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The groundwater fauna of Piani Eterni karstic area (Dolomiti Bellunesi National Park, Southern Limestone Alps, Italy) and its zoogeographic significance*

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ABSTRACT

The groundwater fauna of the Piani Eterni karstic area was surveyed in 2004-2007. Piani Eterni is a 14 km^2 karstic plateau, ranging from 1700 to 1900 m a.s.l., located inside a National Park (Dolomiti Bellunesi) in the Southern Limestone Alps. The Piani Eterni Complex is the largest and deepest cave system in the Dolomites and currently includes over 17 km path length, with a maximum depth of 971 m below the surface. The groundwater fauna, collected from pools, rivulets and small lakes in the vadose zone of Piani Eterni Complex and the nearby Isabella Cave includes 27 species (11 Nematoda, 7 Annelida, 7 Crustacea and 2 Diptera). Ten species are stygobionts, and five of them are new to Science. The importance of Diptera in this subterranean karstic system is noteworthy; several larvae of the chironomid *Eukiefferiella gracei* group were found both in groundwater bodies of Piani Eterni Complex and in a spring (Fontanon) which likely drains part of the aquifer, suggesting that this stygoxene chironomid may be considered as a "biological tracer". Some other stygoxene species (most of Nematoda and Annelida and some Copepoda) live in wet soil, mosses, and interstitial habitats as well as in some surface environments; they are probably recent (i.e. post-glacial) colonizers of the cave system. The presence of stygobionts (37% of the fauna) in an area completely covered by the Alpine glaciers during Pleistocene is noteworthy.

The presence of a possible relict fauna in this area may be explained either by a re-colonization following the retreat of Würmian glaciers, or by their survival in the deep groundwater aquifer during the Quaternary glaciations. Finally, the observed habitat segregation of subterranean species within the caves studied, together with a narrow range of variation of water chemistry, indicating a homogeneity of physico-chemical parameters, suggests that habitat complexity may have played a major role, together with historical factors, in shaping the groundwater biodiversity in this cold, oligotrophic cave system.

Key words**:** Piani Eterni, ground waters, Nematoda, Annelida, Crustacea, Diptera

INTRODUCTION

Groundwater biodiversity in high-elevation Alpine caves is poorly known (Stoch 2008). This may be partly due to the lack of research efforts, influenced by the idea that stygobionts in areas covered by Quaternary glaciations are very rare or absent. Recent researches carried out in Italy in glaciated areas of Trentino (Stoch 2000) and Eastern Alps (Colla and Stoch 2002; Stoch 2004) demonstrated that the percentage of stygobiont, cave-dwelling species in the groundwater fauna of those areas is not as low as expected, ranging between 60% and 62%, i.e. only slightly lower than in Pre-Alpine, non-glaciated areas (72-75%: Stoch 2008). However, even in these studies, few high-elevation, cold caves were sampled, and most of the data deal with a limited number of taxa, mainly crustaceans.

In this study we present an exhaustive biospeleological survey of all taxonomic groups carried out in the complex cave system of the high elevation karstic plateau of Piani Eterni (Southern Dolomites). The aims of this research are: (1) to study the composition and distribution of the groundwater fauna of the karstic massif, assessing its species richness; (2) to define habitat preferences of groundwater species and characteristic species assemblages in different microhabitats with-

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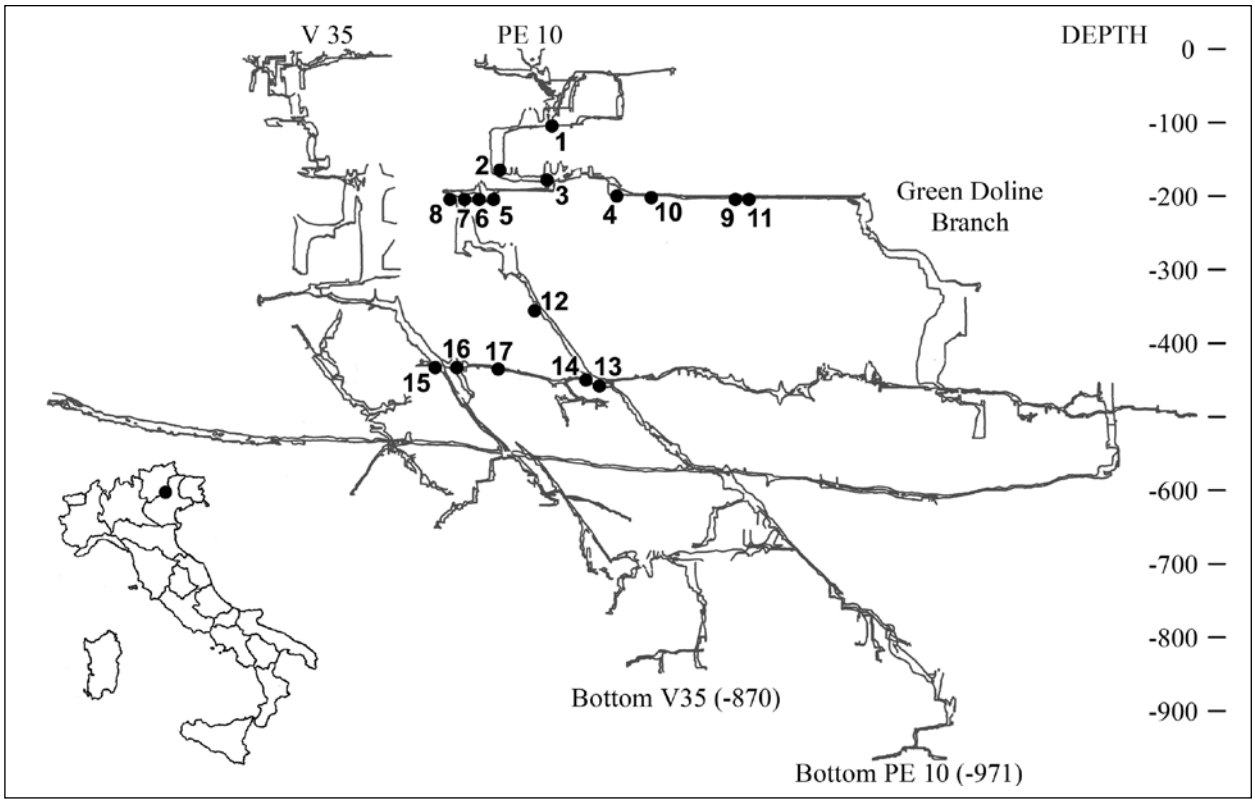

