Genomic characterization of two novel pathogenic avipoxviruses isolated from pacific shearwaters (Ardenna spp.)

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Abstract

Background: Over the past 20 years, many marine seabird populations have been gradually declining and the factors driving this ongoing deterioration are not always well understood. Avipoxvirus infections have been found in a wide range of bird species worldwide, however, very little is known about the disease ecology of avian poxviruses in seabirds. Here we present two novel avipoxviruses from pacific shearwaters (Ardenna spp), one from a Flesh-footed Shearwater (A. carneipes) (SWPV-1) and the other from a Wedge-tailed Shearwater (A. pacificus) (SWPV-2).

Results: Epidermal pox lesions, liver, and blood samples were examined from A. carneipes and A. pacificus of breeding colonies in eastern Australia. After histopathological confirmation of the disease, PCR screening was conducted for avipoxvirus, circovirus, reticuloendotheliosis virus, and fungal agents. Two samples that were PCR positive for poxvirus were further assessed by next generation sequencing, which yielded complete Shearwaterpox virus (SWPV) genomes from A. pacificus and A. carneipes, both showing the highest degree of similarity with Canarypox virus (98% and 67%, respectively). The novel SWPV-1 complete genome from A. carneipes is missing 43 genes compared to CNPV and contains 4 predicted genes which are not found in any other poxvirus, whilst, SWPV-2 complete genome was deemed to be missing 18 genes compared to CNPV and a further 15 genes significantly fragmented as to probably cause them to be non-functional.

Conclusion: These are the first avipoxvirus complete genome sequences that infect marine seabirds. In the comparison of SWPV-1 and SWPV-2 to existing avipoxvirus sequences, our results indicate that the SWPV complete genome from A. carneipes (SWPV-1) described here is not closely related to any other avipoxvirus genome isolated from avian or other natural host species, and that it likely should be considered a separate species.

Keywords: Avipoxvirus, Poxvirus, Next generation sequencing, dermatitis, Ardenna, Shearwater

Background

The Avipoxvirus genus includes a divergent group of viruses that cause diseases in more than 278 species of wild and domestic birds in terrestrial and marine environments worldwide [1, 2]. Relatively little is known about the origins, worldwide host distribution and genetic diversity of avipoxviruses [3]. In affected birds, avipoxviruses typically cause proliferative ‘wart-like’ growths that are most commonly restricted to the eyes, beak or unfeathered skin of the body (so-called ‘dry’ pox), but infections can also develop in the upper alimentary and respiratory tracts (‘wet’ or ‘diptheritic’ pox) [2]. The incubation period and magnitude of avipoxvirus infection is variable, and is rarely fatal although secondary bacterial or fungal infections are common and cause increased mortality [2]. Such conditions in naive populations can reach a much higher prevalence with substantial fatality [4, 5].

Avipoxviruses belong to the subfamily Chordopoxvirinae (ChPV) of the Poxviridae family, which are relatively large double-stranded DNA (dsDNA) viruses that replicate in the cytoplasm of infected cells [6]. Although poxviruses have evolved to infect a wide range of host species, to date
only six avipoxvirus genomes have been published; a pathogenic American strain of *Fowlpox virus* (FPVUS) [7], an attenuated European strain of *Fowlpox virus* (FP9) [8], a virulent *Canarypox virus* (CNPV) [9], a pathogenic South African strain of *Pigeonpox virus* (FeP2), a *Penguinpox virus* (PEPV) [3], and a pathogenic Hungarian strain of *Turkeypox virus* (TKPV) [10]. Although these genome sequences demonstrate that avipoxviruses have diverged considerably from the other chordopoxviruses (ChPVs), approximately 80 genes have been found to be conserved amongst all ChPVs and to comprise the minimum essential poxvirus genome [11]. These genes tend to be present in the central core of the linear genome with the remainder presumed to be immunomodulatory and host specific genes located towards the terminal regions of the genome [3]. With the exception of TKPV (188 kb), avipoxvirus genomes (266–360 kb) tend to be bigger than those of other ChPVs due in part to multiple families of genes.

Over the past two decades, the status of the world’s bird populations have deteriorated with seabirds declining faster than any other group of birds [12]. On Lord Howe Island in eastern Australia, the Flesh-footed Shearwater *Ardenna carneipes* has been declining for many years and is therefore listed as Vulnerable in the state of New South Wales [13]. The ongoing threat of plastic pollution, and toxicity from the elevated concentration of trace elements such as mercury could be confounding drivers of this declining species [14]. Infectious diseases, including those caused by avipoxviruses, have also been identified as an important risk factor in the conservation of small and endangered populations, particularly in island species [15–18]. The impact of the introduction of avipoxviruses has been severe for the avifauna of various archipelagos [19]. The emergence of distinctive avipoxvirus with a high prevalence (88%) in Hawaiian Laysan Albatross (*Phoebastria immutabilis*) enabled one of the first detailed studies of the epidemiology and population-level impact of the disease in the seabirds [20]. However, relatively little is known about the general prevalence or effects of poxviruses in seabird species, including for shearwaters (*Ardenna* or *Puffinus* spp.). Therefore, the aim of the present study was to identify and characterize pathogens associated with clinical disease in breeding colonies of Flesh-footed Shearwater and Wedge-tailed Shearwater sourced from Lord Howe Island in 2015.

**Results**

**Identification of fungal pathogens**

In the sample from *A. pacificus* (15–1526, and 15–1527), there were multifocal areas of inflammation and exudation associated with serocellular surface crust that contained abundant branching fungal hyphae and aggregations of bacteria (Fig. 1c). A PCR screening was conducted for the presence of fungal pathogen using the ITS region to amplify a segment of approximately 550 bp. Two samples (out of 6) were positive for fungal pathogens, and direct Sanger sequencing of the purified gel bands resulted in a 550 bp sequence after trimming off primer sequences (data not shown). These sequences were further verified using high-throughput NGS, and generated con tigs of 3,430 bp (15–1526; GenBank accession KX857213) and 5,188 bp (15–1527; GenBank accession KX857212). A BLASTn search for the bird coinfected with fungal pathogen (15–1526) returned multiple hits to various fungal species, all with very similar scores; however, the best match (88%) was to the *Phaeosphaeria nodorum* (GenBank Accession EU053989.1, and value ≤ e-153), a major necrotrophic fungal pathogen of wheat [21]. Similar search model for the fungal pathogen of bird 15–1527, demonstrated a highest hit (96%) to the *Metarhizium anisopliae* var. *anisopliae* (GenBank Accession AY884128.1, and value ≤ e-173), an entomopathogenic fungus [22].

**Identification of virus**

Samples from six shearwater chicks of two different species, *A. carneipes* and *A. pacificus*, with evidence of gross well circumscribed, popular, crusting lesions across the feather skins (Fig. 1a), were biopsied, with blood and liver samples also collected. Histological examinations of the skin demonstrated focal to diffuse full thickness necrosis of the epidermis and a thick serocellular surface crust. A marked heterophilic rich inflammatory cellular response and exudation was present alongside abundant macrophages and perifollicular fibroplasia. In some areas there was focal proliferation of the adjacent epidermis associated with ballooning degeneration of keratinocytes with eosinophilic intracytoplasmic inclusions (Fig. 1b). A PCR screening was conducted for the presence of poxvirus, circovirus and reticuloendotheliosis virus, which are likely to cause this type of skin lesions. Two birds (*A. pacificus* 15–1526 and *A. carneipes* 15–1528) were positive by PCR targeting the 4b gene that encodes a core protein of ChPV, however, there were no evidence of either circovirus or reticuloendotheliosis for any of the samples used in this study. Direct Sanger sequencing of the purified gel bands resulted in a 578 bp sequence after trimming off primer sequences (data not shown). A BLASTn search with these sequences returned multiple hits to the 4b core gene from a variety of poxviruses, all with very similar scores; however, the best match was to the *Canarypox virus* 4b core protein gene ((bird 15–1526; similarity with AY318871 was 99% and identity score ≤ e-162), and bird 15–1528; similarity with LK021654 was 99% and identity score ≤ e-157)).
Genome sequence and annotation of viruses

The Shearwaterpox virus complete genomes were assembled using CLC Genomics workbench 9.5.2 under La Trobe University Genomics Platform. The assembled complete genomes of SWPV-1 and −2 were 326,929 and 351,108 nt, respectively. The SWPV-1 and −2 complete genomes were annotated as described in the methods using CNPV as a reference genome (Additional file 1: Table S1 and Additional file 2: Table S2). We took a conservative approach to the annotation in order to minimize the inclusion of ORFs that were unlikely to represent functional genes. Table 1 lists the 310 and 312 genes annotated for SWPV-1 and −2, respectively. For the most part, these two new complete genomes are colinear to CNPV although there are a number of rearrangements of blocks of 1–6 genes in addition to insertions and deletions with respect to CNPV (Table 1). Comparison of the predicted proteins of SWPV-2 to orthologs in CNPV reveal the vast majority are >98% identical (aa), with more than 80 being completely conserved. In contrast, the orthologs of SWPV-1 only have an average aa identity of 67% to CNPV. However, with the lower average identity, greater genetic distance, comes a much greater range of variation in the level of identity and a significant number of predicted proteins are 80 – 90% identical (aa) to CNPV orthologs.

