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Francesco Saponi and [M. Antonio Todaro](#)*

Posted Date: 27 November 2023

doi: 10.20944/preprints202311.1659.v1

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Article

Status of the Italian Freshwater Gastrotricha Biodiversity, with the Creation of an Interactive GIS-Based Web Map

Francesco Saponi ¹ and M. Antonio Todaro ^{1,2,*}

¹ Department of Life Sciences, Università di Modena e Reggio Emilia, via G. Campi, 213/D, 41125 Modena, Italy

² National Biodiversity Future Center (NBFC), 90133 Palermo, Italy

* Correspondence: antonio.todaro@unimore.it

Abstract: Gastrotricha are microscopic benthic animals found in almost all water bodies. To date, about 870 species distributed in 71 genera, 18 families, and two orders are known. Freshwater species are 359, several of which found also in Italy; however, a compendium of the studies carried out so far and a georeferenced distribution of the species still needs to be provided. This project aims to summarize information about Italian gastrotrichs' diversity and geographic distribution. Diversity data, acquired over 239 years, were reviewed and corrected based on taxonomic and nomenclatural updates and, in addition to distribution information, were organized into a data matrix useful for statistical analysis and to be feed to a Geographic Information System software to easily understand the overall figures. Results indicate Italy as one best-known country regarding freshwater gastrotrich, with 91 species in 17 genera and 3 families from 61 investigated localities; the Nation counts 17 type localities and 19 endemic species. Despite its censed biodiversity, many Italian regions remain still poorly or not investigated where future research should focus. Implementing a web mapping plugin enabled the creation of interactive maps for an easy and modern sharing of the work done and information acquired.

Keywords: benthos; biodiversity; gastrotrichs; meiofauna; taxonomy

1. Introduction

The phylum Gastrotricha (from Greek gaster, stomach, and thrix, hair, refers to the extensive ventral locomotor ciliature), includes microscopic, free-living, aquatic organisms. Marine and brackish-water species lead a wandering life among the interstitial spaces of poorly compacted sandy sediments; freshwater gastrotrichs, on the other hand, live mostly on the surface of the muddy-sand bottom of lakes and streams (epibenthic) or among aquatic plants (periphytic); few species are interstitial, and even less live in the water column (planktonic) [1].

Gastrotrichs have a more or less transparent body spanning 80 μm to 3.5 mm in length, convex dorsally and flattened ventrally [2]. Gastrotrichs are triblastic, acoelomate organisms, with unsegmented tissues and organs, characterized by locomotor cilia and a well-developed, bistratified outer cuticle often forming variously arranged plates, scales, spines. The cuticle of the gastrotrichs is unique in that it enwraps the entire body, including of the cilia [3].

The phylogenetic position of the Gastrotricha has been a long debated [4]; however, recent robust phylogenomic analyses based on transcriptomic data found these micrometazoans allied with the Phatyhelminthes forming a subclade (Rouphozoa) within the spiralian protostomes [5–7].

To date, over 870 species of gastrotrichs have been described, which are divided into the two orders, Macrotrichida and Chaetonotida, whose representatives may differ biology and ecology and are easily distinguished on morphological basis (Figure 1).



Figure 1. Photomicrographs of gastrotrichs belonging to the two orders. (A) *Macrodasys* sp., order Macrodasysida; notice the elongate body shape with several adhesive tubes along the sides (arrows). (B) *Musellifer* sp., order Chaetonotida, notice the peculiar tenpin body shape, with the furcate caudum, showing a single adhesive tube at each end (arrows). Interferential contrast microscopy (DIC).

The order Macrodasysida (about 400 species, divided into 37 genera and 10 families; Table 1) includes almost exclusively marine and estuarine gastrotrichs (only 4 species are freshwater). They are vermiform-shaped, ranging in size from 150 μm to 3.5 mm, and generally have a smooth cuticle, sometimes differentiated into scales and/or spines with protective function. Macrodasysida generally have many adhesive tubes located along the body sides, which give the animal a remarkable ability to adhere to the substrate, essential for an effective life in the interstitial environment. Locomotor cilia may cover the whole ventral surface or be arranged into two longitudinal ciliary bands.

The mouth opening is often very large, the myoepithelial pharynx, has a triradiate lumen, typically in the shape of an inverted Y, and possesses, in the posterior region two pores that allow the exit of excess water ingested during feeding. Macrodasysida are usually insufficient hermaphrodites, with the exception of about ten species that are parthenogenetic instead [8].

The order Chaetonotida (about 480 species, divided into 34 genera and 8 families; Table 2) includes the gastrotrichs most commonly found in freshwater; however, there are many species that live in marine and estuarine environments, and three families (Muselliferidae, Neodasyidae and Xenotrichulidae) are exclusively marine [2].

Chaetonotidans are generally smaller than macrodasysidans (length is generally between 100 and 300 μm) and their body is tenpin-shaped, with the caudal region furcate (called furca), except for the species affiliated with the genus *Neodasys*, which are vermiform in appearance. The cuticle of Chaetonotida is almost always differentiated into plate, scales and spines of highly variable shape and size. Locomotor cilia are, in general, arranged to form two longitudinal ciliary bands.

In almost all species there are only two adhesive tubes, forming the distal part of the caudal furca. The mouth of Chaetonotida is small and the pharynx has a triradiate, Y-shaped, lumen devoid of pores. Reproduction generally occurs by parthenogenesis in freshwater species while marine forms are usually hermaphroditic with the exception of members of the family Chaetonotidae, which reproduce by parthenogenesis [3].

Table 1. High-ranking classification of the Gastrotricha Macrodasysida.

