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Host whole-genome sequencing reveals *Scutavirus chelonidalpha5* infection in sea turtles from Singapore

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Fibropapillomatosis (FP) is a tumor-forming disease affecting sea turtles worldwide, linked to *Scutavirus chelonidalpha5* (formerly known as chelonid alphaherpesvirus 5, ChHV5) and influenced by environmental stressors. Despite extensive studies elsewhere, FP has not been confirmed in wild sea turtles in Singapore, a highly urbanized city-state with heavily modified coastlines. Here, we report the occurrence of lesions suggestive of FP in Singapore observed in two stranded green turtles (*Chelonia mydas*) with external and internal tumors. To assess viral presence, we analyzed whole genome sequencing datasets generated from blood and tissue samples of four green turtles and four hawksbill turtles (*Eretmochelys imbricata*), including individuals with and without visible tumors. Two complementary approaches were used: reference-based analysis (BWA-MEM) and metagenomic analysis (Kraken2). *Scutavirus chelonidalpha5*-associated reads were detected in all eight genome datasets, with both approaches providing consistent and cross-validated detection of viral sequences. Viral sequences were detected in all genome datasets, with no significant difference between turtles with and without tumors, suggesting that additional host and environmental cofactors such as immune status, microbiome composition, and anthropogenic stressors likely modulate disease expression. This study illustrates the value of genomic surveillance for detecting latent viral infections in both clinically affected and apparently healthy turtles and supports the need for integrated genomic and ecological surveillance of sea turtle health which can inform conservation strategies, improve ecosystem management, and support the resilience of sea turtle populations in Southeast Asia.

KEYWORDS
environmental health, marine turtle conservation, metagenomic surveillance, urbanization, WGS

Introduction

Fibropapillomatosis (FP) is an epizootic disease primarily affecting green turtles (*Chelonia mydas*) but has been recorded in all species of sea turtles (Herbst, 1994; Huerta Rodriguez et al., 2002; Jones et al., 2016; Robben et al., 2023; Whilde et al., 2024). It is characterized by cutaneous tumors that can impair vision, movement, and feeding, and may

even lead to mortality (Foley et al., 2005). The exact etiology of FP has not yet been elucidated, but previous research suggests that FP has been associated with *Scutavirus chelonidalpha5* (previously referred to as chelonid alphaherpesvirus 5, ChHV5), and its emergence and prevalence are believed to be influenced by environmental factors, particularly degraded water quality, eutrophication, and nutrient enrichment (Dos Santos et al., 2010; Dujon et al., 2021).

Fibropapillomatosis is recognized as a significant infectious disease affecting sea turtles worldwide (da Cruz et al., 2024), with prevalence varying widely across regions and populations (Alfaro-Núñez et al., 2014). While FP-associated tumors can cause severe illness and mortality, numerous studies have demonstrated that *Scutavirus chelonidalpha5* can be detected in clinically normal turtles (Page-Karjian et al., 2012; Alfaro-Núñez et al., 2014; Mashkour et al., 2021), suggesting that infection alone is unlikely to trigger disease expression. This supports the hypothesis that FP is a multifactorial disease, where viral infection interacts with host immunity and environmental stressors to drive tumor development.

Recent advances in genomic sequencing have enabled deeper insight into the viral and host genetic factors involved in FP. Whole genome analyses of *Scutavirus chelonidalpha5* from different geographic locations have revealed large genetic diversity between regions (Whitmore et al., 2021), which may influence the virus's pathogenicity and the clinical manifestations of FP in sea turtles. Additionally, genome-wide studies of green turtles have begun to identify host genetic variation that could affect susceptibility or resistance to infection and tumor development (Bentley et al., 2023; Nash and Ryan, 2023). Such genomic approaches are critical to understanding the complex interactions between *Scutavirus chelonidalpha5*, the host genome, and environmental stressors that together drive FP pathogenesis.

