RESEARCH ARTICLE

Genomic sequencing of Troides aeacus nucleopolyhedrovirus (TraeNPV) from golden birdwing larvae (Troides aeacus formosanus) to reveal defective Autographa californica NPV genomic features

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Abstract

Background: The golden birdwing butterfly (Troides aeacus formosanus) is a rarely observed species in Taiwan. Recently, a typical symptom of nuclear polyhedrosis was found in reared T. aeacus larvae. From the previous Kimura-2 parameter (K-2-P) analysis based on the nucleotide sequence of three genes in this isolate, polh, lef-8 and lef-9, the underlying virus did not belong to any known nucleopolyhedrovirus (NPV) species. Therefore, this NPV was provisionally named \"TraeNPV\". To understand this NPV, the nucleotide sequence of the whole TraeNPV genome was determined using next-generation sequencing (NGS) technology.

Results: The genome of TraeNPV is 125,477 bp in length with 144 putative open reading frames (ORFs) and its GC content is 40.45%. A phylogenetic analysis based on the 37 baculoviral core genes suggested that TraeNPV is a Group I NPV that is closely related to Autographa californica nucleopolyhedrovirus (AcMNPV). A genome-wide analysis showed that TraeNPV has some different features in its genome compared with other NPVs. Two novel ORFs (Ta75 and Ta139), three truncated ORFs (pcna, he65 and bro) and one duplicated ORF (38.7 K) were found in the TraeNPV genome; moreover, there are fewer homologous regions (hrs) than there are in AcMNPV, which shares eight hrs within the TraeNPV genome. TraeNPV shares similar genomic features with AcMNPV, including the gene content, gene arrangement and gene/genome identity, but TraeNPV lacks 15 homologous ORFs from AcMNPV in its genome, such as ctx, host cell-specific factor 1 (hcf-1), PNK/PNL, vp15, and apsup, which are involved in the auxiliary functions of alphabaculoviruses.

Conclusions: Based on these data, TraeNPV would be clarified as a new NPV species with defective AcMNPV genomic features. The precise relationship between TraeNPV and other closely related NPV species were further investigated. This report could provide comprehensive information on TraeNPV for evolutionary insights into butterfly-infected NPV.

Keywords: Troides aeacus, Troides aeacus nucleopolyhedrovirus, TraeNPV
Background

The golden birdwing butterfly, *Troides aeacus formosanus* (Rothschild) (Lepidoptera: Papilionidae), is one subspecies of five known *T. aeacus*; it is distributed throughout tropical areas and is also endemic to Taiwan [1]. Golden birdwing butterflies have a large body size and a wingspan that exceeds 15 cm [2]. The population of the golden birdwing butterfly has been declining due to commercial activity and a loss of habitat fitness, i.e., a loss of host plants [1, 3]. Therefore, this butterfly species is protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and the public will have to make more effort in the conservation management of the *T. aeacus formosanus* population [1]. From our previous investigation, a liquefaction symptom was found in the population of rearing golden birdwing butterfly larvae, and this symptom was similar to that of nuclear polyhedrosis. Polyhedral inclusion bodies (PIBs) were observed, and they filled in the body fluid of moribund larvae. A positive signal indicating a polyhedrin gene fragment was detected by PCR. Apparently, the polyhedrosis of the golden birdwing butterfly larvae is caused by nucleopolyhedrovirus (NPV) infection [4].

There are four genera in the *Baculoviridae*, including *Alphabaculovirus* (lepidopteran-specific nucleopolyhedrovirus, NPV), *Betabaculovirus* (lepidopteran-specific granulovirus), *Gammadaculovirus* (hymenopteran-specific NPV) and *Deltabaculovirus* (dipteran-specific NPV) [5]. The phylogenetic analysis based on the polyhedrin (*polh*) genes could further divide the lepidopteran-specific NPVs into group, I and II [6]. To date, more than 78 complete NPV genomes have been deposited in the NCBI GenBank, and most of them are lepidopteran-specific NPVs. However, the occurrence of NPV epizootics in butterfly species is uncommon. Among these sequenced NPV genomes, only *Catopsilia pomona* NPV (CapoNPV) was reported as a butterfly-infecting NPV, and it was clarified as a distinct species in Group I *Alphabaculovirus* [7].

To understand the NPV from the golden birdwing butterfly larvae, the Kimura 2-parameter (K-2-P) distances between the alignment of the *polh*, *lef-8* and *lef-9* nucleotide sequences were performed as described by Jehle et al. for baculovirus identification and species classification [8]. According to the analysis of K-2-P distances from these three genes, this NPV belongs to the group I baculoviruses and is highly closely related to the *Autographa californica* nucleopolyhedrovirus (AcMNPV) group [4]. However, most of the distances between this NPV and other closely related NPVs were higher than 0.015. The K-2-P results also showed an ambiguous taxonomic position for this virus; therefore, the taxonomic status of this virus still requires further clarification. Thus far, we could conclude that this NPV belongs to neither the BmNPV group nor the AcMNPV group. Therefore, this NPV was provisionally named “TraeNPV” [4].