Family CEPHALODASYIDAE Hummon & Todaro, 2010
Genus <i>Cephalodasys</i> Remane, 1926
Genus <i>Dolichodasys</i> Gagne, 1977
Genus <i>Mesodasys</i> Remane, 1951
Genus <i>Pleurodasys</i> Remane, 1927
Genus <i>Paradasys</i> Remane, 1934
Genus <i>Pleurodasys</i> Remane, 1927
Family DACTYLOPODOLIDAE Strand, 1929
Genus <i>Dactylopodola</i> Strand, 1929
Genus <i>Dendrodasys</i> Wilke, 1954
Genus <i>Dendropodola</i> Hummon, Todaro & Tongiorgi, 1993
Family HUMMONDASYIDAE Todaro, Leasi & Hochberg, 2014
Genus <i>Hummondasys</i> Todaro, Leasi & Hochberg, 2014
Family LEPIDODASYIDAE Remane, 1927
Genus <i>Lepidodasys</i> Remane, 1926
Family MACRODASYIDAE Remane, 1926
Genus <i>Kryptodasys</i> Todaro, Dal Zotto, Kånneby, Hochberg, 2019
Genus <i>Macrodasys</i> Remane, 1924
Genus <i>Thaidasys</i> Todaro, Dal Zotto & Leasi, 2015
Genus <i>Urodasys</i> Remane, 1926
Family PLANODASYIDAE Rao & Clausen, 1970
Genus <i>Crasiella</i> Clausen, 1968
Genus <i>Megadasys</i> Schmidt, 1974
Genus <i>Planodasys</i> Rao & Clausen, 1970
Family REDUDASYIDAE Todaro et al. 2012
Genus <i>Anandrodasys</i> Todaro et al., 2012
Genus <i>Redudasys</i> Kisielewski, 1987
Family THAUMASTODERMATIDAE Remane, 1927
Subfamily Diplodasyinae Ruppert, 1978
Genus <i>Acanthodasys</i> Remane, 1927
Genus <i>Diplodasys</i> Remane, 1927
Subfamily Thaumastodermatinae Remane, 1927
Genus <i>Chimaeradasys</i> Kieneke & Todaro, 2020
Genus <i>Hemidasys</i> Claparède, 1867
Genus <i>Oregodasys</i> Hummon, 2008
Genus <i>Pseudostomella</i> Swedmark, 1956
Genus <i>Ptychostomella</i> Remane, 1926
Genus <i>Tetranchyroderma</i> Remane, 1926
Genus <i>Thaumastoderma</i> Remane, 1926
Family TURBANELLIDAE Remane, 1927

Genus *Desmodasys* Clausen, 1965
 Genus *Dinodasys* Remane, 1927
 Genus *Paraturbanella* Remane, 1927
 Genus *Prostobuccantia* Evans & Hummon, 1991
 Genus *Pseudoturbanella* d'Hondt, 1968
 Genus *Turbanella* Schultze, 1853

Family **XENODASYIDAE** Todaro, Guidi, Leasi & Tongiorgi, 2006

Genus *Chordodasiopsis* Todaro et al.ii, 2006
 Genus *Xenodasys* Swedmark, 1967

INCERTAE SEDIS

Genus *Marinellina* Ruttner-Kolisko, 1955

Table 2. High-ranking classification of the Gastrotricha Chaetonotida.

Suborder **PAUCITUBULATINA** d'Hondt, 1971
 Family **CHAETONOTIDAE** Gosse, 1864
 Subfamily **CHAETONOTINAE** Kisielewski, 1991
 Genus *Arenotus* Kisielewski, 1987
 Genus *Aspidiophorus* Voigt, 1903
 Genus *Bifidochaetus* Koliccka & Kisielewski, in Koliccka et al. 2016
 Genus *Caudichthyidium* Schwank, 1990
 Genus *Cephalionotus* Garraffoni et al., 2017
 Genus *Chaetonotus* Ehrenberg, 1830
 Genus *Fluxiderma* d'Hont, 1974
 Genus *Halichaetoderma* Rataj Križanová & Vdacný, 2023
 Genus *Halichaetonotus* Remane, 1936
 Genus *Heterolepidoderma* Remane, 1927
 Genus *Ichthyidium* Ehrenberg, 1830
 Genus *Lepidochaetus* Kisielewski, 1991
 Genus *Lepidodermella* Blake, 1933
 Genus *Polymerurus* Remane, 1926
 Genus *Rhomballichthys* Schwank, 1990
 Subfamily **UNDULINAE** Kisielewski, 1991
 Genus *Undula* Kisielewski, 1991
 Family **DASYDYTIDAE** Daday, 1905
 Genus *Anacanthoderma* Marcolongo, 1910
 Genus *Chitonodytes* Remane, 1936
 Genus *Dasydytes* Gosse, 1851
 Genus *Haltidytes* Remane, 1936
 Genus *Ornamentula* Kisielewski, 1991
 Genus *Setopus* Grünspan, 1908
 Genus *Stylochaeta* Hlava, 1904
 Family **DICHAETURIDAE** Remane, 1927
 Genus *Dichaetura* Lauterborn, 1913
 Family **MUSELLIFERIDAE** Leasi & Todaro, 2008
 Genus *Diuronotus* Todaro, Kristensen & Balsamo, 2005
 Genus *Musellifer* Hummon, 1969
 Family **NEOGOSSEIDAE** Remane, 1927
 Genus *Kijanebalola* Beauchamp, 1932
 Genus *Neogossea* Remane, 1927
 Family **PROICHTHYDIDAE** Remane, 1927
 Genus *Proichthyidium* Cordero, 1918

Genus *Proichthydioides* Sudzuki, 1971
 Family **XENOTRICHULIDAE** Remane, 1927
 Subfamily **DRACULICITERINAE** Ruppert, 1979
 Genus *Draculiciteria* Hummon, 1974
 Subfamily **XENOTRICHULINAE** Remane, 1927
 Genus *Heteroxenotrichula* Wilke, 1954
 Genus *Xenotrichula* Remane, 1927
 Suborder **MULTITUBULATINA** d'Hondt, 1971 [Hummon, 1982]
 Family **NEODASYIDAE** Remane, 1929
 Genus *Neodasys* Remane, 1927

The phylum Gastrotricha has a cosmopolitan distribution (i.e., species of the phylum have been found in at least two oceans and/or continents), as are the two orders and almost all the species-rich families and genera. As other meiobenthic animals, the “meiofauna paradox” [9] applies also to gastrotrichs since many species seem to have a wide distributional range and some are even considered to be cosmopolitan.

As for marine species, dispersal by longshore currents over long periods of time (geologic scale) combined with oceanic dispersal and continental drift can be invoked to explain the biogeographic distribution patterns of Gastrotricha in many marine systems; however, passive dispersal by phoresy, rafting, and within a ballast medium (water or sand) may also play a relevant role.

For the dispersal of freshwater species, a key role can be attributed to the resting eggs, which are spread accidentally by the wind or by particularly vagile animals (e.g., migratory birds). In suitable condition, these eggs coupled with the parthenogenetic reproduction allows for the genesis of new populations in disjunct habitats.

Within freshwater Chaetonotida, the families Dasydytidae, Neogosseidae and especially Chaetonotidae (subfamily Chaetonotinae) are relatively common and are present in the various continents with many species, several of which having anphi-atlantic or a cosmopolitan distribution [10]. By contrast, Dichaeturidae, Proichthydiidae and also the subfamily Undulinae (Fam. Chaetonotidae) are fairly rare, they include 1-4 species whose distribution appear confined to single sites/continent. More specifically, Dichaeturidae includes two species that have been found in Europe, one species in the USA and one species in Japan. Proichthydiidae contains one species that found in South America (Uruguay) and another in Asia (Japan). The subfamily Undulinae (Fam. Chaetonotidae) embraces a single species found in the Amazon [11].

