

Article

Exploring Ice Cave Biodiversity in Northeastern Italy

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Abstract: The ice stored in caves is a widespread yet neglected cryospheric component. The cold-adapted biodiversity of ice caves has received very little attention from research, despite the potential abundance of endemic troglobiotic and cryophilic species and their consequent sensitivity to the changing climate. In this study, we investigated the invertebrate diversity of two ice caves in Northeastern Italy (Bus delle Taccole and Caverna del Sieson, Veneto Region). During 2022 and 2023, we sampled, using pitfall traps, the invertebrates dwelling at different locations in each cave: the shaft base, an intermediate hall, and the cave bottom. At each cave location, we also collected ice samples, on which we measured the stable isotopes of oxygen and hydrogen ($\delta^{18}\text{O}$, $\delta^2\text{H}$), and monitored the air temperature with data-loggers. The two caves had different invertebrate communities, both dominated by a combination of troglobiotic and cryophilic taxa. Despite a low taxonomic richness, which was higher at Taccole (15 taxa) than at Sieson (11 taxa), both caves hosted rare/endemic species, four of which are not described yet. At each cave, the ice water isotopic signatures differed among cave locations, suggesting the ice had formed under different climatic conditions, and/or resulted from different frequencies of thawing/freezing events. The occurrence of summer melt at both caves suggests that these unique ecosystems will quickly disappear, along with their specialized and unique biodiversity.

Keywords: ice cave; subterranean biology; speleology; glaciology; invertebrate communities; Veneto



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1. Introduction

The recent special report from the Intergovernmental Panel on Climate Change (IPCC) on the ocean and cryosphere in a changing climate highlighted a global contraction of permafrost, glaciers, seasonal snow cover, and Arctic sea ice [1]. Rising temperatures are responsible for the current widespread cryospheric loss, and climate models suggest that warming will continue unabated, leading to the loss of most glaciers in the coming decades, paralleled by a widespread permafrost warming and degradation. Small glaciers are most affected by this recent melt, with climate scenarios suggesting losses of up to 80 percent by the end of this century [1]. However, the same report does not include information on the perennial ice hosted in caves, which is the least-visible and -studied component of the global cryosphere.

The ice hosted in caves can originate from different processes, strongly dependent on the conformation of the cave and the microclimatic context. These include snow metamorphism, the freezing of infiltrating spring/rain/snowmelt water, and/or water vapor condensation [2]. These processes can be inferred with the use of the stable isotopes of oxygen and hydrogen in the water molecule. Isotopic ratios ($\delta^{18}\text{O}$, $\delta^2\text{H}$) are a useful tool to investigate the formation and internal dynamics of cave ice [3–6]. The future of this ice will strongly depend on the percolation of warm spring/rainfall water within the karst system and/or on enhanced ablation caused by increased air temperature within the cave [7,8]. Therefore, a rapid reduction in cave ice is predicted to occur during the next few decades,

since the rates of ablation are up to two–three times faster than those of formation and lead to persistently negative annual mass balances [9]. Cave ice deposits are facing the risk of disappearing completely within a decade, with the irreparable loss of historical data on climate, environmental conditions, and the life they host [8].

Most caves that host perennial ice are located in central and southeastern Europe, a region that has experienced some of the most rapid glacier ice losses in recent decades [8]. In Italy, more than 1600 caves are classified as cryo-caves, due to the presence of multiyear snow, firn, or ice. At least 10% of such caves can be included in the ice cave classification (i.e., have a perennial ice deposit), testifying how widespread these cryospheric features are [10]. In Italy, the heterogeneous geology has enabled not only caves formed in limestone, dolomite, and marble terrains, in the Alps as well as in the Apennines, but also in lava tubes on Mount Etna [10]. Most of the Italian ice caves are in the Alpine arc and no caves with perennial ice deposits are known in the Apennines, except for the Abisso Revel in Tuscany [11].

Despite their considerable number, there are only a few studies on ice caves in Italy, and none of these concern the fauna inhabiting such peculiar environments. Ice caves represent unique ecosystems characterized by extreme cold and low light conditions [12]. Troglobionts and stygobionts, already adapted to life in subterranean environments, implement strategies to survive in constantly frozen environments [13]. In addition to these taxa, species typical of glacial or nival environments and that can bear darkness, even if not fully adapted to subterranean life, are commonly found in ice caves, even in their deepest areas. Still, very little is known about the physiological and morphological adaptations of ice cave species, and given the rate at which these ecosystems are disappearing, research about these unique environments needs to be intensified.

For this reason, the Natural History Museum and speleological groups in Verona began to study the ecology and fauna of ice caves in the Alps and Pre-Alps [14–17], establishing the first comprehensive biological research on this kind of environment.

In this study, we explored the ice cave fauna in two caves in Northeastern Italy to test the hypothesis that these environments host a combination of troglobiotic (due to the cave habitat) and cryophilic (due to the ice presence) taxa, including endemic species due to the geographical isolation of such caves.

2. Materials and Methods

2.1. Study Area

The two investigated caves are located in the western part of the Veneto Prealps (Southeastern Italy; Figure 1) [18] in two different mountain ranges: the Baldo-Altissimo group (Verona Province) and the Asiago Plateau (Vicenza Province).

The Baldo-Altissimo group, on which Bus delle Taccole (Taccole Cave, called Taccole hereafter; Figure 2) is located, has a surface area of 398 km² and a maximum elevation of 2200 m above sea level (a.s.l.). This mountain range is predominantly composed of carbonatic rocks; Dolomia Principale, Calcarei Grigi, Calcarei oolitici di S. Vigilio, Rosso Ammonitico, Scaglia Rossa, and Maiolica [19,20]. Caverna del Sieson (Sieson Cavern, called Sieson hereafter; Figure 3) is located in the Asiago Plateau, which has a surface of about 1000 km². In the Asiago Plateau, four main carbonatic formations can be distinguished: the Dolomia Principale Formation (Norian–Rhaetian), the Mount Zugna Formation (Hettangian–Sinemurian), the Loppio Oolitic Limestone Formation (mid-Sinemurian), and the Rotzo Formation (Sinemurian–Pliensbachian). The last three formations belong to the Calcarei Grigi Group [21].