Fig. 1 - Section of Piani Eterni Complex with the location of sampling sites (map redrawn from Sauro et al 2009). Site codes are reported in Table 1.

in the cave system; (3) to identify the main historical and ecological factors shaping groundwater biodiversity in the area.

THE STUDY AREA

Piani Eterni is a wide karstic plateau located in the Dolomiti Bellunesi National Park, in the eastern Italian Alps. It extends over an area of about 14 km^2 and is characterized by slightly inclined rocky surfaces, ranging from 1700 to 1900 m a.s.l., marked by exokarst morphology (dolines, glacio-karst depressions, karren). Piani Eterni presents also several glacial scours, like glacial cirques, which are evidence for the former presence of a large Würmian glacier.

Piani Eterni hosts one of the longest and deepest cave systems in Italy, consisting of more than 300 caves formed in limestone and dolomite. The main cave system, named the Piani Eterni Complex, has four entrances, named PE3, PE10, PE25, V35. The PE10 (cadaster number of Veneto Regional Administration: 3640 V/BL; longitude and latitude, WGS84 datum: E 12°52'42.6''; N 46° 9'42.5'') opens at an altitude of 1890 m a.s.l. and currently includes over 17 km of path length, with a maximum depth of 971 m: it is the deepest cave explored in the Dolomites (Fig. 1). Two main abysses develop inside the system, lying along a fault; they are connected by a net of conduits and sub-horizontal galleries mainly at -270, -450 and -700 m (Sauro et al 2009). The top 100 m are partially filled with snow and perennial ice (Fig. 2); beneath, a complex network of galleries hosts pools, streams, and small lakes. The reaction of groundwater flow to rainfall is quite rapid; infiltrating rainfall tends to flow vertically through the vadose zone of the karstic massif and reaches the deepest part of the cave in a few hours (Sebenello 1994; Bortolas 1998; D'Alberto et al 1998; Salogni 2004; Ferrarese et al 2006; Sauro et al 2009).

The Isabella cave (4798 V/BL; E 12°53'26.6''; N 46°9'18.5''), explored for over 3 km in length, opens on the eastern rim of Piani Eterni plateau (Val Falcina slope)

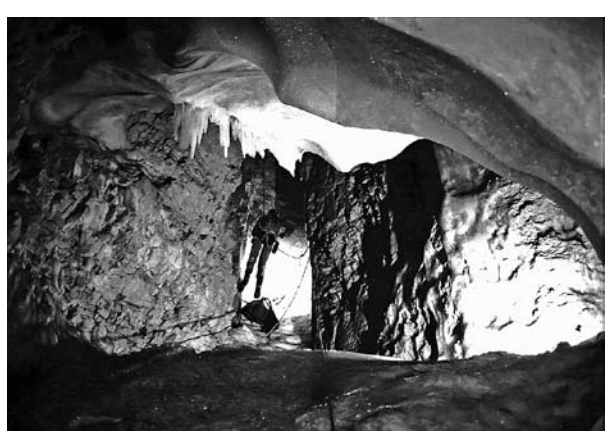

Fig. 2 - Fossil ice at depth -70 m in Piani Eterni Complex (photo: Franco Capretta).

Table 1 - List of sampling sites in the Piani Eterni Karst complex.