The order Macrodasyida includes four freshwater species distribute in two genera whose species have been found either at a single site, such as *Marinellina flagellata* Ruttner-Kolisko, 1955 in Austria [12] or at a few sites, such as *Redudasys fornerise* and *R. brasiliensis* in Brazil [13–15], and *R. neotemperatus* Kånneby & Kirk, 2017 in the USA [16,17].

Knowledge about the diversity, and especially the distribution of the Italian freshwater species, needs to be more detailed than that of marine gastrotrichs [18]. The first gastrotrich in Italian fresh waters was reported by Corti [19], who speaks of a “hairy soft animal” probably referring to a Chaetonotida. Information of the same anecdotal nature continued till the early 1900s and concerned a few individuals found in different localities of the northern area of the Nation [20–22].

The first Italian freshwater gastrotrichs described in some details are by Marcolongo [23,24], who studied the fauna of the Astroni Lake near Naples (southern Italy). Subsequently, a significant contribution came from the studies performed in Sardinia by Mola [25] and especially by Balsamo [26–30], who investigated freshwater biotopes in northeastern and Central regions. The most recent taxonomic research involving the Italian freshwater gastrotrich dates back 25 years and deals with the Interstitial gastrotrichs from lotic Italian waters [31]. More recently, some freshwater species have been used in phylogenetic studies based on molecular markers (e.g., Kånneby et al. [32,33]).

Several checklists of the Italian freshwater gastrotrich species have been proposed over the time [34,35] but none of them provide georeferenced information, nor summaries regarding e.g., distribution at a regional level or specifies the type localities.

The present work aims to fill these gaps. The study is part of a large Italian national biodiversity project (NBFC- National Biodiversity Future Center) and falls under the mission of Spoke 3, focusing on terrestrial and inland water biodiversity. This prodromal research on gastrotrichs primarily involves acquiring and georeferencing of published information on freshwater taxa. The biodiversity data will be checked and corrected according to recent taxonomic and nomenclatural updates and, in addition to a series of information, including distribution data by region, province, locality, and others, will be organized in a georeferenced data matrix that will serve to obtain basic statistics and a database to be exploited by a GIS (Geographic Information System) software enabling the visualization of the information and a more straightforward interpretation of the data. Implementing a web mapping plugin will allow the creation of interactive maps for easy and modern worldwide sharing of the work done and information acquired to benefit taxonomists, conservation biologists, policymakers, and the wider public [36].

2. Materials and Methods

2.1. Gastrotrichs

Information about the diversity and distribution of Italian freshwater gastrotrichs derived from the following published papers listed in ascending temporal order: Corti [19], Maggi [20], Issel [21], Zacharias [22] Grünspan [37], Marcolongo [23,24], Manfredi [38], Mola [25], Balsamo [26–29], Balsamo & Kisielewski [39], Balsamo & Todaro [40], Bertolani & Balsamo [41], Balsamo [30], Balsamo et al. [42], Balsamo & Fregni [43], Balsamo & Todaro [44], Fregni et al. [31], Leasi et al. [45], Leasi & Todaro [46], Kånneby et al. [32,33]. Bibliographic data were carefully checked and, if necessary, corrected based on taxonomic and nomenclatural updates that have occurred over the years e.g., Schwank [47] Kisielewski [11], see also WoRMS [48]. As the subgeneric grouping of *Chaetonotus* and *Ichthydium* species has found to be non-monophyletic [33,49] in this work species are classified to genus level.

In many cases, the geographical coordinates of the investigated sites were not indicated in the consulted papers; consequently, this information was obtained through freely accessible satellite images (Google Earth and ESRI satellite), based on the toponyms and indication reported in the publications. In cases where the geographical coordinates were reported in the original works, satellite maps made it possible to correct errors or improve the precision of the georeference, which in the past was often limited to degrees and minutes only.

Finally, Prof. M. Balsamo kindly provided unpublished information about the sampling sites of some species initially reported in a previous checklist of Italian gastrotrich species [34] (Supplementary Table S1).

2.2. Data Matrix

The information obtained from the bibliographic consultation was collected in the spreadsheet software MS Excel®, and the dataset used to obtain basic statistics (see below) and to build a database exploitable by the open access GIS software QGIS (<https://www.qgis.org/en/site/>) [50]. Therefore, the data were coded in a “columns by rows” matrix in which the columns represent the variables and the rows the cases. The final dataset was made up of 26 columns and 306 rows, for a total of 6527 cells. The variables of the matrix constituted the QGIS attributes (or fields), based on which the program allows to operate targeted visualizations, searches and analyses. The attribute we coded fall into the three categories: **i**) data relating to the species such as high-ranking classification, images, Aphia ID, endemism; **ii**) data relating to the location where the species was found such as administrative Region, Province, Locality, Type locality, national faunistic provinces and geographic coordinates, in both DMS (degrees, minutes and seconds) and DD (decimal degrees) systems, the latter of which are used by the program, and **iii**) data regarding the researchers involved and the original source of the data (i.e., bibliographic reference).

2.3. Statistical Analysis

Presence/absence faunal data were processed to obtain a similarity matrix using the Bray-Curtis algorithm. The similarity matrix was then subjected to multivariate statistical Cluster Analysis and nonmetric Multi-Dimensional Scaling (nMDS). Both statistical analyses and preparation of the similarity matrix were conducted using Past (version 4.07).

2.4. QGIS

2.4.1. Version and plugins used and software preparation

For the analysis and representation of the data relating to the Italian freshwater gastrotrichs after few trials, we select QGIS 3.14 Hannover version (release 3.16.8), as it proved to be the most stable. Distributional data are georeferenced according to WGS 84 reference system (encoded in QGIS as EPSG:4326) as it is the most used by the geolocalization systems. Moreover, the QuickMapService plugin was applied to import the reference map (ESRI satellite) and the map ENSRI BoundariesPlace (by ESRI); the qgis2web plugin was used to produce the web map.

2.4.2. Creation of the data shapefile layers

The data matrix in CSV format was imported in QGIS to produce a comprehensive vector shapefile layer containing points-like geometry, with points representing the investigated locations to which all the other attributes are associated (see above). This shapefile (called Italy-All-Species) was subsequently used to create the shapefiles involving: **i**) Localities, **ii**) the single species, **iii**) the type localities, and **iv**) the endemic species. A further layer, containing the summary information regarding the single Italian administrative regions, was created starting from a pre-existing shapefile downloaded from the Italian National Institute of Statistics (ISTAT) website (<https://www.istat.it/it/archivio/222527>)[51] and modified to suit our purpose. More specifically, the original attributes pertaining to the demography and the territorial features were erased and new ones including the number of species present in a given region, number of sites sampled, number of type localities, average number of species in each site, were created.

