

Article

Planarians, a Neglected Component of Biodiversity in Groundwaters

Benedetta Barzaghi ^{1,2,*}, Davide De Giorgi ¹, Roberta Pennati ¹ and Raoul Manenti ^{1,2}

¹ Department of Environmental Science and Policy, Università degli Studi di Milano, via Celoria 26, 20133 Milano, Italy; d.degiorgi97@gmail.com (D.D.G.); roberta.pennati@unimi.it (R.P.); raoul.manenti@unimi.it (R.M.)

² Laboratorio di Biologia Sotterranea “Enrico Pezzoli”, Parco Regionale del Monte Barro, Località Eremo, 23851 Galbiate, Italy

* Correspondence: benedetta.barzaghi@unimi.it

Abstract: Underground waters are still one of the most important sources of drinking water for the planet. Moreover, the fauna that inhabits these waters is still little known, even if it could be used as an effective bioindicator. Among cave invertebrates, planarians are strongly suited to be used as a study model to understand adaptations and trophic web features. Here, we show a systematic literature review that aims to investigate the studies done so far on groundwater-dwelling planarians. The research was done using Google Scholar and Web of Science databases. Using the key words “Planarian cave” and “Flatworm Cave” we found 2273 papers that our selection reduced to only 48, providing 113 usable observations on 107 different species of planarians from both groundwaters and springs. Among the most interesting results, it emerged that planarians are at the top of the food chain in two thirds of the reported caves, and in both groundwaters and springs they show a high variability of morphological adaptations to subterranean environments. This is a first attempt to review the phylogeny of the groundwater-dwelling planarias, focusing on the online literature. The paucity of information underlines that scarce attention has been dedicated to these animals. Further revisions, including old papers and books, not available online will be necessary.



Citation: Barzaghi, B.; De Giorgi, D.; Pennati, R.; Manenti, R. Planarians, a Neglected Component of Biodiversity in Groundwaters. *Diversity* **2021**, *13*, 178. <https://doi.org/10.3390/d13050178>

Academic Editors: Luc Legal and Michael Wink

Received: 5 March 2021

Accepted: 20 April 2021

Published: 22 April 2021

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Invertebrates embrace the mainstream of metazoan diversity, but only a very small portion of invertebrate species is generally considered in environmental conservation actions [1]. Excluding species of direct importance for human feeding and other economic activities, for example crustacean and mollusks rearing and fishing, the proportion of neglected invertebrates is even much higher [1]. Not only the human concern lessens as organisms get smaller and/or show an increased number of appendages, but often human feeling moves from a constructive concern for their conservation to a negative concern about possible dangers or disgusting reactions [2]. The human preference for furrier and larger animals [3] and the main conservation efforts that are generally focused on vertebrates [2] end in neglecting invertebrates also in terms of preservation, belying the fundamental role that these organisms play in ecosystems and for humans themselves [4]. In freshwater habitats, there is a high invertebrate diversity that mirrors the miscellaneous array of micro-habitat typologies that are available in the aquatic environments [5]. These micro-habitats have various sizes ranging from the small interstices and crevices of the streams substrate, that host unique assemblages of invertebrates enhancing the occurrence of beta diversity [6,7], to large river connections and systems, characterized by ancient drainages that allowed astounding species radiation in different invertebrate groups [8]. In freshwater ecosystems, invertebrates provide several ecological functions of fundamental importance [5]. Detritivore and filtering invertebrates allow water purification and organic

matter processing in both lentic (aquatic habitat with calm water) and lotic (aquatic habitat with running water) habitats [9–12]. Moreover, many freshwater invertebrate species furnish highly considered goods, mainly constituted by food resources (as several crustaceans and mollusks), but also by medicinal and ornamental products [13]. However, despite being a fundamental source of biodiversity, several highly diverse taxa of aquatic invertebrates, like planarians, remain still poorly known and poorly considered in research and conservation projects.

The planarias are Platyhelminthes belonging to the class of Turbellaria, order of Tricladida. They are widespread on a global scale and new species continue to be discovered and described [13–15]. Often, they occur in such large numbers in lakes, streams and springs, as to be a significant component of freshwater communities [16–18].

Commonly referred to as “flatworms” because of their dorso-ventral flattening, freshwater planarians are found mainly in surface waters [19,20], however an unexpected diversity of planarians occurs also in groundwaters [21,22]. Nearly 200 species of planarians have been recorded in groundwater environments [23]. Cave-dwelling planarians are distributed worldwide in subterranean habitats [21,24,25], but most of the described species have extremely narrow ranges, being frequently known for a single cave [22,26]. The study of groundwater-dwelling planarians started relatively late with respect to other invertebrates. *Dendrocoelum cavaticum* was the first subterranean planarian described in 1873 in a cave of Jura Souabe, in South-East Germany [24]. Only 30 years later, some surveys extended its range to other caves and some spring habitats of the same German area [27]. Since then, the number of discovered and described groundwater-dwelling species has regularly increased together with the fauna assessments of caves. However, planarians finding appeared, especially in the past, as a side effect of sampling campaigns devoted to other more charismatic animals [24]. As a consequence, the description of new species of groundwater-dwelling planarians has been rarely combined with information on species habitats [25]. For example, until 2018, the locality type was the only information available for all the Italian cave-dwelling planarians that, in most cases, were sampled only once and not by the scientist who described the species [26,28]. The first studies assessing the features of the habitat of a groundwater-dwelling planarian were performed at the whole cave scale on the species *Atrioplanaria notadena* [29]. Groundwater-dwelling planarians are a promising model group to study the general patterns allowing exploitation and adaptation to a difficult environment. In temperate regions, groundwaters are usually oligotrophic environments: They show limited nutrient content [30], posing serious constraints to the exploitation of these habitats [31]. At the same time, groundwaters may offer shelter from UV radiation and predators [32–35]. Constraints and advantages caused the evolution of different morphological, physiological and behavioral features associated with animals exploiting them [36,37]. Most groundwaters’ specialist animals, named stygobionts (the suffix “stygo” comes from the river Styx flowing in the mythological Greek underworld), show typical features, such as blindness, depigmentation, and elongation of tactile sensory structures, metabolism reduction, resistance to starving, and low oxygen contents [38,39]. Blindness, depigmentation, and elongation of tactile sensory structures are commonly referred to as troglomorphisms [40]. Most of planarian species collected in caves have been considered as stygobionts and are totally blind and depigmented, showing a strong adaptation to groundwaters [41]. Nevertheless, also pigmented species with eyes have been reported in groundwater habitats [26,42]. Moreover, the planarians’ position in the trophic web of groundwaters is unclear. Generally planarians are zoophagous, feeding mainly on small living invertebrates [18], especially oligochaetes, cave-dwelling amphipods or isopods but they are also scavengers and they can also feed on drowned arthropods like crickets and dipterans [17,23]. At the same time, in subterranean habitats, planarians have been reported to serve as food for cave fish, crayfish and salamanders [17,43]. In Europe, there are observations of troglobile terrestrial salamanders preying upon *Dendrocoelum* sp. groundwater-dwelling planarians [44] and studies showing that stygobiont planarians do not coexist in groundwaters with predator fire salamanders larvae [17,45]. In small

