

Biodiversity of groundwater oligochaetes from a karst unit in northern Iberian Peninsula – Ranking subterranean sites for conservation management.

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Abstract

The present study suggests that the Santa Eufemia-Ereñozar karst unit in northern Iberian Peninsula is a biodiversity hotspot for groundwater oligochaetes, due to (1) the presence of a high number of stygobiotic species (corresponding to 18% of the total stygobionts known in southern Europe); (2) the comparatively high number of oligochaete species collected (corresponding to 35% of the total epigeal and hypogean oligochaete species in the region); and (3) the presence of 5 species endemic to the region. A list of the oligochaete taxa found in the karst unit is presented and a conservation ranking of the cavities in the karst is proposed, based on the application of four biodiversity indices (Species richness, Rarity, Vulnerability and Complementarity) to the oligochaete taxa. Vulnerability was evaluated for the first time for groundwater oligochaete taxa and it provided a useful tool to assess the protection status of oligochaetes in karstic systems. Groundwater conservation management strategies could incorporate biodiversity data from the present study.

Introduction

Over the past years, the number of studies on groundwater oligochaetes has increased, but the knowledge about this fauna is still very incomplete if compared with its epigean counterparts. Cernosvitov was the first to create a catalogue of groundwater oligochaetes in 1939, where he mentioned 19 stygobiotic species worldwide. Juget and Dumnicka completed the catalogue and reached up to 65 species in 1986. More recently, Giani et al. (2001) reported up to 96 stygobiotic species worldwide, among which about 58 were present in southern Europe. In the Iberian Peninsula, Hrabe (1963), Delay (1973), Giani & Martinez-Ansemil (1981), Rodriguez & Giani (1987, 1989) and Giani & Rodriguez (1988, 1994) described new species of oligochaetes inhabiting groundwaters, either associated with the hyporheos or with caves and springs. Some of them (*Rhyacodrilus gernikensis* Giani & Rodriguez, 1988, *Rhyacodrilus okamikae* Giani & Rodriguez, 1988 and *Aktedrilus argatxae* Giani & Rodriguez, 1988) are endemic to the Santa Eufemia-Ereñozar karst area.

In the present work, we used the term *stygobiont* for groundwater-limited species; *stygophile* for those usually found in subterranean waters, but able to complete their life cycle either in epigean and or subterranean waters; and *stygoxene* for circumstantial inhabitants of subterranean habitats that are more common in epigean water bodies (Giani et al., 2001; Gibert & Deharveng, 2002). The biodiversity of groundwaters is threatened, and stygobionts in particular are considered to be vulnerable to environmental oscillations due to their high specialisation for the subterranean medium (Bellés, 1987; Sket, 1999a, b). Studies measuring and mapping subterranean fauna over the world have revealed an unexpectedly high biodiversity, and have identified several *hotspots*, *i.e.* areas particularly rich in species, with rare or threatened species, or with some combination of these attributes (Reid, 1998), (*e.g.* Culver & Sket, 2000; Danielopol et al., 2002; Castellarini et al., 2007; Ferreira et al., 2007). Recently, several researchers in southern Europe have joined efforts to study the groundwater invertebrate fauna (PASCALIS, 2002). Despite the inclusion of caves into the European Directive for protection of Habitats (EEC, 1982), there is a general lack of protection measures for the subterranean biodiversity in Spain. Conservation-focused studies concerning biodiversity of subterranean invertebrates are lacking, even though the Cantabric area is situated in a region of high biodiversity, hypothesized by Culver et al., (2006) for

terrestrial cave fauna along the latitudinal band between *ca.* 42° and 46° N in Europe. The region also holds promise of harbouring a high biodiversity in groundwaters. Finally, it is important to note that invertebrates are the main components of biodiversity in subterranean habitats (Juberthie, 2005) and thus, they cannot be ignored in biodiversity assessments. The objectives of the present work were (1) to examine the aquatic oligochaete fauna of the Santa Eufemia-Ereñozar karst unit, looking for new records and new taxa of oligochaete worms; (2) to compare the resulting biodiversity of oligochaetes in groundwaters with that in epigeal waters and also with other karst areas, assessing the potential of the karst unit as a hotspot for groundwater oligochaetes; and (3) to generate a conservation-focused ranking of springs and cave streams in the study karst based on the application of several biodiversity indices to the aquatic oligochaete taxa list.

Study area

The study area is the karst unit of Santa Eufemia-Ereñozar (Fig. 1), which is located in the north of Spain (Biscay Province). The karst occupies a region that comprises 22 municipalities with about 25.000 inhabitants (<http://www.eustat.es>). The karst is mainly composed by Urgonian limestone (Early Cretaceous) and its proximity to the sea shore results in a tidal influence on some of the study sites (sites 4 and 5). Karstification in the area has resulted in complex drainage systems (ADES, 2007). About one third of the karst area, and most of the study sites, are within the Urdaibai Biosphere Reserve (UBR), which has been protected since 1989 (BOPV, 1989) and is included into the NATURA 2000 network of European protected natural areas (<http://www.ingurumena.ejgv.euskadi.net>).

Materials and methods

Sampling and collections

Groundwater oligochaetes and other benthic invertebrates were collected with a Surber sampler (30 x 30 cm²), having a net of 200 µm mesh size, at springs and endogenous cave streams (never resurgences) during 2005-2006: sites 1, 2, 3, 5, 6, 8 and 9 on Figure 1, most of which were sampled at least twice. Habitats sampled were shallow lotic and

lentic waters, and sediment showed an average composition of 80±10% coarse sand, 11±5% medium sand, 5±4% fine sand and 3±3% silt and clay. The material was fixed in the field with 10% formaldehyde and washed through a 100 µm sieve before sorting under a stereomicroscope at the laboratory. Then, specimens were preserved in 70% ethanol and oligochaetes were determined using a Nomarski interference contrast microscope. Worms were whole mounted in glycerol and, when necessary, they were stained with Ehrlich's hematoxylin and dissected or whole mounted in Canada balsam after dehydration and clearing in creosote. Specimens of other invertebrate groups were identified at higher taxonomic levels, and the oligochaete abundance was expressed as percentage of the total individuals at the site. Data from another collection of aquatic oligochaetes from the same karst unit (sites 1, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14 and 15 on Figure 1), studied by Narcisse Giani and Pilar Rodriguez between 1983 and 1986, were also included in the present analysis. The sampling method in this previous survey was qualitative (hand net) and the most relevant findings were published in several contributions (Rodriguez & Giani, 1987; Giani & Rodriguez, 1988; Giani et al., 2001).

