Title page

The stenoendemic cave-dwelling planarians (Platyhelminthes, Tricladida) of the Italian Alps and Apennines: conservation issues

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Abstract

Despite being a fundamental component of biodiversity, several highly diverse taxa of aquatic invertebrates are still poorly known and poorly considered in protection programs. This is the case especially of several invertebrate species that inhabit groundwater. In this environment, invertebrates play significant roles in ecosystem services closely connected to the usefulness of these systems for human welfare and survival. The groundwater biodiversity of continental Italy is largely unknown and its importance is neglected in national and regional legislation. One of the most poorly studied groups of Italian groundwater fauna are planarians (Platyhelminthes, Tricladida). Most known species are endemic to small, single karst areas or a single cave, their geographic range never having been investigated in detail after the original description. The aims of this study are *i*) to provide the first conservation assessment of cave-dwelling planarians in the Italian Alps and Apennines, whose status is at present Not Evaluated in IUCN categories and *ii*) to evaluate which environmental constraints, including potential threats, possibly affect the occurrence of the species within different cave systems. Our results suggest that most of the cave-dwelling planarian species of continental Italy are threatened by water pollution and habitat destruction/alteration; moreover, datasets underline that there is a considerable conservation issue concerning stenoendemic planarians that may involve other cave-dwelling invertebrates with narrow geographic ranges. Generally, the underground habitat of most surveyed species appears to be deeply compromised and changed since the first species description.

Keywords

Triclads, subterranean biodiversity, freshwater flatworms, invertebrate, stygobious, IUCN

Introduction

Biodiversity loss has been one of the major conservation issues in the last decades (Naggs, 2017). Biodiversity represents a priceless resource for the planet, as it is the engine that allows proper ecosystem functioning (Rumeu et al., 2017). Despite multiple calls and efforts of conservationists, we are still far from a proper understanding of the real magnitude of the Earth's biodiversity, and thus many species are disappearing without us even knowing about their existence (Hochkirch, 2016; Regnier et al., 2015). This is mostly linked to a biased consideration of neglected species, which in turn leads to an inadequate method of assessing threats (Hayward, 2009). Indeed, the target of conservation actions are usually charismatic animals, such as mammals, birds, and butterflies (Lydeard et al., 2004; Naggs, 2017). In contrast, most invertebrate species, even if they represent the majority of known biodiversity, are neglected and often considered as a potential threat to public health (Hochkirch, 2016). Therefore, a very small portion of invertebrate species is considered in conservation actions, generally only those that have a well-known economic value (Cardoso et al., 2011; Koperski, 2011; Paillex et al., 2013).

Invertebrates occur worldwide in all environments and represent the keystone of many ecological mechanisms, such as pollination, organic matter decomposition, nutrient cycling and biological pest control (Bush et al., 2012; Thomson & Hoffmann, 2009). The high diversity of invertebrates likely depends on their relatively small size and limited capability for dispersal , which confine them to small areas where they can become extremely specialized, and thus undergo speciation events (Kunin & Gaston, 1997). This fact leads to an evident high frequency of endemism and higher vulnerability to risk of extinction, a feature that should promote conservation prioritisation (Davies et al., 2004; Leroux & Schmiegelow, 2007; Swenson et al., 2012).