2.4.3. Web map preparation and exportation

All the produced layers were selected to be part of the final web map, which was created in the Leaflet format. This format was chosen as it is the most widespread open-source JavaScript library for mobile-friendly interactive maps and it is easier to use. The reference map and comprehensive layer were the only ones set to be active at the opening of the map (Figure 2). All the other layers were set to be activated by the final users (Figure 3). The Popup and the cluster options were selected for the comprehensive layer, while the popup option was selected for all the other points vector layers. For clearness, record points of the main layer were choosing to be shown clustered at the default small scale; the single locations making up each given cluster could be visualized at a larger scale by progressive zoom-in actions (Figure 4). The popup boxes were devised to show significant information related to the specific layer (Figure 5).

Once all the necessary settings were established, the web map export command was evoked and the obtained folders and files were uploaded to the server dedicated to Gastrotricha (www.gastrotricha.unimore.it) which is hosted at the meiofauna laboratory of the Department of Life Sciences, University of Modena and Reggio Emilia. The map is available at the following ULR www.????????????). A redundant copy of the map has been posted on a commercial server provider at the following URL www.??????.

A note for the reviewers: complete web addresses for the interactive map will be made available at the acceptance of the manuscript (i.e., reported in the published paper); for reviewing purposes, a simplified map is available at the following URL <https://provisionalfreshwatermap.altervista.org/>

3. Results

3.1. Gastrotrichs

Records of Italian freshwater gastrotrichs apparently cover a 239-year time frame. However, initial data come from simple species lists, lacking any morphometric information on the reported taxa. Consequently, these accounts should be treated with caution. For instance, Marcolongo [24] believed the first recorded Italian freshwater gastrotrich to be the “hairy soft animal” that Bonaventura Corti found in Modena (see Corti, 1774, Tav. II, Figure XI) [19], and that Eherenberg [52] identifies with *Chaetonotus maximus* Eherenberg, 1838. However, the animal depicted by Corti [19] hardly can be identified as a gastrotrich; its elongate body without the trademark caudal furca makes it resembling most to a microturbellarian instead (a copy of Corti’s paper can be found at the following url: <https://archive.org/details/osservazionimic00corti/page/n209/mode/2up>).

Similarly, we could not confirm the finding of *Lepidodermella squamata* (Dujardin, 1841) near Trieste (Region Friuli Venezia Giulia) attributed to Grünspan, 1908 [37] by Marcolongo [24] and Mola [25]. On the other hand, based on our bibliographic search the following historical records are confirmed: presence of *Ichthyidium podura* (Müller, 1773) and *Chaetonotus larius* Müller, 1773 in Valcuvia (Lombardia region) Maggi [20]; occurrence of *Heterolepidoderma ocellatum* Metschnikoff, 1865 in the Euganean thermal waters (Veneto region) Issel [21]), and of *Chaetonotus chuni* Voigt, 1901 near Pavia (Lombardia region) Zacharias [22].

The first information on the Italian species including morphometric data comes from the taxonomic study by the Italian researcher Ines Marcolongo, who investigated the Astroni crater lake near Naples and reported 17 species, eight of which were described as new to science [23,24]. The most recent published records of Italian freshwater species can be found in two phylogenetic works by Kånneby et al. [32,33], which list *Chaetonotus schultzei*, *Lepidochaetus zelinkai* (Grünspan, 1908) and *Polymerurus nodicaudus* (Voigt, 1901), all found in Lake Pratignano, in the province of Modena [32,33].

Although the period covered by the published literature is relatively broad, the intensity of the studies has remained not constant over time. Relatively long periods of stasis were followed by an energetic and productive investigative activity, although often restricted to single regions (e.g., Manfredi [38] in Lombardia; Mola [25] in Sardinia). It is only since 1977 that the Italian gastrotrichs have become the subject of constant and more extensive studies [26–29,39], and the last decade of the past century proved particularly fruitful in shedding light on the surprising diversity of gastrotrichs in the Italian inland waters. During that period, a series of investigations carried out by researchers of the University of Modena and Reggio Emilia (Italy) headed by professors Maria Balsamo and Paolo Tongiorgi, significantly contributed to the increase in the number of species and of Italian regions involved in the survey [30,31,42–44].

From our analysis, it emerges that the number of freshwater gastrotrich species currently known for the Italian inland waters amounts to 91 (Tables 3 and S1), all belonging to the order Chaetonotida. The 91 Italian species, distributed in 17 genera and three families, were found by investigating 61 localities/biotopes and 81 sites (i.e., sampling points). However, of the 20 Italian regions, less than half (nine regions) have been investigated to some extent with regard to the freshwater gastrotrichofauna (Tables 3 and S1).

The number of species found in the investigated locations (loc) range between 1 and 33, with an average of 5.33 ± 6.13 spp/loc (Table 4). The location for which the highest number of species has been reported (33 species) is Monte Bondone, in the Trentino Alto Adige region. This figure is reached by pooling data from samples collected from several circular water bodies locally known as Viotte or Viote. These are craters created by the explosion of artillery shells during World War I, which were then filled with rainwater [44].

Currently, 19 species are considered endemic to Italy (i.e., so far recorded only in this country), of which 12 have their distribution limited to only one of the nine investigated regions. Italy counts 17 type localities concerning the freshwater gastrotrich species, some of which are such for more than one species (Table 3). *Lepidodermella squamata*, found in 27 localities, appears to be the most frequently found species in the Italian inland waters (Table 4).

The distribution of the investigated localities, as well as the richness and diversity of the fauna, appears uneven. The most studied regions are Emilia Romagna, Sardinia and Tuscany, while the others have been little or not at all investigated (Table 4). The most surveyed region by far is Sardinia, with 21 localities investigated and 34 species found (averaging 1.81 ± 0.93 spp/loc). Most information on the rich gastrotrich fauna of Sardinia dates back to more than 90 years and no additional records have been added since the work by Mola [25], with the exception of three species new to Sardinia reported by Fregni et al. [31].

Table 3. Summary data by Italian regions regarding the freshwater Gastrotricha. Number of investigated localities, taxa and type localities.

Region	n. localities and (sites)	n. species	n. genera	n. families	n. type localities
Abruzzo	-	-	-	-	-
Basilicata	-	-	-	-	-
Calabria	-	-	-	-	-
Campania	1 (1)	17	4	2	1
Emilia Romagna	14 (18)	28	8	1	3
Friuli Venezia Giulia	-	-	-	-	-
Lazio	5 (7)	22	7	1	3
Liguria	1 (1)	2	1	1	0
Lombardia	3 (3)	12	5	2	0
Marche	-	-	-	-	-
Molise	-	-	-	-	-
Piemonte	-	-	-	-	-
Puglia	-	-	-	-	-
Sardegna	21 (24)	34	13	3	0
Sicilia	-	-	-	-	-
Toscana	10 (12)	41	11	2	5
Trentino Alto Adige	4 (14)	27	9	2	4
Umbria	-	-	-	-	-
Valle d' Aosta	-	-	-	-	-
Veneto	2 (2)	15	4	1	1
Italy	61 (81)	91	17	3	17

In his seminal work on the Sardinian gastrotrichs, Mola [25] also proposed an interesting new classification for the group, including new subclasses, orders, families, genera etc. However, Mola's classification was not followed/adopted by succeeding specialists, probably because of the language barrier (the article is written in Italian); of the taxa proposed by Mola [25], only the name *Wolterecka* figures in the current classification of the Gastrotricha, to designate a subset of the vast genus *Chaetonotus* [53].

