

# Mapping biodiversity hotspots and conservation priorities for the Euro-Mediterranean headwater ecosystems, as inferred from diversity and distribution of a water beetle lineage

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Received: 30 May 2014 / Revised: 25 August 2014 / Accepted: 10 September 2014 /

Published online: 16 September 2014

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**Abstract** Euro-Mediterranean running water ecosystems represent a highly threatened habitat due to draining, construction, agriculture, eutrophication, pollution and climate changes. Thereby, the associated biodiversity is often more threatened compared to any other ecosystem and the identification of biodiversity hotspots and conservation priorities for running water fauna is therefore a primary target. Here we focused on invertebrates, as important indicators of ecosystem functions, modelling a lineage of water beetles and investigating the conservation status of Euro-Mediterranean headwater ecosystems. We selected the “*Haenydra*” lineage (Hydraenidae, genus *Hydraena*) as representative taxon (92 species overall, known to occur from Iberian Peninsula to W Iran), analysing a geographic database including literature, field-collecting and Museums data in order to map the distribution of biodiversity hotspots in Euro-Mediterranean headwater systems and locate the running water sites potentially important for invertebrates conservation. We

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Communicated by Dirk Sven Schmeller.

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**Electronic supplementary material** The online version of this article (doi:[10.1007/s10531-014-0798-z](https://doi.org/10.1007/s10531-014-0798-z)) contains supplementary material, which is available to authorized users.

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hence assessed the conservation priorities assigning IUCN threat Categories—following IUCN guidelines—to all the species of the lineage, together with a herein introduced Conservation Score (CS) computed using a procedure tailored specifically for water beetles, also analysing historical series. We found that threatened categories (VU or higher), with high CSs, should be assigned to >70 % of the species, suggesting that, considering this lineage a representative model, the headwaters conservation status in Euro-Mediterranean areas is alarming. Cantabria, Pyrenees, Alps and Central Apennines were identified as biodiversity hotspots, whereas the combination of IUCN categories and CSs revealed that the areas with highest conservation concern are distributed in W-Alps, E-Alps, Apennines, Central Spain, Greece and N-Turkey. Our analyses suggested a dramatic worsening of running water ecosystems in several Mediterranean regions and in particular in Italy, Greece and Turkey. The comparison between the IUCN categories and the newly introduced CSs evidenced the importance of taking into account the ecological, hydrogeological and historical features when assessing conservation strategies for running water organisms.

**Keywords** Hotspots distribution · Freshwater biodiversity · Conservation scores · Hydraenidae · IUCN threat categories

## Introduction

Freshwater ecosystems include a wide range of habitats such as rivers, lakes, wetlands, and the riparian zones associated with them. Their boundaries are constantly changing, being related to the seasonality in the hydrological cycles (Revenga et al. 2000) and to long-term macroclimatic, geo-morphological and hydrological phenomena. Although freshwater systems occupy less than 0.8 % of the Earth surface, they are often characterised by high species richness, hosting more than 45,000 known animal species (Reaka-Kudla et al. 1997). Considering Europe, a recent estimate (<http://www.faunaeur.org>; Audisio et al. 2014) suggests that about 10 % of the whole terrestrial fauna is represented by freshwater Metazoans, corresponding to some 13,000 species over the nearly 130,000 known for Europe (Fontaine et al. 2012).

Freshwater biodiversity is probably more threatened compared to any other ecosystems in the world (Abellán et al. 2007; Domisch et al. 2013). In fact, freshwater ecosystems, and in particular running waters, have been altered since historical times, with strong anthropogenic impacts starting at the beginning of the last century and growing exponentially until the present days (Revenga et al. 2000; Saunders et al. 2002; Nijboer et al. 2004; Abellán et al. 2007, 2013; D'Amen et al. 2010; Balint et al. 2011; Alofs et al. 2014). As a consequence, a high percentage of stream headwaters should be assigned to a threat category (VU, EN or CR) in the IUCN Red List for threatened ecosystems (Rodríguez et al. 2011), especially considering the Euro-Mediterranean areas where the level of human impact is often stronger than in other regions of the world (Hoekstra et al. 2005).

Recent syntheses about the biology and ecology of freshwater insects (Batzer and Wissinger 1996; Keiper et al. 2002) have increased the knowledge of their functional roles, stressing in particular their importance as nutrient recyclers, primary and secondary consumers, food for wildlife, and indicators of ecosystem functions (Euliss et al. 1991; Anderson and Vondracek 1999; Batzer et al. 1999; De Szalay and Resh 1997; Nijboer et al.

2004). Furthermore, in recent years, the interest in running waters fauna conservation increased because many of the world's freshwater ecosystems have been partially lost or completely destroyed following draining, captation, construction, agriculture, eutrophication, and pollution (NRC 1992; Mitsch and Gosselink 2000; Stoate et al. 2001). Moreover, future climate change is projected to have an important impact on freshwater biodiversity, considering both vertebrates (Cianfrani et al. 2011) and invertebrates (Sauer et al. 2011; Domisch et al. 2011, 2013), with also important and usually overlooked effects at the intraspecific level (Balint et al. 2011).

The identification of biodiversity hotspots and conservation priorities for freshwater European invertebrates is therefore a primary target for research. Furthermore, most Euro-Mediterranean freshwater habitats characterised by high concentration of endemic and ecologically specialized species are located in restricted areas which continuously existed since the last interglacial, and possibly going as far back as the Tortonian and Messinian ages (8–5 Mya; Ribera et al. 2011; Trizzino et al. 2011a). These species-rich habitats and their associated communities are dramatically exposed to present-day anthropogenic perturbations due to habitat loss or degradation, and climate changes (e.g. Domisch et al. 2013), resulting respectively in short- and mid-term loss of biodiversity (Kostoski et al. 2010; Tierno de Figueroa et al. 2013).

Therefore, biological monitoring of freshwater ecosystems must go beyond the conventional measures of water quality to address questions of ecosystem functions and integrity (Tilman et al. 1997; Revenga et al. 2000; Santoul et al. 2005; Sondergaard and Jeppesen 2007; Leira et al. 2009). In this framework, the methods that are commonly proposed (e.g., Extended Biotic Index) usually focus on invertebrates in general, often considering taxonomic levels higher than species, and seldom taking into consideration water beetles. However, with an estimated total number of 8,000 species of freshwater insects in Europe (out of the total 13,000 species of freshwater Metazoans, <http://www.faunaeur.org>), Coleoptera are second only to Diptera in terms of number of species being more or less strictly associated with running waters (more than 1,000 species; Audisio et al. 2014). Therefore, Coleoptera should represent a natural focus for the identification of the main Euro-Mediterranean regions representing priority areas for monitoring and conservation of running water ecosystems (Abellán et al. 2005a).

Since at the Euro-Mediterranean scale there is a general paucity of inventory data for running freshwater invertebrates, we believe that analysis of even a single representative, widespread and species-rich taxon could provide useful indications in order to focus the attention of the scientific community over areas potentially important to prevent biodiversity loss in freshwater ecosystems. This is certainly true at least if the selected taxon shows richness pattern, rate of endemism, and responses to water pollution and perturbation markedly and positively correlated to those found in other large groups of running water invertebrates. Therefore, we selected a single lineage (hereafter “*Haenydra*”), belonging to the hyper-diverse water beetle genus *Hydraena* Kugelann 1794 (Coleoptera, Hydraenidae), as the model taxon for our analysis. In fact, at the Country level, species richness and endemism for the genus *Hydraena* are highly correlated with species richness and endemism for basically all the main groups of freshwater macrobenthonic invertebrates (e.g., Plecoptera, Ephemeroptera, Trichoptera, Diptera, and benthic Acarina; <http://www.faunaeur.org>). Furthermore, a rich literature and crossed data banks from specialists of several running water insect taxa supports these correlations even at the local scale of single drainage systems or single study area (Baldari et al. 1983; Compin and Céréghino 2003; Baldi 2003; Abellán et al. 2005a, b; Sánchez-Fernández et al. 2006; Haidekker and Hering 2008; Silveri 2008; Audisio, Belfiore and Fochetti unpublished data).