groundwater habitats, where simple trophic webs occur, we hypothesize that planarians can occupy top positions, and that they could contribute in top-down control of potential prey populations.

As already stated, planarians are generally neglected being neither charismatic nor easily noticed by humans. Studies on groundwater-dwelling planarians are even more scarce and sparse than those on surface freshwater ones. At a first sight, current information on the distribution, on the real level of exploitation and adaptation to subterranean environments, on the morphological and behavioral features of groundwater-dwelling planarians and on their trophic role appears highly fragmentary and out of date. In this paper, we want to provide a review of the existing online literature on groundwater-dwelling planarians to understand: (I) how widespread is the study of stygobiont planarians? (II) How relevant is the occurrence of troglomorphisms in stygobiont planarians? (III) Do planarians occupy top predator positions in groundwaters?

2. Material and Methods

Reviews of the existing scientific literature are fundamental to understand the state of knowledge and to provide future perspectives, especially when the field of research is fragmented. However, covering the whole spectrum of literature is almost impossible, especially in disciplines like zoology and subterranean biology. In fact, these scientific fields have a long history and many historic papers are confined in the so called “grey literature”. Moreover, arbitrary reviews performed without applying an impartial and easily repeatable method may bring to biased conclusions. For these reasons the use of systematic evidence reviews is now largely widespread in different scientific fields [46]. Here we performed a systematic evidence review to answer the three questions detailed in the aims; as not negligible researches on stygobiont planarians have been published last century in local papers and books, but are not retrievable with a standardized and not biased research, we made in the discussion section a comparison between the results of our systematic evidence review and the classical scientific texts that drove planarian research in 19th century. To assess how widespread the study of stygobiont planarians is, we employed the PRISMA (Preferred Reporting Items of Systematic reviews and Meta-Analyses) guidelines [47] and we searched the Web of Science (WoS) and Google Scholar (GS) databases. In WoS, we performed search by topics using the key words “Planarian Cave” and “Flatworm Cave”; in GS, we used only the key “Planarian Cave” because at a preliminary analysis it yielded more reliable results than “Flatworm Cave” and we avoided repetitions of the same literature source. The search was performed in November 2020 from Milan (Italy).

The collected references went through two distinct selection steps. We initially cleaned the dataset by discarding all the articles reporting information that was not clearly related to flatworms and/or groundwaters. This selection was made by reading the introduction; we kept only the papers in which one or more of the following words appeared in the text: “planarian”, “flatworm”, “subterranean”, “hypogean”, “stygobiont”, “troglobitic”, “deep-dwelling”, “want of eye”, “lack of eye”, “absence of”, “blind”, “anophthalmia”, “unpigmented”, “whitish”, “pigmentation”, “adaptive strategy”, “adhesive organ”, “cave”, “spring”, “well”, “waterhole”, “pool”, “stream”. We discarded also papers not written in English. Second, we fully read all the selected papers and we kept only those clearly mentioning one or more species of flatworms along with the location or habitat of occurrence.

From the final dataset, we recorded for each paper the following information: name of the planarian species, place of sampling (name of the locality, country and geographical coordinates), habitat of occurrence distinguishing between subterranean (cave, mine, well etc.) and surface (spring habitats and adjacent environments) ones. For every planarian species, we also recorded presence/absence of troglomorphic characters (blindness, depigmentation) and sympatry with other taxa. If the mentioned species were not accompanied by a morphological description, the occurrence of troglomorphic features was assessed, when possible, from other publications expressly searched, including species’ descriptions.

The taxonomic status of each mentioned species was verified and adapted to current knowledge if needed.

Moreover, we recorded papers' typology, distinguishing between ecology, distribution, phylogeny and taxonomy categories on the basis of the information contained and considering that the same paper could belong to multiple ones.

We performed also a metanalysis to assess the ecological role of flatworms in the food chain of caves. For the metanalysis, we considered only ecological papers reporting finding/observations of planarians in subterranean habitats. For every subterranean mentioned site, we searched GS to collect information on other reported species. We recorded the total number of other reported species in the scientific literature for each site and we divided them according to their trophic role, distinguishing between potential predators of and potential prey for flatworms. Then we built two generalized linear models (GLMs) with binomial error distribution using as dependent variable the fact that the planarian species were or not the only predator known for each site. As fixed factors, we used in one model the number of potential prey species, and in the other one the total number of other reported species. We assessed the significance of the fixed factors with a likelihood ratio test [48]). Metanalysis was performed in R 3.6.3 environment [49] using *glm* and *drop1* functions.

