Parasites of an Arctic scavenger; the wolverine (Gulo gulo)

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ABSTRACT

Parasites are fundamental components within all ecosystems, shaping interaction webs, host population dynamics and behaviour. Despite this, baseline data is lacking to understand the parasite ecology of many Arctic species, including the wolverine (Gulo gulo), a top Arctic predator and scavenger. Here, we combined traditional count methods (i.e. adult helminth recovery, where taxonomy was confirmed by molecular identification) with 18S rRNA high-throughput sequencing to document the wolverine parasite community. Further, we investigated whether the abundance of parasites detected using traditional methods were associated with host metadata, latitude, and longitude (ranging from the northern limit of the boreal forest to the low Arctic and Arctic tundra in Nunavut, Canada). Adult parasites in intestinal contents were identified as Baylisascaris devosi in 72% (n = 39) of wolverines and Taenia spp. in 22% (n = 12), of which specimens from 2 wolverines were identified as T. twitchelli based on COX1 sequence. 18S rRNA high-throughput sequencing on DNA extracted from faeces detected additional parasites, including a pseudophyllid cestode (Diplogonoporus spp. or Diphyllobothrium spp.), two metstrongyloid lungworms (Angiostrongylus spp. or Aelurostrongylus spp., and Crenosoma spp.), an ascidian nematode (Ascaris spp. or Toxocara spp.), a Trichinella spp. nematode, and the protozoan Sarcocystis spp., though each at a prevalence less than 13% (n = 7). The abundance of B. devosi significantly decreased with latitude (slope = −0.68; R² = 0.17; P = 0.004), suggesting a northerly limit in distribution. We describe B. devosi and T. twitchelli in Canadian wolverines for the first time since 1978, and extend the recorded geographic distribution of these parasites ca 2000 km to the East and into the tundra ecosystem. Our findings illustrate the value of molecular methods in support of traditional methods, encouraging additional work to improve the advancement of molecular screening for parasites.

1. Introduction

Representing over 50% of all organisms on Earth, parasites are a fundamental component within all ecosystems –shaping interaction webs as well as host population dynamics and behaviour (Dobson et al., 2008; Hudson et al., 2006; Marcogliese, 2001). Even at high latitudes where biological diversity is purported to be generally low (such as the Arctic), parasite communities can be diverse and often more species-rich than those of their vertebrate hosts (Hoberg et al., 2008; Kutz et al., 2009). For example, over 60 species of parasite are described in four ungiulate species of High Arctic Greenland and North America (Kutz et al., 2012). Such descriptions highlight the extensive distribution of parasites throughout Arctic hosts.

The Arctic is undergoing some of the most rapid rates of climate change and is therefore at high risk of parasite emergence, which may shift baseline data (Brooks and Hoberg, 2007; Kutz et al., 2009; McLaughlin, 2011). It has been forecast that increases in air temperature will be most dramatic at high latitudes (Dobson et al., 2015) and already the Arctic has experienced the 10 warmest years in the past 2 millennia (Kaufman et al., 2009; Hoegh-Guldberg et al., 2018). One expected consequence of warming air temperatures and increased precipitation in the northern hemisphere is a significant increase in the northern expansion of parasites and their hosts into regions that were previously inhospitable to them (Brooks and Hoberg, 2007; Kutz et al., 2009;
Taenia martis. Alaska and the Northwest Territories of Canada, including; moschatus, hare (Lepus sp.), Arctic ground squirrels (Spermophilus par..., and migratory bird species (Koskela et al., 2013; L'Herault et al., 2018). Dissections were conducted on gastrointestinal tracts of 54 wolverines (pylorus to anus, excluding the stomach) at the Universite de Moncton (February 2018). Each intestinal tract was partially defrosted until pliable. A faecal sample and a small intestine sample were collected from near the rectum of each individual (54 faecal and 54 small intestine samples; 108 samples total) and immediately frozen at –20 °C for later 18S parasite profiling. Starting from the stomach end, the intestinal wall of the tract was then systematically cut open, washed through a series of sieves (minimum mesh size of 0.01 mm), and examined for intestinal helminths by naked eye and then using a 40× hand lens. The mucosa was scraped with a spatula into a Petri dish and also examined. Any helminths discovered were removed, counted, and stored in 70% ethanol at –20 °C until further analysis.

