










Testate amoebae (Amorphea, Amoebozoa, Cercozoa) as bioindicators: a scientometric review

Amebas testáceas (Amorphea, Amoebozoa, Cercozoa) como bioindicadores: uma revisão cienciométrica

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Abstract: Aim: The free-living protists testate amoebae are recognized as being bioindicators, able to reveal environmental alteration via remodeling richness, diversity and abundance of species. To assess the scientific production on the use of testate amoebae as bioindicators, a scientometric analysis was undertaken from 1960 to 2020. **Methods:** The sourcing of scientific articles derived from Google Academic, Scielo, Science Direct and Online Library research platforms. All selected papers were factored according to chronology, journal of publication, country, authors' affiliation, whether the study was empirical or experimentation, if it included solely testate amoebae or other groups of organisms, and aspects of the environment. **Results:** A total of 215 papers from ninety-two journals revealed a notable increase in publication numbers over the last decades. The two journals that most published data on this theme were Microbial Ecology and Ecological Indicators. Whilst the largest number of papers was published in the European Continent, the countries that most contributed to the subject were Canada in the temperate region and Brazil in the tropics. Edward A.D. Mitchell published the largest number of studies and regarding institutions the Franche-Comté University. The majority of papers that associated testaceans as bioindicators were empirical, conducted with Thecamoebian inhabiting moss and as such, dissociated from other groups. **Conclusions:** Despite a suggested uptick in research of testaceans as bioindicators, our results indicate a concentration of studies to limited regions of the globe. In another aspect, a great number of studies assess testaceans' community as-a-whole, showing a shortage of in-depth knowledge into species and functional groups. Therefore, our survey points to a wide variety of aquatic ecosystems devoid of study matter, indicating the need to foster the research on testate amoeba's role as bioindicators especially in the tropical regions.

Keywords: protozoa; thecamoebians; bioindication; environmental monitoring.

Resumo: Objetivo: As amebas testáceas são protistas de vida livre reconhecidas como bioindicadores, capazes de revelar alterações ambientais apresentando variação de riqueza, diversidade e abundância de espécies. Para avaliar a produção científica relativa ao uso de amebas testáceas como bioindicadores, foi realizada uma análise cienciométrica no período de 1960 a 2020. **Métodos:** A seleção de artigos científicos foi feita nas plataformas Google Acadêmico, Scielo, Science Direct



e Online Library. Todos os artigos selecionados foram categorizados por cronologia, periódico de publicação, país, afiliação dos autores, se o estudo era empírico ou experimental, se incluía apenas amebas testáceas e aspectos do meio ambiente. **Resultados:** Um total de 215 artigos de noventa e dois periódicos revelou um aumento notável no número de publicações nas últimas décadas. Os dois periódicos que mais publicaram dados sobre o tema foram *Microbial Ecology* e *Ecological Indicators*. Embora o maior número de trabalhos tenha sido publicado no continente europeu, os países que mais contribuíram para o tema foram o Canadá na região temperada e o Brasil nos trópicos. Edward A.D. Mitchell publicou o maior número de estudos e em termos de instituição, a Universidade Franche-Comté. A maioria dos trabalhos que associaram amebas testáceas como bioindicadores foram empíricos, realizados tecamebas habitantes de musgos e, como tal, dissociadas de outros grupos. **Conclusões:** Apesar do aumento verificado nas publicações com testáceas como bioindicadores, nossos resultados indicam uma concentração de estudos em regiões limitadas do globo. Sob outro aspecto, um grande número de estudos avaliou a comunidade de testáceas como um todo, mostrando uma escassez de conhecimento aprofundado sobre espécies e grupos funcionais. Com isso, nosso levantamento aponta para uma grande variedade de ecossistemas aquáticos desprovidos de estudos sobre o assunto, indicando a necessidade de fomentar a pesquisa sobre o papel das amebas testáceas como bioindicadoras, em especial nas regiões tropicais.

Palavras-chave: protozoários; amebas testáceas; bioindicação; monitoramento ambiental.

1. Introduction

Currently, due to the countless and ever-growing anthropic intervention within natural ecosystems, the increasing disposal of various materials and toxic substances cast into the aquatic environment is sadly ongoing throughout the world (Nasser et al., 2020). This scenario is even worse in developing countries, where waste treatment costs are frequently unviable for local economies and recycling strategies are still incipient. Thereby, the search for more efficient monitoring tools becomes essential to access the changes caused in ecosystems by human activity, and to mandate strategies for environmental protection, management and recovery (Oertel & Salánki, 2003).