Biodiversity indices

We calculated four biodiversity indices: Species Richness, Rarity, Vulnerability and Complementarity. Species richness (S) was calculated by counting the total number of species determined at each site for all sampling occasions. Immature individuals were frequent, but usually were difficult to identify to species level; thus, for each site they were ascribed to the species identified at that site which was most similar in terms of somatic morphological characters (mainly based on chaetal morphology). When the somatic characters did not match any of the identified species, the immature worm was counted as a different species.

Based on the local-scale geographic range of the species, we calculated a Rarity index

(R) for each site as $R = \sum_{i=1}^S (1/n_i)$, where n_i is the number of sites where each species (i)

was found (Kerr, 1997). A corrected Rarity index (R/S') (Crisp, 2001) was also calculated by dividing R by the number of species which were used in the calculation of R for each site (S'). S' was used instead of S because some immature specimens could not be determined to species at each site.

We calculated a Vulnerability index (V) for each site, applying a new function used to identify areas of high biodiversity (Benayas & de la Montaña, 2003; Benayas et al., 2006; Abellán et al., 2005a): $V = \sum_{i=1}^S V_i$, where V_i is the vulnerability score for each species. A corrected Vulnerability index (V/S^2) was also calculated. We determined V_i as the arithmetic sum of the scores for 6 criteria that we used to determine the vulnerability of groundwater oligochaete species: global-scale geographic distribution, local-scale geographic distribution, endemism, habitat specificity, local population size, and risk of habitat loss. The selection of criteria was based on those proposed by Rabinowitz et al., (1986) and by Abellán et al., (2005b). The scores applied to each criterion ranged from 0 to 3 and are explained below:

1.- *Global-scale geographic distribution* (GD): taking into account that biogeographical regions for groundwater fauna are not defined yet, we made an approach based on the freshwater faunal regions of Banarescu (Fig.9/1: 1992). Thus, we defined 4 global areas, which may not match those for the underground fauna due to marine transgression/regressions could have played an important role in shaping the extant distribution of this fauna, mainly for those taxa of probable marine origin, such as phalloporins. The highest score (3) was given to those species restricted to the Euro-Mediterranean subregion (Id); a score of 2 was assigned to those also inhabiting subregions Ie, Ig and/or Ih; species occurring in both the Holarctic region (I) and Sino-Indian region (II) were scored as 1; and if the species distribution also included at least one of the regions III, IV, V, VI, VII or VIII, it was scored as 0. Species distributions were based on the literature.

2.- *Local-scale geographic distribution* (LD): we gave the highest score (3) to those species that inhabited only one site in the study area; a score of 2 was assigned if it inhabited two sites; a score of 1 if inhabited three sites; and 0 if inhabited more than three sites.

3.- *Endemism* (E): we scored only the species endemic to the Euro-Mediterranean area. The following scores were used: Biscay Province (3), northern Spain and southern France (2); widespread in south-western Europe, as defined in Giani et al., (2001) (1); and widespread in the Euro-Mediterranean area (0).

4.- *Habitat specificity* (H): we scored species following the classification by Juget & Dumnicka (1986), Giani et al., (2001) and our own experience. The highest score (3)

was given to stygobionts; a score of 2 was given to stygophiles; rare species that have been found in both subterranean and epigeal habitats, but still of uncertain ecological preferences, were scored as 1; and stygoxene species as 0.

5.- *Local population size* (P): the highest score (3) was given to the smallest local populations, which had less than 2 individuals per sample (*i.e.* about 20 individuals per m²) in $\geq 70\%$ of the cases and never more than 9 individuals per sample (*i.e.* less than 100 individuals per m²). A score of 2 was assigned if less than 2 individuals per sample were collected in 50-69% of the cases; a score of 1, if it was in 30-49% of the cases; and if $< 30\%$, they were scored as 0.

6.- *Risk of Habitat Loss* (RL): two steps were necessary to calculate this index. First, a site risk score was calculated based on three criteria: human disturbances (tourism in caves or use of springs for water supply, based on our own field data), evidence of agricultural pollution (data from EVE, 1996) and proximity to towns or any important infrastructure (less than 0.5 km). The maximum score (3) was given to a site if all criteria were fulfilled; if only 2 criteria, a score of 2; and so on. For each species, the mean of the site RL scores where the species was found was calculated. Then, a species RL score of 0 was given to a mean value of 0; a score of 1 to 0.1- 1; a score of 2 to 1.1 - 2; and a score of 3 to 2.1 -3.

A simple complementarity method based on the species richness was applied to the entire study area as follows: first, the site with the highest species richness was selected; then, the site with the highest number of species not represented in the previously selected site was chosen; and so on, until all species were represented at least once. If there were ties (sites with equal scores), we selected sites among ties with the greatest R value. Finally, a Complementarity index (C) was obtained for each site as the percentage of newly incorporated species (n) of the total observed species richness for the study area (S_{obs}) ($C = n * 100 / S_{obs}$) (Baquero, 2001).

Correlation analyses were done to search relationships between site biodiversity indices and between species vulnerability criteria using SPSS 14.0 statistical package (SPSS, 2005).

Species rarefaction curve and richness estimators

A sample-based species rarefaction curve with 95% confidence intervals was generated for the karst unit using the Mao Tau function by Colwell et al. (2004) in the software package EstimateS (Colwell, 2006). Rarefaction curves represent the statistically expected accumulation curves, calculated by re-sampling repeatedly at random (Gotelli & Colwell, 2001). We also estimated the total expected number of species using diverse estimators: Chao's function, first-order jack-knife by Burnham & Overton (1978), second-order jack-knife and bootstrap by Smith & van Belle (1984) (all referenced in Gotelli & Colwell, 2001). The functions are presented below:

$$\text{Chao's function (S}_{\text{Chao2}}\text{): } S_{\text{Chao2}} = S_{\text{obs}} + \left(\frac{m-1}{m} \right) \left(\frac{Q_1(Q_1-1)}{2(Q_2+1)} \right)$$

$$\text{First-order jack-knife estimator of species richness (S}_{\text{jack1}}\text{): } S_{\text{jack1}} = S_{\text{obs}} + Q_1 \left(\frac{m-1}{m} \right)$$

Second-order jack-knife estimator of species richness (S_{jack2}):

$$S_{\text{Jack2}} = S_{\text{obs}} + \left[\frac{Q_1(2m-3)}{m} - \frac{Q_2(m-2)^2}{m(m-1)} \right]$$

$$\text{Bootstrap estimator of species richness (S}_{\text{boot}}\text{): } S_{\text{boot}} = S_{\text{obs}} + \sum_{k=1}^{S_{\text{obs}}} (1-p_k)^m$$

where S_{obs} is the total observed number of species, m is the total number of samples, Q_i is the number of species that occur in exactly i samples (Q₁ is the frequency of uniques and Q₂ the frequency of duplicates), and p_k is the proportion of samples that contain the species k.

