Latin American Journal of Aquatic Research, 50(3): 343-353, 2022
DOI: 10.3856/vol50-issue3-fulltext-2787

Review

What do we know about sea turtle fibropapillomatosis studies in the American continent? A bibliographic review

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ABSTRACT. Fibropapillomatosis (FP) is a debilitating neoplastic infectious disease that affects sea turtles globally. Researchers on the American continent have generated valuable information about FP that can serve as the current source of biological knowledge on the disease, but this information is scattered throughout many scientific journals, books, thesis, and conference proceedings. Through a systematic literature review, the present study intends to summarize the current state of knowledge available on the American continent and highlight the knowledge gaps regarding FP in sea turtles. We reviewed 192 studies published from 1938 to 2021 performed on the American continent. The maximum annual number of publications occurred in 2019 with 15 publications, and there is an increasing trend in studies published over the past 25 years. One hundred thirty studies were performed in North America, 43 in South America, 14 in the Caribbean region, and 5 in Central America. The USA, Brazil, and Mexico were the most productive countries for FP research. The main topics addressed were pathological findings, records/incidences of FP, and molecular characterization. The most studied species with FP is the green turtle (*Chelonia mydas*). Our results show a lack of geographical information about FP, particularly in the Central American region and the Pacific coast of South America. While there has been a significant increase in FP knowledge in the last two decades, most of the studies focused on pathological findings, while viral replication and transmission of this disease remains unknown, highlighting the need for studies that describe immune characteristics, stressful environmental conditions, or disorders in blood chemical values in organisms affected by FP.

Keywords: turtles; fibropapillomatosis; conservation, disease; health; herpesvirus; prevalence; trends

Fibropapillomatosis (FP) is a debilitating neoplastic infectious disease that affects sea turtles globally. It is manifested by a single cutaneous tumor or multiple tumors found anywhere on the turtle's body (Alonso-Aguirre et al. 1998, Page-Karjian 2019, Rossi et al. 2021). This disease may lead to the growth of external lesions on the skin, cornea, carapace, and in the sutures between the scutes of the plastron (Norton et al. 1990, Adnyana et al. 1997, Work et al. 2004), as well as internally in the oral cavity, esophagus, heart, lungs, liver, spleen, kidneys, gastrointestinal system, and skeletal muscles of the affected turtle (Work et al. 2004, Flint et al. 2010, Reséndiz et al. 2019, Oriá et al. 2021). FP tumors are histologically benign (Page-Karjian 2019); however, depending on their size, number, anatomical position, and degree of invasiveness, they can interfere with locomotion, growth, and reproduction (Adnyana et al. 1997, Herbst et al. 1998, Rossi et al. 2016). Addi-

Corresponding editor: Joanna Alfaro

tionally, they can cause starvation due to impairment of sight or cause partial or total obstruction of the glottis, epiglottis, pharynx, larynx, and esophagus, leading to progressive debilitation (Jacobson et al. 1989, Williams et al. 1994, Alonso-Aguirre et al. 2002, Alonso-Aguirre & Lutz 2004) that in severe cases reduces the probability of survival (Herbst 1994, Rossi et al. 2021).

FP was first described in green turtles (*Chelonia mydas*) from Key West, Florida (Smith & Coates 1938). It has been reported among all seven species of sea turtles (Hashberger 1991, Limpus et al. 1993, Barragan & Sarti 1994, Alonso-Aguirre et al. 1999, D'Amato & Moraes 2000, Huerta et al. 2002) inhabiting coastal and shallow waters. Thus, it has a circumtropical distribution (Alonso-Aguirre & Lutz 2004).

Chelonid herpesvirus 5 (ChHV5) has been identified as the etiological agent of FP (Quackenbush et al. 2001, Rodenbusch et al. 2014, Marshang 2019, Page-Karjian 2019). This agent is characterized by a linear, double-stranded DNA virus belonging to the Alphaherpesvirinae subfamily and the *Scutavirus* genus (Lu et al. 2000). Different lineages of ChHV 5 have been observed, varying among the regional origins of the host; such viral lineages diverged long before FP emerged as panzootic, indicating that environmental or ecological factors play a role in its global distribution (Herbst et al. 2004, Patricio et al. 2012). However, FP is a complex disease with a multifactorial etiology. In addition to ChHV 5 infection, environmental, microbial, and immune-related cofactors influence its pathogenesis (Page-Karjian et al. 2017, Marshang 2019).

