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#### Nematodes research: state of the art, prospects, and future directions. A 5 6 network analysis approach.

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#### 20 Abstract

21 Nematodes are among the most successful metazoans inhabiting the Earth and they are pivotal components 22 as in terrestrial as in aquatic (both in marine and freshwater) environments providing important ecosystem 23 services. The aim of this study was to understand major research trends and topics on free-living nematodes 24 inhabiting soil, marine and freshwater environments and to highlight possible differences among them. To 25 achieve this objective, a bibliometric analysis was performed using Scopus database. The indexed global 26 scientific literature on free-living nematodes from 1912 to 2021 was explored using VOSviewer software, 27 allowing a comprehensive overview of the topic. The analyses of co-authorship (among researchers and 28 countries), the co-occurrence of keywords and the analysis of citation of journals were performed. Overall, 29 free-living soil nematodes found a wider audience in high ranked journals especially when compared with 30 freshwater nematodes. Marine nematodes stand in between them and many aspects of biodiversity research 31 in marine ecosystems are covered by high-medium ranked journals (i.e. taxonomy, systematic, phylogeny, 32 morphological and genetic diversity). Although, the estimation of the taxonomic diversity of the phylum 33 Nematoda enumerated a high number of documents, an increasing attention emerged for the investigation of 34 pollution effects (i.e. nematodes as bioindicators of environmental status) and the use of nematodes as model 35 organisms for addressing scientific questions in line with the Eco-Evo-Devo (Ecological Evolutionary 36 Developmental biology) approach. These fundamental themes were indirectly confirmed by the co-authorship 37 analysis, which revealed that taking integrative approaches between taxonomy (both morphological and 38 molecular), ecological and evolutionary aspects attracted a higher number of citations.

39 Keywords: Free-living nematodes; soil; marine; freshwater; VOSviewer; bibliometric analysis

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#### 43 1. Introduction

44 The simple nematode bauplan (i.e. generalized structural body plan) and the low number of body cells belie in 45 their molecular complexity, which have led to unrivalled success amongst the metazoans (Maule and Curtis,

46 2011). About ninety per cent of all the metazoans in the Earth are supposed to be nematodes (Andrássy,

47 2005), but only a fifth of their biodiversity is currently known (Appeltans et al., 2012) with about 12,868 species

48 of free-living nematodes so far described (Nemys eds. 2022).

49 Nematodes have colonized all climatic areas and all types of environments, from aquatic (marine to freshwater) 50 to soil ecosystems. They have also developed a multitude of parasitic lifestyles: obligate or facultative, external 51 or internal parasites with one or two hosts (Zullini, 2012) and that may cause numerous human diseases and 52 large financial losses when involved in agriculture and livestock rearing (Manzanilla-Lópes et al., 2004).

Free-living nematodes dominate all ecosystems for biomass and abundance (see Andrássy, 2005; Giere, 2009 for review) surviving to all the most extreme environmental conditions: e.g. *Halicephalobus mephisto* was found at 1,3 km beneath the soil surface (Borgonie et al., 2011) and *Oncholaimus dyvae* in deep sea hydrothermal vents (Zeppilli et al., 2019). "Contrary to the popular opinion, nematodes do not all look the same...", so Platt and Warwick (1980) stated in their chapter. Zullini (2012) even gave an almost paradoxical but empirically correct diagnosis of the phylum emphasizing the wide variability of the look and lifestyles of these animals.

Free-living nematodes occupy different trophic levels, they provide important ecological functions by connecting other components of the ecosystems and participating in sedimentary, trophic and ecological processes. They stimulate nutrients cycling especially nitrogen, they regulate decomposition processes by grazing on microbes. Nematodes can enhance soil and sediment biodiversity by contributing to the maintenance of healthy environments, both in land and in aquatic systems (Ingham et al., 1985; Freckman, 1988; Neher, 2001; Balsamo et al., 2010; Jiang et al., 2017; Schratzberger and Ingels, 2018).

