The first record of *Pseudanthobothrium hanseni* Baer, 1956 (Cestoda: Echeneibothriidae) in the White Sea

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**ABSTRACT:** Cestodes are fairly common in teleost fishes in the White Sea but have never been previously recorded there in elasmobranchs. We found seven individuals of the cestode *Pseudanthobothrium hanseni* in a single specimen of the starry ray *Amblyraja radiata* caught in the White Sea. Molecular analysis based on partial 28S rDNA sequence confirmed that our specimens belonged to this species. Their morphological and morphometric features were compared with the descriptions of *P. hanseni* available in the literature. Specimens of *P. hanseni* from the White Sea were smaller than those from the other areas, had fewer testes, and the vitelline follicles in their mature terminal proglottids did not reach the proglottid’s posterior end. The reasons of these differences are unclear, but the low salinity of the White Sea may play a certain role. To sum up, in this study we provided the first record of a cestode in an elasmobranch in the White Sea and added a new point to the distribution of *P. hanseni*.

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Первая находка *Pseudanthobothrium hanseni* Baer, 1956 (Cestoda: Echeneibothriidae) в Белом море

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РЕЗЮМЕ: Цестоды являются одними из наиболее распространенных паразитов костистых рыб в Белом море, однако ранее ни разу не были найдены у хрящевых рыб этой акватории. В данной работе мы приводим сведения о первой находке семи особей *Pseudanthobothrium hanseni* в Белом море у ската *Amblyraja radiata*. Филогенетический анализ по 28S подтвердил видовую принадлежность наших цестод. Проведено сравнение их морфологических и морфометрических признаков с описа-
Cestodes are broadly distributed helminths parasitizing as adults a broad range of hosts including various fishes. The fauna of cestodes parasitizing teleost fishes in the White Sea is studied fairly well. It is rather poor, comprising only a few common species such as Diplocotyle olrikii Krabbe, 1874, Bothriocephalus scorpii (Müller, 1776) Cooper, 1917 species complex, Pyramicocephalus phocarum (Fabricius, 1780) Monticelli, 1890, Schistocephalus solidus (Müller, 1776) Steenstrup, 1857, Eubothrium crassum (Bloch, 1779) Nybelin, 1922 and Proteocephalus longicollis (Zeder, 1800) Nufer, 1905, as well as various cestode larvae of uncertain generic affiliation (Shulman, Shulman-Albova, 1953; Glukhova, 1956; Timofeeva, Marasaeva, 1984; Tchesunov et al., 2008). However, no cestodes from elasmobranchs have ever been reported in the White Sea.

Elasmobranchs are rare in the White Sea. Only two species of rays (the starry ray Amblyraja radiata (Donovan, 1808) and the thornback ray Raja clavata Linnaeus, 1758) and two species of sharks (the picked dogfish, Squalus acanthas Linnaeus, 1758 and the Greenland shark Somniosus microcephalus (Bloch et Schneider, 1801) inhabit its cold low-salinity waters (Tchesunov et al., 2008). The salinity in the White Sea has sharp seasonal fluctuations, dropping below 15‰ in some shallow areas (Babkov, 1998).

In this paper we report a finding of the cestode Pseudanthobothrium hanseni Baer, 1956 in the starry ray A. radiata in the White Sea, thus adding a new point to the distribution of this parasite in the Arctic. We also provide morphometric data on our specimens and confirm their species affiliation with the help of molecular analysis based on the partial 28S rDNA sequence.
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1294.C.3m.v8 — separate strobila; 1295.C.3m.v9 — separate strobila) were deposited in the collection of marine parasites in the A.O. Kovalevsky Institute of Biology of the Southern Seas (IBSS), Sevastopol; sample data are available at http://marineparasites.org (Dmitrieva et al., 2015). Parasitological indices follow Bush et al. (1997).