3. Results

From WoS, the search with both keys returned 43 distinct papers, while from GS we gathered 2230 results. The first selection step allowed us to reduce the dataset to a total number of 119 papers potentially dealing with groundwater-dwelling planarians. The second step of selection reduced to only 48 the number of papers actually relevant for the revision. From these 48 papers, we retrieved a total of 113 observations of 107 different planarian species in both groundwaters and adjacent epigean habitats (Figure 1; Supplementary Table S1).

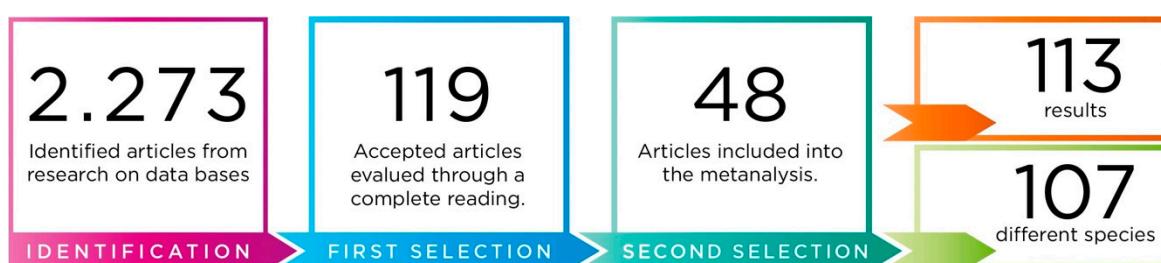


Figure 1. Results of systematic review selection recommended by PRISMA (Preferred Reporting Items of Systematic reviews and Meta-Analyses) guidelines.

3.1. Spread of Studies on Stygobiont Planarians

The typologies of studies covered by the papers retrieved with the systematic evidence review were mainly taxonomic (65%) and ecologic (21%); less frequent were studies on flatworms' distribution (4%), and phylogeny (3%). We did not detect any behavioral study; 7% of the papers did not match any of the typologies considered. 80% of the papers have sampling sites located in the northern hemisphere, while the southern hemisphere appears poorly represented (Figure 1). Moreover, 50 of the 113 observations came from North America (all in the United States), 31 from Europe (21 in Italy), nine from Asia, and two from North Africa. Among southern continents, South America was the most represented (18 observations), followed by Australia and Tahiti with three observations each (Figure 2).



Figure 2. Number of caves related to overhauled articles on Web of Science divided between southern and boreal hemisphere.

3.2. Trophic Functional Role of Planarians in Groundwaters

Sympatry with other taxa was reported in 24 observations out of the 89 referred to subterranean habitats. Only five of these 24 observations reported also the occurrence of potential predators of flatworms. In all the other cases (84%), only the occurrence of potential prey species was reported and planarians were the top predators. However, considering only ecological studies with observations reported for strictly subterranean habitats, the metanalysis revealed that the occurrence of groundwater-dwelling planarians as lone (top) predators is negatively associated with the total number of other species reported for the site ($\chi^2 = 10.28$; $p < 0.001$), but it has no correlation with the potential prey species reported ($\chi^2 = 0.39$; $p = 0.53$).

3.3. Relevance of Troglomorphisms in Stygobiont Planarians

Of the 113 observations collected with the systematic review, 89 were related to 86 planarian species sampled in subterranean habitats, while 24 observations were referred to 21 planarian species sampled in springs or adjacent epigean habitats. Most of the species (78%) reported for groundwaters were fully troglomorphic showing both depigmentation and lack of eyes (Figure 3A). Further, 6% of the species was neither depigmented nor eyeless. The other species showed only one troglomorphic feature: 10% were only depigmented and 6% were blind, but pigmented. Considering species reported in springs and adjacent habitats (Figure 3B), all the species mentioned showed at least a troglomorphic feature. In addition, 42.8% were both depigmented and eyeless, while 47.6% were depigmented but retained eyes, and 9.6% were eyeless but pigmented.

