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Research Article

The Unexplored Biodiversity of 'Glacier Fleas' (Hexapoda: Collembola): Taxonomy, Distribution and Ecology in the European Alps and Apennines

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Springtails (Hexapoda: Collembola) are unique among Alpine arthropods for including cryophilic species, able to live in contact with glacial ice, the so-called 'glacier fleas'. Despite being historically recorded, their taxonomy and distribution are largely unknown. In this article, we present the first comprehensive study of ice-dwelling springtails (family Isotomidae) of the European Alps and Apennines. Morphological and molecular analyses of two mitochondrial genes (*cox1* and *16S*) were performed after an extensive field sampling across 48 European sites. Five new species were identified and described on the Alps: *Desoria orobica* sp. nov., *Vertagopus glacialis* sp. nov., *V. psychrophilus* sp. nov., *V. glacieinigrae* sp. nov. and *V. fradustaensis* sp. nov., together with the already known *D. saltans* and *V. alpinus*. The evidence for two further new species was also reported, with the first occurrence of *Gnathisotoma bicolor* for the Alpine chain. *Desoria calderonis* occurs on the only glacier of the Apennines. Among the new species, *V. glacialis* and *V. psychrophilus* exhibit a wide range distribution, while the other species show a narrow endemic distribution. The study highlighted the unexplored diversity of Alpine 'glacier fleas' and their ecological and biogeographic interest, together with the conservation concern in the context of the present warming cycle.

Keywords: *16S*; cold-adapted species; *cox1*; cryophilic; *Desoria*; glaciers; ice-dwelling springtails; integrative taxonomy; species description; *Vertagopus*

1. Introduction

The cryosphere, especially mountain glaciers, faces significant threats from climate change [1], undergoing profound transformations that impact biotic communities [1–3]. As a consequence of intense melting, many glaciers are increasingly covered by stony debris [4–6]. By the end of the 21st century, most glaciers in the Alps are expected to disappear [7–9]. Permafrost and related landforms (for example, rock glaciers) are also experiencing heavy degradation, for instance through the thickening of the active layer and the reduction of ice volume [10].

Glacial and periglacial landforms host a unique biodiversity of highly specialised and often endemic organisms, adapted to low temperatures [11]. The potential disappearance of these habitats poses a significant risk to this biodiversity, underscoring the urgency for their study and conservation [2, 3, 12–14]. Studying glaciers and rock glaciers, as well as their associated biodiversity, is a necessary prerequisite for future monitoring and management actions [2, 15].

Springtails (Hexapoda: Collembola) are edaphic arthropods that colonise a wide spectrum of habitats, from Equatorial forests to cold biomes [16]. They are among the few terrestrial animals able to colonise Antarctica [17] and,among Alpine arthropods, they are unique for including cryophilic, ice-dwelling species, that is thriving in direct contact with glacial ice [2, 14, 18]. Such species belong to the family Isotomidae [11, 18, 19] which represent 'true ice-dwelling' cryophilic species [11]. Such species should be distinguished from 'firn- and debris-dwelling' cryophilic species that occupy iceless snow patches and the habitat at the margin of the ice. Similarly to snow-dwelling springtails [20], the main period of activity of ice-dwelling springtails occurs during the winter months [21], when they occupy the microhabitat at the interface between ice and snow [11]. Only when snow starts melting, they move up to the snow surface and, during summer, after snow disappearance, they move on the ice and into the ice interstices, down to a depth of about 30 cm [11]. Some species are also obligate ice dwellers, unable to survive elsewhere.

The physiological and behavioural ecology adaptation of springtails to survive under freezing conditions has been the object of many studies (for example, [11, 22–28]). Steinböck [29] demonstrated that ice-dwelling species have extreme adaptations to cold conditions, falling into a chill coma only at −16°C, whereas less adapted, non-glacial species become inactive at −6°C. Furthermore, Schaller and Zinkler [30] found that ice-dwelling springtails have comparatively high respiration rates close to 0°C, demonstrating active metabolism around the freezing point.

Springtails are key components of glacier food webs [31, 32], feeding primarily on in-blown organic material, like pollen granules [21, 28], and on biofilm [32] and serving as prey for numerous predators, like spiders and ground beetles [21, 33, 34].

In high mountain areas, glaciers and permafrost-related landforms (for example, rock glaciers) probably act for ice-dwelling species like islands of ice in a matrix of rocky grounds, prairies, woods and other ice-free landforms, like the so called 'sky islands' [35]. In this situation, the patchily distributed ice-dwelling springtail populations are good candidates for studying the biogeography and ecology of Alpine glacial environments [18].