The aims of the present paper are:

- 1) To identify the main biodiversity hotspots in Euro-Mediterranean running water systems, based on literature, field-collecting and museums data focusing on the “*Haenydra*” lineage, detecting running water systems and sites potentially important for conservation (Gaston 1994; Santoul et al. 2005), using the “*Haenydra*” lineage as a model for the European invertebrates inhabiting the headwater streams.
- 2) To assess conservation priorities (sensu Heiner et al. 2011) and IUCN threat categories for all species of the “*Haenydra*” lineage, assigning also to each species a herein introduced Conservation Score (hereafter CS) specifically tailored for water beetles. CS was computed considering several descriptive parameters important for freshwater invertebrates following the proposals by Keith and Marion (2002), Abellán et al. (2005a, b) and Bennis et al. (2009) and introducing also hydrological and ecological parameters.
- 3) To compare historical records of “*Haenydra*” collected during the 1980s (1980–1992), with recent data collected in the same sites between 2007 and 2010 in order to estimate possible trends in response to water quality and stream structure perturbations in the last 30 years.

## Methods

### Gathering biological and distributional data

The lineage “*Haenydra*” is a monophyletic group of 92 species included in the large genus *Hydraena*, which includes more than 850 species worldwide (Trizzino et al. 2013a). The “*Haenydra*” lineage is distributed exclusively in the Western Palaearctic, from Portugal to Iran, while it’s absent in North Africa. The large majority of the “*Haenydra*” species are distributed in the southernmost portion of Western Palaearctic: Iberian Peninsula, Alps, Apennines, Balkans, Central-European mountain systems and Turkey (Jäch 2004; Ribera et al. 2011; Trizzino et al. 2011a, 2013a, b). Only two species (*H. gracilis* and *H. excisa*) were able to colonize areas north of the main range (Northern Europe, Great Britain, Scandinavia, NW Russia).

Overall, “*Haenydra*” species are small beetles (1.5–3.0 mm), mostly associated with headwater streams and springs. These insects strictly require clean and cold running waters. Furthermore, “*Haenydra*” species are frequently endemic to extremely restricted areas (Jäch 2004; Ribera et al. 2011; Trizzino et al. 2013b; Bilton 2013). This group thus fits well most of the criteria identifying optimal indicator taxa to estimate and preserve biodiversity (Pearson 1994; Pullin 2002; Linke et al. 2011), exhibiting in fact a well-known and relatively stable taxonomy and a well-studied ecology, being easily surveyed by standard sampling methods, occurring over a broad geographic range, exhibiting several entities specialized to each habitat type of running water ecosystems, and their patterns being potentially reflected in several other running water benthic invertebrates.

The knowledge about the “*Haenydra*” lineage was enforced by a series of recent contributions (Jäch 2004; Audisio et al. 2009; Ribera et al. 2011; Trizzino et al. 2011a, b, 2012, 2013a, b; Jäch and Díaz 2012; Stanković and Jäch 2012; Bilton 2013). We considered for our analyses all the 92 species described so far and produced a dataset on species distribution based on bibliographic data (Appendix S2), museum and private collections (see Appendix S4 for a list of visited museum and private institutions), and field-sampling (Appendix S1; Trizzino et al. 2013b).

For field-sampling, we used a standard technique commonly used for freshwater benthic macroinvertebrates (see Trizzino et al. 2013b). Between 2007 and 2010, we sampled 115 sites distributed in Italy (70 sites), France (7 sites), Greece (16 sites) and Turkey (22 sites) (Appendix S1). Furthermore, Ribera et al. (2011) provided further field data for some of the Iberian endemic species. All the Italian, Greek and Turkish sites sampled for the present work had been also previously investigated by PA and collaborators between 1980 and 1992, using the same methodologies (Appendix S1).

To verify if our dataset covers the whole potential distribution range of “*Haenydra*” and to exclude a possible relationship between sampling effort and number of species, we modelled the distribution of the lineage using a maximum entropy algorithm (MaxEnt) through the “dismo” package available in R (Hijmans et al. 2013). To calibrate the model we considered all presence locations and a set of three environmental variables (annual mean temperature, total precipitation, and elevation) available from the WorldClim database (<http://www.worldclim.org/current>) at a resolution of 5 arc-minutes.

Taking into account the large number of entomologists which intensively collected *Hydraena* s.l. during the last 150 years in almost all Countries from Iberian Peninsula to the Middle East, we believe that inventories of each  $50 \times 50$  km cell (see below) could be considered fairly complete. These samplings efforts covered, in fact, most European drainage systems and small rivers, maybe with the exception of some northern Countries (where only a couple of widespread species, obviously representing post-Würmian colonizers, are known to occur) and certain regions such as western Iran, SE Turkey, and Caucasian areas.

### Conservation analyses

We assigned IUCN threat categories to each “*Haenydra*” species following the IUCN Red List guidelines and specifically the “B2” criterion (Standards and Petitions Working Group 2014) which is based on the measurement of Area of Occupancy (AOO) as the main item in order to assess threat categories. We therefore measured AOO as suggested by IUCN Red List guidelines and as previously done for other beetle groups (e.g. Fattorini 2014), computing the number of  $10 \times 10$  km UTM cells occupied by a species as a measure of AOO.

In addition to IUCN categories we assigned a CS to each “*Haenydra*” species: the higher is the CS, the higher is the species conservation concern. CSs were computed on the basis of distributional markers and of some ecological traits as described in Appendix S5 and summarized in Table 1. Finally, some other ecological traits and a few specific conservation items, despite had not been utilized to compute CS, were instead used in a descriptive way, in order to better characterize each “*Haenydra*” species (Table 1, Appendix S5).

Some of the ecological traits used to compute CS, and in particular Habitat Vulnerability (HV), Habitat Rarity (HR) and species Observed Sensitivity to water quality (OS) [See Table 1 for a specific description of the parameters], were relatively problematic to be assessed without field observations and with just a few indications on the ecology of species and on species population trends. Sánchez-Fernández et al. (2008a), in the absence of an obvious quantitative way to evaluate variables like these simply basing on literature data, suggest to rely of a “panel of experts” aiming to assign scores for these kind of covariates.

We used here the same kind of “panel of experts”, involving several European Hydraenid specialists, combined, in a few cases, with a slightly different approach that we

**Table 1** Summary of categorization parameters used for assigning species' Conservation Scores (CS)

Categorization of species' distributional range, measured as Area of Occupancy (AOO)

| AOO—Area of Occupancy (km <sup>2</sup> )                                         | Assigned Score |
|----------------------------------------------------------------------------------|----------------|
| >5,000                                                                           | 0              |
| ≤5,000                                                                           | 1              |
| ≤2,000                                                                           | 2              |
| ≤500                                                                             | 3              |
| <10 or a single known locality (i.e. 100 km <sup>2</sup> with a 10 × 10 km grid) | 4              |

Categorization of species' Average Rarity (AR) in each site of recorded occurrence<sup>a</sup>

| Average rarity | Assigned score |
|----------------|----------------|
| ≤1 %           | 4              |
| >1 %, ≤ 3 %    | 3              |
| >3 %, ≤ 9 %    | 2              |
| >9 %, ≤ 27 %   | 1              |
| >27 %          | 0              |

Categorization of species' Occurrence Frequency (OF) among all sampled sites within species' distributional range<sup>b</sup>

| Occurrence frequency | Assigned score |
|----------------------|----------------|
| ≤10 %                | 4              |
| >10 %, ≤ 20 %        | 3              |
| >20 %, ≤ 40 %        | 2              |
| >40 %, ≤ 80 %        | 1              |
| >80 %                | 0              |

Categorization of habitat's rarity (HR) within species' distributional range and its area of occupancy

| Habitat rarity | Assigned score |
|----------------|----------------|
| ≤10 %          | 4              |
| >10 %, ≤ 20 %  | 3              |
| >20 %, ≤ 40 %  | 2              |
| >40 %, ≤ 80 %  | 1              |
| >80 %          | 0              |

Categorization of species' suitable habitat vulnerability

| HV—estimated species' suitable habitat vulnerability | Assigned score |
|------------------------------------------------------|----------------|
| Very high                                            | 4              |
| High                                                 | 3              |
| Middle-high                                          | 2              |
| Middle                                               | 1              |
| Relatively low                                       | 0              |

**Table 1** continuedCategorization of species' Persistence (SP) among all sampled sites within species' distributional range<sup>c</sup>

| SP—persistence                                         | Assigned score |
|--------------------------------------------------------|----------------|
| No data after 1970 in >50 % of the sampled sites       | 4              |
| No data after 1980 in >50 % of the sampled sites       | 3              |
| No data after 1990 in >50 % of the sampled sites       | 2              |
| No data after 2000 in >50 % of the sampled sites       | 1              |
| Data recorded after 2000 in >50 % of the sampled sites | 0              |