Results

List of taxa and relative abundances

A total of 47 taxa of aquatic oligochaetes were determined from the Santa Eufemia-Ereñozar karst unit, of which 37 were identified to species level (Table 1). Taxa named as "sp" were clearly separated from others included in the table for the purpose of calculation of the indices. Twenty six genera and five families (Tubificidae, Enchytraeidae, Lumbriculidae, Haplotaxidae and Lumbricidae) were represented. Immature individuals were frequent in the collections and only naidines and a few

tubificines (*Tubifex ignotus*, *Psammoryctides barbatus*, *Limnodrilus udekemianus* or *Spirosperma velutinus*) could be identified to species level when immature. Thus, many immature tubificids in this study could be identified only to subfamily level, such as Rhyacodrilinae, Tubificinae, or Phallo-drilinae; or family level, such as Haplotaxidae, Lumbriculidae or Lumbricidae. In the case of enchytraeids, most immatures could be identified to genus level. Tubificids were dominant mainly in the springs, where they could reach the 100% of the total oligochaetes (Fig. 2). They were also the most diverse group, with 25 species. Four subfamilies of tubificids were represented in the study area, and among them, tubificines were the most widely distributed; rhyacodrilines were most abundant in caves; naidines were abundant in springs when present; and phallo-drilines were scarce and were found only at 2 sites (note that most of these records were site 5, a spring which is occasionally flooded by brackish water for about 1 to 2 hours only a few days per year, when a low flow is combined with the maximum tide height of the year –own observations-). Both lumbriculids and enchytraeids were more abundant in caves than in springs. Finally, it is interesting to note that we did not find any haplotaxids in the 2005-06 survey, whereas they were present in the previous survey of 1985-86.

Oligochaetes represented the 3.48 to 66.67% of the total invertebrate fauna in the groundwater community in the 2005-2006 survey, with densities between 33 and 164 individuals/m² in the caves and between 82 to 2732 individuals/m² in the springs and at the entrance of caves. Crustaceans were dominant in relative abundance among the invertebrates in most of the sampling occasions (> 39%). Other components of the community were nematodes, planarids, aelosomatids, hirudineans, gastropods, bivalves, insect larvae, and Hydracarina.

Biodiversity indices

The 15 sites were scored according to the species Richness (S), the corrected Rarity index (R/S') and the corrected Vulnerability index (V/S') (Table 2). The latter was calculated in two ways: considering all the taxa and considering only the taxa identified to species level; results were very similar, resulting in a maximum difference of 3.08%. S values ranged from 2 species (site 10) to 19 species (site 5); R/S' ranged from 0.16 (site 4) to 0.65 (site 3); and V/S' ranged from 2.33 (site 4) to 9.00 (site 10). The results

of the Complementarity index showed that 8 of the 15 study sites would comprise the total oligochaete species richness known up to date in the karst unit.

The correlation coefficients between the different indices were significant in 8 cases (Table 3). As expected, S showed a significant positive correlation with both R ($r=0.955$) and V ($r=0.956$). For this reason, the correction of the indices R and V by S' seems reasonable for a comparative evaluation of richness and rarity between different sites. R/S' and V/S' were not correlated with S, therefore the proportion of rare and vulnerable species is not necessarily associated with the richest sites.

Oligochaete species were evaluated according to their rarity ($1/n_i$) and vulnerability scores (V_i) (Table 1). We considered 5 species to be rare among the stygobionts (*Trichodrilus tenuis*, *T. strandi*, *Aktedrilus argatxae*, *Gianius aquaedulcis* and *Rhyacodrilus gernikensis*) following the criterion proposed by Gaston (1994), who classified a species to be rare if it had a value of $n_i < 25\%$ of the maximum n_i obtained for a species. Regarding local-scale distribution, 16 species inhabited one site exclusively. In contrast, *Pristina jenkinsae*, *Spirosperma velutinus* and *Pristina aequisetata* inhabited 8, 7 and 6 sites, respectively, of the 15 study sites. Twenty taxa had a worldwide geographic distribution, while 13 were restricted to the Euro-Mediterranean region, and 5 were endemic to the Biscay Province. Eleven species were classified as stygobionts and only one as stygophile. The species *Marionina sp.* and *Lamadrilus sp.* are probably new to Science and will be described elsewhere; therefore, they were scored as stygobionts and endemic species. Eighteen species had a small population size in the karst unit; however, the abundance of *Pristina jenkinsae* (up to 2033 individuals/m²), *Tubifex ignotus* (up to 1519 individuals/m²) and *Nais communis* (557 individuals/m²) was very high. *P. jenkinsae* inhabited, in general, sites with high risk of habitat loss. The maximum possible vulnerability score (V_i) for a species in the present study was 18 (6 criteria multiplied by a score of 3), but the maximum score obtained was 15 and the minimum was 2. One enchytraeid (*Marionina sp.*), 3 lumbriculids (*Trichodrilus diversisetosus*, *T. tenuis* and *T. strandi*) and 4 tubificids (*Aktedrilus argatxae*, *Troglo-drilus galarzai*, *Rhyacodrilus okamikae* and *R. gernikensis*) were the most vulnerable species.

The correlation coefficients between the vulnerability criteria plus the rarity values for the species were significant in 13 cases (Table 4). It is interesting to note that for the study area, the rarest species had the smallest populations but do not inhabit the sites with the highest risk of habitat loss. High positive correlation of Habitat specificity (H) with Endemicity (E) implies that most endemics are associated exclusively with groundwaters (*i.e.* they are stygobionts).