Desor [36] cited for the first time *Desoria saltans* Nicolet, 1841, noteworthy for its showy, swarming and large assemblages on Alpine glaciers. For the following years, this species was considered the only Alpine ice-dwelling springtail, the so-called 'glacier flea'. Haybach [37] described a new species of ice-dwelling Isotomidae—*Vertagopus helveticus* Haybach, 1980—on the Morteratsch glacier (Bernina, Switzerland), under supraglacial stony debris. This species is taxonomically related to *Vertagopus alpinus* Haybach, 1972, which had been discovered a few years before in the snowy and high-altitude habitats of Grossglockner, close to the Pasterze glacier (Austria). Only recently another ice-dwelling *Desoria* species has been described from the last remaining glacier of the Apennines (Abruzzo, Italy), *D. calderonis* Valle, 2021 [18], while *Vertagopus nunataki* Potapov & Kremenitsa, 2020 was described from Caucasus [38], although this latter was collected close to a glacier rather than on it.

Thus, in Europe, at least two genera of ice-dwelling Isotomidae are currently thought to be associated with the supraglacial habitat, *Desoria* Nicolet in Desor, 1841 and *Vertagopus* Bagnall, 1939. A third one, *Gnathisotoma* Cassagnau, 1957, is a debris-dwelling cryophilic springtail known to occur in Europe on Pyrenees and Cantabrian mountains and, possibly, a key genus of ice-dwelling alpine springtail evolution [39].

The diversity and distribution of ice-dwelling springtails in the European Alps, however, have been relatively understudied, with existing research suggesting that their diversity is likely underestimated [11, 18, 24].

This article aims to address this gap by presenting the first comprehensive description of ice-dwelling springtails of the European Alps and Apennines employing an integrative taxonomy approach (using morphology and DNA barcode, integrated with biogeographical and ecological information), based on extensive field sampling in 48 sites on Alps, Apennines and Pyrenees.

2. Material and Methods

2.1. Study Area. The sampling was performed in 48 sites in the European Alps (45), Apennines (1) and Pyrenees (2) (Table A1, Figures 1A–H, 2). In the European Alps, we included a wide spectrum of glacier size throughout the Alpine chain. *Desoria calderonis* specimens were sampled by Valle et al. [18] on the Calderone glacier, the last glacier of the Apennines (Table A1).

2.2. Sampling Activity. The sampling activity (Figures 1A–H) was performed during the summers of 2020–2023, in the period July–September, on glaciers and rock glaciers in the European Alps and Apennines (Table A1). Snowfields in extinct glacial sites were also included (i.e., areas formerly occupied by glaciers where perennial or semi-perennial snow cover is still present; Figure 1D). Specimens were searched in the supraglacial habitats, in contact with the ice. Sampling consisted of hand-catching where springtails made consistent assemblages on snow or under

FIGURE 1: (A) Thick supraglacial continuous debris where *V. alpinus* was found (Pasterze glacier), (B) Thin supraglacial continuous debris inhabited by *V. glacialis* sp. nov. (Amola glacier), (C) *V. glacialis* sp. nov. assemblages under a stone on bare ice (Forni glacier), (D) linear transect of 1.5 m at the border of the snow patch where *D. orobica* sp. nov. was sampled (Trobio Est glacier), (E) sampling of *D. saltans* in ice fractures on Glacier Blanc, (F) *V. psychrophilus* sp. nov. assemblages on firn (Agola glacier), (G) *V. glacialis* sp. nov. (Forni glacier), (H) *D. orobica* sp. nov. (Trobio Ovest glacier).

the stones on bare ice (Figure 1C,E,F), whereas the flotation method [40, 41] was used to collect the specimens occurring inside the supraglacial stony debris (i.e., frontal or medial moraines, dirt cones) (Figure 1A,B,D). For rock glaciers, it was not possible to reach the ice because of the deep debris layer, and only sub-surface interstitial habitats were inspected. *Gnathisotoma* specimens of the Pyrenees were sampled with the flotation method in the stony debris at the margin of snow patches in relict glacial areas (Table A1).

2.3. Morphological Analysis

2.3.1. Specimen Preparation and Conservation. Specimens were preserved in 96% ethanol at −20°C. For slide preparation, they were passed in boiling alcohol to remove fats and cleared by a short immersion in 10% KOH solution. Then, specimens were passed in chloralphenol and finally mounted on permanent slides using Swann medium as a preservative solution [42]. Other specimens were initially cleared through a short

immersion in 10% KOH solution and then mounted on depression slides using lactic acid as a preservative solution for better observing the body shape and other diagnostic parts. The specimens fixed in ethyl alcohol are deposited at the Science Museum of Bergamo, Italy. Holotypes and paratypes of new species and material examined for known species are lodged in the following collections: Senckenberg Museum of Natural Sciences, Görlitz, Germany (SMNG), Science Museum of Bergamo, Italy (MCSNBG), MUSE—Science Museum of Trento, Italy (MUSE). Other topotypic material is deposited in thesecollections.