65 In light of the above, nematodes have clearly aroused interest amongst researchers worldwide over the years. 66 Numerous studies and research programs have been carried out to promote their use in the ecological 67 assessment, to develop new indices and indicators, to estimate nematode biodiversity and to increase our 68 knowledge about their great evolutive and ecological success (e.g. Eyualem-Abebe et al., 2006; Danovaro et 69 al., 2008; Miljutin et al., 2010; Höss et al., 2011; Moreno et al., 2011; Schmidt-Rhaesa, 2014; Xie and Zhang, 70 2022). Meiofauna, and in particular nematodes, have been largely recognized as useful tools to assess the 71 environmental quality. Due to their characteristics, nematodes are efficient bioindicators of environmental 72 conditions for several habitats, from estuaries to deep-sea ecosystems (Moreno et al., 2011; Alves et al., 2013; 73 Semprucci et al., 2015). They are highly responsive to different types of impacts such as physical and chemical 74 disturbances and anthropogenic pressures, reflecting variation of the environment with changes in community 75 composition (Höss, 2011; Schratzberger and Ingels, 2018; Biswal, 2022). Despite the good characteristics of 76 meiofauna, and mostly of nematodes, no species or taxa belonging to these groups are included in the 77 environmental directives of the European Union. After the Water Framework Directive (WFD, 2000/60/EC) in 78 2000, many directives have been implemented by the European Parliament and the European Union Council, 79 such as the Marine Strategy Framework Directive (MSFD, 2008/56/EC) in 2008 that was designed to reach 80 the Good Environmental Status by the year 2020. The MSFD directive is focused on ensuring sustainable use 81 of the seas, and management and conservation of marine waters and its resources, using an ecosystem-82 based approach. To do this, some species are monitored as bioindicators, but they all belong to macrofauna. 83 Nowadays, it appears clear that it is important to enrich and refine the knowledge about nematodes ecology 84 in order to integrate MSFD using these organisms as bioindicators.

85 The present paper aims to seize the trends in scientific studies concerning free-living nematodes inhabiting 86 three distinct environments: soil, freshwater and marine sediments. In details, we explore on a global scale: 87 (1) the main temporal trends of the literature on free-living nematodes; (2) main topics of investigation and 88 favorite journals chosen for publication through time; and (3) the evolving collaboration among experts in 89 different countries. 90 The bibliometric analysis by means of software can quantitatively and accurately process large numbers of 91 documents according to several aspects (title, keywords, word frequency, citation, authors, journal, etc.) 92 without the deviations derived by the inevitable subjectivity of a human selection. Among the most widely used 93 bibliometric analysis software, VOSviewer is one of the more convenient to users because the results can be 94 easily visualized with heat and network density maps and because it is relatively accessible to everyone 95 (Buonocore et al., 2018; Zhou et al., 2021; Rendina et al. 2022).

96 This study systematically analyzed the scientific indexed literature related to free-living nematodes in the 97 Scopus database by means of VOSviewer software (Van Eck and Waltman, 2010) to produce an overview on 98 this issue. The bibliometric analysis was performed in two steps: firstly, by considering free-living nematodes 99 in general and, secondly, by including free-living nematodes from soil, freshwater and marine sediment 100 environments specifically.

## 101 2. Methodology

The scientific literature about free-living nematodes was explored by analyzing the course of research through
 the years, authors and countries relations, trends in keywords and research topics. Finally the indexed journals
 concerned with publishing studies on free-living nematodes were examined.

Documents were collected on January 24<sup>th</sup>, 2022, by searching on Scopus the keywords "nematodes" AND "free living", AND NOT "parasite" "human" "disease" "medical", in order to include only publications on freeliving nematodes. Results were exported as .csv files after selecting all the possible information and including the references. Review documents were excluded. The same criteria were used to create three independent databases, one for each environment, adding respectively "soil", "marine" and "freshwater" to the keywords aforementioned in order to analyze the three environments separately. The list of the papers are provided as Supplementary Material (Files S1-S4).

112 The search produced 2,255 results ranging from 1912 to 2021, and the database has been processed using 113 the VOSviewer software (version 1.6.16). The main technical terms used in the software VOSviewer are 114 explained in Table 1 (Van Eck and Waltman, 2018).