Biomonitoring is a scientific technique for assessing information on a quality of a given environment based on living organisms, considered as bio-indicators (Markert et al., 1999). This approach utilizes species, taxonomic assemblage, morphological or functional groups of species, and indexes of integrity based on community (Reynolds et al., 2002; Souza & Vianna, 2020) or multiple-communities (Rooney & Bayley, 2012) to access condition and changes in the environment caused - *or not* - by anthropic action. According to Sládeček (1979), aquatic organisms can serve as indicators, therefore being possible to assess the water quality according to its presence, and in isolated cases, even in recognition of its absence. But testate amoebae have been used as bioindicators through other habitats, including peatlands and soils (Freitas et al., 2022; Tran, 2020; Swindles et al., 2020)

Since the end of the 19th century, the application of biological indicators to water features assessment is standard practice in Europe (Kolkwitz & Marsson, 1909). Presently, the assessment of a series of biological quality elements (BQEs) assists in evaluating the structure and functioning of aquatic ecosystems according to the EU Water Framework Directive (WFD). The procedures of WFD include macroinvertebrates, fish, phytoplankton, macrophytes and phytobenthos, although other communities also present the potential to be employed for such assessment (Jeppesen et al., 2011).

The use of bioindicators for environmental evaluation, especially those from inland aquatic environments, present greater advantages when compared to monitoring that incorporates physical and chemical variables. Most freshwater organisms can impart physical and biological characteristics of locality, being witness to local or surroundings events and thus providing information on past and present environmental conditions (Howe et al., 2007).

Testate amoebae are a polyphyletic group of free-living amoeboid protozoa, whose primary feature consists of a protective shell, known as test, in which a single cell is inserted (Miranda & Mazzoni, 2015; Smith et al., 2007; Souza, 2008). Since the 19th century, several species of testate amoebae have been recorded in various countries in temperate (Ehrenberg, 1840) and tropical regions (Daday, 1905; Prowazek, 1910). Mostly studied from taxonomic perspectives, the accumulation of knowledge regarding the distribution and

ecology of this group furthered their awareness as being environmental bioindicators (Walker, 1982; Nasser et al., 2016, 2020).

The properties of testaceans that deem them suitable for biological indicators are many and diverse in number. The test formation (endogenous or exogenous origin) and composition (proteinaceous, agglutinate-mineral, siliceous, calcareous, agglutinate-mineral-siliceous, and agglutinate-siliceous), test size and morphology, pseudostome shape and position can reveal effects of the environment on species' and or the organism's impact on ecosystem processes (Schwind et al., 2016; Violle et al., 2007; Krashevska et al., 2020). When in contact with environmental fluctuation, testaceans can adapt and recast their richness, diversity, abundance, biomass and species size (Laggoun-Défarge et al., 2008). Their short life cycle contributes to quick response to environmental changes (Souza, 2008); their ability to form cysts renders them capable of resisting different environmental variations; their ability to inhabit diverse environments, such as lakes, rivers, mosses and soils thus provides studies on various biotopes; the test strength enables over time the tracking of environmental changes over long-temporal scales (Souza, 2008; Kajukała et al., 2016). Additionally, the identification by the morphology of their, for example, test allows a classification at lower taxonomic levels, and as such, becomes a friendly and attractive motivation for the use of the testaceans as a bioindicator (Tolonen, 1986).

In view of the increased potential of testate amoebae as a tool for bioindication, we hereby present a scientometric analysis aimed at assessing the studies that use the group for this purpose, aiming to provide a greater view on the usage of testate amoebae as bioindicators. We addressed the following questions: (I) which journals most frequently published articles on the subject testaceans as bioindicators, and what are the temporal trends of these articles? (II) which authors, institution and countries have focused more on this subject? (III) are the studies empirical or experimental, and are only testate amoeba subject of study or, are there other groups included in the mix? and finally, (IV) what types of environments are predominantly studied?

2. Material and Methods

For data collection and scientometric analysis scientific articles that applied testate amoebae as instruments for environmental bioindication were

chosen. The research was carried out using resources from Google Scholar, Scielo, Science Direct, Online Library, Web of Science (Thomson Reuters), CAPES, Lilacs and Scopus, within the time frame from 1960 to 2020. The keywords used were: "testate amebae", "thecamoebian", "biomonitoring", "bioindication", "bioindicator", "paleoecology", and "transfer function", linked to the Boolean operator "AND" and "OR". Subsequently, the selected articles were inspected regarding the content on the applicability of testate amoebae as a bioindication tool. The articles were organized according to the year, publication period, first authors and institutions, group (s) studied, the country of study and type of the environment. Research that occurred in more than one location and that included study of more than one environment were ultimately included in all covered categories. The statistical treatment was performed using the GraphPad Prisma software, version 8.0.2. for Windows; GraphPad software, San Diego, California USA, www.graphpad.com.