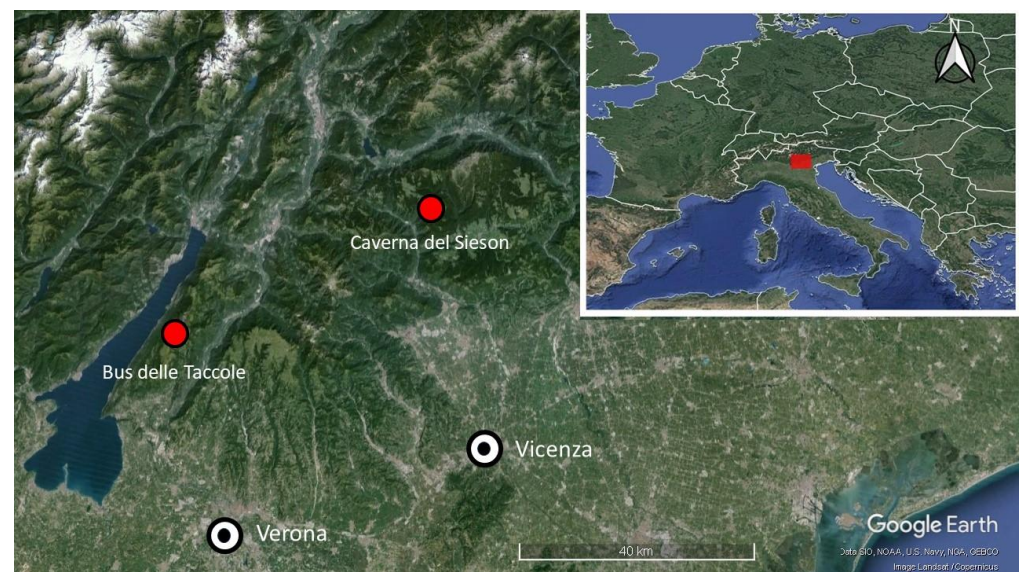


Figure 1. Location of the two studied caves (red dots) in the Lessinia Mountains (the red rectangle in the inlet box indicates the area in Italy).

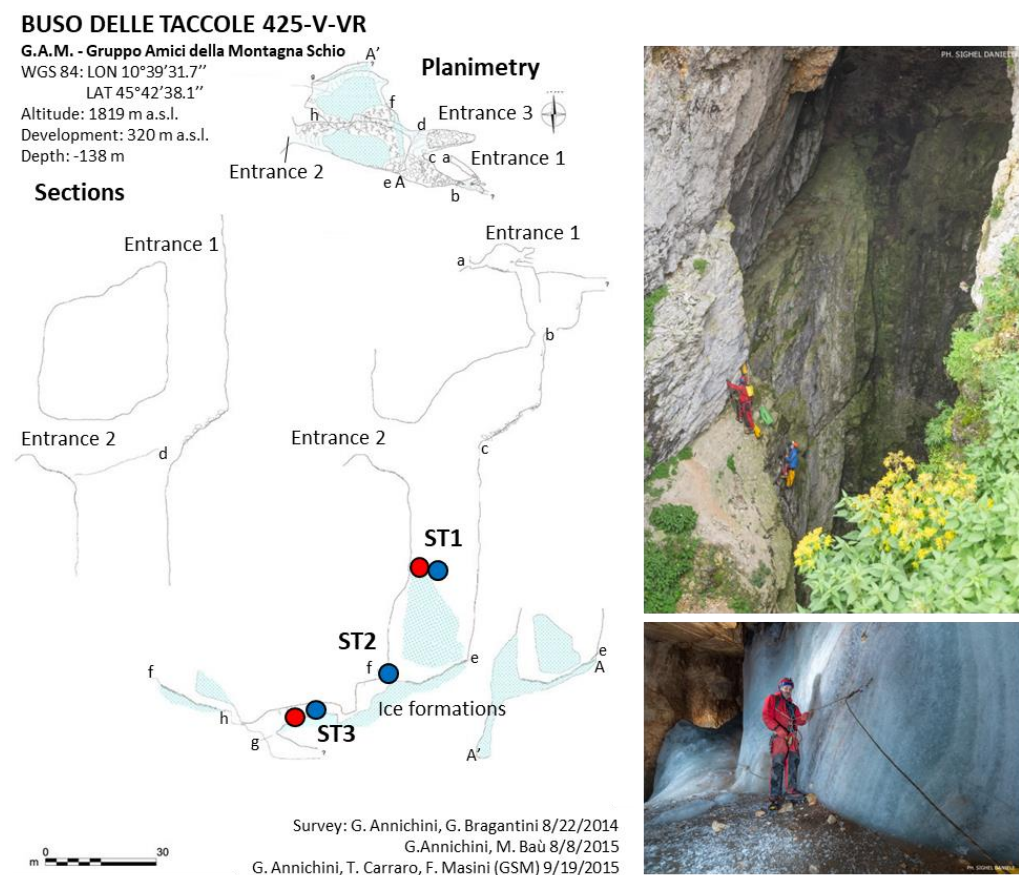


Figure 2. Cave map of Bus delle Taccole, with the locations of the sampling locations (blue dots) and the positions of dataloggers (red dots) used in the study. The extension of the permanent ice coverage inside the cave is illustrated in light blue. On the right: main entrance of the cave, and ice mass at the cave bottom (photos: speleoclick/Sighel D.). NOTE: letters in the map indicate the landmarks.