Term	Description			
Items	Objects of interest (e.g., publications, researchers, keywords, authors).			
Link	Connection or relation between two items (e.g., co- occurrence of keywords).			
Link strength	Attribute of each link expressed by a positive numerical value. In the case of co-authorship links, the higher the value, the higher the number of publications the two researchers have co-authored.			
Network	Set of items connected by their links.			
Cluster	Sets of items included in a map. One item can belong only to one cluster.			
Number of links	The number of links of an item with other items.			
Total link strength	The cumulative strength of the links of an item with other items.			

**Table 1.** Main technical terms used in the software VOSviewer

117 In this study the following analyses were carried out: i) the co-authorship among researchers and countries to 118 create networks in which the items are linked to each other according to the number of jointed publications; ii)

the co-occurrence of keywords in the title, abstract or keyword list of papers; and iii) cited scientific journals,

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120 where two items are linked if at least one cites the other. For each analysis, it is possible to set a threshold in 121 order to produce a clear map and highlight most represented items. In this case, the thresholds were set as 122 follows: minimum 10 documents and 5 citations per author in co-authorship among researchers, minimum 10 123 documents per country in co-authorship among countries, minimum 10 occurrences per keyword in co-124 occurrence analysis, minimum 10 documents per journal in citation analysis. In the keywords co-occurrence 125 analysis, it was necessary to create a thesaurus file, in order to avoid synonyms and to merge terms, when 126 appropriate (e.g. 'Nematoda' and 'nematodes' or singular and plural). In addition, the temporal trend analysis 127 of publication provided by Scopus is reported.

128 The overlay visualization provided by VOSviewer was used to display the main results obtained. In this way, 129 it is possible to score the items by average citation or average publication year and the size of items circles 130 depends on the number of documents.

131 3. Results and discussion

#### 132 3.1 Temporal trend analysis

133 In Figure 1, the temporal trend of publication from 1912 to 2021 is shown. This outcome highlighted an 134 increasing and constant interest for nematodes in the scientific literature over time, even if with an addition of 135 relatively few documents per year (maximum: 102 in year 2015) compared to other topics such as marine 136 microplastics, which reached the number of 518 scientific publications in 2018 (Pauna et al., 2019). How can 137 we explain this low contribution in terms of papers produced? Free-living nematodes are natural components 138 of the ecosystems and they do not represent a threat for humans, ecosystems, or ecosystem services such 139 as the emerging problem of microplastics or the widely known problem of plant-parasitic nematodes (PPNs) 140 that threaten the crop (McSorley, 2011). The basic aspect to explore is related to their huge biodiversity. Being 141 inhabitants of almost all kinds of environments, the sole sampling of a virgin area might lead to the discovery 142 of numerous new species for the science (Hodda et al., 2009). More direct benefits for us arrive just later as a 143 second step. For example, the usefulness of free-living nematodes as bioindicators for the assessment of the 144 environmental quality status is possible only if the species that composed the assemblage are described and 145 therefore known (e.g. Derycke et al., 2008, 2010; Semprucci et al., 2015; Sahraeian et al., 2020; Franzo et al., 146 2022). Despite the low contribution in terms of scientific literature produced, we assited atan increasing interest 147 in the ecological papers and research in the 21st century as recent nematological literature confirmed its focus 148 on parasitic nematodes (Majdi and Traunspurger, 2021). Consequently, in terms of the type of habitats studied 149 more attention has been devoted to soils, whereas free-living marine and freshwater nematodes remained 150 rather marginal (Majdi and Traunspurger, 2021).



152 Fig. 1 Temporal trend analysis of number of publications derived from Scopus database on free-living153 nematodes

#### 154 **3.2 Co-authorship analysis: researchers**

Of the 4,959 resulting authors, 57 met the threshold and were divided into 18 clusters, which were likely to reflect the various research teams and collaboration networks. The top 10 authors ranked by number of documents are reported in Table 2. In Figures 2a and 2b, a focus on the average publication year of each author (2a) and on the average citation (2b) is represented. The focus was made by excluding all isolated items and keeping the central core of connected authors. In Supplementary Material Fig. S1 a, b the complete overlay visualization displaying the average publication year of each author (Supplementary Material Fig S1a) and the average citation (Supplementary Material Fig S1b) are shown.