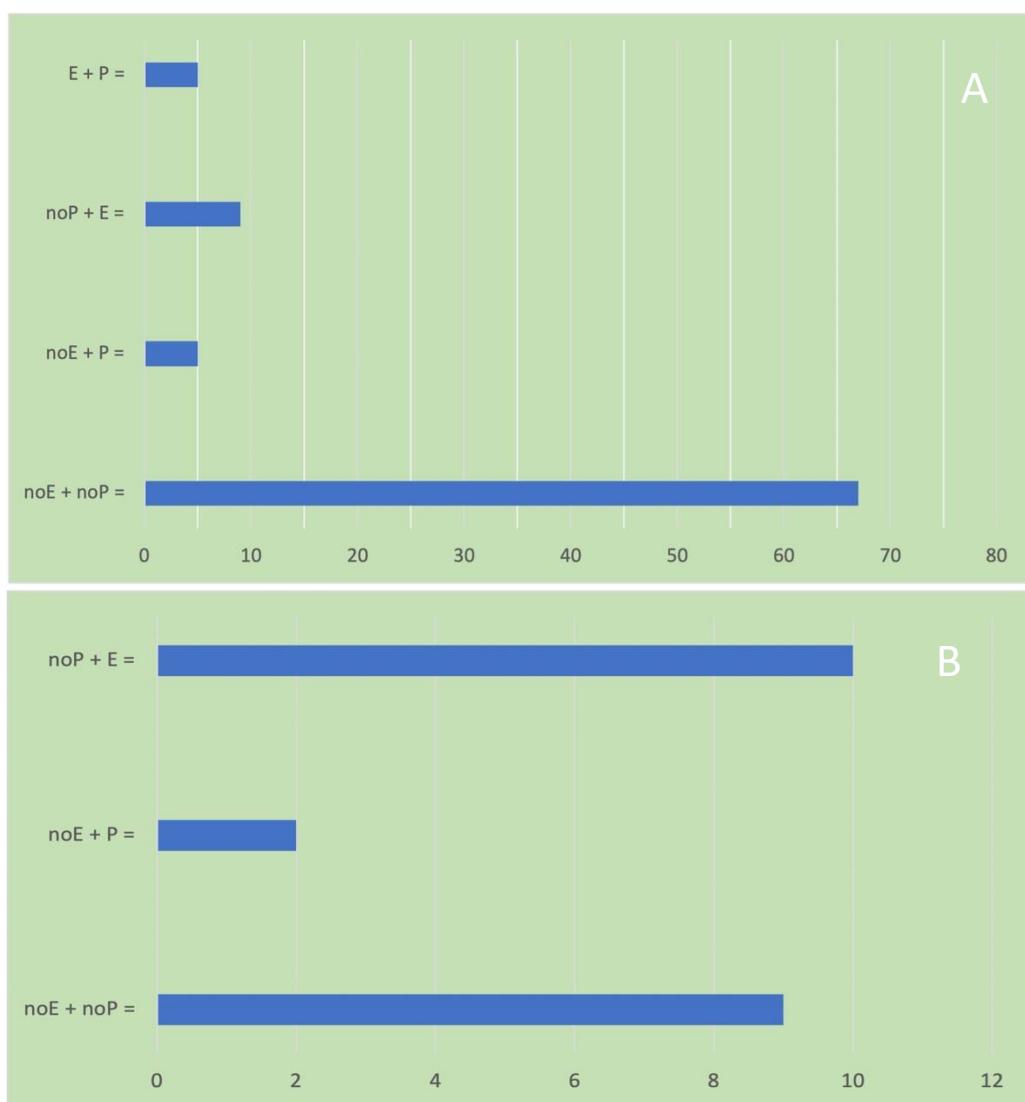


Figure 3. Number of planarians species reported in the selected papers for groundwaters (A) and adjacent surface habitats (B) with both eyes and pigmentation (E + P), eyes but depigmented (E + noP), pigmentation but eyeless (noE + P) and showing complete troglomorphism being both depigmented and eyeless (noE + noP).

4. Discussion

Our review of the literature on groundwater-dwelling planarians reveals that, despite the potential high number of available data, the number of papers effectively providing exhaustive information of planarians in groundwaters and in groundwaters' associated habitats like springs is very limited. We were able to assess only 48 papers.

4.1. Spread of Studies on Stygobiont Planarians

The data about the geographical distribution allowed us to identify the least represented locations and to identify the deficient study areas, which are likely to be the ones with the greatest margins of improvement in terms of knowledge of the environment and description of new species. A strong geographical bias of the studies emerges; most investigations were performed in Europe and North-America and very little information is available for some Asian countries and for South-America, Oceania, and Southern Africa. For Asian and South-American countries, it is possible that studies in the local languages that we did not find or assess, are available online. As an example, several papers written in Japanese are available in GS, but we were not able to assess them for this study. Con-

sidering the extension of karst areas worldwide and the relative frequency of flatworms' occurrence in groundwaters, it is likely that the lack of papers that we underlined especially for the southern hemisphere reflects the few researches performed and the low attention payed to flatworms during fauna surveys in cave environments. It is also possible that studies of this type have been conducted and never published or are not available.

With this review, we found that most studies are mainly taxonomical investigations describing new species. Papers investigating planarian ecology are rarer and we were not able to retrieve any paper dealing with behavior. These results prompted the need of further studies assessing planarians species occurrence in caves and other groundwater habitats together with investigations on main behavioral patterns, such as foraging behavior and circadian rhythm performed at a small scale or at the single population level. In the past, some behavioral tests have been performed on planarians' reaction to light [39]. They reported that subterranean planarians are highly sensitive to light and can be strongly damaged when exposed to it. However, a study performed on the cave flatworm *Sphallopiana percaeca* showed that this species is indifferent to light and does not react when its intensity is not harmful [50].

These results are confirmed by the reading of classical books and papers published in the 19th century. These old papers and books are mainly taxonomical monographies or biological reviews, often published in local languages and providing only sparse information on the site/habitat of collection and on the possible relationships among the species. We detected only one experimental paper dealing with the ecology of a non-troglomorphic planarian species [51]. Categorizing this old literature can be challenging and provide biased results because it is in most cases older than modern scientific branches. However, they constitute a fundamental source of information to retrieve the localities for planarian species that after their description have never been studied further. Assessing if these species still exist and characterizing the features of their habitat could provide fundamental information for studies on conservation and assessing effects of habitat changes in subterranean organisms.

The lack of information on the habitat requirements has been complained since the beginning of the zoological investigations on groundwater-dwelling planarians [24]. Most samplings were performed by cavers and explorers that collect the animals for zoologists during single visits of the caves. Usually they did not report exactly the microhabitat features of the collection site and even did not assess if planarians occur also in other habitats [26]. This established method, even if it increased the comprehension of planarians morphological differentiation in distinct karst areas, prevented the comprehension of the processes that allowed it. It must also be pointed out that the streams and the pools that can be sampled in caves may be only a portion of the whole habitat occupied by planarians that are likely to be also interstitial [52,53].

4.2. Trophic Functional Role of Planarians in Groundwaters

Thanks to our systematic review, some main ecological studies, not detectable in the 19th century literature, emerged and allowed us to retrieve some indications on the position of planarians in the groundwaters' trophic web. First, our results show that planarians are likely the top predators of the sites they inhabit since no other predators were reported in most of the 113 assessed observations. However, the metanalysis showed no correlation between planarians and the number of potential prey and a negative relationship with the total number of species reported for the sites. This negative relationship between the status of top predator and the general diversity of the sites suggests that our hypothesis on a possible top-down control of potential prey populations should be rejected because at least in terms of diversity groundwater-dwelling planarians seem not having large effects upon communities. However, in the total number of other species, we also included species that only occasionally enter into the caves and that likely do not have interactions with groundwaters because the information retrieved in some cases did not allow to verify with certitude the level of connection with subterranean environment and groundwaters.