This difference in similarity between the new viruses and CNPV is easily visualized in complete genome dot-plots (Fig. 2a and b). Significantly more indels are present in the SWPV-1 vs CNPV dotplot (Fig. 2a). However, when the phylogenetic relationships of these viruses were examined together with the other available complete genomes, SWPV-1 was still part of the CNPV clade (Fig. 3a). From this alignment, CNPV is 99.2%, 78.7%, 69.4%, 69.5%, 68.8% and 66.5% identical (nt) to SWPV-2, SWPV-1, FeP2, PEPV, FWPV and TKPV, respectively. A greater selection of viruses was included in the phylogenetic tree by using other fragments of incompletely sequenced avipoxvirus genomes. For example, Vultur gryphus poxvirus (VGPV), Flamingo poxvirus (FGPV) and Hawaiian goose poxvirus (HGPV) are all more similar to SWPV-2 and CNPV than SWPV-1 (Fig. 3b), this confirms that other poxviruses are as closely related to CNPV as SWPV-2. By also building phylogenetic trees with partial nucleotide sequences from the p4b gene (Fig. 4) and DNA polymerase gene (Fig. 5), we discovered that several other viruses are within the SWPV-1, SWPV-2 and CNPV clade. This includes a poxvirus isolated from Houbara Bustards (Chlamydotis undulata) in captive-breeding programs in Morocco [23], but named CNPV-morocco, and avipoxviruses isolated from American crow (Corvus...
| SWPV1 synteny | SWPV2 synteny | CNPV synteny | CNPV BLAST hits | SWPV1 % identity | SWPV2 % identity | SWPV1 AA size | SWPV2 AA size | Reference AA size | notes |
|---------------|---------------|--------------|-----------------|------------------|------------------|---------------|---------------|------------------|-------|
| SWPV2-001     | CNPV002       | CNPV001      | hypothetical protein | 92.941           | 171              | 171           |                |                  |       |
| SWPV1-001     | CNPV003       | CNPV002      | hypothetical protein | 32.044           | 181              | 208           | 204           |                  |       |
| SWPV1-002     | CNPV004       | CNPV003      | C-type lectin-like protein | 85.99           | 468              | 514           |                |                  |       |
| SWPV1-003     | CNPV005       | CNPV004      | ankyrin repeat protein | 99.55           | 220              | 222           | 222           |                  |       |
| SWPV1-004     | CNPV006       | CNPV005      | conserved hypothetical protein | 87.387          | 134              | 182           |                | SWPV2: C-terminus fragment, not likely translated |       |
| SWPV1-005     | CNPV007       | CNPV006      | hypothetical protein | 88.71            |                  |               |                |                  |       |
| SWPV1-006     | CNPV008       | CNPV007      | ankyrin repeat protein | 50              | 174              | 169           | 169           |                  |       |
| SWPV1-007     | CNPV009       | CNPV008      | C-type lectin-like protein | 99.564          | 688              | 688           |                |                  |       |
| SWPV1-008     | CNPV010       | CNPV009      | ankyrin repeat protein | 99.147          | 586              | 586           |                |                  |       |
| SWPV1-009     | CNPV011       | CNPV010      | ankyrin repeat protein | 100             | 189              | 189           |                |                  |       |
| SWPV1-010     | CNPV012       | CNPV011      | ankyrin repeat protein | 98.81           | 168              | 168           |                |                  |       |
| SWPV1-011     | CNPV013       | CNPV012      | ankyrin repeat protein | 99.184          | 490              | 490           |                |                  |       |
| SWPV1-012     | CNPV014       | CNPV013      | ankyrin repeat protein | 97.538          | 528              | 528           |                |                  |       |
| SWPV1-013     | CNPV015       | CNPV014      | ankyrin repeat protein | 35.556          | 138              | 134           |                |                  |       |
| SWPV1-014     | CNPV016       | CNPV015      | ankyrin repeat protein | 58.932          | 487              | 514           |                |                  |       |
| SWPV1-015     | CNPV017       | CNPV016      | ankyrin repeat protein | 52.128          | 117              | 168           | 168           |                  |       |
| SWPV1-016     | CNPV018       | CNPV017      | ankyrin repeat protein | 64.471          | 425              | 479           | 486           |                  |       |
| SWPV1-017     | CNPV019       | CNPV018      | ankyrin repeat protein | 64.61           | 277              | 396           |                |                  |       |
| SWPV1-018     | CNPV020       | CNPV019      | ankyrin repeat protein | 90.805          | 190              | 191           |                |                  |       |
| SWPV1-019     | CNPV021       | CNPV020      | ankyrin repeat protein | 99.083          | 436              | 436           |                |                  |       |
| SWPV1-020     | CNPV022       | CNPV021      | ankyrin repeat protein | 56.311          | 412              | 419           | 419           |                  |       |
| SWPV1-021     | CNPV023       | CNPV022      | ankyrin repeat protein | 31.656          | 483              | 469           |                |                  |       |
| SWPV1-022     | CNPV024       | CNPV023      | ankyrin repeat protein | 62.313          | 528              | 535           | 535           |                  |       |
| SWPV1-023     | CNPV025       | CNPV024      | ankyrin repeat protein | 65.642          | 356              | 358           | 358           |                  |       |
Table 1  Shearwaterpox virus (SWPV) genome annotations and comparative analysis of ORFs relative to CNPV genomes (Continued)

| SWPV1-014 | PEPV260 ankyrin repeat protein | 53.158 | 190 | 192 |
| SWPV1-015 | CNPV011 ankyrin repeat protein | 34 | 530 | 586 |
| SWPV2-019 CNPV023 CNPV023 vaccinia C4L/C10L-like protein | 98.595 | 424 | 427 |
| SWPV2-020 CNPV024 hypothetical protein | 96.629 | 178 | 178 |
| SWPV1-016 SWPV2-021 CNPV025 CNPV025 alpha-SNAP-like protein | 57.491 | 98.667 | 304 | 300 | 300 |
| SWPV1-017 SWPV2-022 CNPV026 CNPV026 ankyrin repeat protein | 54.271 | 98.953 | 397 | 382 | 382 |
| SWPV1-018 SWPV2-023 CNPV027 CNPV027 ankyrin repeat protein | 59.375 | 98.722 | 646 | 626 | 626 |
| SWPV1-019 SWPV2-024 CNPV028 ankyrin repeat protein | 57.618 | 99.164 | 408 | 365 | 362 |
| SWPV1-020 SWPV2-025 CNPV029 CNPV029 C-type lectin-like protein | 50.35 | 99.296 | 142 | 142 | 142 |
| SWPV1-021 SWPV2-026 CNPV030 CNPV030 ankyrin repeat protein | 63.72 | 98.529 | 345 | 340 | 340 |
| SWPV1-022 SWPV2-027 CNPV031 C-type lectin-like protein | 60.331 | 97.479 | 120 | 119 | 119 |
| SWPV1-023 SWPV2-028 CNPV032 CNPV032 Ig-like domain putative IFN-gamma binding protein | 44.048 | 168 | 168 |
| SWPV1-024 SWPV2-028 CNPV032 CNPV032 Ig-like domain putative IFN-gamma binding protein | 51.837 | 92.149 | 242 | 242 | 242 |
| SWPV1-025 SWPV2-029 CNPV033 CNPV033 Ig-like domain protein | 48.095 | 93.496 | 238 | 246 | 246 |
| SWPV2-030 CNPV034 CNPV034 ankyrin repeat protein | 99.848 | 659 | 659 |
| SWPV2-031 CNPV035 C-type lectin-like protein | 94.776 | 133 | 134 |
| SWPV1-026 SWPV2-032 CNPV036 CNPV036 conserved hypothetical protein | 48.235 | 98.947 | 88 | 95 | 95 |
| SWPV1-027 SWPV2-033 CNPV037 CNPV037 conserved hypothetical protein | 63.068 | 99.441 | 178 | 179 | 179 |
| SWPV1-028 SWPV2-034 CNPV038 CNPV038 vaccinia C4L/C10L-like protein | 54.523 | 99.516 | 411 | 413 | 413 |
| SWPV1-029 SWPV2-035 CNPV039 CNPV039 G protein-coupled receptor-like protein | 67.284 | 97.859 | 323 | 327 | 327 |
| SWPV1-030 SWPV2-036 CNPV040 CNPV040 ankyrin repeat protein | 57.36 | 93.401 | 589 | 591 | 591 |
| SWPV1-031 SWPV2-037 CNPV041 CNPV041 ankyrin repeat protein | 66.284 | 98.605 | 432 | 430 | 430 |
| SWPV1-032 SWPV2-038 CNPV042 CNPV042 ankyrin repeat protein | 72.712 | 99.339 | 608 | 605 | 605 |
| SWPV1-033 SWPV2-039 CNPV043 CNPV043 conserved hypothetical protein | 74.627 | 99.005 | 202 | 201 | 201 |
| SWPV1-034 SWPV2-040 CNPV044 CNPV044 ankyrin repeat protein | 67.316 | 99.583 | 470 | 480 | 480 | SWPV2: High SNP Density |