162	Table 2. List of top 10 authors ranked by number of documents. For each author, the gender (M= male; F=
163	female), the number of citations and the total link strength are provided.

Author	Gender	Documents	Citations	Total link strength
	Gender	Doodmonto	Chations	rotar inne strongen
Gagarin V.G.	Μ	86	282	58
Huang Y.	Μ	54	383	14
Traunspurger W.	Μ	40	987	3
Vanreusel A.	F	34	711	19
Mahmoudi E.	Μ	32	555	101
Rothstein M.	Μ	31	815	0
Aïssa P.	F	30	577	95
Beyrem H.	Μ	27	556	88
Yushin V.V.	Μ	27	156	23
Moens T.	Μ	26	1,426	24



Fig. 2 Overlay visualization of map for co-authorship authors. Thresholds were set as minimum 10 documents
 and 5 citations per author in co-authorship among researchers. a) plot ranked by average publication year; b)
 plot ranked by average citations. The colors go from blue as minimum up to yellow as maximum values

168 As clearly shown in Figure 2 and reported in Table 2, although holding the highest number of publications, 169 V.G. Gagarin was the second least cited author. Similarly, Y. Huang, the second most productive author 170 identified, was the third least cited one. The scientific production of these authors is almost exclusively 171 taxonomic (e.g., discovery and descriptions of new species, emended diagnoses, systematic), e.g., V.G. 172 Gagarin described many new species from lake Baikal that attracted a poor number of citations both in Scopus 173 and in the grey literature. In particular, the grey literature being published in non-indexed journals and often of 174 local importance, is practically 'invisible' to the international scientific community due to its limited distribution. 175 These results open the controversial and long-standing issue of taxonomy research line. Although this branch 176 of science is essential for providing the baseline for any type of investigation on nematode biodiversity and 177 ecology, it is often considered of scarce appealing especially after the coming of the so-called 'omics' (i.e. 178 genomics, proteomics, metabolomics, metagenomics and transcriptomics) (Bhadury, 2012). Traditional 179 taxonomy is certainly a time-consuming activity and, in a world dominated by the rule 'publish or perish', the 180 limited reward obtainable from a scientific production based only on this approach discourages many young 181 scientists from taking this path. This problem was recently emphasized by Gleason (2022) who, in the last