As aforementioned, we attempted to sequence the whole genome of TraeNPV. Furthermore, a phylogenetic analysis based on 37 baculovirus core genes of 77 sequenced baculoviruses will be analysed to clarify the TraeNPV taxonomic issue. The genomic features of the whole genome, including the gene structure, orientations and genome density will be described in this report. Comparative genomic analyses were also performed, and the genome sequences were further compared in detail with the previously published group I NPV type species including AcMNPV [9], *Bombyx mori* NPV (BmNPV) [10], *Maruca vitrata* MNPV (MaviMNPV) [11], group II NPV type species LdMNPV [12] and one Betabaculovirus, the *Cydia pomonella* granulosis virus (CpGV) [13]. This report provides new insight into evolutionary aspects of butterfly-infecting NPVs. Therefore, the precise relationship between TraeNPV and other closely related NPV species could be further investigated.

Results and discussion

General characteristics of the TraeNPV genome

The TraeNPV genome is 125,477 bp in length and has a G + C content of 40.35% (see Additional file 1: Table S1). The complete genomic sequence with gene annotation information was submitted to GenBank (accession number: MH077961). The open reading frames (ORFs) were predicted according to the initial criteria for further study. A total of 144 ORFs were identified for further analysis (Fig. 1; Additional file 1: Table S2), and the nucleotides in the TraeNPV genome were numbered sequentially, beginning with the A (designated position 1) of the polyhedrin start codon (ATG). The arrows indicate the directions of the transcripts. The ratio of the ORF orientations was approximately 1:1.06 [clockwise (70/144): anticlockwise (74/144)] for those oriented clockwise with respect to the orientation of the *polh* gene (ORF1) [14]. The TraeNPV genome had a high number of ORFs, which ranked 18.99% (15/79) compared to the other 78 sequenced baculovirus genomes (Additional file 2: Figure S1). Among these putative ORFs, 40.97% (59 ORFs) showed overlap in the genome, and the length of the overlap ranged from 1 bp to 158 bp. Four pairs of ORFs that had a larger overlap than that found in TraeNPV were identified, namely, Ta59 (lef-3)/Ta60 (ac68), Ta72 (ac81)/Ta73 (tp20), Ta106 (ac121)/Ta107 (ac122) and Ta5 (38.7 K)/Ta6 (lef-1). Ta59 overlaps with Ta60 by 52 aa in the opposite ORF direction. Ta72 overlaps with Ta73 by ca. 50 aa. There were ca. 36 aa of overlap between the ORFs Ta106/Ta107 and Ta5/Ta6. There were 37 conserved genes in...
all the baculovirus genomes, including the dipteran and hymenopteran baculoviruses [15–18], and all of these genes were found in the TraeNPV genome. Except in the TraeNPV genome, the Ac108 was found in all the alpha- and betabaculovirus genomes [19]. Moreover, two baculovirus-repeated ORFs (the bro genes bro-a and bro-a) were also identified in this genomic sequence. Most of the 144 TraeNPV ORFs had related homologues in other baculoviruses except for two unique ORFs (Ta75 and Ta139), which were identified in the TraeNPV genome (Fig. 1; Additional file 1: Table S2).

In addition to these 144 predicted ORFs, other internal spaces were made up of intergenic spaces and common DNA non-coding functional elements (nfes), i.e., homologous regions (hrs). The TraeNPV genome exhibited 8 hr (hr1 ~ 8) (Fig. 1; Additional file 1: Table S2), and the orientations of the hrs were similar to those of AcMNPV. A conserved non-protein-coding genomic element (CNE, 156 bp), which was identified as a member of the Alphabaculovirus genus and was speculated to play a role in viral replication, was also found in the TraeNPV genome [20]. The CNE of TraeNPV is located from 118,740 bp to 118,895 bp. For the CNE structures, the seven conserved nucleotide clusters (C1~C7) in the CNE were also found in the CNE of TraeNPV. According to the structure and nucleotide composition, the conserved nucleotide clusters could also be further divided into dyad symmetry elements (DSs) and TAT-containing sequences (Fig. 2a). In the CNE of TraeNPV, three inverted repeats (IR) are presented in the DS left (DSl), DS central (DSc) and DS right (DSr) regions (Fig. 2a). Regarding the orientation of the CNE in TraeNPV, the location of the CNE lacked any ORF overlap in the TraeNPV genome; by contrast, the AcMNPV CNE overlapped with Ac152 (Fig. 2a). The identity of the CNE showed the highest shared sequence identity (96%) with that of AcMNPV, while the sequence composition of the TraeNPV CNE (AT content 73.8%) revealed a higher AT content than that of AcMNPV (AT content 68.6%).
Based on the experimental data obtained using a CNE-deficient AcMNPV bacmid, the CNE was demonstrated to be a polyfunctional genomic element involved in an essential role in AcMNPV pathogenesis [20]. Moreover, it also demonstrated that the CNE position would not impact the function of the CNE, suggesting that the CNE of TraeNPV might share a similar pathogenesis ability.