3. Results

From a search result of 3,268 publications performed until December 20th, 2020, were discarded those that do not fit the proposal (reviews, state of the art, abstracts, theses, brief mentions), and a total of 215 articles were selected for the analyses (Figure 1), following the Preferred Reporting Items for Systematic Reviews (PRISMA) model (Page et al., 2021).

These 215 articles addressing testaceans and bioindication were published in 92 journals being the first recognized study worldwide published in Brazil in the 1980s, by the National Institute for Research of the Amazon (Walker, 1982). Considering the decades, there was a growing production of articles on the subject (Figure 2a). From the eighties, the number of publications remained stable, whilst reducing somewhat between 1 and 3 articles per year until a peak in 2000, in which 9 articles were published (Figure 2b). Post 2004, there was a notable increase in the number of publications that oscillated between 3-5 articles, until reaching yet another peak in 2008, in which there were 13 published articles. In 2020, 20 articles were published, the most productive year.

Since the 1960s, the majority (70.65%, 65 magazines) of scientific journals have published at least one article on the subject whilst there were just six journals that included more than eight published studies; these being: Journal of

Quaternary Science, Ecological Indicators, The Holocene, European Journal of Protistology, Microbial Ecology and Journal of Paleolimnology with the latter presenting the highest number with 19 articles on bioindication and testaceans.

In relation to study location, the European continent produced the higher number of publications (129 papers) (Figure 3), followed by North America (65), Asia (44), South America (19), Oceania (3), Africa (3) and Antarctica (2). Fifty-two countries presented studies on bioindication and testaceans and among these, Canada was prominent with 41 papers (16,47% of the total), followed by Switzerland (16, or 6,43% of the total), Poland (15, or 6,02%), United States (15 or 6,02%), France (13 or 5,22%), China (12 or 4,82%), Brazil (11 or 4,42%) and Russia (11 or 4,42%). Combined, these eight countries accounted for 53.82% of subject matter. Brazil was the most representative country in South America, furnishing 11 of the 19 articles published. Canada and Switzerland figured prominently whilst North America and Europe proffered 41 of the 65 and 16 of the 129 articles published, respectively. In Europe however, more countries published on the subject, while in other continents, there was a clear concentration of publications.

The authors who produced the higher number of publications on the subject were R. J. Payne and G. T. Swindles (10 and 9 articles, respectively) (Figure 4a). When accounting for the universities

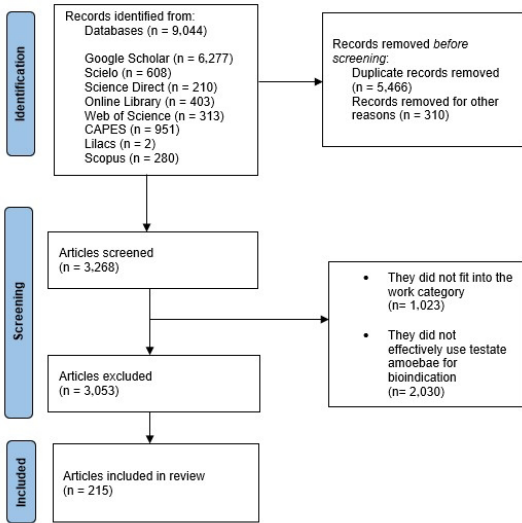


Figure 1. Scheme used to filter the results obtained in the search, based on Flow Diagram for Systematic Reviews (PRISMA).