Despite increasing recognition of FP as a global conservation concern, large geographic data gaps remain in disease surveillance, particularly in Southeast Asia. This region encompasses some of the most heavily urbanized coastlines globally, where sea turtles are exposed to several anthropogenic stressors including habitat modification and pollution. Such conditions may facilitate both viral persistence and disease emergence, yet investigations of FP and *Scutavirus chelonidalpha5* in the region remain scarce (Hargrove et al., 2016). The earliest known record in the region, to our knowledge, dates back to 1958 when FP was reported to affect nesting green turtles from the Sarawak Turtle Islands, Malaysia (Hendrickson, 1958). Subsequent studies have documented FP in Indonesia (Adnyana et al., 1997; Devi et al., 2021) and Taiwan (Li et al., 2017). More recently, molecular evidence of *Scutavirus chelonidalpha5* infection has been recorded in green, hawksbill, and olive ridley (*Lepidochelys olivacea*) turtles in Borneo, Malaysia, including in individuals without visible tumors (Robben et al., 2023). However, there has been no prior confirmed report of FP in wild sea turtles in Singapore.

Singapore is a densely populated city-state known for its rapid urban development and highly industrialized coastline (Sin et al., 2016). Despite its small land area, Singapore's coastal waters support diverse marine life, including populations of green turtles and hawksbill turtles (*Eretmochelys imbricata*), both of which forage in its waters, with hawksbill turtles also nesting on suitable sandy beaches (Cham et al., 2024). However, the intense urbanization and land reclamation activities over the past decades have drastically altered Singapore's natural coastline (Sin et al., 2016), leading to habitat loss, fragmentation, and chronic pollution.

Given Singapore's highly urbanized coastline and persistent marine pollution challenges (Sin et al., 2016), the potential emergence of FP is of ecological and conservation concern. Pollution associated with urbanization can weaken marine organisms' immune systems, increasing their susceptibility to diseases such as FP (Sepp et al., 2019). Additionally, anthropogenic nutrient enrichment and degraded water quality have been linked to higher FP prevalence, suggesting that heavily impacted coastal ecosystems may present elevated disease risks (Jones et al., 2016). In this study, we document the first detection of *Scutavirus chelonidalpha5* in sea turtles from Singapore, alongside lesions suggestive of FP. Leveraging host whole genome sequencing, we screened green and hawksbill turtles, both with and without visible tumors, to assess viral occurrence and explore host-pathogen dynamics in this urbanized marine environment.

Methods

Sample collection

Samples were collected from four stranded green turtles (*Chelonia mydas*), and four live hawksbill turtles (*Eretmochelys imbricata*) that were found stranded or nesting in Singapore between 2020 and 2022 (Figure 1). Of the green turtles, two individuals were recovered alive with visible tumors, found floating near the coasts of East Coast Park and Lazarus Island, Singapore, in August 2021 and August 2022, respectively (Figure 1). They were collected and transported by the National Parks Board (NParks) to Singapore Oceanarium, Resorts World Sentosa (formerly S.E.A. Aquarium) for veterinary health assessment.

Histopathological confirmation of tumors was not possible due to lack of access to veterinary diagnostic records; therefore, lesions were classified based on gross morphology. Sample collection varied among individuals due to logistical and sampling constraints. Blood samples were collected from the six live individuals, while liver and muscle tissues were obtained opportunistically from the dead turtles depending on sample availability and condition (Table 1). As a result, not all sample types were available for every individual.

DNA extraction and sequencing

All samples were stored at −20 °C prior to DNA extraction. For blood samples, DNA was extracted using the QIAGEN Blood and Cell Culture DNA Kit (QIAGEN, Germany) and the Genomic-tip procedure (QIAGEN, Germany). For muscle and liver tissues, DNA was extracted using the QIAGEN Puregene Tissue Kit (QIAGEN, Germany) following the manufacturer's protocols. DNA quantity and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and Qubit fluorometer (Invitrogen, USA), respectively. DNA integrity was verified by

agarose gel electrophoresis prior to sequencing. The genomic DNA samples were used to construct Illumina TruSeq Nano (150 bp paired-end) libraries that were sequenced on the Illumina HiSeq X platform (Illumina, USA), targeting approximately 30x genome coverage. The generated raw sequencing reads were assessed for quality using FastQC v. 0.11.8 (Andrews, 2010) and subsequently trimmed to remove adapter sequences using cutadapt v. 2.10 (Martin, 2011).