Taxonomic position and phylogenetic analysis of TraeNPV

The phylogenetic analyses of TraeNPV were performed using NJ and ML methods, and the results were inferred from a data set that combined the amino acid sequences of the 37 baculovirus core genes from 77 whole genomic sequenced baculoviruses (Additional file 1: Table S3) [5, 16]. Both of the phylogenetic trees showed a similar result, and the ML trees revealed higher bootstrap values and are shown in Fig. 3. The family Baculoviridae consists of five major clades, the NPVs infecting Lepidoptera (including groups I and II), the GV, the hymenopteran-specific NPVs, and CuniNPV. This analysis reflected the current systematic assignment of the viruses. Moreover, two subclades within lepidopteran NPV group I resembled the AcMNPV and OpMNPV. The result also indicated that TraeNPV was grouped together with AcMNPV (Fig. 3).

From our previous data, although we attempted to clarify the classification of TraeNPV and its closely related NPVs by K-2-P analysis based on the sequences of polh, lef-8 and lef-9, TraeNPV apparently had an ambiguous relationship with its closely related viral species. The results revealed that TraeNPV belonged to the group I baculoviruses and was highly closely related to the BmMNPV and AcMNPV groups [4]. By contrast, the distances for polh between TraeNPV and the PlxyNPV, RoNPV, AcMNPV groups exceeded the thresholds of the different viral species, and for all the concatenated polh/lef-8/lef-9 sequences, the distances were apparently much greater than the threshold of the same viral isolates; therefore, the limited data indicate an ambiguous situation for TraeNPV [4, 8].

From the comparative genomic studies, the conservation of the general mechanisms underlying baculoviral biology could be speculated; thus, the 37 core genes shared by all the sequenced baculovirus genomes might not only represent the similar function in the mode of viral infection, but they could also reflect the most realistic taxonomic position [20, 21]. Through the whole genome sequencing and the phylogenetic analysis based on 37 baculoviral core genes, it was revealed that TraeNPV is closely related to AcMNPV rather than BmNPV.
Genome-wide comparisons

Comparisons of whole genomes and the gene arrangements of the selected ORFs were performed with CGView, Mauve and a gene parity plot analysis. For the whole genome comparisons, TraeNPV showed a highly similar genomic fragment identity compared to AcMNPV and BmNPV, while a lower shared genomic identity was found between TraeNPV and MaviNPV (Additional file 3: Figure S2). In addition, compared to the TraeNPV genome, there are three locations flanked by the ORFs of Ta22/Ta24, Ta74/Ta76 and Ta132/Ta141, which showed a lower shared identity with those of other baculoviruses (Additional file 3: Figure S2). A graphical interpretation of the homologous blocks in viral genomes from alphabaculoviruses from group I and II and from CpGV is shown in Fig. 4. This information also revealed that the conserved segments appeared to be internally free from the genome rearrangement of other baculoviruses; however, a locally collinear block (LCB) deletion between alk-exo (Ta118) and p35 (Ta119) was found in TraeNPV (Fig. 4). Moreover, the gene arrangement of the TraeNPV genome was highly collinear with that of AcMNPV, BmNPV and MaviNPV. For the gene parity plot analysis, the gene arrangement of the TraeNPV genome showed lower collinearity with LdMNPV and CpGV and the ORFs displayed a much more dispersed pattern (Fig. 5).
**Fig. 4** Mauve (multiple alignment of conserved genomic sequence with rearrangements) representation of alphabaculoviruses from group I and II and CpGV. The alignment was performed on collinear sequences in which NPV was a reference sequence and the polh gene was considered as a first ORF (Except AcMNPV). Coloured sections (bordered with a curve that indicates the level of nucleotide similarity) represent the homologous fragments of compared genomes. The section that is located beneath the X-axis shows the inversion of this genome fragment in comparison to the reference.

**Fig. 5** Gene parity plot analysis of TraeNPV in comparison with (a) AcMNPV, (b) BmNPV, (c) MaviNPV, (d) LdMNPV and (e) CpGV, as indicated. Axes: the relative position of each ORF; dots: ORFs.
A further comparison of the genomic fragments from Ta132 to Ta141 to that of AcMNPV revealed a 1576 bp DNA fragment insertion from the nucleotide positions 121,403 bp to 122,979 bp in the TraNPV genome (Fig. 2b). Within the inserted DNA fragment, one novel gene (Ta139) and one duplicate gene were found; in addition, the restriction enzyme profile also revealed a difference in the Ta132/Ta141 fragment relative to that of AcMNPV (Fig. 2b). Although TraNPV was similar to AcMNPV and BmNPV in terms of gene organization, the presence of a different region was found upon genome-wide analysis.

According to the comparative analysis of baculoviral genomes, baculoviruses are highly diverse in terms of their GC content, genome length, gene content, and gene organization. These characteristics could reflect the evolutionary history of baculoviruses in adapting to different hosts [21, 22]. Based on the gene content (two novel ORFs were found in TraNPV and were lacking 15 AcMNPV homologous ORFs) and genomic length (shorter than AcMNPV), TraeNPV might be distinct from AcMNPV.