Categorization of species' average sensitivity to water quality

| OS—estimated species' sensitivity to water quality | Assigned score |
|----------------------------------------------------|----------------|
| Very high                                          | 4              |
| High                                               | 3              |
| Middle-high                                        | 2              |
| Middle                                             | 1              |
| Relatively low                                     | 0              |

<sup>a</sup> The Average Rarity was measured as the average % of sampled specimens of each species over the total number of *Hydraena* s. l. specimens locally sampled in each sampling site. This is a measure of the species "local rarity" sensu Gaston (1994)

<sup>b</sup> The Occurrence Frequency was measured as the observed % of sampled sites within a single species distributional range where occurrence the single species was currently recorded. This is a measure of the species overall "geographic rarity" sensu Gaston (1994)

<sup>c</sup> Extension of time span from the last recorded specimen collected in all sites of recorded occurrence within species' distributional range

however believe to be reliable as well. The "*Haenydra*" lineage includes in fact several groups of both allopatric and parapatric sibling species; within each of these groups, the included species are usually characterized by extremely similar ecological features: they live in the same habitat typologies, at the same altitudes, with similar vegetation coverage and with the same lithological substrates. They are characterized by similar patterns of rarity and they have comparable habitat ranges. Therefore, it could be assumed with good confidence that also parameters as HV, HR and OS could be shared among sibling species, at least in the few cases where recent information coming from local specialists or data from authors' personal samples was pending. Since, in the great majority of cases, we collected and observed on field (or we obtained from local specialists reliable information on) at least one (or most, if not all) species for each group of siblings, we believe that our approach to compute CS could be considered reliable.

Furthermore, there is a huge number of existing papers dealing with both "*Haenydra*" and *Hydraena* s.l. species in Western Palearctic (starting from 1,800 to present day; including both specialized papers dealing with *Hydraena* faunistics, taxonomy, and ecology, and more general ecological/hydrobiological papers mostly dealing with water quality assessment of single rivers or drainage systems). Especially the most recent ones provide also detailed information on species ecological features and could hence support inferences on these parameters, as well as on relationships between ecological and biogeographic signals coming from *Hydraena* s.l. and from other large, relevant, and sensitive co-occurring running water insect taxa such as Ephemeroptera and Plecoptera (Hynes 1976;

Landolt and Sartori 1997; Elliott and Humpesch 2010; Audisio, Belfiore and Fochetti, unpublished data).

### Spatially explicit analysis

Distribution data of “*Haenydra*” species were stored in a relational databank and analyzed using the Quantum GIS version 2.0 Geographic Information System (QGIS Development Team 2014). Explicit point location coordinates of each species presence site, when not already available, were obtained using the software Google Earth.

AOO computations and analyses of biodiversity hotspots were performed with the “sp” package (Pebesma and Bivand 2005; Bivand et al. 2013) of R software (R Core Team 2013). For estimating AOO, presence points have been overlapped to a  $10 \times 10$  km grid with European extent (downloaded from the EEA website; European Environmental Agency, 2003).

In order to identify biodiversity hotspots, we produced a  $50 \times 50$  km grid with European extent and we superimposed the “*Haenydra*” presence points aiming to measure the number of species within each square, producing a map evidencing therefore the European areas harbouring the highest number of “*Haenydra*” species.

## Results

### Conservation analyses

According to our analyses (Table 2), 34 “*Haenydra*” species were assigned to the IUCN category Endangered (EN), 32 species to Vulnerable (VU), 21 species to Near Threatened (NT) and four species to Least Concern (LC). Finally, *H. sappho*, which has never been collected again after its description in 1965 and considering that the only known site of presence in Lefkas island (NW Greece) has been recently destroyed, could be reasonably classified as Extinct (EX). The detailed measures of the AOO for each species are provided in the supplementary materials (Appendix S3).

Moreover, ten species, including *H. sappho*, reached a  $CS > 20$  (Table 2). All of these species have a very limited distribution range, being endemics of small areas: *H. pangaei* is known with certainty from two springs on Pangaeon Mt. (N Greece); *H. pelops* is known from a few streams in Southern Peloponnesus; *H. sanfilippo* is distributed in a few sites in NW Apennines; *H. solarii* is currently known with certainty from a few sites in Central Apennines; *H. carniolica* is known from three localities (the type locality in Slovenia and two sites in NE Italy near Treviso) where the species is exceedingly rare; four species (*H. altamirensis*, *H. madronensis*, *H. rosannae* and *H. kucinici*) are apparently endemic to their type localities in CW Spain (the first two species), SW Sardinia and Macedonia respectively.

Eight species scored between 16 and 20. More specifically, two of these species (*H. decolor* and *H. occitana*) are endemic to Maritime Alps (in W Italy and SE France); one species (*H. czernohorskyi*) is endemic to Slovenia plus a few localities in NE Italy (SE Trieste province), the latter sites being strongly altered by human impact; *H. bononiensis* is endemic to very few sites in Italian Apennines; two species (*H. crepidoptera* and *H. sinope*) are endemic to N Turkey; finally, *H. epeirosi* is endemic to SW Greece and N Peloponnesus, whereas *H. lotti* is known from a few localities in S Peloponnesus.

**Table 2** Evaluation of conservation status and risk of threat for each species

| Species ( <i>H.</i> ) | Distribution                | Distributional and ecological markers |           |           |           |           |           |           |           |           |           | CS | Other traits |           |      |    | DF | IUCN |
|-----------------------|-----------------------------|---------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----|--------------|-----------|------|----|----|------|
|                       |                             |                                       |           |           |           |           |           |           |           |           |           |    |              |           |      |    |    |      |
|                       |                             | <i>AOO</i>                            | <i>AR</i> | <i>OF</i> | <i>HR</i> | <i>HV</i> | <i>SP</i> | <i>OS</i> | <i>LS</i> | <i>AL</i> | <i>HT</i> |    | <i>VC</i>    | <i>IP</i> |      |    |    |      |
| <i>akbesiana</i>      | SE Turkey                   | 3                                     | 2         | 1         | 2         | 2         | 0         | 2         | 12        | CA        | II-III    | SR | F            | N         | C, R | EN |    |      |
| <i>alpicola</i>       | Central Europe              | 2                                     | 2         | 3         | 2         | 3         | 0         | 3         | 15        | NP        | II, III   | SR | E            | N         | C, P | VU |    |      |
| <i>altamirensis</i>   | Extremadura (SW Spain)      | 4                                     | 3         | 4         | 4         | 3         | 0         | 3         | 21        | NP        | II        | SR | F            | N         | P, C | EN |    |      |
| <i>anatolica</i>      | N and SE Turkey             | 1                                     | 1         | 1         | 1         | 1         | 0         | 1         | 6         | NP        | II, III   | SR | E            | N         | R, A | NT |    |      |
| <i>aroensis</i>       | Peloponnesus (S Greece)     | 3                                     | 1         | 2         | 2         | 2         | 1         | 3         | 14        | NP        | II, III   | SR | F            | N         | C, R | VU |    |      |
| <i>belgica</i>        | Central Europe              | 1                                     | 2         | 1         | 1         | 3         | 0         | 3         | 11        | NP        | I, II     | SR | E            | N         | R, A | NT |    |      |
| <i>bensae</i>         | Maritime Alps               | 2                                     | 3         | 2         | 2         | 2         | 1         | 3         | 15        | NP        | I-III     | SR | E            | N         | R, P | VU |    |      |
| <i>berthelemyana</i>  | Lebanon                     | 3                                     | 2         | 1         | 2         | 2         | 0         | 2         | 12        | NP        | III       | SR | E            | N         | C, R | EN |    |      |
| <i>bicuspidata</i>    | Central Massif (C-E France) | 3                                     | 2         | 1         | 2         | 1         | 0         | 2         | 11        | NP        | I, II     | SR | E            | N         | C, P | EN |    |      |
| <i>bitruncata</i>     | Spain & French Pyrenees     | 2                                     | 3         | 2         | 2         | 2         | 0         | 2         | 13        | NP        | II, III   | SR | E            | N         | C, P | VU |    |      |
| <i>bononiensis</i>    | Tosco/Emiliano Apennine     | 2                                     | 4         | 3         | 2         | 2         | 1         | 3         | 17        | Ca        | I-II      | CA | N            | N         | R, P | VU |    |      |
| <i>bosnica</i>        | Bosnia, Serbia, Montenegro  | 2                                     | 3         | 2         | 2         | 2         | 1         | 2         | 14        | Si        | II        | SR | E            | N         | R, C | VU |    |      |
| <i>carniolica</i>     | NE Italy, Slovenia          | 3                                     | 4         | 4         | 3         | 3         | 4         | 3         | 24        | Ca        | I         | SR | N            | Y         | C, D | EN |    |      |
| <i>cata</i>           | W Turkey, Lesbos Island     | 3                                     | 2         | 2         | 2         | 2         | 0         | 2         | 13        | Si        | I, II     | SR | E            | N         | C, R | EN |    |      |
| <i>catalonica</i>     | Catalonia (NE Spain)        | 2                                     | 2         | 1         | 2         | 2         | 0         | 2         | 11        | NP        | II, III   | SR | E            | N         | C, P | VU |    |      |
| <i>caucasica</i>      | NE Turkey, Georgia          | 3                                     | 2         | 1         | 2         | 2         | 1         | 2         | 13        | NP        | I-III     | SR | E            | N         | C, R | VU |    |      |
| <i>christinae</i>     | Peloponnesus (S Greece)     | 2                                     | 3         | 2         | 2         | 2         | 1         | 2         | 14        | NP        | II, III   | SR | E            | N         | C, R | VU |    |      |
| <i>crepidoptera</i>   | C Pontic Chain (N Turkey)   | 3                                     | 3         | 3         | 3         | 3         | 2         | 3         | 20        | NP        | III       | SS | N            | N         | C, R | EN |    |      |
| <i>czernohorskyi</i>  | NE Italy, Slovenia          | 2                                     | 1         | 3         | 3         | 3         | 2         | 3         | 17        | Ca        | I         | SR | N            | N         | C, P | VU |    |      |
| <i>dalmatina</i>      | N Balkans                   | 3                                     | 2         | 1         | 2         | 2         | 1         | 2         | 13        | Si        | II, III   | SR | N            | N         | C, R | VU |    |      |
| <i>decolor</i>        | Maritime Alps               | 2                                     | 4         | 3         | 2         | 2         | 2         | 4         | 19        | Ca        | I-III     | CA | N            | N         | R, P | VU |    |      |
| <i>dentipes</i>       | Central Europe              | 1                                     | 1         | 1         | 2         | 1         | 0         | 2         | 8         | Si        | I-III     | SR | E            | N         | C, P | NT |    |      |
| <i>devillei</i>       | Italian Peninsula           | 0                                     | 1         | 1         | 2         | 1         | 0         | 1         | 6         | Si        | I, II     | NP | E            | N         | R, P | NT |    |      |
| <i>devincta</i>       | NE Italy, Slovenia          | 1                                     | 1         | 1         | 2         | 1         | 0         | 1         | 7         | Si        | I         | SR | E            | N         | R, P | NT |    |      |