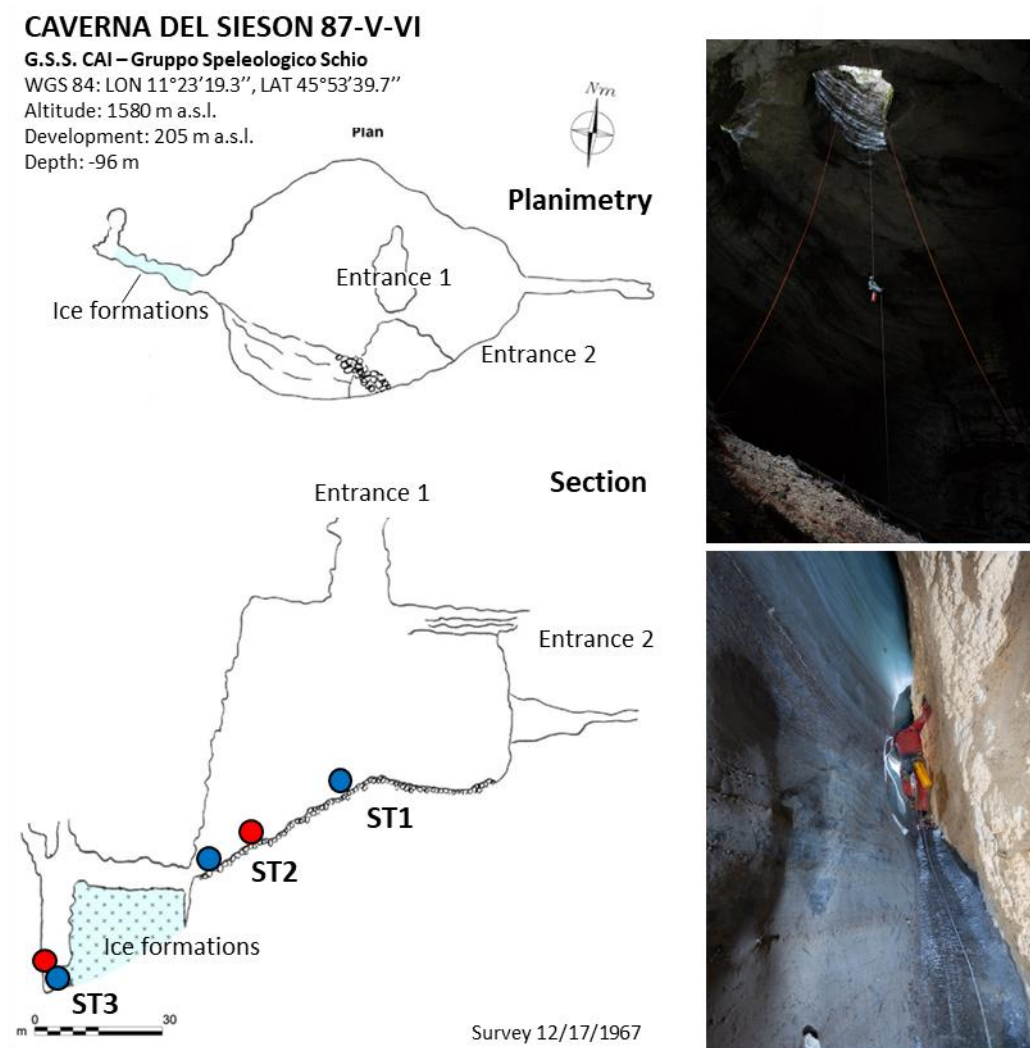


Figure 3. Cave map of Caverna del Sieson with the locations of the selected stations (blue dots) and the positions of dataloggers (red dots) used in the study. The extension of the permanent ice coverage inside the cave is illustrated in light blue. On the right: abseiling at the cave shaft base and descent along the cave ice. (Photos: speleoclick/Sighel D.).

2.1.1. Bus Delle Taccole

Cadastral number: 425 VVR

Locality: Baldo Mountain, Cima Telegrafo, Brenzone District, Verona Province

Coordinates WGS84: long. 10°89'31.7"–lat. 45°42'38.1"

Altitude: 1819 m a.s.l.

Development: 320 m

Depth: -138 m

The cave consists of three large shafts offset (Figure 2). A -40 m shaft departs from the upper entrance and connects to the top of the lower one, or enters a meander that, after about 15 m, past a landslide, enters a -40 m shaft, also in communication with the one below. Going up a few meters instead, it is possible to continue in the meander. The lower entrance is a large crack about 20 m high and 10 m wide that enters through a debris chute into a -65 m shaft, with a regular diameter of 20 × 15 m. The base widens considerably and is encumbered by a large accumulation of ice that bisects the shaft. Between the wall and the glacial mass, in a northerly direction, it is possible to descend for about -20 m between ice and rock. Continuing, it is possible to descend a few meters below the ice vault.

At the base of the entrance shaft, there are accumulations of soil and bird guano (a small colony of *Pyrhacorax graculus* (Linnaeus, 1766) nests on the upper part of entrance shaft walls), which are an evident trophic supply for the cave.

2.1.2. Caverna del Sieson

Cadastral number: (87 V–VI)

Locality: Casare Campolongo, Rotzo District, province of Vicenza

Coordinates WGS84: long. 11°23'19.3"–lat. 45° 53'39.7"

Altitude: 1580 m a.s.l.

Development: 205 m

Depth: −96 m

The cave consists of a large shaft with an upper entrance of about ten meters that then enters an lower one, with a very wide opening that leads into a shaft about 50 m in diameter and 40 m deep (Figure 3). At the base of the shaft, one descends along a debris cone for another 30 m until reaching the beginning of the glacier, which one descends for 20 m in a narrow passage between rock and ice. The mass of ice in the cave has evidently been decreasing in recent years. There are no bat or bird colonies at the entrance or in the inner areas of the cave, so the trophic input from outside is mainly from organic material dropped on the bottom of the pit.

2.2. Specimens Sampling

For each cave, we selected three sampling locations: Station 1 (ST1) at the entrance shaft base, Station 2 (ST2) in a deeper area but not in direct contact with the ice, Station 3 (ST3) at the bottom of the cave in contact with the ice mass (Figures 2 and 3).

The trophic supply, in terms of animal or plant organic material, was very different among the sampling locations of both caves. It was abundant at the base of the entrance shaft, due to the presence of organic material from outside and from the top of the shaft, and was extremely limited in the deeper areas occupied by ice.