Depth from entrance (m)	Sampled habitat	Station number	Sampling dates
-105	Pool of percolating water	01	23/07/07
-175	Pools of percolating water	03	23/07/07
-175	Rivulet	02	23/07/07
-200	Pool Sole che Ride	09	11/08/05; 23/07/07; 11/07/07
-200	Rivulet near Quadrivio	04	23/07/07
-200	Pools from Quadrivio to Dolina Verde	10	23/07/07
-208	Lake Berto	05	11/08/05; 23/07/07
-208	Lake Berto effluent	06	12/12/04; 11/08/05; 23/07/07
-210	Lake Berto effluent	07	12/12/04; 23/07/07
-210	Pool near effluent	08	23/07/07
-340	Rivulet along P140	12	23/07/07
-430	Small lake at V35 connection	15	23/07/07
-450	Pools of percolating water near camp	14	23/07/07
-430	Pool fed through conductive fractures	16	23/07/07
-430	Pools of percolating water near camp	17	23/07/07
-460	Lake below P140	13	23/07/07

and is probably connected to the Piani Eterni Complex (Fig. 3): speleologists are searching the "human-sized" link between the two systems that should unify them in a complex more than 25 km long (Sauro et al 2009).

Piani Eterni groundwaters are drained by springs located at an elevation below 600 m a.s.l. The inclination of the strata suggests that waters of PE10 cave system flow to northeast towards the resurgence named Fontanon. Unfortunately, due to the restrictive rules of the Dolomiti Bellunesi National Park, no water tracing experiments were authorized. Fontanon (3631 V/BL; E 12°1'6.3'', N 46°12'6.1'') is a perennial karstic spring located at 550 m a.s.l on the very steep Northeastern side of the Piani Eterni plateau. Pontin & Gargini (2009) studied the hydrodynamics of this spring. The mean annual discharge, temperature and specific conductivity at 20°C of Fontanon were 179 l/s (range 58-1231 l/s), 6.1°C (5.7-6.7°C), and 195 μS/cm (134-210 μS/cm) respectively.

MATERIALS AND METHODS

Four surveys in Piani Eterni Complex were carried on during December 2004, August 2005, July 2007, and August 2007, sampling 17 different sites in order to collect groundwater fauna (Fig. 1 and Table 1). Rimstone pools within the first kilometre from the entrance of the Isabella cave were investigated in August 2007. Rimstone pools, trickles, and rivulets were sampled with small hand pumps or by sieving sediments through a hand net (100 µm mesh size). In larger rivulets and lakes, invertebrates were collected using the same hand net to filter the water; where present, the sediment was stirred before netting in order to remove burrowers and interstitial fauna.

The Fontanon spring was sampled in February 2005 and April 2005, a period of low and mild discharge. The sampling site was located in the final stretch of subterranean stream about 10 m above the spring mouth. In February, the sample was collected filtering for 30' the water with a drift net; in April the drift net was left *in situ* for 15 days.

Temperature, specific conductivity at 25°C and pH were measured using a portable multi-probe analyzer by Hanna Instruments. Hardness was measured in selected sites by a titrimetric method with EDTA.

Faunal samples were fixed in the field with 4% formaldehyde solution and sorted in the laboratory using a stereo-microscope; the collected specimens were stored in vials and preserved in 70% ethanol. Taxonomic groups were identified by Aldo Zullini (Nematoda), Enrique Martìnez-Ansemil and Beatrice Sambugar (Annelida), Fabio Stoch (Copepoda, Amphipoda), Uberto Ferrarese (Diptera). Nematoda were mounted in glycerine following the