Species accumulation curve and richness estimators

The species rarefaction curve of the karst unit (Fig. 3) did not reach an asymptote, but it seemed to start levelling out. After 7 sampled sites, the estimated number of species reached 70% of the maximum value obtained with 15 sites and after 11 samples, 90%. The curve rose slowly due to the high number of species that occurred exclusively at one site (16 species). This fact suggested that there was still a number of species to be discovered in the karst. The application of four different functions estimated the species richness in the study karst to be 7 to 16 species higher than the observed value (47 species) (Table 5).

Discussion

The species identified in the karst unit of Santa Eufemia-Ereñozar (78 km² of limestone in a karst unit of approximately 760 km²) represent a comparatively high richness for subterranean aquatic oligochaetes, since they constitute about the 35% of the total number of the oligochaete species known in both epigean and hypogean waters of the Basque Country and neighbouring areas (*ca.* 15.000 km²) (Rodriguez, 1986a, b; Rodriguez & Armas, 1983; Rodriguez & Giani, 1984, 1986). Nevertheless, the stygobiotic species richness of the karst unit only represents about 10% of the total aquatic oligochaete fauna (epigean + hypogean). We calculated a similar proportion at the scale of the Iberian Peninsula, where only the 12% of the total 166 aquatic oligochaete species known are stygobionts (own data). At the scale of southern Europe, the proportion of stygobiotic species increases to about 23% (calculated on data from Giani et al., 2001 and own data). Sket (1999a, b) proposed the lower food resources, the reduced number of habitats (due to lack of green plants, no temperature fluctuations and permanent darkness) and the limited accessibility to hypogean habitats for epigean

populations as limitations for the richness of European stygobiotic fauna compared with surface aquatic fauna. Some of these factors are probably limitations for aquatic oligochaete species too; on the other hand, limited food resources could explain low densities but not necessarily low diversity. The results of the present study suggest that the actual richness of subterranean oligochaetes in the region is likely to be higher than that of the samples (54 to 63 species are expected to be found only in the karst area studied). Taking into account that subterranean habitats in the area are less prospected than epigeal habitats (79 species have been reported in running waters of the Basque Country and neighbouring areas), we could expect a higher groundwater oligochaete biodiversity, comparable to the epigeal one.

The species richness and the number of stygobiotic species recorded in the karst of Santa Eufemia-Ereñozar is relatively high compared with other karst areas of northern Iberian Peninsula. For example, a study of the epikarst fauna of Ojo Guareña Cave (over 40 km length) resulted in 15 oligochaete species, only two of them being stygobionts (Camacho et al., 2006). Another example from southern Europe is a study of the aquatic oligochaete fauna in wells of diverse regions of Italy and Greece, which resulted in 5 to 21 taxa and 1 to 4 stygobiotic species (Dumnicka, 1990). The estimated aquatic oligochaete species richness for the present study area is considerably higher (54 to 63 species). In addition, a high number of stygobionts was collected (11), which represents 86 % of the total stygobiotic species known in the Iberian Peninsula and 19% of those in southern Europe; 5 of these species are endemic to the Iberian Peninsula. Therefore, at least for the oligochaete fauna, the karst unit seems to be a hotspot of biodiversity. Culver & Sket (2000) used an arbitrary cutoff of 20 or more obligate subterranean species for a site to be a subterranean hotspot, and the caves and karst wells selected by these authors from around the world had between 0 and 7 stygobiotic aquatic oligochaetes. The Santa Eufemia-Ereñozar karst is also inhabited by other interesting stygobiotic taxa, e.g. the endemic crustacean *Pseudoniphargus guernicae* Notenboom, 1986. However, the general lack of knowledge of most invertebrate taxa does not allow us to evaluate the total biodiversity in the area, or to classify the karst unit as a hotspot of biodiversity for subterranean fauna (aquatic and terrestrial) in general. Nevertheless, future research would probably increase species richness of oligochaetes and other subterranean taxa in the karst area.

The high number of stygobionts in some areas can be a consequence of the geographic location and the geological past (Sket, 1999b), the number of caves (as a measure of karstification; see Culver et al., 2003) and the sampling intensity (Culver et al., 2004). We sampled only 15 of the about 230 cavities known in the UBR area (ADES, 2007); and samplings were made on relatively few occasions, thus this cannot be considered to be an intensive sampling. Although not all these cavities contain aquatic habitats, the subterranean aquatic system in the study karst has developed over the last one million years (Aranburu, pers. com.), which is a reasonable time frame for evolution of stygobiotic fauna. The occurrence of some Tubificidae with marine phyletic affinities (e.g. *Aktedrilus argatxae* and *Gianius aquaedulcis*) suggests that a geological past related to a marine environment could also have influenced the evolution of some stygobiotic oligochaete taxa. We hope that future molecular studies provide insight into this interesting question.

Considering the karst area as a hotspot of biodiversity for oligochaete fauna, conservation of the subterranean aquatic medium is important. Culver and Sket (2000) suggested that protection and concern for the subterranean fauna of a region often begin with interest in a particular cave. Thus, we propose a conservation-focused ranking of the cavities based on the results of the Complementarity index: Sites 5 > 13 > 9 > 2 > 3 > 6 > 8 > 7 (from highest to lowest conservation value). We selected the Complementarity index for the ranking because conservation of the proposed 8 sites would protect all species known in the karst unit so far. The Complementarity index showed a significant correlation with the Rarity index, thus the sites with the highest value of rarity (sites 3, 2 and 9) were also included in this ranking. However, the sites with the highest value of vulnerability (1 and 10) were excluded; they harbour very few species, and their high vulnerability could be due to their proximity to intensive human activity. Appropriate management to improve the habitat quality in sites 1 (within the UBR) and 10 would be desirable.

Biodiversity indices are useful tools for ranking sites, but we observed some limitations in the present study of the aquatic oligochaete fauna. First, the fact that most of the specimens were immature (e.g. 76% of the total number of individuals in the 2005-2006 survey) was a handicap for the taxonomic study. This frequently occurs in studies of groundwater oligochaetes (Belles, 1987). The difference between the observed and

estimated number of species emphasizes the need for more surveys to collect all of the species in a cavity, or for sampling all cavities (Schneider & Culver, 2004), some of which are difficult to access. Neither haplotaxids nor the endemic species *Rhyacodrilus gernikensis* nor *Troglodrilus galarzai* were found in the last survey (2005-2006) at the sites where they were initially collected (type localities for the two latter species). This fact is worrying since it could indicate the possibility of local extinctions. Elliot (2005) discussed the possibility of disappearance of cave-limited species endemic to a single cave, due to the disturbance or pollution of the cave. A criterion for species persistence should be added if future surveys are made in the karst, to better evaluate vulnerability. Finally, analysis of the biodiversity of groundwater oligochaetes in other karst units in the Cantabric area would be an interesting subject for future research that would allow for a better comparison of the uniqueness and importance of the karst of Santa Eufemia – Ereñozar.