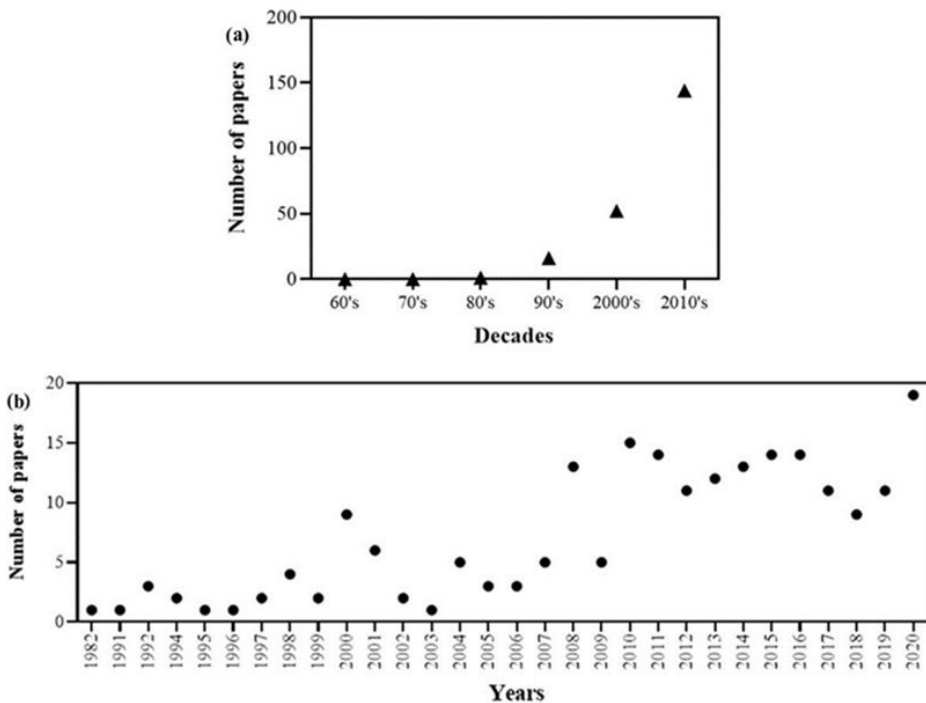


Figure 2. (a) Number of articles on testate amoebae and bioindication published per decade and (b) number of articles on testaceans and bioindication published annually. Years in which there were no publications on the topic were not represented on the axis.

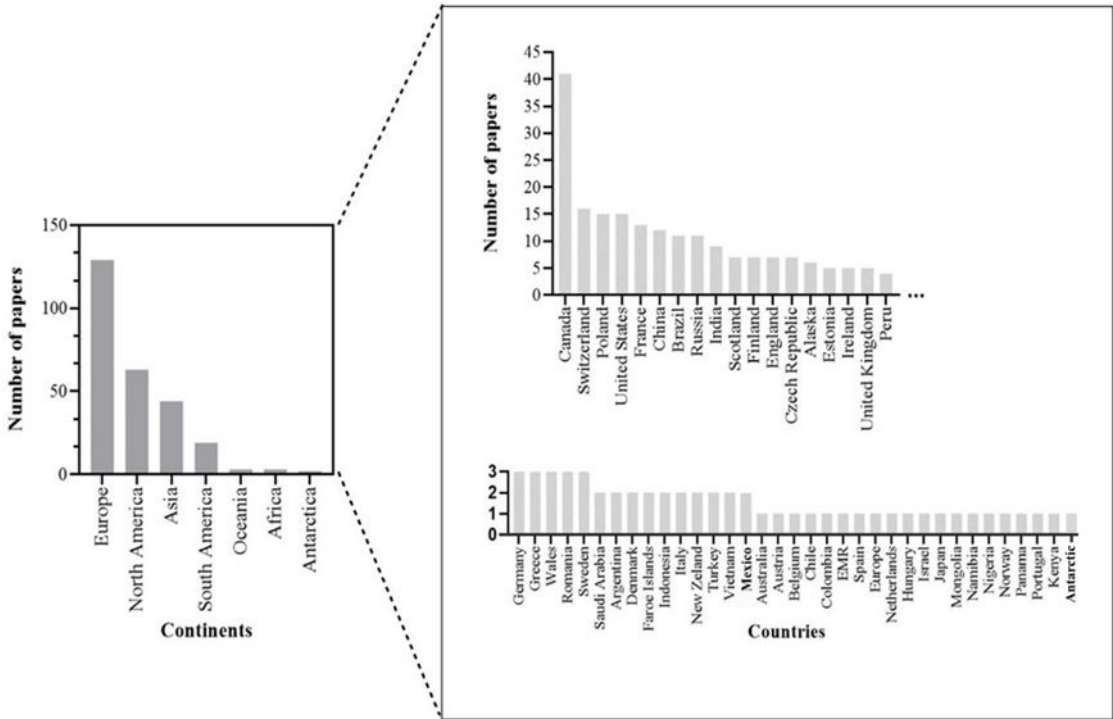


Figure 3. Number of papers published by continent and by country.