182 international conference of nematology, underlined as in United States, the country of the founder of the 183 nematology (N.A. Cobb), there are a few classically trained plant nematologists to teach higher level courses. 184 Thus, in light of the above, can we abandon the traditional morphological taxonomic approach? The response 185 is negative. Since the 50s we are searching for "short cuts" (e.g. functional traits, Wieser 1953) that might 186 reduce the time required for making a taxonomical identification or for obtaining the response of the nematode 187 community to environmental changes. An additional solution came in the 2000s, when barcoding technique 188 found a growing application in the integrative taxonomy followed more recently by the metabarcoding 189 approach. However, there is no right or wrong approach, researchers should make a choice strictly depending 190 on the purpose of the project or in relation to the available resources. There is evidence that even cryptic 191 species can have different roles in the ecosystem functioning (Guden et al., 2018, 2021) and only DNA 192 barcoding can detect them (Armenteros et al., 2014; Shenk et al., 2020). On the other hand, it is inconceivable 193 to completely avoid the morphological approach because the DNA (eDNA) metabarcoding is a technique that 194 still needs improvement (possible biodiversity assessment bias by capturing signals from dead organisms and 195 extracellular DNA; false readings due to taxonomic selectivity and restricted sensitivity of primers; 196 unavoidability of primers and amplification bias; lack of comprehensive genetic databases) (Ruppert et al., 197 2019). Again, approaches based on combinations of morpho-functional traits (sensu Semprucci et al., 2022) 198 might greatly speed the analyses of nematodes and might be used by unexperienced people in biomonitoring 199 programs, especially when a low financial budget is available. Even if free-living meiofaunal nematodes are 200 not vet officially included in the MSFD, the contribution of nematodes to essential ecosystem processes (e.g. 201 nutrients cycling especially nitrogen, regulation of decomposition processes by grazing on microbes, soil 202 enrichment) is widely recognized (Höss, 2011; Schratzberger and Ingels, 2018). All these key functions 203 contribute to the maintenance of a healthy environment, both in land and aquatic systems (Freckman, 1988; 204 Neher, 2001; Jiang et al., 2017). Nematodes could be suitable indicators to assess pollution impact because 205 of their biological characteristics, their strong connection with sediments and tolerance to pollutants (Heininger 206 et al. 2007; Höss et al. 2011). Alterations in the structure of the soil nematode community have been studied 207 to create measurable indices that can be used to assess soil and marine sediments health (Bongers, 1999; Lu 208 et al., 2020; Ridall and Ingels, 2021). Thus, an integrative approach and a community of nematologists able to 209 integrate their expertise, remain the only long-term strategy to promote the nematology in all its aspects and 210 the only way to find a link between a "sequence" and a "life strategy" or "ecological notes". When the 211 zoology/taxonomy is integrated with the ecological perspectives, the number of citations and the relative 212 visibility of the nematologists notably increase. Two clear examples of this are represented by T. Moens and 213 W. Traunspurger. Moens was the last of the top ten authors listed for number of documents (26), but he had 214 the highest number of citations (1,426) thanks to the relevant appealing of his research topics that include 215 marine benthic food webs, biodiversity – ecosystem functioning relationships, population genetics and (micro) 216 evolution, nematode - bacteria interactions (e.g. Derycke et al., 2013; De Meester et al., 2016; Guden et al., 217 2021; Francolino et al., 2021). Similarly, Traunspurger ranked not only the second most productive author in 218 terms of publication number (40), but also one of the most cited (987). His main research lines are focused 219 both on nematode population dynamics, interspecific competition, functional response and microcosm 220 experiments (e.g. on the impacts of microplastics, heavy-metals, crude oil water-soluble fractions, fungicides 221 on nematodes) (e.g. Haegerbaeumer et al., 2018; Monteiro et al., 2018, 2019; Fueser et al., 2020). His highly 222 interdisciplinary research in zoology and taxonomy has been demonstrated also by his studies in which all 223 three morphological, DNA barcoding and metabarcoding approaches are combined (Fonseca et al., 2008; 224 Schenk et al., 2022). All these aspects have certainly contributed to increase the visibility of this researcher.

225 Among the top 10 authors of Table 2 only two women were listed, i.e. A. Vanreusel and P. Aïssa. Similarly, 226 less than half of the authors in Figures 2 and S2 are women. Although the gender was not one of the factors 227 considered in the present study because of being out of its scope, such evidences are nothing but surprising 228 because they confirm the well-known problem of gender inequality in scientific careers (Barret et al. 2019; 229 Huang et al. 2020). Although discussing the issue considering all the STEM disciplines (science, technology, 230 engineering and mathematics) and not only strictly the biology fields, Huang and coauthors (2020) 231 demonstrated that the gradual increase of women in STEM in the last 60 years was accompanied, 232 paradoxically, by an increase in the gender disparities expressed as productivity and impact. The main causes 233 of such inequality are the career length and the dropout rate, this latter defined as the yearly fraction of authors

in the population who have just published their last paper. Overall, men tend to have longer careers and lower dropout rates than women. In other words, each year, women scientists have a higher risk to leave academia than male colleagues, giving male authors a major cumulative advantage over time. Moreover, the authors demonstrate that the dropout gap is not limited to junior researchers but persists at similar rates throughout scientific careers. These trends sadly explain why the most pronounced gender gap is among the highly productive authors, i.e. those who train the new generations of scientists and serve as models for them, reducing furthermore the role and the contribution of women in science.