Eight herpesviruses have been identified in the sea turtles' populations. However, the current knowledge focuses on just five of them (marine herpesviruses). These five are ChHV 1 (grey-patch disease), ChHV 5 (FP), ChHV 6 (lung, eye, and tracheal disease), LGRHV (loggerhead genital respiratory herpesvirus), and LOHV (loggerhead orocutaneous herpesvirus). Three of these five marine herpes viruses are well studied due to frequent outbreaks, while the remaining two are very infrequently reported (Rao et al. 2020).

Most of the studies on sea turtles' threats focus on fishing-related mortalities, egg poaching, meat consumption, illegal marketing of turtle shells, and the impacts of temperature-dependent sex determination (Hamman et al. 2013, Godley et al. 2020), among others. However, the knowledge of mortality-causing diseases spread by viruses is less studied and reported (Rao et al. 2020), even though diseases are likely a contributing or primary factor in the decline of world populations of marine turtles and pose challenges to long-term conservation efforts that monitor the health status of populations (Mashkour et al. 2020). The role of viral infections in marine turtles' pathology is poorly understood, but it is a burgeoning field of investigation (Sarker et al. 2021).

Until now, Jones et al. (2016), Page-Karjian (2019), and Rao et al. (2020) have provided essential overview information on etiology, pathology, pathogenesis, diagnosis, and disease prevalence, along with highlighting the treatment of FP. Nonetheless, an analysis compiling this knowledge in sea turtle areas of importance of the American continent is needed, including the distribution of the disease and the continent's epizootic. On the other hand, researchers on the American continent have generated valuable information about FP that can serve as the current state of biological knowledge of this disease, but this information is scattered throughout many scientific journals. Additionally, the number of studies related to FP has increased notably throughout the continent in recent years, but there are still significant geographical knowledge gaps about FP. Through a systematic literature review, the present study intends to summarize the current state of knowledge generated in the Americas and highlight the information gaps regarding FP in sea turtles in the continent.

Selection of bibliographic material

Scientific publications were selected from searches for sea turtle FP on the American continent in different academic databases and search engines (Elsevier-Scopus, Scielo, JStor, Worldwide Science, Google Scholar, Research Gate). Each search was delimited using several combinations of keywords in English and Spanish, such as: "fibropapillomatosis," "FP tumor," "FP prevalence," "herpesvirus 5," "ChHV 5," "neoplastic disease," "Chelonid alphaherpesvirus 5," "sea turtle cancer," "sea turtle oncogenesis," "epizootic tumors," "pollution," "pollutant," "Spirorchiidiasis," and "blood fluke". We selected English, Spanish and Portuguese because, in more than 90% of the countries on the American continent, they are the first or second languages. The search encompassed the period from 1938 to 2021. We started the review in 1938 when the etiological agent was discovered. For the selection of publications, only articles, chapters of books, thesis, and conference proceedings that met the following requirements were considered: 1) studies conducted on the American continent, 2) studies with registers of sea turtle FP, 3) studies that addressed pathologies, molecular evidence, and characterizations of ChHV5, in addition to immune function and blood values affected by FP. We excluded research in which the study area was not mentioned or described, as well as studies that were only focused on general concepts or reviews about FP. References cited in each publication

were also meticulously checked to locate any neglected scientific publications that matched our criteria.

Characterization of literature

Based on the criteria described above, 192 publications were selected. For practicality, we divided the American continent into four regions based on political divisions (Echeverría & Capuz 2010): 1) North America, 2) Central America, 3) South America, and 4) the Caribbean region (Fig. 1). For this work, we consider the Hawaii region as part of the American territory as a state of the USA, even if it is within the limits of Oceania.

Likewise, all publications gathered were classified as research articles (RA), chapters of books (CB), philosophical doctorate dissertations (Ph.D.), master's dissertations (MD), bachelor's thesis (BT), or conference proceedings (CPR). In this analysis, we did not consider the final technical reports submitted by institutions because they lack arbitral review and are treated as gray literature.

For each publication, we noted the species, general topic, and country in which the study was performed. Since there is no previous classification of FP-related topics, we proposed an initial classification of 18 topics (see Table 1 for the definition of our criteria), according to the content expressed in the title, abstract, and the main text.

The studies were analyzed on a continental level, by regions, country, and by the most studied topics and species. However, when a study was conducted in more than one country, we considered each country independent; therefore, the number of studies is higher than the total of studies reviewed. Additionally, we grouped the studies analyzed by 10-year periods between 1960 and 2019 and a smaller group from 2020 to 2021.