2.3.2. Species Identification. Identification was performed through the keys reported in Potapov [43]. Morphological observations and pictures were made with light microscopes Leica DM2500 with phase and DIC contrasts and drawing arm, a Nikon Eclipse Ci and Carl Zeiss Axiolab 5 with phase contrast, a Carl Zeiss AXIO Zoom V16 stereomicroscope and a Quanta400 (FEI) scanning electron microscope.

2.4. Genetic Analysis

2.4.1. DNA Sequencing. Whole genomic DNA was extracted from 6 to 10 specimens (entire organism) from each of the sampled species found in each site, using the Wizard® SV Genomic DNA Purification System (Promega, Madison, WI, USA) according to the manufacturer's instructions. Due to limited sample availability, a smaller number of individuals was extracted for some localities (Table A1). DNA extraction of individuals from ORN and PEL was performed on the body while the head was prepared in slides, to verify, by comparing morphology and genetics (i.e., the *cox1* and/or *16S* sequence or amplification efficacy with specific primers)—the efficacy of head diagnostic characteristic for discriminating among *V. alpinus*, *V. glacialis* and *V. psychrophilus* based on the presence/absence of seta among OMMA A, B and C, D and of seta *e7* on labium. Overall, 17 specimens were analysed, 7 *V. glacialis* (ORN20, 27; PEL 20,29,31,34,36), 6 *V. psychrophilus* (ORN 21,22,23,24,26,31) and 4 *V. alpinus* (PEL 20,24,26,28).

For each specimen, we amplified and sequenced two mitochondrial markers. The *cytochrome c oxidase subunit 1*, 5P fragment (*cox1*) was amplified using the primers 5′ GGTCAACAAATCATAAAGATATTGG 3′ (LCO1490, forward) and 5′- AAACTTCAGGGTGACCAAAAAATCA - 3′ (HCO2198, reverse) [44]. The mitochondrial ribosomal large subunit RNA (*16S*) was amplified using the primers 5′-CCGGTCTGAACTCAAATCATGT - 3′ (LR-J-12887M, forward) and 5′- CGACTGTTTAACAAAAACAT - 3′ (LR-N-13398M, reverse) (Simon et al. [45], modified after Zhang et al. [46]).

Due to the non-specific amplification for *cox1* in *D. orobica* sp. nov. (TRO), *G. bicolor* (TGN and TOU_14), *V. alpinus* (MIA_3-4, MDG, and PAS) and *V. glacieinigrae* sp. nov. (GLN), we designed a different primer pair for this locus and employed it for populations of these four species. Specifically, a new forward primer (LCO_ALPI; 5′ TCAACAAACCACAAAGATATTGG 3′) was obtained from the correction of LCO1490 using the BOLD sequence of *Desoria tigrina* (OD987622.1). Two new HCO primers were specifically designed using a partial sequencing of the neighbour fragment of cytochrome c oxidase subunit 1 achieved through the partial amplification of the adjacent locus through the primers COIf (5′ - CCTGCAGGAGGAGGAGATCC - 3′, forward) COIa (5′-AGTATAAGCGTCTGGGTAGTC - 3′, reverse; [47]) for *D. orobica* sp. nov. (TRO), *G. bicolor* (TGN and TOU_14), *V. alpinus* (MIA_3–4, MDG, and PAS) and *V. glacieinigrae* sp. nov. (GLN). As such, two distinct reverse primers were obtained: HCO_PAS (5′- TATACTTCAGGRTGGCCAAAAAATCA - 3′), used with LCO_ALPI to amplify and sequence *V. alpinus* (PAS, MIA, MDG) and *V. glacieinigrae* sp. nov.; and HCO_TRO (5′- TAYACTTCTGGGTGCCCAAAAAATCA - 3′), employed with LCO_ALPI to amplify and sequence *D. orobica* sp. nov. (TRO) and *G. bicolor* and (TGN, and TOU_14).

PCRs were prepared with AmpliTaq Gold 360 Master Mix in a 10-μL reaction volume containing: 2 μL of whole genomic DNA and 0.5 μL of both forward and reverse primers (10 μM). Amplifications were run with the following conditions for each of the 35 cycles: a denaturation step at 95°C for 1 min, an annealing step at 50°C for 1 min and an elongation step at 72°C for 90s. An additional initial denaturation step was set at 95°C for 5 min as well as a final extension step at 72°C for 7 min [18, 47]. For TGN *cox1* amplification, PCR cycles were extended up to 40 cycles. For *D. calderonis* (CAL), the *16S* marker was sequenced from the same nine specimens of the original description [18].