Results are thus mainly preliminary and need more investigations; the methodology used in our systematic review, however, could be useful to be applied at national or regional scales where detailed biological surveys of groundwaters exist and may be used to make correlations with planarians occurrence. Moreover, further field studies, considering the whole groundwater-dwelling community in both deep and spring-closed sectors, could increase the understanding of the role played by planarians in shaping stygofauna features. In any case, it could be very difficult to understand the functional role of the planarians in the trophic webs of groundwaters as only little information from sparse observations exists on their trophic niche [23,28,54]. On the contrary, in epigean freshwaters planarians, both fundamental and realized trophic niches have been extensively studied [18]. There is much overlap in terms of diet among the species that eat mainly isopod and amphipod crustaceans, oligochaetes, and snails [18]. Globally our results suggest that future field investigations comparing groundwater sites with and without planarians, even inside the same cave system, may reveal new insights.

4.3. Relevance of Troglomorphisms in Stygobiont Planarians

The understanding of the relevance of troglomorphic features is one of the most interesting results of this review. Most species reported in subterranean environments and all species reported in springs showed at least a troglomorphic character. However, our analysis reveals that there are exceptions, with planarian species observed in caves that are neither eyeless nor depigmented.

Similar results can be obtained if we consider some of the most relevant papers and books published in the 19th even not in English language, particularly from the books and papers of De Beauchamp [22], Gourbault [24], Benazzi [55], Benazzi and Gourbault [56], Carpenter [21], Christian and Spötl [57], De Vries and Benazzi [58], Del Papa [59], Elliot and Mitchell [60], Ginet and Puglisi [30], Lunghi, et al. [61], Pateé and Gourbault [62], Puccinelli and Benazzi [63], René and Christian [64], Sluys [65], Ullyott [51], Viali [66], and Von Heinz [67], it is possible to retrieve mentions of 115 mentions 83 species, including 14 reports of epigean species in subterranean environments. Most of the mentions [51] refer to blind and depigmented planarians, but in this old literature there is also a not negligible number of species [22] for which it is impossible to assess the occurrence of troglomorphic features.

The study of troglomorphic features, especially from a taxonomical point of view, has been a core point of biological studies of subterranean animals for many years [23]. However, in the last decade it has been shown that troglomorphisms may be highly variable, as in the case of cave fishes [68]. In the past years, a more evolutionarily oriented concept of troglomorphic features has been developed: that of features whose presence/absence may reflect a real adaptive meaning [40,69]. Our results show that the occurrence of eyes in depigmented stygobiont planarians is particularly high in springs, such as at the interface with groundwaters. Capacity of detect light stimuli has been described for other troglobiont species; eyes reduction but not complete loss occurs in different species considered strictly cave adapted but occurring also at the border with surface [70,71]. This is the case of the stygobiont planarian *Polyclelis benazzii*, which was described only for a pool at the entrance of a cave and it was never found in deeper habitats [27]. Light-detection in cave-dwelling animals has been associated with the necessity of detecting and avoiding surface risky habitats [72]. Anyway, this capability may also allow to distinguish night from day and favor the exploitation of springs and other border habitats during night, when UV radiation is not a constraint for depigmented animals [73]. The high variability in the occurrence of troglomorphic features that we detected suggests that planarians could be a very useful model to understand the evolutionary processes underlying adaptation to cave environments. Most experimental studies on cave-dwelling animals have been performed on cave fish [74–76]. Considering that planarians rearing requires less space and attention than fish, the high variability that we detected should allow to extend the hypotheses till now tested in cave fishes to different suitable species for comparable experimental studies.

5. Conclusions

This review is a first attempt to summarize the extant knowledge on groundwater-dwelling planarians. The paucity of papers and information that we gathered underlines that low attention has been dedicated to these animals which occurrence and importance in groundwaters could be larger than thought. Further revisions will be necessary to enlarge the comprehension of the main distributional, developmental, and evolutionary trends of groundwater-dwelling planarians. As having access to the large amount of information that is actually confined in the so called “grey literature” is demanding and, without a specific and easily repeatable method, its review may lead to biased conclusions, we hope that the systematic review proposed here could provide a first and unbiased synthesis stimulating for future investigations on a neglected compound of biodiversity such as planarians. We hope that by drawing together this compound of papers, we can begin to exploit the diversity of planarians in groundwaters to identify the fundamental trends and forces that underlie adaptations to unusual habitats.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13050178/s1>, Table S1: List of all the mentions of groundwater-dwelling planarian species retrieved within the 48 papers selected during the systematic review process carried out.

Author Contributions: Conceptualization B.B.; literature review D.D.G. and B.B.; first draft B.B. and R.M.; writing—review and editing R.M., B.B., and R.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the retrieved data occur in Table S1.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