Table 2 continued

| Species ( <i>H.</i> )  | Distribution                | Distributional and ecological markers |           |           |           |           |           |           |           |           |           | CS | Other traits |           |      |    | DF | IUCN |
|------------------------|-----------------------------|---------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----|--------------|-----------|------|----|----|------|
|                        |                             | <i>AOO</i>                            | <i>AR</i> | <i>OF</i> | <i>HR</i> | <i>HV</i> | <i>SP</i> | <i>OS</i> | <i>LS</i> | <i>AL</i> | <i>HT</i> |    | <i>VC</i>    | <i>IP</i> |      |    |    |      |
| <i>diazi</i>           | Spanish and French Pyrenees | 2                                     | 2         | 1         | 2         | 2         | 0         | 2         | 11        | NP        | I-III     | SR | E            | N         | C, R | VU |    |      |
| <i>discreta</i>        | NW Italy                    | 1                                     | 3         | 2         | 3         | 1         | 3         | 2         | 15        | Ca        | I, II     | SR | F            | N         | C, A | NT |    |      |
| <i>elisabethae</i>     | Thassos Island (Greece)     | 3                                     | 0         | 1         | 2         | 2         | 0         | 2         | 10        | NP        | II        | SS | E            | N         | C, A | EN |    |      |
| <i>emarginata</i>      | NW Spain, French Pyrenees   | 1                                     | 2         | 1         | 2         | 2         | 0         | 2         | 10        | NP        | II, III   | SR | F            | N         | C    | NT |    |      |
| <i>epitrosi</i>        | W and S Greece              | 3                                     | 3         | 2         | 3         | 3         | 2         | 4         | 20        | Ca        | III       | CA | E            | N         | C, P | EN |    |      |
| <i>evanescens</i>      | Corsica                     | 3                                     | 1         | 1         | 2         | 2         | 0         | 2         | 11        | Si        | II        | SR | N            | N         | C, P | VU |    |      |
| <i>exasperata</i>      | Iberian Peninsula           | 0                                     | 2         | 1         | 1         | 1         | 0         | 1         | 6         | NP        | I-III     | NP | E            | N         | –    | NT |    |      |
| <i>excisa</i>          | Central E Europe            | 1                                     | 2         | 1         | 1         | 1         | 0         | 2         | 9         | NP        | I-IV      | NP | E            | N         | –    | NT |    |      |
| <i>fontiscarsavii</i>  | SE Turkey, NW Iran          | 3                                     | 1         | 1         | 2         | 2         | 0         | 3         | 12        | Ca        | IV        | CA | E            | N         | R    | EN |    |      |
| <i>fosterorum</i>      | Demanda (N Spain)           | 2                                     | 1         | 2         | 2         | 2         | 0         | 2         | 10        | NP        | III       | SR | F            | N         | C    | VU |    |      |
| <i>gaditana</i>        | SW Spain                    | 3                                     | 2         | 1         | 2         | 2         | 0         | 2         | 12        | Si        | I, II     | SR | E            | N         | C    | VU |    |      |
| <i>gracilidelphis</i>  | Cantabrian Mts. (NW Spain)  | 1                                     | 0         | 1         | 2         | 0         | 0         | 2         | 6         | Ca        | I-III     | NP | E            | N         | P, R | NT |    |      |
| <i>gracilis</i>        | Europe                      | 0                                     | 0         | 0         | 1         | 1         | 0         | 1         | 3         | NP        | I-IV      | NP | E            | N         | –    | LC |    |      |
| <i>graciloides</i>     | W Pontic Chain (N Turkey)   | 4                                     | 2         | 1         | 2         | 3         | 0         | 2         | 14        | NP        | III       | SS | F            | N         | C    | EN |    |      |
| <i>gynaephila</i>      | Lesbos Island (NE Greece)   | 4                                     | 1         | 1         | 2         | 2         | 0         | 2         | 12        | NP        | II        | SR | N            | N         | C, R | EN |    |      |
| <i>heterogyna</i>      | Italy, SE France            | 0                                     | 0         | 1         | 2         | 2         | 0         | 2         | 7         | NP        | I-III     | SP | F            | N         | C, P | LC |    |      |
| <i>hispanica</i>       | N–C Iberian Peninsula       | 1                                     | 2         | 1         | 2         | 2         | 0         | 2         | 10        | NP        | I, II     | SR | N            | N         | C, R | NT |    |      |
| <i>hosseinieorum</i>   | NW Iran                     | 3                                     | 1         | 1         | 3         | 3         | 0         | 1         | 12        | NP        | V         | PL | N            | N         | A    | EN |    |      |
| <i>hungarica</i>       | Carpathian Mts.             | 2                                     | 2         | 1         | 2         | 2         | 1         | 2         | 12        | Si        | II        | SR | E            | N         | R, C | VU |    |      |
| <i>iberica</i>         | Iberian Peninsula           | 1                                     | 0         | 1         | 2         | 1         | 0         | 1         | 6         | NP        | II, II    | NP | E            | N         | R, P | NT |    |      |
| <i>integra</i>         | N Turkey, Georgia           | 2                                     | 0         | 0         | 1         | 1         | 0         | 1         | 5         | NP        | I-III     | NP | E            | N         | R, A | VU |    |      |
| <i>jaechiana</i>       | Peloponnesus (S Greece)     | 2                                     | 1         | 1         | 2         | 2         | 0         | 2         | 10        | Ca        | I-III     | SR | E            | N         | C    | VU |    |      |
| <i>khazoriani</i>      | E Turkey, Armenia           | 3                                     | 2         | 1         | 2         | 2         | 0         | 2         | 12        | Si        | III       | SR | F            | N         | C, R | EN |    |      |
| <i>krastnodarensis</i> | NW Caucasus (SW Russia)     | 4                                     | 2         | 1         | 2         | 2         | 0         | 1         | 12        | NP        | I         | SR | N            | N         | C, R | EN |    |      |