The second region based on localities investigated is Emilia Romagna, with 14 biotopes studied and 28 species collected (averaging 4.57 ± 3.08 spp/loc). Data from this region are among the most recent, the latest of which date back to 2013 [33]. Although Tuscany ranks only third in terms of localities investigated (10 locations), it occupies the first position regarding the number of species found (41 spp). The most recent information from Tuscany dates back about 25 years and comes from surveys conducted in lotic waters in the province of Pisa [31]. Despite the low number of sites surveyed, Campania, Trentino Alto Adige, and Lazio host a relatively high number of species found and occupy the first, second, and third places, respectively, in terms of species abundance (number of species per locality, Table 4). The Campania records dates more than a century ago, and the reported simplified anatomy of some of the described species leaves doubts as to their reliability, also considering the poor optical instrumentation in use at that time [23,24]. In contrast, the information from Trentino Alto Adige and Lazio is relatively recent and derived from methodologies in line with current taxonomic practices (e.g., interferential contrast microscopy [44]).

The diversity of the Italian gastrotrich fauna, understood as the number of species, does not seem to be related to the number of localities investigated as much as to the type of biotope studied; eutrophic environments characterized by rich and varied aquatic vegetation seem to offer the best conditions for the development of a highly diversified biocoenosis [27]. However, the peculiarity of fauna associated with oligohaline environments at high altitudes or with biotopes whose waters are characterized by a low pH value, such as pit moss, and even the interstitial environments should be emphasized [29,31]. Planning future research should take all these considerations into account.

Table 4. Italian regions with summary data regarding the freshwater Gastrotricha. Number of specie per locality, most common species and number of endemites.

Region	n. specie per locality (Mean \pm s.d.)	Most common species (% localities)	n. endemites
Abruzzo	-	-	-
Basilicata	-	-	-
Calabria	-	-	-
Campania	17 \pm N.A.	N.A.	4
Emilia Romagna	4.57 \pm 3.08	<i>C. aemilianus</i> (12.5%)	3
Friuli Venezia Giulia	-	-	-
Lazio	9.40 \pm 5.50	<i>L. squamata</i> (14.9%)	1
Liguria	2 \pm N.A.	N.A.	0
Lombardia	4.67 \pm 5.51	N.A.	0
Marche	-	-	-
Molise	-	-	-
Piemonte	-	-	-
Puglia	-	-	-
Sardegna	1.81 \pm 0.93	N.A.	0
Sicilia	-	-	-
Toscana	7.60 \pm 6.24	N.A.	3
Trentino Alto Adige	12.75 \pm 15.24	<i>C. hystrix</i> (13.7%)	0
Umbria	-	-	-
Valle d'Aosta	-	-	-
Veneto	8 \pm 9.90	<i>H. ocellatum</i> (12.5%)	1
Italy	5.33 \pm 6.13	<i>L. squamata</i> (8.4%)	19

Multivariate statistical analyses based on presence/absence data indicate a higher faunal similarity between Emilia Romagna, Tuscany, Trentino Alto Adige, and Lazio than the other investigated regions (Figure 14 and Figure 15). However, it must be taken into account that these results are greatly affected by the different sampling efforts applied to the different regions and by the different taxonomic techniques that have occurred over the long period between the collection of the first data [23] and the most recent ones [31].

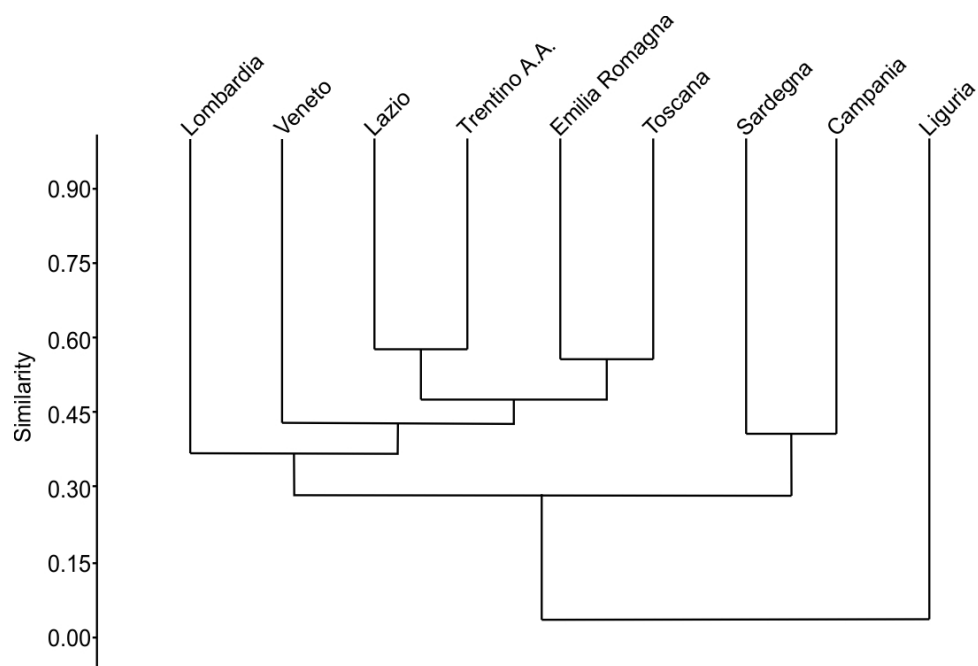


Figure 2. Cluster analysis result showing hierarchical clustering of the investigate regions based on Bray-Curtis similarity index on presence/absence of freshwater gastrotrich species.

