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Research Development on Horseshoe Crab: A 30-Year Bibliometric Analysis

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This review provides the updated information and analysis of research development on horseshoe crabs over the past 30 years and raises some suggestions for future studies on horseshoe crabs. Horseshoe crab is a unique marine species and attracts many scholars in various research fields. However, to date, the development of horseshoe crab research has not been analyzed and reported from a bibliometric perspective. Bibliometric analysis is a unique tool for evaluating the development of a specific research area by analyzing relevant publications and researchers from all over the world. In this study, Web of Science, Google Scholar, and PubMed were used to collect relevant data on horseshoe crabs. VOSviewer software was used to visualize cooperation and major keywords in horseshoe crab research. From 1989 to 2019, scholars emphasized the physiological characteristics, medical value, and ecological conservation of horseshoe crabs. Some topics in this field have gradually developed because of some reasons explained in this review. Some important or recent studies have been discussed, and some potential research topics for future research have been suggested.

Keywords: bibliometric analysis, marine biology, horseshoe crab, VOSviewer, physiology, conservation, Limulus polyphemus, Tachypleus tridentatus

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INTRODUCTION

Horseshoe crab belongs to phylum Arthropoda, subphylum Chelicerata, class Merostomata, order Xiphosura, and family Limulidae. It is a crab-like animal with blue hemolymph and a brown body composed of the cephalothorax, abdomen, and sword tail. As ancient creatures (Cartwright-Taylor et al., 2009; Delvaeye and Conway, 2009) that lived earlier than dinosaurs (Rudkin and Young, 2009), they first appeared in the Devonian period of the Paleozoic era, about 450 million years ago (Rudkin and Young, 2009). For 450 million years, they maintained their original traits and lived in the benthic zone and were thus famous living fossils (Sekiguchi and Sugita, 1980; Rudkin and Young, 2009). Horseshoe crab types include only *Limulus polyphemus* (American horseshoe crab), *Tachypleus tridentatus* (trispine horseshoe crab), *Tachypleus gigas* (southern horseshoe crab), and *Carcinoscorpinus rotundicauda* (mangrove horseshoe crab; Carmichael and Brush, 2012; Mashar et al., 2017). *L. polyphemus* is mainly distributed in the American Atlantic coast and the Gulf of Mexico. *T. tridentatus* is spread largely in the southeast and east coastal areas of Asia, including the Beibu Gulf of Guangxi, Hongkong, Taiwan, Japan, and Malaysia. *T. gigas* and *C. rotundicauda* are present in the waters of south and southeast Asia (Sekiguchi, 1988; Vestbo et al., 2018).

Horseshoe crabs are widely utilized as biological resources in various fields. *L. polyphemus* has been mass harvested for use as eel and whelk bait in commercial fishery in the last century (Berkson and Shuster, 1999; Smith et al., 2009). This species is still caught for use in fertilizer and livestock feed. In China and southeast Asia, *T. tridentatus* and *T. gigas* are regarded as food and traditional Chinese medicine and thus consumed by local people or travelers (Fu et al., 2019). With the progress in scientific research, specifically, biomedical research (Shuster, 1962), researchers explored and revealed the visual, endocrine, and physiological processes of horseshoe crabs (Berkson and Shuster, 1999; Zaldívar-Rae et al., 2009; Carmichael and Brush, 2012). Horseshoe crabs have medical importance given that they produce reagents for testing bacterial endotoxins (Levin and Bang, 1964; Cooper et al., 1971; Novitsky, 1984; Berkson and Shuster, 1999; Swan, 2002; Kreamer and Michels, 2009) and vaccines (Maloney et al., 2018). In the marine ecological community, horseshoe crabs form prey–predator relationships with many animals and play a vital role in estuarine and coastal communities (Botton, 2009). Horseshoe crabs can be used to detect endotoxin concentration in the environment (Novitsky, 2009) and are regarded as an indicator of coastal ecology (Chen et al., 2004).

However, horseshoe crabs have faced multiple threats (Maloney et al., 2018). The abuse of horseshoe crab biological resources, along with other factors, such as environmental pollution and shoreline retreat, has caused the population of horseshoe crabs to severely decline (Rudloe, 1982; Widener and Barlow, 1999; Cartwright-Taylor et al., 2011; Smith et al., 2017; Vestbo et al., 2018). In the International Union for Conservation of Nature (IUCN) Red List, the status of the American horseshoe crab (Vulnerable) and the trispine horseshoe crab (Endangered) has been recorded; their population is decreasing (Smith et al., 2016; Laurie et al., 2019). Therefore, for conserving horseshoe crabs and promoting further biological research in this field, previous studies on horseshoe crabs should be reviewed.

Bibliometrics, which was established in 1958 (Thelwall, 2008), has been used to analyze various types of information in publications (Borgman, 1989). Norton defined bibliometrics as the measurement of texts and information (Wilson, 2001). Bibliometrics is an important and efficient method to evaluate scientific research. With the external characteristics of literature (including word frequency analysis and simple document counting) as research objects and applying certain mathematical statistical methods for quantitative analysis, bibliometrics can objectively reflect the research status and developmental trends of relevant disciplines (Morris et al., 2002; Daim et al., 2006; Nederhof, 2006). Bibliometrics is performed to generally analyze from two perspectives: the influence of a researcher or a group in a certain field and the internal situation of a research field (including communication, cooperation, and hot topics; Borgman, 1989; Thelwall, 2008). Bibliometrics has been applied in various fields, such as psychology, biodiversity, ecological restoration, and biotechnology (Daim et al., 2006; Godin, 2006; Leydesdorff, 2007; Lv et al., 2015; Cao et al., 2016).

The Web of Science (WOS) is a global authoritative scientific literature retrieval platform (Falagas et al., 2008; Wang et al., 2019). WOS contains approximately 11,000 source journals (Bornmann et al., 2016). Although it is a multidisciplinary database (Bar-Ilan, 2008), WOS is biased toward the natural and life sciences (Bornmann et al., 2016). It is also a common and useful tool for bibliometric analysis (Mongeon and Paul-Hus, 2016). The databases of PubMed and Google Scholar were also applied to obtain additional information on horseshoe crab research. The bibliometric analysis of horseshoe crabs helped researchers understand hot topics and develop potential directions for research on this animal. Thus, this research aimed to provide a framework for understanding the development of studies on horseshoe crab-related topics, including ecology, conservation, and physiology.