2.3. Identification of adult parasites using COXI

In total, 44 recovered adult helminths from 34 hosts were sent for identification at the Jenkins Lab, Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, via molecular methods. DNA from a subset of representative specimens was extracted individually using the DNeasy Blood and Tissue Kit (Qiagen, Toronto, Canada) following manufacturer instructions. The primer set COX1-F (5′— TTITTGGGCTTCCTGAAGTTATTT—3′) and COX1-R (5′— TAAAGAGAAACATAATGAAAATGY—3′) (Bowles...
et al., 1992) was used to target a ~366 base pair region of the cytochrome c oxidase subunit 1 (COX1) mitochondrial gene following methods detailed in Lavikainen et al. (2003). For each sample, 25 μL reaction mix was prepared by mixing 12.5 μL 2 × Taq FroggaMix (FroggaBio, Toronto, Canada), 1 μL forward and reverse primer mixture (10 μM of each primer), 3 μL template DNA and 7.5 μL nuclease-free water. The thermal conditions used were: preheating at 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C (30 s), annealing at 55 °C (30 s) and extension at 72 °C (1 min). This was followed by a 10 min final extension at 72 °C. PCR products were separated by gel electrophoresis on 1% agarose gels in TAE buffer. PCR products of 15 samples, representing samples which yielded bands at different positions, were purified and sent for sequencing at Macrogen, South Korea. All nucleotide sequences were compared to sequences from the NCBI GenBank database using BLAST. All nucleotide sequences were compared to sequences from the NCBI GenBank database using BLAST.

### 2.4. 18S parasite profiling using high-throughput sequencing

Total genomic DNA was extracted from 54 faecal and small intestine samples using a QIAamp DNA Stool Mini Kit (Qiagen, Toronto, Canada). Five of the samples were extracted and processed a second time, to check for metabarcoding consistency. Methods followed the manufacturer’s instructions for pathogen detection using 250 mg of sample, with the addition of a 6 min homogenization step to enhance bacterial cell lysis after the addition of buffer ASL (Step 2 in manufacturer handbook). Homogenization was achieved using a Tissuelyser II (Qiagen) for 6 min at 5.5 Hz with the following combination of glass beads per tube: 0.3 g of 106 μm beads, 0.5 g of 425–600 μm beads, and x1 3 mm bead (Sigma-Aldrich, Ontario, Canada).

For parasite detection within faeces and small intestine samples, a ~260 base pair region of the V9 fragment of the 18S rRNA gene was amplified using the primer set Euk_1391f (5’—TCTAAA-GATTAAGGCCATGC—3’) and EuKBr (5’—TTTACCGCTCAAGACTAGGG—3’) (Amaral-Zettler et al., 2009) in conjunction with the mammal blocking primer: GCCCGTGGCTACTACCGATTGIIIIITTAGT-GAGGCCCT-(C3 Spacer) (Vestheim and Jarman, 2008) following methods described in the Earth Microbiome Project (http://www.earthmicrobiome.org/). In brief, targeted PCR reactions were used whereby a sequence tag was added to the 5’ end of each primer. The tag sequence was used to bind primers in a second PCR reaction during which individual sample barcodes and Illumina adapters were annealed. All barcoded products were run on 2% agarose gels. DNA concentrations of all samples were measured using PicoGreen, allowing pooling of samples at equimolar amounts. The pool (library) was cleaned using AMPure® beads. The library was then quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). Average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Illumina MiSeq PE250 high-throughput sequencing of 18S libraries was conducted with the MiSeq Reagent Kit v2 (500 cycles) at Génome Québec, Montréal, Canada. The raw sequencing data can be found at the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) [Accession number: PRJNA662086].