Among geographic areas/biotopes with high endemicity and a wealth of species, karst systems represent a key hotspot for groundwater biodiversity. The occurrence of a highly diversified karst area gives rise to a wide range of underground aquatic systems, which host highly specialized invertebrate fauna with unique, unusual, and sometimes even inexplicable morphological, behavioural and ecological adaptations (Culver & Pipan, 2009; Romero, 2009). In several cases, both stygobionts (aquatic specialised cave-dwelling organisms) and troglobionts (terrestrial specialized cave-dwelling organisms) (Sket, 2008; Trajano & De Carvalho, 2017) are micro-endemic invertebrate species which have been found in one or only a few caves (Culver & Pipan, 2014; Mammola & Isaia, 2016; Wei et al., 2017). Moreover, most specialized cave species are often considered to be numerically rare. This rarity may be linked to the low levels of trophic resources occurring underground, although this should not be the case in species inhabiting shallow subterranean habitats (SSHs) (Culver & Pipan, 2014) where food income is higher (Gers, 1998). The rarity of cave-dwelling organisms may also be strongly overstated. According to different recent overviews, the main habitat of most subterranean species is the network of fissures and interstices occurring underground, which in karst areas is interconnected with caves (Culver & Pipan, 2014; Mammola et al., 2016; Romero, 2012). This habitat is difficult to be investigated by humans, for which caves are just windows to the more complex and mostly inaccessible subterranean domain (Mammola et al., 2016; Romero, 2012). As a consequence, most often populations of cave-dwelling species are just estimated to be small without true evidence. The conservation of cave ecosystems is affected both by processes acting on the surface and by local factors linked to human activity. At a global scale climate change (Mammola et al., 2018), deforestation (Trajano, 2000) and epigean invasive species (Wynne et al., 2014) may threaten the environmental features and the biodiversity of caves. Locally, also touristic exploitation, pollution and quarrying may be highly detrimental for cave conservation (Doran et al., 1999; Romero, 2009).

Therefore, cave-dwelling organisms need to be targeted by specific conservation actions (Williams et al., 2009). Besides their well-known importance as biodiversity hotspots, underground environments (and associated cave-specialized fauna) are still underrepresented (or even neglected) in conservation actions, with the exception of marine caves (Manconi et al., 2009; Gerovasileiou & Voultsiadou, 2012).

Among groundwater fauna, the taxon Tricladida is one of the most poorly studied (Collier et al., 2016; Stocchino et al., 2013). Planarians are small, free-living flatworms, generally predators, feeding on small living invertebrates and decaying organisms (Reynoldson & Young, 2000). Most of the species are particularly sensitive to organic matter pollution, water quality and environmental features (Reynoldson & Young, 2000), therefore representing an optimal bioindicator for the world's largest underground freshwater supply (Culver & Pipan, 2014). Most stygobiont planarians show extremely narrow ranges, often being confined to a single cave (De Beauchamp, 1932; Gourbault, 1972; Benazzi, 1982; Stocchino et al., 2013, 2017a) and show adaptations (e.g. anophthalmia, depigmentation) to the subterranean environments. Besides their original description, no further information is usually available for these species (Gourbault, 1972). As an example, for the Italian peninsula ten species of stygobiont triclads have been described (Benazzi, 1982; Stocchino et al., 2017a) for which only old and mainly morphological information is available, except for recently described species (Stocchino et al., 2017a) (Tables 1, 2). No conservation status assessments exist for these micro-endemic species, which might be potentially good bioindicators of subterranean aquifers and karst areas.

The aims of this study are *i*) to provide the first conservation assessment of cave-dwelling planarians in the Italian Alps and Apennines, whose status is at present Not Evaluated based on IUCN categories, and *ii*) to evaluate which environmental constraints, including potential threats, possibly affect the occurrence of the species within different cave systems.

Materials and Methods

Target species

We focused on eight cave-dwelling planarian species of the Italian Alps and Apennines: *Dendrocoelum italicum* Vialli, 1937; *D. benazzii* De Beauchamp, 1995; *D. beauchampi* Del Papa, 1952; *D.* cf. *beauchampi* Sluys & Benazzi, 1992; *D. collini* (De Beauchamp, 1919); *Polycelis benazzii* De Beauchamp, 1955; *Atrioplanaria morisii* Benazzi & Gourbault, 1977; and, *Dugesia brigantii* De Vries & Benazzi, 1983. Also considered here is the population of *Dendrocoelum* sp. reported for a Ligurian cave (Benazzi, 1982). We did not collect data on *Dendrocoelum leporii* Stocchino & Sluys, 2017, which was described after the beginning of our study, and on *D. spelaeum* (Kenk, 1924), occurring in the Dinaric Massif between Italy and Slovenia. Most of the planarian species surveyed were reported exclusively for single caves and represent excellent models of micro-endemic ranges (Fig. 1, Table 1).