Data analysis

To assess the presence of *Scutavirus chelonidalpha5*, two approaches were used. First, a reference-based analysis was performed by aligning quality-filtered paired-end reads from each turtle's whole-genome sequence against the *Scutavirus chelonidalpha5* reference genome (GenBank Accession: HQ878327.2) using the BWA-MEM algorithm (Li and Durbin, 2009). Given the large difference in genome size between *Scutavirus chelonidalpha5* (~170 kb) and sea turtle host genomes (~2.1 Gb), viral sequences were expected to comprise only a very small fraction of total reads in the turtle whole-genome sequencing datasets. Default scoring parameters were applied, including a match score of 1, mismatch penalty of 4, gap open penalty of 6, gap extension penalty of 1, and minimum seed length of 19. Post-alignment filtering was performed using SAMtools (Li et al., 2009) to retain only primary mapped read pairs (flag -F 2308), ensuring that unmapped, secondary, and supplementary alignments were excluded from the final count. The whole sequence reads, including turtle sequences, were aligned to the viral genome using BWA-MEM in order to maximize sensitivity for detecting low-abundance viral sequences potentially sequenced together with the host genomic DNA.

A complementary metagenomic analysis was also performed using Kraken2 (Wood et al., 2019). Sequence reads classified as host (*Chelonia mydas* or *Eretmochelys imbricata*) were removed, and the remaining host-subtracted reads were queried against the RefSeq viral database using default parameters, where the number of matches with the genus *Scutavirus* were retrieved and counted.

From the metagenomic analysis, we examined the taxonomic composition of the 40 most abundant taxa of each sample (Supplementary Figures 1, 2). The sample obtained for G21B exhibited a markedly different composition characterized by a high proportion of unclassified reads and biased taxonomic representation relative to all other samples. This is most likely due to the high contamination of the muscle sample as it was collected from a deceased stranded turtle. As this prevented reliable assessment of viral loads, G21B was excluded from downstream statistical analyses.

To account for variations in sequencing effort between individuals, both reference-mapped and metagenomic match read counts were normalized to the smallest number of total sequence reads. Statistical analyses were conducted to evaluate whether the count of reference-based viral reads differed by species and tumor presence. Because of the small and uneven sample sizes, non-parametric tests were used. Differences between two groups (species and tumor presence) were assessed using the Wilcoxon rank-sum exact test. To assess concordance between the reference-based (BWA-MEM) and metagenomic (Kraken2) detection approaches, a Spearman's rank correlation test was performed between normalized *Scutavirus* reads across the seven individuals. All tests were two-tailed, and statistical significance was determined at $p = 0.05$. Analyses were performed in R (version 2024.09.1 + 394; R Core Team, 2024).

Results

The stranded green turtles recovered from East Coast Park (G21A) and Lazarus Island (G22A) exhibited multiple external lesions on the neck and flippers. Internal examination revealed extensive pulmonary tumors that compromised lung integrity and buoyancy. The remaining green turtles and hawksbill turtles showed no visible external tumors at the time of sampling.

Using the reference-based analysis approach, *Scutavirus chelonidalpha5*-associated sequences were detected in all eight individuals, including those without visible tumors. Raw mapped reads were normalized to the smallest library size (223,847,886 reads) to allow for direct comparison across samples, with normalized read counts ranging from 30,869 to 98,326 reads (Table 2). The highest reference-based viral read count was detected in sample G22A, derived from a green turtle with visible external and internal tumors (Figure 2). Comparable levels of *Scutavirus chelonidalpha5*-mapped reads were detected in green turtles with and without visible tumors (G21A and G20A), while the lowest proportion was observed in a green turtle found with no tumors that was removed from analysis because of the high proportion of unclassified reads (Supplementary Materials). Viral sequences were detected at lower abundances in all four hawksbill turtles, none of which exhibited visible tumors.

The complementary metagenomic analysis using Kraken2 (Wood et al., 2019) corroborated these findings, yielding normalized reads between 178 and 488 (Table 2). Similar to the reference-alignment approach, higher normalized metagenomic reads were generally observed in green turtles compared to hawksbill turtles, with the highest counts detected in samples G21A and G22A, green turtles with tumors. To assess concordance between the two detection approaches, Spearman's rank correlation was performed between normalized reference-based viral reads (BWA-MEM) and metagenomic viral *Scutavirus* reads (Kraken2). A significant positive correlation was observed between approaches (Spearman's $\rho = 0.79$, $p = 0.048$) (Figure 2A). This concordance suggests that both methods are capturing the same underlying signal of viral presence, supporting the robustness of *Scutavirus chelonidalpha5* detection across independent analytical pipelines.