Table 2 continued

| Species ( <i>H.</i> ) | Distribution                   | Distributional and ecological markers |    |    |    |    |    |    |     |    |         | CS | Other traits |    |      |    | DF | IUCN |
|-----------------------|--------------------------------|---------------------------------------|----|----|----|----|----|----|-----|----|---------|----|--------------|----|------|----|----|------|
|                       |                                | AOO                                   | AR | OF | HR | HV | SP | OS | LS  | AL | HT      |    | VC           | IP |      |    |    |      |
| <i>kucinici</i>       | Macedonia                      | 4                                     | 4  | 4  | 3  | 2  | 0  | 4  | 21  | Si | II      | SR | E            | N  | R, C | EN |    |      |
| <i>lapidicola</i>     | Alps, Central Europe           | 0                                     | 0  | 1  | 1  | 2  | 0  | 2  | 6   | Si | II, III | NP | E            | N  | –    | LC |    |      |
| <i>larissae</i>       | N Italy                        | 1                                     | 1  | 1  | 3  | 3  | 1  | 3  | 13  | NP | IV      | SS | F            | N  | C    | NT |    |      |
| <i>lazica</i>         | E Pontic Chain (N Turkey)      | 3                                     | 2  | 1  | 2  | 2  | 0  | 3  | 13  | NP | IV      | SR | E            | N  | C, R | VU |    |      |
| <i>leonhardi</i>      | Bulgaria, N Greece             | 3                                     | 2  | 2  | 2  | 2  | 0  | 2  | 13  | Si | II      | SR | E            | N  | R, C | VU |    |      |
| <i>lotti</i>          | S Peloponnesus (S Greece)      | 3                                     | 0  | 4  | 4  | 4  | 0  | 4  | 19  | Ca | III     | SP | F            | N  | C, D | EN |    |      |
| <i>lusitana</i>       | Portugal, NW Spain             | 2                                     | 2  | 1  | 2  | 2  | 0  | 3  | 12  | NP | II, III | NP | E            | N  | P, C | VU |    |      |
| <i>madronensis</i>    | Sierra Madrona (CW Spain)      | 4                                     | 1  | 4  | 4  | 4  | 0  | 4  | 21  | NP | II      | SR | F            | N  | P, C | EN |    |      |
| <i>magnessa</i>       | W Turkey                       | 4                                     | 2  | 2  | 2  | 2  | 0  | 2  | 14  | NP | II      | SR | N            | N  | C    | EN |    |      |
| <i>manfredjaechi</i>  | S Spain                        | 3                                     | 2  | 0  | 2  | 2  | 0  | 2  | 11  | Si | II, III | SR | E            | N  | C    | VU |    |      |
| <i>monstruosipes</i>  | NW Spain                       | 3                                     | 2  | 2  | 2  | 2  | 0  | 3  | 14  | Si | II, III | SR | E            | N  | C    | EN |    |      |
| <i>muelleri</i>       | NE Italy, Slovenia             | 1                                     | 2  | 1  | 2  | 2  | 0  | 2  | 10  | NP | I       | SP | F            | N  | C, P | NT |    |      |
| <i>nike</i>           | Samothraki Island              | 3                                     | 0  | 0  | 2  | 3  | 0  | 2  | 10  | NP | I       | SS | N            | N  | C, A | EN |    |      |
| <i>nilguenae</i>      | SE Turkey                      | 3                                     | 4  | 3  | 2  | 1  | 0  | 2  | 15  | Ca | II      | SR | N            | N  | R    | EN |    |      |
| <i>occitana</i>       | Maritime Alps, NW Apennine     | 1                                     | 3  | 3  | 3  | 3  | 2  | 3  | 20  | Ca | I-III   | CA | N            | N  | R, P | NT |    |      |
| <i>orthostia</i>      | Lebanon                        | 4                                     | 2  | 1  | 2  | 2  | 0  | 1  | 12  | NP | III, IV | SP | E            | N  | C, R | EN |    |      |
| <i>pangaei</i>        | Mt. Pangeon (NE Greece)        | 4                                     | 1  | 4  | 4  | 4  | 0  | 4  | 21  | Ca | III     | SP | F            | N  | C, D | EN |    |      |
| <i>pelops</i>         | S Peloponnesus (S Greece)      | 4                                     | 4  | 4  | 3  | 3  | 0  | 3  | 21  | NP | III, IV | SS | F            | N  | C    | EN |    |      |
| <i>phallica</i>       | SE Europe                      | 1                                     | 2  | 2  | 2  | 2  | 2  | 2  | 13  | NP | I, II   | SR | E            | N  | C, R | NT |    |      |
| <i>planata</i>        | Caucasus                       | 3                                     | 2  | 1  | 2  | 2  | NA | NA | >10 | –  | –       | –  | –            | –  | –    | EN |    |      |
| <i>plastica</i>       | W Pontic Chain (N Turkey)      | 3                                     | 2  | 2  | 2  | 3  | 0  | 3  | 15  | Si | III     | SR | F            | N  | C, R | EN |    |      |
| <i>plumipes</i>       | Tosco/Emiliano Apennine        | 2                                     | 2  | 1  | 2  | 1  | 0  | 2  | 10  | NP | I-III   | SR | F            | N  | C, P | VU |    |      |
| <i>polita</i>         | C–W Europe, N Spain            | 1                                     | 3  | 2  | 2  | 1  | 0  | 3  | 12  | Ca | II, III | SR | E            | N  | –    | NT |    |      |
| <i>producta</i>       | Central and SE France, N Spain | 2                                     | 2  | 1  | 2  | 2  | 0  | 2  | 11  | NP | II, III | SR | E            | N  | C, P | VU |    |      |