Surveys

Multiple surveys were performed from October 2016 to September 2017 in 33 caves (minimum surveys per species = 3; average (±SD) = 7.69 ± 2.7). Eight caves correspond to the already known localities of the previously known eight stygobiont planarian species. We also included a Ligurian cave for which *Dendrocoelum* sp. was reported; 24 caves hosting accessible freshwater sites were selected in the surroundings of the known locality of each species/taxon (generally at a maximum distance of 6 km, except for *Dendrocoelum benazzii* for which we extended the research over a larger karst area at 20 km from the known locality). All caves were fully explored, focusing on all freshwater habitats (streams, creeks, drip pools, dripping layers on the walls). Planarians were first searched by visual census, i.e. observing each habitat for 30 minutes, and subsequently by disturbing the substrate and removing possible shelters under which the worms may hide. For each cave we recorded the minimum distance from the cave entrance to the first freshwater biotope in which we found planarians. In caves in which planarians were not found we recorded the minimum distance from the cave entrance to the first freshwater habitat that we encountered. For caves that were dry during our surveys, we recorded the minimum distance from the cave entrance to the first collection site that was indicated in the planimetric map. We also recorded as water quality indicators the abundance of periphyton in the main freshwater bodies, the level of habitat alterations in the cave, and the occurrence of aquatic crustaceans that could be potential prey items for the planarians. The abundance of periphyton over the substrate was visually assessed using a rank scale (1 = periphyton absent or substrate cover < 5%; 2 = 5% ≤ periphyton cover < 40%; 3 = 40% ≤ periphyton cover < 60%; 4 = 60% ≤ periphyton cover < 80% and 5 = periphyton cover ≥ 80% of the substrate). The habitat alteration level of the caves was assessed considering the occurrence/absence of three main indicators: touristic pathways; artificial lighting; water catching or other man-made structures altering the subterranean freshwater bodies. The level of habitat alteration was scored using a rank scale from 0 to 3, where 0 means no signs of any anthropogenic habitat alteration, while we assigned a point to each habitat alteration recorded.

We used a binomial Generalized Linear Mixed Model (GLMM) to assess the relationship between the occurrence of planarians and the recorded environmental variables. Planarians were considered present in the caves if we found them in at least one survey per cave. In particular, the occurrence of planarians was considered as a dependent variable. As independent variables we included periphyton cover, level of habitat alteration, water depth, and the occurrence of crustaceans. To compare different sites of different species ranges, species identity was used as a random factor. We built models representing all possible combinations of independent variables and we selected only the best model using the Akaike Information Criterion for small samples (AICc) values (Rolls, 2011). Variance inflation factor (VIF) was calculated within each model and only models with a VIF value < 5 were considered. The best model contained only a variable and the random factor. To test the performance of this model we applied a Hosmer Lemeshow goodness-of-fit (GoF) test which was not significant (χ^2= 13.6; P = 0.09). The significance of the variable in the best model was assessed with a Wald test (Bolker et al., 2008). The analysis was performed in R 3.3-2 environment (R Development Core Team, 2016) using the packages MuMIn 1.15-6 (Barton, 2016), multcomp 1.4-6 (Hothorn et al., 2008), lmerTest 2.0-33 (Kuznetsova et al., 2016), car 2.1-4 (Fox & Weisberg, 2011) and ResourceSelection 0.3-2 (Subhash et al., 2017).