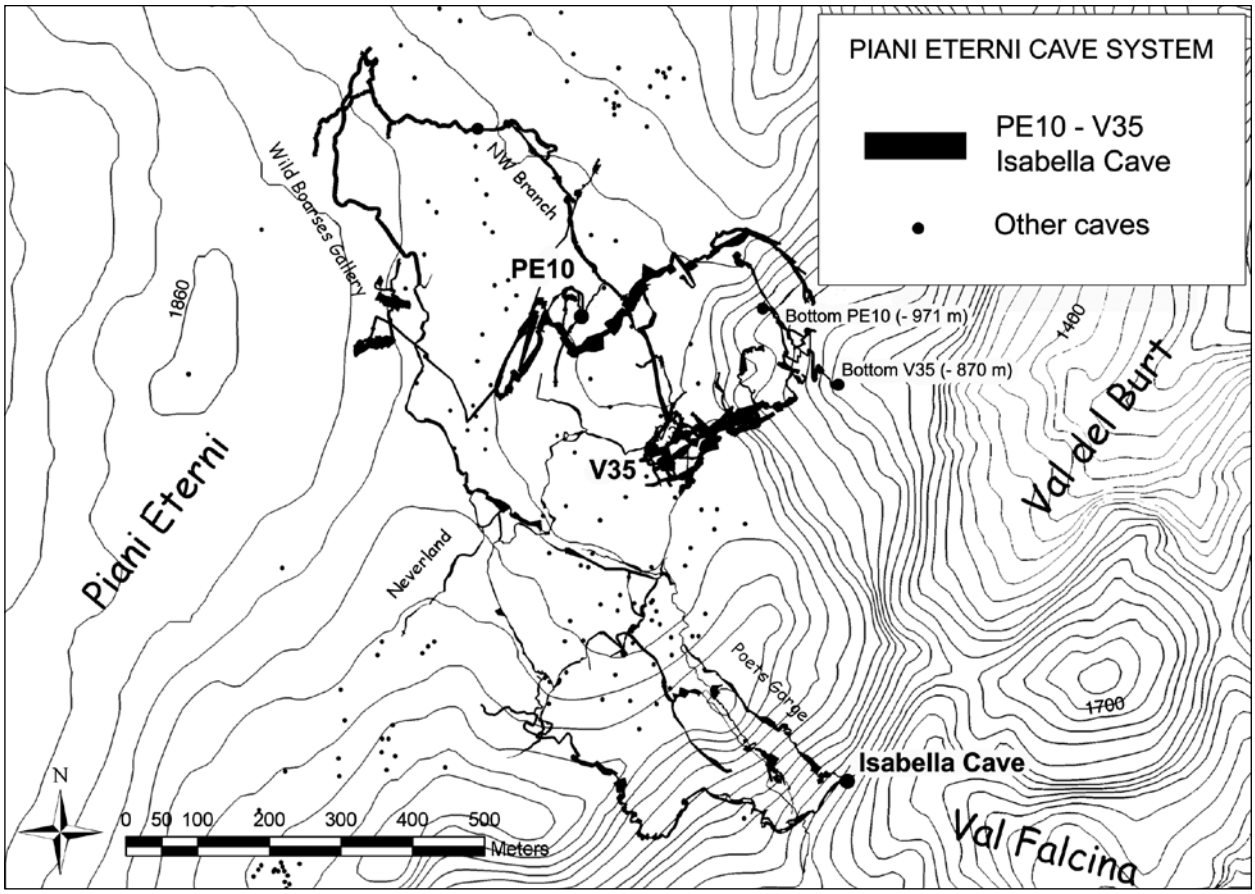

Fig. 3 - Plant of the cave system in the Piani Eterni karst plateau (map redrawn from Sauro et al 2009).

standard slow procedure. Annelida were mounted whole on slide in glycerin; mature specimens of oligochaetes were stained with Paracarmin and either dissected under a stereo-microscope and then mounted in Canada Balsam, or mounted whole in Canada Balsam. Immature specimens were stored in ethanol. Copepods were dissected on a slide in a drop of glycerin and permanent mounts were obtained sealing the coverslip with hepoxyds: the techniques followed Stoch (2007). Amphipods were mounted temporarily on slides in glycerine and stored after identification in ethanol. Dipteran larvae were prepared following Pinder (1983) and Schlee (1966).

Relationships between the composition of stygobiotic assemblages of different microhabitats within Piani Eterni complex were investigated by means of principal coordinate analysis (PCO), applied on the symmetric, square matrix of Euclidean distances (Pielou 1984) based on the species × sites matrix (Table 2), using the software package MVSP® 3.01a, Kovach Computing Services.

RESULTS

Physico-chemical characterisation of the habitats

Physico-chemical data taken in the subterranean habitats are very similar to each other and contribute to classify the Piani Eterni Complex and Isabella caves as a unique complex of high mountain cold caves belonging to the same karstified plateau. Air temperature spanned between 2.1° and 3.1°C, pH between 7.5 and 7.9, electric conductivity between 155 μS/cm and 196 μS/cm, and hardness between 160 and 204 mg/l $CaCO_3$. Water temperature increased with depth (Pearson's $r = 0.937$, $p < 0.01$, depth log-transformed) during the sampling survey in Summer 2007 (Fig. 4), also in relation to the geothermal gradient. The ice present in the top 100 m freezes percolating water (at depth -105 m, the water temperature is 0.8°C), which, flowing along walls and crevices, gets warmer, reaching, below 430 m depth, more than 3°C. Direct observations of microhabitat structure and very localized, small accumulations of vegetable debris suggest that the cave system may be classified as oligotrophic.

Fontanon spring showed in February and April 2005 the same water temperature (6.4°C); small differences in pH values (8.4 and 8.2 respectively), specific conductivity (196 and 186 μS/cm) and hardness (216 and 171 mg/l) confirm the ranges observed by Pontin and Gargini (2009). The estimated discharges were 50 l/s in February and 200 l/s in April.

Faunal data

The Piani Eterni Complex fauna is listed in Table 2.

Table 2 - List of species and number of individuals collected in the groundwater microhabitats of the Piani Eterni Complex. Stygobiont species are marked with an asterisk; two asterisks indicate that the species is new to Science as well.