Table 2 continued

| Species ( <i>H.</i> ) | Distribution              | Distributional and ecological markers |    |    |    |    |    |    | CS  | Other traits |         |    |    | DF | IUCN |    |
|-----------------------|---------------------------|---------------------------------------|----|----|----|----|----|----|-----|--------------|---------|----|----|----|------|----|
|                       |                           | AOO                                   | AR | OF | HR | HV | SP | OS |     | LS           | AL      | HT | VC |    |      | IP |
| <i>prokini</i>        | NW Caucasus               | 3                                     | 1  | 1  | 2  | 2  | 0  | 1  | 10  | NP           | I       | SR | N  | N  | C, P | EN |
| <i>rosannae</i>       | SW Sardinia               | 4                                     | 1  | 4  | 4  | 4  | 0  | 4  | 21  | Si           | I       | SR | F  | N  | P    | EN |
| <i>saga</i>           | Central and E Europe      | 1                                     | 2  | 1  | 2  | 2  | 0  | 2  | 10  | NP           | II, III | SR | E  | N  | C    | NT |
| <i>samnitica</i>      | Abruzzo (Central Italy)   | 2                                     | 0  | 2  | 2  | 3  | 2  | 4  | 15  | NP           | II, III | SP | F  | N  | C, P | VU |
| <i>sasiflippii</i>    | Liguria, Toscana          | 3                                     | 4  | 4  | 2  | 3  | 4  | 4  | 24  | NP           | II      | SR | N  | N  | R, P | EN |
| <i>sappho</i>         | Lefkas Island (NW Greece) | 4                                     | 4  | 4  | 4  | 4  | 4  | NA | >24 | Si           | I       | SS | N  | N  | –    | EX |
| <i>schuleri</i>       | Central and E Europe      | 1                                     | 2  | 2  | 3  | 2  | 0  | 3  | 13  | Ar           | I-II    | SR | E  | N  | C    | NT |
| <i>scitula</i>        | NW Turkey                 | 3                                     | 2  | 1  | 2  | 2  | 0  | 2  | 12  | NP           | I       | NP | E  | N  | R, C | VU |
| <i>septemlacuum</i>   | W Pontic Chain (N Turkey) | 3                                     | 2  | 2  | 2  | 2  | 1  | 3  | 15  | NP           | I, II   | SR | F  | N  | R    | EN |
| <i>sinope</i>         | W–C Pontic Chain (Turkey) | 3                                     | 2  | 2  | 2  | 2  | 2  | 3  | 16  | NP           | II      | SR | F  | N  | R    | VU |
| <i>solarii</i>        | Central and S Italy       | 2                                     | 4  | 4  | 2  | 3  | 2  | 4  | 21  | Ca           | I-II    | CA | N  | N  | R, P | VU |
| <i>solodovnikovii</i> | NW Caucasus (SW Russia)   | 3                                     | 1  | 1  | 2  | 2  | 0  | 1  | 10  | NP           | I, II   | SR | F  | N  | C, R | EN |
| <i>subintegra</i>     | Balkans (to N Greece)     | 1                                     | 2  | 2  | 2  | 2  | 0  | 2  | 11  | NP           | II, III | SR | F  | N  | C, A | NT |
| <i>tarvisina</i>      | NE Italy                  | 2                                     | 2  | 2  | 3  | 2  | 0  | 2  | 13  | Ca           | I, II   | SS | N  | Y  | C, D | VU |
| <i>tatii</i>          | Sierra Nevada (S Spain)   | 4                                     | 2  | 1  | 2  | 2  | 0  | 2  | 13  | Si           | III, IV | SR | E  | N  | C    | EN |
| <i>truncata</i>       | S Europe                  | 0                                     | 0  | 0  | 1  | 0  | 0  | 0  | 1   | NP           | I-IV    | NP | E  | N  | –    | LC |
| <i>tyrrhena</i>       | Central and N Sardinia    | 2                                     | 1  | 2  | 2  | 3  | 1  | 3  | 14  | Si           | II, III | SR | E  | N  | C, P | VU |
| <i>valentini</i>      | Greece                    | 2                                     | 2  | 2  | 2  | 3  | 0  | 2  | 13  | NP           | II, III | SR | F  | N  | C, A | VU |
| <i>vedrasi</i>        | S Balkans                 | 1                                     | 1  | 0  | 1  | 1  | 0  | 1  | 5   | NP           | I-III   | LR | E  | N  | C, P | NT |
| <i>zezerensis</i>     | Central Portugal          | 3                                     | 2  | 3  | 2  | 2  | 0  | 3  | 15  | NP           | II      | SR | N  | N  | C    | EN |

The majority of “*Haenydra*” species (55/92) scored between 10 and 15: they are mostly species with narrow distribution, living in different streams/springs typologies, with variable lithological substrates at different altitudes.

Finally, 19 species get a CS < 10. They are all species widely distributed, adapted to live in several freshwater typologies, including both streams and springs as well as larger rivers, at different elevations, even in partially human-influenced environments (Trizzino et al. 2013b).

The analyses of available historical series of “*Haenydra*” records in Italy, Greece and Turkey (Appendix S1) revealed that:

- Italy: only in 25 of the 70 original sites visited in the 1980s all of the species were found again. Furthermore, 17 of the 70 sites no longer host neither water beetles nor most of the other previously recorded macro-invertebrates (i.e. Plecoptera, Ephemeroptera, Trichoptera and other Coleoptera);
- Greece: in nine of the 16 sites all of the species collected in the 1980s were found again. Four of the 16 sites no longer host neither water beetles nor most of the other previously recorded macro-invertebrates;
- Turkey: in 12 of the 22 sites all of the species collected in the 1980s were found again. Six of the 22 sites no longer host neither water beetles nor most of the other previously recorded macro-invertebrates.
- In none of the three Countries (Italy, Turkey, Greece), species appearing in the current sampling were absent in the sampling carried out in the 1980s.

### Spatially explicit analysis

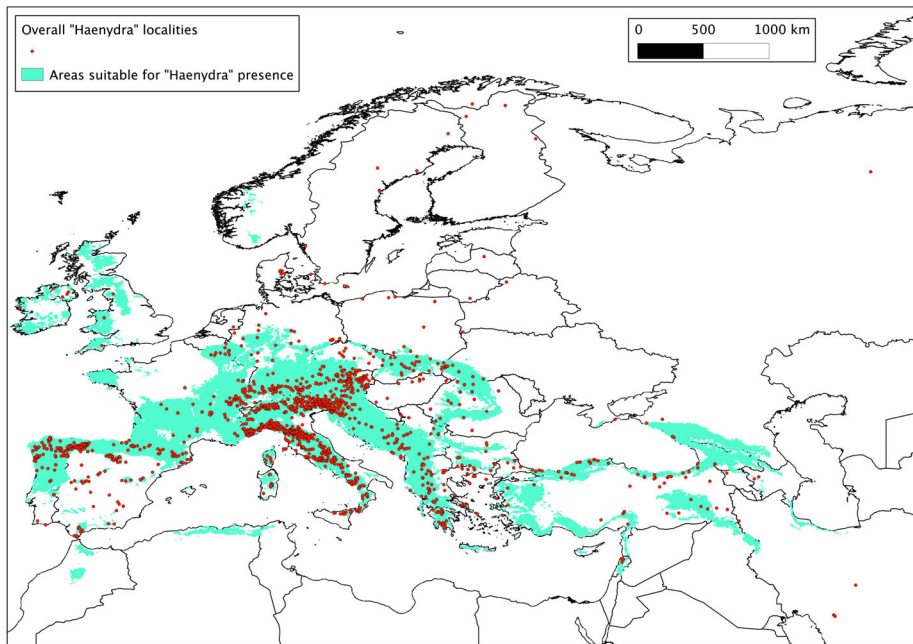
The fieldwork provided new data on the presence of 49 out of the 92 species so far described. Furthermore, Ribera et al. (2011) provided updated presence data for 15 Iberian endemic species. The remaining species distributions were determined using only literature and museum/private collections data. Overall, we produced a database including ca. 3,300 records for all the 92 species along the entire geographic range of the lineage (Fig. 1; Appendix S2; see also Trizzino et al. 2013b).

The MaxEnt analysis suggested that our dataset covers the large majority of the European areas suitable for the lineage (Fig. 1). This analysis, also considering the large amount of bibliographical data on this investigated lineage, seems to exclude a possible relationship between the sampling effort and the number of retrieved species in the different Western Palaearctic areas.

Great Britain and NW France, seemingly showing a lack of sampling (Fig. 1), were instead intensely investigated by entomologists in the last two centuries; therefore, the lack of “*Haenydra*” species in those areas (excluding the very widespread *H. gracilis*, that is the only species known for Great Britain) is likely due to biogeographic reasons. Similarly, it is worth noting that Turkey, despite the extensive sampling in the last decades and although known to harbour more than 100 *Hydraena* s.l. species (Jäch 1992), seems to effectively host a reduced number of “*Haenydra*” beetles compared to other Countries in the SW Palaearctic (e.g., Greece, Italy or Spain).

The Cantabrian Mountains, the Pyrenees, the Alps (in particular Western and Eastern slopes) and the Central Apennines were identified as the regions hosting the highest number of species per 50 × 50 km square (Fig. 2).

Figure 3 shows the geographic distribution of the “*Haenydra*” species considered endangered based on IUNC B2 criterion and having at the same time a high CS (>20).