### 2.5. Bioinformatic analysis

All bioinformatic analyses were conducted using QIIME2, version 2018.11 (Bolyen et al., 2018). Briefly, paired-end reads were joined using VSEARCH (Rognes et al., 2016) and quality filtered using the default settings of q-score-joined (Bokulich et al., 2013). Data reads were denoised using DADA2 (Callahan et al., 2016) with a minimum phred quality score of 28 (below which data quality tailed off). Taxonomic assignments of representative sequences from each Amplicon Sequence Variant (ASV) were performed using the SILVA reference database (Quast et al., 2013) at 99% identity so as to minimize potential identification mismatch. Taxonomic assignment was conducted in conjunction with the alignment-based taxonomy consensus classifier, BLAST+ (Camacho et al., 2009). To additionally evaluate taxonomic assignment, the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) was used to compare representative sequences of parasite taxa against the NCBI database. As lower taxonomic identifications are less certain, parasite identifications are reported at genus level.

### 2.6. Statistical analysis

All statistical analyses were conducted in R version 3.5.0 (R Core Team, 2018). Generalized Linear Models (GLMs) were used to investigate whether i) total B. devosi abundance, ii) total parasite abundance of both B. devosi and Taenia spp. and iii) coinfection of B. devosi and Taenia...
spp. (i.e. 0, 1, ≥2 parasite species present) were associated with sex, age class, body length, carcass mass, latitude, and longitude. An outlier, identified via a Cook’s distance residuals versus leverage plot, was omitted from the models. The coinfection model did not include parasite species detected using molecular methods owing to the low prevalence identified and difficulty in distinguishing true wolverine infections from secondary parasite detection from infected individuals. To follow GLM assumption, a negative binomial error family with a log link function was used for B. devosi abundance and for total parasite abundance of both B. devosi and Taenia spp. A Poisson family and identity link function was used for coinfection of B. devosi and Taenia spp., as based on residual plots. No interacting terms were included due to small sample size limiting the numbers of individuals represented in each group. For 18S data, a parasitic infection was considered present if a parasite was detected in either the faecal or small intestine sample. Due to the low prevalence of detection and because the range of dietary items consumed by wolverines makes it difficult to discern whether parasite detection indicated a true infection of wolverines or is instead a secondary detection from an infected prey item, parasites detected through 18S rRNA high-throughput sequencing were not included in the statistical models.

3. Results

3.1. Parasite diversity and abundance

Based on adult helminth recovery from intestines, 83% of wolverines (n = 45) were parasitised; nine individuals had no visible parasite infection, 34 were infected with one parasite species, and 11 were coinfectected. Baylisascaris devosi (Nematoda) was found in 72% (n = 39) of individuals at an average of 3.6 worms per individual (SD: ± 4.6, abundance range: 0–21; Fig. 2a), and Taenia spp. (Cestoda, Cyclophyllidea) were found in 22% (n = 12) individuals at an average of 1.8 worms per individual (SD: ± 6.5, abundance range: 0–45). Based on COX 1 primers, two taeniid cestodes from two hosts were identified as T. twitchelli (99% similarity to Genbank accession number EU544598) and seven nematodes from seven hosts were identified as B. devosi (99% similarity to Genbank accession number KM216978). When using 18S rRNA high-throughput sequencing, six parasite genera were detected, at a low overall prevalence (22% total, n = 12; ≤13% prevalence for any parasite). The prevalence of each parasite genus against associated host metadata can be found in Supplementary Table 1. Although a higher parasite diversity was detected using molecular methods, it is uncertain whether or not the parasites are secondary detections from infected prey items as opposed to true infections of wolverines and, in some instances, there was no consensus in taxonomic identification between the two reference databases used, and likely identifications for wolverine parasites are seldom present in these databases. For example, a metagonial lungworm (detected at a prevalence of 2%; n = 1) was identified as Angiostrongylus sp. in accordance with the SILVA reference database (99% similarity) but Aelurostrongylus sp. when using the NCBI database (99.4% similarity). Similarly, an ascarid nematode was detected in 6 small intestinal and 1 faecal sample at a prevalence of 13% (n = 7), and identified as an Ascaris sp. in the SILVA database (99% similarity) but as Toxocara sp. in the NCBI database (99.4% similarity). A pseudophyllid cestode (Subclass: Eucestoda) which was identified as Diplogonoporus sp. according to the SILVA database (99%) or Diphyllobothrium sp. according to NCBI (99.4%) was detected at a prevalence of...
2% (n = 1). Further, the following parasitic genera were each detected in 2% (n = 1), the identification of which were consistent between the SILVA and the NCBI databases (>99% similarity); Crenosoma spp., Trichinella spp. (Nematoda) and Sarcocystis spp. (Protozoa). In addition to these 6 parasites for which wolverine could serve as potential definitive hosts, two parasites were detected which are likely parasites detected from infected prey items. For example, Gregarina sp. (detected in both the SILVA and NCBI databases; 99% and 96.5% respectively), a parasite of insects, was recovered at a prevalence of 7% (n = 4). Similarly, Bodonidae sp. (detected in both the SILVA and NCBI databases; >99%), an ectoparasite of fish, was recovered at a prevalence of 4% (n = 2). The Baylisascaris sp. and Taenia sp. detected using traditional methods were not detected by 18S rRNA high-throughput sequencing. When using 18S rRNA sequencing, a greater diversity of parasites was recovered from faecal samples (n = 8) as opposed to small intestine samples (n = 2). However, a greater number of Ascaris sp. detections were recorded in small intestine samples (n = 6) compared to faecal samples (n = 1). Only Ascaris sp. and Bodonidae sp. were detected in both a faecal and a small intestine sample.

