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BRIEF COMMUNICATION

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First recorded occurrence of the parasitic barnacle (Anelasma squalicola) on a Greenland shark (Somniosus microcephalus) in the Canadian Arctic

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Abstract

A solitary Anelasma squalicola specimen was collected from the cloaca of a Greenland shark (Somniosus microcephalus), the first time this association has been recorded. The specimen's identity was confirmed through morphological and genetic assessment (mitochondrial markers: COI and control region). A. squalicola is a species typically associated with deep-sea lantern sharks (Etmopteridae) and, until the present observation, had never been observed at a sexually mature size in the absence of a mating partner. Given the reported negative effects of this parasite on its hosts, monitoring Greenland sharks for additional cases is recommended.

KEYWORDS

cirripedia, genetics, marine, parasite, sharks

1 INTRODUCTION

Parasites play diverse roles in aquatic ecosystems and can in many instances drive changes to the behaviour, physiology and population dynamics of their hosts (Barber et al., 2000; Ferrón & Palacios-Abella, 2022; Krkošek et al., 2011; Slavík et al., 2017). As with most teleost fishes, elasmobranchs globally are known to host numerous ecto- and endoparasite species (Caira & Healy, 2004). For the large and long-lived Greenland shark [Somniosus microcephalus (Bloch & Schneider, 1801)], which inhabits Arctic and North Atlantic waters, Ommatokoita elongata (Grant, 1827) is a well-documented parasite reported on most sampled individuals (MacNeil et al., 2012, but see Harvey-Clark et al., 2005). This species of copepod anchors directly to an individual shark's cornea, causing epithelial lesions which likely result in reduced vision (Benz et al., 2002; Borucinska et al., 1998). Research into the prevalence of other parasites associated with Greenland sharks, however, is limited in the literature. Given that the

GenBank accession numbers for the reported on A. squalicola specimen. COI: OQ890737 Control region: OQ926372.

latitudinal diversity gradients for parasites are often weak or opposite to the general trend of higher richness at low latitudes (Johnson & Haas, 2021; Poulin, 2010), it is possible that increased sampling of polar elasmobranchs such as Greenland sharks could reveal a greater diversity of parasites than is presently known.

Anelasma squalicola (Lovén, 1844) is a species of barnacle with unique features that is reported to parasitize several species of shark in the Etmopteridae and Scyliorhinidae families, primarily the velvet-belly lantern shark [Etmopterus spinax (Linnaeus, 1758), Rees et al., 2019; Figure 1a]. Although many barnacle species anchor themselves to larger marine animals, most rely on filter-feeding to obtain food. In contrast, A. squalicola has evolved root-like appendages on its peduncle which embed into its host's muscle tissue to directly draw nutrients (Ommundsen et al., 2016; Rees et al., 2014). To date, examined stomachs of A. squalicola have all been empty, and their vestigial cirri lack the setae necessary for filter-feeding (Ommundsen et al., 2016). This, combined with recent molecular analyses showing minimal genetic variation across globally sampled A. squalicola, suggests this species evolved its parasitic lifestyle relatively recently (Ommundsen et al., 2016; Rees et al., 2019).

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FIGURE 1 (a) Simplified evolutionary tree depicting relatedness of all known host species of *Anelasma squalicola* (Angulo & Sibaja-Cordero, 2021; Kazunari Yano & Musick, 2000; Long & Waggoner, 1993; Rees *et al.*, 2019), including a novel host, the Greenland shark (*Somniosus microcephalus*). Species are grouped by order, family and genus. Branch lengths are arbitrary. (b) Sampling locations of the A. *squalicola* specimen collected on a Greenland shark in Tremblay Sound, Nunavut (red), and the specimen with a COI haplotype match collected from a *Galeus melastomas* in the Rockall Trough, near Scotland (gold; Rees *et al.*, 2019). (c) Photograph of the A. *squalicola* specimen collected from a 275 cm total length female Greenland shark in Tremblay Sound, Northern Baffin Island in 2018 (Photo credit: E. Ste-Marie). —, Scyliorhinidae; —, Somniosidae; —, Etmopteridae