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| SWPV1-035 | SWPV2-041 | CNPV045 | CNPV045 G-protein-coupled receptor-like protein | 65.231 | 100 | 331 | 332 | 332 |
| SWPV1-036 | SWPV2-042 | CNPV046 | CNPV046 ankyrin repeat protein | 68.08 | 98.667 | 452 | 450 | 450 |
| SWPV1-037 | SWPV2-043 | CNPV047 | CNPV047 conserved hypothetical protein | 65.6 | 99.194 | 125 | 124 | 124 |
| SWPV1-038 | SWPV2-044 | CNPV048 | CNPV048 alkaline phosphodiesterase-like protein | 68.238 | 98.502 | 804 | 801 | 801 |
| SWPV1-039 | SWPV2-045 | CNPV049 | CNPV049 hypothetical protein | 72.667 | 100 | 148 | 150 | 150 |
| SWPV1-040 | SWPV2-046 | CNPV050 | CNPV050 ankyrin repeat protein | 67.422 | 98.864 | 352 | 352 | 352 |
| SWPV1-041 | SWPV2-047 | CNPV051 | CNPV051 DNase II-like protein | 63.683 | 96.75 | 398 | 408 | 401 |
| SWPV1-042 | SWPV2-048 | CNPV052 | CNPV052 C-type lectin-like protein | 50 | 100 | 182 | 171 | 171 |
| SWPV1-043 |  |  | FWPV ankyrin repeat protein | 45 | 329 | 406 |
| SWPV1-044 | SWPV2-049 | CNPV053 | CNPV053 conserved hypothetical protein | 68.148 | 100 | 135 | 146 | 146 |
| SWPV1-045 | SWPV2-050 | CNPV054 | CNPV054 conserved hypothetical protein | 62.59 | 99.286 | 141 | 140 | 140 |
| SWPV1-046 | SWPV2-051 | CNPV055 | CNPV055 conserved hypothetical protein | 74.534 | 100 | 162 | 163 | 163 |
| SWPV1-047 | SWPV2-052 | CNPV056 | CNPV056 dUTPase | 80.986 | 98.621 | 155 | 145 | 145 |
| SWPV1-048 | SWPV2-053 | CNPV057 | CNPV057 putative serpin | 63.107 | 99.02 | 301 | 306 | 306 |
| SWPV1-049 | SWPV2-054 | CNPV058 | CNPV058 bcl-2 like protein | 51.744 | 98.857 | 174 | 180 | 175 |
| SWPV1-050 | SWPV2-055 | CNPV059 | CNPV059 putative serpin | 71.302 | 99.704 | 338 | 338 | 338 |
| SWPV1-051 | SWPV2-056 | CNPV060 | CNPV060 conserved hypothetical protein | 46.939 | 95.098 | 236 | 206 | 316 |
| SWPV1-052 | SWPV2-057 | CNPV061 | CNPV061 DNA ligase | 80.995 | 98.761 | 567 | 565 | 565 |
| SWPV1-053 | SWPV2-058 | CNPV062 | CNPV062 putative serpin | 70.94 | 100 | 349 | 350 | 350 |
| SWPV1-054 | SWPV2-059 | CNPV063 | CNPV063 hydroxysteroid dehydrogenase-like protein | 71.348 | 99.441 | 359 | 358 | 358 |
| SWPV1-055 | SWPV2-060 | CNPV064 | CNPV064 TGF-beta-like protein | 56.897 | 98.587 | 272 | 283 | 282 |
| SWPV1-056 | SWPV2-061 | CNPV065 | CNPV065 semaphorin-like protein | 69.735 | 99.485 | 573 | 583 | 583 |
| SWPV1-057 | SWPV2-062 | CNPV066 | CNPV066 hypothetical protein | 37.349 | 98.519 | 139 | 399 | 405 |
| SWPV2-063 | CNPV067 | CNPV067 hypothetical protein | 100 | 57 | 57 |
| SWPV1-058 |  |  | no significant BLAST hits |  |  | 239 |
| SWPV1-059 | SWPV2-064 | CNPV068 | CNPV068 GNS1/SURH4-like protein | 84.825 | 99.611 | 257 | 257 | 257 | \(\text{SWPV1: Possible Unique ORF}\) |
| SWPV1-060 | SWPV2-065 | CNPV069 | CNPV069 late transcription factor VLTF-2 | 87.5 | 100 | 154 | 155 | 155 |
| SWPV1-061 | SWPV2-066 | CNPV070 | CNPV070 putative rifampicin resistance protein, IMV assembly | 88.065 | 100 | 553 | 551 | 551 |
| SWPV1-062 | SWPV2-067 | CNPV071 | CNPV071 mRNA capping enzyme small subunit | 89.273 | 100 | 289 | 289 | 289 |
| SWPV2-068 | CNPV072 | CNPV072 CC chemokine-like protein | 96.262 | 132 | 312 | \(\text{SWPV2: N-terminus fragment}\) |
| SWPV1-063 | SWPV2-069 | CNPV073 | CNPV073 hypothetical protein | 45.263 | 100 | 110 | 109 | 109 |
| SWPV1-064 | SWPV2-070 | CNPV074 | CNPV074 NPH-I, transcription termination factor | 92.756 | 99.685 | 635 | 635 | 635 |
| SWPV1-065 | SWPV2-071 | CNPV075 | CNPV075 mutT motif putative gene expression regulator | 79.295 | 100 | 226 | 228 | 228 |
| SWPV1-066 | SWPV2-072 | CNPV076 | CNPV076 mutT motif | 84.549 | 99.569 | 233 | 232 | 232 |
| SWPV1-067 | CNPV077 | CNPV077 hypothesised | 29.806 | 435 | 586 |
| SWPV1-068 | SWPV2-073 | CNPV078 | CNPV078 RNA polymerase subunit RNApR018 | 82.39 | 100 | 161 | 160 | 160 |
| SWPV2-074 | CNPV079 | CNPV079 Ig-like domain protein | 94.161 | 160 | 160 | 160 | 160 |
| SWPV1-069 | SWPV2-075 | CNPV080 | CNPV080 early transcription factor small subunit VETFS | 96.682 | 100 | 633 | 633 | 633 |
| SWPV2-076 | CNPV081 | CNPV081 Ig-like domain protein | 97.006 | 160 | 160 | 160 | 160 |
| SWPV1-070 | SWPV2-077 | CNPV082 | CNPV082 NTPase, DNA replication | 88.818 | 99.748 | 794 | 794 | 794 |
| SWPV1-071 | SWPV2-078 | CNPV083 | CNPV083 CC chemokine-like protein | 60.352 | 91.855 | 223 | 221 | 221 |
| SWPV1-072 | CNPV084 | CNPV084 uracil DNA glycosylase | 30.994 | 195 | 204 |
| SWPV1-073 | SWPV2-079 | CNPV085 | CNPV085 putative RNA phosphatase | 86.364 | 97.706 | 220 | 218 | 218 |
| SWPV1-074 | SWPV2-080 | CNPV086 | CNPV086 CC chemokine-like protein | 67.895 | 94.312 | 245 | 303 | 403 |
| SWPV1-075 | CNPV087 | CNPV087 conserved hypothetical protein | 39.225 | 398 | 404 |

