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An iridovirus from the Antarctic seaspider *Pentanymphon antarcticum* (Pycnogonida)

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Abstract: The Antarctic seaspider *Pentanymphon antarcticum* is a benthic species in the Southern Ocean, but little is known about its pathogen profile. In this study, we provide a draft genome for a new iridovirus species that has been identified using metagenomic techniques. The draft genome totals 157 260 bp and encodes 188 protein-coding genes. The virus shows greatest protein similarity to a 'carnivorous sponge-associated iridovirus' from a deep-sea sponge host. This study represents the first discovery of a pycnogonid iridovirus and the first iridovirus from the Antarctic region.

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Seaspiders (Chelicerata: Pycnogonida) are a diverse group of marine invertebrates that inhabit benthic marine ecosystems throughout the world (Arnaud & Bamber 1987). Up to 20% of the ~1400 described pycnogonid species (Bamber *et al.* 2023) are found in the Southern Ocean, and ~270 species are endemic (Munilla & Soler-Membrives 2009). Seaspiders consume sponges, cnidarians, molluscs, echinoderms and polychaetes, as well as biofilms and algae (Dietz *et al.* 2018). Several genera have larval forms, which are parasitic (employing endo- and ecto-parasitism) upon hydroids and molluscs (Dietz *et al.* 2018). To date, few parasitic associations have been made with the Pycnogonida, although they have been recorded hosting leeches as well as epifauna (hydroids, bryozoans, arthropods, forams and sponges; Maxwell *et al.* 2022). No viruses are known to occur in these animals.

We present a novel iridovirus (*Iridoviridae*), a large family of dsDNA viruses typically packaged in an icosahedral capsid within a lipid membrane (Chinchar *et al.* 2017). The *Iridoviridae* sit within the nucleo-cytoplasmic large DNA virus (NCLDV) group (*Varidnaviria* (realm), *Bamfordvirae* (kingdom), *Nucleocytoviricota* (phylum), *Megaviricetes* (class) and *Pimascovirales* (order)). Two subfamilies (*Alphairidovirinae* and *Betairidovirinae*) contain multiple genera (seven in total) housing 22 viral species accepted by the International Committee for the Taxonomy of Viruses (ICTV; Chinchar *et al.* 2017). Iridovirus hosts are found throughout the animal kingdom (reptiles, fish, amphibians, crustaceans, insects and sponges). Our new discovery of an iridovirus from a pycnogonid increases their known host range to seaspiders. The region of this discovery increases their known geography to Antarctica.

Pentanymphon antarcticum Hodgson, 1904, a Southern Ocean endemic pycnogonid, was collected from a depth of 870 m using an Agassiz trawl in the Prince Gustav Channel, east of the Antarctic Peninsula (-63.80603, -58.06523) in March 2018 (research cruise JR17003a, RRS *James Clark Ross*). Specimens were placed in pre-cooled 96% ethanol and stored at -20°C, before being stored at the British Antarctic Survey (Cambridge) at ambient temperature. Specimens were identified using morphological characters and confirmed via the metagenomic data collected herein, which resulted in a comparable mitochondrial 16S (GenBank accession: OQ748071).

DNA extraction was conducted separately for 10 individuals using a Wizard DNA extraction kit (Promega). A total of 10 µl (50–100 ng/µl) of the resulting DNA template was pooled into a single 100 µl volume. The pooled sample was prepared into a metagenomic library using an Illumina TruSeq DNA PCR-Free library preparation kit. The library was quality-screened using a bioanalyzer, quantified using a QuantiFluor fluorimeter and denatured using NaOH

Table I. Reagents and concentrations/volumes necessary for the use of the seaspider iridovirus diagnostic. The polymerase chain reaction experiments were conducted in 50 µl total reaction volumes.

Reagents	Concentration (volume added) sample^{-1} (50 µl solution)
Water	35.75 µl
Flexi buffer	5X (10 µl)
$MgCl_2$	1 mMol (2.5 µl)
dNTP mix	2.5 mMol (0.5 µl)
SSIr-F	100 nMol (0.5 µl)
SSIr-R	100 nMol (0.5 µl)
Taq polymerase	0.25 µl