To explore geographic areas with and without studies related to FP on the American continent, using QGIS 3.16 software and our literature review, we prepared a map to identify the gaps for both the Pacific and Atlantic coasts. Finally, a timeline was designed using Adobe Illustrator 2021 software to identify the main contributions to the FP knowledge in the continent.

Microsoft Excel was used to record all the information compiled and provide descriptive analyses (country rankings, number of articles, years, scientific journals, regions).

Fibropapillomatosis (FP) on the American continent

The bibliographic search yielded 192 studies published between 1938 and 2021. The highest number of studies (35.4%) were published in the 2010s, followed by 28.6% in the 2000s, 19.6% in the 1990s, and smaller proportions in the 1980s (1.1%) and 1970s (2.1%). The highest annual number of publications on FP in sea turtles occurred in 2019, with 15 publications. The beginning of the 2020s exhibited a considerable number of studies, continuing a remarkable trend of increasing research over the past 25 years (Fig. 1).

The number of articles on FP could reflect the increased investment in research, more human capacities (e.g. trained veterinarians), and the recent interest in multidisciplinary approaches in conservation. The year 2000 is especially significant in the history of this field due to the increase in the role that veterinarians began to play in conservation initiatives, as well as their contributions to gathering the baseline health data necessary for a better understanding of how changes in landscapes and habitats, wildlife population dynamics, and disease ecology are affecting wildlife health (Deem et al. 2000). Consequently, in recent years the term 'conservation medicine' has been used to examine the interaction between pathogens and their linkages with the synergies between species and ecosystems (Alonso-Aguirre et al. 2012). The study of FP is an example of this approach.

Number of studies by region

A total of 130 (67.7%) studies were conducted in North America, 43 (22.4%) in South America, 14 (7.9%) in the Caribbean region, and 5 (2.6%) in Central America. The most productive countries for FP research were the USA (117), Brazil (40), and Mexico (13) by the number of articles (Fig. 2). Within North America, more literature was found in Florida and Hawaii (Fig. 3). However, even with the recent increase in sea turtle FP research in the Americas, the research trend mentioned above is likely due to financial limitations and ongoing social and political challenges in the countries lacking studies (ECLAC 2022).

In addition, the small number of articles from certain regions can be explained by the shortage of human resources, financial limitations, or their low interest in the subject. This finding reveals the need to implement courses and programs to develop this discipline (conservation medicine) or to apply the discipline in the curriculum of courses related to biodiversity and conservation (Rejane et al. 2015).

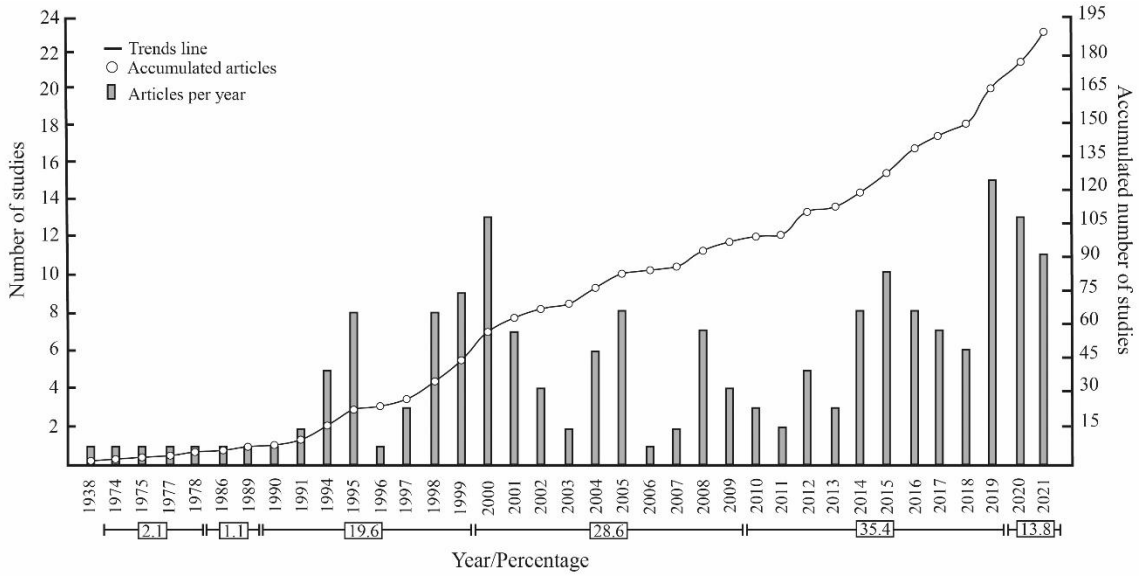

Figure 1. Trends on sea turtle fibropapillomatosis studies published yearly on the American continent from 1938 to 2021. The percentages of studies per decade are shown in the lower boxes

Table 1. Eighteen categories were used to classify the topics studied in the reviewed studies.