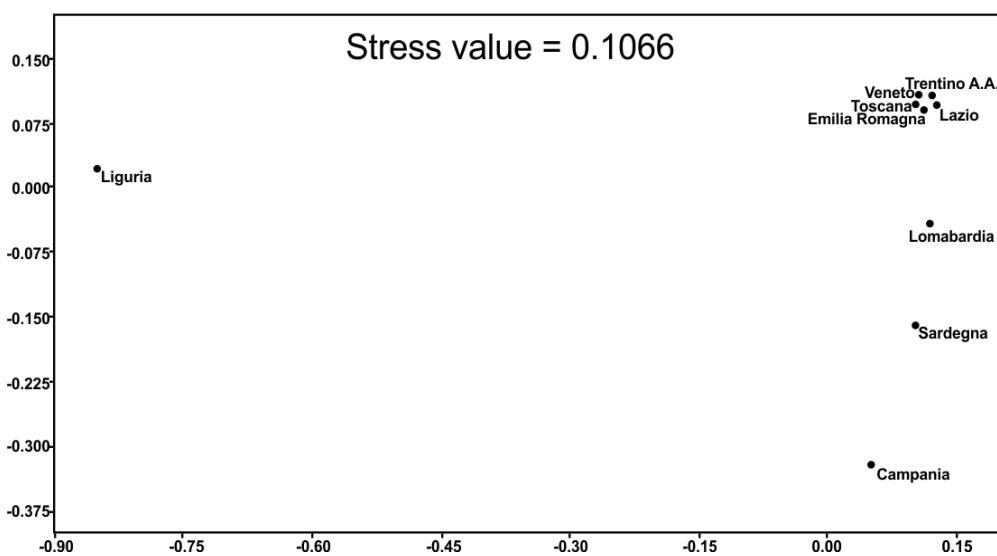


Figure 3. Result of the nMDS analysis showing the ordination of the investigated regions based on the Bray Curtis similarity index on presence/absence of freshwater gastrotrich species.

3.2. QGIS and Web Map

The first screen of the interactive map that is offered to the user shows a map containing two panels through which specific searches can be made: i) the panel zoom, through which the user can zoom in and out the areas of interest, and ii) the panel layers, through which the user can query the system in search for the information of interest (Figure 4).

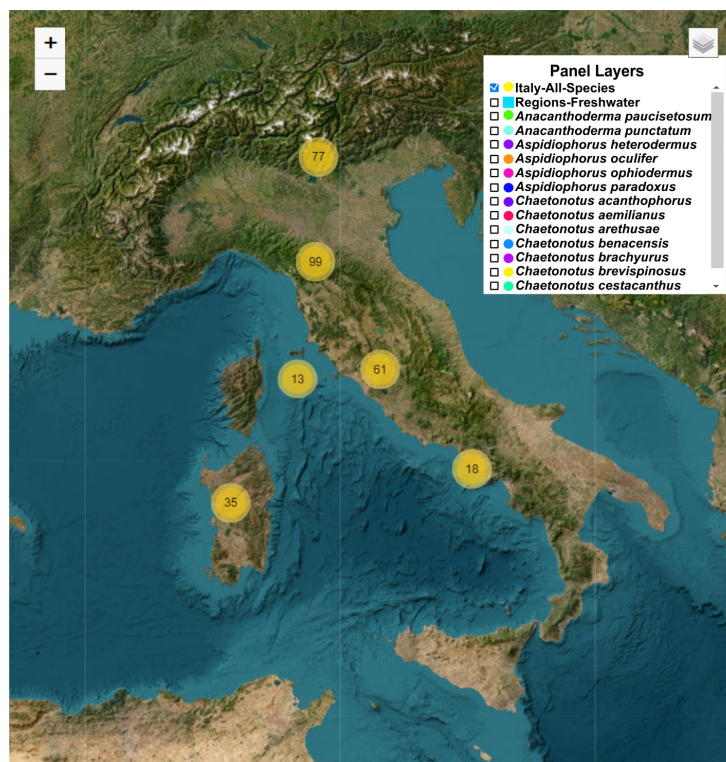


Figure 4. Example of the interactive map dedicated to the Italian freshwater Gastrotrocha. Screenshot of the initial view of the web map with indications for interactivity: the panel zoom at the top left, and the panel layers at the top right (sovrainposed is an image showing some of the contained layers). Records appear clustered in six large groups based on reciprocal distance. Numbers at the center of the groups indicate the records associated to each cluster.

Regarding interactivity, the most important panel is probably the panel layers (Figure 4). It contains 98 layers in total, one of which represents the reference map (eSRI satellite). All the other layers, except one, can effectively express their contents only if activated in conjunction with the reference map (e.g., Figure 5 and Figure 7). The exception is the layer containing the summary data of each Italian region, which may fully show its information even in the absence of the reference map (see below).

For this research, the comprehensive layer **Italy-All-Species** assumes particular relevance. Activating this layer, in conjunction with the reference map, allows the visualization of all the vector points within the Italian political boundaries, i.e., it visualizes all the points/locations in which gastrotrich species have been found.

Since it was chosen the points to be shown clustered, the initial screen shows six large clusters, in which the vector points are grouped based on their mutual geographical distance (Figure 4). Progressive zooming moves to increasingly smaller clusters until one can visualize the individual sampling points (Figure 5A–C).

Once the point (location) of interest has been exposed, a number at the disk's center indicates how many records (i.e., species) are associated with that location. Clicking on the disk makes it possible to “explode” the records, which will be displayed surrounding the point itself (Figure 5D). A click on the single record opens a popup box containing all the salient information about that record, e.g., the individual species name, locality, geographical coordinates, bibliographic reference etc. (Figure 6). In short, the popup box reports the 15 attributes chosen to be visualized to the end user, as they are the most valuable. The remaining 11 attributes (see data matrix above) are visible only to the authors (us) on the QGIS desktop platform to facilitate the housekeeping of the dataset.