METHODS AND MATERIALS

Data Collection

In the WOS database, the core collection was chosen to extract relevant information. With "horseshoe crab" as the theme of the search, 1,248 results were found on July 26, 2019. The time range was from 1989 to July 26, 2019. The bibliometric plug-in in WOS was used to count the number of the publications of authors, organizations, journals, countries, or regions. All the information of relevant papers (e.g., title, authors, affiliation, address, and keywords) was selected and exported in a tab-delimited format to construct bibliometric maps by using VOSviewer (Version 1.6.11). Bibliometric tools in WOS were also used to extract other relevant information (the number of publications of each author, country, region, organization, field, and journal; the total number of times the publications were cited; and the proportion of all publications).

The bibliometric plug-in in WOS can list the top 20 authors on the basis of the number of publications. Given that WOS may omit relevant information, relevant data were also collected from PubMed and Google Scholar. Two approaches were used to search relevant publications in Google Scholar. The method for collecting publications that included the word "horseshoe crab" only in titles was designated as G1, and the method for collecting publications that included the word "horseshoe crab" in whole articles was designated as G2. The numbers of publications on horseshoe crabs by the top 20 authors were searched in Google Scholar via G1 and G2. In PubMed, the number of publications on horseshoe crab by these 20 authors over the last 30 years was collected.

Important Settings for Bibliometric Map Construction

VOSviewer 1.6.11 was used to generate bibliometric maps. VOSviewer is a free computer program (available in http://www.vosviewer.com/) used primarily for bibliometric visual analysis (van Eck and Waltman, 2010; Vezyridis and Timmons, 2016; Sweileh, 2018). The bibliometric maps generated by VOSviewer, which is supported by the VOS mapping technique, perform well (Van Eck and Waltman, 2007). The VOSviewer can be used to analyze cooperative relationships within a research field and for hot keyword analysis and clustering analysis (van Eck and Waltman, 2017).

The counting method is full counting, which means each link has the same weight. The maximum number of lines is 1,000, and the minimum strength of lines is zero.

The type of analysis was set as coauthored publication and the units of analysis were set as authors, organizations, and countries (regions included) to visualize the research cooperation in this field. Studies that "published by a large number of authors (country or organization)" were not selected. The following thresholds were set: the minimum number of documents of an author (country or organization) was five, and the minimum number of citations of an author (country or organization) was zero. Thus, in this research, the degree of cooperation was defined as the extent of the coauthorship of publications.

"Co-occurrence" and "all keywords" were chosen to identify the hot keywords of papers on horseshoe crabs. The threshold or the minimum number of occurrences of a keyword was five.

RESULTS

Publications Per Year

The annual number of publications on horseshoe crabs in Google Scholar (using method G2) increased from 1989 to 2014 (**Figure 1A** and **Table 1**). Although a declining trend has been shown since 2015, the annual number of publications on horseshoe crabs in Google Scholar (using method G2) is higher than that of publications on other topics.

In the WOS database, the number of total publications about horseshoe crabs fluctuates roughly (**Figure 1B** and **Table 1**). Before 1995, few publications on horseshoe crabs were available. In 1995, the proportion of all publications about horseshoe crabs increased sharply. Afterward, the number fluctuated.

The annual number of publications about horseshoe crabs in PubMed decreased. In PubMed, the highest number of publications about horseshoe crabs was recorded in 1994. In **Figure 1B**, the annual number of publications about horseshoe crabs from 1989 to 1995 in Google Scholar (using method G1) ranged from 20 to 26. It then fluctuated after 1995 and showed a similar trend to WOS.

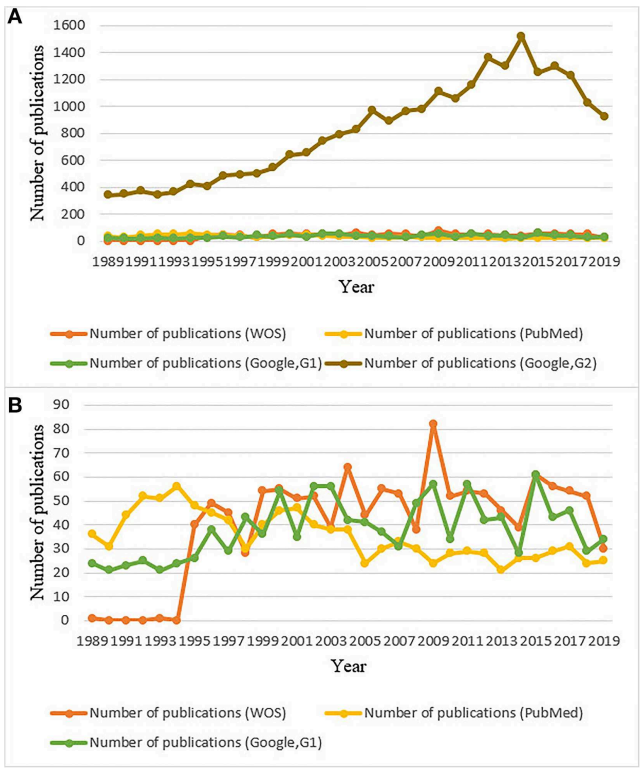

FIGURE 1 | Number of publications of each year from 1989 to 2019 based on WOS, PubMed, and Google Scholar. G1: Publications in Google that include "horseshoe crab" only in titles. G2: Publications in Google that include "horseshoe crab" in whole articles. The number of publications from WOS, PubMed, G1, and G2 are all included in **(A)**, the number of publications excluded G2 are listed in **(B)**.

TABLE 1 | Number of publications of each year from 1989 to 2019 based on WOS, PubMed, and Google Scholar.

Year	Number of publications (WOS)	Number of publications (PubMed)	Number of publications (Google, G1)	Number of publications (Google, G2)
1989	1	36	24	342
1990	0	31	21	349
1991	0	44	23	373
1992	0	52	25	347
1993	1	51	21	368
1994	0	56	24	425
1995	40	48	26	408
1996	49	45	38	488
1997	45	42	29	497
1998	28	30	43	505
1999	54	40	36	548
2000	55	46	54	639
2001	51	47	35	657
2002	52	40	56	746
2003	39	38	56	792
2004	64	38	42	828
2005	44	24	41	970
2006	55	30	37	891
2007	53	33	31	965
2008	38	30	49	980
2009	82	24	57	1110
2010	52	28	34	1060
2011	54	29	57	1160
2012	53	28	42	1360
2013	46	21	43	1300
2014	39	26	28	1520
2015	61	26	61	1250
2016	56	29	43	1300
2017	54	31	46	1230
2018	52	24	29	1030
2019	30	25	34	925

G1: Number of publications that include the word "horseshoe crab" only in titles.
G2: Number of publications that include the word "horseshoe crab" in whole articles.