Each station was sampled using standard pitfall traps, consisting of a glass cup with an open diameter of 10 cm, filled with propylene glycol, in which was placed a tube containing an attracting bait of blue mold cheese. Traps were left on site for 13 months in total at Sieson (April 2022–May 2023) and for 16 months in total at Taccole (August–October 2020, June 2022–October 2023). The traps were changed every three months between spring and autumn and every six months in winter. The collected specimens were fixed in 75% ethanol for morphological study. All specimens sampled in this study are preserved in the collections of the Museo di Storia Naturale of Verona, Italy.

2.3. Temperature Measurement

Two Tinytag Plus temperature dataloggers (−30 °C to +50 °C, accuracy 0.01 °C) were positioned inside each cave, one at the base of the shaft and one at the cave bottom (Figures 2 and 3), where they recorded the air temperature (T_{air} , °C) at 12 h intervals.

2.4. Ice Sampling and Isotopic Analyses

During each sampling campaign, we used an ice screw to sample ice aliquots belonging to the external part of the ice mass (0–15 cm). Sampling spots included the cave trap locations, at the floor (0.5 m) and at breast height (1.5 m), where screws were inserted horizontally. The ice was collected into 50 mL polyethylene containers with double caps. Samples were transported in thermal bags to the laboratory of the Faculty of Science and Technology, University of Bolzano, where they were stored at 4 °C. At Sieson, we also collected samples of snow and of water dripping from the cave walls and from a pond at the cave bottom. The ratios of oxygen and hydrogen stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$) of the melted ice water were determined with a laser spectrometer (Cavity Ring-Down Spectroscopy Picarro L2130i, Santa Clara, CA, USA) after filtration (0.45 μm). The precision of the analyzer was 0.5 ‰ for $\delta^2\text{H}$, and 0.25 ‰ for $\delta^{18}\text{O}$. The memory effect [22] was minimized,

following the procedure reported in Penna et al. [23]. All isotopic results were referred to the Vienna Standard Mean Oceanic Water and expressed in ‰ notation.

2.5. Data Analysis

To visually represent the dissimilarities among the communities belonging to different cave locations, we used the “vegan” package [24] in R (version 4.2.2) to perform a non-metric multidimensional scaling (NMDS). We estimated community dissimilarity distances based on the Bray–Curtis index on log-transformed taxa abundances. Before the analyses, we discarded the larvae (identified at a broad taxonomic detail), and taxa found with only one specimen (*Hymenaphorura* sp., *Lepidocyrtus* sp., and *Pogonognathellus flavescens*). We identified the cryophilic and troglobiotic taxa (see Table 1) to plot them on a bidimensional ordination. Finally, we tested if communities from the two caves were significantly different based on the ANOSIM (analysis of similarity) test in “vegan”.

Table 1. List of the known taxa found in the studied caves. Tb—troglobiont; Cy—cryophilic; 1—present; 0—absent.

Class	Order	Family	Genus/Species/Subspecies	Status	Tac. St 1	Tac. St 2	Tac. St 3	Sie. St 1	Sie. St 2	Sie. St 3
Arachnida	Trombidiformes	Rhagidiidae	Gen. sp.	Tb	1	1	1	0	0	0
Arachnida	Trombidiformes	Fam.	Gen. sp.		1	1	0	0	0	1
Diplopoda	Chordeumatida	Neoatractosomatidae	<i>Osellasoma caoduroi</i> Mauries, 1984	Tb	1	1	1	0	0	0
Collembola	Poduromorpha	Onychiuridae	<i>Deutheraphorura</i> n. sp.	Tb	1	1	0	0	0	0
Collembola	Poduromorpha	Onychiuridae	<i>Onychiuroides</i> n.sp.	Tb	0	0	0	1	1	1
Collembola	Poduromorpha	Onychiuridae	<i>Hymenaphorura</i> sp.		0	1	0	0	0	0
Collembola	Poduromorpha	Hypogastruridae	<i>Ceratophysella cf macrocantha</i>		0	1	1	0	0	0
Collembola	Poduromorpha	Hypogastruridae	<i>Ceratophysella bengtssoni</i> (Agren, 1904)		0	0	1	0	0	0
Collembola	Poduromorpha	Neanuridae	<i>Pseudachorudina alpina</i> Stach, 1949	Cy	1	0	0	0	0	0
Collembola	Entomobryomorpha	Isotomiidae	<i>Folsomia nigrimaculata</i> Najt, 1981	Cy	1	0	0	0	0	0
Collembola	Entomobryomorpha	Isotomiidae	<i>Desoria</i> n. sp.	Cy	0	0	0	1	1	1
Collembola	Entomobryomorpha	Tomoceridae	<i>Pogonognathellus flavescens</i> (Tullberg, 1871)		0	0	0	0	0	1
Collembola	Entomobryomorpha	Entomobryidae	Gen. sp.		0	0	1	0	0	0
Collembola	Entomobryomorpha	Entomobryidae	<i>Lepidocyrtus</i> sp.		0	1	0	0	0	0
Collembola	Entomobryomorpha	Entomobryidae	<i>Pseudosinella concii</i> Gising, 1950	Tb	0	0	0	0	1	1
Collembola	Neelipleona	Neelidae	<i>Megalothorax carpaticus</i> Papáček and Kováč, 2013		0	0	0	0	1	0
Insecta	Coleoptera	Staphylinidae	Gen. sp.		0	1	0	1	0	0
Insecta	Coleoptera	Staphylinidae	<i>Pselaphinae</i> Gen. sp.		0	0	0	1	0	0
Insecta	Coleoptera	Leiodidae	<i>Halberria</i> n. sp.	Tb	1	1	1	0	0	0
Insecta	Coleoptera	Cucujidae	Gen. sp.					1		
Insecta	Diptera	Trichoceridae	<i>Trichocera maculipennis</i> Meigen, 1818	Cy	1	1	1	1	1	1
Insecta	Diptera	Limoniidae	<i>Chionea araneoides</i> (Dalman, 1816)	Cy	0	0	1	0	1	1

Based on the isotopic ratios, we calculated for each water sample the parameter d-excess [25], defined as follows:

$$\text{d-excess (‰)} = \delta^2\text{H} - 8 \delta^{18}\text{O}$$

We estimated the correlation between $\delta^{18}\text{O}$ and d-excess with the non-parametric Spearman’s rank correlation coefficient (ρ).

