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TRENDS AND GAPS IN SCIENTIFIC PRODUCTION ON FRESHWATER SPONGES

Marcelo Rodrigues Freitas de Oliveira[1], Cintia da Costa[2] & Evanilde Benedito[*3]*

[1]Universidade Estadual de Maringá, Programa de Pós-Graduação em Biologia Comparada, Avenida Colombo, 5790, Bloco G-80, Sala 201, CEP: 87020-900, Maringá, Paraná, Brazil.

[2] Universidade Estadual de Maringá, Departamento de Biologia, Avenida Colombo, 5790, Bloco G-80, Sala 201, CEP: 87020-900, Maringá, Paraná, Brazil.

[3] Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura (Nupélia) /PEA/PGB, Universidade Estadual de Maringá (UEM), Avenida Colombo, 5790, Bloco H-90, CEP: 87020-900, Maringá, Paraná, Brazil

Emails: m_marcelo_bio@yahoo.com.br, cintiacosta1503@gmail.com, eva@nupelia.uem.br (*corresponding author).

Abstract: Researchers working with freshwater sponges are faced with old, unresolved issues and fragmented knowledge. In the present study, a systematic search of the studies on continental sponges, from the 21st century, was carried out to identify trends and gaps in this restricted area of knowledge, using the scientific productions available on the Web of Science. The publication of scientific papers involving studies with freshwater sponges has increased over the years, with a decline in the last two years (2016-2017). The focus of the Brazilian studies included taxonomy and ecology, while Russian researchers contributed with knowledge about the genetic, phylogenetic and molecular biology of endemic sponges of the region. Eight sub disciplines were identified: Biochemistry and Physiology, Genetics and Phylogenetics, Cellular and Developmental Biology, Taxonomy and Morphology, Ecology, Paleontology and Anthropology, Biotechnology and Materials Science and Human Health. It was concluded that freshwater sponges still deserve a significant effort in the elucidation of taxonomic issues, the realization of biodiversity inventories and research in the different lines of knowledge, aiming at their biotechnological potential.

Keywords: Taxonomy; Ecology Demospongiae; Spongillida; continental sponges.

INTRODUCTION

The Phylum Porifera is considered to be the most basal group among the taxa of kingdom Animalia, and are the oldest living metazoans. They correspond to sessile, marine or freshwater, filter-feeding benthic macroinvertebrates which present an aquifer system and a complex network of water conductive canals and cavities. The reference work for the study of sponges is the Systema Porifera (Hooper & van Soest 2002). As indispensable as this work, which constitutes a pivotal search device for researchers, an online database is maintained, the World Porifera Database (van Soest *et al.* 2018), which is constantly updated and until December 2017 recorded 251 freshwater species.

The Neotropical biogeographic region is the most diverse in the world, considering the number of freshwater sponges, with more than 65 species, followed by the Palearctic region, with approximately 60 species and the Ethiopian region, with 49 species (van Soest *et al.* 2012). Smaller richness is found on islands in the Pacific Ocean and the Caribbean (five species each). The precise geographic boundaries for various genera and species, however, are still uncertain (Manconi & Pronzato 2008).

Researchers working with freshwater sponges

are faced with old unresolved issues, such as phylogenetic relations, which have been receiving considerable attention (Cardenas *et al.* 2012). The concentration of efforts in this area and the consequent rising number of molecular phylogenetics publications culminated in a recent review of the class Demospongiae, suggesting the elevation of the suborder Spongillina to order Spongillida, comprising the current eight families (Morrow & Cardenas 2015).

Since 1970, experts from around the world meet every four or eight years to present and discuss advances in the studies of sponges. In the last conference in 2013 (XI World Sponge Conference, Australia), it was highlighted the high frequency of studies that, using molecular techniques, investigated questions of taxonomy to phylogeny or evolution to physiology, and that have been used in the growing field of biotechnology (Schonberg *et al.* 2016).

Nonetheless, a quantitative synthesis of researches on freshwater sponges will be useful as a guide for the development of scientific policies and will favor, more readily, a knowledge advancement about freshwater sponges in different areas of Science. Establishing indicators of scientific activity and the areas of interest in a given subject allow us to understand how and how much scientists communicate from the perspective of the relations between the advancement of science and technology on one hand, and social and economic progress on the other (Macias-Chapula 1998).

In this context, this work intended to identify trends and gaps in the research field of continental sponges, and investigated the following hypotheses: (i) research carried out on freshwater sponges has included all areas of knowledge; (ii) the most diverse regions correspond to those with the highest number of studies. The results may not only serve as a basis for further investigations but also direct efforts to elucidate questions and to propose new hypotheses that promote scientific and biotechnological development in the use of the potential of this taxonomic group.

MATERIAL AND METHODS

The search for studies was performed on the Web of Science ™ (WofS) platform, in the following databases: Web of Science ™, Derwent Innovations Index℠, KCI - Korean Journal Database, Russian Science Citation Index e SciELO Citation Index. According to Unesco (2010) the most constantly used indicator is Thomson Reuters Science Citation Index (SCI), due to its large number of registered scientific publications. Using keywords, boolean and approximation operators, the term was formed: ((freshwater* or continental*) near/15 sponge*) or "fresh-water* sponge*" or cauxi*. For the period 2000-2017, a result of 446 studies was obtained in a search performed until October 2017.