**Table 1** Shearwaterpox virus (SWPV) genome annotations and comparative analysis of ORFs relative to CNPV genomes (Continued)
| SWPV1-076 | SWPV2-081 | CNPV086 | CNPV086 TIR-like protein | 67.327 | 71.569 | 103 | 112 | 117 |
|-----------|-----------|--------|--------------------------|--------|--------|-----|-----|-----|
| SWPV2-082 | CNPV087   |        | CNPV087 glutathione peroxidase | 98.473 |        | 131 | 198 |     |
| SWPV1-077 | CNPV227   | N1R/p28-like protein | 74.638 | 256 | 359 |
| SWPV1-078 | CNPV088   |        | CNPV088 conserved hypothetical protein | 55.769 | 97 | 104 | 100 | 100 |
| SWPV1-079 | CNPV089   |        | CNPV089 conserved hypothetical protein | 64.935 | 100 | 164 | 159 | 159 |
| SWPV1-080 | CNPV090   |        | CNPV090 conserved hypothetical protein | 62.393 | 100 | 124 | 127 | 127 |
| SWPV1-081 | CNPV091   |        | CNPV091 HT motif protein | 64.634 | 100 | 77 | 83 | 83 |
| SWPV1-082 | CNPV092   |        | CNPV092 conserved hypothetical protein | 64.901 | 97.945 | 140 | 146 | 146 |
| SWPV1-083 | CNPV093   |        | CNPV093 virion protein | 60.37 | 99.625 | 270 | 267 | 267 |
| SWPV1-084 | CNPV094   |        | CNPV094 T10-like protein | 75 | 98.909 | 282 | 275 | 275 |
| SWPV1-085 | CNPV095   |        | CNPV095 conserved hypothetical protein | 71.111 | 100 | 47 | 45 | 45 |
| SWPV1-086 | CNPV096   |        | CNPV096 ubiquitin | 100 | 100 | 77 | 85 | 85 |
| SWPV1-087 | CNPV097   |        | CNPV097 conserved hypothetical protein | 70.031 | 99.705 | 298 | 339 | 339 |
| SWPV1-088 | CNPV098   |        | CNPV098 hypothetical protein | 67.442 | 98.75 | 61 | 80 | 80 |
| SWPV1-089 | CNPV099   |        | CNPV099 beta-NGF-like protein | 62.162 | 97.949 | 186 | 195 | 195 |
| SWPV1-090 | CNPV100   |        | CNPV100 putative interleukin binding protein | 51.176 | 98.225 | 211 | 168 | 169 |
| SWPV2-096 | CNPV101   |        | CNPV101 hypothetical protein | 98.824 |        | 85 | 85 |
| SWPV1-091 | CNPV097   |        | CNPV097 conserved hypothetical protein | 54.167 | 99.048 | 102 | 105 | 105 |
| SWPV1-092 | CNPV103   | N1R/p28-like protein | 54.167 | 99.048 | 102 | 105 | 105 |
| SWPV1-093 | CNPV104   | putative glutaredoxin 2, virion morphogenesis | 86.4 | 99.2 | 125 | 125 | 125 |
| SWPV1-094 | CNPV105   |        | CNPV105 conserved hypothetical protein | 77.35 | 98.718 | 234 | 234 | 234 |
| SWPV1-095 | CNPV106   |        | CNPV106 putative elongation factor | 76.829 | 98.039 | 103 | 102 | 102 |
| SWPV1-096 | CNPV107   |        | CNPV107 hypothetical protein | 64 | 444 | 336 |
| SWPV1-097 | CNPV108   |        | CNPV108 putative metalloprotease, virion morphogenesis | 85.489 | 100 | 633 | 632 | 632 |
| SWPV1-098 | CNPV109   |        | CNPV109 NPH-II, RNA helicase | 86.05 | 99.706 | 681 | 681 | 681 |
| SWPV1-099 | SWPV2-105 | CNPV110 | CNPV110 virion core proteinase | 87.441 | 99.763 | 421 | 422 | 422 |
| SWPV1-100 | SWPV2-106 | CNPV111 | CNPV111 DNA-binding protein | 80.612 | 99.488 | 391 | 391 | 391 |
| SWPV1-101 | SWPV2-107 | CNPV112 | CNPV112 putative IMV membrane protein | 81.481 | 100 | 81 | 81 | 81 |
| SWPV1-102 | SWPV2-108 | CNPV113 | CNPV113 thymidine kinase | 75.978 | 99.441 | 181 | 179 | 179 |
| SWPV1-103 | SWPV2-109 | CNPV114 | CNPV114 HT motif protein | 69.62 | 100 | 79 | 82 | 82 |
| SWPV1-104 | SWPV2-110 | CNPV115 | CNPV115 DNA-binding phosphoprotein | 71.429 | 82.353 | 282 | 289 | 289 |
| SWPV1-120 | SWPV2-115 | CNPV120 | CNPV120 putative IMV redox protein, virus assembly | 83.969 | 100 | 131 | 131 | 131 |
| SWPV1-105 | SWPV2-111 | CNPV116 | CNPV116 unnamed protein product | 73.913 | 98.551 | 66 | 69 | 69 |
| SWPV1-106 | SWPV2-112 | CNPV117 | CNPV117 DNA-binding virion protein | 88.854 | 99.677 | 314 | 310 | 310 |
| SWPV1-107 | SWPV2-113 | CNPV118 | CNPV118 conserved hypothetical protein | 75.762 | 99.387 | 656 | 652 | 653 |
| SWPV1-108 | SWPV2-114 | CNPV119 | CNPV119 virion core protein | 83.969 | 100 | 131 | 131 | 131 |
| SWPV1-109 | SWPV2-115 | CNPV120 | CNPV120 putative IMV redox protein, virus assembly | 80.851 | 100 | 94 | 93 | 93 |
| SWPV1-110 | SWPV2-116 | CNPV121 | CNPV121 DNA polymerase | 89.17 | 99.899 | 988 | 988 | 988 |
| SWPV1-111 | CNPV122 | CNPV122 putative membrane protein | 83.088 | 100 | 273 | 274 |
| SWPV1-112 | SWPV2-117 | CNPV123 | CNPV123 conserved hypothetical protein | 82.312 | 85.336 | 571 | 502 | 571 |
| SWPV1-113 | SWPV2-118 | CNPV124 | CNPV124 variola B22R-like protein | 67 | 98.957 | 1906 | 1916 | 1918 |
| SWPV1-114 | SWPV2-119 | CNPV125 | CNPV125 variola B22R-like protein | 71.669 | 99.66 | 1742 | 1767 | 1767 |
| SWPV1-115 | SWPV2-120 | CNPV126 | CNPV126 variola B22R-like protein | 64.456 | 98.847 | 1902 | 1839 | 1951 |
| SWPV2-121 | | | CNPV126 variola B22R-like protein | 96 | 153 | 1951 | SWPV2: C-terminus fragment, not likely translated |
| SWPV1-116 | SWPV2-122 | CNPV127 | CNPV127 RNA polymerase subunit RPO30 | 96.154 | 100 | 182 | 182 | 182 |
| SWPV1-117 | SWPV2-123 | CNPV128 | CNPV128 conserved hypothetical protein | 77.072 | 99.752 | 742 | 721 | 721 |
| SWPV1-118 | SWPV2-124 | CNPV129 | CNPV129 poly(A) polymerase large subunit PAPL | 83.898 | 99.788 | 472 | 472 | 472 |
| SWPV1-119 | SWPV2-125 | CNPV130 | CNPV130 DNA-binding virion core protein | 76.471 | 100 | 114 | 119 | 119 |
| SWPV1-120 | SWPV2-126 | CNPV131 | CNPV131 conserved hypothetical protein | 64.115 | 99.517 | 212 | 207 | 207 |
| SWPV1-121 | SWPV2-127 | CNPV132 | CNPV132 conserved hypothetical protein | 81.081 | 99.324 | 151 | 148 | 148 |
| SWPV1-122 | SWPV2-128 | CNPV133 | CNPV133 conserved hypothetical protein | 73.737 | 100 | 90 | 99 | 99 |
| SWPV1-123 | SWPV2-129 | CNPV134 | CNPV134 variola B22R-like protein | 65.517 | 99.001 | 1774 | 1801 | 1801 |
| SWPV1-124 | SWPV2-130 | CNPV135 | CNPV135 putative palmitylated EEV envelope lipase | 89.418 | 99.735 | 378 | 378 | 378 |
| SWPV1-125 | SWPV2-131 | CNPV136 | CNPV136 putative EEV maturation protein | 75.602 | 99.68 | 622 | 625 | 625 |
| SWPV1-126 | SWPV2-132 | CNPV137 | CNPV137 conserved hypothetical protein | 62.26 | 98.925 | 467 | 462 | 465 |
| SWPV1-127 | SWPV2-133 | CNPV138 | CNPV138 putative serine/threonine protein kinase, virus assembly | 83.632 | 100 | 445 | 444 | 444 |
| SWPV1-128 | SWPV2-134 | CNPV139 | CNPV139 conserved hypothetical protein | 81.69 | 100 | 213 | 213 | 213 |
| SWPV1-129 | SWPV2-135 | CNPV140 | CNPV140 conserved hypothetical protein | 78.788 | 100 | 65 | 66 | 66 |
| SWPV1-130 | SWPV2-136 | CNPV141 | CNPV141 HAL3-like domain protein | 88.333 | 100 | 182 | 184 | 184 |
| SWPV1-131 | no significant BLAST hits | 28 | 101 | 571 | SWPV1: Possible Unique ORF |
| SWPV1-132 | SWPV2-137 | CNPV142 | CNPV142 N1R/p28-like protein | 48.266 | 98.442 | 314 | 321 | 321 |
| SWPV1-133 | SWPV2-138 | CNPV143 | CNPV143 ankyrin repeat protein | 54.103 | 98.361 | 634 | 671 | 671 |
| SWPV1-134 | SWPV2-139 | CNPV144 | CNPV144 ankyrin repeat protein | 59.011 | 99.281 | 562 | 556 | 556 |
| SWPV1-135 | SWPV2-140 | CNPV145 | CNPV145 conserved hypothetical protein | 75.814 | 100 | 439 | 440 | 440 |
| SWPV1-136 | SWPV2-141 | CNPV146 | CNPV146 RNA polymerase subunit RPO7 | 88.525 | 100 | 66 | 62 | 62 |
| SWPV1-137 | SWPV2-142 | CNPV147 | CNPV147 conserved hypothetical protein | 80.851 | 100 | 188 | 188 | 188 |
| SWPV1-138 | SWPV2-143 | CNPV148 | CNPV148 virion core protein | 86.533 | 100 | 347 | 348 | 348 |
| SWPV2-144 | CNPV149 | CNPV149 putative thioredoxin binding protein | 99.673 | 306 | 306 |
| SWPV2-145 | CNPV150 | CNPV150 ankyrin repeat protein | 85.683 | 351 |
| SWPV2-146 | CNPV151 | CNPV151 ankyrin repeat protein | 99.029 | 412 | 412 |
| SWPV2-147 | CNPV152 | CNPV152 hypothetical protein | 98 | 149 | 187 |
| SWPV2-147 | CNPV153 | CNPV153 Rep-like protein | 99.359 | 312 | 312 |
| SWPV1-139 | CNPV159 | CNPV159 N1R/p28-like protein | 78.488 | 333 | 337 |
| SWPV1-140 | FWPV121 | FWPV121 CC chemokine-like protein | 46 | 93 | 121 |
| SWPV1-141 | SWPV2-148 | CNPV154 | CNPV154 variola B22R-like protein | 90.067 | 98.286 | 1939 | 875 | 1928 | SWPV2: N-terminus fragment/SWPV1: Low SNP Density |
| SWPV1-142 | SWPV2-149 | CNPV155 | CNPV155 variola B22R-like protein | 82.427 | 99.454 | 1810 | 1831 | 1830 |
| SWPV2-150 | CNPV156 | CNPV156 hypothetical protein | 96.287 | 834 | 832 |
| SWPV2-151 | CNPV157 | CNPV157 TGF-beta-like protein | 87.679 | 343 | 349 |
| CNPV158 | CNPV158 TGF-beta-like protein | 172 |
| CNPV159 | CNPV159 N1R/p28-like protein | 337 |
| CNPV160 | CNPV160 N1R/p28-like protein | 396 |
| SWPV2-152 | CNPV161 | CNPV161 TGF-beta-like protein | 99.441 | 358 | 358 |
| SWPV2-153 | CNPV162 | CNPV162 TGF-beta-like protein | 97.987 | 149 | 149 |
| CNPV163 | CNPV163 hypothetical protein | 92 |
| CNPV164 | CNPV164 hypothetical protein | 98 |
| SWPV2-154 | CNPV165 | CNPV165 N1R/p28-like protein | 98.75 | 320 | 346 |
| SWPV1-143 | SWPV2-155 | CNPV166 | CNPV166 Ig-like domain protein | 96.812 | 95.652 | 345 | 345 | 345 |
| SWPV1-144 | SWPV2-156 | CNPV167 Ig-like domain protein | 94.767 | 88.372 | 172 | 168 | 171 |
| SWPV2-157 | CNPV168 | CNPV168 N1R/p28-like protein | 96 | 350 | 358 |
| SWPV1-145 | CNPV169 | CNPV169 N1R/p28-like protein | 83.578 | 337 | 332 |
| SWPV1-146 | SWPV2-158 | CNPV170 thymidylate kinase | 100 | 100 | 121 | 212 | 212 |
| SWPV1-147 | SWPV2-159 | CNPV171 late transcription factor VLF-T1 | 96.923 | 100 | 260 | 260 | 260 |
| SWPV1-148 | SWPV2-160 | CNPV172 putative myristylated protein | 83.125 | 99.403 | 336 | 335 | 335 |
| SWPV1-149 | SWPV2-161 | CNPV173 putative myristylated IMV envelope protein | 91.358 | 98.354 | 243 | 243 | 243 |
| SWPV1-150 | SWPV2-162 | CNPV174 conserved hypothetical protein | 47.917 | 100 | 303 | 303 | 303 |
| SWPV1-151 | SWPV2-163 | CNPV175 conserved hypothetical protein | 84.158 | 100 | 253 | 252 | 252 |
| SWPV1-152 | SWPV2-164 | CNPV176 DNA-binding virion core protein | 87.747 | 100 | 131 | 130 | 130 |
| SWPV1-153 | SWPV2-165 | CNPV177 conserved hypothetical protein | 84.733 | 100 | 131 | 130 | 130 |
| SWPV1-154 | SWPV2-166 | CNPV178 | CNPV178 putative IMV membrane protein | 85.135 | 100 | 148 | 148 | 148 |
| SWPV1-155 | SWPV2-167 | CNPV179 | CNPV179 poly(A) polymerase small subunit PAPS | 88.667 | 100 | 300 | 302 | 302 |
| SWPV1-156 | SWPV2-168 | CNPV180 | CNPV180 RNA polymerase subunit RPO22 | 87.634 | 99.462 | 186 | 186 | 186 |
| SWPV1-157 | SWPV2-169 | CNPV181 | CNPV181 conserved hypothetical protein | 82.353 | 100 | 136 | 136 | 136 |
| SWPV1-158 | SWPV2-170 | CNPV182 | CNPV182 RNA polymerase subunit RPO147 | 93.866 | 99.922 | 1288 | 1288 | 1288 |
| SWPV1-159 | SWPV2-171 | CNPV183 | CNPV183 putative protein-tyrosine phosphatase, virus assembly | 85.542 | 100 | 166 | 166 | 166 |
| SWPV1-160 | SWPV2-172 | CNPV184 | CNPV184 conserved hypothetical protein | 91.534 | 100 | 190 | 189 | 189 |
| SWPV1-161 | SWPV2-173 | CNPV185 | CNPV185 ankyrin repeat protein | 32.632 | 96.341 | 337 | 328 | 328 |
| SWPV1-162 | SWPV2-174 | CNPV186 | CNPV186 IMV envelope protein | 100 | 100 | 329 | 330 | 330 |
| SWPV1-163 | SWPV2-175 | CNPV187 | CNPV187 RNA polymerase associated protein RAP94 | 91.114 | 99.75 | 799 | 799 | 799 |
| SWPV1-164 | SWPV2-176 | CNPV188 | CNPV188 late transcription factor VLTF-4 | 70.115 | 92.941 | 170 | 170 | 170 |
| SWPV1-165 | SWPV2-177 | CNPV189 | CNPV189 DNA topoisomerase | 88.608 | 99.684 | 316 | 316 | 316 |
| SWPV1-166 | SWPV2-178 | CNPV190 | CNPV190 conserved hypothetical protein | 77.124 | 99.346 | 153 | 153 | 153 |
| SWPV1-167 | SWPV2-179 | CNPV191 | CNPV191 conserved hypothetical protein | 70.874 | 99.029 | 103 | 103 | 103 |
| SWPV1-168 | SWPV2-180 | CNPV192 | CNPV192 mRNA capping enzyme large subunit | 88.221 | 99.764 | 848 | 846 | 846 |
| SWPV1-169 | SWPV2-181 | CNPV193 | CNPV193 HT motif protein | 72.619 | 100 | 104 | 106 | 106 |
| SWPV1-170 | SWPV2-182 | CNPV194 | CNPV194 virion protein | 71.223 | 100 | 139 | 140 | 140 |
| SWPV1-171 | SWPV2-183 | CNPV195 | CNPV195 hypothetical protein | 51.2 | 98.611 | 139 | 144 | 144 |
| SWPV1-172 | SWPV2-184 | CNPV196 | CNPV196 conserved hypothetical protein | 62.963 | 100 | 189 | 190 | 190 |
| SWPV1-173 | SWPV2-185 | CNPV197 | CNPV197 N1R/p28-like protein | 61.679 | 97.818 | 279 | 275 | 275 |
| SWPV1-174 | SWPV2-186 | CNPV198 | CNPV198 C-type lectin-like protein | 55.844 | 99.359 | 159 | 156 | 156 |
| SWPV1-175 | SWPV2-187 | CNPV199 | CNPV199 deoxycytidine kinase-like protein | 79.111 | 100 | 222 | 225 | 225 |
| SWPV1-176 | SWPV2-188 | CNPV200 | CNPV200 Rep-like protein | 72.903 | 97.59 | 152 | 166 | 166 |
| SWPV1-177 | SWPV2-189 | CNPV201 | CNPV201 conserved hypothetical protein | 60 | 97.661 | 197 | 167 | 192 |
| SWPV1-178 | SWPV2-190 | CNPV202 | CNPV202 N1R/p28-like protein | 69.203 | 99.638 | 275 | 276 | 276 |
| SWPV1-179 | SWPV2-191 | CNPV203 | CNPV203 N1R/p28-like protein | 64.935 | 99.738 | 380 | 382 | 382 |
| SWPV1-180 | SWPV2-192 | CNPV204 | CNPV204 conserved hypothetical protein | 53.226 | 100 | 53 | 61 | 61 |
| SWPV1-181 | SWPV2-193 | CNPV205 | CNPV205 N1R/p28-like protein | 71.885 | 99.371 | 317 | 318 | 318 |
| SWPV1-182 | SWPV2-194 | CNPV206 | CNPV206 putative photolyase | 84.989 | 99.364 | 464 | 472 | 472 |
| SWPV1-183 | | | CNPV081 Ig-like domain protein | 53.988 | 332 | 333 |
| SWPV1-184 | SWPV2-195 | CNPV207 | CNPV207 N1R/p28-like protein | 64.535 | 98.235 | 193 | 173 | 183 |
| SWPV1-185 | SWPV2-196 | CNPV208 | CNPV208 conserved hypothetical protein | 52.239 | 97.5 | 172 | 200 | 200 |
| SWPV1-186 | SWPV2-197 | CNPV209 | CNPV209 N1R/p28-like protein | 65.686 | 100 | 311 | 310 | 310 |
| SWPV1-187 | SWPV2-198 | CNPV210 | CNPV210 N1R/p28-like protein | 74.419 | 99.237 | 130 | 131 | 131 |
| SWPV1-188 | SWPV2-199 | CNPV211 | CNPV211 conserved hypothetical protein | 49.02 | 98.148 | 54 | 54 | 54 |
| SWPV1-189 | SWPV2-200 | CNPV212 | CNPV212 N1R/p28-like protein | 76.136 | 98.295 | 175 | 176 | 176 |
| SWPV1-190 | | no significant BLAST hits | 70 | | | |
| SWPV1-191 | SWPV2-201 | CNPV213 | CNPV213 deoxycytidine kinase-like protein | 58.768 | 99.539 | 216 | 216 | 217 |
| SWPV2-202 | CNPV214 | CNPV214 vaccinia C4L/C10L-like protein | 99.438 | 356 | 356 |
| SWPV1-192 | | CNPV012 conserved hypothetical protein | 37.41 | 165 | 189 |
| SWPV1-193 | | CNPV223 ankyrin repeat protein | 31.579 | 674 | 847 |
| SWPV1-194 | SWPV2-203 | CNPV215 | CNPV215 CC chemokine-like protein | 49.751 | 96.078 | 202 | 204 | 204 |
| SWPV2-204 | CNPV216 | CNPV216 conserved hypothetical protein | 98.762 | 401 | 404 |
| SWPV2-205 | CNPV217 | CNPV217 N1R/p28-like protein | 95.152 | 330 | 330 |
| SWPV1-195 | | CNPV223 ankyrin repeat protein | 38.474 | 729 | 847 |
| SWPV1-196 | SWPV2-206 | CNPV218 | CNPV218 N1R/p28-like protein | 66.667 | 99.522 | 318 | 223 | 437 |
| SWPV1-197 | | CNPV228 N1R/p28-like protein | 53 | 161 | 371 |
| SWPV1-198 | | CNPV160 N1R/p28-like protein | 79.293 | 367 | 396 |
| SWPV1-199 | | CNPV160 N1R/p28-like protein | 66.582 | 360 | 396 |