**Comparison of TraeNPV ORFs with other baculoviruses**

TraeNPV shares 142 ORFs with AcMNPV, 136 ORFs with BmNPV, 124 ORFs with MaviMNPV, 90 with LdMNPV and 74 with CpGV. The average shared amino acid sequence identity between TraeNPV and AcMNPV, BmNPV, MaviMNPV, LdMNPV and CpGV were 90.96, 86.61, 78.71, 33.20, and 25.61%, respectively. Based on the presented data, TraeNPV is closely related to AcMNPV; of the 142 ORFs that are common to TraeNPV and AcMNPV, only 2 ORFs sharing 100% identity and 97 ORFs sharing > 95% identity were found. Of the other 43 ORFs, 18 ORFs sharing 95–90% identity, 12 ORFs sharing 89–80% identity and 13 ORFs sharing < 80% identity were found. It is notable that there were three ORFs, Ta95 (Ac106–107), Ta103 (Ac118) and Ta126 (odv-e18), that had low shared identities (39, 52 and 61%, respectively) compared to those of the AcMNPV homologues due to the variations in the amino acid lengths, suggesting that there might be amino acid variations between TraeNPV and AcMNPV.

In fact, the further analysis showed that variations were found in the amino acid lengths and identities between TraeNPV, AcMNPV and BmNPV (Figs. 1 and 6; Additional file 1: Table S2). In addition, it also showed clear amino acid length differences compared to those of MaviMNPV, LdMNPV and CpGV. TraeNPV lacks 15 ORFs in AcMNPV and 7 ORFs in BmNPV (Table 1). In addition, there are two pairs of adjacent AcMNPV ORFs (Ac58/Ac59 and Ac106/Ac107) that were fused together into single ORFs (Ta51 and Ta95, respectively) in TraeNPV. As reported for Rachiplusia ou MNPV-R1, the re-sequencing of these regions in AcMNPV-C6 indicated that the ORF pairs occurred as a single ORF in the AcMNPV-C6 stock [23]. Homologues of these ORFs were also found in other baculovirus genomes in which they were fused into a single ORF (Additional file 1: Table S2).

**TraeNPV structural genes**

TraeNPV contains 35 baculovirus structural genes, which were listed by Hayakawa et al. (2000), Jehle et al. (2006) and Thumbi et al. (2013) [5, 21, 24], and only the p15 (Ac87) gene was absent from the TraeNPV genome (Table 2). Of the 35 structural proteins, the P74 protein is associated with occluded virions and is required for oral infectivity [25, 26]; the VP1054 protein is required for AcMNPV nucleocapsid formation [27]; the P10 protein has been shown to be involved in the formation and stability of polyhedra and may influence cell lysis late in infection [28–30]; VP80 is associated with both ODV and BV in AcMNPV and OpMNPV [31, 32]; and ORF1629 is associated with the basal end of nucleocapsids and is essential for AcMNPV viability [33, 34]. The GP64 protein is the envelope fusion protein of the budded virus, and it is specific to the group I NPVs [35, 36]. Another envelope fusion protein that is functionally analogous to the GP64 protein called Ld130 is present in all lepidopteran and dipteran baculoviruses that have been completely sequenced, including those that contain gp64. The TraeNPV genome also contains these proteins, and it encodes both GP64 (Ta113) and Ld130 (Ta14). It has been suggested that Ld130 homologues may play a role in the ancient envelope fusion protein, and its fusion function was substituted by gp64; the co-existence of this gene with gp64 might occur because it has other essential functions [36]. There are several genes that encode capsid-associated proteins (vp39 and vp91), ODV envelope proteins (odv-e18, -e25, -e56, and -e66), DNA binding protein (p6.9), and the tegument protein (gp41) that is also associated with BV production [37, 38]. Most of these structural genes have highly shared identities in AcMNPV, specifically > 95% shared identities, suggesting that the structure of the TraeNPV may be similar to that of AcMNPV. The structural protein of TraeNPV shared a high similarity with that of AcMNPV, and there are four structural genes with slightly lower shared identities with AcMNPV, namely, polh (Ta1; 88%), gp64 (Ta113; 92%), odv-e18 (Ta126; 61%) and odv-e26 (Ta8; 89%) (Additional file 1: Table S2). It has been reported that the AcMNPV polh consists of a mosaic of group I and group II NPV-specific sequences and it has a chimerical structure [39]. Interestingly, a low shared identity (88%) for polh was found between TraeNPV and AcMNPV, suggesting that this difference may be related to a process in baculovirus evolution.
Transcription-specific genes

A total of 13 genes involved in late baculovirus gene transcription that are all present on other baculovirus genomes [5, 21, 24] are also present in the TraeNPV genome, including lef-4~12, 39 K, p47, vlf-1 and pe38 (Table 2). Of these genes, 10 genes (lef-4~6, 8~12, 39 k, and p47) are required for optimal levels of late gene transcription in the AcMNPV genome [40, 41]. These 10 proteins play a role in the virus-encoded RNA polymerase that recognizes a late promoter element, RTAAG (R = A, T, or G) [42]. Moreover, lef-4, lef-8, lef-9, and p47 form a minimal complex with late polymerase activity [43]. Additionally, a conserved gene, vlf-1 might, regulates very late gene transcription and may be involved in DNA processing [44–46]. These genes had high shared identities with AcMNPV, at 84–98%, suggesting that a similar mechanism for late gene transcription might occur in the Baculoviridae group.