before being diluted to 10 pmol in Illumina HT1 hybridization buffer for paired-end sequencing on an Illumina NovaSeq. The resulting raw read data were trimmed using *Trimmomatic* v.0.36 (Bolger *et al.* 2014) to result in paired files and unpaired files (BioProject: PRJNA954604). The trimmed data were assembled using *SPAdes* v.3.15.3 (Bankevich *et al.* 2012; N50: 2551, N75: 1462, L50: 58 485, L75: 123 220). The contiguous sequences were screened for viruses using *BlastX* (NCBI Viral RefSeq, February 2022). This highlighted 57 contiguous sequences (contigs) that encoded one or more proteins that were *Iridoviridae*-like. These contigs were annotated in *GeneMarkS* (Besemer *et al.* 2001) and vetted using *BlastP* to remove non-viral sequences. One contig was used to develop a diagnostic polymerase chain reaction (PCR) so that the original sample could be identified and sequenced (following the same process above) alone instead of in a pool. The diagnostic PCR (Table I) targeted gene 31 (Supplemental Table 1). Two primers, SSIr-F (5'-CTGTTTTTCAAAAT GGAAAAGTGT-3') and SSIr-R (5'-CAGACTTAAT CCTTACGCATC-3'), result in an 891 bp amplicon in positive cases under the following thermal conditions: 94°C (5 min); 30 cycles (94°C (1 min); 52°C (1 min); 72°C (1 min)); 72°C (7 min). The prevalence was 1/10 (10%).

After re-sequencing, the total next-generation sequencing data were reassembled as above, and the contigs were screened as above. The final contig list included 18 iridovirus segments (OQ791181–OQ791198). The sum of the contig lengths totalled 157 260 bp and encoded 188 protein-coding genes, as assessed via *GeneMarkS* annotation (Fig. 1a & Supplemental Table 1; Besemer *et al.* 2001). *BlastP* determined that the new iridovirus was most similar overall to a 'carnivorous sponge-associated iridovirus' (csaIV; ON887238; Supplemental Table 1; Canuti *et al.* 2022).

A concatenated phylogeny of 'Group I' iridoviruses determined that the new isolate clades with csaIV and 'Cherax quadricarinatus iridovirus' (CqIV; Fig. 1b; Li *et al.* 2017). Within this group, we see three main environmental clusters: an 'aquatic' cluster with the marine seaspider iridovirus (Pycnogonida host), deep-ocean csaIV (Porifera host) and freshwater CqIV (crustacean host); a 'semi-aquatic' group containing semi-aquatic insect iridoviruses (Diptera hosts); and a terrestrial group with an 'Armadillidium vulgare iridescent virus' (crustacean host) and several terrestrial insect iridoviruses (Orthoptera hosts; all 100% bootstrap confidence). We compared the DNA polymerase protein sequences from all iridoviruses, resulting in a similar topology (Fig. 1c,d). Overall, the environment appears to be the greatest evolutionary influence in the diversity of the iridoviruses we know of being in Group I.

Despite the incomplete genome, we consider the virus not to be endogenous as the diagnostic PCR did not provide a positive result for all 10 animals. The 18 contigs show little protein similarity to groups outside of the *Iridoviridae*. The new iridovirus was present at a prevalence of 10% in our dataset, but the true prevalence and impact of the virus on Southern Ocean ecology remain unknown and require further sampling and diagnostic application. The ecological impacts of csaIV and CqIV are also little known. csaIV originates from the deep-ocean Atlantic species *Chondrocladia grandis* and *Cladorhiza oxeata*. Canuti *et al.* (2022) hypothesized that the virus may infect the sponge's prey, as it closely resembled a member of the Decapodiridovirus. CqIV is from red claw crayfish in Fujian, China (Xu *et al.* 2016). Xu *et al.* (2016) confirmed the presence of virions, and in a follow-up study in 2017 (Li *et al.* 2017), the genome of the virus was sequenced (165 695 bp). This crayfish virus poses a significant risk to crayfish aquaculture.

In conclusion, we present the first genomic data for a pycnogonid iridovirus and the first iridovirus from the Antarctic region. The *Iridoviridae* can drive host mortality in a variety of species globally (Williams & Barbosa-Solomieu 2005), and this new knowledge highlights the vital importance of exploring viral diversity in the Southern Ocean around Antarctica (Breitbart *et al.* 2007). As our seas warm and our climate changes, we must have a strong baseline understanding of both the marine Antarctic ecosystem and Antarctic disease ecology to support our ongoing exploration of the area. Through this discovery, we hope to highlight the potential for virological discovery in Antarctic fauna.

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Competing interests

The authors declare none.