Table 1 Shearwaterpox virus (SWPV) genome annotations and comparative analysis of ORFs relative to CNPV genomes (Continued)
| SWPV1-200  | CNPV161 TGF-beta-like protein | 36.882  | 256  | 358  |
| SWPV1-201  | CNPV162 TGF-beta-like protein | 50      | 141  | 149  |
| SWPV1-202  | no significant BLAST hits     | 98      |      |      |
| SWPV2-207  | CNPV219 N1R/p28-like protein | 99.713  | 349  | 349  |
| SWPV2-208  | CNPV220 N1R/p28-like protein | 80.263  | 85   | 178  |
| SWPV2-209  | CNPV221 N1R/p28-like protein | 94.231  | 213  | 281  |
| SWPV2-210  | CNPV222 N1R/p28-like protein | 99.649  | 285  | 285  |
| SWPV2-211  | CNPV223 ankyrin repeat protein | 98.819  | 847  | 847  |
| SWPV2-212  | CNPV224 hypothetical protein  | 50.382  | 100  | 239  |
| SWPV2-213  | CNPV225 N1R/p28-like protein | 74.038  | 94   | 159  |
| SWPV2-214  | CNPV226 N1R/p28-like protein | 96.825  | 126  | 134  |
| SWPV2-215  | CNPV227 N1R/p28-like protein | 359     |      |      |
| SWPV2-216  | CNPV228 N1R/p28-like protein | 371     |      |      |
| SWPV1-203  | CNPV229 ankyrin repeat protein | 44.498  | 97.926 | 434  | 434  |
| SWPV1-204  | CNPV230 hypothetical protein  | 98.462  | 65   | 65   |
| SWPV1-205  | CNPV231 MyD116-like domain protein | 72.222  | 98.101 | 158  | 158  |
| SWPV2-217  | CNPV232 CC chemokine-like protein | 59.024  | 93.137 | 205  | 204  |
| SWPV1-206  | CNPV233 ankyrin repeat protein | 56.936  | 99.788 | 476  | 471  |
| SWPV1-207  | CNPV234 ankyrin repeat protein | 100     | 508  | 508  |
| SWPV2-220  | PEPV008 vaccinia C4L/C10L-like protein | 55      | 420  | 411  |
| SWPV2-221  | CNPV235 conserved hypothetical protein | 88.426  | 432  | 432  |
| SWPV2-222  | CNPV236 ribonucleotide reductase small subunit | 83.282  | 95.666 | 323  | 323  |
| SWPV2-223  | CNPV237 ankyrin repeat protein | 97.732  | 441  | 441  |
| SWPV1-210  | CNPV238 late transcription factor VLTF-3 | 95.111  | 100  | 225  |
| SWPV1-211  | CNPV239 virion redox protein | 80.282  | 100  | 72   | 75   |

Table 1: Shearwaterpox virus (SWPV) genome annotations and comparative analysis of ORFs relative to CNPV genomes (Continued).
| SWPV1-213 | SWPV2-226 | CNPV240 | SWPV240 virion core protein P4b | 88.788 | 99.848 | 660 | 659 | 659 |
| SWPV1-214 | SWPV2-227 | CNPV241 | CNPV241 immunodominant virion protein | 47.368 | 99.07 | 242 | 215 | 215 |
| SWPV1-215 | SWPV2-228 | CNPV242 | CNPV242 RNA polymerase subunit RPO19 | 88.166 | 98.817 | 169 | 169 | 169 |
| SWPV1-216 | SWPV2-229 | CNPV243 | CNPV243 conserved hypothetical protein | 81.501 | 98.928 | 373 | 373 | 373 |
| SWPV1-217 | SWPV2-230 | CNPV244 | CNPV244 early transcription factor large subunit VETF | 95.91 | 100 | 709 | 709 | 709 |
| SWPV1-218 | SWPV2-231 | CNPV245 | CNPV245 intermediate transcription factor VIF-3 | 90.667 | 99.667 | 300 | 300 | 300 |
| SWPV1-219 | SWPV2-232 | CNPV246 | CNPV246 putative IMV membrane protein | 80 | 98.667 | 76 | 75 | 75 |
| SWPV1-220 | SWPV2-233 | CNPV247 | CNPV247 virion core protein P4a | 81.494 | 99.664 | 897 | 893 | 893 |
| SWPV1-221 | SWPV2-234 | CNPV248 | CNPV248 conserved hypothetical protein | 78.723 | 100 | 281 | 279 | 279 |
| SWPV1-222 | SWPV2-235 | CNPV249 | CNPV249 virion protein | 74.269 | 99.405 | 167 | 168 | 168 |
| SWPV1-223 | SWPV2-236 | CNPV250 | CNPV250 conserved hypothetical protein | 36.082 | 94.595 | 73 | 56 | 99 |
| SWPV1-224 | SWPV2-237 | CNPV251 | CNPV251 putative IMV membrane protein | 69.565 | 100 | 69 | 69 | 69 |
| SWPV1-225 | SWPV2-238 | CNPV252 | CNPV252 putative IMV membrane protein | 68.478 | 98.913 | 92 | 92 | 92 |
| SWPV1-226 | SWPV2-239 | CNPV253 | CNPV253 putative IMV membrane virulence factor | 73.585 | 98.113 | 53 | 53 | 53 |
| SWPV1-227 | SWPV2-240 | CNPV254 | CNPV254 conserved hypothetical protein | 75 | 98.958 | 96 | 96 | 96 |
| SWPV1-228 | SWPV2-241 | CNPV255 | CNPV255 predicted myristylated protein | 84.282 | 99.728 | 368 | 368 | 368 |
| SWPV1-229 | SWPV2-242 | CNPV256 | CNPV256 putative phosphorylated IMV membrane protein | 81.006 | 100 | 188 | 192 | 192 |
| SWPV1-230 | SWPV2-243 | CNPV257 | CNPV257 DNA helicase, transcriptional elongation | 87.229 | 99.784 | 462 | 462 | 462 |
| SWPV1-231 | SWPV2-244 | CNPV258 | CNPV258 conserved hypothetical protein | 77.647 | 100 | 86 | 89 | 89 |
| SWPV1-232 | SWPV2-245 | CNPV259 | CNPV259 DNA polymerase processivity factor | 81.86 | 100 | 432 | 434 | 434 |
| SWPV1-233 | SWPV2-246 | CNPV260 | CNPV260 conserved hypothetical protein | 91.071 | 99.77 | 112 | 112 | 112 |
| SWPV1-234 | SWPV2-247 | CNPV261 | CNPV261 Holliday junction resolvase protein | 80.405 | 100 | 151 | 152 | 152 |