TABLE 2 | Numbers of publications, the proportion of all publications, and the total number of times the top 10 countries or region are cited and ranked by the number of publications based on WOS.

Rank	Country/ Region	Number of publications	Proportion of all publications (%)	Average number of citations
1	USA	578	46.314	23.80
2	Japan	184	14.744	44.38
3	China	111	8.894	10.45
4	Germany	74	5.929	28.51
5	England	64	5.128	23.89
6	Singapore	53	4.247	31.19
7	Canada	46	3.686	38.87
8	France	46	3.686	60.09
9	India	39	3.125	13.46
10	Taiwan	29	2.324	16.38

TABLE 3 | Numbers of publications, the proportion of all publications, and the total number of times the top 10 source journals are cited and ranked by the number of publications based on WOS.

Rank	Organizations	Number of publications	Proportion of all publications (%)	Average number of citations
1	Kyushu University	71	5.689	45.28
2	University of Florida	63	5.048	15.57
3	National University of Singapore	49	3.926	33.00
4	Rutgers State University	38	3.045	15.84
5	Marine Biological Laboratory—Woods Hole	37	2.965%	25.46
6	University of California Davis	30	2.404	22.23
7	United States Geological Survey	28	2.244	18.14
8	City University of Hong Kong	24	1.923	6.21
9	University of New Hampshire	24	1.923	11.00
10	Chinese Academy of Sciences	23	1.843	15.04

Research Intensity According to Regions

Countries or Regions

The contribution of the USA is particularly significant with the most publications on horseshoe crabs (**Table 2**). However, the average number of times each article was cited in the USA did not reach the horizontal dotted line. The average number of times each article was cited in Japan, France, Canada, and Singapore was higher than that of the average level. Japan might be the pioneer in horseshoe crab studies because it has the highest number of publications and number of citations per article.

Organizations

Kyushu University had the highest number of publications, and the University of Florida had the second-highest number of publications, followed by the National University of Singapore (**Table 3**). The number of publications of these three organizations was higher than the average. Kyushu University still had the highest number of average citations per article. In addition, Kyushu University, the National University of Singapore, Marine Biological Laboratory (Woods Hole), and the University of California Davis had a higher number of average citations per article than the average.

Authors

WOS, Google Scholar, and PubMed were applied to analyze the publication records of authors worldwide. As shown in **Table 4**, the publications on horseshoe crabs by authors have been recorded widely by Google Scholar. PubMed has fewer records of horseshoe crab publications by authors than the two other databases.

Journals

The Journal of Biological Chemistry had released 53 papers, which was the highest number among journals. "*Biology and Conservation of Horseshoe Crabs*" published by Springer in 2017 had the second-highest number of articles. Biological Bulletin had the third-highest number of articles. Only the numbers of publications of these three journals/books were higher than the average. The average number of times each article of the Journal of Biological Chemistry was cited was the highest. The average number of times each article of four journals was cited was higher than the average (**Table 5**).

Research Cooperation Intensity

Countries or Regions

Close cooperative relationships among countries or regions in this field were observed. The United States is the core of academic exchange and cooperation in this field because the dot representing the United States is the largest and is surrounded by numerous lines. The first country to study horseshoe crabs is China, which published articles on horseshoe crabs in 1990. The following countries are Singapore, Japan, France, and Denmark, which mainly published relevant articles between 2000 and 2010. Most countries or regions published articles in 2010. Malaysia, Poland, Wales, and Italy published articles in 2019. In the chart, almost every dot can be connected to another dot with a different color, indicating that each country or region cooperated with other countries at different time spans (**Figure 2**).

Organizations

The relationship among organizations has two situations. In the first situation, some organizations rarely or never cooperated with others in horseshoe crab studies. In the second situation, numerous organizations cooperated with Kyushu University. Among the organizations that seldom or never cooperated with others, the first to study horseshoe crabs may be Florida State University and the Indian Institute of Chemical Biology. They published articles in 2000. The University of Hong Kong, the University of British Columbia, and Boston University published articles from 2000 to 2010. Shenzhen Polytech, Johns Hopkins

TABLE 4 | The numbers of horseshoe crab publications of top 20 authors based on WOS, PubMed, and Google Scholar.

Author	Average times getting cited in WOS	Number of publications			
		Web of Science#	Google Scholar*, G1	Google Scholar*, G2	PubMed*
Armstrong PB	24.13	30 (5)	31 (4)	70 (5)	37 (5)
Battelle BA	16	27 (7)	14 (12)	45 (13)	37 (5)
Berkson J	21.93	14 (15)	17 (10)	25 (19)	2 (14)
Botton ML	14.92	25 (8)	35 (3)	59 (7)	6 (10)
Brockmann HJ	15.4	30 (5)	25 (7)	56 (8)	5 (11)
Chabot CC	12.6	20 (11)	20 (9)	33 (17)	9 (9)
Cheung SG	7.61	23 (9)	26 (6)	54 (10)	2 (13)
Ding JL	34.04	45 (2)	16 (11)	103 (4)	42 (3)
Fujii N	51.5	16 (14)	1 (14)	61 (6)	6 (10)
Ho B	35.16	43 (3)	16 (11)	114 (3)	39 (4)
Iwanaga S	74.93	41 (4)	59 (2)	116 (2)	72 (1)
Kawabata S	46.68	53 (1)	62 (1)	130 (1)	54 (2)
Loveland RE	17.46	13 (16)	16 (11)	29 (18)	2 (13)
Muta T	54.78	18 (13)	27 (5)	51 (11)	35 (6)
Niles LJ	51.46	13 (16)	11 (13)	43 (14)	4 (12)
Osaki T	42.31	13 (16)	11 (13)	24 (20)	11 (8)
Shin PKS	8.63	19 (12)	22 (8)	40 (15)	0 (16)
Smith DR	18.68	28 (6)	25 (7)	55 (9)	1 (15)
Tamamura H	46.71	14 (15)	0 (15)	34 (16)	6 (10)
Watson WH	12.36	22 (10)	26 (6)	49 (12)	14 (7)

Ranks are shown in brackets.
G1: number of publications that included the word "horseshoe crab" only in titles.
G2: number of publications that included the word "horseshoe crab" in whole articles.
#The authors in the table are the top 20 authors ranked by the number of publications on horseshoes in Web of Science core collection.
**Google Scholar and PubMed lack the function of ranking the number of publications of authors. The ranked authors are the top 20 authors based on WOS.*

University, University of Stirling, and University Putra Malaysia published articles after 2010 (**Figure 3**).