- Cardoso, P.; Erwin, T.L.; Borges, P.A.V. The seven impediments in invertebrate conservation and how to overcome them. *Biol. Conserv.* **2011**, *144*, 2647–2655. [[CrossRef](#)]
- Collen, B.; Böhm, M.; Kemp, R.; Baillie, J.E.M. *Spineless: Status and Trends of the World’s Invertebrates*; Zoological Society of London: London, UK, 2012.
- Ceríaco, L.M.P. Human attitudes towards herpetofauna: The influence of folklore and negative values on the conservation of amphibians and reptiles in Portugal. *J. Ethnobiol. Ethnomed.* **2012**, *8*, 8. [[CrossRef](#)]
- Decaëns, T.; Jiménez, J.J.; Gioia, C.; Measey, G.J.; Lavelle, P. The values of soil animals for conservation biology. *Eur. J. Soil Biol.* **2006**, *42*, S23–S38. [[CrossRef](#)]
- Collier, K.J.; Probert, P.K.; Jeffrie, M. Conservation of aquatic invertebrates: Concerns, challenges and conundrums. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2016**, *26*, 817–837. [[CrossRef](#)]
- Collier, K.J.; Smith, B.J. Distinctive invertebrate assemblages in rockface seepages enhance lotic biodiversity in northern New Zealand. *Biodivers. Conserv.* **2006**, *15*, 3591–3616. [[CrossRef](#)]
- Stubbington, R.; Wood, P.J.; Reid, I. Spatial variability in the hyporheic zone refugium of temporary streams. *Aquat. Sci.* **2011**, *73*, 499–511. [[CrossRef](#)]
- Scalici, M.; Bravi, R. Solving alpha-diversity by morphological markers contributes to arranging the systematic status of a crayfish species complex (Crustacea, Decapoda). *J. Zool. Syst. Evol. Res.* **2012**, *50*, 89–98. [[CrossRef](#)]
- Gupta, K.; Sharma, A. Macroinvertebrates as indicators of pollution. *J. Environ. Biol.* **2005**, *26*, 205–211.
- Koperski, P. Diversity of freshwater macrobenthos and its use in biological assessment: A critical review of current applications. *Environ. Rev.* **2011**, *19*, 16–31. [[CrossRef](#)]
- Dauba, F.; Lek, S.; Mastrorillo, S.; Copp, G.H. Long-term recovery of macrobenthos and fish assemblages after water pollution abatement measures in the River Petite Baise (France). *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 277–285. [[CrossRef](#)]
- Kiffney, P.M.; Richardson, J.S.; Bull, J.P. Responses of periphyton and insects to experimental manipulation of riparian buffer width along forest streams. *J. Appl. Ecol.* **2003**, *40*, 1060–1076. [[CrossRef](#)]

13. Araujo, A.P.G.; Carbayo, F.; Riutort, M.; Alvarez-Presas, M. Five new pseudocryptic land planarian species of Cratera (Platyhelminthes: Tricladida) unveiled through integrative taxonomy. *PeerJ* **2020**, *8*. [[CrossRef](#)]
14. Hellmann, L.; Ferreira, R.L.; Rabelo, L.; Leal-Zanchet, A.M. Enhancing the still scattered knowledge on the taxonomic diversity of freshwater tricladids (Platyhelminthes: Dugesiidae) in caves from two Brazilian Biomes. *Stud. Neotrop. Fauna Environ.* **2020**. [[CrossRef](#)]
15. Mateos, E.; Jones, H.D.; Riutort, M.; Alvarez-Presas, M. A new species of alien terrestrial planarian in Spain: Caenoplana decolorata. *PeerJ* **2020**, *8*. [[CrossRef](#)]
16. Manenti, R.; Barzaghi, B. Is landscape of fear of macroinvertebrate communities a major determinant of mesopredator and prey activity? *Knowl. Manag. Aquat. Ecosyst.* **2020**. [[CrossRef](#)]
17. Manenti, R.; Lunghi, E.; Barzaghi, B.; Melotto, A.; Falaschi, M.; Ficetola, G.F. Do Salamanders Limit the Abundance of Groundwater Invertebrates in Subterranean Habitats? *Diversity* **2020**, *12*, 161. [[CrossRef](#)]
18. Reynoldson, J.D.; Young, J.O. *A Key to the Freshwater Tricladida of Britain and Ireland with Notes on Their Ecology*; Freshwater Biological Association: Ambleside (Cumbria), UK, 2000; p. 72.
19. Knezovic, L.; Milisa, M.; Kalafatic, M.; Rajevic, N.; Planinic, A. A key to the freshwater tricladids (Platyhelminthes, Tricladida) of Herzegovina watercourses. *Period. Biol.* **2015**, *117*, 425–433. [[CrossRef](#)]
20. Navarro, B.S.; Jokela, J.; Michiels, N.K.; D’Souza, T.G. Population genetic structure of parthenogenetic flatworm populations with occasional sex. *Freshw. Biol.* **2013**, *58*, 416–429. [[CrossRef](#)]
21. Carpenter, J.H. Observations on the biology of cave planarians of the United States. *Int. J. Speleol.* **1982**, *12*, 9–28. [[CrossRef](#)]