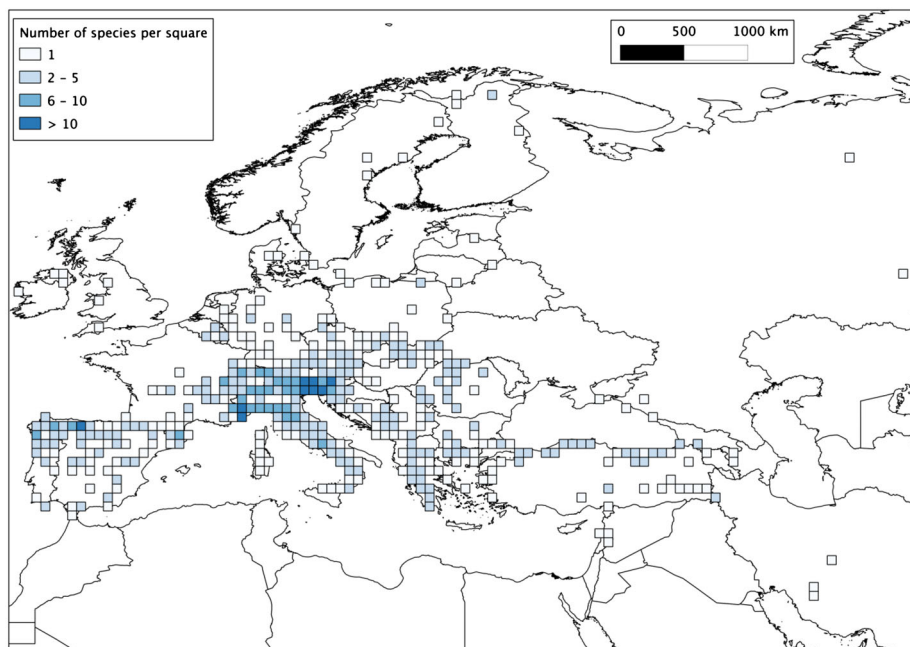
**Fig. 1** Regions suitable for the presence of “*Haenydra*” beetles; overlaid points are “*Haenydra*” species overall presence points (Datum WGS84; EPSG: 4326). MaxEnt AUC = 0.9473571

Figure 4 depicts instead species having highest CSs (moderately high if  $15 < X < 20$ , high if  $>20$ ), without considering IUCN categories: as evidenced by the two figures, Western Alps, Eastern Alps, Apennines, Central Spain, Greece (including Peloponnesus), and Northern Turkey were identified as the region with highest conservation concerns.

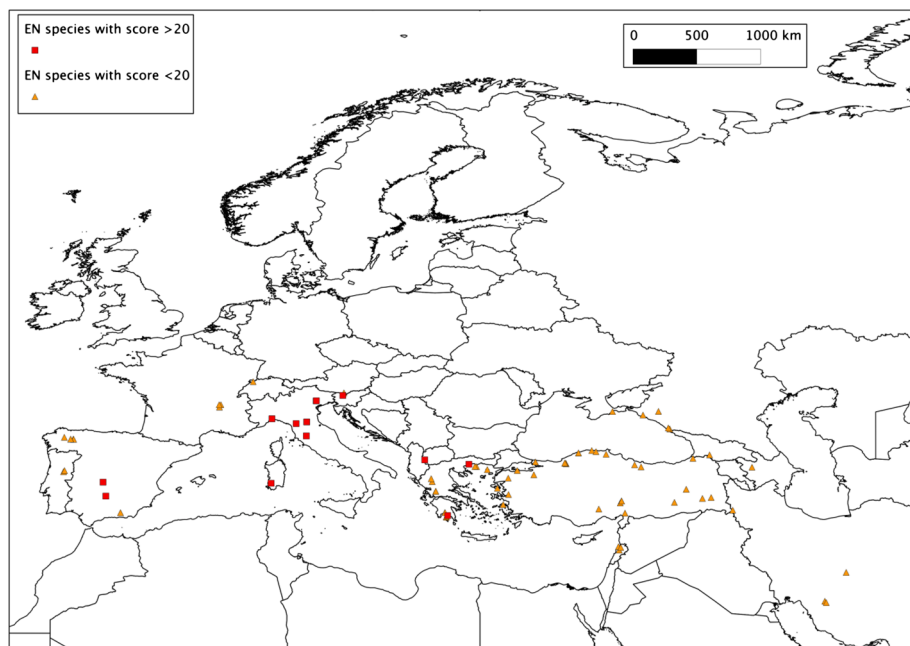
## Discussion

### “*Haenydra*” species conservation status

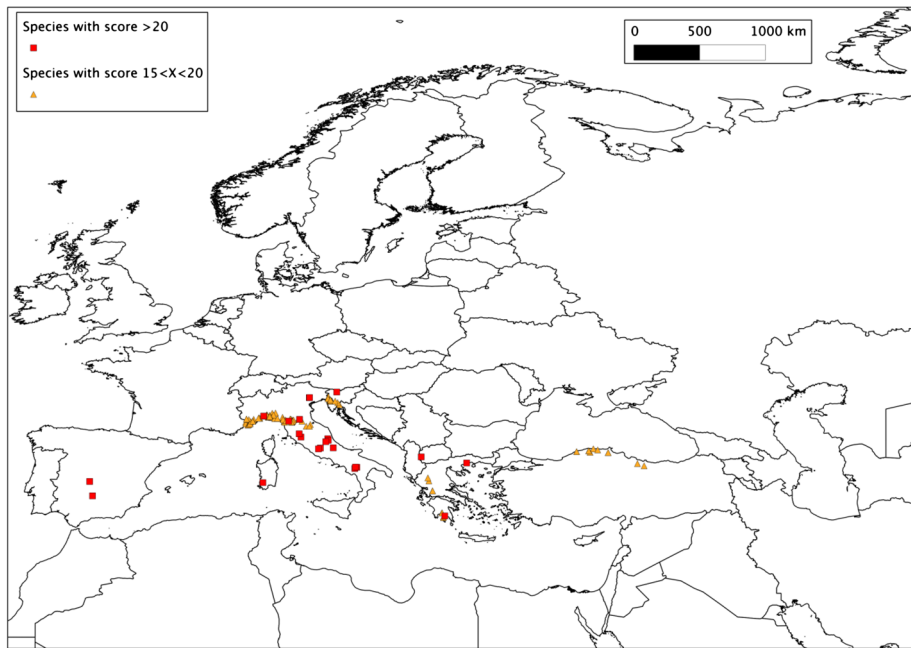
The size of geographic range is obviously the key factor for “*Haenydra*” species conservation: a narrow geographic distribution is in fact the only trait shared by most of the VU and EN “*Haenydra*” species. According to Gaston and Fuller (2009), the range size, as well as its evolution through time, is effectively one of the most important ecological and evolutionary characteristics of living species, and several studies defined the size of geographic range as a strong predictor of extinction risk (Fortin et al. 2005; Waldron et al. 2006; Gove et al. 2008; Davies et al. 2013). Considering also other ecological traits, small streams at low elevations (0–500 m a.s.l) and small mountain springs could be considered as the two most threatened habitat types for this lineage of water beetles: the former is clearly a habitat markedly altered by human impact (urbanization, pollution, infrastructures etc.), the latter is usually considered an ephemeral environment mainly because of water capture for agriculture and other human needs, especially in Southern Europe, and in the islands less rich in water. More specifically, ecological traits associated with EN species



**Fig. 2** Biodiversity hotspots map, showing the number of “*Haenydra*” species within each 50 × 50 km square (Datum WGS84; EPSG: 4326)



**Fig. 3** Geographic distribution of Endangered “*Haenydra*” species (Datum WGS84; EPSG: 4326)



**Fig. 4** Geographic distribution of Endangered “*Haenydra*” species having highest conservation scores (Datum WGS84; EPSG: 4326)

(Table 2) indicate that most of these species share a preference for calcareous substrates delimited by deep canyons where limestone is dominant and for small streams or mountain springs with different typologies of vegetation cover, and strictly require very clean and not polluted waters.

Looking to CSs, species characterized by highest scores (i.e.  $>20$ ) are all associated with small streams at lower altitudinal range (0–500 m a.s.l.) or with mountain springs ( $> 1000$  m a.s.l.).

#### Biodiversity hotspots and regions with conservation priorities

The Cantabrian Mountains, the Pyrenees, the Alps (in particular Western and Eastern slopes) and the Central Apennines were identified as the regions hosting the highest number of species per  $50 \times 50$  km square and could therefore be considered as biodiversity hotspots for this lineage of water beetles (Fig. 2). Peloponnesus represents instead a focal point for endemic species concentration (Table 2). Several authors already pointed out the importance of the Mediterranean areas as a biodiversity hotspot for water beetles (Ribera et al. 1998; Ribera 2000; Millán et al. 2002; Sánchez-Fernández et al. 2006, 2008a, b). As evidenced by Picazo et al. (2010), the main reason explaining such a high level of water beetle diversity deals with the presence of several different typologies of water bodies (fresh, saline, temporary, permanent, karstic, endorheic) in a suitable climatic condition. In the same way, the Alps are known as a hotspot for biodiversity, with a huge quantity of endemic animal and plant species, both because of the several diverse biotopes and natural landscapes they offer and, especially, due to the impact of Plio-Pleistocene

Glacial Cycles that certainly had a huge influence on biogeography and evolution of species characterised by relatively low vagility such as water beetles.