**DNA extraction, amplification, sequencing, alignment and phylogenetic analysis**

The total DNA was extracted from 96% ethanol-fixed adult worm using Wizard SV Genomic DNA Purification System (Promega), as recommended by the manufacturer. The nuclear 28S rRNA gene was amplified using the polymerase chain reaction (PCR) with the primers ZX-1 (5'-ACCCGCTGAATTTAAGCATAT-3'), 1500R (5'-GCTATCCTGAGGGAACTTTCG-3'), LSU_300F (5'-CAAGTCGTCAGGAAAAGTTG-3'), 1090F (5'-TGAAACACGGACCAAGG-3'), LSU_1200F (5'-CCCGAAAGATGGTGAACTATGC-3'), ECD2 (5'-CTTGGTCCGTGTTTCAAGACGGG-3'), which were described earlier (Waeschchenbach, Littlewood, 2017). The initial PCR was performed in a total volume of 20 µl that contained 0.25 mM of each primer pair, 1 µl DNA in water, 1× Taq buffer, 1.25 mM dinucleotide triphosphates (dNTPs), 1.5 mM MgCl2 and 1 unit of Taq polymerase. The amplification was carried out by CJSC Eurogen (Moscow) with a 3-min denaturation hold at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at 55 °C and 2 min at 72 °C, and a 10-min extension hold at 72 °C. Negative and positive controls were amplified using all primers. The PCR products were directly sequenced using the ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit, as recommended by the manufacturer, with the PCR primers. The PCR products were analyzed by CJSC Eurogen (Moscow). The obtained sequence of *P. hanseni* has been submitted to GenBank (NSBI) with accession number MT777179.

Partial sequence of the 28S rRNA gene, used in our study to evaluate the phylogenetic connections of our specimen, were assembled using the Geneious ver. 10.0.5 software and aligned with sequences retrieved from the GenBank database (Table 1) using the ClustalW DNA weight matrix within the MEGA 10.0.5 software alignment explorer (Kumar et al., 2018). Phylogenetic analysis of the nucleotide sequences was undertaken using the maximum likelihood (ML) and Bayesian (BI) methods. Phylogenetic trees using ML and BI methods were reconstructed using the MEGA 10.0.5 (Kumar et al., 2018) and MrBayes v. 3.6.2 software (Ronquist, Huelsenbeck, 2003), respectively. Best nucleotide substitution model for the dataset was estimated using jModelTest version 0.1.1 software (Posada, 2008). In both methods, the general time-reversible model GTR+G+I was used based on the Aikake Information Criteria (AIC). A Bayesian algorithm was performed using the Markov chain Monte Carlo (MCMC) option with ngen = 10,000,000, nruns = 2, nchains = 4 and samplefreq = 100. The burnin values were 2,500,000 for the ‘sump’ and ‘sumt’ options. The robustness of the phylogenetic relationship was estimated using bootstrap analysis with 1000 replications (Felsenstein, 1985) for ML and with posterior probabilities for BI (Ronquist, Huelsenbeck, 2003). Outgroup choice follows Healy et al. (2009).

**Results**

Seven cestode specimens were found in the spiral valve of the dissected starry ray. As evidenced by 28S gene-based phylogenetic analysis, all of them belonged to *P. hanseni* (intensity of infection, 7) (Fig. 1). Many morphological characteristics of our specimens (Fig. 2) corresponded to the redescription of *P. hanseni* from *A. radiata* collected in the Western Passage (Bay of Fundy, Canada) (Randhawa et al., 2008). However, after an analysis of morphological and morphometric features of the White Sea specimens some differences were also revealed (Table 2). It is important to note that the cestodes at our disposal were mature, being represented by proglottids with a formed uterus but without eggs, while the descriptions of *P. hanseni* in the literature are based on gravid speci-
Table 1. Data on the 28S rDNA sequences used in the phylogenetic analysis.
Таблица 1. Данные о последовательностях 28S рДНК, использованных в филогенетическом анализе.