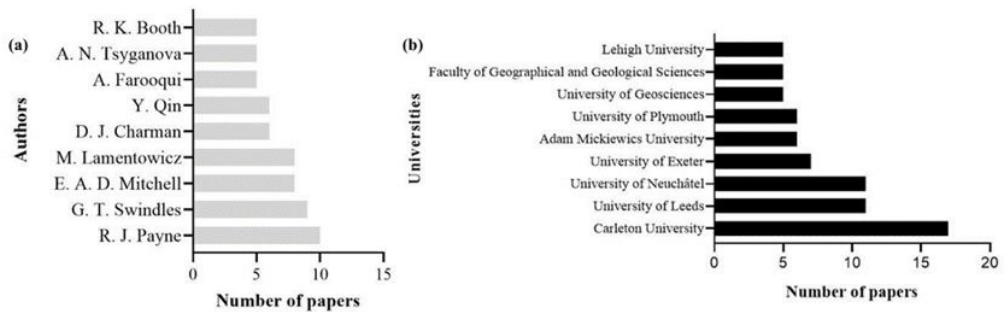


Figure 4. (a) Number of articles published by authors five or more publications on bioindication and testate amoebae; (b) Number of articles published by universities with five or more publications on bioindication and testate amoebae.

that were host institutions of the research, the Carleton University (Figure 4b) produced the highest number (17), with studies conducted by R.T. Patterson (4 out of 17 published), K. Holcová (2 out of 17), L. A. Neville (2 out of 17), N. A. Nasser (2 out of 17) and others.

The highest number of publications included only testaceans to document results (Figure 5a). In three articles, testacean amoebae were included in a large group (zooplankton). The higher number of studies that addressed more than one group examined simultaneously testaceans and

foraminifera (10 articles), testaceans and pollen (9 articles), testaceans and diatoms (8 articles) and testaceans and fossils (7 articles) (Figure 5b). The great majority of publications (96.71%) addressed the community of testaceans as a whole (Figure 5c), and as such, chose not profile a specific genus or species and, as a matter course, were empirical (90.61%) (Figure 5d).

Among 26 biotopes addressed, most studies were on moss (62 studies, or 29.38% of the total) (Figure 6), followed by lakes (41, or 19.43%), peatlands (29, or 13.74%) and bogs (16, or 7.58%).

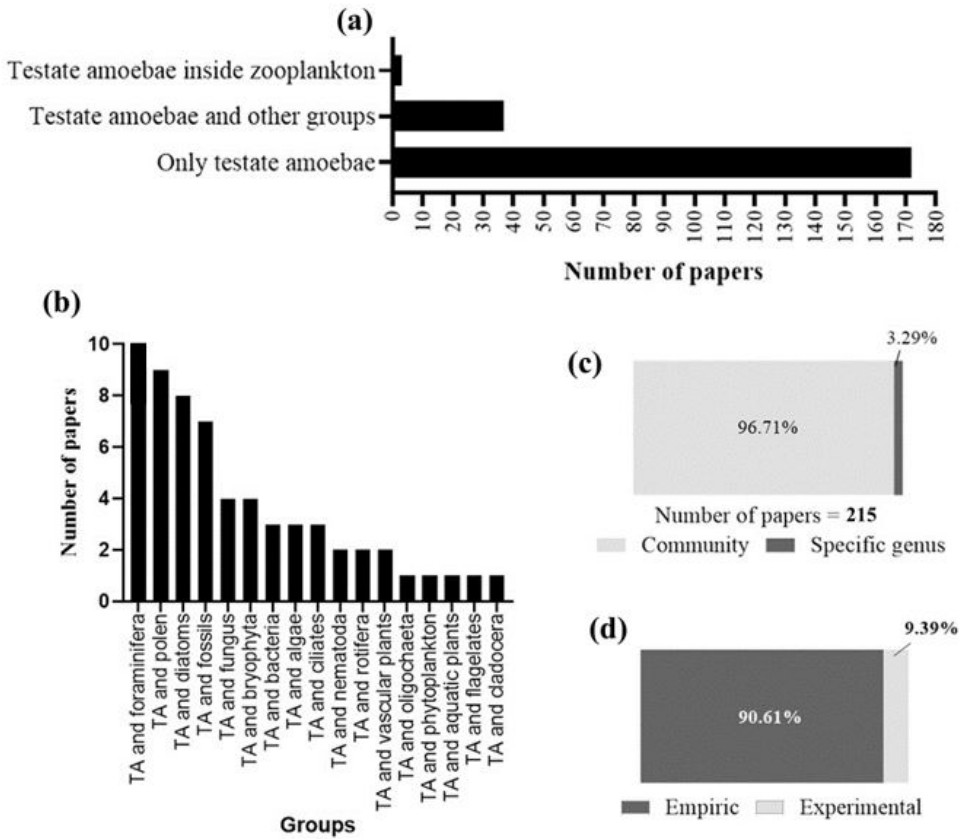


Figure 5. (a) Number of articles on bioindication that used only the testate amoebae for their results that mentioned this group within zooplankton and that use testaceans and other groups; (b) Subdivision of studies with more than one group; (c) Taxonomic cut studied. Percentages in approximate values; (d) Type of the articles. Percentages in approximate values.