Since no isotopic data on local precipitation were available, we used as a reference those from a close-by pre-Alpine catchment, Ressi (721 m a.s.l., Vicenza province; reference period: 2012–2022 [26]), located 15 km and 35 km away from Sieson and Taccole, respectively. When producing a dual isotope plot of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ with the collected data, we plotted the local meteoric water line (LMWL) of Ressi, as described by Marchina et al. [27] and Zuecco et al. [28].

3. Results

3.1. Subterranean Fauna

The fauna sampled in the two caves consisted of 22 taxa of invertebrates. Of these, 15 were found at Taccole and 11 were found at Sieson. Six trogllobiotic species were sampled, four of them at Taccole and two of them at Sieson. Five species can be considered cryophilic, two of which were found in both caves (Table 1).

Regarding Diplopoda, in Taccole, we sampled the Neoatractosomatidae *Osellasoma caoduroi* Mauries, 1984 (Figure 4). Although present throughout the cave, it was particularly abundant at the bottom.



Figure 4. A specimen of the Diplopoda *Osellasoma caoduroi* collected in the Buso del Vallon (Photo: L-Latella).

Collembola were the most represented order within the two caves. Nine species were found at Taccole, with one trogllobiotic and two cryophilic species. Inside Sieson, five Collembola species were present, one trogllobiotic and one cryophilic (Table 1). Among Neanuridae, *Pseudachorudina alpina* Stach, 1949, was found at Taccole, with a few specimens. Onychiuridae included a new species belonging to the genus *Deutheraphorura* n. sp., which was abundant in Taccole, especially at the shaft base and in the intermediate zone of the cave; the trogllobiotic *Onychiuroides* n. sp. was abundant at all sampling locations in the Sieson cave (B. Valle pers. com.). For the Isotomidae, one species belonging to the genus *Folsomia nigrimaculata* Najt, 1981 was found at Taccole. At Sieson, Isotomidae were represented by *Desoria* n. sp., a probable new species related to *D. fjellbergi* (Najt, 1981), a species typical of nival environments (B. Valle pers. com.). At Sieson, it was abundant at the cave bottom near the ice mass. To the Entomobryidae belongs *Pseudosinella concii*

Gisin, 1950, quite common in the innermost areas of Sieson. The trogllobiotic Neelidae *Megalothorax carpaticus* Papáç and Kováč, 2013 was found only in ST2 of Sieson.

In addition to Collembola, the most interesting trogllobiotic species was the Leiiodidae Cholevinae *Halberria* n. sp. This is a new species related to *Halberria carlini* Vailati, 2017, present at all stations sampled within the Taccole cave.

Diptera were represented by two cold-adapted species. The Trichoceridae *Trichocera maculipennis* Meigen, 1818, was sampled in both caves at the adult stage, as well as at the larval stage in Taccole. The Limoniidae *Chionea araneoides* Dalman, 1816 (snow fly) was present in both caves as well.

3.2. Distinct Cave Communities

The NMDS (stress < 0.001, $k = 2$) showed distinct communities in the two caves, even though the ANOSIM test did not show significant differences ($R = 1$, $p = 0.09$), which was very likely due to the low sample size. The cryophilics *T. maculipennis*, *P. alpina*, and *F. nigrimaculata*, as well as the trogllobiotics *Halberria* n. sp., Rhagidiidae, *O. cauduroi*, and *Deuteraphorura* n. sp. were more aligned with the Taccole samples, whereas the cryophilic *Desoria* n. sp. and the trogllobiotics *Pseudosinella concii* and *Onychiuroides* sp. were more aligned with the Sieson ones (Figure 5). The communities belonging to different locations of the cave were more homogeneous at Sieson. Contrastingly, the abundance of *C. araneoides* and *T. maculipennis* increased, and that of *Desoria* n. sp. decreased, when moving from ST1 to ST2 and ST3 in Taccole (Figure 5).

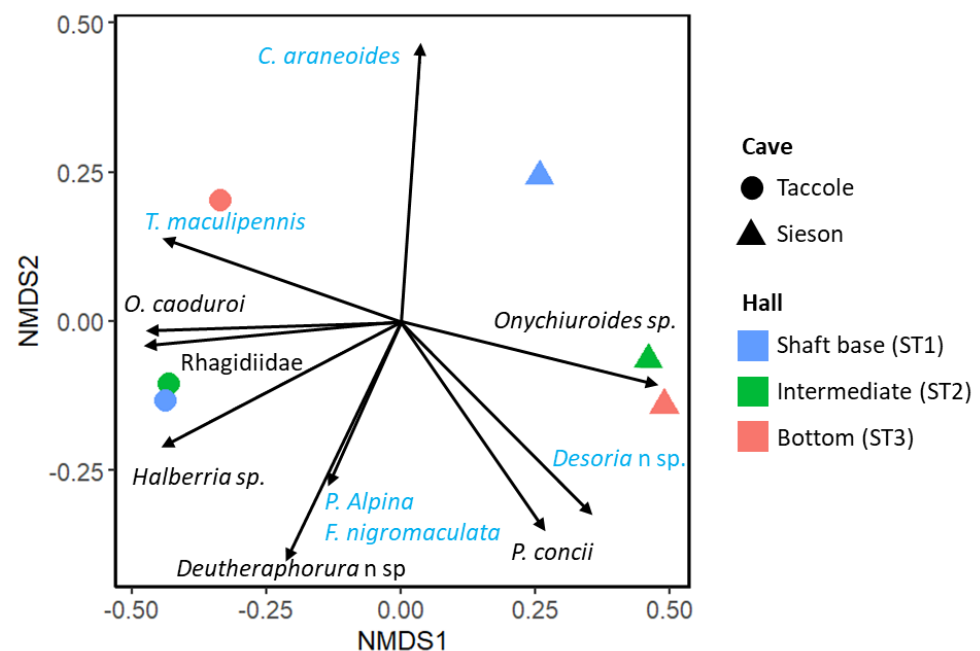


Figure 5. NMDS biplot, based on the Bray–Curtis index, of the communities belonging to different trap locations, focusing on cryophilic (light blue) and trogllobite (black) taxa.