Three collaboration cores, namely, Kyushu University, University of Florida, and Rutgers State University, were identified. These three organizations published articles mainly from 2000 to 2010. Kyoto University, Kyushu University, and other minor organizations published articles mainly from 1990 to 2000. The City University of Hong Kong, the University of New Hampshire, Yale University, the University of Bristol, the University of Kansas, Aarhus University, the University of California Riverside, and the Chinese Academy of Sciences published articles about horseshoe crabs after 2010. As shown in the figure, each dot is mostly connected to dots of a similar color, which means that organizations cooperated with one another in about 5 years (**Figure 3**).

Authors

As illustrated in **Figure 4**, dots and lines are dense in two areas. In the area with Kawabata S as the main core, most authors published articles related to horseshoe crabs in 1999. In the area with Watson and Shin as the main core, most of the papers have been published since 2010. Many authors who have not collaborated with other authors existed outside of these two areas. The dots representing these authors are relatively small, suggesting that they have not published many papers on horseshoe crabs. Some of these authors published articles between 2000 and 2010, whereas others published articles after 2010. To explore the cooperative relationship between authors in detail, we enlarged the image and carefully observed the two dense areas for further discussion. Most authors published horseshoe crab papers in 2000 or earlier (**Figure 4**). Iwanaga, Kawabata, Ding, Armstrong, Botton, and Battelle were highly prolific authors in 2000. The relationship between Iwanaga and Kawabata was close. Ibuka, Otaka, Yamamoto, Fujii, Tamamura, and Waki had a close relationship. The lines between their dots are dense, and each line is thick (**Figure 4B**).

Chabot, Watson, Brockman, Smith, Shin, and Cheung were the authors with a high number of published articles in 2010 (**Figure 4C**). Chabot, Watson, and Brockman have few cooperative relations with other authors but have a large number of published papers; this result may indirectly indicate that these three authors had a strong scientific research performance. Hu, Shin, and Cheung's studies likely represented the latest studies and frontiers in this field given that most of their papers were published after 2010. These three authors also cooperated with one another often because they were all at the City University of Hong Kong in 2010.

Research Intensity According to Hot Keywords

The phrase "horseshoe crab" had the highest frequency because it was the name of the study object (**Figure 5**). In 2000, hot terms were population, beach, photosensors, muscle, and immunodeficiency-virus activity. These results indicated that researchers focused on the vision, immune function, and population distribution of horseshoe crabs during this period. In 2005, the main hot key words were hemocytes, *Drosophila*, vision, hemolymph, cDNA cloning, specificity, signal transduction, *Tridentatus*, and *Limulus*. During this period, researchers emphasized the immune ability and vision of horseshoe crabs and began to study the molecular biology of this species. Hot keywords that appeared in 2010 were horseshoe crab, *Limulus-polyphemus*, antimicrobial peptides, evolution, growth, temperature, eggs, shorebirds, *Arthropoda*, lectin, bacterial lipopolysaccharides, and behaviors. In 2015, the hot key words were heavy metals, black tiger shrimp, seasonal movements, phylogeny, arthropod trackways, genomic organization, and *Xiphosurida*. In recent years, scholars focused on the physiology, phylogeny, and behavior of horseshoe crabs.

TABLE 5 | Numbers of publications, the proportion of all publications, and the total number of times the top 10 source journals are cited and ranked by the number of publications based on WOS.

Rank	Source journals or books	Number of publications	Proportion of all publications (%)	Average number of citations
1	Journal of Biological Chemistry	53	4.247	62.75
2	Biology and Conservation of Horseshoe Crabs (book)	40	3.205	10.85
3	Biological Bulletin	38	3.045	8.95
4	Developmental and Comparative Immunology	23	1.843	43.87
5	Fish Shellfish Immunology	23	1.843	25.61
6	Comparative Biochemistry and Physiology B Biochemistry Molecular Biology	14	1.122	13.14
7	Integrative and Comparative Biology	14	1.122	1.36
8	Current Zoology	13	1.042	14.08
9	PLOS ONE	13	1.042	10.62
10	Biochemistry	12	0.962	49.33

Fields

We could infer that studies on horseshoe crab have mainly focused on the biochemical reaction, immune ability, medical application, ecology, and conservation of this animal. Biochemistry molecular biology is the hottest field (**Table 6**). Marine freshwater biology, zoology, environmental sciences ecology, and immunology have also been highly explored.

DISCUSSIONS

Status of Horseshoe Crab Research

Google Scholar (using G2 method) might include articles that mentioned horseshoe crabs but did not focus on them, resulting in much more publications found in Google Scholar G2 than in WOS, PubMed, and Google Scholar G1. For example, through