Studies with non-pertinent themes, which did not involve freshwater sponges, were excluded and the valid documents obtained, it resulted in 258 articles. Publications related to i) publication year, ii) authors and their number of participations in published works, iii) country of origin of each author/co-author, iv) institutions of origin of each author/co-author, v) title of the source/journal of publication and their respective impact factor (data from the edition of 2015 of Journal Citation Reports®) were analyzed. The number of results for the aforementioned analyzes varied due to documents that did not contain information for some fields and synomonous authorship as author name (in this case they were summed to the same author).

Finally, the studies were grouped according to the previously proposed research area involving continental sponges, which were confirmed during the search. Documents were differentiated observing the organization level proposed by Odum (1983) in which it predominated their scientific investigation (for example: molecular, cellular, ecologic). The articles, grouped in eight areas of knowledge, were analyzed (A - Biochemistry and Physiology, B - Genetics and Phylogenetics, C - Cellular and Developmental Biology, D - Taxonomy and Morphology, E - Ecology, F - Paleontology and Anthropology, G - Biotechnology and Material Sciences and H - Human Health), considering the number of citations of works to identify research trends and knowledge gaps.

RESULTS

Temporal variation of scientific production

Although from the 21st century onwards, the publication of scientific articles involving studies on freshwater sponges has increased over the years,

there was a decline lately, 2016-17 (Figure 1). The highest number of publications occurred in 2013 with 31 studies (12%) and in 2015 with 25 studies (9%).

Research on freshwater sponges

Among the 258 documents surveyed, it was verified the participation of approximately 485 authors and co-authors. At the top of this ranking stands out the Brazilian researcher Cecília Volkmer-Ribeiro (Figure 2), participating in 9 % of the works (25), usually focused on the taxonomic and ecological areas. Also noteworthy are the Russian researchers Oxana V. Kaluzhnaya (8 %, 22 publications), Sergey I. Belikov (7 %, 18 publications) and Valeria B. Itskovich (7 %, 18 publications), who published several works in partnership between them, acting mainly in lake Baikal (Russia), contributing with the knowledge about genetics, phylogenetics and molecular biology of endemic sponges.

Studies with continental sponges were carried out in 42 countries (Figure 3). There was a higher concentration of researchers in Europe and North America (62% of the studies), with research efforts focused mainly on the composition of assemblages and temporal variation of species, corroborating the revision of Manconi & Pronzato (2008). However, from 2008 onwards, this scenario has undergone modifications. An increment of new researchers in South America, mainly Brazilian researchers focused on questions of taxonomy and biogeography, have made important contributions to the knowledge of the continental poriferous fauna.

The number of Asian studies also increased. The Russians, as well as the Japanese, have committed themselves to the area of molecular biology and cytogenetics, while the Indians have mainly devoted themselves to ecotoxicology. The Israelites, usually in partnership with Russian researchers, presented results in the fields of biochemistry and chemical composition of these sponges.

Among 262 registered institutions, the main research institution for continental sponges (Figure 4) was the Russian Academy of Sciences, followed by the Brazilian institution Zoobotanical Foundation of Rio Grande do Sul. In most of the other countries, researchers do not concentrate on a single institution, but are in greater numbers distributed among several institutions. The main example is noted for the US that, despite being the third country with the highest number of publications, researchers are from several institutions. Thus, presenting no main researcher nor main institution.