Topics used	Scope and description
Associated pathology	Studies focused on the knowledge of pathogen agents or diseases associated with the presence of fibropapillomatosis FP (comorbidities).
Blood chemistry	Studies focused on disorders' knowledge in blood chemical values related to the presence of FP.
Causal agent (by consumption)	Studies focused on the relationship between tumour presence and diet, considering characteristics such as eutrophication, the presence of amino acids, and diet type.
Causal agent (by pollutants)	Studies refer to the effect of pollutants (including heavy metals, toxins, organochlorinated, and perfurans) as possible causal agents of FP.
Causal agent (by a vector)	Studies address parasites' presence as vectors that contribute to the transmission of FP.
Diagnostic	Studies focused on diagnosing FP by techniques that do not include molecular characterization.
Environmental stressors	Studies that include stressful environmental conditions as a probable cause of FP.
Epidemiology	Studies focused on describing the epidemiology and epizootiology related to FP.
Hematology	Studies that describe hematic values or blood alterations as a cause of FP.
Histopathology	Studies that describe the histological characteristics of FP.
Immunology	Studies that describe immune characteristics through the presence of antibodies, immunogenic proteins, and immune disorders present in organisms affected by FP.
Molecular characterization	Studies that describe viral identification and characterization using molecular biology techniques.
Pathological findings	Studies that describe pathological findings, presence of injuries, clinical signs, and affected body areas in organisms with FP.
Prevalence estimation	Studies that describe the prevalence of FP, degree of severity, and higher prevalence in body areas.
Record/Incidence of FP	Studies that describe the registration, incidence, and growth rate of FP.
Treatment	Studies focused on describing treatments applied to individuals with FP.
Viral replication	Studies that describe or determine the viral replication in vitro of the virus responsible for FP.
Viral transmission	Studies that describe diverse viral transmission types, not including vectors, diet consumption, or toxic influence.

The current state of knowledge

A total of 167 studies (88%) were classified as RA, followed by CPR (16 or 8.4%), MD (4 or 2.1%), and Ph.D. thesis (3 or 1.6%). Based on our revision, the 10 most common academic journals are listed in Table 2. Of the 79 journals in which articles related to FP have been published, 10 made up more than 75% of the knowledge. The Journal of Diseases of Aquatic Organisms (n = 23), Journal of Wildlife Diseases (n =

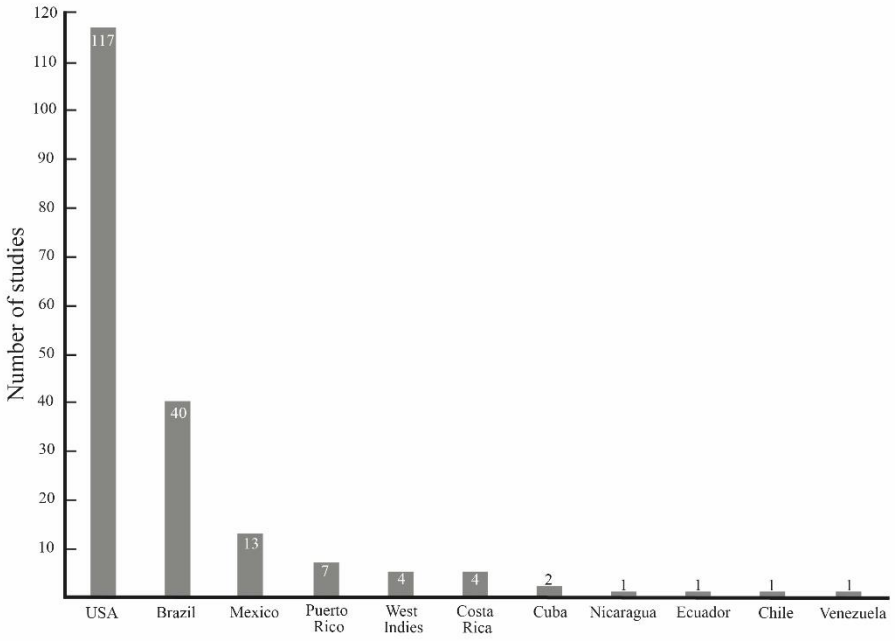

Figure 2. The number of studies related to sea turtle fibropapillomatosis realized on the American continent by country.