Results

General conservation status of cave-dwelling planarians

The presence of three out of the eight Italian cave-dwelling planarians species was reconfirmed at their type/known locality (Table 2). The presence of *Dendrocoelum* sp. in the “Grotta Grande di Pignone” cave was also reconfirmed. Only two caves showed no signs of threat for the planarians, while in all other caves we recorded habitat alterations linked to pollution, touristic activity, artificial lighting, and quarrying activities (Fig. 2, Table 2). The type localities of *A. morisii*, *P. benazzii*, *D. beauchampi* and *D.* cf. *beauchampi*, were completely dry during at least one survey although they are listed in the speleological registers as perennial emitting caves or hosting perennial freshwater habitats. The species that we still detected at their type locality were *D. italicum* and *A. morisii*. We also detected *D. collini* and *Dendrocoelum* sp. in their known caves. No planarians were found in the type localities of *D. beauchampi*, *D.* cf. *beauchampi*, *D. benazzii*, *Dugesia brigantii* and *Polycelis benazzii*. (Table 2).

Dendrocoelum italicum

The species *D. italicum* was the first endemic cave-dwelling flatworm described for the fauna of Italy. Maffo Vialli discovered this species in the “Bus del Budrio” cave (speleological land registry number: LO BS 71) in the Italian Prealps and described it on the basis of 20 specimens collected on 13 October 1936 (Vialli, 1937). The original description also reports some second-hand data on the collection site and habitat of *D. italicum* which was found exclusively in a large pool below a small waterfall (Vialli, 1937). No other data is presently available for this species.

During our surveys we detected on average (± SD) 30±1.6 planarians, all localised in small dripping pools, while the large pools described in Vialli’s paper no longer existed because of a water catching structure that was installed in the cave (Table 2).

Dendrocoelum collini

This species was reported for the first time in France in wells and springs in some localities of Côte-d’Or in Bourgogne. Del Papa (1959) ascribed some planarians from the “Grotta Nuova di Villanova” cave (northeaster Italy, slrn: FR 656) to *D. collini*. Since then no other studies or surveys have been performed on this species in its Italian range.

The “Grotta Nuova di Villanova” cave is nowadays open to tourists. On average (± SD), we detected 48 ± 8.4 planarians at each survey in the natural part of the cave, while none occurred in the part with artificial lighting (Table 2). Numerous still unidentified stygobiont dendrocoelids were found in a cave nearby.

Atrioplanaria morisii

The planarian *Atrioplanaria morisii* was found in 1974 in the “Tana di San Luigi” (slrn: 112 pi/cn) cave in Piedmont (northwestern Italy; Fig. 1) (Benazzi & Gourbault, 1977). No further studies have been performed on this cave. This species was also reported in the “Grotta di Bossea” cave (Morisi, 1991), a touristic cave situated 8 km from the type locality of *A. morisii*.

During our surveys the occurrence of this species was confirmed at the type locality, where on average (± SE) 27 ±4.2 planarians were recorded.

The record of *A. morisii* for the “Grotta di Bossea” cave is to be considered a misinterpretation of the first data reporting the occurrence of specimens presumably belonging to the genus *Dendrocoelum* (see Morisi, 1972). A recent histological study performed by one of us (G.A. Stocchino pers. obs.) on specimens collected in 2011 by E. Lana confirmed the presence of only *Dendrocoelum* sp. from the “Grotta di Bossea” cave. Unfortunately, absence of fully sexually developed individuals prevented a detailed assessment of their taxonomic status. No planarians were found during our surveys in the “Grotta di Bossea” cave (Table 2).

Dendrocoelum sp. from “Grotta Grande di Pignone” cave

Individuals of *Dendrocoelum* sp. were found in the “Grotta Grande di Pignone” cave (slrn: LI SP 36) in the district of La Spezia (Liguria, northwestern Italy; Fig. 1) (Benazzi, 1982). The asexual condition of the specimens prevented a detailed assessment to species level. No further surveys or studies were ever performed on this cave-dwelling planarian.

During our surveys we recorded animals in a very shallow perennial stream 80 m from the cave entrance. In this habitat we counted on average 12 planarians during each survey (Table 2). Moreover, during a study on the diet of the salamander *Hydromantes ambrosii* Lanza, 1954 (Lunghi et al., 2018), the stomach flushing performed on several individuals revealed that planarians may represent a potential prey item for these salamanders; in this specific case, a planarian specimen was found among the stomach contents of one adult male. The salamander was found 21 m from the cave entrance, much closer to the surface than the small creek in which planarians were regularly observed; this may mean that planarian topographic distribution inside this cave is wider than previously thought.