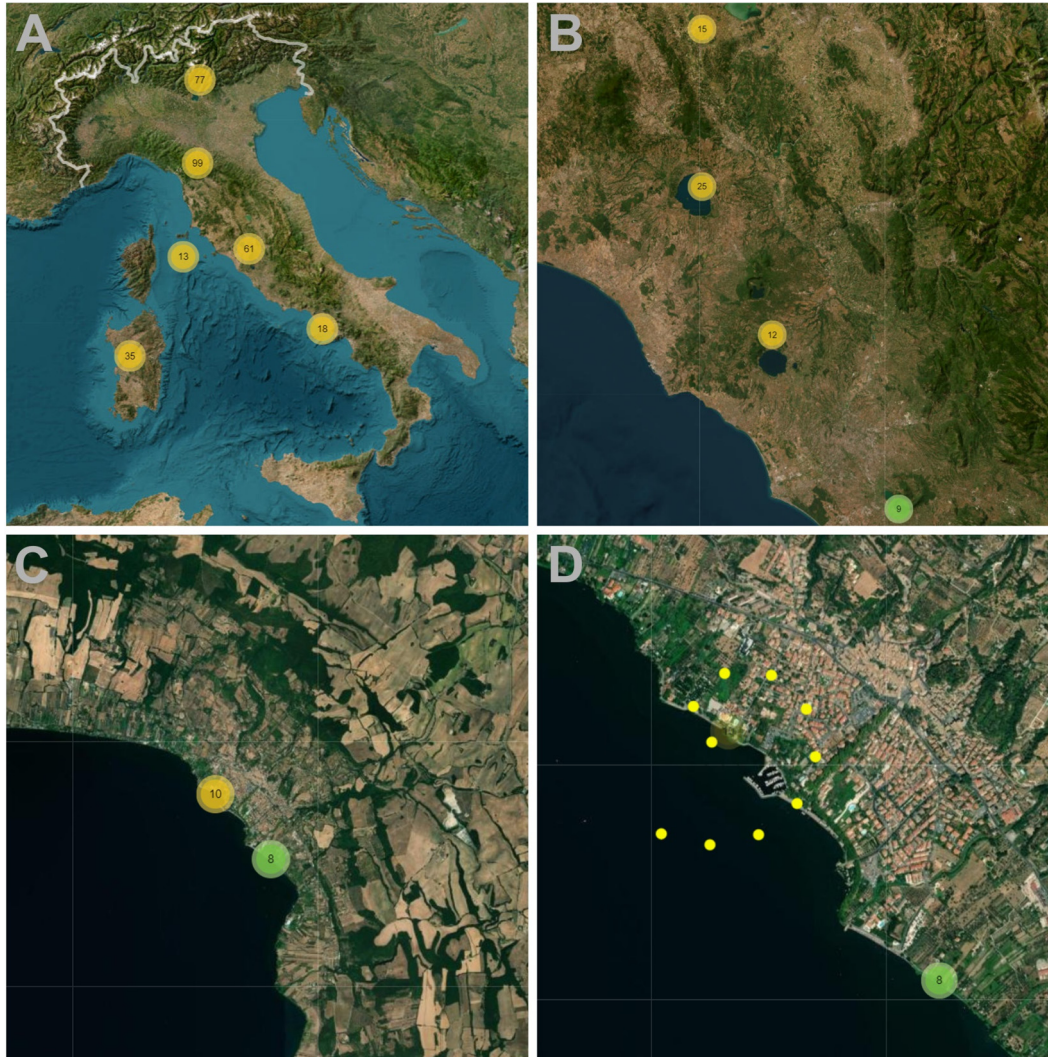


Figure 5. Examples of the interactive map dedicated to the Italian freshwater Gastrotricha. Screenshots of the web map with the layers Italy-All-Species and the reference map active. The images show the vectors grouped into more or less comprehensive clusters depending on the scale (zoom), from the smallest scale (A) up to progressively larger scales (B–D). The numbers at the center of the groups indicate the records associated with the individual clusters. At the largest scale, the point (location) of interest is reached/shown, and clicking it will display the associated records surrounding the point itself (D).

If activated, the layer **Localities** offers the overall glimpse of the distribution of the investigated localities and along the Italian territory (Figure 7). Clicking on the individual vector points activates a popup card with the relevant information of the location, including geographical and bibliographic information (Figure 7).

The layers dedicated to the individual species appear to be highly effective in conveying helpful information; by activating them in conjunction with the reference layer, the web map shows the known distribution on the Italian territory of the species of interest; it is, therefore, possible to immediately understand whether it is widespread or confined to a single water body. Paradigmatic examples are shown in Figure 7, where the distribution of a rare (Figure 8A) and of a relatively common species (Figure 8B) is shown.

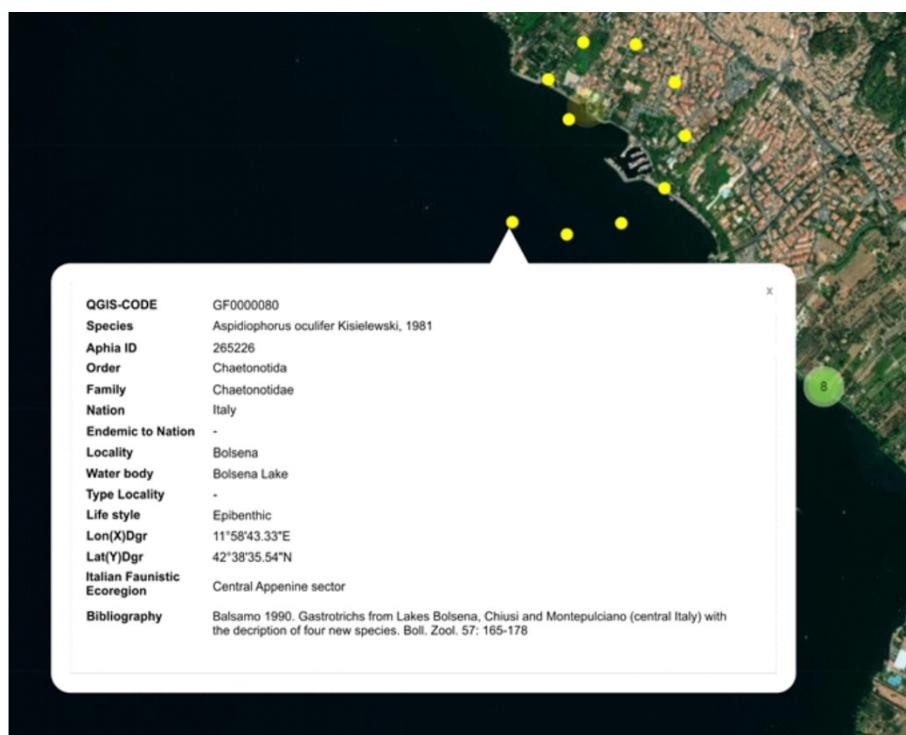


Figure 6. Example of the interactive map dedicated to the Italian freshwater Gastrotricha. The same image as Figure 5D, showing the popup box associated with one of the records.



Figure 7. Example of the interactive map dedicated to the Italian freshwater Gastrotricha. Screenshot of the interactive map originating from the layer Localities, showing the distribution of the investigated locations along the national boundaries.