The partnership between researchers from

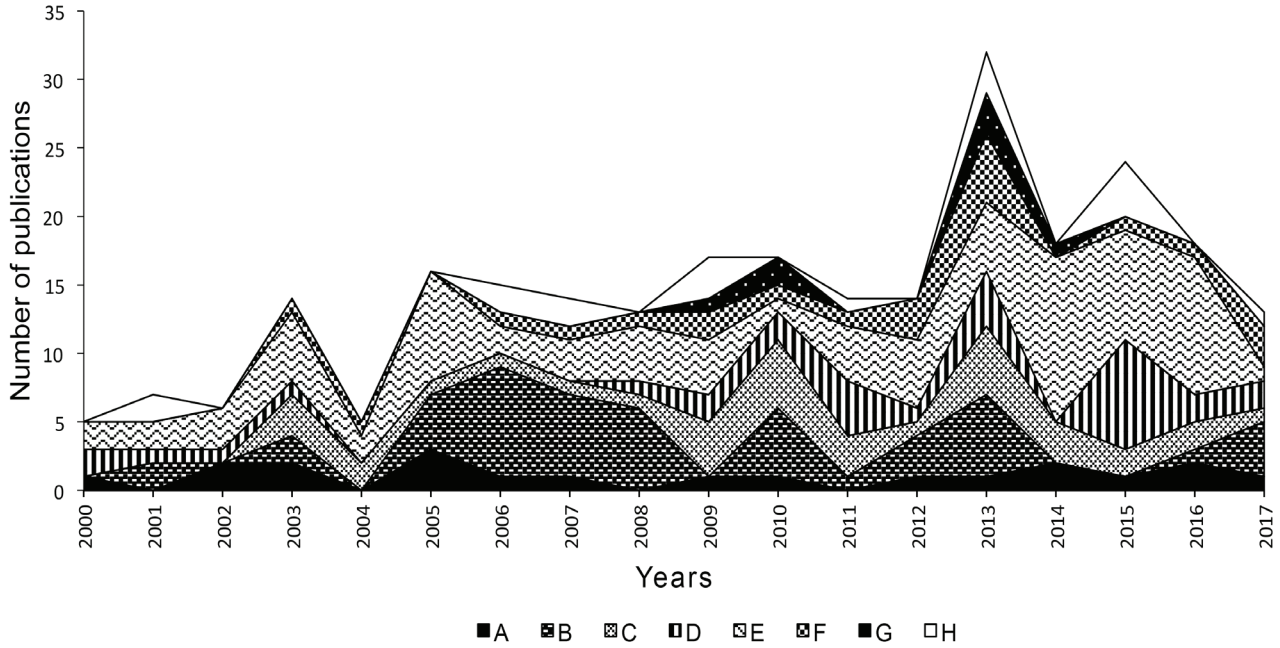

Figure 1. Annual number of papers published on freshwater sponges from 2000 to 2017 and annual variation of publications by areas of knowledge identified in freshwater sponge research. A) Biochemistry and Physiology, B) Genetics and Phylogenetics, C) Cell and Developmental Biology, D) Taxonomy and Morphology, E) Ecology, F) Paleontology and Anthropology, G) Biotechnology and Materials Science and H) Human Health

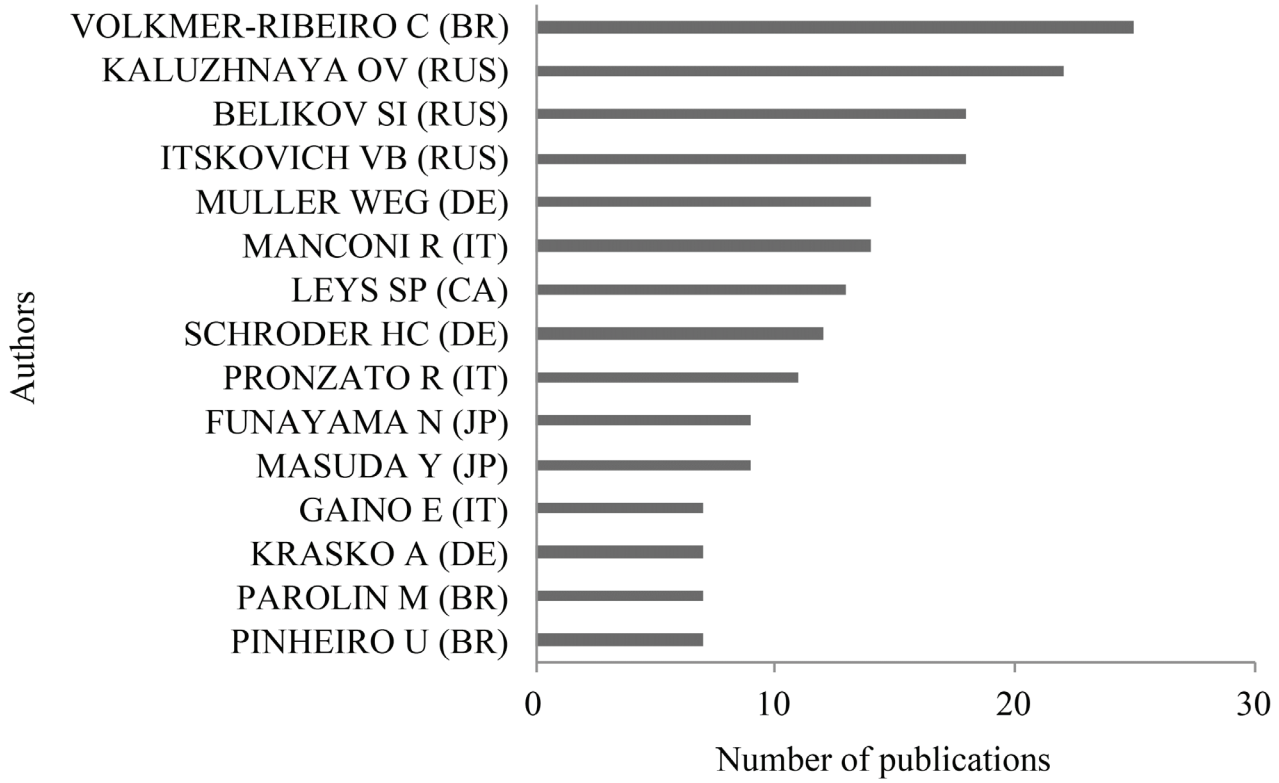

Figure 2. Researchers with the largest number of papers published on freshwater sponges (N = 240 papers) (BR = Brazil; RUS = Russia; DE = Germany; IT = Italy; CA = Canada; JP = Japan)