Dendrocoelum beauchampi

This species was described on the basis of some individuals collected in 1950 by the speleologist Nino Sanfilippo (Del Papa, 1952). The species is endemic to the “Grotta di Cavassola” cave (slrn: LI GE 125), near Genoa (Liguria, northwestern Italy; Fig. 1). Some information on the habitat was reported by Sanfilippo (1950) who stated that the access to this cave was obtained through two artificial openings made during the Second World War. The author found the planarians in a small subterranean spring in the 10 m natural part of the cave. At present, it is reported in the speleological register of the Region Liguria that the natural configuration of the cave has collapsed due to digging activities.

During our surveys we recorded no planarians in the “Grotta di Cavassola” cave, which was completely dry during summer (Table 2). However, we found a population of *Dendrocoelum* sp. in the deepest sectors of a cave nearby. We detected on average (± SD) 10.3 ±8.1 planarians during 12 surveys. Some individuals were collected and are currently being taxonomically identified.

Dendrocoelum cf. *beauchampi*

Dendrocoelum cf. *beauchampi* was reported by Sluys & Benazzi (1992) from the "Tann-a da Suja" cave (Genoa district, Liguria, northwestern Italy; Fig. 1, slrn: LI GE 5). This cave is relatively close (4.5 km) to the *D. beauchampi* type locality ("Grotta di Cavassola" cave).

The "Tann-a da Suja" cave was completely dry during summer and autumn of 2017 (Table 2), except for some small pools in which the leech *Erpobdella octoculata* Linnaeus, 1758 (Arhynchobdellida, Erpobdellidae) occurred.

Dendrocoelum benazzii

The species *Dendrocoelum benazzii* was described on the basis of several individuals collected by Valerio Sbordoni in 1971 from the "Grotta di Stiffe" cave (Apennines, central Italy; Fig. 1, slrn: 7 ab/aq) (Del Papa, 1973).

The "Grotta di Stiffe" cave is quite developed and now open to tourists in its accessible parts, with artificial lighting and extensive alteration of the stream that crosses the cave through dam construction and stream bed modifications (Table 2). During our surveys we also detected evidence of water pollution with extensive periphyton cover on the substrate of the stream (Table 2). We detected no planarians, but we did observe an abundant population of the leech *Erpobdella octoculata*.

Dugesia brigantii

Dugesia brigantii is known only from the "Grotta di Bocca Lupara" cave in the city of La Spezia (Liguria, northwestern Italy; Fig. 1, slrn: LI SP 74). It was described by De Vries & Benazzi (1983) and a subsequent paper provided second-hand information on the location (Puccinelli & Benazzi, 1985). Planarians were found only inside the cave at 16 m from the entrance. Also for this species no other studies have been performed. Although endemic to the cave, *D. brigantii* shows no troglomorphic features, exhibiting both eyes and pigmentation.

During our surveys we detected no planarians, but we found evidence of strong organic water pollution, with extensive periphyton cover of the substrate (Table 2) and a dense population of the leech *Erpobdella testacea* (Savigny, 1820).

Polycelis benazzii

Polycelis benazzii was described by De Beauchamp (1955) on the basis of individuals collected by Franciscolo during 1952 (Franciscolo, 1955). The species is endemic to the "Tana di Spettari" cave (Liguria, northwestern Italy; Fig. 1, slrn: LI SA 183) and was found at its entrance only in a small dripping pool (Franciscolo, 1955).

We found no planarians in the "Tana di Spettari" cave. The dripping pool described by Franciscolo (1955) no longer existed and the entire cave showed extensive signs of vandalism (Table 2). Moreover, it is likely that the subterranean aquifer has been modified by quarrying activity in an adjacent watershed.