	01 Pool of percolating water	**02 Rivulet**	**03 Pools of percolating water**	**04 Rivulet near Quadrivio**	**05 Lake Berto**	**06 Lake Berto effluent**	**07 Lake Berto effluent**	**08 Pool near effluent**	**09 Pool Sole che Ride**	**10 Pools from Quadrivio to Dolina Verde**	**12 Rivulet along P140**	**13 Lake below P140**	**14 Pools of percolating water near camp**	**15 Small lake at V35 connection**	**16 Pool fed through conductive fractures**	**17 Pools of percolating water near camp**
NEMATODA																
Epidorylaimus humilis (Thorne & Swanger, 1936)					1											
Eudorylaimus sp. 1					1											
Eudorylaimus sp. 2						1					2		1			
Eudorylaimus sp. 3		2				1										
(*) Mylonchulus andrassyi Loof, 1993		7							1	2		2	1			11
Eumonhystera longicaudatula (Gerlach & Riemann, 1973)														1		
Prodorylaimus filiarum Andrássy, 1964					1											
(*) Theristus athesinus Andrássy, 1962		2			6							2		1		
(*) Theristus vesentiniae Andrássy, 1962												5		3		
(**) Theristus sp.	1				1						1			1		
Tripyla filicaudata De Man, 1880		2			41	1	2	1			2	56		8		
ANNELIDA																
Polychaeta																
Aeolosoma sp.	1	3	2	3					4	1	16	4		2	3	
Oligochaeta																
(*) Trichodrilus cernosvitovi Hrabe,1937		1												2	5	
Cernosvitoviella minor Dózsa-Farkas, 1990							2					7				
Cernosvitoviella sp. (immature)				1	1		1				1					
Buchholzia simplex Nielsen & Christensen, 1963	1				1											
Fridericia sp.	3												1			1
Marionina argentea (Michaelsen, 1889)		5		5	14	8	1				2	9		11	1	
Marionina sp. (immature)		14														
Enchytraeidae gen. sp. (immature)	5		2	4				1			3	2	2	5		
(**) Rhyacodriloides sp.		21	1						2		4	3				

	01 Pool of percolating water	02 Rivulet	03 Pools of percolating water	04 Rivulet near Quadrivio	05 Lake Berto	06 Lake Berto effluent	07 Lake Berto effluent	08 Pool near effluent	09 Pool Sole che Ride	10 Pools from Quadrivio to Dolina Verde	12 Rivulet along P140	13 Lake below P140	14 Pools of percolating water near camp	15 Small lake at V35 connection	16 Pool fed through conductive fractures	17 Pools of percolating water near camp
CRUSTACEA																
Copepoda Harpacticoida																
Bryocamptus (*Arcticocamptus*) *vandouwei* (Kessler, 1914)					1							2				
(**) Bryocamptus (*Arcticocamptus*) sp.	9		6		21		2		1					3		
Bryocamptus (Limocamptus) echinatus (Mrázek, 1893)														14		
(**) Lessinocamptus sp.		13		52	14	3	2		2		13	15		6	7	
(*) Parastenocaris sp.															2	
Copepoda Cyclopoida																
(**) Speocyclops sp.		1			2						1					
Amphipoda																
(*) Niphargus similis Karaman & Ruffo, 1989		9		5	3	1			3		2	2		5	1	
HEXAPODA																
Diptera Chironomidae																
Eukiefferiella gracei group		2				14	10	4			1	2		15		
Orthocladiinae gen. sp.									1							

In Table 2 stygobiont species are marked with an asterisk, while two asterisks indicate stygobiont species new to Science. The faunal list includes Nematoda (11 species, 2 of them stygobiont), Polychaeta (1 species), Oligochaeta (6 species with 2 stygobiont), Copepoda (6 species with 4 stygobiont), Amphipoda (1 stygobiont species) and Dipteran larvae (2 stygoxenic species) for a total of 634 specimens examined. Ten species out of 27 (i.e. 37%) are stygobiont, and five of them are new to Science. The fauna of Isabella cave is represented by specimens of the stygobiont amphipod *Niphargus similis* and the stygobiont copepod *Lessinocamptus* sp., which colonised all the sampled habitats. In Fontanon spring the fauna is largely dominated by stygoxenic larvae of the chironomid orthocladiine *Eukiefferiella gracei* group, found also in many subterranean habitats of Piani Eterni Complex, including the deepest sampled sites. The larvae were sampled in great numbers, both in February (89 specimens) and in April (458), when the drift net was left in situ for 15 days. Another orthocladiine specimen was found: a juvenile larva of *Corynoneura*. Beside Chironomidae, one specimen of *Hapalothrix lugubris* Loew, 1876 (Blephariceridae) was found. Larvae of Plecoptera (*Protonemura nitida* (Pictet, 1835), *P. lateralis* (Pictet, 1836)) and Ephemeroptera (*Baetis* sp.) were also collected.

The 17 sampling sites of Piani Eterni Complex were arranged by PCO analysis (Fig. 5) along a gradient (first axis, 39.8% of the total variation), which clearly separates microhabitat typology (pools, small streams and small lakes). This gradient is not linked to depth below cave entrance (Fig. 6).