*SWPV2: N-terminus fragment*
Table 1 Shearwaterpox virus (SWPV) genome annotations and comparative analysis of ORFs relative to CNPV genomes (Continued)

| SWPV1-235 | SWPV2-248 | CNPV262 | CNPV262 intermediate transcription factor VITF-3 | 86.126 | 100 | 383 | 383 | 383 |
| SWPV1-236 | SWPV2-249 | CNPV263 | CNPV263 RNA polymerase subunit RPO132 | 94.301 | 100 | 1158 | 1157 | 1157 |
| SWPV1-237 | SWPV2-250 | CNPV264 | CNPV264 A type inclusion-like protein | 81.015 | 99.502 | 602 | 601 | 603 |
| SWPV1-238 | SWPV2-251 | CNPV265 | CNPV265 A type inclusion-like/fusion protein | 67.015 | 99.789 | 471 | 475 | 475 |
| SWPV1-239 | SWPV2-252 | CNPV266 | CNPV266 conserved hypothetical protein | 89.286 | 99.286 | 140 | 140 | 140 |
| SWPV1-240 | SWPV2-253 | CNPV267 | CNPV267 RNA polymerase subunit RPO35 | 77.558 | 99.016 | 303 | 305 | 305 |
| SWPV1-241 | SWPV2-254 | CNPV268 | CNPV268 conserved hypothetical protein | 73.529 | 100 | 72 | 75 | 75 |
| SWPV1-242 | SWPV2-255 | CNPV269 | CNPV269 conserved hypothetical protein | 70.796 | 100 | 113 | 113 | 113 |
| SWPV1-243 | SWPV2-256 | CNPV270 | CNPV270 conserved hypothetical protein | 70.588 | 100 | 119 | 120 | 120 |
| SWPV1-244 | SWPV2-257 | CNPV271 | CNPV271 DNA packaging protein | 89.963 | 99.648 | 272 | 284 | 284 |
| SWPV1-245 | SWPV2-258 | CNPV272 | CNPV272 C-type lectin-like EEV protein | 76.136 | 99.448 | 182 | 181 | 181 |
| SWPV1-246 | | CNPV012 | CNPV012 conserved hypothetical protein | 30.147 | 100 | 172 | 189 |
| SWPV1-247 | SWPV2-259 | CNPV273 | CNPV273 conserved hypothetical protein | 62.816 | 99.635 | 276 | 274 | 274 |
| SWPV1-248 | SWPV2-260 | CNPV274 | CNPV274 putative tyrosine protein kinase | 63.197 | 99.628 | 286 | 269 | 269 |
| SWPV1-249 | SWPV2-261 | CNPV275 | CNPV275 putative serpin | 72.271 | 99.408 | 340 | 338 | 338 |
| SWPV1-250 | SWPV2-262 | CNPV276 | CNPV276 conserved hypothetical protein | 56.667 | 100 | 227 | 252 | 252 |
| SWPV1-251 | SWPV2-263 | CNPV277 | CNPV277 G protein-coupled receptor-like protein | 90 | 99.677 | 310 | 310 | 310 |
| SWPV1-252 | SWPV2-264 | CNPV278 | CNPV278 conserved hypothetical protein | 89.691 | 98.958 | 97 | 96 | 96 |
| SWPV1-253 | SWPV2-265 | CNPV279 | CNPV279 beta-NGF-like protein | 63.415 | 100 | 167 | 169 | 169 |
| SWPV1-254 | SWPV2-266 | CNPV280 | CNPV280 HT motif protein | 67.692 | 99.231 | 134 | 130 | 130 |
| SWPV1-255 | SWPV2-267 | CNPV281 | CNPV281 conserved hypothetical protein | 71.728 | 99.533 | 192 | 214 | 214 |
| SWPV1-256 | SWPV2-268 | CNPV282 | CNPV282 HT motif protein | 71.552 | 100 | 118 | 120 | 120 |
| SWPV1-257 | SWPV2-269 | CNPV283 | CNPV283 CC chemokine-like protein | 63.208 | 99.408 | 182 | 111 | 111 |
| SWPV1-258 | SWPV2-270 | CNPV284 | CNPV284 putative interleukin binding protein | 37.405 | 90.769 | 192 | 193 | 195 |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | EGF-like protein |
|-------|-------|-------|-------|------------------|
| 1-259 | 2-271 | 285   | 285   | 62.992 99.206    |
|       |       |       |       | 123 126 126      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | putative serine/threonine protein kinase |
| 2-260 | 2-272 | 286   | 286   | 76.744 99.672    |
|       |       |       |       | 303 305 305      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | conserved hypothetical protein |
| 2-261 | 2-273 | 287   | 287   | 73.248 98.758    |
|       |       |       |       | 165 160 161      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | C-type lectin-like protein |
| 2-262 | 2-274 | 288   | 288   | 52.414 88.435    |
|       |       |       |       | 163 147 147      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | putative interleukin binding protein |
| 2-263 | 2-275 | 289   | 289   | 58.993 99.281    |
|       |       |       |       | 132 139 139      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | conserved hypothetical protein |
| 2-264 | 2-276 | 290   | 290   | 84 83.784 75      |
|       |       |       |       | 75 75 75          |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | ankyrin repeat protein |
| 2-265 | 2-277 | 291   | 291   | 48.067 98.99      |
|       |       |       |       | 613 594 594      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | hypothetical protein |
| 2-266 | 2-278 | 292   | 292   | 37.209 100        |
|       |       |       |       | 101 74 74         |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | ankyrin repeat protein |
| 2-267 | 2-279 | 293   | 293   | 55.634 99.648    |
|       |       |       |       | 305 284 284      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | ankyrin repeat protein |
| 2-268 | 2-280 | 294   | 294   | 68.447 99.07     |
|       |       |       |       | 424 430 430      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | pIPV223 host range protein |
| 2-269 |       |       |       | 51 138 143       |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | FWPV217 hypothetical protein |
| 2-270 |       |       |       | 50 330 328       |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | ankyrin repeat protein |
| 2-271 | 2-281 | 295   | 295   | 57.736 100        |
|       |       |       |       | 264 396 396      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | ankyrin repeat protein |
| 2-272 | 2-282 | 296   | 296   | 67.195 99.127    |
|       |       |       |       | 438 458 458      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | ankyrin repeat protein |
| 2-273 | 2-283 | 297   | 297   | 54.972 99.457    |
|       |       |       |       | 717 737 737      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | ankyrin repeat protein |
| 2-274 | 2-284 | 298   | 298   | 64.591 99.825    |
|       |       |       |       | 573 571 571      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV299 ankyrin repeat protein |
| 2-275 | 2-285 | 299   | 299   | 67.893 99.333    |
|       |       |       |       | 303 300 300      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV300 ankyrin repeat protein |
| 2-276 | 2-286 | 300   | 300   | 75.82 98.77      |
|       |       |       |       | 253 244 244      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV219 N1R/p28-like protein |
| 2-277 |       | 301   | 301   | 28.467 142       |
|       |       |       |       | 142 349          |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV228 N1R/p28-like protein |
| 2-278 |       | 302   | 302   | 43.038 87        |
|       |       |       |       | 87 371           |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | TKPV163 ankyrin repeat protein |
| 2-279 |       | 303   | 303   | 40 432           |
|       |       |       |       | 432 434          |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV301 ankyrin repeat protein |
| 2-280 | 2-287 | 304   | 304   | 59.546 99.241    |
|       |       |       |       | 510 527 527      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV302 conserved hypothetical protein |
| 2-281 | 2-288 | 305   | 305   | 45.026 100       |
|       |       |       |       | 175 193 193      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV303 ankyrin repeat protein |
| 2-282 | 2-289 | 306   | 306   | 68.938 99.4      |
|       |       |       |       | 499 500 500      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV304 ankyrin repeat protein |
| 2-283 | 2-290 | 307   | 307   | 62.105 99.785    |
|       |       |       |       | 476 466 466      |
| SWPV1 | SWPV1 | CNPV1 | CNPV1 | CNPV305 N1R/p28-like protein |
| 2-284 | 2-291 | 308   | 308   | 54.545 100       |
|       |       |       |       | 261 262 262      |
| SWPV1-285 | SWPV2-292 | CNPV306 | CNPV306 |
|-----------|-----------|---------|---------|
|          |          | hypothetical protein | 30.769 |
|          |          | CNPV307 C-type lectin-like protein | 55.828 |
|          |          | CNPV308 ankyrin repeat protein | 58.757 |
|          |          | CNPV309 ankyrin repeat protein | 69.388 |
|          |          | CNPV310 ankyrin repeat protein | 47.359 |
|          |          | CNPV311 EFc-like protein | 54.4 |
|          |          | CNPV312 conserved hypothetical protein | 53.704 |
|          |          | CNPV313 Ig-like domain protein | 69.43 |
|          |          | CNPV314 ankyrin repeat protein | 71.552 |
|          |          | CNPV315 G protein-coupled receptor-like protein | 59.17 |
|          |          | CNPV316 ankyrin repeat protein | 35.294 |
|          |          | CNPV317 Ig-like domain protein | 35.556 |
|          |          | CNPV318 ankyrin repeat protein | 98.054 |
|          |          | CNPV319 ankyrin repeat protein | 97.638 |
|          |          | PIPV253 EFc-like protein | 69 |
|          |          | CNPV320 Ig-like domain protein | 76.858 |
|          |          | CNPV321 EFc-like protein | 99.194 |
|          |          | CNPV322 ankyrin repeat protein | 98.408 |
|          |          | CNPV323 ankyrin repeat protein | 35.556 |

SWPV1-290: C-terminus fragment, not likely translated

**Table 1** Shearwaterpox virus (SWPV) genome annotations and comparative analysis of ORFs relative to CNPV genomes (Continued)
brachyrhynchos) and American robin (Turdus migratorius) [24], which is almost identical to CPNV-1 within this relatively small fragment of the genome.