Determinants of planarian occurrence

Of the eight already known caves, two showed a high degree of periphyton cover (Table 2). They correspond to the type localities of *Dendrocoelum benazzii* and *Dugesia brigantii*, which were no longer detected (Table 2). Only habitat alteration was included in the best model. Planarians occurrence was negatively related to caves with a high level of alteration ($F_{1,28} = 6.9$, $p = 0.01$).

Discussion

Our research underlines that since their description, no or very little information on the status of continental Italian cave-dwelling planarians has been collected; in 75% of the cases the environmental conditions of the type locality of a species has been altered and 50 % of the species is no longer present at its type locality. Our results show that habitat destruction through watercourse alteration, water catching and artificial lighting are important threats to cave-dwelling planarians (Table 2).

Generally, pools and streams occurring in caves are considered only one of the habitats that can be inhabited by planarians adapted to subterranean environments (Ginet & Puglisi, 1964; Gourbault, 1972). In many cases, planarians also exploit interstices under the perennial groundwater table and the hyporheic biotopes (Culver & Pipan, 2014; Ginet & Puglisi, 1964; Gourbault, 1972). These are environments in which cave-dwelling planarians have occasionally been found but which are very difficult to sample (Gourbault, 1972). For this reason, the fact that we did not find some of the planarian populations does not necessarily mean that they are extinct. However, in the case of water pollution it is likely that the conditions of the whole aquifer are unsuitable for planarian survival and persistence. Freshwater planarians are an important component of the community of unpolluted lakes, springs and streams (Knakievicz, 2014). Planarians are generally sensitive to organic pollution and water quality, as in the case in the genera *Polycelis* and *Crenobia* (Manenti, 2010; Alonso & Camargo, 2011; Wu et al., 2012), indeed cave-dwelling species generally require oligotrophic waters (Gourbault, 1972). With respect to the species that we no longer found to be present in caves, further research in other portions of the aquifer will be necessary to assess whether they have really become locally extinct.

It is relevant that in most of the investigated caves, the freshwater habitat has been greatly modified without any evaluation by authorities, environmental managers or zoologists on the impact that these changes may have on both planarians and all other underground freshwater invertebrate fauna. Most cave invertebrates belong to species with very narrow distributional ranges which have attracted the interest of taxonomists but, at the same time, are scarcely considered in general zoological and ecological studies. This situation, as exemplified by cave-dwelling planarians, reveals a great lack of knowledge, especially for the early described species. Planarians are bioindicators with a key role in the trophic web of both epigean and underground freshwater habitats (Reynoldson & Young, 2000). Nearly 200 species of Platyhelminthes, mostly planarians, have been recorded in underground environments (Romero, 2009). Most of these species are blind and unpigmented, thus showing a strong adaptation to subterranean life (Harrath et al., 2012, 2016; Stocchino et al., 2013, 2017a,b). In subterranean biotopes, planarians hold an intermediate position in the food web: they represent occasional prey for cave fishes, crayfishes and salamanders (Gillespie, 2013; Manenti, 2014; present paper), while in turn they feed on living or dead cave-dwelling animals such as amphipods, isopods and drowned arthropods such as crickets and dipterans (Romero, 2009). From a conservation point of view, planarians are generally neglected, being neither charismatic nor noticed by humans (Sluys, 1999). Our study is the first extensive assessment of the conservation status of multiple freshwater planarian species. Among freshwater triclads, only one species has a high conservation profile, viz. the pink planarian *Kenkia glandulosa* (Hyman, 1956), inhabiting a cave in Missouri, USA. This latter species is the only cave-dwelling planarian regularly monitored for conservation purposes and for which recent research has been done at the level of micro-habitat preferences (Wicks et al., 2010). The situation revealed by our study for continental Italy may arouse interest in cave-dwelling planarians worldwide. Planarian conservation and ecological studies after the first taxonomic description of the species remain rare and a general lack of conservation information affects most cave-dwelling planarians worldwide. The description/record of a species is only a first step, after which there is the necessity, especially for not particularly attractive species such as flatworms, to promote awareness of their important role and their inclusion in ecological and conservation studies when surveys of their habitat are performed. Our research on the conservation status of planarians could be extended to other karst areas to understand the actions needed to preserve these key organisms for the subterranean environment. The fact that three cave type localities that were previously defined and officially listed as perennial emitting caves or as having perennial freshwater habitats were found completely dry during at least one of our surveys is noticeable. Planarians are highly sensitive to dryness, even though some species may show some resistance (Ginet & Puglisi, 1964; Gourbault, 1972); therefore, the impact of prolonged periods of drought may be detrimental to population survival and fitness.