3.3. Environmental and Isotopic Conditions

The two caves had similarly cold air conditions (Table 2; Figure 6). Their bottoms had a larger thermal variability than the shaft base, and a lower T_{max} . Daily T_{air} generally exceeded 0°C during the period of August–November at the cave bottom, and during July–November at the shaft base (Table 2). The collection of records during different periods did not allow the drawing of any comparisons of the thermal profiles at the two caves.

Table 2. Main thermal features of the two caves at their bottoms and shaft bases. For each cave, we refer here to the daily values of the period when dataloggers were recording at both locations.
* Freezing index refers to the number of days during which T_{avg} was higher than 0°C .

Thermal Parameter	Sieson Shaft Base	Sieson Bottom	Taccole Shaft Base	Taccole Bottom
Reference period	9/14/22–5/16/23	9/14/22–5/16/23	9/13/20–5/8/21 9/11/21–12/15/21	9/13/20–5/8/21 9/11/21–12/15/21
T_{min}/T_{max}	$-5.5/4.2^{\circ}\text{C}$	$-2.9/4.3^{\circ}\text{C}$	$-3.1/1.2^{\circ}\text{C}$	$-2.5/1.0^{\circ}\text{C}$
$T_{avg} \pm T_{sd}$	$-0.9 \pm 1.3^{\circ}\text{C}$	$-0.4 \pm 0.8^{\circ}\text{C}$	$-0.7 \pm 1.0^{\circ}\text{C}$	$-0.5 \pm 0.7^{\circ}\text{C}$
T_{med}	-0.8°C	-0.5°C	-0.3°C	-0.2°C
T_{aug}	na	na	$0.14 \pm 0.16^{\circ}\text{C}$	$0.01 \pm 0.03^{\circ}\text{C}$
Freezing index *	163	161	192	181

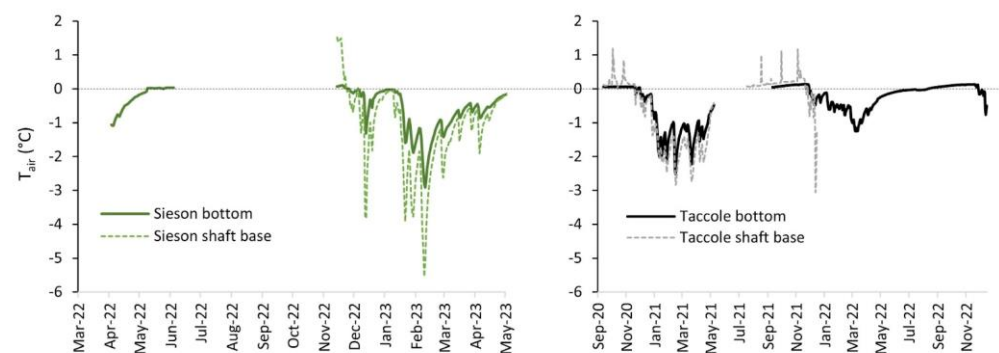


Figure 6. Time series of average daily air temperature (T_{air}) at the shaft base and bottom locations of the two caves. The 0°C threshold is provided (dashed black line) to aid interpretation.

Overall, the two caves had similar isotopic conditions, with $\delta^{18}\text{O}$ in the range of $-6/-9\text{‰}$ and d-excess in the range of $9-14\text{‰}$. At each cave, the isotopic composition of the ice generally differed among cave locations (Figure 7). At Sieson, there was a tendency towards a depletion in heavy isotopes when moving at depth and, at each location, the breast height ice was more depleted in heavy isotopes than the floor ice. By contrast, at Taccole, different locations had only slight isotopic differences, except at the shaft base, where the breast height ice was more enriched in heavy isotopes when compared with that of the other sampling locations/heights. D-excess only slightly differed between different locations at Sieson, where the larger differences were found between the floor and breast height ice (Figure 7). Indeed, at each location, breast height ice had higher d-excess when compared with the floor ice. In contrast, at Taccole, d-excess increased when moving from the shaft base to the bottom location, with slighter differences between floor and breast height when compared to Sieson. In the ice of both caves, we found a strong negative correlation between $\delta^{18}\text{O}$ and d-excess ($\rho = -0.8$, $p < 0.001$).

When plotted in the dual isotope plot (Figure 8), the ice samples shaped freezing lines with a lower slope and intercept than those of the global meteoric water line ($\delta^2\text{H} = 8 \delta^{18}\text{O} + 10$), and of the local meteoric water line (LMWL) of the close-by Ressi catchment. The samples of water and snow that were collected at Sieson more evidently aligned along this LMWL than those of Taccole. The isotopic range of cave ice reflected the values of spring/autumn rainfall occurring in the Ressi catchment (highest affinity with the months of April and September), and it was isotopically more enriched with heavy isotopes than the snow collected above the Sieson (Figure 8).