3.2. Parasitism and host metadata

No association was found between B. devosi abundance and wolverine sex, age class, body length, carcass mass or longitude, but a significant decrease in latitude (slope = -0.68; R² = 0.17; P = 0.004). Specifically, a total of 52 B. devosi nematodes (mean: 5.8) were recovered at 61°N, compared to a total of 5 (mean: 1.6) at 69°N, a mean decrease of 0.5 per degree North. The geographic region of detection for B. devosi recorded here extends the known distribution recorded from previous research and demonstrates the presence of this species within the Arctic tundra (Fig. 2a). Due to the number of individuals infected with Taenia spp. (22%, n = 12), it was not possible to compare abundance of this species alone with any host metadata; therefore both parasites were examined together (i.e. total parasite abundance). However, T. twitchelli is present in wolverines in a region where it was previously unrecorded, and also demonstrates the presence of this species within the Arctic tundra (Fig. 2b).

No association was found between total parasite abundance (i.e. abundance of both B. devosi and Taenia spp.) and wolverine sex, age class, body length, carcass mass, longitude or latitude (P > 0.05). Similarly, no association was found between the presence of a coinfec­tion (0, 1 or ≥2 parasite species present) and sex, age class, body length, carcass mass, longitude or latitude (P > 0.05).

4. Discussion

Within this study, we combined adult helminth recovery (where taxonomy was confirmed by molecular identification using the COX1 mtDNA locus) and 18S rRNA high-throughput sequencing of DNA extracted from faecal and small intestinal samples to provide comprehensive insight into the gastrointestinal parasite community of wolverines. Two parasite species were detected by adult helminth recovery followed by molecular identification, Baylisascaris devosi and Taenia twitchelli, both of which have been recovered in wolverines previously, although the latest published study to do so is over 40 years old (Addison and Boles, 1978). Sequences for B. devosi from morphologically confirmed specimens from Canadian wolverines are, however, present in Genbank (Accession numbers KM216978-985). In addition, 18S rRNA targeted high-throughput sequencing detected threefold the diversity of parasite species (n = 6) compared to gross adult parasite recovery (n = 2), suggesting that combined molecular and adult parasite recovery represents an important tool for characterising the parasite community of a given host. Comparative studies in other species, including wild rats (Rattus norvegicus and R. rattus), found 18S rRNA targeted high-throughput sequencing to be at least as sensitive as traditional count methods (Hino et al., 2016; Tanaka et al., 2014), and detects a broad diversity of intestinal eukaryotes in long-tailed macaques (Macaca fascicularis) and spotted hyenas (Crocuta crocuta) (Heitlinger et al., 2017; Wilcox and Hollocher, 2018).