| GenBank ID | Parasite                | Host                  | Locality            | Reference              |
|------------|-------------------------|-----------------------|---------------------|------------------------|
| MT525320   | Pseudanthobothrium hanseni | Amblyraja radiata     | White Sea           | this study             |
| MH688744   | Pseudanthobothrium hanseni | Malacoraja senta      | Canada: Passamaquoddy Bay | Beer et al., 2019      |
| MH688743   | Pseudanthobothrium hanseni | Amblyraja doellojurado | Atlantic Ocean: Falkland Islands | Beer et al., 2019      |
| MH688745   | Pseudanthobothrium hanseni | Amblyraja radiata     | North Sea           | Beer et al., 2019      |
| MH688741   | Pseudanthobothrium purtoni | Leucoraja erinacea    | Canada: Passamaquoddy Bay | Beer et al., 2019      |
| MH688740   | Pseudanthobothrium purtoni | Leucoraja ocellata    | Canada: Passamaquoddy Bay | Beer et al., 2019      |
| KF685750   | Pseudanthobothrium sp.    | Leucoraja erinacea    | Canada: St. Andrews, New Brunswick | Caira et al., 2014    |
| MH913263   | Phormobothrium affine     | Zearaja nasuta        | New Zealand         | Bennett et al., 2019  |
| MH688748   | Echeneibothrium multiloculatum | Zearaja chilensis   | Atlantic Ocean: Falkland Islands | Beer et al., 2019      |
| MH688750   | Echeneibothrium vernetae  | Leucoraja erinacea    | Canada: Passamaquoddy Bay | Beer et al., 2019      |
| MH688751   | Echeneibothrium canadensis | Amblyraja radiata     | Canada: Passamaquoddy Bay | Beer et al., 2019      |
| KF685901   | Scyphophyllidium cf. giganteum | Galeorhinus galeus  | New Zealand: Chatham Rise | Caira et al., 2014    |
| AF286930   | Litobothrium janovyi      | Alopias superciliosus | Mexico              | Waeschenebach et al., 2007 |

mens (Williams, 1966; Randhawa et al., 2008). Therefore, only the morphology of mature proglottids was taken into account in our further analysis.

**Discussion**

Both molecular and morphological analyses confirmed that our specimens belonged to *P. hanseni*. However, some differences from the descriptions of *P. hanseni* available in the literature were also found. To note, in this study we used only some of the numerous sequences of *P. hanseni* and other representatives of the family Echeneibothriidae available in GenBank (Fig. 1). Therefore, our tree cannot be used for discussing the systematics of the genus *Pseudanthobothrium*. 
Pseudanthobothrium hanseni is recorded in the North Atlantic from the starry ray *Amblyraja radiata* and *Malacoraja senta* in the Atlantic waters of Canada (Randhawa et al., 2007; Randhawa et al., 2008; Randhawa, Burt, 2008), and the spiny-tail skate *Bathyraja spinicauda* (Jensen, 1914) in the north-eastern Norwegian Sea (Rokicki et al., 2001). *Pseudanthobothrium purtoni* Randhawa, Saunders, Scott & Burt, 2008 was primarily described from the same area in the North Atlantic, but according to our own data on helminths of *Bathyraja sp.* (‘*Bathyraja sexoculata*’) in the Simushir Island area (Gordeev, Polyakova, 2020), it also inhabits the North Pacific. Thus, the distribution area of the genus *Pseudanthobothrium* seems to be underestimated. It may be broadly present in the bottom ecosystems in the Arctic seas and elsewhere.
Table 2. Morphological characteristics of *Pseudanthobothrium hansenii* Baer, 1956 ex *Amblyraja radiata* (Donovan, 1808) from different areas.

| Source: Present study | Baer, 1956 | Williams, 1966 | Randhawa *et al.*, 2008 |
|-----------------------|------------|---------------|-------------------------|
| Locality:             | White Sea  | Disko Bay, West Greenland | North Sea | Bay of Fundy, Canada |
| Total length (mm)¹ | 8.2–9.1 | 4 | Up to 20 | 5.1–25.8 |
| Maximum W² | 340 | 250 | 400 | 195–600 |
| No. of proglottids | 135–148 | 40 | 200 | 39–131 |
| Bothridia | | L 108–167 | – | 1000 | 140–380 |
| | | W 56–103 | – | 800 | 135–306 |
| Myzorhynchus | | L 58–110 | – | 1000 | 45–440 |
| | | d 50–63 | 55 | 150 | 60–175 |
| Stalk | | L 55–85 | – | 60–210 | 60–210 |
| | | W 50–85 | – | – | 65–160 |
| Neck | | L 32–35 | – | – | 60–335 |
| | | W 53–71 | – | – | 40–150 |
| Mature proglottids | | L 508–642 | – | – | – |
| | | W 231–323 | – | – | – |
| Gravid proglottids | | L – | – | 1500 | 475–1645 |
| | | W – | – | 400 | 205–600 |
| No. of testes | 13–16 | Few | 16–20 | 19–32 |
| Size of testes | | L 86–105 | Large | – | 50–150 |
| | | W 17–28 | – | – | 38–90 |
| Cirrus-sac | | L 94–114 | – | 280 | 140–255 |
| | | W 52–59 | – | 140 | 60–105 |
| Vitelline follicles | | L 16–29 | Large | – | 16–55 |
| | | W 10–24 | – | – | 15–35 |

¹ All measurements in micrometres unless otherwise indicated.