DNA replication genes

A major group of conserved genes involved in DNA replication was described previously [5, 21, 24, 47]. AcMNPV and OpMNPV contain 5 genes that are essential for transient DNA replication (ie-1, lef-1, lef-2, lef-3 and helicase) and 5 non-essential ones that stimulate transient DNA replication genes (dna-pol, p35, ie-2,
These 10 genes are all present in the TraeNPV genome (Table 2). Six of these 10 genes (ie-1, lef-1, lef-2, lef-3, helicase and dna-pol) have previously been reported as essential DNA replication factors for baculoviruses, indicating that baculoviruses share a common DNA replication mechanism [50]. The other DNA replication genes, such as single-stranded DNA-binding protein (dbp1) and immediate-early gene (me53), which have been implicated in DNA replication, were also found in TraeNPV (Table 2) [51]. During viral infections, the host cell RNA polymerase II is often transactivated by genes such as ie-0, ie-1, ie-2 and pe38. These genes are conserved relative to those of AcMNPV (84–98%); however, a small variant form of the IE-2 protein was found between TraeNPV and other closely related NPVs (Fig. 7).

Although the TraeNPV IE-2 amino acid sequence shared 92% identity with that of the AcMNPV IE-2, the serine-rich and proline/glutamine-rich domains involved in activating a subset of early baculovirus promoters by AcMNPV IE-2 have a short deletion in the TraeNPV sequence (Fig. 7) [52]. A RING finger domain, which is required for cell cycle arrest, E3 ubiquitin ligase activity, and nuclear focus association; and a predicted coiled-coil region (coiled-coil-II), which is involved in self-interaction and association with nuclear foci, were strongly conserved in TraeNPV IE-2 and AcMNPV [53–56].

The TraeNPV genome encodes two PCNA proteins (Ta40 and Ta41), and both proteins had low shared amino acid identities with AcMNPV (53 and 36%). Further investigation revealed that a single DNA base deletion resulted in two truncated forms of PCNA proteins, while a proliferating cell nuclear antigen PCNA protein may be involved in viral DNA replication, DNA recombination or DNA repair, but not the essential function of DNA replication, suggesting that the side effect of DNA replication may present differences between different viral species and hosts [57, 58].

### Genes with auxiliary functions

Auxiliary genes are not essential for viral replication, but they provide a selective advantage for increasing the virus production/survival at either the cellular or organismal level [21]. A total of eighteen auxiliary genes have homologues in TraeNPV (Table 2). These auxiliary genes in TraeNPV were 90–100% identical in terms of amino acid sequences compared to those of AcMNPV, except for alk-exo and arif-1. The TraeNPV alk-exo was 81% identical to AcMNPV and its arif-1 was 72% identical to that of AcMNPV. According to the analysis, the lower shared identities were caused by amino acid length variations. The arif-1, which is involved in the sequential rearrangement of the actin cytoskeleton, is found only in NPVs [59]. Therefore, it may contribute to the morphological differences between different NPV and GV-infected cells.

### Homologous regions (hrs)

Homologous regions (hrs) is one of the feature found in most baculovirus genomes and locate in multiple sites in the genomes [60]. The structure of each hr contains a palindrome, which is flanked by direct repeats. Hrs function as origins of NPVs and GV's replication [61] and also serve as RNA polymerase II-mediated transcription enhancers in early baculovirus promoters in NPVs [62]. Recently, it has been reported that no single homologous repeat region is essential for the DNA replication of AcMNPV [63].

The TraeNPV genome contained eight homologous repeat regions (hr1, hr2, hr3, hr4, hr5, hr6, hr7 and hr8)
that included one to eight palindrome repeats for a total of 30 repeats (Fig. 8a and c) and accounted for 0.72% of the genome. Similar to the AcMNPV palindrome sequence [9], the TraeNPV hr palindrome consensus GHKTTACRAGTAGAATTCTACDNGTAAHVC shows a 23/30 matched palindrome (Fig. 8b) and the palindromic consensus sequence included seven highly variable positions (Fig. 8b). All the nucleotides in the palindrome were conserved, except for the twenty-second nucleotide. In addition, the LdMNPV consensus hr palindrome shared 43.3% of its sequence identity with the TraeNPV consensus hr sequence (Fig. 8b). The genomic positions of the TraeNPV regions hr1 - hr8 were conserved with the genomic positions of AcMNPV [9]; however, a lack of AcMNPV hr2-a was found in the TraeNPV genome (Fig. 8c).