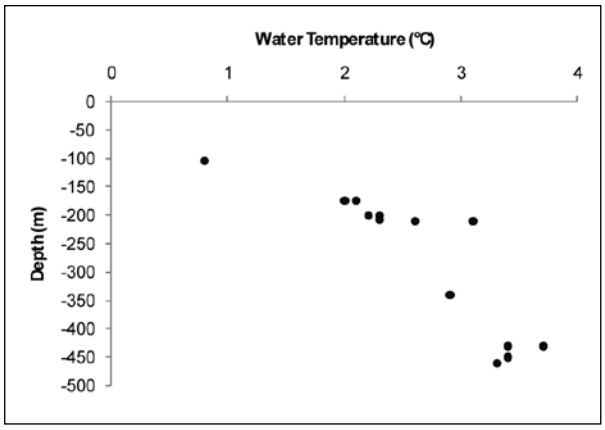

Fig. 4 - Inverse relationship between water temperature and depth of sampling sites from Piani Eterni Complex.

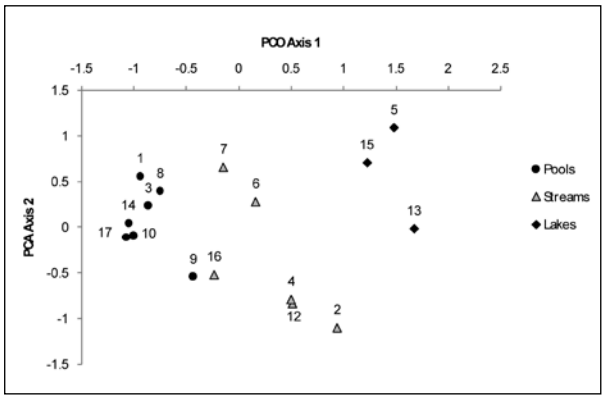

Fig. 5 - Ordination of sampling sites of Piani Eterni Complex on the space defined by the first two axes extracted by Principal Coordinate Analysis (PCO), explaining 56.8% of the total variation in the data set. Site codes are reported in Table 1.

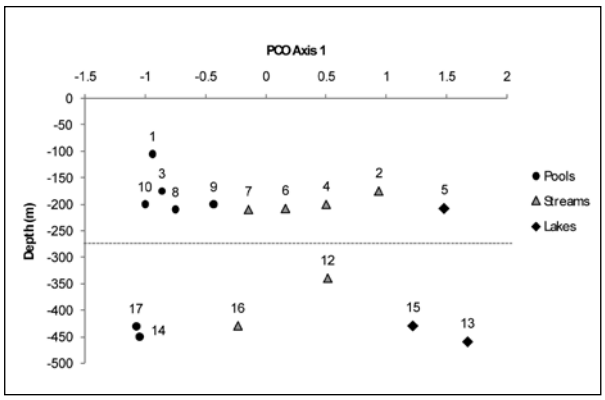

Fig. 6 - Ordination of sampling sites of Piani Eterni Complex on the space defined by the first axis extracted by Principal Coordinate Analysis (PCO), explaining 39.8% of the total variation in the data set, and the depth below cave entrance where the sites are located. Site codes are reported in Table 1. The horizonthal dotted line separates sites sampled in July (up) and August (bottom) 2007 at different depths.

Pools were inhabited by a low number of species (13 out of 29); the oligochaete genus *Fredericia* was found exclusively in pools. The other species were present in the other habitat types as well, but were represented in pools by a very low number of specimens. The chironomid larvae of *Eukiefferiella gracei* group are very rare in this habitat, being well represented in running waters and lakes.

Small streams and rivulets (17 species) hosts the highest numbers of specialized stygobionts (*Rhyacodriloides* sp., *Lessinocamptus* sp.); two species of Nematoda (genus *Eudorylaimus*) as well as the harpacticoid *Parastenocaris* sp. live exclusively in these running waters.

Finally, small lakes have the highest species richness (22 species), most of it in common with running waters; all the species exclusively found in these habitats (two species of *Eudorylaimus*, *Bryocamptus* (*A.*) *vandouwei*, *Bryocamptus* (*L.*) *echinatus* and an unidentified species of Chironomidae Orthocladiinae) are stygoxenic.