A higher number of reference-based viral read counts was observed in green turtles, but this difference was not statistically significant (Wilcoxon rank-sum exact test, $W = 12$, $p = 0.057$, Figure 2B), likely due to the small sample size. Hawksbill turtles exhibited consistently detectable viral sequences despite the absence of visible tumors. Additionally, no significant difference in viral read count was observed between turtles with and without visible tumors (Wilcoxon rank-sum exact test, $W = 1$, $p = 0.1905$). These analyses should be interpreted cautiously given the small and unbalanced sample sizes.

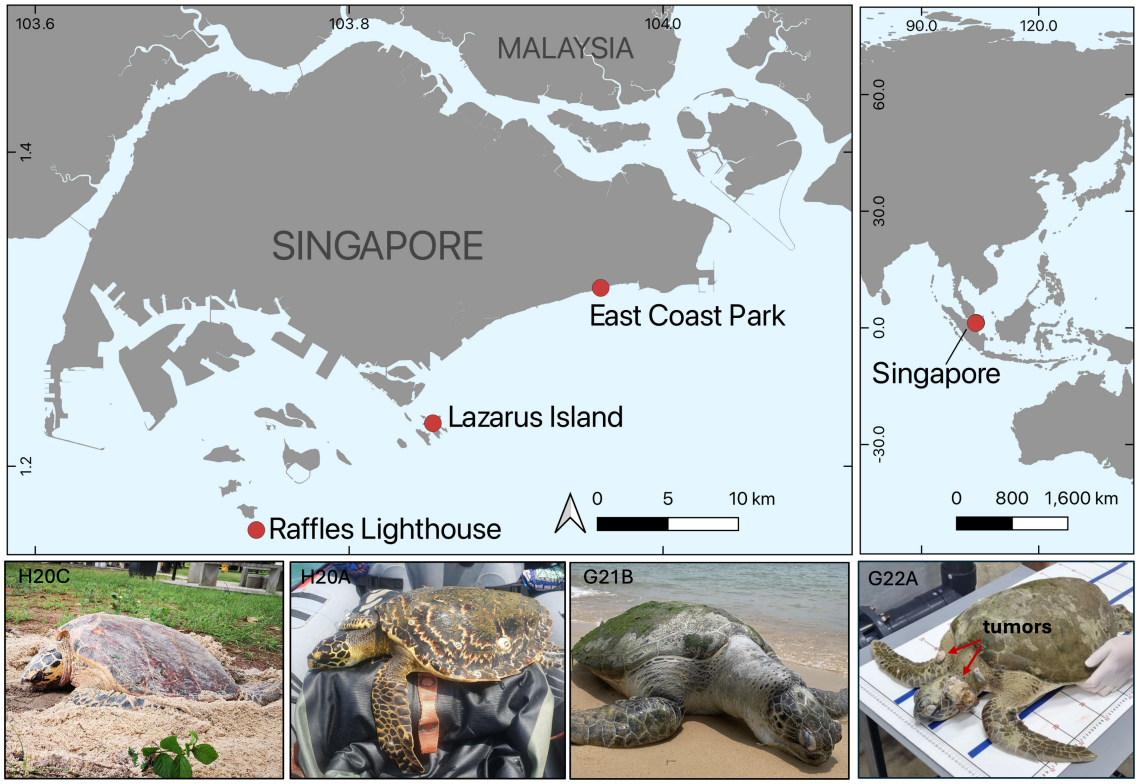

FIGURE 1
Map of Singapore showing the locations where the four stranded green turtles, two stranded hawksbill turtles, and two nesting hawksbill turtles were found. The main map highlights the specific coastal areas of East Coast Park (ECP), Lazarus Island, and Raffles Lighthouse where the turtles were found. The inset map shows Singapore's location within Southeast Asia. Photographs of four turtles, including one individual with visible tumors, are also presented. Photos, labelled as H20C, H20A and G21B, reprinted with permission from Singapore National Parks Board (NParks). Photo, labelled as G22A, reprinted with permission from Singapore Oceanarium, Resorts World Sentosa (formerly S.E.A. Aquarium).