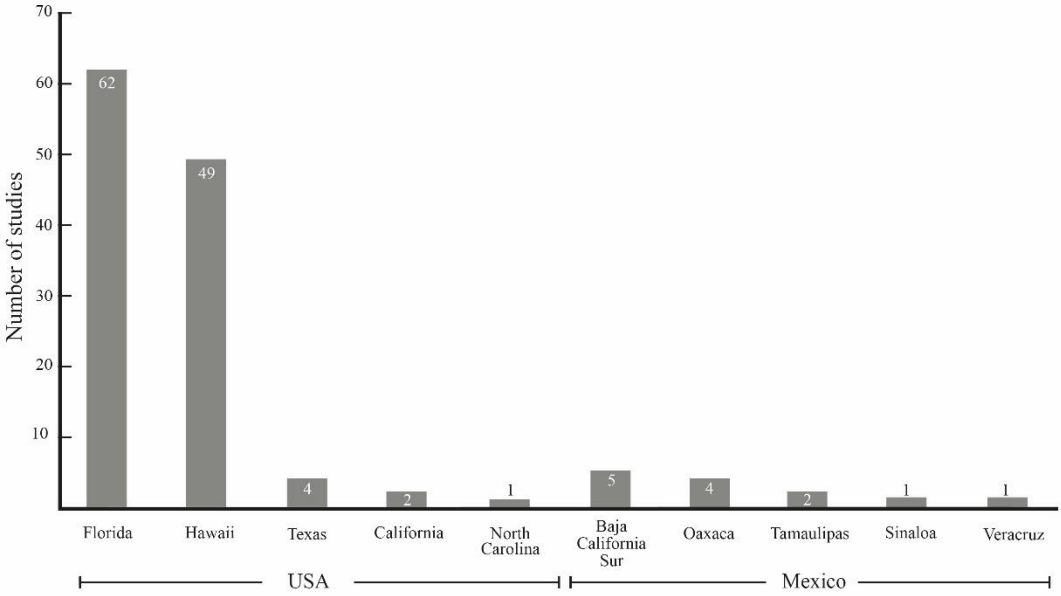

Figure 3. The number of articles related to sea turtle fibropapillomatosis published by states in the North American region.

13), Marine Turtle Newsletter (n = 8), Journal of Aquatic Animal Health (n = 7), and the Journal of Virology (n = 5) comprised more than 50% of the knowledge (Table 2).

Our revision highlights the need for research in FP in some regions of the Americas that could be improved through government funding for universities and local researchers working in conservation medicine (Cuervo & Morales 2009, Ramos & Sarmiento 2021). Of 192 literature reviewed, a considerable percentage (40.5%) of studies were found in peer-reviewed journals. However, the disparity in the number of published scientific articles between regions is possibly associated with the investment in funding and resources for research and training of human resources (Bárcenas et al. 2020, Ramos & Sarmiento 2021).

Table 2. Main peer-reviewed journals in which articles related to fibropapillomatosis (FP) published between 1938-2021 on the American continent. NA: North America, SA: South America, CA: Central America, CR: Caribbean region.

Journal	Number of articles	%	NA	SA	CA	CR
Diseases of Aquatic Organisms	23	13.8	17	6	0	0
Journal of Wildlife Diseases	13	7.8	11	1	1	0
Marine Turtle Newsletter	8	4.8	3	5	0	0
Journal of Aquatic Animal Health	7	4.2	5	0	1	1
Journal of Virology	5	3.0	5	0	0	0
Journal of Zoo and Wildlife Medicine	5	3.0	2	2	0	1
Archives of Virology	4	2.4	4	0	0'	0
Communications Biology	4	2.4	4	0	0	0
Journal of Comparative Pathology	4	2.4	1	2	0	1
Peer J	4	2.4	4	0	0	0

Table 3. Percentage of studies per region on 18 topics associated with fibropapillomatosis (FP) performed on the American continent. NA: North America, SA: South America, CA: Central America, CR: Caribbean region.