22. De Beauchamp, P. Biospeleologie. Turbellariés, Hirudinées, Branchiobdellidés (Deuxième série). *Arch. De Zool. Expérimentale Et Générale Hist. Nat. Morphol. Histol. Évolution Des Animaux* **1932**, *73*, 113–380.
23. Romero, A. *Cave Biology*; Cambridge University Press: New York, NY, USA, 2009. [[CrossRef](#)]
24. Gourbault, N. Recherches sur les Triclades Paludicoles hypogés. *Mémoires Du Muséum Natl. D’histoire Nat. Ser. A* **1972**, *73*, 1–249.
25. Sluys, R.; Kawakatsu, M.; Riutort, M.; Baguna, J. A new higher classification of planarian flatworms (Platyhelminthes, Tricladida). *J. Nat. Hist.* **2009**, *43*, 1763–1777. [[CrossRef](#)]
26. Manenti, R.; Barzaghi, B.; Lana, E.; Stocchino, G.A.; Manconi, R.; Lunghi, E. The stenoendemic cave-dwelling planarians (Platyhelminthes, Tricladida) of the Italian Alps and Apennines: Conservation issues. *J. Nat. Conserv.* **2018**, *45*, 90–97. [[CrossRef](#)]
27. Enslin, E. Dendrocoelum cavaticum Fries: Verbreitung in der schwäbischen Alb. Anatomie nebst Bemerkungen über die Reduktion der Augen. Systematische Stellung. *Jahresh. Des Ver. Für Vaterl. Nat. Im Württember* **1906**, *62*, 312–360.
28. Manenti, R.; Barzaghi, B.; Tonni, G.; Ficetola, G.F.; Melotto, A. Even worms matter: Cave habitat restoration for a planarian species has increased prey availability but not population density. *Oryx* **2019**, *53*, 216–221. [[CrossRef](#)]
29. Ginet, R.; Puglisi, R. Ecologie de *Fonticola notadena* de Beauchamp (Turbellarie, Triclade) dans la grotte de La Balme (Isère, France); survie en période de sécheresse. *Int. J. Speleol.* **1964**, *1*, 203–216. [[CrossRef](#)]
30. Culver, D.C.; Pipan, T. Redefining the extent of the aquatic subterranean biotope-shallow subterranean habitats. *Ecohydrology* **2011**, *4*, 721–730. [[CrossRef](#)]
31. Barzaghi, B.; Ficetola, G.F.; Pennati, R.; Manenti, R. Biphasic predators provide biomass subsidies in small freshwater habitats: A case study of spring and cave pools. *Freshw. Biol.* **2017**, *62*, 1637–1644. [[CrossRef](#)]
32. Culver, D.C.; Pipan, T. *Shallow Subterranean Habitats: Ecology, Evolution, and Conservation*; Oxford University Press: New York, NY, USA, 2014.
33. Culver, D.C.; Pipan, T. *The Biology of Caves and Other Subterranean Habitats*, 2nd ed.; Oxford University Press: New York, NY, USA, 2019.
34. Galassi, D.M.P.; Stoch, F.; Fiasca, B.; Di Lorenzo, T.; Gattone, E. Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. *Freshw. Biol.* **2009**, *54*, 830–847. [[CrossRef](#)]
35. Niemiller, M.L.; Glorioso, B.M.; Fenolio, D.B.; Reynolds, R.G.; Taylor, S.J.; Miller, B.T. Growth, Survival, Longevity, and Population Size of the Big Mouth Cave Salamander (*Gyrinophilus palleucus necturoides*) from the Type Locality in Grundy County, Tennessee, USA. *Copeia* **2016**, *104*, 35–41. [[CrossRef](#)]
36. Romero, A. The Evolution of Cave Life. *Am. Sci.* **2011**, *99*, 144–151. [[CrossRef](#)]
37. Romero, A. Hypogean Communities as Cybernetic Systems. *Diversity* **2020**, *12*, 413. [[CrossRef](#)]
38. Culver, D.C.; Pipan, T. *The Biology of Caves and Other Subterranean Habitats*; Oxford University Press: New York, NY, USA, 2009. [[CrossRef](#)]
39. Vandel, A. *Biospeleologie: La Biologie des Animaux Cavernicoles*; Gauthiers-Villars: Paris, France, 1964; Volume XVIII, p. 619.
40. Pipan, T.; Culver, D.C. Convergence and divergence in the subterranean realm: A reassessment. *Biol. J. Linn. Soc.* **2012**, *107*, 1–14. [[CrossRef](#)]
41. Stocchino, G.A.; Sluys, R.; Marcia, P.; Manconi, R. Subterranean aquatic planarians of Sardinia, with a discussion on the penial flagellum and the bursal canal sphincter in the genus *Dendrocoelum* (Platyhelminthes, Tricladida, Dendrocoelidae). *J. Cave Karst Stud.* **2013**, *75*, 93–112. [[CrossRef](#)]
42. De Beauchamp, P. Nouvelles diagnoses de Triclades obscuricoles. X. *Polyclelis benazzi* n. sp. dans une grotte de Ligurie. *Bull. De La Société Zool. Fr.* **1955**, *80*, 119–124.
43. Gillespie, J.H. Application of stable isotope analysis to study temporal changes in foraging ecology in a highly endangered amphibian. *PLoS ONE* **2013**, *8*, e53041. [[CrossRef](#)]