Discussion

Fibropapillomatosis (FP) represents a growing conservation concern for sea turtle populations worldwide, yet its presence in Singapore's urbanized coastal waters has remained undocumented. Here, we report the first molecular detection of *Scutavirus chelonidalpha5* (previously chelonid alphaherpesvirus 5, ChHV5) in both green (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) in Singapore. Two green turtles exhibited multiple large external and internal tumors that severely impaired mobility and survival. It is important to note that tumors were classified based on gross morphology alone, as histopathological confirmation was not available due to logistical constraints. Consequently, the lesions are described as suggestive of fibropapillomatosis (FP) rather than definitively diagnosed. Interestingly, *Scutavirus chelonidalpha5* DNA was also detected in turtles without visible tumors, indicating that subclinical infections could be relatively common for both sea turtle species in Singapore's waters. No significant difference was observed in the number of *Scutavirus chelonidalpha5* reads between species, however, hawksbill turtles trended toward lower counts. Viral sequences were nonetheless consistently detectable in all hawksbill turtle samples despite the absence of visible tumors. These findings indicate that viral presence alone is insufficient to trigger tumor development and suggest additional factors, such as differences in host genetics and immune responses between the two species, play a contributing role.

Previous studies suggest that additional factors, such as microbiome composition and environmental stressors, may trigger FP tumor development by modulating disease expression (Dos Santos et al., 2010; Nash and Ryan, 2023; Iurk et al., 2024). Shifts in the host-associated microbiome may influence local immune function, inflammation, and viral latency dynamics, potentially facilitating *Scutavirus chelonidalpha5* reactivation or tumor progression. Concurrently, environmental stressors such as pollution, habitat degradation, elevated nutrient loads, thermal stress, and anthropogenic disturbance may impair host immune competence or alter microbial communities, thereby increasing susceptibility to FP (van Houtan et al., 2010; Dos Santos et al., 2010). Together, these findings support the hypothesis that FP emergence reflects an interaction between viral presence, host immune status, microbiome structure, and environmental conditions, rather than simple pathogen exposure alone.

Singapore's coastal waters, while subject to robust environmental management frameworks (Chng et al., 2022), still experience significant pressures from land reclamation, urban runoff, and pollution (Sin et al., 2016). Such stressors have been linked globally to increased FP prevalence and may facilitate viral persistence or disease expression, consistent with global observations linking FP prevalence and eutrophic, polluted coastal habitats (Dujon et al., 2021; Manes et al., 2022; Oduor et al., 2024). These conditions suggest that Singapore's highly urbanized coastline may provide an environment conducive to FP emergence. It is important to note

TABLE 1 Metadata and tissue samples collected from stranded green turtles (*Chelonia mydas*) and stranded or nesting hawksbill turtles (*Eretmochelys imbricata*) in Singapore between 2020 and 2022.

Sample ID	Species	Location	Date collected	Tumor presence	Sample origin	Sample type
G20A	*C. mydas*	East Coast Park	Oct 2020	Absent	Stranding	Liver
G21A	*C. mydas*	East Coast Park	Aug 2021	Present	Stranding	Blood
G21B	*C. mydas*	East Coast Park	Nov 2021	Absent	Stranding	Muscle
G22A	*C. mydas*	Lazarus Island	Aug 2022	Present	Stranding	Blood
H20A	*E. imbricata*	Raffles Lighthouse	Aug 2020	Absent	Stranding	Blood
H20B	*E. imbricata*	East Coast Park	July 2020	Absent	Nesting	Blood
H20C	*E. imbricata*	East Coast Park	July 2020	Absent	Nesting	Blood
H21A	*E. imbricata*	East Coast Park	June 2021	Absent	Stranding	Blood

Sampling locations, dates of collection, presence or absence of visible tumors suggestive of fibropapillomatosis (FP), and the samples collected for DNA extraction (blood, muscle, liver) are shown. Sample G21A and G22A were the only individuals exhibiting visible tumors.

that environmental parameters were not measured in this study, and therefore relationships between pollution, habitat quality, and viral presence cannot be inferred from the present dataset.

Our study leverages host whole genome sequencing (WGS) data from green and hawksbill turtles in Singapore to detect *Scutavirus chelonidalpha5* DNA, representing a novel genomic surveillance strategy that complements existing viral and environmental monitoring efforts. To further assess these detections, host read subtraction was performed using metagenomic analysis, and the resulting *Scutavirus*-classified reads were compared with reference-based alignment read counts across all seven individuals. The two approaches yielded comparable read count patterns, providing an additional layer of methodological cross-checking for the detected viral sequences. With these complementary approaches using whole genome sequencing dataset, BWA-MEM provided a broad, mismatch-tolerant assessment of potential viral load, while Kraken2 offered conservative, high-confidence taxonomic validation, balancing the need for sensitive detection with the specificity required to confirm the presence of *Scutavirus chelonidalpha5* DNA within the host genomic background.