#### 241 Co-authorship analysis of countries in three different environments: soil, marine, freshwater

242 This second step analysis was performed by focusing on free-living nematodes inhabiting different 243 environments: soil, marine and freshwater. The number of documents per country for each environment are 244 visualized in Figure 3 and listed in Supplementary Material Table S1. The majority of countries (17 out of 22 245 countries considered in our analysis) produced papers on free-living nematodes from soil, with an increasing 246 number from the east (e.g. China, Japan) to the west (e.g. Germany, UK and finally USA) of the globe (Figure 247 3). This trend can be ascribed firstly to the long tradition of countries like UK. Germany and USA in the research 248 field of the free-living nematodes and secondly to the economic interest by all countries, which guides the 249 research on nematodes from soil. An opposite trend emerged when the documents on free-living nematodes 250 from marine environment were considered. A decreasing trend appeared moving from the east to the west of 251 the globe. Emerging countries such as China (with the highest number of documents) and, for a lesser extent 252 India and Brazil, are clearly expressing their interest in this topic and consequently they are allocating notable 253 funding to the scientific research. For the Russian Federation the long tradition in marine nematode research 254 puts this country in second place (Supplementary Material Table S1). Less explored is the field of freshwater 255 free-living nematodes. Only 5 out of 22 countries reported documents on this topic. Russian Federation first 256 and Germany as second were the countries with the highest numbers of documents. This result is mostly due 257 to the flourishing paper production in nematode taxonomy by Gagarin V.G. from Russia and ecology by 258 Transpurger W. from Germany (see also Table 2). From the map it was possible to notice a very low number 259 of papers on free-living freshwater nematodes worldwide, except for the ones produced in the Russian 260 Federation and in Germany. Moreover, a remarkable feature of these maps was an almost absent scientific 261 production in most of the 'emerging countries' such as Africa, Central and South America except for Brazil, 262 Indonesia and Middle East related to free-living nematodes from the different investigated environments.



Fig. 3 Co-authorship analysis of countries in the three different environments: soil, marine, freshwater.
 Thresholds were set as minimum 10 documents per country in co-authorship among countries. Each country is coloured according to the number of documents referred to the scale bar. Note that the maximum number of documents for each country varies for the three environments (max. 129 for soil, max. 76 for marine and max. 48 for freshwater)

269

#### 270 3.3 Co-occurrence analysis of keywords in soil, marine and freshwater environments

271 The keyword map shows the average citations and the average publication year. Keywords for each database, 272 ranked by occurrences, are listed in Table S2 of Supplementary Material. Of the 3,565 results for soil database, 273 90 met the threshold and, after the creation of the thesaurus file, 16 keywords are displayed in Figure 4, scored 274 by average citation. It was possible to see how item's size based on the occurrences was independent from 275 the color, which indicated the average citation value: for example, the keyword "ecological indices", definitely 276 smaller than "free-living nematodes" or "soil", was colored in yellow (average citation number 60) and has been 277 cited much more than the other in blue (average citation number 30). To explain this, it should be reminded 278 that many ecological indices for nematodes have been developed in terrestrial environments, mostly to 279 evaluate soil health. Free-living nematodes are, in fact, largely recognized as useful tools to assess the 280 environmental quality. Bacteria and fungi are the main food sources of soil free-living nematodes and the 281 interactions of nematodes with microbial decomposers affect the ecosystem processes such as decomposition 282 and nutrient cycling (Chen et al., 2010), as indicated by the keywords "food web", "bacteria" and "fungi" which 283 were all linked to "free-living nematodes" and "ecological indices".



Fig. 4 Co-occurrence keywords in soil environment (minimum 10 occurrences per keyword in co-occurrence
 analysis). Keywords are ranked by average citation. The colors go from blue as minimum up to yellow as
 maximum values (i.e. 20 and 60 respectively)

288 Also, the keywords "heavy metals", "pollution" and "toxicity" resulted as high-cited, revealing the importance of 289 this topic for the ecological investigations in soil systems. These topics are usually closely associated to C. 290 elegans. Thanks to the feasibility of rearing in controlled conditions, the short generation time, the opportunity 291 to manipulate eggs/embryos and adults, the genome sequencing knowledge, this species is recognized as a 292 model organism in biology and ecotoxicology (Corsi et al., 2015). The occurrence of the term "taxonomy" 293 especially linked to "dna" and "physiology" underlines the routinary role of the molecular analyses in the 294 taxonomy of soil nematodes, combined to the investigation of their ability to survive severe environmental 295 fluctuations (i.e. mechanisms that act to withstand temperature extremes, desiccation, osmotic and ionic 296 stress). In fig. S2 (Supplementary Material) keywords were scored by average publication year from 2004 to 297 2010 and it was possible to notice how "taxonomy", "ecology" and "food web" were colored in yellow (average 298 publication year 2010), underlining the importance in recent times of these three topics in free-living nematodes 299 research, while "C. elegans" has been used more in the past (average publication year 2004).

