsy is greatly appreciated. I also thank the laboratory staff of the Institute of Tropical Aquaculture and Fisheries (AKUATROP) who kindly permitted the use of the laboratory, and staff who assisted in using the equipment.

Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follows, Mohd Ihwan Zakariah; designed study. Mohd Tamimi Ali Ahmad; collected the sample. Mohd Shafiq Razak; collected the sample for genetic. Norainy and Mohd Husin; conducted a genetic analysis. Wahidah Wahab; measured the parasites. Rabi-Atun Adawiah Abdullah; conducted a genetic sequencing. Nor Asma Husna Yusoff; analysed the data. Surzanne Agos; managed the morphological study. Marina Hassan; devised the main conceptual ideas and revised critically. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Funding Information

No funding available for this research.

References

Bass, D., Stentiford, G. D., Littlewood, D. T. J., & Hartikainen, H. (2015). Diverse applications of environmental DNA method in parasitology. *Trends in Parasitology*, 31(10):499-513.

Baylis, H. A. (1932). A list of worms parasitic in Cetacea. Discovery Reports, 6:26.

Costa, G., Chubb, J., & Veltkamp, C. (2000). Cystacanths of *Bolbosoma vasculosum* in the black scabbard fish *Aphanopus carbo*, oceanic horse mackerel *Trachurus picturatus* and common dolphin *Delphinus delphis* from Madeira, Portugal. *Journal of Helminthology, 74*(2):113-120.

Delyamure, S. L. (1955). Helminthofauna of marine mammals (Ecology and Phylogeny). Moscow: Izdatel'stvo Akiademii Nauk SSSR.

Diaz-Delgado, J., Fernandez, A., Sierra, E., Sacchini,

S., Andrada, M., Vela, A. I., Quesada-Canales, O., Paz, Y., Zucca, D., Groch, K., & Arbelo, M. (2018). Pathologic findings and causes of death of stranded cetaceans in the Canary Islands (2006-2012). *Plos One*, 13(10):e0204444.

Diesing, K. M. (1851). Systema Helminthum. Vol. 2. Berlin: Vindobonae Apud Wilhelmum Braumuller. pp. 558.

Golvan, Y. J. (1959). Acanthocephala of the genus *Corynosoma* Luhe 1904, mammalian parasites from Alaska & Midway. *Annales Parasitology Human Comparison*, 34:288-321.

Gregori M, Aznar FJ, Abollo E, Roura Á, González ÁF, Pascual S (2012) *Nyctiphanes couchii* as intermediate host for the acanthocephalan *Bolbosoma balaenae* in temperate waters of the NE Atlantic. *Diseases of Aquatic Organisms*, 99:37-47.

Gubanov, N. M. (1951). Giant nematode from the placenta of Cetacea; *Placentonema gigantissima nov. gen., nov. sp*. *Doklady Akademii Nauk SSSR*, 77(6):1123-1125.

Hasegawa, M., Kishino, H. & Yano, Taka-aki. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22:160–174.

Hermosilla, C., Silva, L. M., Prieto, R., Kleinertz, S., Taubert, A., & Silva, M. A. (2015). Endo- and ectoparasites of large whales (Cetartiodactyla: Balaenopteridae, Physeteridae): Overcoming difficulties in obtaining appropriate samples by non- and minimally-invasive methods. *International Journal for Parasitology: Parasites and wildlife*, *4*(3):414–420.

Ionita, M. M. G., Varela E. T., Lysons. T. R., Spraker. S. C., & Tolliver, S.C. (2008). Hookworms (*Uncinaria lucari*) and Acanthocephalans (*Corynosoma* spp. and *Bolbosoma* spp.) found in dead northern fur seals (*Callorhinus ursinus*) on St. Paul Island, Alaska in 2007. *Parasitology Research*, 103(5):1025-1029.

Jafferson, T. A., Webber, M. A. & Pitman, R. L. (2015). Marine mammals of the world: a comprehensive guide to their identification. (2nd Ed.). San Diego: Academic Press.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6):1547-1549.

Margolis, L., & Pike, G. C. (1955). Some helminth parasites of Canadian Pacific whales. *Journal of the Fisheries Board of Canada*, 12:97-120.

Measures, M. L. (1992). *Bolbosoma turbinella* (Acanthocephala) in a Blue Whale, *Balaenoptera musculus*, stranded in the St. Lawrence Estuary, Quebec. *Journal of the Helminthological Society of Washington*, 59(2):206-221.

Norina, L., Norsharina, A., Nurnadiah, A. H., Sarol, K., Nurliyana, M. T., Fahmi, Aida, M., Noordin, M. M., Nor-Yasmin, A. R., Hassan, M. D., Kamaruddin, I., Munir, M. N., Syed Abdullah, S. A. K., & Tamimi, M. A. A. (2017). First report of decompression sickness (DCS) in a Sei Whale (*Balaenoptera borealis*) stranded in Southern Peninsular Malaysia. *Malaysian Journal of Veterinary Research*, 8:57-64.

Omar, M. A., & Leo, M. (1998). Redescription of *Bolbosoma capitatum* (Acanthocephala: Polymophidae) from False Killer Whale off Vancouver Island, with taxonomic reconsideration of the species and synonymy of *B. physeteris*. *Journal of the Helminthological Society of Washington*, 65(2):179-188.

Pinto, R. M., Muniz-Pereira, L. C., Alves, V. C., & Siciliano, S. (2004). First Report of a Helminth Infection for Bryde's Whale *Balaenoptera edeni* Anderson, 1878 (Cetacea, Balaenopteriadae). *Latin American Journal of Aquatic Mammals*, 3(2):167-170.

Shuko, K., Mizuki S., Kentaro G., Ryo M., Hiroshi K., Minoru N., & Hideo, H. (2019). A case small bowel obstruction due to infection with *Bolbosoma* sp. (Acathocephala: Polymorphidae). *Journal of Parasitology International*, 68:14-16.

Tada, I., Otsuji, Y., Kmaiya, H., Mimori, T., Sakaguchi, Y., & Makazumi, S. (1983). The first case of a human infected with an Acanthocephalan parasite, *Bolbosoma* sp. *Journal of Parasitology*, 69:205-208.

Vongpakorn, M., Pongsopawijit, P., Manawatthana, S., Soison, O., Adulyanukosol, K., Kittiwattanawong, K., and Boontong, P. (2012, May). Identification of helminths in dolphins stranded along the Andaman

coast of Thailand during 1992 – 2006. Paper presented at Conference: Thailand-Japan Joint Conference on Animal Health 2012: The 25th Year An- niversary of National Institute of Animal Health, Bangkok, Thailand.

Yamaguti, S. (1963). Systema helminthum. Vol. 5. Acenthocephala. New York: Interscience Publish-ers.