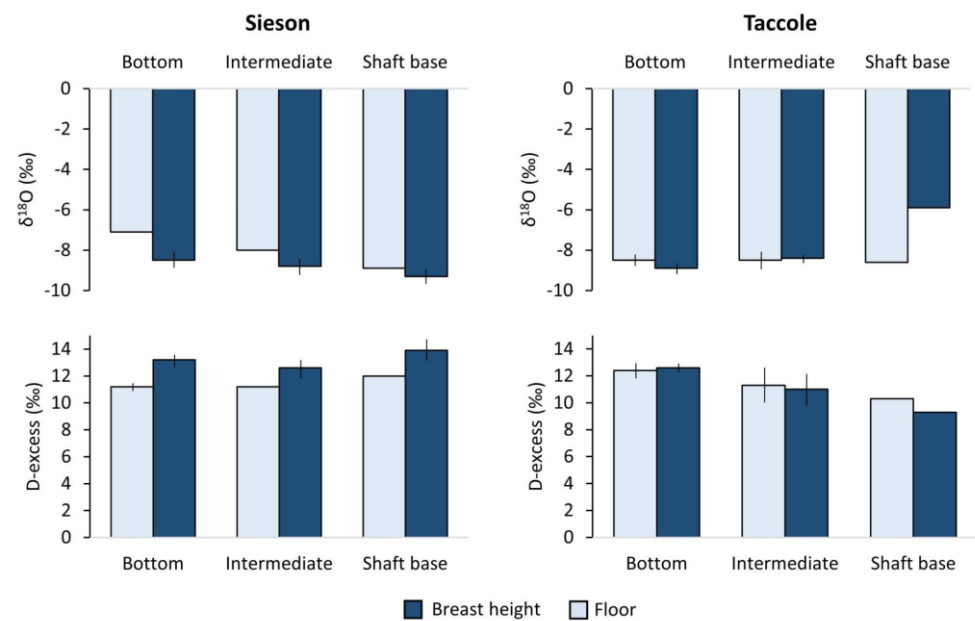


Figure 7. Bar plots of $\delta^{18}\text{O}$ and d-excess values of the ice at the two caves, at different locations and heights of the ice mass. Minimum and maximum values are displayed with vertical bars at each plot.

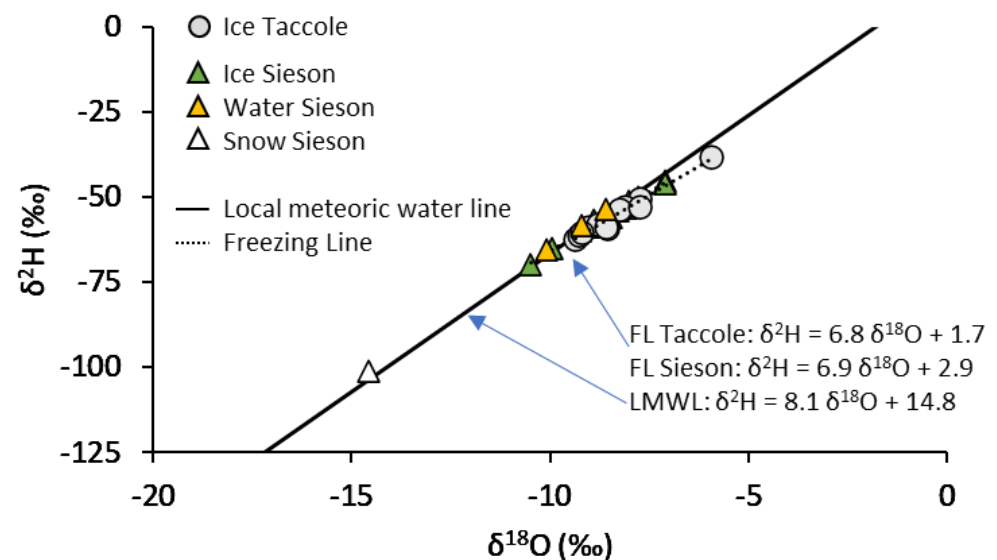


Figure 8. Dual-isotope plot showing the samples of ice at the two caves, the sample of snow that was collected immediately outside from the Sieson, and the water dripping from stalactites or belonging to a pond located at the cave bottom of Sieson. LMWL = local meteoric water line for the Ressi catchment [28]; FL = freezing line.

4. Discussion

4.1. Ice Cave Fauna Composition

In the two investigated caves, the trogllobiotic biodiversity was low and limited to a few taxonomic groups that were mainly represented by Collembola and Coleoptera, as commonly observed in ice caves of temperate zones [13]. Overall, our hypothesis on the presence of a mixture of trogllobiotic and cryophilic taxa, including endemic species, can be retained. In ice caves, typical subterranean-dwelling species co-dwell with a “cryophilic ipogean faunistic association” consisting of elements (mainly Diptera, such as the genera *Trichocera* and *Chionea*) that, although not exclusive of subterranean life, are well adapted to cold conditions [14,29]. At the Taccole cave, we found the Neanuridae *P. alpina*, a

species typical of high mountain environments, and the Isotomidae *F. nigrimaculata*, a rather rare species typical of the cold environments of the Pyrenees [30]. The Trichoceridae *T. maculipennis* (winter crane fly) is a Holarctic cold-tolerant species, known from the Arctic to the southern regions of the Mediterranean zone and the Far East [31]. It has been reported in different cold caves in central Europe [31], and it was sampled in both the study caves at the adult stage, as well as at the larval stage at Taccole. The Limoniidae *C. araneoides* (snow fly) is another cryophilic species found at both caves. Adults of the genus *Chionea* are easily distinguishable due to the absence of the wings (aptery). In alpine and pre-Alpine environments, these flies are visible in winter walking on the snow cover and in caves with a low internal temperature [14].

While some cryophiles (e.g., *T. maculipennis* and *C. araneoides*) were found in both caves, all troglobiotic species were endemic. At Taccole, these included the Neoatractosomatidae *O. caoduroi*. It is an endemic species for the Veneto and Lombardy pre-Alps, known at present only from another cave in Monte Baldo and on the pre-Alps of Bergamo (Lombardy). According to Mauries [32], it may be a Quaternary element related to cold climates that found refuge in the cave after the last glaciations. The Onychiuridae family was represented by a new species belonging to the genus *Deutheraphorura* n. sp., abundant at Taccole, where a second new species related to *Halberria carlini* Vailati, 2017, described from Monte Altissimo (Baldo-Altissimo Group), was found. At Sieson, we found a probable new species of *Desoria* n. sp. (Isotomidae), related to *D. fjellbergi* (Najt, 1981), which is typical of nival environments (B. Valle pers. com.).

More widespread cave taxa were found too. Several genera of rhagid mites include species adapted to life in subterranean habitats [33]. As in the case of the specimens sampled at Taccole, adaptations are usually manifested through the elongation of appendages and the progressive development of sensory organs, such as the increased length and number of rhagidial solenidia on the tarsi and tibiae of the first two pairs of legs [33]. The first pair of elongated legs acts as antennae, while the extremely elongated tactile setae, particularly on the palpi and first pair of legs, are useful for extending the tactile perception zone and create combs that help capture the prey [33]. To the Entomobryidae belongs *Pseudosinella concii* Gisin, 1950, a species distributed in different caves in Italy and Switzerland, and is quite common in the innermost areas of the Sieson. The troglobiotic Neelidae *M. carpaticus* was found only at Sieson.