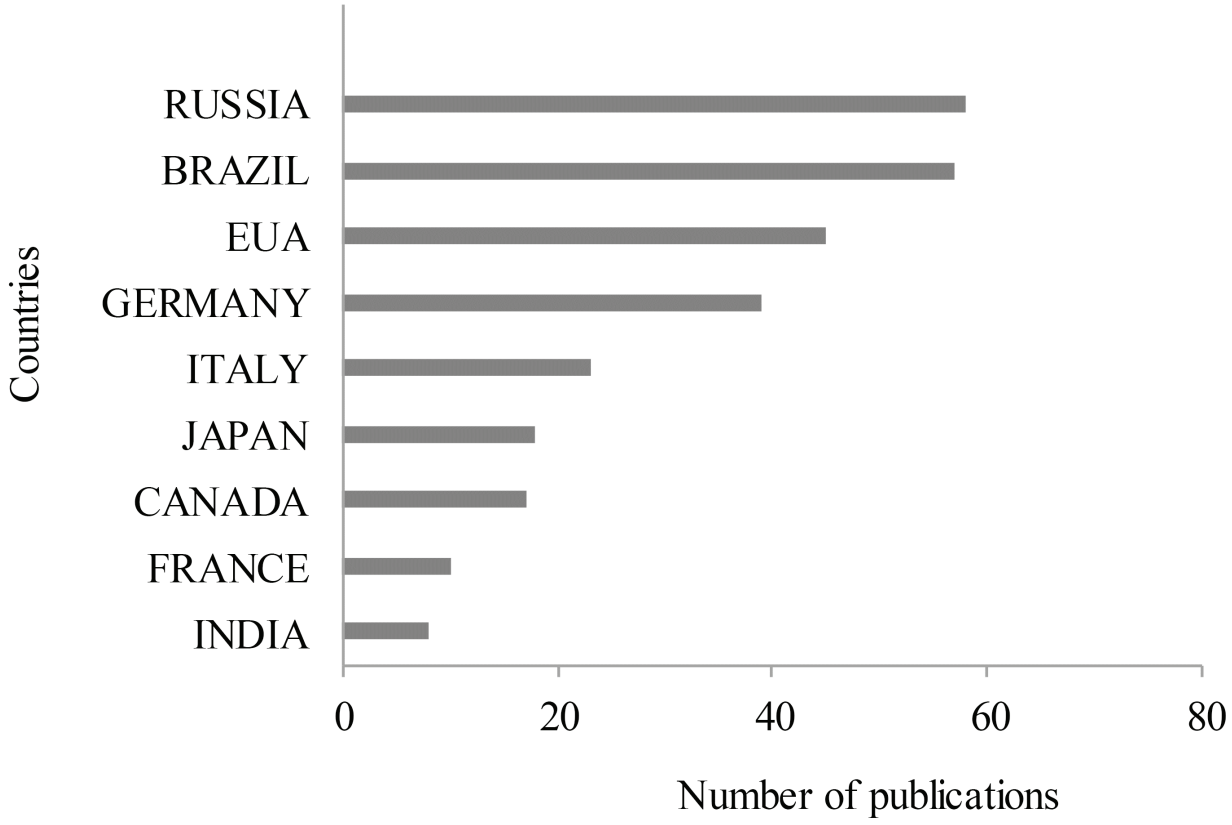

Figure 3. Countries with the most participation in works on freshwater sponges

different countries has allowed the filling of knowledge gaps about the taxonomy, ecology and biotechnological potentialities of sponge species in poorly investigated regions. An example is the cooperation network between Italians and researchers from Asian countries, such as Thailand and Laos (Manconi *et al.* 2013).

The articles were published in 130 journals, with different study approaches on freshwater sponges. For example, the scientific journal Zootaxa, which concentrates articles on taxonomy, and the journals Hydrobiologia and Plos One, important in the investigation of aspects related to ecology (Figure 5). These journals still stand out because they published the most impactful work (2.051 and 3.057 respectively), together with the *Molecular Phylogenetics and Evolution* journal, which presented the highest impact factor (3.792) among the top 15, disseminating the main results related to phylogenetic research.

DISCUSSION

Extensive areas of knowledge presented studies on freshwater sponges as their subject. With this finding we accepted the first proposed hypothesis. However, Ecology was the area with the highest number of articles (81 documents), with an increment between 2014 and 2016 (Figure 1) being divided into topics, since it is a specialty of biology that has a broad domain of issues to be studied. In a generic context, there was a survey of the sponges assembly in the Central Amazon, where they were characterized by habitats (Batista *et al.* 2003). Also noteworthy are studies on sponges as bioindicators of some coastal environments in South America