Topics	Number of studies	%	NA	SA	CA	CR
Associated pathology	4	2.1	4	0	0	0
Blood chemistry	5	2.6	2	3	0	0
Causal agent (by consumption)	4	2.1	4	0	0	0
Causal agent (by pollutants)	11	5.8	7	4	0	0
Causal agent (by a vector)	4	2.1	4	0	0	0
Diagnostic	3	1.6	2	0	0	0
Environmental stressors	4	2.1	1	2	0	1
Epidemiology	7	3.7	4	1	1	1
Hematology	6	3.2	3	3	0	0
Histopathology	7	3.7	3	4	0	0
Immunology	7	3.7	7	0	0	0
Molecular characterization	31	16.4	24	6	0	1
Pathological findings	43	22.8	28	7	1	7
Prevalence estimation	8	4.2	5	2	1	0
Record/Incidence of FP	33	17.5	23	6	1	3
Treatment	7	3.7	3	4	0	0
Viral replication	2	1.1	2	0	0	0
Viral transmission	3	1.6	2	0	1	0

Topics of studies and species targets

We found that 22.8% of the studies on FP on the American continent assessed pathological findings, 17.5% included records/incidences of FP, and 16.4% focused on molecular characterization. The remaining 43.3% included associated pathologies, blood chemistry, causal agents, diagnostics, and other categories used (Table 3). In general, the North American region presented studies on all topics (67.7%), followed by South America (122.2%), the Caribbean region (6.8%), and Central America (2.6%).

From a total of six species registered in this review for the Americas, the most studied species with FP were the green turtle (*Chelonia mydas*, 83.1%), the olive ridley (*Lepidochelys olivacea*, 3.7%), and the loggerhead turtle (*Caretta caretta*, 1.6%), hawksbill turtle (*Eretmochelys imbricata*, 1.7%) and leatherback turtle (*Dermochelys coriacea*, 1.6%). However, some studies were conducted on more than one species (Table 4). A higher proportion of studies were found in the northern region, with the green turtle (*C. mydas*) as the most studied species.

An explanation for the higher number of studies on green turtles could be a wider distribution and a high prevalence of FP infection (Jones et al. 2016). However, FP is a disease that has now been reported in all sea turtle species, but it has only reached a panzootic status in the green turtle (Williams et al. 1994).

Table 4. Percentage of studies realized per region on marine turtle species related to fibropapillomatosis in the American continent. NA: North America, SA: South America, CA: Central America, CR: Caribbean region.

Species	Number of studies	%	NA	SA	CA	CR
Chelonia mydas	157	83.1	112	31	1	13
Lepidochelys olivacea	7	3.7	3	1	3	0
Chelonia mydas / *Lepidochelys olivacea*	5	2.6	3	1	1	0
Chelonia mydas / *Caretta caretta*	3	1.6	3	0	0	0
Caretta caretta	3	1.6	2	1	0	0
Chelonia mydas / *Lepidochelys kempii*	3	1.6	3	0	0	0
Dermochelys coriacea	3	1.6	1	2	0	0
Eretmochelys imbricata	3	1.6	0	3	0	0
Lepidochelys kempii	2	1.1	2	0	0	0
Chelonia mydas / *Eretmochelys imbricata*	1	0.5	1	0	0	0
Chelonia mydas / *Eretmochelys imbricata* / *Dermochelys coriacea*	1	0.5	0	1	0	0
Chelonia mydas / *Caretta caretta* / *Lepidochelys olivacea*	1	0.5	0	1	0	0