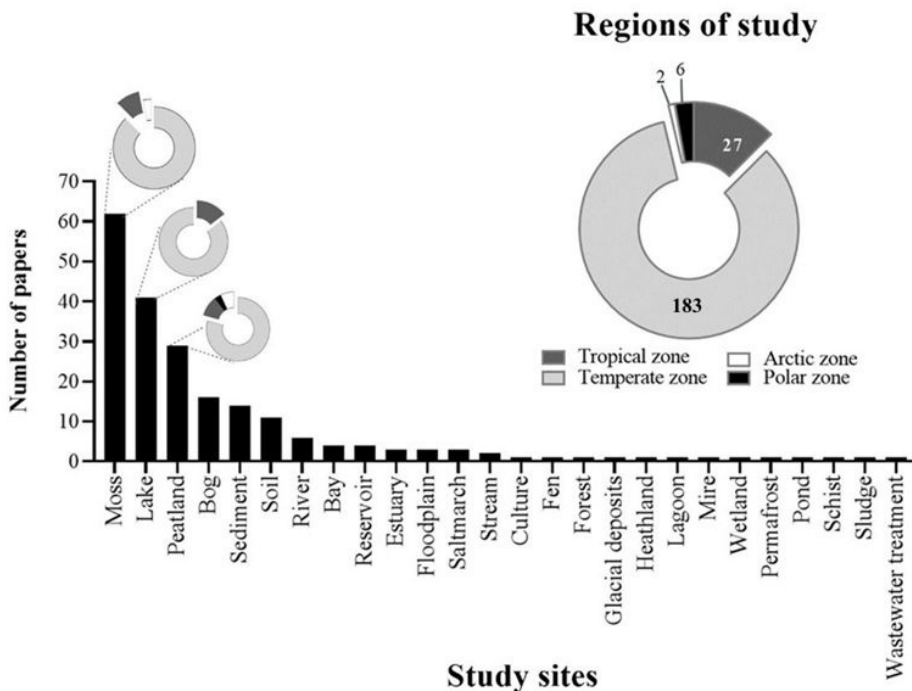


Figure 6. Number of papers per biotope and region of the study.

Most of these biotopes, in special mosses, lakes and peatlands, derive from temperate regions.

4. Discussion

4.1. Number of publications per year

The bioindicator potential of testate amoebae proved to be prominent within most of the studies and this has been confirmed by the increase in the number of global publications that address the theme. The production of articles on biodiversity of testaceans including their potential as bioindicator has increased over the decades, emphasizing their importance as ecological indicators (Lansac-Tóha et al., 2007; Schwind et al., 2016), since the number of publications is important for assessing increasing knowledge on a given subject (Silva & Bianchi, 2001; Brofman, 2018).

The growing number of publications on testaceans reflects the growing trend in research involving the group (Schwind et al., 2013), thus suggesting a greater interest in several countries, and subsequently, their appliance in monitoring studies. In addition, it is worthy of mention that growth patterns in the number of publications is closely associated to the increase in the number of researchers, institutions and universities that have commenced work with the group. The majority of the authors who published three or more articles on the theme did it between the years 2000 and 2010, which further corroborates with the quantitative increase over the last two decades.

4.2. Global publication

The European continent produced the highest number of publications in relation to testaceans and bioindication, followed by North America, Asia and South America. The same pattern was registered for studies solely based on testate amoebae (Schwind et al., 2013), which leads one to presume research articles on bioindication that details this group are closely related to general studies of the community. It is noteworthy that among the nine authors who penned the greater number of publications on the subject, eight published their studies within the European continent, which in turn, provided a healthy distribution of research among their countries. However, we must consider that each research must be linked to a specific project, which in turn may be related to several institutions.

In the American continent, Canada is the country with the largest production output in North America, and Brazil in South America. Some

features of these countries may explain this finding. Both countries contain large swaths of territory that harbours the existence of several biotopes to be studied, especially those in Brazil, being the biggest tropical country in the world and with a high biodiversity. An important aspect however, is the economic imperative that provides differing value allocation for research between developed countries (Canada) and developing countries (Brazil). This can be seen by the number of publications in Brazil being concentrated in some regions, as also reported by Schwind et al. (2013), and associated with institutions with consolidated research groups and receiving greater funding for research. This discussion then takes us back to the importance of the existence of research centers with funded projects focusing on the theme with testaceans to explain the higher number of articles.