**Baculovirus repeated ORFs (bro genes)**

A striking feature of most lepidopteran and dipteran NPVs sequenced to date and in some of the GVs is the presence of one to 16 copies of bro genes. Typically, bro genes are highly conserved, repetitive and widely distributed amongst insect DNA viruses [64]. The function of these genes is unclear, but they have been shown to bind to DNA [65]. These genes have also been found to be associated with the viral genome rearrangement regions [66]. During the process of baculovirus replication, the viruses that synthesize mRNAs in the nucleus and this mRNA should be exported to the cytoplasm, while some viral proteins produced in the cytoplasm must be imported into the nucleus. It was demonstrated that the BRO proteins of BmNPV play a role in the function of the nucleocytoplasmic shuttling proteins that utilize the CRM1-mediated nuclear export pathway [67].

TraeNPV contained two bro genes, which were named bro-a and bro-b based on their order in the genome (Fig. 1; Additional file 1: Table S2). Most BROs contained a core sequence of 41 aa at the N-terminal half and several different domains throughout the sequence. The bro gene family has been divided into four groups based on the similarity of those domains [12]. Both of the TraeNPV bro genes, namely Ta-bro-a (Ta141) and -b (Ta142) (which were homologues of Bm-bro-d) belong to group III. Moreover, two TraeNPV bro genes encoded small fragments of truncated protein (234 aa and 92 aa). It has been reported that mutations in the leucine-rich region of Bm-BRO proteins resulted in the nuclear accumulation of transiently expressed proteins; however, the mutant Bm-BRO-D with an altered nuclear export signal (NES) did not show nuclear accumulation in the infected cells due to a reduction in RNA synthesis [67], suggesting that the truncated BRO protein in TraeNPV may share a similar function as that of Bm-BRO-D.

**Genes involved in host range determination**

Baculoviruses usually showed high specificity to a few, or even individual, insect species [68, 69]. For this reason, a variety of efforts has been made to understand the baculoviral genes that are related to their host range. Many viruses encode a variety of proteins related to the host range; AcMNPV is the most widely researched member of the Baculoviridae. AcMNPV contains several genes that involved in host range determination, including p143 (helicase), hrf-1 (host range factor 1), hcf-1 (host
cell-specific factor 1), ie-2 and p35 [69–72]. Of these genes, p35 and iap (inhibitor of apoptosis), are two major families of anti-apoptosis genes, which are commonly found in baculovirus genomes [73, 74].

The inhibition of vary caspase pathways by the p35 and its homologue p49 have been demonstrated [75]. The p35 and p49 are found in few sequenced baculoviruses, such as AcMNPV and Spodoptera litura MNPV (SpltMNPV) [9, 76]. For another anti-apoptosis gene family, the anti-apoptotic inhibition of IAP proteins has been demonstrated either directly or indirectly during baculovirus infection in permissive cells or heterogeneous insect cells in AcMNPV, Anticarsia gemmatalis MNPV (AgMNPV), Cydia pomonella granulovirus (CpGV), Epiphyas postvittana NPV (EppoNPV), Helicoverpa armigera NPV (HearNPV), Hyphantria cunea NPV (HyCuNPV), Leucania separata MNPV (LeseMNPV), Orgyia pseudotsugata MNPV (OpMNPV), S. littoralis NPV (SpliNPV) and LyxyMNPV [75, 77–88]. Similar to AcMNPV, in the TraeNPV genome, p35 (Ta119) and two iaps, iap1 (Ta18) and iap2 (Ta62), were identified. The amino acid identities of these three proteins are 97, 95 and 84% shared with those of AcMNPV; it is speculated that these proteins might share similar activities in the host cells.

Recently, ld-apsup (ld109), a novel gene that inhibits apoptosis in LdMNPV-infected Ld652Y cells, was identified and its anti-apoptotic activities and mechanism were demonstrated [89, 90]. According to a survey of the genome data, AcMNPV (Ac112–113) and other 17 baculoviruses contained aapsup homologue genes in their genomes [89]. Interestingly, a lack of Ac112–113 was found in the TraeNPV genome (Table 1), and more extensive experiments may be performed to investigate the host range issue.

TraeNPV truncated and duplicate genes
There were three truncated ORFs (pcna-a/pcna-b, he65-a/he65-b and bro-a/bro-b) and one duplicated ORF (38.7 K in the Ta5 and Ta138 locations) located in the TraeNPV genome. All of the truncated ORFs showed low shared identities with their homologues in
AcMNPV. For pcna-a/pcna-b (Ta40/Ta41), the amino acid identities are 53 and 36% shared, respectively, compared to Ac49; 4 and 12% in he65-a/he65-b (Ta93/Ta94) compared to Ac105; and 56 and 16% in bro-a/bro-b (Ta140/Ta141) compared to Ac2. For these truncated genes, the nucleotide deletions leading to the introduction of stop codons were found in both pcna-a/pcna-b (Ta40/Ta41) and bro-a/bro-b (Ta140/Ta141). For pcna-a/pcna-b (Ta40/Ta41), a one-bp deletion was found in the downstream 398 bp (+ 398 bp) of ac- pcna; this deletion resulted in the introduction of a stop codon (TGA) in the + 434 bp, and thus a second pcna-b start codon was found between + 436 bp and the end of this gene. In bro-a/bro-b (Ta140/Ta141), a seven-bp deletion was found 222 bp downstream (+ 222 bp) of ac-bro, and this deletion resulted in the introduction of a stop codon (TGA) in the + 284 bp. Thus, a second bro-b start codon was found between + 283 bp and the end of this gene. For he65-a/he65-b (Ta93/Ta94), instead of the full-length he65 (553 aa) in AcMNPV, TraeNPV encoded two smaller proteins, he65-a (58 aa) and he65-b (72 aa). The HE65 protein is one of the RNA ligase families, and it acts as an early transcription gene involved in RNA replication, transcription and modification as well as in G-actin localization in the nucleus during AcMNPV cell infection. Although truncated he65 was found in the genome, it is considered a nonessential protein for AcMNPV and BmNPV [91, 92].