The overall results of our assessment underline the necessity to establish proper conservation actions for neglected invertebrate species; in particular, our results show the need for stronger protection in terms of laws (Hochkirch, 2016). Cardoso et al. (2011) discussed several different causes currently obstructing a larger inclusion of invertebrates in contemporary conservation actions, which included low public interest in inconspicuous invertebrate species as compared with large vertebrates, together with an incomplete knowledge of the ecology, population dynamics and even distribution and taxonomy of invertebrates. This is particularly evident when we consider freshwater invertebrates: of the 8,000 species currently listed in the IUCN Red List, some 35% are considered as threatened (Collen et al., 2012). Given broadly differing levels of accessible knowledge on the geographic distribution of invertebrate groups, combined with the large number of species, it has so far been possible to assess only the conservation status for a relatively small number of taxonomic groups. Our study indicates that despite their narrow distributional range, micro-endemic species may represent important conservation issues. Indeed, species having a narrow distributional range are more vulnerable to extinction than widespread ones, as any environmental change may be detrimental for the whole species' populations (Davies et al., 2004; Williams et al., 2009; Swenson et al., 2012). Conservation actions focusing on freshwater cave-dwelling micro-endemic species should take into account the whole aquifer and its relationship with the surrounding environment (Culver & Pipan, 2014). As most cities and regions rely on groundwater for their water supply, the conservation of these biotopes is fundamental not only for biodiversity but also for humans themselves (Culver & Pipan, 2014). Our results indicate that different actions are needed to conserve cave-dwelling planarians and other freshwater cave-dwelling invertebrates: protection of water quality in the whole aquifer/watershed; restoration of at least some microhabitats in caves visited by tourists; management of surface habitats; and development of a proper outreach plan to increase interest in neglected invertebrates such as flatworms.

Finally, the fact that we recorded planarians at new sites underlines the necessity to perform further research, which could also include training for caving groups and speleologists to stimulate the recording of flatworms which are generally difficult to observe.

Overall, our study reveals that the conservation status of the populations that we monitored is not good. For many of them our surveys represented the first time that they were searched for after the first record, which often dates back several decades. For four of the eight continental species, the type locality has profoundly changed since their first description.

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Tables

Species	Type/known locality/cave
Atrioplanaria morisii	Grotta Tana di San Luigi (Alps, Prealps)
Dendrocoelum beauchampi	Grotta di Cavassola (Apennine, Genoa district)
Dendrocoelum cf. *beauchampi*	Grotta Tanna da Suja (Apennine, Genoa district)
Dendrocoelum benazzii	Grotta di Stiffe (Apennine, Abruzzo)
Dendrocoelum collini	Grotta Nuova Villanova (Alps, Prealps)
Dendrocoelum italicum	Grotta Bus del Budrio (Alps, Prealps)
Dendrocoelum sp.	Grotta Grande di Pignone (Apennine, La Spezia district)
Dugesia brigantii	Grotta di Bocca Lupara (Apennine, La Spezia district)
Polycelis benazzii	Tana di Spettari (Alps, Maritime Alps)

Table 1. Italian cave-dwelling planarian species (Platyhelminthes, Tricladida) investigated in our conservation status assessment.