The combination of IUCN categories (Fig. 3) and CSs (Figs. 3, 4) revealed that the “*Haenydra*” species with conservation concern are mostly distributed in Western Alps, Eastern Alps, Apennines, Central Spain, Greece (including Peloponnesus), and Northern Turkey. More specifically, most of the species with highest conservation concerns (i.e. EN category plus a CS > 20) are found within the Italian Peninsula (Fig. 4) and this could be likely due to multiple reasons: as a consequence of the several natural landscapes and the diverse biotopes distributed along the whole peninsula, its natural history (Glacial Cycles, the geological formation of Alps and Apennines, the Messinian salinity crisis) and the peculiar climate, Italy harbours in its territory a huge number of endemic species characterised by more or less narrow distributions: ca. the 10–12 % of Metazoan species found in Italy are in fact endemics (Minelli et al. 1993–1995; Stoch 2000; Audisio 2013). The percentage is even larger if looking to water beetles, and in particular to “*Haenydra*” species: 21/92 species (ca. 23 %) are endemic to geographic Italy (Italy and the surrounding regions: E Provence, NW Slovenia, SW Switzerland, Corso-Sardinia; Trizzino et al. 2013b). Unfortunately, such a high rate of water beetles diversity is not correlated to an adequate management of freshwater ecosystems. As discussed above, the analysis of historical sampling-series revealed that only in the 35 % of the recently sampled Italian sites were all of the species that had been collected in the 1980s found again. Furthermore, the 25 % of the sites no longer host water beetles or most of the other previously recorded macro-invertebrates. This remarkable worsening of the biological status of running waters in Italy was likely caused by both physical (captation, draining, construction) and biological (pollution, eutrophication) degradation of most of the sampled sites. As a consequence, some particularly rare species (e.g. *H. solarii*, *H. sanfilippoi*) are now extremely threatened being up to date known with certainty only from a couple of sites each (Trizzino et al. 2013b). The strategies of the Italian Government towards conservation and biodiversity of headwater streams ecosystems have been therefore clearly unsatisfactory so far and much stronger efforts are hence needed, as evidenced also by previous works dealing with the decline of the Italian wetlands fauna (D’Amen et al. 2010). Furthermore Italy, as many other European Countries, is currently suffering from severe landscape and habitat fragmentations (Inclán et al. 2014) and this could certainly influence also freshwater ecosystems.

A different pattern was apparently observed in Spain: despite some Spanish regions representing “*Haenydra*” (and water beetles in general) biodiversity hotspots, only two “red points” (Figs. 3, 4) are found within the Iberian Peninsula, corresponding to the type localities of *H. altamirensis* and *H. madronensis* respectively. These two species are known exclusively for these sites in Central Spain and hence this region must be considered as a conservation priority: physical or biological alteration of these sites would almost certainly result in the extinction of both species. Otherwise, it is worth noting that other Iberian regions considered as “*Haenydra*” biodiversity hotspots, although known to host several narrow endemic species, do not harbour EN species (e.g. Pyrenees) or they harbour EN species but with low CS (N Spain). Therefore, although as evidenced by Abellán et al. (2013) Iberian National Parks perform poorly in the preservation of freshwater phylogenetic diversity, the strategies adopted by the Spanish Government focused on conservation and management of freshwaters, probably combined with a historical impact of human pressure lower than in Italian Peninsula, are likely going toward the right direction.

Finally, based on our observations in the field, Greece and Turkey are characterised by two relatively different situations: we found in fact that many of the Greek sites we

sampled currently share poor ecological quality of running waters: many rivers and streams were eutrophicated or broadly polluted, and springs or headwater portions of several river basins suffered also water capture. In fact, as above mentioned, analyses of historical series revealed that 25 % of the sampled sites currently no longer harbour macrobenthic invertebrates, this representing an additional proof of poor water quality. Otherwise, Turkish headwater streams ecosystems mostly suffer physical alteration, particularly due to capitation of springs and streams for agriculture needs and hydroelectric powers, or due to activities of earth-moving machinery for building of roads, tunnels, and bridges; however, when the habitat was physically preserved, then waters were generally clean. This pattern is confirmed also by the analyses of historical series: when habitat was not altered, the species found by PA and collaborators in the 1980s were found again (ca. 55 % of the sites; Appendix S2), thus indicating a notably good water quality.

#### Suitability of current IUCN category of risk for water beetles

The comparison between the IUCN categories (assigned following IUCN guidelines) and the CSs, the latter computed on the basis of descriptive parameters specific for running water organisms, highlighted the importance of taking into account the ecological, hydro-geological and historical features, as well as the local rarity, when assessing conservation strategies for running waters and their habitat restricted organisms. For example, *H. solarii* was ranked as VU based on the size of its AOO. However, the comparison between recent collecting data (2007–2010) with the previous collecting activities (1980–1992) in the same sites revealed that the species is not now found in more than half of the sites where was previously collected, since the waters are highly polluted and/or eutrophicated. This species is hence currently known with certainty only from a couple of sites in the Central Apennines and in such sites it is extremely rare: therefore, according also to the “C” criterion of IUCN guidelines (which deals with species population size and especially with evidence of declining trend) an EN status should be more appropriate. Moreover, the criteria implemented in the present work for computation of CSs evidenced that certain species relatively widely distributed but particularly rare and with apparently declining populations, should be considered more threatened than species exhibiting a much more restricted AOO, but with locally abundant populations with no indications of decline. As an example, *H. elisabethae* and *H. nike* were ranked as EN, since they are both characterised by very reduced AAO (they are basically endemics to a couple of Greek islands; Trizzino et al. 2013b). However, the sites where they are currently found are almost all in good conservation status, their populations are relatively abundant and do not exhibit particular evidences of decline: for these reasons they were both attributed a low CSs. Finally, it is worth remarking that even species being common and widespread (i.e. high values of AOO, corresponding to LC or NT IUCN categories), but showing peculiarly high degrees of sensitivity to water quality, should be considered somewhat threatened and could thus be used as markers for evaluating the conservation status of running water ecosystems.

#### Conclusions

The conservation status of the studied group of water beetles in Europe-Mediterranean areas is definitely alarming, with 73 % of the species being threatened; assuming that “*Haenydra*” could be considered as good models for the entire ecosystem, the outcome for

running water ecosystems is not going to be much better. Our analyses suggested the importance of using parameters such as geographic and local rarity, sensitivity to pollution, ecological plasticity, and population trends, besides the AOO, as criteria for assigning conservation priorities. Additionally, we believe that since running water insects are characterised by a peculiar ecology, a specific procedure for measuring AOO for running water arthropods should be set in the future, also integrating the recent observations and proposals introduced by Cardoso et al. (2011); an upcoming paper, aimed to more carefully calculate AOO by means of hydrological parameters (Gregory and Walling 1968; Tucker and Bras 1998), will try to address this specific point (Petitta et al. in preparation).

Some European regions, and in particular Italian Alps, peninsular Italy, Greece and Northern Turkey, should be designated as very high conservation priorities in order to prevent the loss of biodiversity in headwater streams ecosystems. Moreover, in the future, special attention must be addressed to Euro-Mediterranean areas characterised by highest rates of freshwater beetles species-richness and endemism (Cantabrian Mountains, Western and Eastern Alps, Central Apennines, southern Greece).

**Acknowledgments** We are indebted to Lucilla Carnevali (Rome, Italy) for preliminary georeferentiation of many “*Haenydra*” localities and Stefano De Felici (Rome, Italy) for preliminary analyses and suggestions. We thank Ignacio Ribera (Barcelona, Spain) for suggestions and for providing information about Iberian “*Haenydra*”. We are grateful to all water beetle specialists that shared ecological and distributional data on “*Haenydra*” species, and in particular M. Jäch (NMW, Wien, Austria), J.A. Delgado (Murcia, Spain), L.F. Valladares (Leon, Spain), D. Bilton (Plymouth, UK), J.A. Díaz (Santiago de Compostela, Spain), J. Garrido (Vigo, Spain), C.E. Sainz Cantero (Granada, Spain) and F. Angelini (Francavilla Fontana, Brindisi, Italy). A special thank to our friend and colleague Giorgio Ferro (Treviso, Italy), recently passed away. We thank all the curators of all the museums visited by PA and MT and all the colleagues helping us in collecting beetles (Appendix S2) and especially Alessio De Biase (Rome, Italy). Thanks to two anonymous reviewers for suggestions and criticisms to early version of the manuscript. A visit of the first author to the NMW was supported by Synthesys (Application AT-TAF-53).

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