DISCUSSION

Detailed faunal remarks

Nematoda

Eleven species of Nematoda were found in the Piani Eterni Complex. Four of them are very uncommon and typical of psammon, subsoil and cave waters. Free-living nematodes, always being interstitial animals, lack the usual morphological markers (e.g. exclusive pigment and eyes reduction) useful to recognize troglobiont species. Among bacterial and algal feeders, *Theristus athesinus* was originally described from the psammon of the Adige River, at about 1 m from the bank, at 15 km S-E from Verona (Andrássy 1962); moreover there is a doubtful report from the psammon of Elbe River at 159 km from its sea outlet (Riemann 1966). *Theristus vesentiniae* is a psammic species also coexisting with the previous one in the Adige River; it was also found in the eulittoral zone of the Léman Lake (Juget, 1969). *Theristus* sp., with very long spicules, is a species new to Science. Genus *Theristus* (= *Cylindrotheristus* following Andrássy 2005) includes six continental species occurring in limnic, brackish and terrestrial (interstitial) habitats, and several marine species. In many cases their presence testifies an ancient relationship of their environment with marine or salt habitats. *Eumonhystera longicaudatula*, as most freshwater nematodes, was found in more than one continent, but it is interesting to note that it often colonizes deep lake sediments and subterranean water. Among predator nematodes, *Mylonchulus andrassyi* is known from subterranean water in Hungary: Baradla cave (11°C); Italy: psammon of Adige River, and in the cave Tomba del Polacco (near Bergamo, 10°C); Austria: 80 cm interstitial, below the bottom of a brook near Lunzer Untersee (Andrássy 1959, 1962; Zullini 1982; Loof 1993). The other nematode species found in Piani Eterni are common in various kinds of surface water, wet soils and mosses, seldom found (*Tripyla filicaudata*) in subterranean water; all these species have a wide distributional range.

Annelida

Polychaeta are represented by the genus *Aeolosoma*, widespread in subterranean environments (Sambugar, unpublished data). In the Piani Eterni Complex, *Aeolosoma* sp. colonizes all the different microhabitats (pools, rivulets, lakes) and is found even in the deepest sampling sites. Oligochaeta are present with 6 taxa, all belonging to the family Enchytraeidae with the exception of *Trichodrilus cernosvitovi* (Lumbriculidae) and *Rhyacodriloides* sp. (Naididae). Enchytraeidae are the most important component of the Piani Eterni Complex assemblage, with 115 specimens found (more than one half of the total annelid species richness). All research in European ground waters confirm that this family is the most frequent in these habitats, and that it constitutes also the most diverse group (Juget and Dumnicka 1986; Giani et al 2001; Martin et al 2008). Enchytreidae are generally amphibiotic, but *Cernosvitoviella* is aquatic, and is frequent and abundant in springs and subterranean waters, where stygobiont species of this genus are known. In a previous research on the subterranean fauna of Lessinian Mountains (a karst unit located in the eastern Alps, near to Piani Eterni plateau) *Marionina* and *Cernosvitoviella* were the most diverse genera found, also including species new to Science (Sambugar, unpublished data). In the Piani Eterni Complex, *Marionina argentea*, a stygophylic species frequently found in European caves, and *Cernosvitoviella minor* are the most abundant and frequent species. The discovery of the lumbriculid *T. cernosvitovi* is the first for the Italian fauna. In the Piani Eterni Complex this species colonizes different habitats till the depth of -460 m (station n. 13). It is a stygobiont species, known from European subterranean waters (Timm 2007).

The most important faunistic and biogeographic finding is that of a new species, belonging to *Rhyacodriloides*, an ancient naidid genus, only known from Lake Baikal. This species will be described elsewhere (in collaboration with Patrick Martin, Brussels), together with material from other localities of Northern Italy and Slovenia. Owing to the large and discontinuous distribution of *Rhyacodriloides*, one can conjecture that this genus had a wide ancestral distribution in freshwater habitats of the Palearctic region, and survived in relict populations in the depths of Baikal Lake and in subterranean habitats. The new species is rather common in the cave of Piani Eterni, where it is found in different habitats and depths.

The finding of cysts in the population of *Rhyacodriloides* sp. sampled in July 2007 is noteworthy, since the reports of cysts in oligochaetes are very rare. Every cyst contained one mature specimen, with mature ovocyte. The cyst formation is an adaptive strategy to disperse, recolonize or survive to habitat constraints, like drought, food deficiency, dehydration, and lower temperatures (Anlauf 1990; Montalto and Marchese 2005). This is the first report of an oligochaete cyst formation in a cave: it can be a response to the desiccation of groundwater microhabitats in the vadose zone, resource shortage, or species dispersal requirements. Other adaptations to the underground habitat life are some morphoanatomical peculiarities observed in *T. cernosvitovi* like the reduction of the body size and the asymmetrical disposition of spermathecae, favouring the movement of the species through small crevices.

Crustacea

Among Copepoda, three species of the genera *Lessinocamptus*, *Bryocamptus* (subgenus *Arcticocamptus*) and *Speocyclops* are new to Science; four out of the six species found are stygobionts. The new species of *Lessinocampus* is the most widespread copepod both in Piani Eterni Complex and in Isabella cave; it was previously found in other cold caves of the central-eastern Alps (Stoch 2000). To date, only three other species are known to belong to this genus (Stoch 1997), which is endemic to the karstic areas of the eastern Alpine chain. A new species of *Arcticocamptus* is quite a surprise, because this subgenus includes almost exclusively surface species widely distributed in mountain areas of the Nearctic region (Dussart and Defaye 1990). The whole subgenus is in urgent need of revision, and the affinities of the new species are difficult to be established. The genus *Speocyclops* is also under revision; the so-called *S. infernus* group, to which the species found on Piani Eterni belongs, includes several undescribed species in northern Italy (Stoch, unpublished data), where it colonizes both karstic and porous groundwater habitats. While smaller stygobiont harpacticoids like *Parastenocaris* and *Lessinocamptus* possess a cylindrical habitus suitable for moving in small karstic fissures (Galassi 2001), the cyclopoids of the genus *Speocyclops* have a flattened body shape which suggests a benthic life style. As a matter of fact, specimens of *Speocyclops* usually colonize small pools of percolating water and are uncommon in crevicular habitats. The other species found in Piani Eterni, *Bryocamptus* (*Arcticocamptus*) *vandouwei* and *Bryocamptus* (*Limocamptus*) *echinatus*, are widespread in Alpine springs (Stoch 2007) and probably stygoxenic in ground waters.