Although the methods used within this study offer an insight into the diversity of parasites in wolverines, it is important to note that the results may not reflect the total diversity, especially since we did not search for parasites in extra gastrointestinal locations that may be shed in faeces. All sequencing primer sets have some level of bias and the small amount of sample from which DNA is extracted for PCR may simply not contain eggs or DNA of all parasites present (Elbrecht and Leese, 2015; Pawluczyn et al., 2015; Pompanon et al., 2012). This may explain why the 18S rRNA targeted high-throughput sequencing approach did not detect Taenia spp.; as well, taenid egg shedding is sporadic, often shed in segments, and it is notoriously difficult to extract DNA from thick-walled taenid eggs (Hidalgo et al., 2018). The same is true of Baylisascaris spp.; Baylisascaris eggs have thick walls, again making it difficult to liberate the DNA (Dangoudoubyiam et al., 2009; Testini et al., 2011). Taxonomic resolution from metabarcoding is limited by the availability of data in reference databases, which is often lacking for wildlife parasites. For example, unless the Ascaris sp. detected was a misidentification of B. devosi (which is possible considering that the 18S gene is highly conserved), the molecular methods used within this study failed to detect the species recovered by traditional methods. Additionally, because the 18S gene is highly conserved, it is important to interpret the metabarcoding results with caution. We report parasite taxonomy at genus level, as lower taxonomic classification is uncertain, but even so there are genera that may be misidentifications. For example, we detected a metastrongylid lungworm that could be Angiostrongylus gubernaculatus or Aelurostrongylus prihadii, both previously identified in mustelids in North America (Anderson, 1963; Dougherty, 1946; Faulkner et al., 2001), or a closely related genus not represented in the reference databases we used. Further, it is important to note that the parasites detected here through 18S rRNA high-throughput sequencing may not be parasites of wolverines, but instead may be a parasite of an infected prey item (Sheppard et al., 2005; reviewed in Pompanon et al., 2012).

The molecular methods used here allow us to comprehensively characterise the broader parasite diversity in wolverines. However, due to the lower prevalence of parasites detected using molecular methods (22% total; no more than 13% prevalence for any given parasite) and because of the added benefit of abundance data associated with traditional methods, only the data obtained from traditional techniques was used when running our models. As reported previously (Addison and Boles, 1978), adult helminths Baylisascaris devosi and Taenia spp., including T. twitchelli dominated the gastrointestinal tract helminth fauna of wolverines, present in 72% and 22% of wolverines in the current study, respectively, compared to 74% and 11%, respectively, in Addison and Boles (1978). Compared to previous studies, we found lower prevalence (2%, n = 1) of Sarcocystis (80%) and Trichinella (88%) (Dubey et al., 2010; Reichard et al., 2008b), although the last was based on larval recovery from muscle rather than intestinal based methods (Reichard et al., 2008b). It is likely that the DNA we detected for Trichinella is either from larvae ingested from a prey item, or from a transient adult nematode. The high parasite prevalence detected in our wolverine samples using count methods may reflect the fact that wolverines travel huge distances across a heterogeneous geographic range, from boreal forests to the Arctic tundra (Coppel and Arel et al., 2010; Dangon et al., 2010), which may lead to high exposure to parasites. It is more likely, however, that diet drives the high parasite prevalence detected. Wolverines consume an intensely varied diet of live prey and carcasses (LoRoith et al., 2007; Krokela et al., 2013), which may lead to elevated infection rates from trophically transferred parasites, such as B. devosi (transmitted directly and through paratenic hosts) and T. twitchelli (transmitted through consumption of intermediate hosts, including ground squirrels (family: Sciuridae), lemmings (family: Cricetidae), etc.).
voles (family: Cricetidae), muskrats (*Ondatra zibethicus*), and porcupine (*Erethizon dorsatum*) (Rausch, 1959).