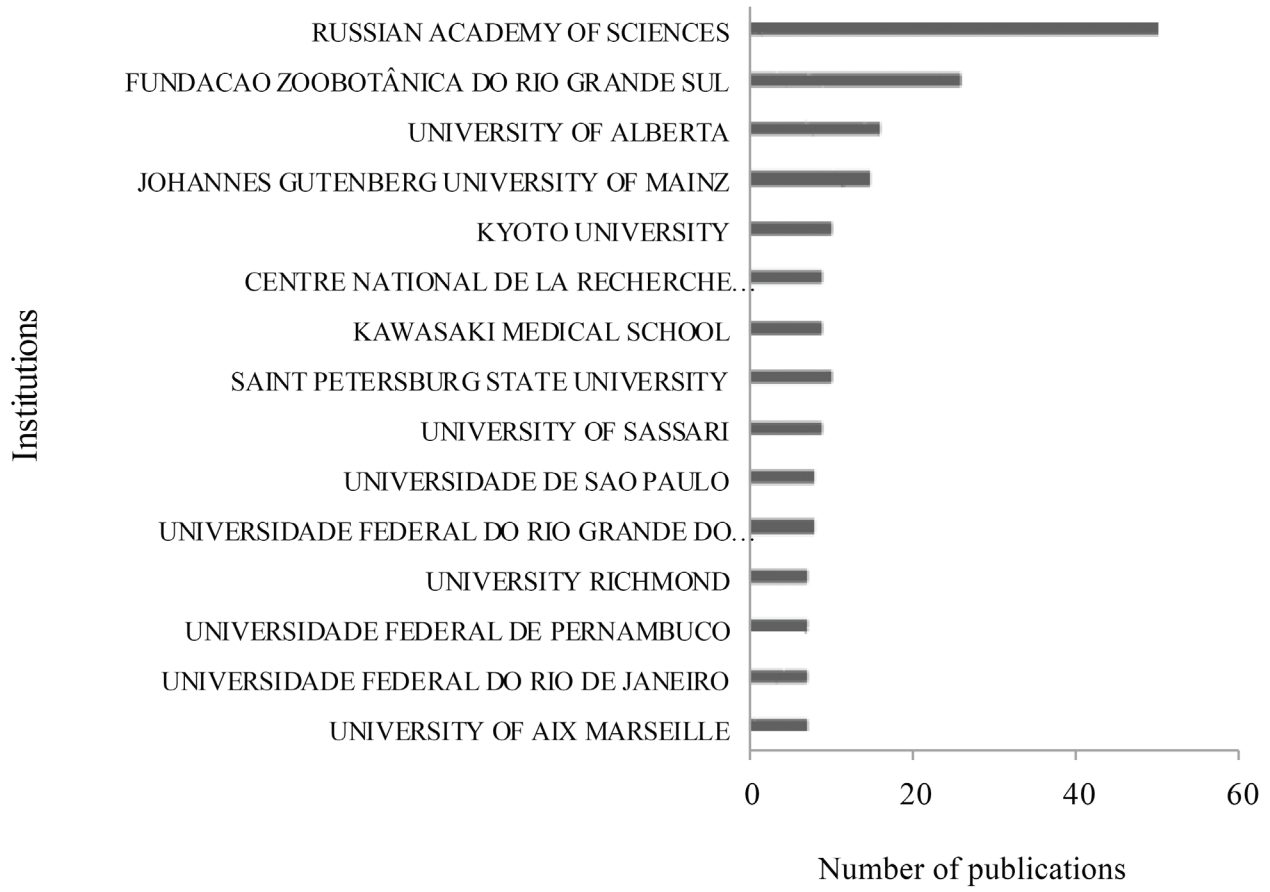

Figure 4. Top 15 institutions with affiliated authors, publishing documents related to research on freshwater sponges (Russian Academy of Sciences = Russia; Rio Grande Do Sul Zoobotanical Foundation = Brazil; University Of Alberta = Canada; Johannes Gutenberg University Of Mainz = Germany; Kyoto University = Japan; National Center of La Recherche Scientifique = France; Kawasaki Medical School = Japan; Saint Petersburg State University = Russia; University of Sassari = Italy; University of Sao Paulo = Brazil; = Brazil; University Richmond = United States; Federal University of Pernambuco = Brazil; Federal University of Rio de Janeiro = Brazil; Aix-Marseille Université = France).