Overall, the coexistence of troglobiotic and cryophilic taxa in ice caves can be explained both by the refugia hypothesis, according to which the caves were used as a refuge during the Plio-Pleistocene climatic alternations [34], and by the paleogeography of the two mountain ranges. Both the Baldo-Altissimo group and the Asiago Plateau were in fact isolated during the climatic oscillations of the Quaternary, and are therefore rich in species that were isolated and thus became endemic, even not in subterranean environments [35].

4.2. Relation to Environmental Variables

The inputs of organic matter can be a key food resource for cave fauna [13]. At Taccole, abundant trophic inputs from the outside (mainly soil, plant, and animal organic matter) slightly influenced cave communities. Indeed, taxa abundance and diversity were higher at the intermediate depth, where the organic matter was also more abundant. At Sieson, where the external organic inputs were much lower than at Taccole (only a few plant fragments were found at the base of the entrance shaft), we did not find different diversity and abundances among sampling locations.

The air temperature was very low within the two caves, with average values below 0 °C at all investigated locations. This explains why cryophilic species did not show preference for a particular cave location and, instead, were found at all depths.

4.3. Heterogeneous Isotopic Fingerprint of Cave Ice

The investigated ice had a large spatial variability of isotopic composition in the two caves. This variability was observed not only among different locations but also within

the same locations, between the upper and the floor samples of the ice mass profile. This spatial pattern was particularly evident for $\delta^{18}\text{O}$ at Sieson.

Since lower $\delta^{18}\text{O}$ values generally indicate the origin of the ice water under colder weather conditions than higher values [5], the positive trend of $\delta^{18}\text{O}$ with depth found for Sieson may suggest that the ice at the bottom had formed in warmer periods (i.e., season and/or years), when compared with that of the shaft base. The wide opening of the Sieson entrance suggests that winter precipitation and snowmelt can more effectively enter the shaft base and quickly freeze, as personally observed. At the bottom of the cave, higher isotopic values of the ice suggest a “seasonality bias” [3] of formation during a warmer season when compared with the ice of the shaft base. Differently, at Taccole, $\delta^{18}\text{O}$ values decreased with depth, but only at breast height. In this cave, the formation of the basal layers may have resulted from the congelation of water with a consistent origin for all depths, whereas the breast height layers (i.e., the younger ones) may have resulted from an inverse “seasonality bias”, when compared with Sieson. Thus, a different internal structure may be responsible for the different vertical isotopic pattern of the ice in the two caves. The upper part of the Taccole shaft base ice might have formed more recently, given the higher isotopic values when compared with those of the other locations in the cave, i.e., the shaft base floor, the intermediate locations, and the cave bottom. According to the thermal profiles of this work, at the Taccole shaft base, the air temperature dropped below 0 °C earlier during autumn, when compared with the cave bottom. Thus, rainfall occurring in this period may freeze more easily at the shaft base than at the bottom, and this may explain the enriched isotopic values observed at the shallower (and likely younger) layer (breast height) of the shaft base.

Despite these spatial differences, similar isotopic ranges suggest comparable seasonal formation of the ice at Sieson and Taccole. From springtime to autumn, rainwater can more easily penetrate at depth before freezing, given the warm conditions found at the shaft base of the caves. Indeed, when compared with decadal data on precipitation collected at the close-by Ressi catchment, the $\delta^{18}\text{O}$ values of the ice were compatible with those of precipitation fallen during springtime and autumn. By contrast, the d-excess values were generally higher in these precipitation samples when compared with those of the ice collected in this study. This suggests ice formation under colder climates than nowadays and/or the occurrence of fractionation processes during the ice formation. Overall, the positive relationship between $\delta^{18}\text{O}$ and d-excess suggests the occurrence of thawing/freezing events prior to the ice formation [6] at both caves.

We suggest that the ice at both caves did not originate from the condensation of water vapor, which would have returned freezing lines with higher intercepts [3,36], nor from snow metamorphism, which would have resulted in much lower isotopic values than those observed. We hypothesize that the ice formation in these caves mainly resulted from cold air traps (“static caves with congelation ice”) [2], rather than from chimney effects. This was also testified by the lack of evidence of thermal anomalies during wintertime, when the shaft bases were colder than the cave bottoms because of the relevant influence from outside temperature.

In general, the limited number of ice samples collected only allowed for a qualitative estimation of a very likely more complex isotopic profile of the cave ice. Furthermore, very little local-scale information is available on the isotopic conditions of precipitation and snowmelt at both sites. Also, Taccole is located at a further distance from the Ressi catchment, compared to Sieson, leading to weaker isotopic comparisons of ice and precipitation.

5. Conclusions

Ice caves are overlooked cold environments at imminent risk of extinction. Life in temperate subterranean environments is driven by certain environmental factors (darkness, constant temperature, lack of primary production, a scarcity of food resources, etc.). In ice caves, these factors are combined with extremely low temperatures and dry air. These environmental constraints allow for the existence of species-poor communities mostly

composed of a few, highly adapted species, which are therefore extremely sensitive to environmental changes.

Our study on two ice caves in northeastern Italy confirms the uniqueness of these environments, hosting a combination of euryoecious, troglobiotic, and cryophilic species. The low local diversity of invertebrates is outpaced by the high conservation value of cave communities, including endemic taxa, three of which are new to science. The ice of these caves likely results from thawing/freezing events after the infiltration of springtime waters, and during different historical periods, depending on the cave location. The rapid pace at which this ice is shrinking was personally observed during the different field visits, and it was testified with temperature records by the unexpectedly large number of days at which melt likely occurs. This raises concerns about the potential loss of a unique and unexplored biodiversity that will be paralleled by the vanishment of cave ice during the upcoming years, in the investigated ice caves as well as those of temperate regions globally.

Our ongoing research in several other ice caves will provide a better understanding of the biodiversity of these cryospheric ecosystems, and of the relationship between ecological features and the isotopic signature of the cave ice. We will also perform radiocarbon dating of the ice to estimate its age and correlate it with paleogeographic events and faunal composition.

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