One pair of genes (Ta5/Ta138) was identified as duplicated homologs of 38,7 K in the TraeNPV genome. This duplicate gene (Ta138) showed low shared identities with the homologues of AcMNPV (15%).

Unique TraeNPV ORFs
Two genes are unique in the TraeNPV genome, including Ta75 and Ta139 (Fig. 1; Additional file 1: Table S2). These unique ORFs were small in size (55–60 aa). Both Ta75 and Ta139 had no any baculovirus homologue and no significant BLAST database hit. However, the promoter region should be predicted in the future to evaluate the transcriptional contribution to TraeNPV.

Comparison of TraeNPV to AcMNPV
Based on the sequence analysis, TraeNPV was highly similar to AcMNPV. The phylogenetic analysis indicated that TraeNPV belonged to Alphabaculovirus Group 1. However, there were still some distinctions in the genomic features and gene content between these two viruses. The most significant difference between TraeMNVP and AcMNPV was that the TraeNPV genome is 8417 bp smaller than the AcMNPV genome (133,894 bp) and it contains 15 fewer ORFs (Table 1), while the TraeNPV genome contained two ORFs that were not found in the AcMNPV genome (Additional file 1: Table S2). Moreover, according to the data on the in silico restriction enzyme fragment length polymorphism (in silico RFLP) pattern using BamHI, TraeNPV showed a different pattern compared to that of AcMNPV (Additional file 4: Figure S3). The AcMNPV genome contains 15 ORFs, which were not found in the TraeNPV genome. Two genes that encode HCF-1 and APSUP were described as the host range determination factors in baculoviruses [89, 90]. It has been demonstrated that the AcMNPV HCF-1 protein is an essential viral factor for the productive NPV infection of TN-368 cells [93, 94]. Recently, a novel anti-apoptotic protein, APSUP, was identified in LdMNPV [95]; moreover, it has been demonstrated that the full-length Ld-Apsup could work against the apoptosis of Ld652Y cells induced by exposure to actinomycin D and UV and could interact with Ld-Dronc to prevent cells from undergoing apoptosis. The baculovirus host range likely involves a complicated array of viral and cellular factors. Based on the data from the genomic analysis, a lack of Ac112–113 was found in the TraeNPV genome (Table 1), and more extensive experiments may be performed to uncover more evidence regarding the host range issue.

There were 142 ORFs in common between TraeNPV and AcMNPV, and their order is mostly identical. However, several of these ORFs had different lengths, as shown in Fig. 6. These genes included arif-1, IAP2, vp91/p95, pp34, alk-exo, odv-e18, and ie-2 as well as other genes with unassigned functions. Moreover, three pairs of truncated genes were found in the TraeNPV genome, namely, pcna-a/pcna-b, he65-a/he65-b and bro-a/bro-b. These truncated genes also showed amino acid length variations between TraeNPV and AcMNPV (Fig. 6). The hrs of TraeNPV are similar to those of AcMNPV in terms of their position, numbers and orientations, while there was no hr2a in TraeNPV. The gene content, ORF length, and hr are possible candidates for regulators of the different virulence levels between two closely relative species [67], which might be the case for TraeNPV and AcMNPV.

Conclusions
In conclusion, TraeNPV showed a high degree of collinearity and shared sequence identity with AcMNPV. However, these two viruses showed different host ranges and geographical distribution. To date, TraeNPV has only been isolated from T. aeacus, which is a native butterfly species under conservation in Taiwan. Furthermore, although the genome sequence analysis revealed that TraeNPV lacks 15 homologous genes from AcMNPV, TraeNPV gained two novel unique genes. Interestingly, there were two host range determination genes, hef-1 and apsup, in AcMNPV (and also in other
alphanbaculoviruses) that were not found in TraeNPV. These findings were very interesting and worthy of further studies to collect more evidence about the host range issue. Based on our analytical data, TraeNPV would be clarified as a new NPV species, which has defective AcMNPV genomic features. The lack of hcf-1 and apsupe in the genomic sequence data for TraeNPV could provide useful information for understanding the baculoviral host ranges and for gaining evolutionary insights.