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LUMBRICULIDAE

| | | | | | | | | | |
|------------------------------------------------------------|-----------------------------------------------------|-------|----|---|---|---|----------------|---|---|
| <i>Stylodrilus heringianus</i> Claparède, 1862 | 13C | 1.00 | 4 | 1 | 3 | 0 | 0 | 0 | 0 |
| <i>Stylodrilus lemani</i> (Grube, 1879) | 5S, 7S, 9C, 13C | 0.25 | 3 | 2 | 0 | 0 | 0 | 0 | 1 |
| <i>Stylodrilus parvus</i> (Hrabe and Cernovitov, 1927) | 5S, 9C, 11C | 0.33 | 4 | 2 | 1 | 0 | 0 | 0 | 1 |
| <i>Trichodrilus campoyi</i> Rodriguez, 1988 | 9C, 9S, 10C, 11C, 15S | 0.20 | 9 | 3 | 0 | 2 | 3 | 0 | 1 |
| <i>Trichodrilus diversisetosus</i> Rodriguez & Giani, 1986 | 9C, 1C | 0.50 | 14 | 3 | 2 | 2 | 3 | 2 | 2 |
| <i>Trichodrilus tenuis</i> Hrabe, 1960 | 9C | 1.00* | 12 | 3 | 3 | 0 | 3 ^S | 3 | 0 |
| <i>Trichodrilus strandi</i> Hrabe, 1936 | 2C | 1.00* | 13 | 3 | 3 | 1 | 3 ^S | 3 | 0 |
| <i>Trichodrilus sp.</i> (moravicus group) | 5S, 15S | 0.50 | 7 | 1 | 2 | 0 | 1 | 2 | 1 |
| <i>Lumbriculidae</i> imm. | 1C, 2C, 5S, 6S, 7S, 8S, 9C, 10C, 11C, 13C, 14C, 15S | - | - | - | - | - | - | - | - |

TUBIFICIDAE**Phallodrilinae**

| | | | | | | | | | |
|------------------------------------------------------|----|-------|----|---|---|---|----------------|---|---|
| <i>Aktedrilus argatxae</i> Giani and Rodriguez, 1988 | 5S | 1.00* | 13 | 3 | 3 | 3 | 3 ^S | 0 | 1 |
| <i>Gianius aquaedulcis</i> (Hrabe, 1960) | 2C | 1.00* | 10 | 1 | 3 | 0 | 3 ^S | 3 | 0 |
| <i>Phallodrilinae</i> indet. | 5S | 1.00 | 7 | 0 | 3 | 0 | 0 | 3 | 1 |
| <i>Phallodrilinae</i> imm. | 5S | - | - | - | - | - | - | - | - |

Tubificinae

| | | | | | | | | | |
|-------------------------------------------------|-----------------|------|----|---|---|---|----------------|---|---|
| <i>Lamadrilus sp.</i> | 2S, 5S, 7S, 12S | 0.25 | 11 | 3 | 0 | 3 | 3 ^S | 0 | 2 |
| <i>Limnodrilus hoffmeisteri</i> Claparède, 1862 | 12S | 0.50 | 7 | 0 | 2 | 0 | 0 | 3 | 2 |
| <i>Limnodrilus udekemianus</i> Claparède, 1862 | 2S, 5S, 12S | 0.33 | 3 | 0 | 1 | 0 | 0 | 0 | 2 |
| <i>Limnodrilus</i> imm. | 13C | - | - | - | - | - | - | - | - |

| | | | | | | | | | |
|------------------------------------------------------------|-------------------------------|-------|----|---|---|---|----------------|---|---|
| <i>Potamothenis bavaricus</i> (Oschmann, 1913) | 3S | 1.00 | 6 | 0 | 3 | 0 | 0 | 3 | 0 |
| <i>Psammoryctides barbatus</i> (Grube, 1861) | 9C, 11C | 0.50 | 7 | 1 | 2 | 0 | 0 | 3 | 1 |
| <i>Spirosperma velutinus</i> (Grube, 1879) | 5S, 7S, 8S, 9C, 12S, 13C, 15S | 0.14 | 3 | 1 | 0 | 0 | 0 | 0 | 2 |
| <i>Troglodrilus galarzai</i> (Giani and Rodriguez, 1988) | 5S, 11C | 0.50 | 14 | 3 | 2 | 2 | 3 ^S | 2 | 2 |
| <i>Tubifex ignotus</i> (Stolc, 1886) | 2S, 2C, 5S, 11C | 0.33 | 2 | 0 | 1 | 0 | 0 | 0 | 1 |
| <i>Tubifex tubifex</i> (Müller, 1774) | 12S, 13C | 0.50 | 4 | 0 | 2 | 0 | 0 | 0 | 2 |
| <i>Varichaetadrilus bizkaiensis</i> Rodriguez & Giani 1984 | 3S, 13C | 0.50 | 9 | 3 | 2 | 3 | 1 | 0 | 0 |
| <i>Tubificinae</i> imm. | 2S, 3S, 5S, 6S, 9C | - | - | - | - | - | - | - | - |
| <u>Rhyacodrilinae</u> | | | | | | | | | |
| <i>Peristodrilus montanus</i> (Hrabe, 1962) | 2S, 9C | 0.50 | 7 | 3 | 2 | 1 | 1 | 0 | 0 |
| <i>Rhyacodrilus falciformis</i> Bretscher, 1901 | 5S, 6S, 8S | 0.33 | 8 | 1 | 1 | 0 | 1 | 3 | 2 |
| <i>Rhyacodrilus gernikensis</i> Giani and Rodriguez, 1988 | 6S | 1.00* | 15 | 3 | 3 | 3 | 3 ^S | 0 | 3 |
| <i>Rhyacodrilus okamikae</i> Giani & Rodriguez, 1988 | 11C, 13C | 0.50 | 14 | 3 | 2 | 3 | 3 ^S | 2 | 1 |
| <i>Rhyacodrilus</i> sp. | 3S | 1.00 | 6 | 0 | 3 | 0 | 0 | 3 | 0 |
| <i>Rhyacodrilinae</i> imm. | 2C, 2S, 6S, 9C, 11C, 14C, 15S | - | - | - | - | - | - | - | - |
| <u>Naidinae</u> | | | | | | | | | |
| <i>Amphichaeta sannio</i> Kallstenius, 1892 | 8S | 1.00 | 5 | 1 | 3 | 0 | 0 | 0 | 1 |
| <i>Nais bretscheri</i> Michaelsen, 1899 | 12S | 1.00 | 6 | 0 | 3 | 0 | 0 | 0 | 3 |
| <i>Nais communis/Nais variabilis</i> Pigué, 1906 | 4S, 6S, 7S, 8S, 13C | 0.20 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| <i>Nais elinguis</i> Müller, 1773 | 5S, 13C | 0.50 | 3 | 0 | 2 | 0 | 0 | 0 | 1 |