Attachment points for A. *squalicola* can vary, but specimens are predominantly found in clusters near the dorsal or pectoral fins in areas where the skin is thinner, or where turbulence may facilitate attachment by their free-swimming larvae (Eliassen, 2016). Host effects are not well understood, although previous studies on several species of lantern shark (Etmopteridae) have reported smaller testes, claspers and ova across individuals that are parasitized compared with those that are not, suggesting that *A. squalicola* hinders the development of reproductive organs in some of its hosts (Eliassen, 2016; Hickling, 1963; Yano & Musick, 2000). Yano and Musick (2000) proposed that *A. squalicola* may divert energy away from their host's reproductive development by drawing resources directly from the host's tissues, though no specific mechanism has been demonstrated empirically. Here, the authors for the first time report the occurrence of an *A. squalicola* barnacle parasitizing a Greenland shark captured in the high Arctic, in Tremblay Sound, Nunavut, Canada.

2 | METHODS

On 14 August 2018, while scientific fishing for Greenland sharks for electronic tagging and metabolic rate studies (see Ste-Marie *et al.*, 2020, 2022 for details on fishing methods), the authors observed the burgundy-coloured mantle of an *A. squalicola* specimen protruding from the cloaca of a 275 cm [total length (TL)] female Greenland shark. The parasite was extracted, which revealed its yellowish-white peduncle and rootlets. The specimen was preserved in ethanol in the field and subsequently frozen for future dissection, identification and molecular analyses.

In the laboratory, the sampled A. squalicola was first examined under a Leica M80 stereo microscope to determine if the specimen's morphology matched the species description (Johnstone & Frost, 1927). The individual was measured using a ruler and the peduncle, and mantle cavity was examined separately. Specifically, the authors assessed the morphological structure of the thorax and the mantle cavity to determine whether the animal was, or had previously been, egg-bearing. Genomic DNA was then extracted from the ethanol-preserved sample following the methods described in Rees *et al.* (2019). Two mitochondrial markers (COI and the control region) were amplified for comparison with existing A. *squalicola* sequence data. PCR primers, amplification conditions and DNA sequencing reactions were as outlined in Rees *et al.* (2019).

3 | RESULTS

The single A. squalicola specimen measured 45 mm from the brownblack mantle tip to the distalmost part of the white peduncle (Figure 1b). Most of the rootlets of the peduncle were damaged during the process of separation from the host, but the proximal parts of the rootlets were still visible. The mantle cavity contained the head, mouthparts and thorax of the barnacle, with six pairs of reduced cirri and an unpaired penis posterior to the last pair of cirri. Eggs were not present in the mantle cavity, nor were there signs indicating the previous presence of eggs. This was likely because only a single individual was present in the shark's cloacal opening, and therefore, it had not had an opportunity to mate with a partner; this may indicate that another individual had never been present and/or detached prior to host capture. Overall, the individual was visually identical to A. squalicola individuals sampled from several other shark species (Rees et al., 2019); nonetheless, the large body size was notable when compared to previously measured solitary

A. squalicola (Eliassen, 2016). Comparison of the COI sequence (532 bp) for this A. squalicola specimen indicated a 100% match to a haplotype previously recorded for A. squalicola sampled from Galeus melastomus (Rafinesque, 1810) from the Rockall Trough, Scotland (Figure 1c). Similarly, control region sequence data showed very close affinity with the same Anelasma individual from Scotland (724 bp; 99.74% identity).

4 | DISCUSSION

Both morphological and molecular data confirm that the pedunculated barnacle sampled from a Greenland shark in the high Arctic belongs to the species A. squalicola. The match of the COI haplotype also links this individual to an A. squalicola population sampled in the Rockall Trough (Rees et al., 2019). The large body size of this solitary A. squalicola tied with its close affinity to individuals sampled from the Rockall Trough raises questions about the commonality of its occurrence on this host and potential connectivity of Greenland sharks between these two regions. Much remains unknown about the connectivity of Greenland shark populations in Baffin Bay and Northern Europe (Edwards et al., 2019), although studies on the movement ecology of this species have demonstrated its capacity for longdistance travel (Campana et al., 2015; Fisk et al., 2012; Hussey et al., 2018). Additionally, although a recent study using speciesspecific microsatellite markers found some evidence for genetic differentiation of Baffin Bay Greenland sharks, the signal was weak, admixture was evident and no European samples were included in the analysis (Swintek et al., in revision). Moreover, next-generation sequencing data revealed genetic homogeneity in Greenland sharks across the sampled range (Timm et al., 2023).