Nevertheless, the study of Alves et al. (2014) in relation to how water quality indices have been addressed within international scientific literature indicated that countries with low rates of sanitation and low water quality such as India, China and Brazil produced more publications on new tools for water monitoring. Therefore, the high number of publications in these countries appears to justify the need for more informed scientific research into water quality. In Brazil, this nature of research has enhanced the importance of testate amoebae for environmental monitoring (Costa et al., 2016; Schwind et al., 2019), a gap yet to be filled in countries such as India and China, according to our present study.

4.3. Authors

The author that alone published more papers about bioindication and testate amoebae was Richard J. Payne. This can be a result of Payne's research line, that go around ecology, transfer function and diversity of soil protists, focusing on testaceans, especially in peat bogs (Payne et al., 2017; The University of York, 2021). This pattern was also consistent for other authors like Graeme Swindles and Edward Mitchell, who are also those with most publishing and work with research areas ecologically connected to soil and, in most part, using testaceans.

4.4. Experimental and empirical studies

Research on potential bioindicator protists can prove challenging as it may require detailed analyses regarding ecological suitability of the organisms, which must be chosen according to the

particularities of habitat (Foissner, 1999). The few experimental studies on bioindication and testate amoebae demonstrates how this area of knowledge remains incipient. Most studies however, solely adopted testaceans for their analysis, demonstrating that the group's potential as a bioindicator is widely acknowledged. The dominance of empirical studies within the delimited framework of our study, did point to the lack of experimental studies. It is worth mentioning that most of these unearthed studies are preliminary positions for their areas. Most finalized their conclusions by suggesting that further tests are necessary, thus opening a myriad of opportunities for future experimental research into testaceans as bioindicator organisms.

4.5. Lack of biomonitoring studies with only testate amoebae

Further to those publications recording data solely on testaceans, there were also selected studies in which they were included within large groups, and there was a cluster of essays in which they were studied as a component along with other groups. Foissner (1997) posited that testaceans are constantly excluded from studies that evaluate the effects of biocides on soil protozoa, even with beneficial characteristics being recognized. Corliss (2002) also reported that the importance of testate amoebae for biodiversity within ecosystems had been neglected, suggesting a possible answer the records of these organisms within larger groups like zooplankton. This factor is possibly linked to the gradual increase since 1999 in the number of studies on zooplankton, which then presented higher yields as the years progressed with more published works post 2010 (Souza et al., 2018).

4.6. Studied groups

The near majority of articles in question addressed the community of testate amoebae as-a-whole, electing not to focus on a genus or species. This result suggests that bioindication studies with testaceans are still at an early stage, and where there is still a lack of specific knowledge regarding the community. Schwind et al. (2013) pointed to an increase in the publication of experimental studies with the group over the years, identifying however, a concentration of descriptive and predictive research. This indicates the existence of an on-going worldwide search for basic knowledge on testate amoebae.

In the present study, the results show the use of testate amoebae with other groups in

paleoenvironmental studies, like foraminifera, diatoms and pollen. An increase application of groups that conserve themselves through thousands of years has been related to studies aimed at recording environment features amplitude and occurrence of estuaries, swamps, seas, and lakes (Scott et al., 1991; Eichler et al., 2006a, 2006b). Groups showing a facility in their preservation enable the elucidation of ecology of past periods, the floristic survey and the characterization of the climatic and hydrological conditions of certain areas and time bands (McCarthy et al., 1995). The existing relationship between the groups opened a wide path for paleoenvironmental and bioindication studies with their applicability.

4.7. Study sites and implications

Testate amoebae were frequently studied in peat bogs, primarily on the European continent (Tolonen, 1986; Foissner, 1997; Charman & Warner, 1997). Historically, ecological studies that reference testaceans have focused on peatlands dominated by *Sphagnum*, and ombrotrophic environments, originated from precipitation water, such as rain and snow, where they are mostly used as indicator of water table depth (Mitchell et al., 2008; Song et al., 2014). Testate amoebae are well distributed, diverse and abundant in swamp bogs (Ogden & Hedley, 1980; Warner & Chmielewski, 1992), where they can constitute between 5 to 30% of microbial biomass and remain important in the cycling of nutrients and carbon (Gilbert et al., 1998b; Mitchell et al., 2003; Swindles et al., 2016; Creevy et al., 2018). Soil moisture is the main controller in the distribution of testaceans in peatlands (Lousier, 1974), rendering them eminently suitable as bioindicators for this biotope (Warner & Chmielewski, 1992). In sphagnum-dominated bogs, testaceans perform a central role in the microbial trophic web, and react rapidly to environmental changes (Gilbert et al., 1998a, b; Mitchell et al., 2000).