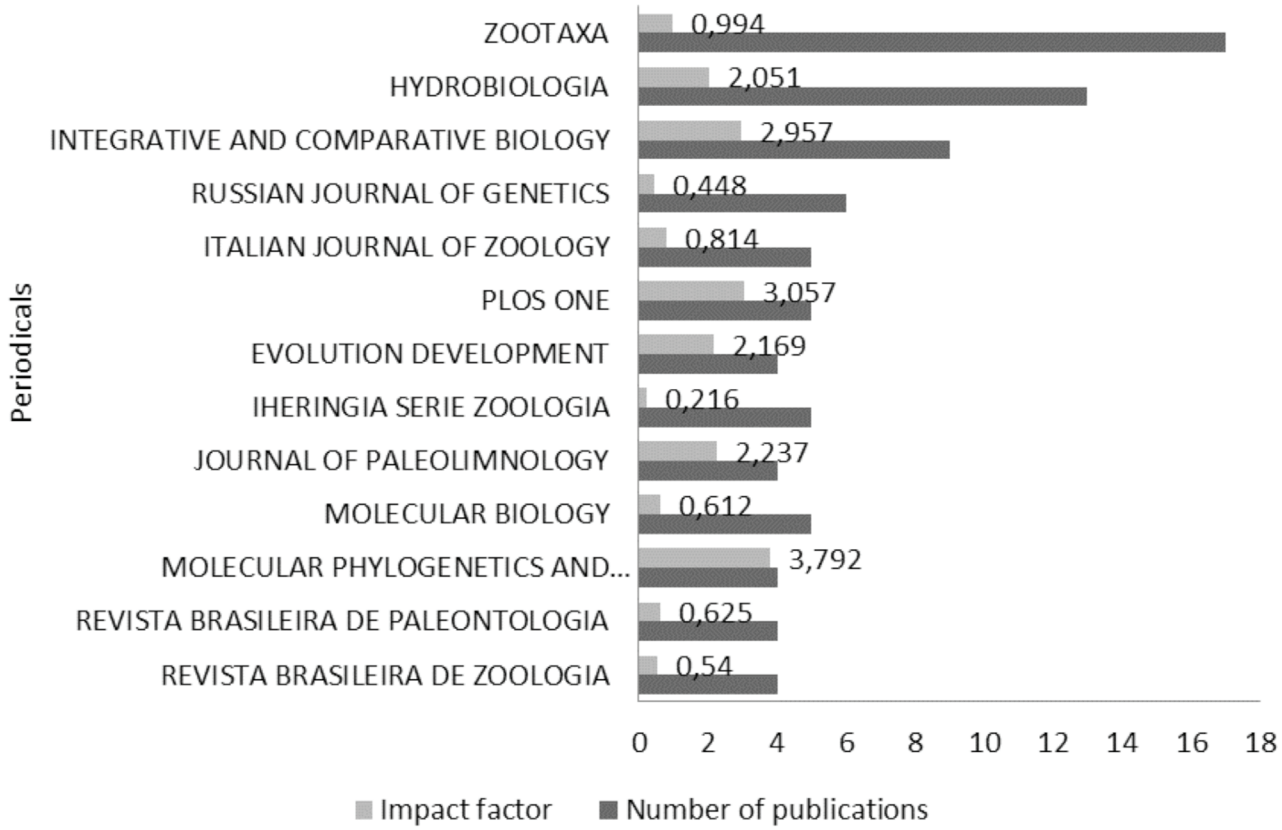

Figure 5. Journals that concentrated the largest volume of publications and their impact factor.

(Volkmer-Ribeiro & Machado 2007), and with environmental factors related to the production of a complex set of spicules (Matteuzzo *et al.* 2015).

Regarding the associated fauna, the relationship between the continental sponges and the zebra mussel, *Dreissena polymorpha* (Pallas 1771) was investigated for Lakes Michigan (USA) and Trasimeno (Italy) (Lauer & Spacie 2013). Other studies such as these were not performed later, or in other regions, which represents a gap in research on the interactions of freshwater sponges with invasive species. In Brazil, there are several publications that involve the Diptera (Chironomidae) inhabitants of sponges. They are mainly descriptions of species of chironomids, most of them being of the genus Oukuriella, in several regions of the country, such as Paraná River (Roque *et al.* 2004, Fusari *et al.* 2008) and Amazon (Fusari *et al.* 2009, Roque *et al.* 2007). There is also a single record of oligochaetes (Corbi *et al.* 2005), suggesting that more information about organophysical interactions is needed.

Another community, commonly studied in interaction with sponges, is that of microorganisms. In this regard, it is important to highlight the evaluation of the composition and identification of this set of microorganisms, mainly in the species *Ephydatia fluviatilis* (Costa *et al.* 2013, Keller-Costa *et al.* 2014), the description of endemic species of Lake Baikal (Kaluzhnaya & Itskovich 2016), in addition to a comparison with species of marine sponges (Gaikwad *et al.* 2016). Other microorganisms involved in studies were microalgae (Chernogor *et al.* 2013), and rotiferous (Bołtruszko & Ejsmont-Karabin 2013). This demonstrates that further studies on other microorganisms associated with sponges, such as protozoa and viruses, should be performed.

Regarding ecotoxicology, Indians analyzed the effects of washing soda (Sodium Carbonate, Na_2CO_3) as a contaminant in freshwater ecosystem. Of these studies, alterations induced by this compound were reported in phagocytic and cytotoxic responses of *Eunapius carteri* (Bowerbank, 1863) (Mukherjee *et al.* 2015a), an estimation of morphological damage, lysosomal membrane integrity, phosphatase activity and apoptosis in *E. carteri* cells (Mukherjee *et al.* 2015b) and, more recently, the negative effect on the immunophysiological status of *E. carteri* was confirmed. Contamination of freshwater aquifers by the compound, therefore, represents

an alarming ecotoxicological threat to sponges (Mukherje *et al.* 2016). There were no other studies conducted in other regions, also presenting a gap in this area.

In a scenario of diversity and conservation, Manconi & Pronzato (2008) evaluated the biodiversity and made a synthesis of the sponges belonging to *Eunapius* from the African continental waters. These authors also conducted a survey of global diversity of freshwater sponges, in addition to reporting the diversity and distribution of Asian continental water sponges. Biodiversity in Southwest Asia is heavily underestimated, as indicated by new recent records and the discovery of new species of freshwater sponges in Thailand (Manconi *et al.* 2013). Bell *et al.* (2015) evaluated the status of the current number of species for continental sponges. There is little information available for most species and more data is needed on the impacts of anthropogenic pressures. This is a critical information gap in understanding the conservation status of sponges.

Genetics and Phylogenetics corresponded to the second greatest area of knowledge explored (48 documents), and the longest period of publication occurred between the years 2006 and 2013. From then on, the number of studies decreased considerably. Those studies that depended on the application of specific techniques and methods in the area of genetics, phylogenetics and molecular biology, to obtain their results, were framed in this area, and many studies grouped in this area of knowledge interpassed through the three areas.