44. Lunghi, E.; Cianferoni, F.; Ceccolini, F.; Zhao, Y.H.; Manenti, R.; Corti, C.; Ficetola, G.F.; Mancinelli, G. Same Diet, Different Strategies: Variability of Individual Feeding Habits across Three Populations of Ambrosi’s Cave Salamander (*Hydromantes ambrosii*). *Diversity* **2020**, *12*, 180. [[CrossRef](#)]
45. Manenti, R. Role of cave features for aquatic troglobiont fauna occurrence: Effects on “accidentals” and troglomorphic organisms distribution. *Acta Zool. Acad. Sci. Hung.* **2014**, *60*, 257–270.
46. Acreman, M.; Hughes, K.A.; Arthington, A.H.; Tickner, D.; Duenas, M.A. Protected areas and freshwater biodiversity: A novel systematic review distils eight lessons for effective conservation. *Conserv. Lett.* **2020**, *13*. [[CrossRef](#)]
47. Page, M.J.; Moher, D. Evaluations of the uptake and impact of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) Statement and extensions: A scoping review. *Syst. Rev.* **2017**, *6*. [[CrossRef](#)]
48. Bolker, B.; Holyoak, M.; Krivan, V.; Rowe, L.; Schmitz, O. Connecting theoretical and empirical studies of trait-mediated interactions. *Ecology* **2003**, *84*, 1101–1114. [[CrossRef](#)]
49. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.
50. Buchanan, J.W. Notes on an American cave flatworm, *Sphalloplana percaeca* (Packard). *Ecology* **1936**, *17*, 194–211. [[CrossRef](#)]
51. Ulliyott, P. The behaviour of *Dendrocoelum lacteum*. Responses at light-and-dark boundaries. *Lab. Freshw. Biol. Assoc. Wind. Zool. Lab. Camb.* **1935**, *13*, 253–264.
52. Sluys, R.; Benazzi, M. A new finding of a subterranean dendrocoelid flatworm from Italy (Platyhelminthes, Tricladida, Paludicola). *Stylogolia* **1992**, *7*, 213–217.
53. Stocchino, G.A.; Sluys, R.; Kawakatsu, M.; Sarbu, S.M.; Manconi, R. A new species of freshwater flatworm (Platyhelminthes, Tricladida, Dendrocoelidae) inhabiting a chemoaerotrophic groundwater ecosystem in Romania. *Eur. J. Taxon* **2017**, *342*, 1–21. [[CrossRef](#)]
54. Mitchell, R.W. Cave adapted flatworms of Texas systematics, natural history and responses to light and temperature. *Am. Zool.* **1970**, *10*, 547.
55. Benazzi, M. Tricladi cavernicoli italiani. *Biogeogr. J. Integr. Biogeogr.* **1982**, *7*. [[CrossRef](#)]
56. Benazzi, M.; Gourbault, N. *Atrioplanaria morisii* N.Sp., A New Cave Planarian from Italy. *Bulletino Di Zool.* **1977**, *44*, 327–335. [[CrossRef](#)]
57. Christian, E.; Spötl, C. Karst geology and cave fauna of Austria: A concise review. *Int. J. Speleol.* **2010**, *39*, 71–90. [[CrossRef](#)]
58. De Vries, E.J.; Benazzi, M. *Dugesia brigantii* n.sp., a freshwater planarian found in an Italian cave. *Bulletino Di Zool.* **1983**, *50*, 263–268. [[CrossRef](#)]
59. Del Papa, R. *Dendrocoelum (Dendrocoelides) benazzii* N. Sp. from the Cave of Stiffe (Abruzzo). *Bulletino Di Zool.* **1973**, *40*, 253–259. [[CrossRef](#)]
60. Elliot, W.R.; Mitchell, R.E. Temperature Preference Responses of Some Aquatic, Cave-adapted Crustaceans from Central Texas and Northeastern Mexico. *Int. J. Speleol.* **1973**, *5*, 171–189. [[CrossRef](#)]
61. Lunghi, E.; Corti, C.; Mulargia, M.; Zhao, Y.; Manenti, R.; Ficetola, G.F.; Veith, M. Cave morphology, microclimate and abundance of five cave predators from the Monte Albo (Sardinia, Italy). *Biodivers. Data J.* **2020**, *8*, e48623. [[CrossRef](#)]
62. Patée, E.; Gourbault, N. Turbellaries triclades paludicoles (planaires d’eau douce). *Société Linnéenne De Lyon* **1981**, *9*, 279–304.
63. Puccinelli, I.; Benazzi, M. Osservazioni sulla ecologia e cariologia della planaria. *Dugesia brigantii*. *Atti Societa Toscana Sci. Nat. Resid. Pisa Mem. Process. Verbali Ser. B* **1985**, *92*, 283–289.
64. René, G.; Christian, J. Le peuplement animal des karsts de France (Éléments de biogéographie souterraine pour les Invertébrés). Première partie: La faune aquatique. *Karstologia Rev. De Karstol. Et De Spéléol. Phys.* **1987**, *10*, 43–51. [[CrossRef](#)]
65. Sluys, R. A new, sibling species of cave flatworm from Switzerland (Platyhelminthes, Tricladida, Dendrocoelidae). *Rev. Suisse De Zool.* **2012**, *119*, 181–188. [[CrossRef](#)]
66. Vialli, M. Una nuova specie di *Dendrocoelum* delle Grotte Bresciane. *Bulletino Di Zool.* **1973**, *8*, 179–187. [[CrossRef](#)]
67. Von Heinz, N. Ökologisch-faunistische Untersuchungen über die Hydrofauna der Lurgrotte zwischen Peggau und Semriach in der Steiermark. *Sitz. Ber. Österr. Akad. Wiss. Math. Naturw. Kl.* **1974**, *1*, 103–139.
68. Romero, A.; Green, S. The end of regressive evolution: Examining and interpreting the evidence from cave fishes. *J. Fish Biol.* **2005**, *67*, 3–32. [[CrossRef](#)]
69. Juan, C.; Guzik, M.T.; Jaume, D.; Cooper, S.J. Evolution in caves: Darwin’s ‘wrecks of ancient life’ in the molecular era. *Mol. Ecol.* **2010**, *19*, 3865–3880. [[CrossRef](#)]
70. Porter, M.L.; Sumner-Rooney, L. Evolution in the Dark: Unifying our Understanding of Eye Loss Introduction. *Integr. Comp. Biol.* **2018**, *58*, 367–371. [[CrossRef](#)] [[PubMed](#)]
71. Protas, M.; Jeffery, W.R. Evolution and development in cave animals: From fish to crustaceans. *Wiley Interdiscip. Rev. Dev. Biol.* **2012**, *1*, 823–845. [[CrossRef](#)] [[PubMed](#)]
72. Fišer, Z.; Novak, L.; Lustrik, R.; Fiser, C. Light triggers habitat choice of eyeless subterranean but not of eyed surface amphipods. *Sci. Nat.* **2016**, *103*, 7. [[CrossRef](#)] [[PubMed](#)]
73. Manenti, R.; Barzaghi, B. Diel Activity of *Niphargus* amphipods in spring habitats. *Crustaceana* **2021**, in press.
74. Jeffery, W.R. Cavefish as a model system in evolutionary developmental biology. *Dev. Biol.* **2001**, *231*, 1–12. [[CrossRef](#)] [[PubMed](#)]
75. Lunghi, E.; Zhao, Y.H. Do Chinese cavefish show intraspecific variability in morphological traits? *Ecol. Evol.* **2020**, *10*, 7723–7730. [[CrossRef](#)]
76. Romero, A.; Green, S.M.; Romero, A.; Lelonek, M.M.; Stropnický, K.C. One eye but no vision: Cave fish with induced eyes do not respond to light. *J. Exp. Zool. Part B-Mol. Dev. Evol.* **2003**, *300B*, 72–79. [[CrossRef](#)]