² Abbreviations: No. — number; L — length; W — width, d — diameter.

Beer *et al.* (2019) recorded two species of *Pseudanthobothrium* in *Amblyraja doellojordoi* (Pozzi, 1935) caught in the Falkland Islands area. Apparently, this genus tends to be associated with the polar and sub-polar waters.

*Amblyraja radiata* is a very common ray in the North Atlantic and the seas adjacent to the Arctic Ocean (Last *et al.*, 2016). Throughout this extensive area, it serves as a host of at least 19 species of onchoproteocephalidean, phyllobothriidean, rhinebothriidean, diphyllidean, and trypanorhynchian cestodes (Pollerspöck, Straube, 2019). The freshened environment of the White Sea makes possible the presence of the euryhaline species in marine communities (Tchesunov *et al.*, 2008), while truly marine fish come from the Barents Sea mostly through poorly studied bottom recesses, in which normal oceanic salinity is preserved. This may be the reason why a parasite of an elasmobranch in the White Sea was recorded in our study for the first time.

Ecological and biological features of the elasmobranchs such as feeding behavior, age, size, depth and geographical distribution, play an important role in their co-evolution with
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Cestodes (Caira, Bardos, 1996; Klimpel et al., 2003; Palm, 2004; Beer et al., 2019). In this study, we cannot explain with certainty the morphometric differences between *P. hanseni* from the White Sea and other areas (Table 2). Some role may be played by salinity, which in the White Sea (Babkov, 1998) could be twice or more lower than in habitats of the previously studied hosts of *P. hanseni* (Baer, 1956; Williams, 1966; Randhawa et al., 2008). The White Sea is not isolated from the ocean, but the capture of rays there is quite rare. This might mean that the environmental conditions there are on the verge of tolerance of marine rays. This, in turn, is likely to affect their parasites.

The length of the strobila of cestodes in the present study could not be used as a diagnostic sign, since the specimens were at different stages of development. However, the mature cestodes from the White Sea in our study had a greater number of proglottids (135–148) than gravid *P. hanseni* (39-131) (Randhawa et al., 2008). Our specimens also had fewer testes, 13–16 vs. 19–32 in Randhawa et al. (2008) vs. 16–20 in Williams (1966). In the mature proglottids of worms under study, the yolk follicles were located in two lateral bands extending from the anterior end of the proglottid to the anterior end of the ovary, without reaching the posterior end of the proglottid (Fig. 2). Moreover, in the figures of mature proglottids in the description of *P. hanseni* in the work of Williams (1966: fig. 95), as well as in mature and gravid proglottids in the work of Randhawa et al. (2008: figs 2, 3), yolk follicles extend from the anterior to posterior end of the proglottid, without interruption at the level of the ovary. However, according to the redescription of *P. hanseni* in Randhawa et al. (2008) “Vitelline follicles ... , arranged in 2 paired lateral bands anterior to ovary, extending as 2 single lateral bands posterior to anterior margin of the ovary along length of proglottis (Fig. 2A).” Thus, the location of yolk follicles in the text of the description of *P. hanseni* are somewhat inconsistent with the details shown in Figure 2 (see Randhawa et al., 2008). In our specimens the yolk vitelline follicles reached the posterior margin of proglottids only in immature proglottids. A relatively low intensity of infection (7 worms/host) must be associated with the small size and, accordingly, age of the examined host specimen. As elasmobranchs grow, their diet changes, and their infection with cestodes increases as a result (Randhawa et al., 2008; Gordeev, Polyakova, 2020). All other cestode species recorded in the White Sea (Tchesunov et al., 2008; see above) parasitize at the adult stage teleosts, birds, and mammals which, unlike rays, are common in the White Sea. It is therefore unsurprising that the starry ray examined in our study harbored only a few individuals of a single parasite species.

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Compliance with ethical standards

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