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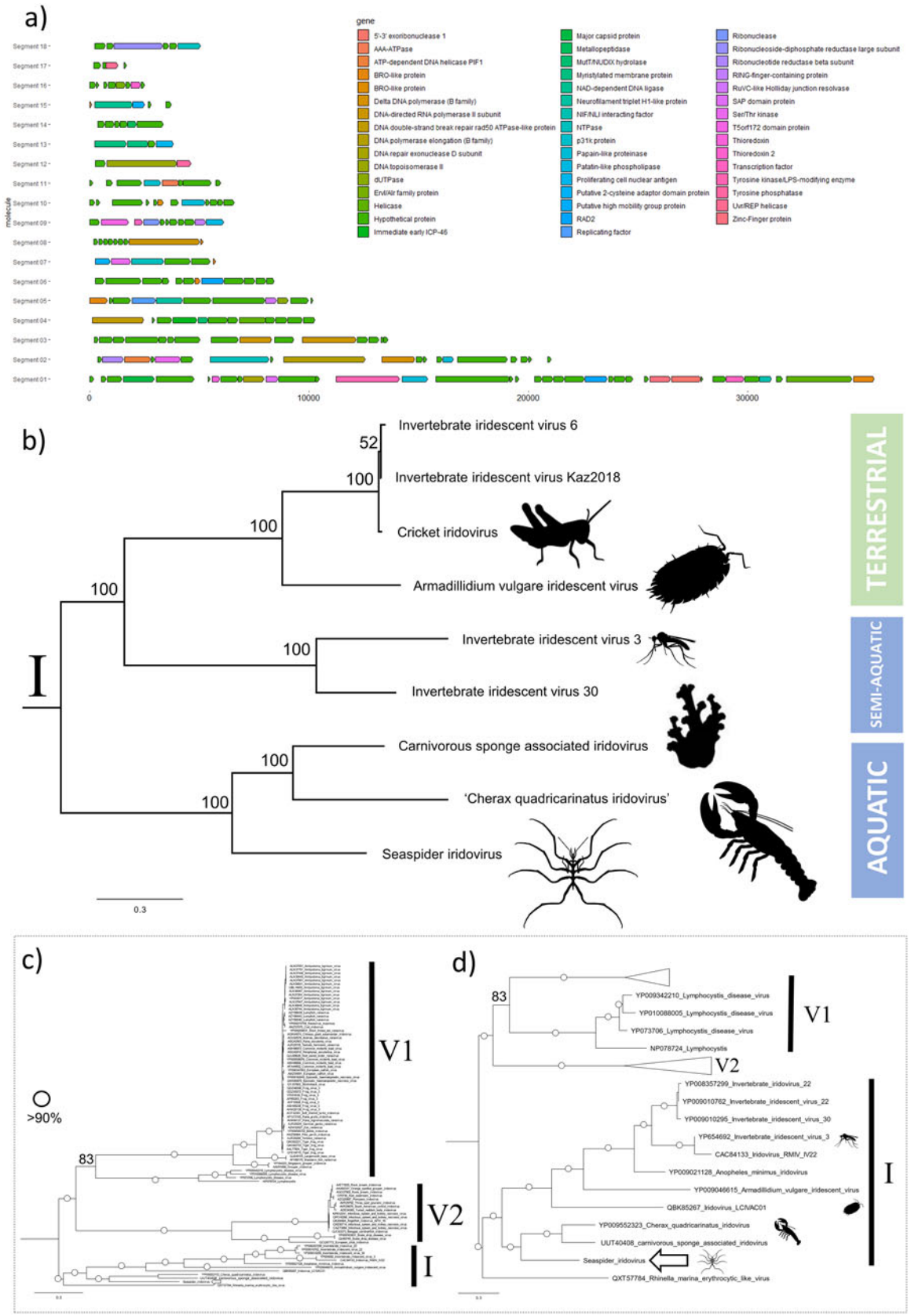

Figure 1. Phylogeny and draft genome architecture for the new iridovirus from the seaspider *Pentanymphon antarcticum*. **a.** The coding regions present on the 18 iridovirus genome segments. **b.** A concatenated phylogeny built using *OrthoFinder* to determine phylogenomic topology and the position of the new iridovirus. The 'seaspider iridovirus' is a strongly supported member of the aquatic cluster. **c.** A maximum-likelihood DNA polymerase phylogeny from a range of iridoviruses, with both complete and partial genomes, un-collapsed. **d.** The same DNA polymerase tree with a greater focus on cluster I. The arrow indicates the presence of the new seaspider-infecting isolate. Larger images of **c.** and **d.** are available as Supplemental Figs 1 & 2.

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Author contributions

JMM, HJG and LA collected the seaspider specimens. JB, ALB and LN conducted the metagenomics, phylogenetics and PCR design. JB and BF developed the discussion around iridoviruses. All authors contributed to the manuscript text.

Supplemental material

A supplemental section including two figures and one table is included in the online version of this publication.

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