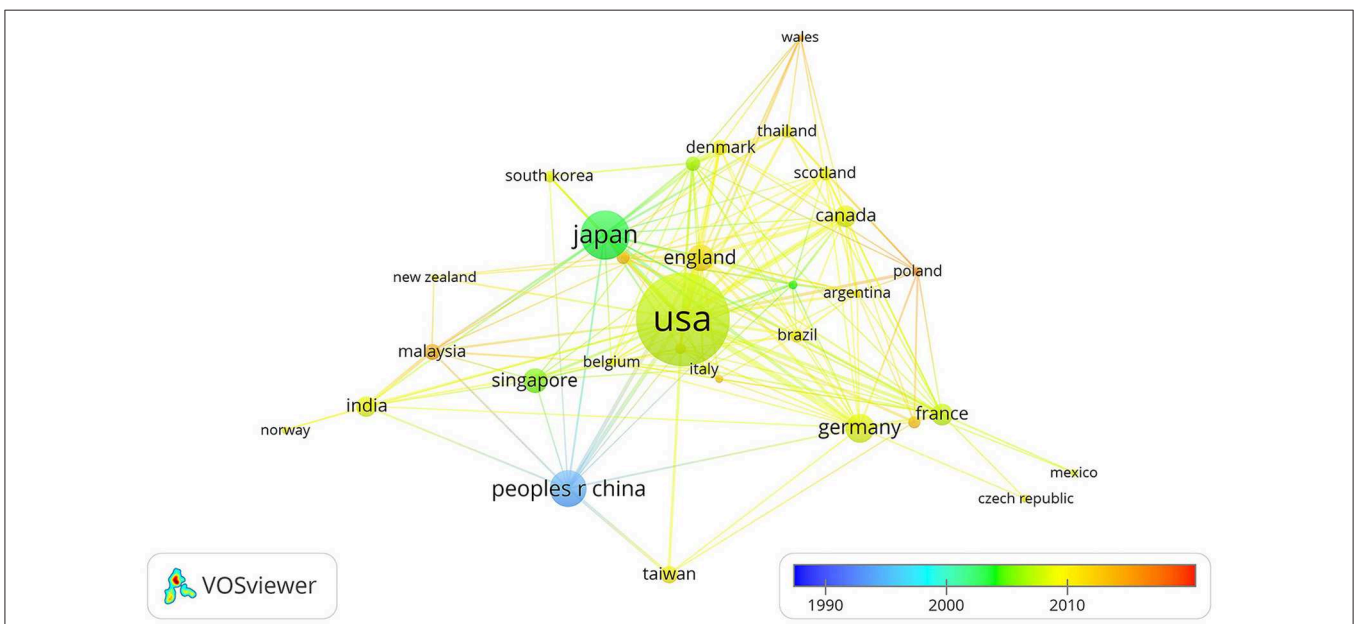

FIGURE 2 | Network showing cooperation among countries. Large dots indicate countries with high numbers of publications. The color of each dot represents when the publication is released. Thick lines between dots indicate cooperation among numerous countries.

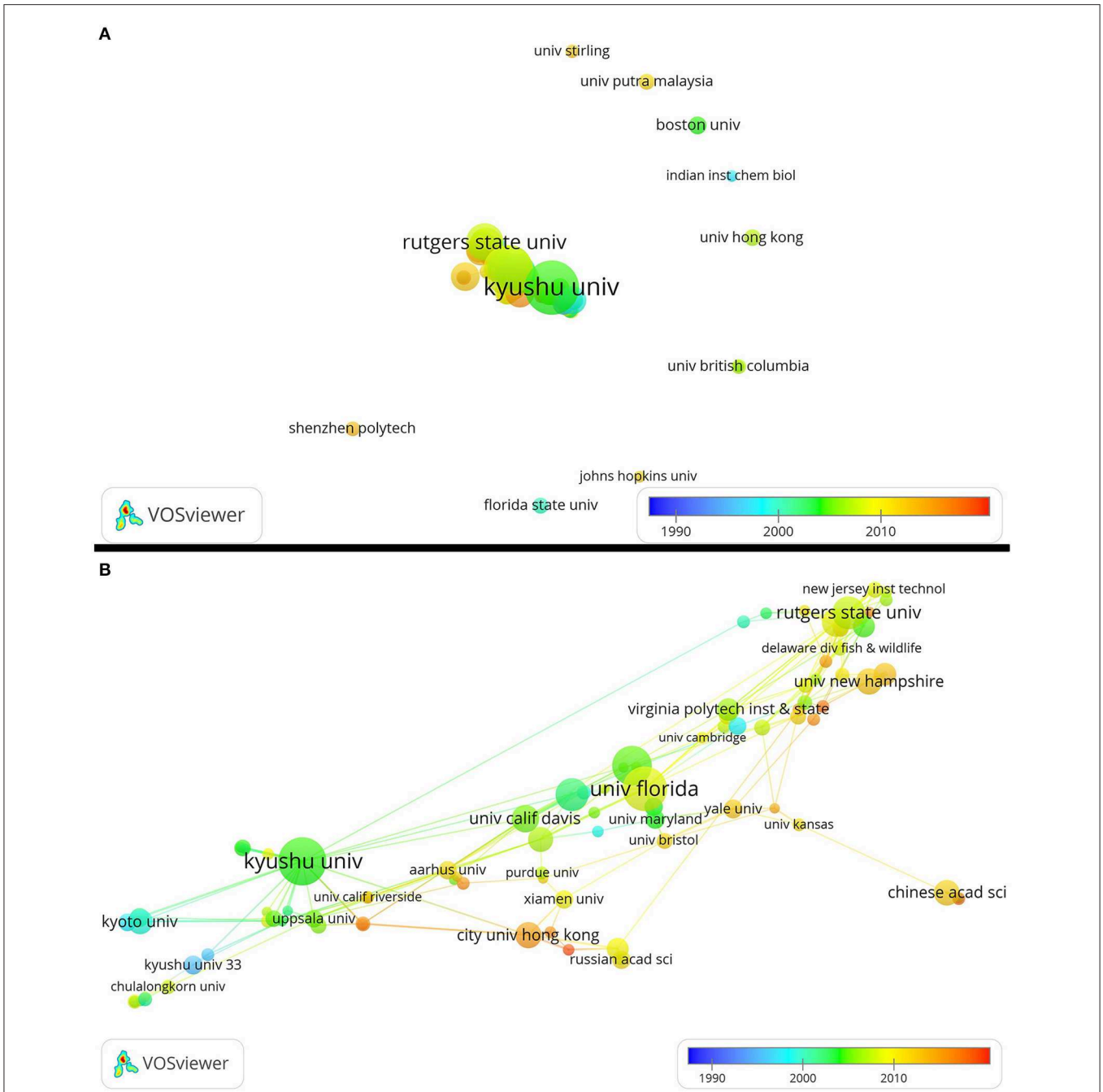

FIGURE 3 | Network showing cooperation among organizations. **(B)** Is the zoomed-in result from **(A)**. Large dots indicate that organizations have a high number of publications. The color of each dot represents when the publication is released. Thick lines indicate cooperation among numerous organizations.