Features of SWPV-2
As noted above, and displayed in the Dotplot (Fig. 2b), SWPV-2 is very similar to CNPV with almost 98% nt identity. However, a 1% difference still gives approximately 10 mutations in an average sized gene any of which could have drastic effects if an early STOP codon is introduced to the gene sequence. Similarly, small changes to promoter regions can significantly alter gene expressions that are impossible to predict in these viruses. With this annotation strategy, 18 CNPV genes were deemed to be missing from the SWPV-2 complete genome and a further 15 genes significantly fragmented as to probably cause them to be non-functional (Table 1). No novel genes were predicted in SWPV-2, and no rearrangement of genes compared to CNPV was observed.

Features of SWPV-1
As expected from the much lower percent nt identity, SWPV-1 was found to be considerably more different to CNPV than SWPV-2 when compared at the level of genes present or absent. (Table 1). 43 CNPV genes are absent from SWPV-1 and a further 6 are significantly fragmented. There are 4 predicted genes in SWPV-1 that are not present in any other poxvirus, nor do they match

Table 1 Shearwaterpox virus (SWPV) genome annotations and comparative analysis of ORFs relative to CNPV genomes (Continued)

| SWPV1-306 | CNPV008 | C-type lectin-like protein | 50   | 174   | 169   |
| SWPV1-307 | SWPV2-309 | CNPV323 | conserved hypothetical protein | 75.61 | 93.651 | 84   | 186   | 182   |
| SWPV1-308 | SWPV2-310 | CNPV324 | conserved hypothetical protein | 87.387 | 99.55 | 220   | 222   | 222   |
| SWPV1-309 | CNPV325 | CNPV325 ankyrin repeat protein | 56.458 | 468   | 514   |
| SWPV1-310 | SWPV2-311 | CNPV326 | CNPV326 C-type lectin-like protein | 32.044 | 85.99 | 181   | 208   | 204   |
| SWPV2-312 | CNPV327 | CNPV327 conserved hypothetical protein | 92.941 | 171   | 171   |
| CNPV328 | CNPV328 hypothetical protein | 72   |

Fig. 2 Dotplots of Shearwaterpox viruses (SWPV-1 and 2) vs CNPV genomes. Horizontal sequence: SWPV-1 (a) and SWPV-2 (b), vertical sequence CNPV. Red and blue boxes represent genes transcribed to the right and left of the genome, respectively.
any sequences in the NR protein database using BLASTP. However, they are all relatively short ORFs and it is possible that they are not functional genes. Additionally, SWPV-1 encodes nine polypeptides that do not match CNPV proteins, but do match proteins from other avipoxviruses (penguinpox, turkeypox, pigeonpox and fowlpox). This could be due to recombination among ancestral viruses, but could also result from the loss of the corresponding ortholog in CNPV leaving another virus to provide the “best match”.

As might be expected given the greater distance between SWPV-1 and CPNV than between SWPV-2 and CNPV, there are more instances of minor rearrangements that created a loss of synteny (Table 1). However, since most of these involve the families of repeated genes, it is also possible that divergence of these
sequences has led to the inability to distinguish between the orthologous and paralogous genes.

**Evidence of recombination among avipoxviruses**

When we reviewed a graph of nt identity between the 2 new complete genomes and CNPV using BBB (not shown), there were several relatively short syntenic regions where 1) SWPV-1 matched CNPV significantly better than the majority of the genome, and 2) SWPV-2 matched CNPV significantly worse than the majority of the genome. To examine these regions in more detail, the **Visual Summary** feature of BBB was used to display individual SNPs for these genome comparisons (Fig. 6a and b). This analysis revealed that SWPV-1 and SWPV-2 were unique in these regions and confirmed that the genome sequences of SWPV-1 and SWPV-2 were not contaminated during their assembly. However, when these regions were used as query sequences in BLASTN searches of all poxvirus sequences the best match remained CNPV suggesting that these sequences originated from avipoxvirus genomes that are not represented in the public databases.

**Discussion**

This paper describes the detection and characterization of two novel avipoxvirus complete genome sequences in a naturally occurring infections of avian pox in a naïve
population of shearwaters. The DNA sequences of SWPV-1 and SWPV-2 are significantly different than each other but nevertheless had closest similarity with *Canarypox virus* (67% and 98%, respectively). Furthermore, the genetic distance and novel genome structure of SWPV-1 from *A. carneipes* considered to be missing 43 genes likened to CNPV and contained 4 predicted genes which are not found in any other poxvirus and is overall sufficiently genetically different to be considered a separate virus species. Whilst, the SWPV-2 complete genome was missing 18 genes compared to CNPV, with a further 15 genes significantly fragmented as to probably cause them to be non-functional. Furthermore, the phylogenetic distribution of SWPV-1 indicates that shearwaters and perhaps other long-lived, vagile marine birds could be important hosts for avipoxvirus dispersal around the globe. The natural hosts of these avipoxviruses maybe this population of shearwaters, other migratory birds that use Lord Howe Island for breeding or resident avian host reservoir species. Species such as the Lord Howe White-eye (*Zosterops tephrophylla*) and Lord Howe Golden Whistler (*Pachycephala petoralis contempta*) are candidate passerine birds that might provide such function.

Examining the phylogenetic relationship between the *Shearwaterpox viruses* and other avipoxviruses, it is evident that the SWPV-2 is most closely related to *Canarypox virus*. The SWPV-1 and SWPV-2 complete genomes both contain several genes that are more closely related to CNPV throughout their entire genome. As shown in Fig. 3 it is reasonable to postulate that these viruses originated from a common ancestor that diverged from a CNPV-like progenitor related to *fowlpox, penguinpox* and *pigeonpox* viruses. Finer resolution of the phylogenetic relationship using partial nucleotide sequences of p4b and DNA polymerase genes of avipoxviruses revealed that SWPV isolated from seabirds also clustered in global clad B consisting of avipoxviruses originating from Canary Morocco, Canarypox and poxviruses from American crow and American robin. Given their genetic diversity, it is perhaps not surprising that Shearwater species can be exposed to multiple avipoxviral infections. Studies such as those by Barnett et al. [25] suggest that the species specificity of poxviruses is variable. Some genera, such as *Suipoxivirus* are highly restricted to individual vertebrate hosts, swinepox for instance, whereas others, such as avipoxviruses demonstrate some evidence of cross-species infection within a predator–prey system [24]. This suggests that the avipoxviruses can infect a diverse range of bird species if they are within a close enough proximity to each other [26]. Thus far, there were no clear patterns regarding species-specificity in the *Shearwaterpox viruses* described here.

While overt and systemic lesions and fatal disease can occur, avian pox tends to be a self-limiting localized infection of apertial skin with full recovery possible. Many bird species experience life-long immunity if the immune system is not weakened and or the birds are not infected by different strains [27, 28]. As shown in our example, secondary infections can occur and these may contribute to morbidity and mortality [29–31]. Similar to the example in shearwaters, Shivaprasad et al. [30] reported evidence of poxvirus infection and secondary fungal pathogens in canaries (*Serinus canaria*). Stressful conditions, poor nutrition, overt environmental contamination and other underlying causes of immunosuppression and ill health may contribute to the pathogenesis of such lesions. This was the primary reason we tested for avian circovirus and other potential pathogens.

Avian pox has not been previously reported in shearwaters (*Ardenna spp.*) from Lord Howe Island, nor has it been documented for any other bird species in this region. So it is difficult to attribute the causality of this unique event in these species. The value of complete genome characterization and analysis is highlighted since a phylogenetic relationship based on single gene studies such as the polymerase gene may have falsely implicated *Canarypox virus* as a potential exotic introduced emerging disease from domesticated birds. Although we cannot trace the actual source of infection in the shearwater chicks, it is more likely that the infection in the birds resulted from parental feeding or arthropod mediated transmission from other island bird species [32]. While, the reservoir host of these novel *Shearwaterpox viruses* is unknown, mosquitoes are suspected to play a part in transmission within the island. Avipoxvirus infection appears to be relatively rare in seabirds, but it has been reported in several species when they occur on human-inhabited islands that harbor mosquito vectors [33]. According to the Lord Howe Island Board, ship rats, mice, cats, humans and other invasive pest species such as owls are implicated in the extinction of at least five endemic birds, two reptiles, 49 flowering plants, 12 vegetation communities and numerous threatened invertebrates [34]. These rodents and invasive pests have also been highlighted for the potential reservoir of poxvirus infections [3, 35]. Transmission of avipoxvirus by prey–predator and other migratory seabirds likely plays a prominent role; however, the mode of avipoxvirus transmission on Lord Howe Island is not completely understood. Studies by Gyuranecz et al. [24], for example, postulated that raptors may acquire poxvirus infection from their avian prey. This suggests that the poxvirus in shearwaters is likely to be transmitted from other island species such as other.
migratory seabirds and/or prey–predator, although, it is difficult to be certain without further studies.

Interestingly, these new shearwaterpox virus complete genomes also provide evidence that supports the hypothesis that recombination may play an important role in the evolution of avipoxviruses. A number of genes in SWPV-1 appear to be rearranged compared to CNPV and blocks of unusual similarity scores were seen in both SWPVs. Software that is designed to look for gross recombination between two viruses, such as two strains of HIV, fails to detect this level of recombination and it is left to the investigator to observe such small events by eye after visualizing the distribution of SNPs between viruses. Such relatively small exchanges of DNA may still exert important influences on virus evolution, and has been predicted to have been a driver in the evolution of smallpox [36].