Amphipoda are represented by a single species, *Niphargus similis*, which is endemic to the Southern Limestones Alps in Italy, living even at high altitudes (Ruffo & Stoch 2006). The species is widespread in Piani Eterni Complex and Isabella cave, where it colonizes almost any kind of microhabitat.

Diptera

In the Piani Eterni Complex and in Fontanon spring almost all the Diptera are represented by abundant immature (maximum third stage) larvae of a species of Chironomidae Orthocladiinae of the *Eukiefferiella gracei* group. This group includes only species living in running waters, sometimes very fast flowing. The massive presence of this single taxon both in the cave and in the spring suggests a connection, by means of a fast

running water, between the upper and the lowest part of the karst massif. The fractures and conduits almost vertical in the rock aquifer of Piani Eterni represent routes for the flux of these insect larvae from the surface to the underground system. The same species was recently collected in another cave system of eastern Alps (La Foos, Friuli: Ferrarese, unpublished data) at a depth of -500 m. These findings suggest that this chironomid could be used as a "biological tracer" in groundwaters (*sensu* Sket & Bole 1982) and may be useful in evaluating the intrinsic vulnerability of karstic aquifers (*sensu* Di Lorenzo et al 2003).

Furthermore, it is noteworthy the finding in August 2005 in the pool named 'Sole che Ride' of a larva of Chironomidae Orthocladiinae with a set of characters different from those of the known genera; it is likely to be a new taxon.

General remarks

The groundwater assemblages of the Piani Eterni Complex – Isabella cave system consists of three main components.

The stygoxenes inhabiting the conductive part of the karstic aquifer include mainly rheophilic dipteran larvae (*Eukiefferiella gracei* group), which colonize the whole cave complex from the surface to the Fontanon spring. Their distribution pattern within the karstic systems depends on the rapid flow of infiltrating rainfall in conduits and main galleries, indicating a high intrinsic vulnerability of this subsystem of the massif (see Di Lorenzo et al 2003).

Other stygoxenes or stygophiles, together with most stygobionts, probably inhabit the complex of crevices in the vadose zone. Some species (most of Nematoda and Annelida and some Copepoda) may live in marginal habitats, like wet soil, mosses, interstitial habitats as well as in some surface environments; they are probably recent (i.e. post-glacial) colonizers of the cave system where they may have occupied the niches which were left empty after the retreat of Quaternary glaciers.

Stygobionts represent approximately 37% of the whole collected fauna, five of them (belonging to the genera *Theristus, Rhyacodriloides, Lessinocamptus, Bryocamptus*, and *Speocyclops*) being new to Science. This fact is noteworthy, because the study area was completely covered by the Alpine glaciers during the Pleistocene. The origin of this remarkable stygodiversity of possible pre-Quaternary origin may be explained either by a recolonisation of the cave system following the retreat of glaciers (hypothesis already stated for *Niphargus similis*, which may have a high dispersal power: Stoch 2000), or by their survival in the deep groundwater aquifer during Quaternary glaciations. The presence of relict species like the oligochaete *Rhyacodriloides* sp., known from the depths of Baikal Lake and from subterranean habitats of the Eastern Alpine chain and with interesting adaptations to the subterranean life, and the copepod *Lessinocampus* sp., belonging to a genus endemic of the caves of the eastern Alps, may be explained following the latter hypothesis; extensive networks of fractures probably allowed stygobionts to move deep down in the aquifer to seek refuge during surface freezing and to recolonise the ancestral habitats after the glaciers retreated. The persistence of an ancient, pre-Quaternary, groundwater fauna under deep glacier covers was hypothesized by Holsinger et al (1983) and Stoch (2000).

Finally, our results demonstrate the habitat preferences of subterranean species within the cave system studied. Habitat segregation does not show any relationship with depth from the surface, indicating that local factors (i.e. microhabitat structure as well as accumulation of vegetable debris following the complex water flow within the karstic network) may be responsible for the observed pattern. Also the range of variation of hardness, specific conductivity and pH is very narrow, indicating a homogeneity of water chemistry within the cave complex. These observations suggest that habitat complexity may have played a major role, together with historical factors, in shaping groundwater biodiversity in this cold, oligotrophic cave system.

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