G2 searching, studies about the exoskeleton structure (Raabe et al., 2005) and lectin (Moura et al., 2006) were included, but these studies were not about horseshoe crabs because "horseshoe crabs" in these articles were used as references but did not examine horseshoe crabs. The data showed that horseshoe crabs have been widely explored (**Figure 1A**), and the trend of the number of publications in PubMed is different from that in WOS and Google Scholar (**Figure 1B**). The number of horseshoe crab publications from 1989 to 1997 in the PubMed database was higher than that in Google Scholar and WOS databases. Google Scholar and WOS database had a high number of horseshoe crab publications. One possible reason was the selective collection of the PubMed database, which tends to focus on the medical field. From 1989 to 1997, medically relevant studies were published and included; from 2003 to 2019, some medically irrelevant horseshoe crab publications (e.g.,

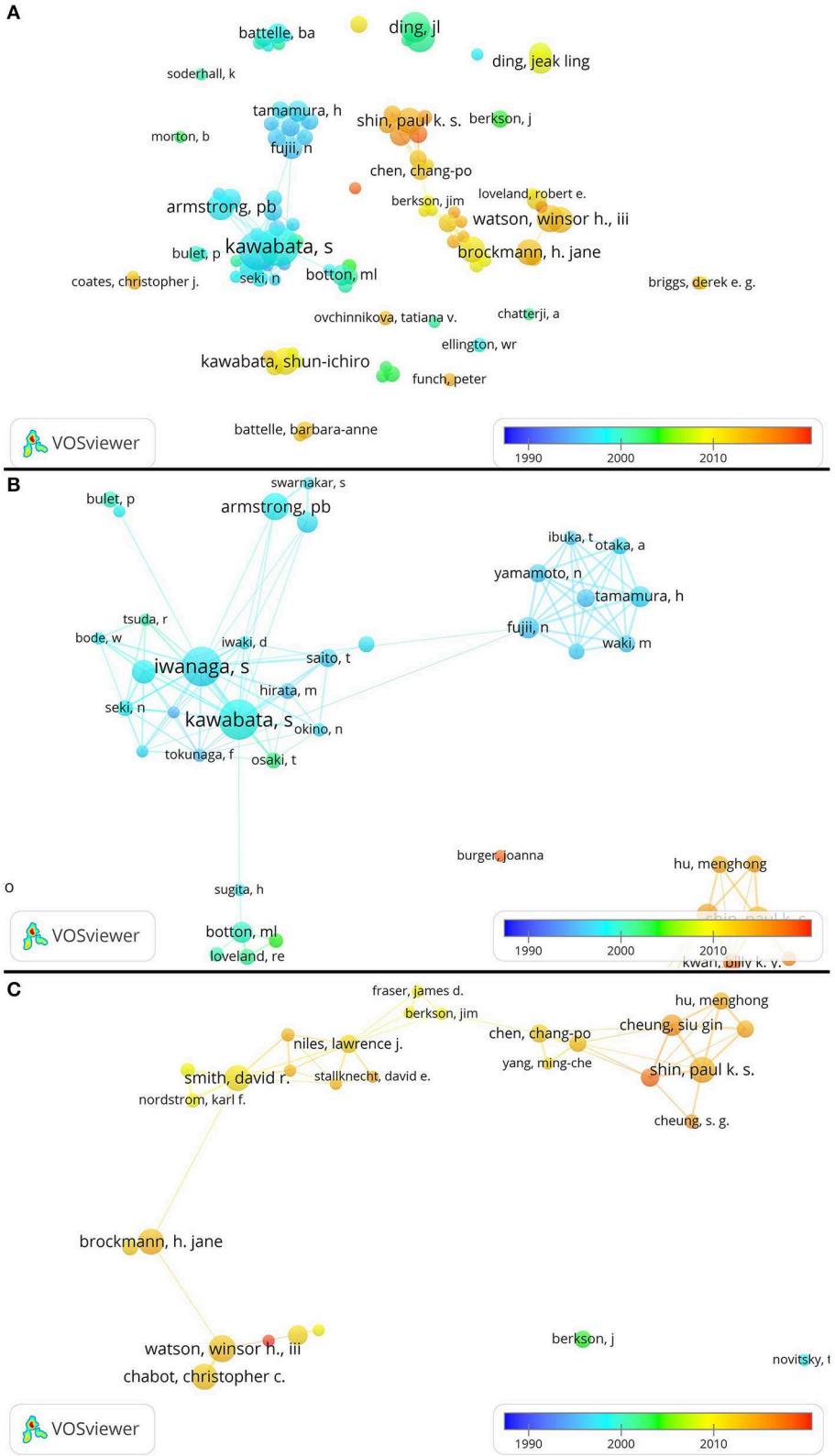

FIGURE 4 | Network showing cooperation among authors. **(B,C)** Are the zoomed-in results from **(A)**. Large dots indicate that authors have a high number of publications. The color of each dot represents when the publication is released. Thick lines between dots indicate cooperation among numerous authors. The same author may write her or his name in different ways. For example, in this figure, "ding, jl" and "ding, jeak ling" may refer to the same author.