Conclusions

These are the first avipoxvirus complete genome sequences that infect marine bird species. The novel complete genome sequences of SWPV-1 and −2 have greatly enhanced the genomic information for the Avipoxvirus genus, which will contribute to our understanding of the avipoxvirus more generally, and track the evolution of poxvirus infection in such a non-model avian species. Together with the sequence similarities observed between SWPV and other avipoxviruses, this study concluded that the SWPV complete genome from A. carneipes (SWPV-1) described here is not closely related to any other avipoxvirus complete genome isolated from avian or other natural host species, and that it likely should be considered a separate species. Further investigations of Shearwaterpox viruses genetic and pathogenesis will provide a unique approach to better assess the risk associated to poxvirus transmission within and between marine bird species.

Methods

Source of sampling

A total of six samples were collected from two different species of shearwater, five were from Flesh-footed Shearwater (ID: 15-1527-31), and other one was from Wedge-tailed Shearwater (ID: 15–1526). Of size birds, two were recoded to have evidence of gross well circumscribed lesions in the beak (Fig. 1a) and ankle, and others had feather defects (fault lines across the vanes of feathers). Samples were collected from fledglings (approximately 80–90 days of age) of both species on Lord Howe Island, New South Wales (32.53S, 159.08E) located approximately 500 km off the east coast of Australia during April-May 2015. Samples were collected with the permission of the Lord Howe Island Board (permit no. LHIB 02/14) under the approval of the University of Tasmania and Charles Sturt University Animal Ethics Committees (permit no. A0010874, A0011586, and 09/046). Samples from one individual of each shearwater species were collected including skin lesions, liver and skin biopsies, as well as blood for identifying the causative agents. Depending on the samples, either 25 mg of skin tissue were cut out and chopped into small pieces or 50–100 μL of blood were aseptically transferred into clean 1.5 mL microcentrifuge tube (Eppendorf), and genomic DNA was isolated using the Qiagen blood and tissue mini kit (Qiagen, Germany). The extracted DNA has been stored at −20 °C for further testing. Histopathological examination of the skin was performed.

Archived viral and fungal pathogen testing

Initially, the extracted DNA was screened for detecting novel circoviruses [37, 38] and reticuloendotheliosis virus [39]. For poxvirus screening, the primers PoxP1 (5′-CAGCAGGTGCTAAACAACAA-3′) and PoxP2 (5′-CGGTAGCTTAACGCAGAATA-3′) were synthesized from published literature and used to amplify a segment of approximately 578 bp from the 4b core protein gene for all ChPV species [40]. Optimized PCR reactions mixture contained 3 μL of extracted genomic DNA, 25 pmol of each primer (GeneWorks, Australia), 1.5 mM MgCl2, 1.25 mM of each dNTP, 1xGoTaq® Green Flexi Reaction Buffer, 1 U of Go Taq DNA polymerase (Promega Corporation, USA) and DEPC distilled H2O (Invitrogen, USA) was added to a final volume of 25 μL. The PCR amplification was carried out in an iCycler thermal cycler (Bio-Rad) under the following conditions: denaturation at 94 °C for 2 min followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, and a final extension step of 2 min at 72 °C.

The internal transcribed spacer (ITS) region was chosen for screening and identification of fungal pathogens [41]. A set of fungus-specific primers ITS1 (5′-TCCGTAGGTGAACCTGCGG -3′) and ITS4 (5′-TCCTCGCTTATTGATATGC -3′) were designed and used to amplify a segment of approximately 550 bp from the fungal ITS gene [42]. The PCR was standardized to amplify ITS genes, and the 25-μL reaction mixture contained 3 μL of extracted genomic DNA, 25 pmol of each primer (GeneWorks, Australia), 1.5 mM MgCl2, 1.25 mM of each dNTP, 1xGoTaq® Green Flexi Reaction Buffer, 1 U of Go Taq DNA polymerase (Promega Corporation, USA). The PCR reaction involved initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min, and with a final step of one cycle extension at 72 °C for 10 min.
Amplified PCR products, together with a standard molecular mass marker (Sigma), were separated by electrophoresis in 2.0% agarose gel and stained with GelRed (Biotium, CA). Selected bands were excised and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer’s instructions. Purified amplicons were sequenced with PCR primers by the Australian Genome Research Facility Ltd (Sydney) using an AB 3730xl unit (Applied Biosystems). For each amplicon, sequences were obtained at least twice in each direction for each isolate. The sequences were trimmed for primers and aligned to construct contigs (minimum overlap of 35 bp, minimum match percentage of 95%) using Geneious Pro (version 10.0.2).

**High throughput sequencing**

Next-generation sequencing (NGS) was used to sequence the poxvirus genomes. Virion enrichment was performed by centrifugation for 2 min at 800 × g to remove tissue debris, and the supernatants were subsequently filtered through 5 μm centrifuge filters (Millipore) [43]. The filtrates were nuclease treated to remove unprotected nucleic acids using 8 μL RNase Cocktail Enzyme Mix (Invitrogen). Viral nucleic acids were subsequently extracted using QIAamp DNA mini (Qiagen). The genomic libraries were prepared with an insert size of 150 paired-end. DNA sequencing (NGS) was performed on a HiSeq4000 sequencing platform (Illumina) by Novogene, China.

**Bioinformatics**

Assembly of the viral genome was conducted according to the established pipeline [44] in CLC Genomics workbench 9.5.2 under La Trobe University Genomics Platform. Briefly, the preliminary quality evaluation for each raw read was generated using quality control (QC) report. The raw data were preprocessed to remove ambiguous base calls, and bases or entire reads of poor quality using default parameters. The datasets were trimmed to pass the quality control based on PHRED score or per base sequence quality score. Trimmed sequence reads were mapped against closely available host genome (Albatross) to remove possible remaining host DNA contamination, and post-filtered reads were mapped against reference Canarypox virus complete genome sequence. Consensus sequences were used to generate the complete poxvirus genome. Avipoxvirus complete genome sequences were aligned using MAFFT [45]. Then the poxvirus specific bioinformatics analyses were performed using the Viral Bioinformatics Resource Centre (virology.uvic.ca) [46], and the further analyses were conducted using the following tools: Viral Orthologous Clusters Database for sequence management (VOCs) [11]; Base-By-Base for genome/gene/protein alignments [47, 48]; Viral Genome Organizer for genome organization comparisons (VGO) [11], and Genome Annotation Transfer Utility for annotation (GATU) [49].

Open reading frames (ORFs) longer than 60 amino acids with minimal overlapping (overlaps cannot exceed 25% of one of the genes) to other ORFs were captured using the CLC Genomics Workbench (CLC) ORF analysis tool as well as GATU, and other protein coding sequence and annotation software described in Geneious (version 10.0.2, Biomatters, New Zealand). These ORFs were subsequently extracted into a FASTA file, and similarity searches including nucleotide (BLASTN) and protein (BLASTP) were performed on annotated ORFs as potential genes if they shared significant sequence similarity to known viral or cellular genes (BLAST E value ≤ e-5) or contained a putative conserved domain as predicted by BLASTp [50]. The final SWPV annotation was further examined with other poxvirus ortholog alignments to determine the correct methionine start site, correct stop codons, signs of truncation, and validity of overlaps.

**Phylogenetic analysis**

Phylogenetic analyses were performed using full poxvirus genome sequences for Shearwater species determined in this study with related avipoxvirus genome sequences available in GenBank database. A selection of partial sequences from seven completely sequenced avipoxvirus genomes and fragments of incompletely sequenced avipoxvirus genomes from Vultur Gryphus poxvirus (VGPV), flamingopox virus (FGPV) and Hawaiian goose poxvirus (HGPV) were also used for phylogenetic analysis. To investigate closer evolutionary relationship among avipoxviruses, partial nucleotide sequences of p4b and DNA polymerase genes were selected. The avipoxvirus sequences were aligned using ClustalO, and then manually edited in Base-by-Base. MEGA7 was used to create a maximum likelihood tree based on the Tamura-Nei method and tested by bootstrapping with 1000 replicates. An additional analysis was performed using complete genome nucleotide sequences of Canarypox virus (CNPV; AY318871), Pigeonpox virus (FeP2; KJ801920), Fowlpox virus (FPV; AF198100), Turkeypox virus (TKPV; NC_028238), Shearwaterpox virus strain-1 (SWPV-1; KX857216), and Shearwaterpox virus strain-2 (SWPV-2; KX857215), which were aligned with MAFFT in Base-By-Base for genome/gene/protein alignments [48]. The program jModelTest 2.1.3 favoured a general-time-reversible model with gamma distribution rate variation and a proportion of invariant sites (GTR + I + G4) for the ML analysis [51].
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Availability of data and materials
The complete genome sequences of the Shearwaterpox virus 1 from a Flesh-footed Shearwater (Ardenna carneipes) and Shearwaterpox virus 2 from a Wedge-tailed Shearwater (Ardenna pacifica) have been deposited in the NCBI database under GenBank accession numbers: [SWPV-1, GenBank: KX857216] and [SWPV-2, GenBank: K00857215].

Authors’ contributions
Conceived and designed the experiments: SS, SRR. Performed the experiments: SS, SRR. Analyzed the data: SS, CU, JI. Contributed reagents/materials/analysis tools: SS, SD, JLL, IHH, KH, CU, JI, SRR. SS, JLL, CU, JI, SRR wrote the initial manuscript. All authors read, edited and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval
Samples were collected with the permission of the Lord Howe Island Board (permit no. LHB 02/14) under the approval of the University of Tasmania and Charles Sturt University Animal Ethics Committees (permit no. A/13836, A0010874, A0011586, and 09/046).

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Additional files

| Additional file 1: Table S1. Summary of SWPV1 genome annotations (DOCX 52 kb) |
| Additional file 2: Table S2. Summary of SWPV2 genome annotations (DOCX 145 kb) |

Abbreviations
CNPV: Chordopoxvirinae; CNPV: Canavox virus; dsDNA: double-stranded; FGPV: Flamingopox virus; FP9: European strain of Fowlpox virus; FPUS: South African strain of Fowlpox virus; GATU: Genome Annotation Transfer Utility; HGPV: Hawaiian goose pox virus; ITS: internal transcribed spacer; ML: Maximum likelihood; NGS: Next-generation sequencing; ORF: open reading frame; PCR: polymerase chain reaction; PEPV: Penguinpox virus; QC: Quality control; SWPV-1: Shearwaterpox virus 1; SWPV-2: Shearwaterpox virus 2; TKPV: Turkeypox virus

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