In total, 10 species of *Baylisascaris* exist worldwide, most of which utilise carnivorous mammals as definitive hosts, with a smaller prey host serving as a paratenic host (Sapp et al., 2017). Some species of *Baylisascaris* incur detrimental health effects on their paratenic hosts; the raccoon roundworm, *B. procyonis*, for example, causes severe or even fatal neurological disease in humans and wildlife, yet little or no clinical disease in raccoon definitive hosts (Sapp et al., 2017). The effect that *B. devosi* infection has on wolverine health remains unknown; however, as they serve as definitive hosts, it is likely to be minimal. The common occurrence of *Baylisascaris* in species of the lower Arctic is attributed to the parasite’s ability to persist in the external environment. It would appear, however, there is perhaps a northern limit of *B. devosi*, indicative from our finding that *B. devosi* abundance decreases with latitude—an important finding considering that increasing temperatures within the Arctic are expected to contribute to the northern expansion of parasites into regions that were previously inhospitable (Brooks and Hoberg, 2007; Kuta et al., 2009; McLoughlin, 2011). This finding resembles what is seen in some ascarid nematodes, such as *Toxocara canis* which does poorly above 60°N (reviewed by Jenkins et al., 2011), but not others, such as *Toxascaris leonina* which is found all the way up to the high Arctic (Andreassen et al., 2017; Kapel and Nansen, 1996). Lindensfors et al. (2007) found latitude to be a primary predictor of parasite species richness in carnivores. *Taenia* spp. have a broad host range in mammalian definitive hosts that occupy northern territories, including brown bears (*Ursus arctos*), wolves (*Canis lupus*), reindeer (*Rangifer tarandus*) and arctic foxes (*Vulpes lagopus*) (Kapel and Nansen, 1996; Lavikainen et al., 2011). The prevalence of *Taenia* spp. in northern environments is again likely owed to the ability of *Taenia* eggs and gravid proglottids to survive for months in the external environment (Ilse et al., 1990), and the ability to transmit between predator-prey cycles. A likely intermediate host for *T. twichellii* consumed by wolverine in Nunavut include ground squirrels, lemmings, voles, muskrats, and porcupine (*Erethizon dorsatum*; Rausch 1954; Dalerner et al., 2009; Kukka and Jung 2015).

Our findings showed that the abundance of *B. devosi*, total parasite abundance of both *Baylisascaris* and *Taenia*, and co-infection of *Baylisascaris* and *Taenia* (i.e. 0, 1 or ≥2 parasite species present) did not differ with sex or age class, a finding that mirrors what is seen with *Sarcocystis* and *T. gondii* infections in wolverines (Dubey et al., 2010; Reichard et al., 2008a). The lack of sex bias, however, challenges what might be expected, as male wolverines occupy a larger home range compared to females (Pasitschniak-Arts and Lariviére, 2008a) which may increase their exposure to parasites. Geographical range size is also considered an important determinant of parasite infection in various other carnivores (Lindensfors et al., 2007). Additionally, male wolverines are larger in both size and mass compared to females (Awan and Szor, 2012; Banci, 1994); larger-bodied organisms require a greater resource intake, potentially increasing exposure to trophically transmitted parasites (reviewed in Morand and Poulin 1998). Alternatively, the increased parasite abundance typically found in males compared to females may be attributable to immunological differences that exist between sexes, which may in turn influence the susceptibility of male hosts (reviewed in Klein 2004).

Wolverines are a culturally important species to northern communities and, as such, it is important to address parasite species that are of concern. As such, it is unlikely that the parasites detected within this paper are of risk to trappers and hunters handling wolverine carcasses. *Trichinella* spp. are zoonotic, but it is important to note that the *Trichinella* spp. detected within our study may be from an ingested prey item, rather than being a true parasite of wolverines, and so may not pose a risk.

Monitoring wildlife plays an important role in identifying changes such that actions can be taken to mitigate or minimize pressure. Here we have filled a knowledge gap in parasite community data and have shown, through the use of combined adult parasite recovery and molecular methods, that the parasite diversity of wolverines is greater than previously observed. To this end, we recognise the value of molecular methods to aid adult parasite recovery, especially in remote species. We encourage more work be done to improve the advancement of molecular screening methods for parasites, including broader databases with sequences from morphologically confirmed specimens, and interpretation of findings in light of the best available understanding of parasite life cycles and known host and geographic distributions.

**Ethical statement**

The authors declare that they have no conflicts of interest. Wolverines are traditionally harvested by Inuit in Nunavut; a subsample of the harvest was collected with the return of carcasses to the Government of Nunavut offices.

**Declaration of competing interest**

None.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at [https://doi.org/10.1016/j.ijppaw.2020.10.004](https://doi.org/10.1016/j.ijppaw.2020.10.004).

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