| | | | | | | | | | |
|---------------------------------------------|----------------------------------------|------|---|---|---|---|---|---|---|
| <i>Pristina aequiseta</i> Bourne, 1891 | 4S, 5S, 6S, 7S, 11C, 13C | 0.17 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| <i>Pristina jenkinae</i> (Stephenson, 1931) | 1C, 3S, 4S, 5S, 6S, 7S, 8S, 11C, 12S | 0.11 | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| <i>Pristina longiseta</i> Ehrenberg, 1828 | 7S, 8S | 0.50 | 6 | 0 | 2 | 0 | 0 | 2 | 2 |
| <i>Pristina menoni</i> (Aiyer, 1906) | 5S | 1.00 | 4 | 0 | 3 | 0 | 0 | 0 | 1 |
| <i>Pristina sima</i> (Marcus, 1944) | 6S, 13C | 0.50 | 9 | 0 | 2 | 0 | 2 | 3 | 2 |
| <i>Tubificidae</i> imm. | 2S, 5S, 6S, 7S, 8S, 10C, 11C, 12S, 13C | - | - | - | - | - | - | - | - |
| <i>Tubificidae</i> indet. | 5S, 10C, 13C | - | - | - | - | - | - | - | - |
| <hr/> | | | | | | | | | |
| LUMBRICIDAE | | | | | | | | | |
| <i>Eiseniella tetraedra</i> (Savigny, 1826) | 7S, 13C | 0.50 | 4 | 0 | 2 | 0 | 0 | 0 | 2 |
| <i>Lumbricidae</i> imm. | 2S, 2C, 4S | - | - | - | - | - | - | - | - |
| <hr/> | | | | | | | | | |

Table 2. Biodiversity indices values for the 15 study sites in the Santa Eufemia-Ereñozar karst unit: S = taxa richness, R/S' = corrected rarity index, V/S' = corrected vulnerability index for all the taxa (1) and only for taxa identified to species level (2), and C = complementarity index. Number of stygobiotic species, number of endemic species to the Iberian Peninsula (IP) and number of genera for each site are also given.

| Index | S | R/S' | V/S' (1) | V/S' (2) | C | No. of stygobiotic species | No. of species endemic to the IP | No. of genera |
|-------|----|------|----------|----------|-------|----------------------------|----------------------------------|---------------|
| 1 | 5 | 0.24 | 8.40 | 8.40 | 0 | 2 | 1 | 4 |
| 2 | 9 | 0.51 | 8.25 | 8.43 | 6.38 | 4 | 2 | 9 |
| 3 | 5 | 0.65 | 6.00 | 6.00 | 4.26 | 0 | 1 | 4 |
| 4 | 4 | 0.16 | 2.33 | 2.33 | 0 | 0 | 0 | 3 |
| 5 | 19 | 0.45 | 6.37 | 6.29 | 40.43 | 4 | 3 | 10 |
| 6 | 12 | 0.37 | 6.50 | 6.33 | 4.26 | 1 | 1 | 9 |
| 7 | 9 | 0.27 | 4.56 | 4.56 | 0 | 1 | 1 | 7 |
| 8 | 11 | 0.32 | 5.33 | 5.33 | 4.26 | 1 | 0 | 8 |
| 9 | 13 | 0.50 | 7.00 | 7.22 | 14.89 | 3 | 0 | 9 |
| 10 | 2 | 0.20 | 9.00 | 9.00 | 0 | 1 | 0 | 2 |
| 11 | 11 | 0.31 | 7.36 | 7.40 | 0 | 4 | 2 | 10 |
| 12 | 7 | 0.41 | 5.29 | 5.29 | 2.13 | 1 | 1 | 6 |
| 13 | 17 | 0.43 | 6.12 | 6.06 | 23.40 | 2 | 3 | 13 |
| 14 | 2 | - * | - * | - * | 0 | 0 | 0 | 2 |
| 15 | 5 | 0.34 | 6.50 | 6.44 | 0 | 1 | 0 | 4 |

* Only immature specimens were found at this site and thus, the corrected indices could not be calculated.

Table 3. Correlation coefficients between site biodiversity indices: S = species richness, R = rarity index, R/S' = corrected rarity index, V = vulnerability index, V/S' = corrected vulnerability index and C = complementarity index.

| | R | R/S' | V | V/S' | C |
|-------------|----------------|---------------|----------------|---------------|----------------|
| S | 0.955** | 0.376 | 0.960** | -0.022 | 0.824** |
| R | | 0.575* | 0.951** | 0.069 | 0.917** |
| R/S' | | | 0.412 | 0.164 | 0.688** |
| V | | | | 0.208 | 0.786** |
| V/S' | | | | | 0.045 |

** Correlation coefficients were significant at $P < 0.01$.

* Correlation coefficients were significant at $P < 0.05$.

Table 4. Correlation coefficients between values of rarity (1/n), vulnerability (V) and criteria of vulnerability (GD = global-scale geographic distribution, LD = local-scale geographic distribution, E = endemism, H = habitat specificity, P = local population size and RL = risk of habitat loss) for oligochaete taxa.

| | V_i | GD | LD | E | H | P | RL |
|------------------------|----------------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| 1/n_i | 0.292* | -0.033 | 0.989** | -0.021 | 0.006 | 0.314* | -0.531** |
| V_i | | 0.632** | 0.274 | 0.686** | 0.813** | 0.428** | -0.075 |
| GD | | | -0.076 | 0.729** | 0.726** | -0.138 | -0.182 |
| LD | | | | -0.052 | -0.001 | 0.332* | -0.531** |
| E | | | | | 0.762** | -0.229 | 0.010 |
| H | | | | | | 0.034 | -0.046 |
| P | | | | | | | -0.227 |

** Correlation coefficients were significant at P < 0.01.

* Correlation coefficients were significant at P < 0.05.