**Methods**

**Viral DNA extraction and DNA sequencing**

Diseased *T. aeacus* larvae samples were homogenized in 1.7 mL microcentrifuge tubes and then examined under a light microscope for viral occlusion bodies (OBs). To obtain the OBs, the samples were centrifuged at 14,000×g at 4°C for 10 min and the supernatants were removed. The pellets were washed in 1× TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 7.6) and centrifuged three times at 14,000×g at 4°C for 10 min. The pellets were then resuspended in 1× TE buffer with a final concentration of 1% (w/v) SDS and then incubated with proteinase K (0.25 mg/ml) at 56°C for 3 h. The total DNA (including the host and viral DNA) was extracted using previously published methods [96]. A sequencing library was prepared following the standard protocol from the NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB) and sequenced with an Illumina MiSeq sequencer with paired-end (PE) technology for 2×300 bp.

**Data pre-processing and bioinformatics analysis**

The total PE reads were conducted for sequencing adapter identification and then trimmed by cutadapt [97]. Ambiguous bases and bases with lower quality values were removed by PRINseq [98] from either the 5’- or 3’-end. The final high quality reads were selected using NGS QC Toolkit [99] with the default parameters (Additional file 1: Table S4). These trimmed reads were then subjected to genome assembly and annotation by bioinformatics analysis (Additional file 5: Figure S4).

The strategy for TraeNPV genome assembly is to employ the longer paired-end (PE) reads. The genome assembly approach used in this study is reference-guided assembly, with benefits from the reference organism. The reference species is identified as the top-ranked assembly types, maps sequencing reads against reference GenBank. MIRA [100], one of the reference-guide assembly types, maps sequencing reads against reference species to generate the genome sequence of target species. Gap elimination was applied using an in-house script programme by mapping the quality PE reads and contigs iteratively until convergence was reached. Contigs are the joined paired-end reads found by using COPE [101] and assembled contigs were found by de novo assembly, with SOAPdenovo [102]. Draft genome gap filling and gene coding region validation were performed by Sanger sequencing to complete the final genome and gene annotation, respectively. The designed primer sets for PCR validation are listed in Additional file 1: Tables S5 and S6.

The genome annotations were performed with both NCBI ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) and Glimmer [103] to identify the open reading frames in the genome. Repetitive sequence regions were detected by RepeatMasker (http://www.repeatmasker.org/). CD-HIT and BLASTN in the NCBI BLAST package were used to identify the correctness of the predicted genes and the corresponding sequence identities. A circular map of the viral genome was generated by CGView [104].

**Phylogenetic analysis**

The phylogenetic tree was inferred from a data set of concatenated amino acid sequences from the 37 baculovirus core genes [5, 16] of the 77 baculoviruses that were completely sequenced at the time of analysis (Additional file 1: Table S3). A maximum likelihood (ML) analysis was performed using MEGA version 7.0 [105]. *Culex nigripalpus* NPV (CuniNPV) [106] was selected as the out-group. A bootstrap analysis was performed to evaluate the robustness of the phylogenies using 100 replicates for ML analysis.

**Comparative genomic analysis**

Both the whole genome and all the putative ORFs of TraeNPV were subjected to a comparative genomic analysis with 4 alphabaculoviruses (3 group 1 NPVs and 1 group 2 NPVs) and 1 betabaculovirus using the CGView Comparison Tool (CCT) [107]. Moreover, the multiple alignment of conserved genomic sequence with rearrangements was performed by Mauve [108].

**Additional files**

*Additional file 1*: Table S1. Characteristics of current sequenced baculovirus genomes. 1n/a = no information is available in either the paper or GenBank file. 2The GenBank file with accession number KX1513952 is not available at the GenBank website. 3Virus name printed in bold were used for further comparison. *Table S2*. ORFs predicted in the TraeNPV genome. *Table S3*. ORF numbers of the 37 baculovirus core genes from 77 baculoviruses. *Table S4*. Sequencing library statistics. *Table S5*. Primer sets for validating the genome assembly and gap filling. 1A total of 103 primer sets were used in this study. The average amplicon size is ca. 1150 bp and the re-sequenced coverage is ca. 1. *Table S6*. Primer sets for validating the gene coding regions. (XLSX 99 kb)

*Additional file 2*: Figure S1. Genome density of TraeNPV compared to 78 sequenced baculoviruses. Genome density = number of ORFs/genome size; ratio of genome density = relative genome density to that of TraeNPV. The number behind the column represents the order of the
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Competing interests

Not applicable.

Ethics approval and consent to participate

read and approved the final manuscript.

THC and YSN purified the virus genomic DNA. YSN, THC, YFH, ZTC, SJL, JCK, YFH and THC carried out the experiments and analysed these sequences.

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Availability of data and materials

GenBank accession number: MH077961.

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YFH and THC carried out the experiments and analysed these sequences. THC and YSN purified the virus genomic DNA, YSN, THC, YFH, ZTC, SJL, JCK, and JSK analysed sequences and confirmed the validation results. CHW, KPC, YFH and YSN carried out the design and draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

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