Given the sampling of A. squalicola from a single Greenland shark, it is unclear whether the observed parasitization represents an atypical occurrence or whether it occurs frequently in nature. Greenland sharks inhabit higher latitudes than any other shark species (MacNeil et al., 2012; Yano et al., 2007), yet southern parts of their geographic range overlap with known host species of Anelasma [e.g., Centroscyllium fabricii (Reinhart, 1825) and Etmopterus princeps (Collett, 1904)]. Moreover, the bentho-pelagic behaviour of Greenland sharks (Andrzejaczek et al., 2022; Campana et al., 2015) likely leads to vertical overlap in distribution with these latter two shark species that could facilitate transfer. Given the larvae of A. squalicola are lecithotrophic and weak swimmers, range overlap of host species represents a likely means of dispersal for this parasite (Frost, 1928; Rees et al., 2019). As a largely understudied species, it is possible that increased sampling of Greenland sharks could reveal that a proportion of individuals are regularly parasitized by A. squalicola. Nonetheless, Greenland sharks have particularly abrasive skin composed of large dermal denticles that could hinder or prevent extensive parasitization (Benz et al., 2002; Feld et al., 2019). The mature-sized A. squalicola specimen described here was located without a partner and inside the opening of the Greenland shark's cloaca where the tissue is much

softer than the surrounding skin, perhaps indicating that this was an opportunistic attachment and not representative of a larger pattern of infestation in this host (Eliassen, 2016).

The measured size (45 mm) of the solitary A. squalicola specimen is notable. A. squalicola, as with many other barnacle species, is hermaphroditic, and all previous observations indicate that each individual, to reproduce, requires a partner in its immediate proximity. Egg-bearing A. squalicola individuals have thus exclusively been observed with one, or in rare cases, more mating partners (Eliassen, 2016). Prior to the present observation, solitary individuals of A. squalicola have all been small (maximum size of 10 mm) and always without eggs (Eliassen, 2016). The size of this individual sampled from a Greenland shark demonstrates that it is possible for this species to achieve reproductive size without a mating partner present. Nonetheless, whether the size of the host (Greenland shark, 275 cm TL), in contrast to a lantern shark species (typical <100 cm TL), provides increased growth opportunities for the parasite, or whether there are other factors at play, requires further study.

The prevalence of ectoparasites is often affected by environmental conditions such as temperature (Bush *et al.*, 2001). Although little is known about the thermal niche of *A. squalicola*, it primarily parasitizes lantern sharks that inhabit deep and cold waters where temperature regimes remain relatively constant (Eliassen, 2016). Unlike typical host species, the bentho-pelagic nature of Greenland sharks results in individuals experiencing temperatures ranging from -1.8 to 17.2° C depending on depth, location and season (Campana *et al.*, 2015; MacNeil *et al.*, 2012). This could potentially limit the extent of parasitization, though a more thorough understanding of the temperature preferences of adult and larval *A. squalicola* is needed. This is particularly important given the continuing increases in Arctic sea temperatures resulting from climate change (Carvalho & Wang, 2020).

Previous studies have suggested that A. *squalicola* stunts the gonadal development of some Etmopterid hosts by extracting metabolic resources from their tissues (Eliassen, 2016; Hickling, 1963; Yano & Musick, 2000). Given the much larger body size of Greenland sharks (total length of mature females >400 cm, Nielsen *et al.*, 2020) relative to previously studied host species, it is possible that this effect would be reduced. Nonetheless, further research into the specific mechanism by which *A. squalicola* affects reproductive development in sharks, and if this is transferable to large-bodied species, is required.

Future work by scientists and fisheries observers interacting with Greenland sharks (or related species in the Somniosidae family) should include monitoring for parasitization by *A. squalicola* to determine its frequency of occurrence and if its presence results in negative impacts on these understudied sharks. Given the growing number of known host species for *A. squalicola* worldwide, its uniquely evolved feeding strategy, minimal genetic variation across global sampling locations and novel presence in the Canadian high Arctic, further investigation into the parasite's biology is needed. Specifically, research into *A. squalicola*'s thermal preferences, feeding behaviour and

reproductive biology could potentially reveal insights into this parasite-host relationship in the context of climate change and be used as a marker to elucidate the broad-scale connectivity of host species.

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