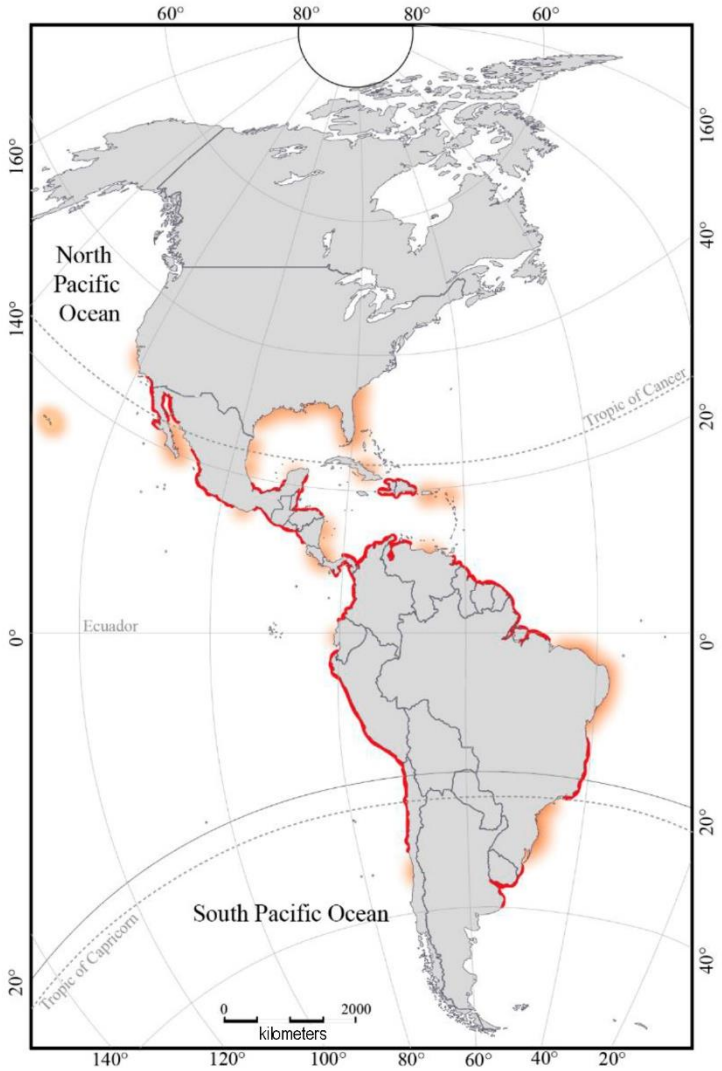

Figure 4. Geographical gaps in knowledge about fibropapillomatosis on the American continent. Areas with information are shown with orange shading, and areas without information are shown with red shading.