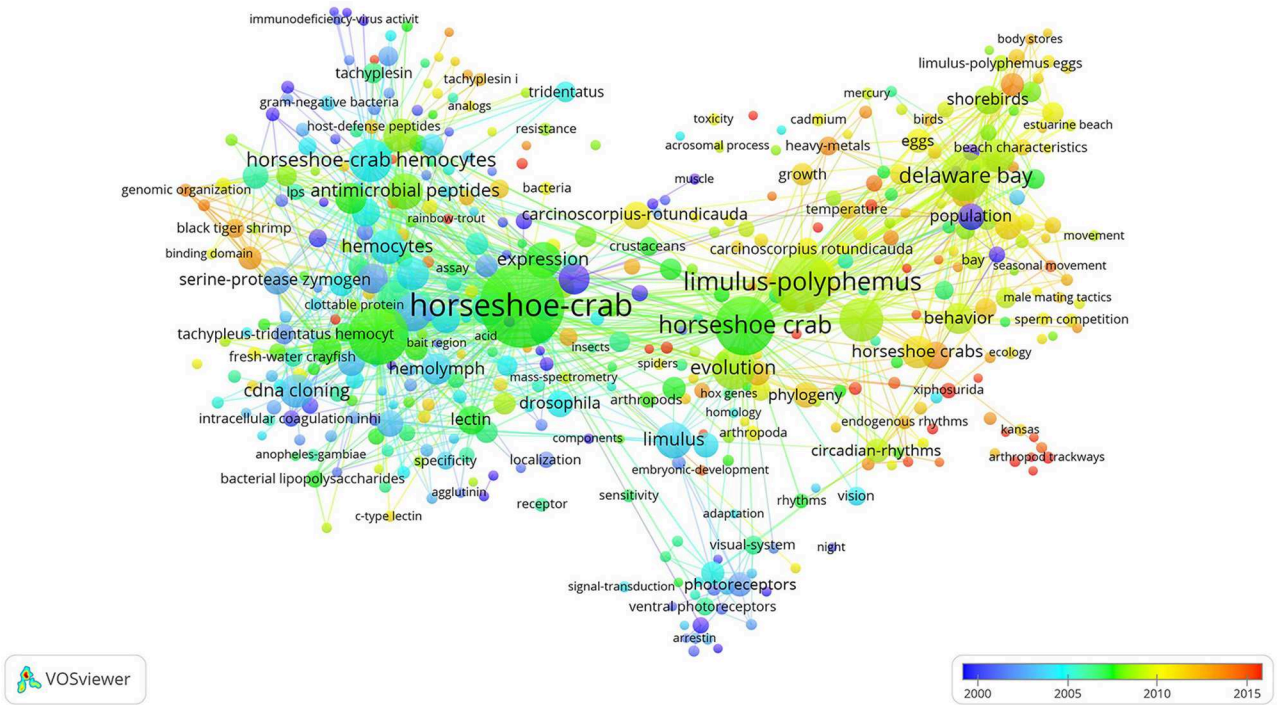

FIGURE 5 | Network showing co-occurrence among keywords. Large dots indicate a high number of publications containing a specific keyword. The color of each dot represents when the publication is released. Thick lines indicate the co-occurrence of a high number of keywords.

TABLE 6 | Numbers of publications and the proportion of all publications of the top 10 fields ranked by the number of publications based on WOS.

Field	Number of publications	Proportion of all publications (%)
Biochemistry Molecular Biology	319	25.561
Marine Freshwater Biology	234	18.750
Zoology	174	13.942
Environmental Sciences Ecology	149	11.939
Immunology	119	9.535
Fisheries	100	8.013
Life Sciences Biomedicine Other Topics	88	7.051
Biophysics	65	5.208
Biodiversity Conservation	62	4.968
Pharmacology Pharmacy	61	4.888

conservation and basic physiology researches) were excluded by PubMed. According to WOS, the top 20 pioneers have provided many important studies (**Table 4**). Thus, new learners who are interested in horseshoe crabs are suggested to read the articles of these pioneers. As shown in **Table 4**, Google Scholar can provide scholars with a large number of publications.

The large number of publications may indicate efforts in research, whereas a high number of citations per article may indicate that these studies are hot spots and attract considerable attention. According to the results, the USA and Japan finished numerous works in horseshoe crab studies (with the first and second-highest number of publications). Publications from France, Japan, Canada, and Singapore have attracted the attention of researchers (a high number of times each article was cited). Kyushu University and the National University of Singapore have released a large number of publications, which may attract more attention from researchers. Articles in the Journal of Biological Chemistry, Developmental and Comparative Immunology, Fish Shellfish Immunology, and Biochemistry have been widely explored. Publications from these countries, organizations, and journals can be helpful as references for people who want to conduct further research on horseshoe crabs.

Cooperation

Cooperation among countries or regions has improved since 1989. However, many authors and organizations rarely cooperate with others. The USA is the most active in cooperation. Kyushu University, the University of Florida, and Rutgers State University may be cooperation cores. Kawabata, Iwanaga, Watson, and Shin often collaborated with others.

Global cooperation was connected by four rounds of the International Workshop on the Science and Conservation of Horseshoe Crabs, which has been held by the Horseshoe Crab Specialist Group of the International Union for Conservation of Nature (IUCN) every 4 years since 2007. As a result, global cooperation and communication have greatly improved in this field. The first International Symposium on the Science and

Conservation of Horseshoe Crabs was held in New York, and proceeding papers were collected in the book "Biology and Conservation of Horseshoe Crabs," which was edited by Tanacredi, Botton, and Smith (Tanacredi et al., 2009). The second, third, and fourth international workshops on the Science and Conservation of Asian Horseshoe Crabs were held in Hongkong, Nagasaki, and Guangxi, respectively. The change in conference subject indicated that researchers were encouraged to focus on Asian horseshoe crabs. The book "Changing Global Perspectives on Horseshoe Crab Biology, Conservation, and Management" edited by Carmichael, Botton, Shin, and Cheung reported a significant progress on scientific research on horseshoe crabs within the past 10 years and collected proceeding papers from the second and third international conferences (Carmichael et al., 2015). As cochairs of the Horseshoe Crab Specialist Group of the IUCN, Botton and Shin provided considerable contributions to this event and the editing of conference proceedings, which accelerated global collaboration on horseshoe crabs.

Hot Research Orientation

The hot key words and fields indicated that scholars focused on physiology, medical value, and ecological conservation. Before and in 2000, researchers focused on the vision, immune function, and population distribution of horseshoe crabs. In 2010, researchers continued to focus on the immune ability and vision of horseshoe crabs and began to study the molecular biology of this animal. Scholars focused on the physiology and behavior of horseshoe crabs, and the effects of environmental stress on the horseshoe crabs were also investigated. The trend of variations in research focus showed that horseshoe crab physiology, especially the immune system, has been most widely explored, followed by medical application and conservation.

Horseshoe crab is unique with a strong immune system and famous for the utilization of their hemolymph in endotoxin detection (Fennrich et al., 2016). Over the past 30 years, studies have been conducted to identify the mechanism of this strong immunity. Iwanaga and Kawabata discovered the molecular basis for the innate immunity of horseshoe crab and attributed it to the application of horseshoe crabs' inner defense molecules in medicine (Kawabata et al., 1996; Iwanaga et al., 1998; Beisel et al., 1999; Iwanaga, 2002). Ding and Ho explored the hemolymph protein of horseshoe crabs and the mechanism of immune molecules, which greatly contributed to bacterial pyrogen testing (Tan et al., 2000a,b; Ding and Ho, 2001; Frecer et al., 2004; Zhu et al., 2004; Ng et al., 2007; Yu et al., 2009). Armstrong mainly focused on alpha (2)-macroglobulin and found that it is important for horseshoe crab hemolysis and immune system because of its special function (Armstrong et al., 1998; Armstrong and Quigley, 1999). The high citation frequency of these authors' works proved the high degree of concern for the immune system of horseshoe crabs (**Table 4**).