Table 5. Estimates of total taxa richness (S) in the Santa Eufemia-Ereñozar karst unit, using different estimators (S_{Chao2} , S_{Jack1} , S_{Jack2} and S_{Boot}) and number of uniques (number of taxa that occur at only one site). See text for details.

| Item | Total species richness |
|----------------|------------------------------|
| Observed S | 47.0 |
| No. of uniques | 16.0 |
| S_{Chao2} | 54.0 |
| S_{Boot} | 54.7 |
| S_{Jack1} | 61.9 |
| S_{Jack2} | 63.7 |

Figure 1.

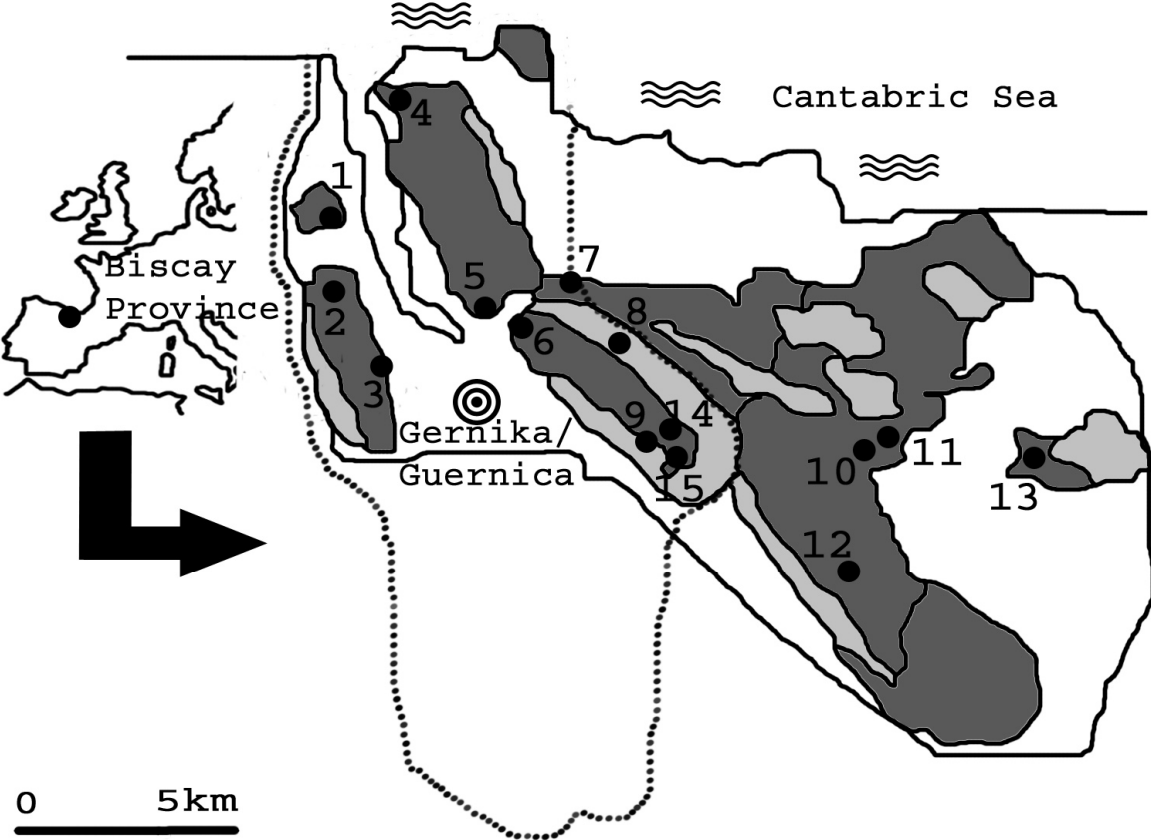
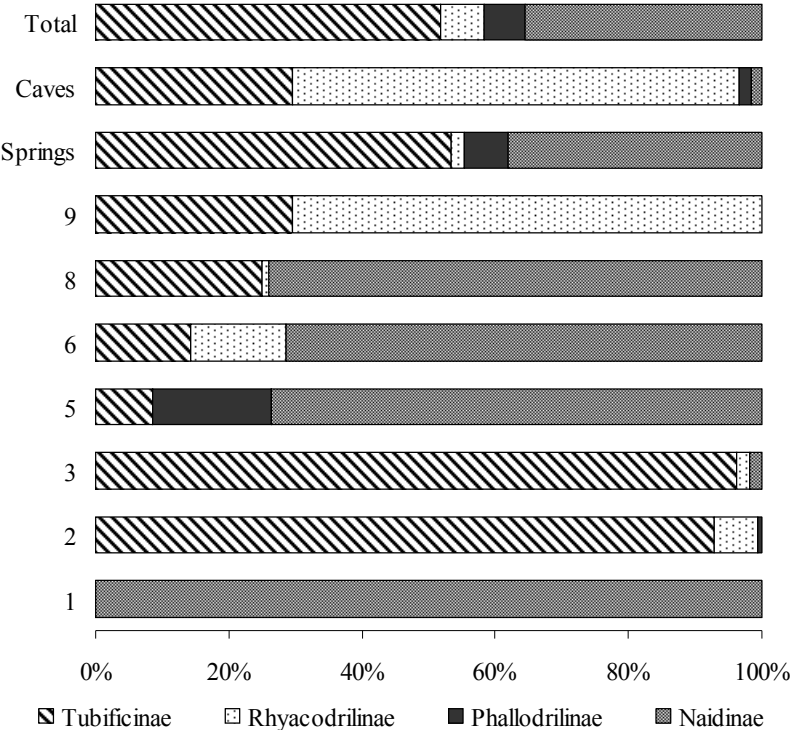
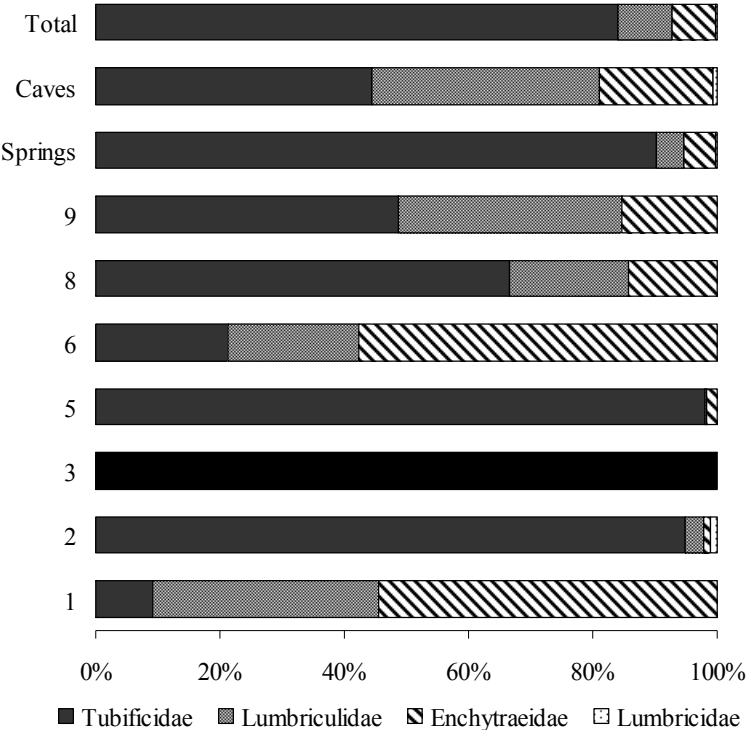