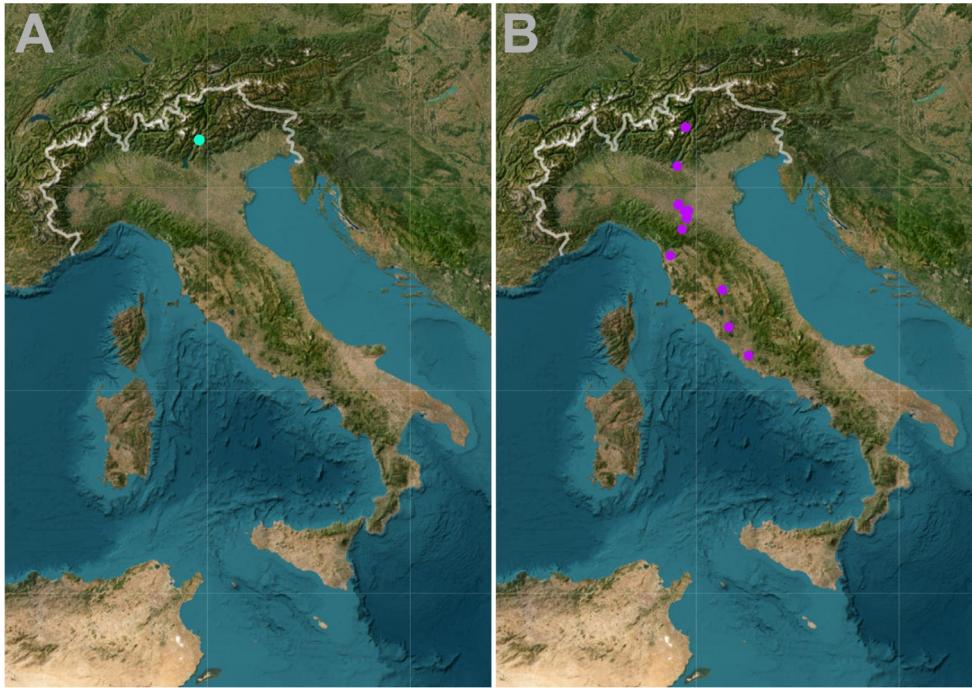


Figure 8. Examples of the interactive map dedicated to the Italian freshwater Gastrotricha. Maps showing the distribution of individual species. (A) *Chaetonotus arethusae*, with distribution limited to a single biotope; (B) *Chaetonotus aemilianus*, with a wide distribution.

The layer **Type Localities** is significant because it indicates the locations where one or more species were originally described from, and therefore, these sites could be the focus of conservation projects (Figure 9A). Similarly, the layer **Endemic Species** is exceptionally informative because it indicates sites where the Italian endemic species have been found, so this layer can also be used to direct possible conservation efforts (Figure 9B).

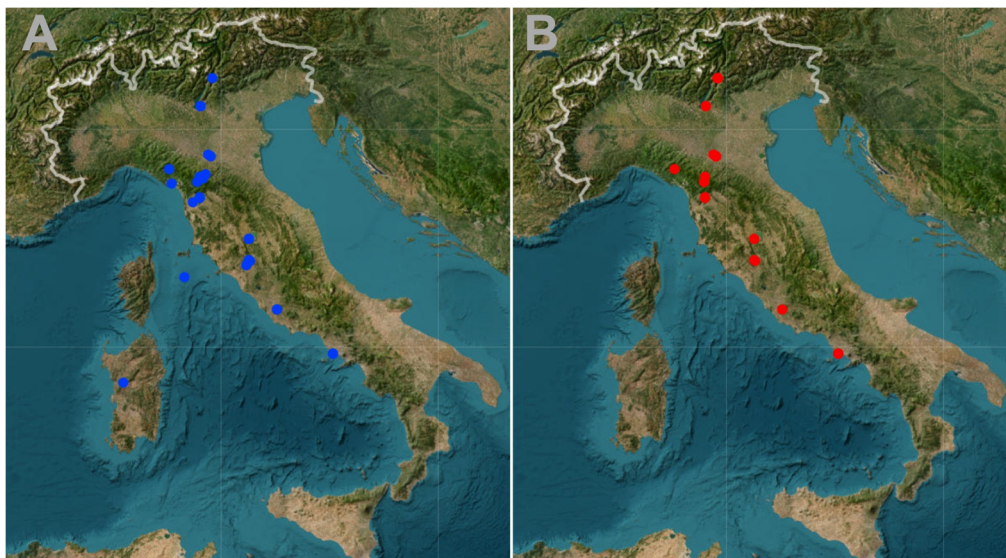


Figure 9. Examples of the interactive map dedicated to the Italian freshwater Gastrotricha. Screenshots of the map showing the distribution of type localities (A) and type locality of the endemic species (B).

The layer **Regions-Freshwater** is also of great interest because it allows rapid access to summary information on the taxa and the sampling effort regarding the individual Italian regions. The map shows the regional borders and each region can be activated to show a popup box containing

information relating to the selected region, including the number of species found, locations investigated, etc. (Figure 9). The interactive map can provide all the valuable information quickly and effectively, like (and probably more than) a detailed scientific article.



Figure 8. Layer Regions-Freshwater, by clicking on one of the investigated regions (in the example the Emilia Romagna region), a pop-up box appears with the summary data relating to the investigated locations and the species found, as well as the most common species at regional level and its image.

4. Conclusions

4.1. Gastrotrichs

This research has highlighted that knowledge of the Italian freshwater gastrotrichs needs to be completed. In 239 years, 25 studies have been carried out, thanks to which 61 locations, distributed across 9 regions, have been investigated; in total, 91 species were found, distributed across 17 genera and three families; 19 species are endemic to the country, while the type localities are 17.

Comparing the data from other nations (Table 5), it also emerges that Italy is one of the best-known countries concerning the freshwater gastrotrich fauna, despite research being restricted to only a few regions. To better understand Italian biodiversity and the biogeography of Italian species, future investigations should focus primarily on areas that have been poorly or not investigated at all. It would also be interesting to screen out genetically disjunct populations of widely distributed species in order to confirm the status of these taxa or, conversely, to establish whether they are reproductively isolated but morphologically indistinguishable populations, thus identifying phenomena of cryptic speciation [54].

Table 5. Known freshwater gastrotrichofauna in selected nations.

Nation	n. species	n. genera	n. families	Reference
Denmark	27	6	2	[55]
France	36	10	3	[56]
Germany	91	13	3	[57]
Italy	91	17	3	Present study
Poland	107	11	3	[58]

Romania	90	15	3	[59]
Sweden	54	9	2	[60]
South Korea	16	6	3	[61]
Canada	39	10	2	[62]
U.S.A.	60	13	4	[17,63,64]
Brazil	98	18	4	[14,15,65–73]

4.2. QGIS

QGIS is a compelling informatics platform that offers numerous data management and analysis prospects but requires in-depth knowledge of IT resources. However, this has not prevented the purpose of this study from being achieved, by offering through a web map a wide public access to data on diversity and distribution of the Italian freshwater gastrotrichs. In addition to the effective dissemination of data, maps make it possible to easily highlight gaps in knowledge and offer an immediate indication of the areas of the peninsula in which future research should focus, depending on specific goals.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1. List of Italian freshwater Gastrotricha.

Author Contributions: Conceptualization M.A.T.; methodology, resources, data curation and writing F.S. and M.A.T.; funding acquisition M.A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This project was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union—NextGenerationEU. Project Code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP E93C22001090001, Project title “National Biodiversity Future Center—NBFC”.

Conflicts of Interest: The authors declare no conflict of interest.

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