300 Of the 1,926 results for marine database, 70 met the threshold and, after the creation of the thesaurus file, 18 301 keywords are displayed in Figure 5. The keywords map resulting from the analysis on marine nematodes 302 revealed a high heterogeneity. The right part of the figure shows the keywords that attracted less citations and 303 that are associated to the spheres of taxonomy and biogeography: "taxonomy", "China" (where many marine 304 species have been described), "distribution" and "dispersal". The central part was related to general ecology 305 that is also associated with the general "meiofauna" appearing in the lower part of the plot. The most attractive 306 topics are on the assessment of pollution effects, since "diversity", "density", "richness" and "community 307 structure" can be considered as descriptors of environmental changes, both in laboratory ("microcosm") (e.g. 308 Monteiro et al., 2014; Boufahja and Semprucci, 2015) and in the field ("sediments") (e.g. Alves et al., 2013; 309 Losi et al., 2021; Franzo et al., 2022). In contrast, the keyword "biomass" has metless popularity, showing an 310 intermediate citation rank. In fig. S3 (Supplementary Material) keywords map is displayed scored by average 311 publication year: "pollution" appears as a more recent topic even if with high citation rank, followed by 312 "taxonomy", "diversity" and "china" keywords underlining again the importance of this country in free-living

- 313 marine nematodes studies. The blue colour of the term "biomass" is the minimum value of the map (average
- 314 publication year 2016), which indicates together with the intermediate citation rank, the low interest for this
- 315 topic being, as aforementioned, nematode biomass estimation a highly time-consuming analysis.



Fig. 5 Co-occurrence keywords in marine environment (minimum 10 occurrences per keyword in co-occurrence analysis). Keywords are ranked by average citation. The colors go from blue as minimum up to yellow as maximum values (i.e. 5 and 9, respectively)

320 Of the 1,241 results for freshwater database, 29 met the threshold and after the creation of the thesaurus file 321 8 keywords are displayed in Figure 6. As in marine systems, "taxonomy" keyword was the second one listed 322 for the number of documents, but it attracts a low citation number along with "eurasia", "lake" and "biodiversity" 323 that likely remained mainly related to taxonomic literature, while "community structure" and the general 324 "meiofauna" remained the most cited ones (Fig. 6). Indeed, in both marine and freshwater habitats, free-living 325 nematodes are a permanent component of the benthos, representing the most diverse and abundant taxon 326 (Giere, 2009, Zeppilli et al., 2017; Semprucci and Sandulli, 2020). In Fig. S4 (Supplementary Material)where 327 keywords were ranked by average publication year, both "meiofauna" and "community structure" appeared 328 colored in blue to indicate the oldest publication year, while biodiversity Eurasia and lake were in green and 329 yellow, being more recent.



Fig. 6 Co-occurrence keywords in freshwater environment (minimum 10 occurrences per keyword in co-occurrence analysis). Keywords are ranked by average citation. The colors go from blue as minimum up to yellow as maximum values (i.e. 10 and 20, respectively)

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#### 335 3.4 Citation analysis of journals in soil, marine and freshwater environments

The citation analysis of journals performed separately for each database (soil, marine and freshwater) is displayed in Figure 7.



338

Fig. 7 List of journals for soil, marine and freshwater environments ranked by number of documents fromcitations-source analysis. Each journal shows the quartile to which it belongs in brackets

The results show five journals dedicated to soil. The three belonging to the first quartile (Q1), and especially *Applied soil ecology* and *Soil biology and biochemistry*, although dealing with ecology, biology and biochemistry, reached a high citation value per document. The two journals dedicated to all aspects of nematological research, i.e. *Journal of nematology* and *Nematology*, both Q2, revealed an overall high number of citations (931 and 371, respectively).