Cave	Latitude	Longitude	Cave surveyed length (m)	Species	Threats recorded	Surveys N
Cave group 1						
Tana di San Luigi	44.296	7.889	50	*Atrioplanaria morisii*	D	3
Grotta di Bossea	44.240	7.812	700	*Dendrocoelum* sp.	D, Al, Tp, Cw	3
Tana di Camplass	44.295	7.881	110	-	D	3
Tana delle Fontanelle	44.294	7.893	10	-	D	3
Cave group 2						
Grotta di Cavassola Cave	44.452	9.035	70	-	D, Al	10
Tanna da Scaggia	44.435	9.056	210	*Dendrocoelum* sp.	D	7
Pertuzo do Paolin	44.413	9.029	15	-	D	2
Grotta Inferiore do Paolin	44.414	9.026	6	-	D	2
Pertuzo do Canté	44.401	9.030	24	-	D	2
Vivagna do Fontanin	44.401	9.018	15	-	D	2
Cave group 3						
Tann-a da Suja	44.421	9.035	67	-	D	4
Cave group 4						
Grotta di Stiffe	42.256	13.545	640	-	D, Al, Tp, Cw, P	3
Grotta della Vacca Morta	42.256	13.541	350	-		2
Unnamed artificial cave	42.258	13.293	10	-		2
Cave group 5						
Grotta Nuova di Villanova e; low entrance	46.253	13.280	300	*Dendrocoelum collini*	Al, Tp, Cw	6
Grotta Nuova di Villanova e; high entrance	46.257	13.282	640	*Dendrocoelum collini*	P	6
Tirfore	46.248	13.285	930	*Dendrocoelum* sp.		2
Abisso Vigant	46.244	13.289	30	-		2
Risorgiva Cimitero	46.247	13.284	10	-		2
Cave group 6						
Bus del Budrio	45.591	10.347	60	*Dendrocoelum italicum*	Cw, P	15
Artificial unnamed cave	45.592	10.363	6	-		3
Draining gallery	45.582	10.361	50	-		6
Cave group 7						
Grotta Grande di Pignone e	44.176	9.723	200	*Dendrocoelum* sp.	M	9
Griotta Seconda di Pignone	44.176	9.724	56	-	D	8
Faggiona 1 mine	44.196	9.704	80	-		2
Fornace	44.175	9.721	42	-	D	4

Faggiona 2 mine	44.196	9.703	65	-		2
Grotta di Sant'Antonio	44.183	9.724	12	-	D	3
Cave group 8						
Grotta di Bocca Lupara	44.121	9.796	42	-	P, Cw	9
Ninpharum Domus	44.122	9.797	44	-	P, Cw	2
Cave group 9						
Tana di Spettari	44.139	8.169	136	-	D, Q	5
Grotta di Rio Mezzane	44.136	8.165	8	-	Q, D	2
Risorgente Acquaranda	44.162	8.172	6	-	D	2

Table 2. Complete list of the Italian caves surveyed during our study, considering both the type/known localities of cave-dwelling planarians and surrounding caves never surveyed for the occurrence of planarians. Each cave group refers to caves, including the type/known locality of a species/population and the surrounding caves surveyed. Cave group 1 = type locality area of *Atrioplanaria morisii*; Cave group 2 = type locality area of *Dendrocoelum beauchampi*; Cave group 3 = type locality area of *Dendrocoelum* cf. *beauchampi*; Cave group 4 = type locality area of *D. benazzii;* Cave group 5 = area of the Italian known locality of *D. collini;* Cave group 6 = type locality area of *D. italicum*; Cave group 7 = area including *Dendrocoelum* sp. from "Grotta Grande di Pignone" cave; Cave group 8 = type locality area of *Dugesia brigantii*; Cave group 9 = type locality area of *Polycelis benazzii*. Assessed threats indicated as Al = artificial lighting; Cw = catchwater systems collecting the subterranean streams or aquifers inside the cave; D = dryness (i.e. no water occurring in caves in which pools, streams or other water bodies were officially inserted in the speleological land registry): P = pollution; Q = quarrying activities; Tp = tourist pathways.

Figures captions

Figure 1. Geographic distribution of the studied species. Filled red circles show the location of each surveyed type/known locality; black circles identify the surrounding investigated area.

Figure 2. Frequency of threats recorded in the type localities and previously known caves for the investigated eight species.