Recent studies have demonstrated the utility of genomic and transcriptomic methods to characterize the abundance and transcriptional state of *Scutavirus chelonidalpha5* in tumor tissues, as well as environmental DNA (eDNA) approaches to assess viral shedding dynamics (Farrell et al., 2022). By enabling unbiased viral detection in both clinically affected and apparently healthy individuals, host WGS extends these approaches and enhances sensitivity for identifying latent infections.

The detection of *Scutavirus chelonidalpha5* DNA in green and hawksbill turtles without visible tumors highlights the limitations of disease surveillance based solely on external FP lesions. This finding is consistent with reports from other regions, where *Scutavirus chelonidalpha5* has been detected in clinically normal green and hawksbill turtles (Robben et al., 2023), indicating that virus prevalence based on FP tumors alone likely underestimates true infection rates. Because *Scutavirus chelonidalpha5* infection can remain latent, viral loads may fall below detection thresholds depending on tissue type, infection stage, and host species (Alfaro-Núñez and Gilbert, 2014), further limiting the reliability of visual surveys. Notably, the lowest viral read proportion occurred in the sole

TABLE 2 Presence of visible tumors suggestive of fibropapillomatosis (FP) and the quantity of reads mapping to the *Scutavirus chelonidalpha5* genome.

Sample ID	Species	Tumor presence	Sample type	Total Reads	Reference-based viral read counts (BWA-MEM)	Normalized reference-based viral read counts	Metagenomic viral read counts (Kraken2)	Normalized metagenomic viral read counts
G22A	*C. mydas*	Present	Blood	266,500,475	117,061	98,326	548	460
G20A	*C. mydas*	Absent	Liver	229,962,330	96,417	93,852	413	402
G21A	*C. mydas*	Present	Blood	286,016,413	115,622	90,490	624	488
G21B	*C. mydas*	Absent	Muscle	289,305,063	39,896	30,869	242	187
H21A	*E. imbricata*	Absent	Blood	237,173,742	80,196	75,690	438	413
H20B	*E. imbricata*	Absent	Blood	256,912,489	85,055	74,108	301	262
H20A	*E. imbricata*	Absent	Blood	223,847,886	73,402	73,402	282	282
H20C	*E. imbricata*	Absent	Blood	422,776,351	134,730	71,336	337	178

Data are shown for green turtles (*Chelonia mydas*; G) and hawksbill turtles (*Eretmochelys imbricata*; H). Raw mapped reads were normalized to the smallest library size. Although G21B was excluded from statistical analyses, its read counts are retained in the table for completeness.