The six journals sorted by VOSviewer for marine nematodes were distributed in four quartiles. Among them only *Ecological indicators* is Q1 with only 9 documents. *Zootaxa*, a taxonomy journal, belongs to the second quartile and is the richest in both the number of documents (55) and of citations (180). *Journal of the marine biological association of the United Kingdom* (Q3) and *Marine biodiversity* (Q2) are two journals that deal with all the aspects of biodiversity research in marine ecosystems, included studies on taxonomy, systematics and phylogeny as well as morphological and genetic diversity. Two journals dedicated to nematodes, *Nematology* (Q2) and *Russian journal of nematology* (Q4) are again among the sorted journals.

Only three journals result for freshwater free-living nematodes. *Inland water biology* is strictly dedicated to this type of environment and belongs to Q4, while *Zootaxa* and *Nematology* are both in Q2. Overall, free-living soil nematodes found a wider audience in high ranked journals especially when compared with freshwater nematodes. *Nematology* is the specialist journal that occurs in the top list of all the three environments, while the aspects of systematic zoology covered by the international journal *Zootaxa* are mainly focused on aquatic nematodes, especially marine ones.

### 359 4. Conclusions

Pathogenic nematodes get most attention in biology because of their relevant impact on crop production,
 livestock rearing or human health. However, free-living nematode biodiversity contributes to the ecosystem
 quality status and resilience (i.e. capacity to suppress diseases or alterations) in a complex interaction between
 biological, chemical and physical properties.

364 The temporal trends of publication clearly showed an overall increasing and constant interest of the scientific 365 community for the free-living nematodes, although their potential in ecological research needs to be fully 366 recognized. The co-occurrence of similarities and divergences in the nematology trends of all three 367 environments reveals a complex scenario. Overall, the Russian Federation holds the highest number of 368 publications on free-living nematodes, but the topic of these papers is almost exclusively taxonomic with 369 description of new species found in freshwaters. The highly specificity of the subject as well as the local nature 370 of the sampling area (i.e. restricted spatial scale of investigation) may justify the low number of citations. 371 However, also some emergent countries such as China, India and Brazil show to have an increasing weight 372 in terms of paper production in these three environments (soil, marine and freshwater). According to the citation 373 analysis of journals performed on Scopus and according to the thresholds we have chosen, free-living soil 374 nematodes found a wider audience in high ranked journals (i.e. 3 Q1, 2 Q2) compared with freshwater 375 nematodes (2 Q2, 1 Q4), while marine nematodes found a more heterogeneous audience of journal (i.e. 3 Q2; 376 1 Q1, Q3, Q4). However, the case of freshwater nematodes deserves a further consideration. In fact, only if 377 the threshold in the minimum number of documents is reduced from 10 to 1, the Q1 journals Hydrobiologia 378 and Freshwater Biology appeared.

379 The environmental assessment and the estimation of the taxonomic diversity of the phylum Nematoda are 380 among the most frequent topics of the documents, but the most successful one is certainly related to the 381 investigation of the pollution effects on the free-living communities. Both these aspects are fundamental for 382 the growth of the nematology, and biodiversity investigations are pivotal to fill in the gaps of knowledge on one 383 of the most important, abundant and diversified phyla of the biosphere. We are facing with increasing 384 environmental changes and accurate disturbance assessments require the building of a catalogue of free-385 living organisms against which to measure future changes and biodiversity losses. Furthermore, the use of the 386 nematodes as bioindicators may be effective only knowing the real biology and life-strategy of as many species 387 as possible. Thus, as confirmed by the co-authorship analysis, an integrative approach between taxonomy 388 (both morphological and molecular) and ecology led by a community of 'well diversified' and collaborative

nematologists seems to be the only long-term strategy to promote the knowledge on free-living nematodes. In fact, when the zoology/taxonomy is integrated with the ecological aspects, the number of citations and relative appealing of the nematologist research lines increase building new possible perspectives for the biology including new frontiers of biology such as Eco-Evo-Devo (Ecological Evolutionary Developmental biology) for which nematode model organisms (e.g. *Caenorhabditis elegans, Pristionchus pacificus* and *Litoditis marina*) might be crucial.

395

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