Figure 2.



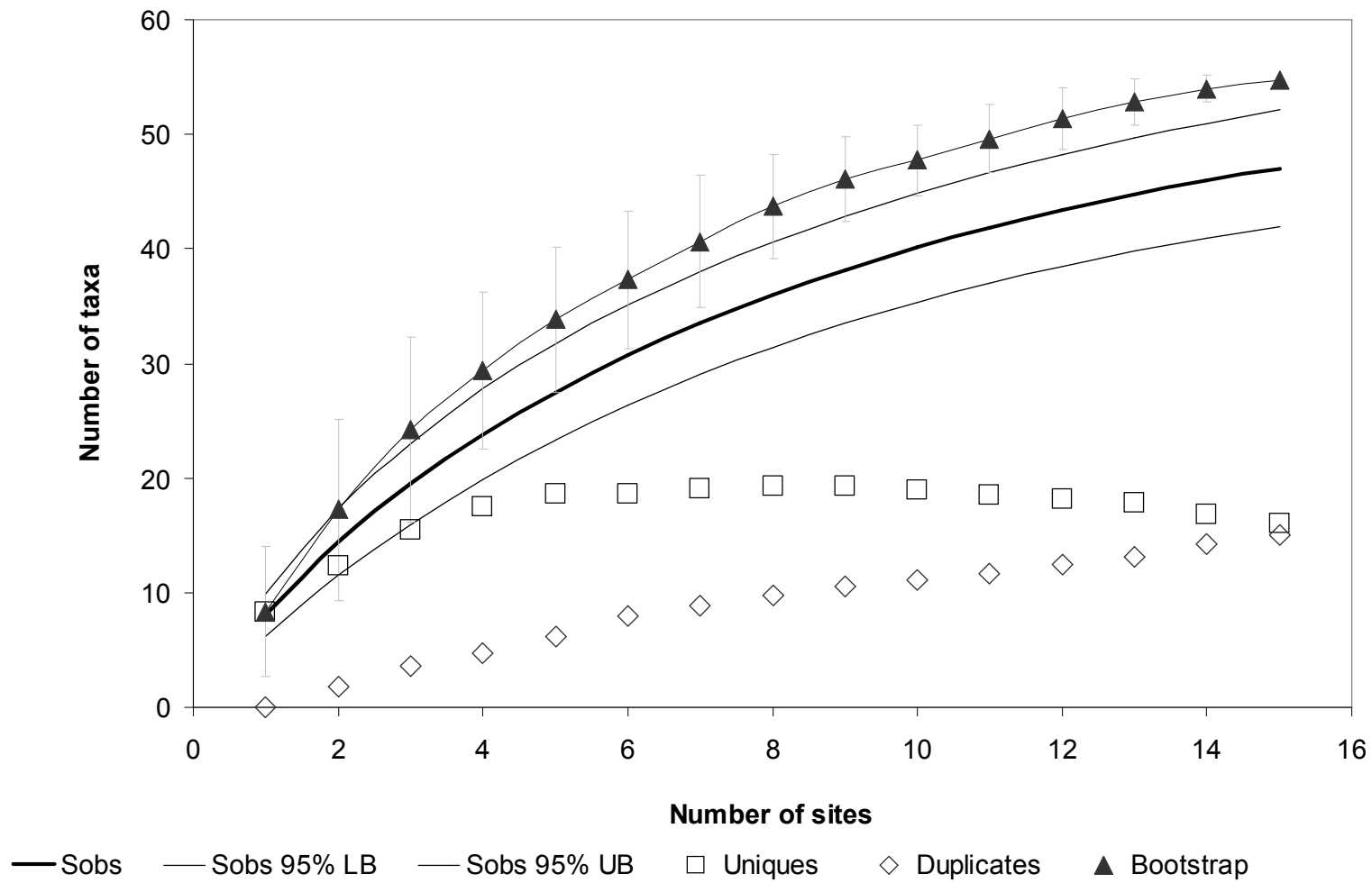


Figure 3.

Figure captions

Figure 1: Map of Santa Eufemia-Ereñozar karst unit showing karst subunits in dark grey, subunits basins in light grey and the Urdaibai Biosphere Reserve (UBR) area outlined by a dotted line. Sampling sites are numbered and marked in black.

Figure 2. Relative abundances of families (Tubificidae, Lumbriculidae, Enchytraeidae and Lumbricidae) and subfamilies of tubificids (Tubificinae, Rhyacodrilinae, Phallodrilinae and Naidinae) in the Oligochaeta community in the survey of autumn 2005.

Figure 3. Oligochaeta taxa rarefaction curve and Bootstrap estimator for 15 study sites calculated based on functions by Colwell et al., (2004). Key definitions are: Sobs, observed species richness; Sobs 95% LB, lower bound of 95% confidence interval for Sobs; Sobs 95% UB, upper bound of 95% confidence interval for Sobs; Uniques, number of species each known from only one sample; Duplicates, number of species each known from exactly two samples; Bootstrap, Bootstrap richness estimator and standard deviation.