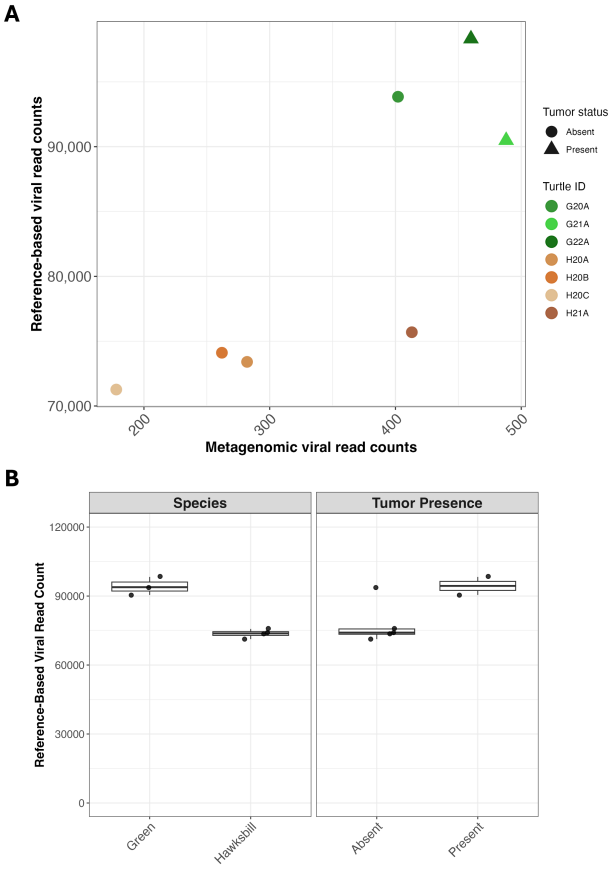

FIGURE 2
(A) Relationship between the number of reads detected by the reference-based alignment (BWA-MEM) and metagenomic analysis (Kraken2) normalized to the smallest library size. Points are colored by turtle ID and shaped by tumor status (circle = absent, triangle = present). Green shades represent green turtles (*Chelonia mydas*) and brown shades represent hawksbill turtles (*Eretmochelys imbricata*). A significant positive correlation was observed between approaches (Spearman's ρ = 0.79, p = 0.048). **(B)** Number of *Scutavirus chelonidalpha5* reads detected by the reference-based alignment (BWA-MEM) across sea turtle species and tumor status. Boxplots show the median, interquartile range (IQR), and 1.5 × IQR whiskers, with individual samples overlaid as jittered points. There was no significant difference in the read counts between species (Wilcoxon rank-sum test, W = 12, p = 0.057), nor between turtles with and without tumors (Wilcoxon rank-sum test, W = 1, p = 0.1905).

muscle-derived sample, which also contained the highest proportion of unclassified reads. However, this sample originated from a deceased stranded individual, and post-mortem degradation may have compromised DNA integrity and reduced viral detectability, independent of tissue type. As such, cross-tissue comparisons should be interpreted cautiously given the differences in sample condition across individuals. Collectively, these findings suggest that many turtles may act as cryptic carriers of *Scutavirus chelonidalpha5*, facilitating viral persistence within populations.

Beyond individual health impacts, FP and *Scutavirus chelonidalpha5* may also serve as biological indicators of cumulative environmental stress in urbanized marine systems (Garcês and Pires, 2022). The integration of sensitive molecular tools, such as whole genome sequencing, with traditional diagnostics and environmental monitoring offers a powerful framework for early detection of disease risk and broader evaluation of ecosystem health. Looking forward, there is a critical need for comprehensive health assessments of sea turtle populations across Singapore and the wider Southeast Asian region. Integrating environmental monitoring of water quality and pollutant loads with molecular and clinical surveillance of FP will help elucidate environmental cofactors driving disease emergence.

Future work would benefit from more balanced and systematic sampling across species, life stages, tissue types, and health conditions to better contextualize the prevalence and distribution of *Scutavirus chelonidalpha5* within Singapore's sea turtle populations. In the present study, sample size and types are limited, which may influence detection patterns and limit broader inference. Expanding sample sizes and ensuring more even representation, particularly of clinically normal individuals and those presenting with lesions, will be important for distinguishing true prevalence from sampling effects. Additionally, while tumors in this study were classified based on gross morphology, definitive diagnosis of fibropapillomatosis (FP) requires histopathological confirmation. Incorporating standardized diagnostic approaches, including biopsy and histological assessment where feasible, would strengthen future studies by improving lesion classification and clarifying the relationship between viral presence and disease manifestation.

In conclusion, this study provides the first molecular evidence of *Scutavirus chelonidalpha5* in a subset of Singapore's sea turtle populations, including in clinically normal individuals. Viral DNA was detected in both turtles with and without tumors, demonstrating the value of whole-genome sequencing as a sensitive tool for

identifying infections independent of clinical tumor presentation. Importantly, the detection of *Scutavirus chelonidalpha5* in both tumored and asymptomatic turtles and both turtle species reinforces that viral presence alone is insufficient to drive tumor formation, highlighting the likely role of additional host and environmental cofactors such as immune function, microbiome composition, pollutant exposure, and other physiological stressors. Together, these results position *Scutavirus chelonidalpha5* as both a wildlife health concern and a potential sentinel of environmental stress, underscoring the need for integrated genomic, ecological, and environmental surveillance to support sea turtle conservation in Singapore and across Southeast Asia.

Data availability statement

The data presented in the study are deposited in the NCBI SRA repository, BioProject number PRJNA1491560, BioSample accession numbers SAMN61464342-SAMN61464349.

Ethics statement

The animal study was approved by National Parks Board (NParks), Singapore under permit NP/RP19-103-5a. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

LT: Methodology, Visualization, Writing – original draft, Writing – review & editing. AE: Writing – review & editing, Writing – original draft, Formal analysis, Methodology. RT: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. HK: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/famrs.2026.1814588/full#supplementary-material

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