There are broad variety of correlations between testate amoebae and non-climatic variables in peatlands, such as atmospheric pollution, contamination by metals, sulfur, oil sands and peat acidification (Neville et al., 2011; Nguyen-Viet et al., 2007, 2008; Payne, 2010; Payne et al., 2010; Yang et al., 2011). All these studies point to testaceans as being bioindicators of ecological features and climate conditions in peatland environments (Turner & Swindles, 2012). In this aspect, testate amoebae are capable of showing

changes in peatland restructuring. The community is also linked to the local hydrology, understanding the depth of the water table through knowledge of transfer functions based on hydrological tolerances characteristic of the group (Wilmshurst et al., 2003; Charman & Blundell, 2007). This happens due to species distribution influenced by changes in pH, water chemistry and amount of available nutrients (Lamentowicz et al., 2008).

Studies testing bioindication capability of testate amoebae to assess air pollution, also employed moss samples. Due to their anatomy and physiology, mosses are highly permeable to water and solutes, including ions and trace elements (Nguyen-Viet et al., 2007). For this reason, they have been used as bioindicators for monitoring heavy metals in the atmosphere, and mainly in bodies of water (Reinhardt et al., 1998; Yang et al., 2011). In non-aquatic mosses, testaceans live in a sub-aerial condition, being somewhat exposed to the atmosphere and its pollutants (Nguyen-Viet et al., 2004). Besides indicating the conditions of a specific location, bioindication complements both physical and chemical analysis in measuring atmospheric pollution, as it indicates long-term changes even when measurements of environmental variables are sporadic (Nguyen-Viet et al., 2004).

Nevertheless, testate amoebae are widely used as bioindicators in paleoenvironmental studies (Hendon & Charman, 1997; Mitchell et al., 2008), in lakes (McCarthy et al., 1995; Booth, 2002) and swamps (Warner & Charman, 1994; Booth, 2002; Charman et al., 2004; Charman & Blundell, 2007; Booth et al., 2008). In North America, for example, the Holocene's greatest source of climatological data emanates from studies of testaceans in small lakes (Booth, 2002). Thus, part of the number of studies with testaceans on bioindication in lakes and swamps that contained a paleoenvironmental approach is possibly linked to their presence in these locations, which already have been significantly studied in terms of testaceans (Mitchell et al., 2008; Charman & Blundell, 2007).

A current concern in relation to the degradation of water bodies and loss of biodiversity is growing throughout the world. Thus, biomonitoring strategies are increasingly being put into practice. Among aquatic communities, both benthic and zooplankton communities present favorable characteristics to be utilized as bioindicator tools of water conditions (Picapedra et al., 2021), this including testate amoeba (2013, 2016). In lakes, most of the studies involving testaceans purposed

them as tools for studying contaminants in this biotope (Reinhardt et al., 1998; Neville et al., 2013; Nasser et al., 2016, 2020). They are also used to monitor the consequences of eutrophication including test size and shape (Macumber et al., 2020), as indicators of water quality (Roe et al., 2009; Roe & Patterson, 2014; Qin et al., 2013; 2016; Radhakrishnan & Jayaprakas, 2015; Schwind et al., 2017), pH conditions (Patterson et al., 2013), contamination by trace elements (Misailidis et al., 2018), and inorganic suspended materials (Schwind et al., 2019). The functional traits of testate amoeba were also used as indicator of floodplain restoration success (Fournier et al., 2012).

5. Conclusion

The present scientometric analysis showed the growth over decades in the number of articles regarding the use of testate amoeba for environmental bioindication, and the publications in several journals demonstrates the interest on this topic of research. Important to note that most of the publications remain concentrated in regions where knowledge of the group is more consolidated. Large part of studies was undertaken in temperate region reaffirming the importance of Canada and part of the European continent in the study of this theme. In the tropics, Brazilian studies stand out despite the scarcity of publications from most the tropical regions particularly from the African continent.

Notwithstanding being the temperate region the origin of a greater number of publications, when referencing reservoirs and lagoons, a contrary finding was detected with fewer studies on these biotopes in the north hemisphere. This finding was possibly due to the lack of research specialized on testate amoebae as biomonitors in the aquatic ecosystems and by the main authors on the theme being working mostly in moss and peatlands. In another aspect, a great number of studies assess the testaceans community as-a-whole, which suggests a shortage of more consolidated and in-depth knowledge into species and functional groups. Therefore, our survey points to a large variety and numerous freshwater ecosystems devoid of study matter, indicating the need of efforts to foster the study of testate amoeba's role as bioindicators in special in the tropical region.

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Data availability

All data used in the article is available and can be requested to the authors.

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