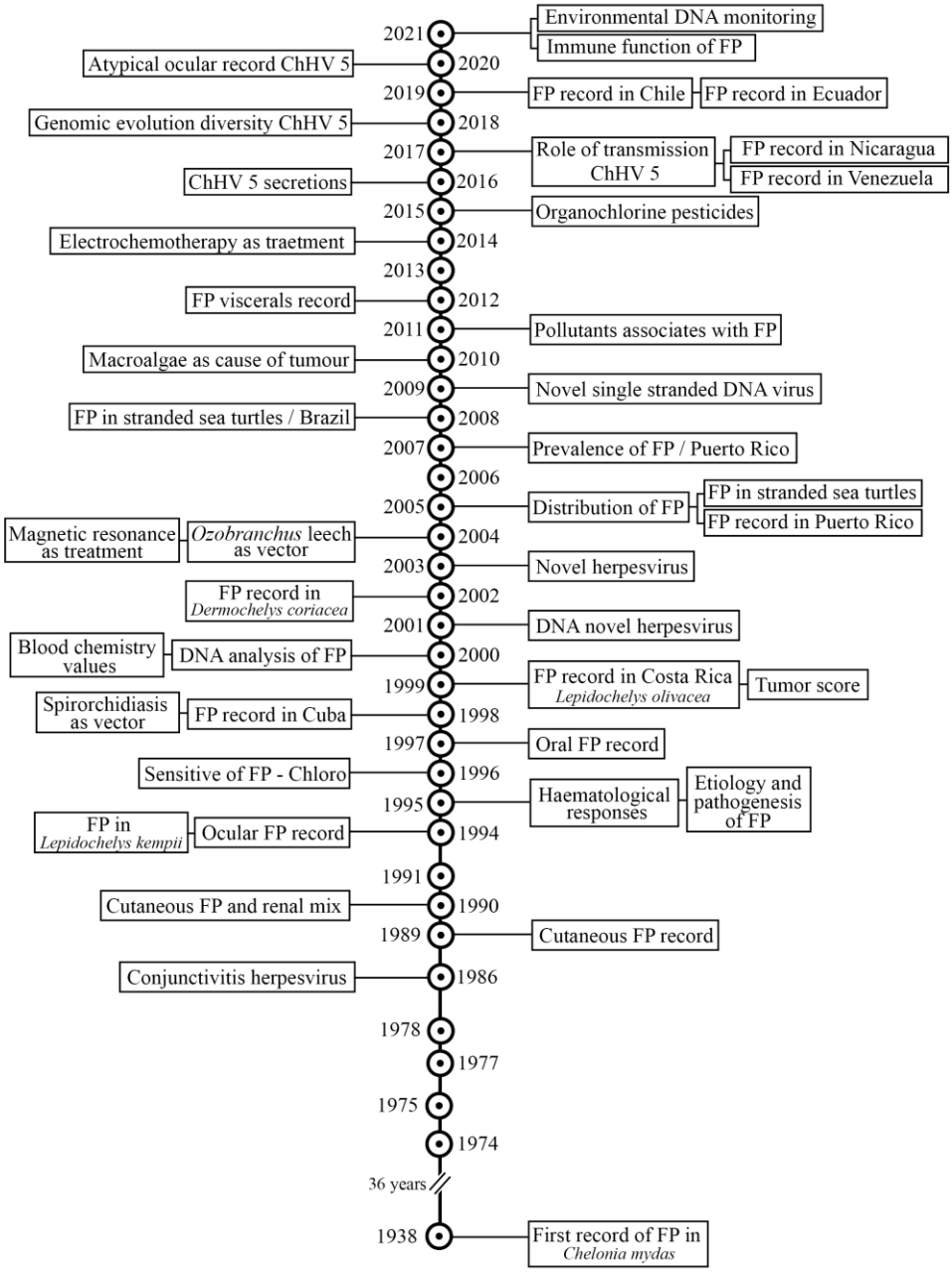

Figure 5. Timeline showing the main fibropapillomatosis (FP) studies on the American continent from 1938 to 2021. ChHV5: chelonid herpesvirus 5.

Geographical gaps of knowledge on fibropapillomatosis (FP)

Our results show a lack of geographical information about FP on the American continent, particularly for Central America and the Pacific coast of South America. As demonstrated in Figure 4, most studies have been concentrated in specific regions of the continent (i.e. Florida and Hawaii); and there is limited information for many countries such as Belize, Guatemala, El Salvador, Honduras, Panama, Colombia, Peru, Uruguay, Suriname, and Guyana, where there is no report of the presence of the disease. The relative lack of data about FP from the Central American region and the South Pacific coast may reflect limited capacities (e.g. human resources, technology, logistics) and low financial support for research. In response to

these factors, our study provides an overview of FP knowledge and highlights geographical gaps in specific regions where future efforts can be applied.

Timeline on principal FP knowledge

A comprehensive understanding of topics associated with FP (e.g. pathology, diagnosis) could determine the direction of future investigations on the virus. It would also provide a better understanding of the prevalence of diseases in marine turtles, their well-being, and the relationship between ecosystem conditions and turtle health (Jones et al. 2016). For example, since 2000, the number of studies related to molecular analysis of the etiological agent has increased, and consequently, now it is possible to recognize viral variants of ChHV5, which indicates that FP is geographically specific (Herbst et al. 2004, Ene et al. 2005, Patricio et al. 2012). In addition, although pathological findings and records/incidences of FP are the main topics studied over time (Fig. 5), these are the initial step of beginning to assess the health of sea turtle populations, and the baseline health data will enable managers to understand pathogen distribution, effects, and epidemiology (Page-Karjian & Perrault 2021).

According to the literature survey, a significant increase in FP knowledge has occurred during the last 25 years; however, there are still some gaps in geographic areas that require attention identified through our study. Regarding the topics assessed (Table 1), Central and South America (except for Brazil) show an absence of valuable information knowledge (Table 3). Topics related to FP knowledge, such as blood chemistry and treatments, are the least commonly studied, possibly due to the costs and logistics involved in their conduction.

Understanding FP's prevalence and incidence could help develop effective conservation strategies for at-risk species like marine turtles. As highly migratory species, sea turtles do not have geopolitical borders, and establishing a well-connected network of researchers would increase our knowledge of how widespread FP is in the American continent. However, most of the studies focused on pathological findings. At the same time, viral replication and transmission of this disease remain unknown, highlighting the need for studies that describe immune characteristics, stressful environmental conditions, or disorders in blood chemical values in organisms affected by FP.

ACKNOWLEDGMENTS

We thank the Universidad Autónoma del Estado de México (UAEMex) and its Division of Postgraduate Studies (PCARN) for the logistics and facilities provided for this study. In addition, thanks go to A.T. Rosewicz for her revisions and suggestions that improved the English of this manuscript. JG-G, PS-N, and MLRG thank the Sistema Nacional de Investigadores (SNI) for their recognition and support.

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Received: July 1, 2021; Accepted: June 19, 2022

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