Given the ability of amebocytes to instantaneously react with endotoxins, horseshoe crabs are hunted for use in safety tests in medical production (Gauvry, 2015; Fennrich et al., 2016; Krisfalusi-Gannon et al., 2018). Tachyplesin in horseshoe crab hemocytes exerts a strong antimicrobial effect on Gram-positive and Gram-negative bacteria (Dorrington and Gomez-Chiarri, 2008) and has an antimicrobial activity against multidrug-resistant pathogens (Liu et al., 2018). Tachyplesin kills bacteria by targeting FabG, which is the conserved β-ketoacyl-acyl carrier protein reductase in unsaturated fatty acid biosynthesis (Liu et al., 2018). However, tachyplesin may target homologous enzymes in mammalian cells, causing cytotoxicity (Liu et al., 2018). In view of the mechanism of tachyplesin, more researchers should focus on the discrimination between bacterial and mammalian FabG proteins to select specific inhibitory ligands and develop safe antibiotics (Liu et al., 2018). Tachyplesin I from horseshoe crabs shows an antitumor activity; however, the action mode of tachyplesin I in tumor cells remains unclear (Li et al., 2017). The new antimicrobial peptide polyphemusin III can induce cell death by destroying the plasma membrane (Marggraf et al., 2018). Purified recombinant Factor C may be applied to inhibit inflammation and septic shock (Li et al., 2007). The 18-mer peptide T22 in horseshoe crab can target cells quickly through a receptor-specific endosomal route, showing a promising capacity for intracellular delivery (Unzueta et al., 2012). CrSPI, a serine protease inhibitor in horseshoe crabs, can modulate immune responses, including complements and PPO-mediated antimicrobial activities (Jiang et al., 2009). The outer shells of horseshoe crabs can be utilized as burn dressings to accelerate wound healing (Kumar et al., 2016).

The population of horseshoe crabs has declined because of marine environment changes and overhunting for feed and medical use (Tanacredi and Portilla, 2015; Krisfalusi-Gannon et al., 2018). Their spawning grounds and habitat have degraded (John et al., 2018). High acid volatile sulfide values contribute to a decline in the horseshoe crab population in Yamaguchi Bay, Japan (Moqsud et al., 2017). In 2015, 583,000 horseshoe crabs were harvested as eel and whelk bait, and 559,903 horseshoe crabs were harvested for biomedical production (Krisfalusi-Gannon et al., 2018). Although horseshoe crabs are released after medical production or studies, they would become vulnerable, and their mortality rate might increase (Walls and Berkson, 2003; Leschen and Correia, 2010). Female horseshoe crabs released back to the sea encounter difficulties in spawning (Anderson and Chabot, 2013). A green alga of the family Ulvaceae might cause harm to horseshoe crabs in their natural habitats (Braverman et al., 2012). Moreover, population decline would affect other organisms in food webs involving horseshoe crabs. Horseshoe crabs and their eggs are the food of many animals, such as shorebirds (Berkson and Shuster, 1999; Smith and Berkson, 2005), American eel, and sand shrimp (Perry, 1931; Price, 1962; Botton and Haskin, 1984; Botton and Ropes, 1989; Botton, 2009). Adult horseshoe crabs feed on bivalves (Botton and Ropes, 1989). Therefore, protecting horseshoe crabs is a matter of concern. Using recombinant Factor C as a synthetic alternative to horseshoe crab blood may be a partial solution for horseshoe crab conservation (Maloney et al., 2018), and placing limitations on the exploitation of this animal and the reasonable management of their habitats are keys to conservation.

Global marine environmental problems, such as ocean acidification and warming, drew increased attention in the twenty-first century. Thus, many studies have focused on the

effect of these environmental stresses on marine organisms. As shown in **Figure 5**, in 2010, researchers began to focus on the effects of the changes in temperature on horseshoe crabs and the advantage of horseshoe crab biological adaptation to environmental stress. Botton et al. contributed valuable research on the distribution and developmental ecology of *L. polyphemus* (Botton and Loveland, 2003; Botton et al., 2003, 2006, 2010; Botton, 2009) and determined the effect of heavy metals, such as copper and cadmium, on horseshoe crab embryos and larvae (Botton et al., 1998; Itow et al., 1998; Botton, 2000). Shin and Cheung cooperated on many studies on horseshoe crab aquaculture and ecology (Hu et al., 2009, 2013; Shin et al., 2009; Kwan et al., 2015, 2016, 2017). They further explored the effect of stressful habitat conditions, such as starvation and hypoxic exposure, on horseshoe crabs (Hu et al., 2010, 2011; Shin et al., 2014). Studies have provided valuable chromosome-level genome or transcriptome data on horseshoe crab (Gong et al., 2019; Liao et al., 2019b) and may be useful for horseshoe crab research.

A large number of studies in various fields have focused on horseshoe crabs. However, research progress on horseshoe crabs is not so rapid for some reasons. First, more cooperation should be encouraged. Our analysis revealed that authors, countries, regions, and organizations mostly become involved in cooperation (**Tables 2–4** (indicating more publications), e.g., the USA and Japan, Kyushu University, and Rutgers State University, Kawabata, and Iwanaga. Cooperation would accelerate and contribute to research on horseshoe crabs by providing more studies. Second, wild horseshoe crabs are difficult to obtain. The habitats of horseshoe crabs are not global and only include a few areas; in addition, the horseshoe crab population is declining (Adibah et al., 2012; Liao et al., 2019a). Captive breeding and artificial rearing should be explored, and some researchers searched for good sperm donors (Sheikh et al., 2019). Lastly, conducting research on horseshoe crabs in labs may be difficult. Time would decelerate our research because of the life span of horseshoe crabs. Horseshoe crabs take 10 years to sexually mature (Smith and Berkson, 2005). Mortality is high during the development of juvenile horseshoe crabs into adults (Gauvry, 2015). Relevant studies have shown that growth and survival decrease over 128 days regardless of diet composition (Carmichael et al., 2009). The survival rate of horseshoe crabs in labs may vary in practice. The hemolymph of horseshoe crabs can clot easily. The addition of LPS-free reagents at low and consistent temperatures may prevent clotting (Armstrong and Conrad, 2008). In the future, attempts to solve these problems are worth making.

CONCLUSIONS AND FUTURE DIRECTIONS

We need additional cooperation to discuss and improve horseshoe crab research. The survival of horseshoe crabs is the most important, or we may even have no horseshoe crabs for scientific studies. The conservation of horseshoe crabs is a major cause of concern. The search and widespread application for the substitutes of horseshoe crab blood will benefit the medical industry and the survival of horseshoe crabs. Scientific studies, medical industry, commercial value, and agricultural use may develop rapidly and sustainably.

AUTHOR CONTRIBUTIONS

ZL collected the data, performed bibliometric analysis, and wrote the manuscript. FM provided important relevant information about horseshoe crabs and wrote the manuscript. MH and YW conceived the idea and structure and wrote the manuscript.

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