Genetic and molecular level research on skeleton formation processes (spicules) in Demospongiae during the early 21st century revealed the role of the silicatein enzyme as a catalyst in the formation of biogenic silica (Muller *et al.* 2004, Kaluzhnaya *et al.* 2005), subsequently several studies thoroughly investigating the silicatein were published. Studies range from their use in the investigation of sclerocytes cellular differentiation (Funayama *et al.* 2005), to the identification of the expression genes in several species. Hence, due to the large difference in length found between these genes, they began to be seen as a promising tool to be used as markers to distinguish sponge species, or even in phylogenetic analyses (Kalyuzhnaya *et al.* 2011).

In the field of cellular and developmental biology (35 studies), investigations focused on understanding the processes of cell differentiation. The publications were continuous over the years, with their apogee in the years 2009 to 2014 (Figure 1). Japanese were distinguished with studies involving *Ephydatia fluviatilis*, (Linnaeus, 1758) who committed themselves to clarify the mechanisms involved in the regulation of differentiation of archaeocytes to choanocytes (Funayama *et al.* 2005). Canadians were devoted to the embryogenesis and larvae differentiation (Leys & Ereskovsky 2006), epithelial differentiation (Adam s*et al.* 2010, Eerkes-Medrano *et al.* 2015), in addition to discussing elements of a "nervous system" in sponges (Leys 2015). Studies conducted by Russians ranged from research on the origin of a neuro-sensory system (Renard *et al.* 2009), to research on the ultrastructure and oogenesis of germinative determinant cells (Gonobobleva & Efremova 2017).

Studies devoted to the meticulous description of external morphology, with photographs under the scanning electron microscopy (SEM) and/or drawings to the lucid chamber of the spicules clusters and/or the gemmules; besides the registration of new species, were grouped in the area of knowledge taxonomy and morphology (32 documents). These studies, similarly, involved the characterization of the areas where the specimens were found. The number of publications that approached this area was constant over the years, but its peak occurred in the year 2015 (Figure 1).

In Brazil, the studies covered sponges communities from different localities (Batista & Volkmer-Ribeiro 2002, Tavares *et al.* 2003,Volkmer-Ribeiro *et al.* 2009); studies with specimens of different genera such as Radiopongilla Penney & Racek, 1968 (Nicacio *et al.* 2011), *Corvoheteromeyenia* Ezcurra de Drago, 1979 (Pinheiro e*t al.* 2015, Calheira & Pinheiro 2016) and a survey on the biodiversity of the freshwater sponges of northeastern Brazil (Nicacio & Pinheiro 2015). Nonetheless, it is important to highlight the limitation on morphological traits for taxonomy, and the scarcity of records for the systematic and its distribution, besides the fact that several Brazilian localities are unexplored or poorly sampled.

In relation to the discovery of new species, new records continue to occur in other parts of the world, as in the United States of America (Peterson & Addis 2000, Annesley *et al.* 2008, Copeland *et al.*

2015); in Southeast Asia (Lim & Tan 2013, Manconi *et al.* 2012) and in the African continent (Manconi & Pronzato 2015), in Venezuela (Quintero *et al.* 2017) and in Northeast Australasia (Ruengsawang *et al.* 2017). Despite these records, continental sponges are poorly explored in these parts of the planet, evidencing a gap to this area of knowledge.

In the Paleontology area, the research (23 documents) involved, mainly, taxonomic and morphological analyses of the accumulation of siliceous spicules (spongiolites) and interpretations of the paleoenvironmental conditions of lakes. In 2013, the highest number of publications on this subject was recorded (Figure 1). This is a subject that deserves prominence in Brazilian studies due to the occurrence of massive quaternary deposits of spongiolites, providing economically important biomining resources.

For Anthropology, there were only two publications made with pre-colonial Amazonian civilizations and Amerindians of Minas Gerais, reporting the incorporation of sponge spicules (commonly called cauxi) in clay for the manufacture of ceramics (Natalio *et al.* 2015, Rodrigues *et al.* 2017). Because they are the only publications, we emphasize the existence of a gap in researches that involve the area of Paleontology.

The Brazilian studies that approached the taxonomic analysis of the spicules occurred in the northwest of Minas Gerais (Almeida *et al.* 2009, Almeida *et al.* 2010) and in the paleolake Cemetery, state of Goiás (Machado *et al.* 2012). While those which comprised paleoenvironmental investigations were carried out in a coastal area in Rio Grande do Sul (Volkmer-Ribeiro *et al.* 2006), in the state of Minas Gerais (Almeida et al. 2009), in the region of Iguape (Silva *et al.* 2012), in the south of the Pantanal (Kuerten *et al.* 2013) and Upper Paraná (Zviejkovski *et al.* 2017). Furthermore, Pronzato *et al.* (2017) conducted a review where they provided an overview of the fossils of continental sponges, with temporal space distribution, and inferred that most fossil species belong to the cosmopolitan family Spongillidae. These authors also argue that in Paleontology there is a discrepant proportion between marine and freshwater sponges in the rare records until the Miocene, being less than 0.4% of all known fossil sponges. They reported that only a few taxa were correctly described by renowned taxonomists at the level of genus and species.

Studies that are characterized mainly by describing chemical compounds and the synthesis processes of these compounds in sponges, or unraveling the physiological processes present in these organisms totaled 20 studies, grouped in subdisciplines Biochemistry and Physiology. Japanese investigated lipid components on sponges and presented the fatty acid composition for the species *Heterorotula multidentata* (Weltner, 1895) and *Spongilla Alba* (Carter, 1849) (Sata *et al.* 2002), besides other works on the same theme from the sponges of Syria and Israel, mainly *Ephydatia syriaca* (Topsent, 1910) (Rezanka & Dembitsky, 2002, Rezanka *et al.* 2006).

For the Neotropical region, biochemical researches were concentrated on studies of sterols and volatile constituents of sponges from the Amazon region (De Barros *et al.* 2013, De Barros *et al.* 2015). However, since the Neotropical region concentrates the greatest diversity of freshwater sponges (approximately 65 species), the number of studies found for the area of knowledge Biochemistry and Physiology of freshwater sponges in this region can be considered scarce, and new research efforts must be considered.

As depicted above, many of the studies focused on the evolutionary, ecological, taxonomic and structural aspects of sponges. Based on what is known about their structure, sponges have unique characteristics. As a consequence, they have drawn the interest of scientists aimed at improving biomaterials (Wang et al. 2010), since the study can lead to new applications for raw materials of animal origin products. In this regard, Biotechnology and Material Sciences is one of the most recently developed areas (since 2009), and that has the smallest number of published articles (seven documents) (Figure 1). De Barros *et al.* (2013), conducting studies in Amazonian rivers (Brazil), concluded that the mechanisms involved in the process of incorporating chemical elements by sponges, suspended or dissolved material, is still an unresolved issue, because the selectivity in this process represents a possible tool to be used in chemotaxonomy and to be explored both in the biomonitoring of its environments, in bioaccumulation and bioremediation. German researchers have made the identification and first insights into the structure and biosynthesis of chitin by the continental sponge *Spongilla lacustres*

(Linnaeus, 1758) (Ehrlich *et al.* 2013), which offers many opportunities for new practical applications and cultivation for biotechnology purposes.

Other studies have drawn the attention of scientists by involving the use of mesoporous amorphous silica (SiO_2), which due to its extraordinary applications such as molecular sieves, heat-proof materials, optical communication fibers and catalysts in organic synthesis can be useful in several areas of science. Jensen *et al.* (2009) showed that SiO_2 can be synthesized from the spicules of the skeleton of freshwater sponge. In Brazil, Da Silva Lacerda *et al.* (2013) managed to synthesize MCM-4 – widely used molecular sieves as catalysts and supports of high absorption capacity and ion exchange – from the freshwater sponge *Drulia browni* (Bowerbank, 1863).

Finally, 12 publications were counted for the Human Health area, including researches and patents (Figure 1), especially applications for dermatological purposes (Volkmer-Ribeiro & Batista 2007, Magalhães *et al.* 2011). On the other hand, a broad bioprospecting, exploring new possibilities for the implementation of natural products in medicine and pharmacy, is necessary, since this group of organisms potentially presents new therapeutic agents (Dos Santos *et al.* 2011; Cruz *et al.* 2013) to be used in the treatment of skin diseases (Marilha, 2007; Marilha, 2009a; Marilha, 2009b), of the urinary tract (Zhang 2015a, 2015b), in addition to pancreatic and prostate cancer (Ottinger *et al.* 2012).

For the coverage period of this research (last 18 years), a large gap in research efforts from Oceania was noticeable. The small number of papers found from this region were those in partnership with researchers from other continents, and that the research of the evolution and phylogeny of sponges was focused, in general, on marine sponges, only including some data on freshwater sponges (Erpenbeck *et al.* 2011, Adamska *et al.* 2011). The knowledge gap on freshwater poriferous, especially regarding the biodiversity existing in this region, has been filled by foreign researchers, such as the Italians who recently described a new Australian species. In addition to providing information on the biogeography of inland water poriferous to the country (Manconi & Pronzato 2015).

No researchers linked to institutions on the African continent were found. The small number of works for this region were also conducted by researchers from Italy, who contributed mainly to the taxonomic knowledge and biogeography of the genera *Eunapius* and *Metania*, (Manconi *et al.* 2008, Manconi *et al.* 2015), including an identification key for the species of these genera found in the continent and in Madagascar.

Therefore, hypothesis 2 was accepted because the most diverse regions – Neotropical and Palearctic – were those with more studies involving species of freshwater sponges. This scenario highlights Brazil, a representative of the Neotropical region, with 55 publications and Russia, a representative of the Palearctic region, with 57 publications (Figure 3).

It was concluded that the researches carried out with freshwater sponges have included broad areas of knowledge. The main knowledge gaps are concentrated within the ecology area, highlighting the deficit of information for understanding the conservation status of sponges, the interactions of freshwater sponges with invasive species, as well as information on organophysical interactions, gaps in ecotoxicological threats and the effects of pollutants on sponges.

Unfortunately, environmental degradation is at an accelerated pace and surely many species have not even been sampled. As in Brazilian territory, many aquatic courses around the world are compromised by deforestation, damming and invasion of non-native species from the most different taxa (Garcia *et al.* 2017). For research to intensify on freshwater sponges, this study emphasizes that, in addition to new experts, greater support for inventories and experimentations with a focus on freshwater sponges is essential. Moreover, the need for the establishment of priority conservation areas in diverse ecosystems around the world is urgent.

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