PCR products were then purified using the kit Wizard SV Gel and PCR Clean-up (Promega, Madison, WI, USA), sequenced on both strands with a 3730 x l DNA Analyzer (Applied Biosystems) at the BMR Genomics laboratory (Padova, Italy) and assembled using Benchling [48]. Final corrected sequences were deposited in Genbank (NCBI) under accession numbers: PP441687–PP441920 (*cox1*) and PP465209–PP465442 (*16S*) (see Table A3 for details).

2.4.2. Phylogenetic Analysis. The final datasets included 232 sequences for both the *cox1* and the *16S* markers. To understand the phylogenetic relationships of the sampled species, three outgroups (*Cryptopygus terranovus*, *Folsomia candida* as in Valle et al. [18] replacing *Parisotoma notabilis*, for which both *cox1* and *16S* from the same specimens are not available, with *Folsomotoma octooculata* due to its proximity with studied ingroups [49]) were added to these two datasets and independently aligned. The *cox1* dataset was aligned using macse v. 2.07 (*-gc_def 5* [50],) to preserve codon structure, while the *16S* dataset was aligned using MUSCLE [51] as implemented in Aliview v. 1.28 [52]. The two datasets were then trimmed in Gblocks (*-t = c -b1 = $var -b2 = $var -b3 = 6 -b4 = 10 -b5 = h*; where $var = number of sequences/2 + 1; [53]) and concatenated through catsequences (https://github.com/ChrisCreevey/catsequences). Sequences were collapsed into haplotypes using DNAcollapser as implemented in the FaBox web server (https://birc.au.dk/~palle/php/fabox/dnacollapser.php). The resulting final matrix was 985 nucleotides in length and accounted for 111 haplotypes (including the outgroups). Haplotype sequence headers were renamed

according to all the haplotype site names. The alignment, along with the scheme partition file divided into blocks by gene and codon position (only for the *cox1*), was used for an IQTREE2 v. 2.2.0 (*-B 1000 -m MFP+MERGE;* [54]) analysis, with model estimated using the BIC criterion GTR+F+I+G4 for all the three partitions.

To unravel the possible phylogenetic relationships with other species belonging to the subfamily Isotominae, the published dataset used in Valle et al. [18] *cox1* fragment only was downloaded from BOLD using the bold R package [55]. The longest sequence for each species studied in this work was added to the Valle et al. [18] dataset. The final trimmed alignment accounted for 97 sequences with 657 nucleotides in length. The matrix was partitioned as Valle et al. [18]- that is excluding the third position — and run in IQTREE2 with previously reported parameters. Both phylogenetic trees were visualised in iTOL v.6 [56].

2.4.3. Genetic Distances. To quantify genetic distances within and among species in the genera of interest, all *Desoria* and *Vertagopus* COI-5P sequences were downloaded from BOLD using the bold R package [55] and aligned through macse v.2.07, as previously described. Unaligned sequences, possibly belonging to another *cox1* fragment and erroneously deposited in BOLD as COI-5P, were removed. Similarly, all *cox1* sequences of Alpine springtail species were aligned with macse v.2.07, as previously described. Both matrices were separately imported in R v.4.3.2 [57]. Genetic distances were separately calculated for both matrices with the ape package [58] using the dist.dna function (model = 'raw', pairwise.deletion = T) and plotted with ggplot2 [59].

2.4.4. Species Delimination Analyses. Two methods of species delimitation were employed to assess species number and species boundaries within the COMBINED dataset. In detail, the glacial springtail phylogenetic tree was processed with bPTP (bayesian Poisson Tree Processes; available at: https://species.h-its.org/) under default parameters and applying a burn-in of 0.25 [60]. The corresponding sequence alignment was analysed in ASAP (assemble species by automatic partitioning; available at: https://bioinfo.mnhn.fr/abi/public/asap/) using default parameters [61]. The best partition was identified according to the lowest asap-score.

2.5. Nomenclatural Acts. The new names contained in this article are available under the International Code of Zoological Nomenclature. This work and the nomenclatural acts it contains have been registered in ZooBank. Zoobank Life Science Identifier (LSID) for this publication is: urn:lsid:zoobank.org:pub:CF4D6E35-869A-4677-B8C7-3577F6C95144. The LSID registration and any associated information can be viewed in a web browser by adding the LSID to the prefix 'https://zoobank.org/'

3. Results

Ice-dwelling springtails were found in 32 sites of the 45 sites in the investigated European Alps (Table A1, Figure 2), and on the only glacier of the Apennines. All the ice-dwelling springtails were found on clean-ice glaciers and debris-covered glaciers, and not on snow patches and rock glaciers (Table A1), where only ground-dwelling species were found. In most of the cases, one single species was found on each glacier, but in eight sites two or three species were found (Table A1, Figure 2).

3.1. Morphological Analyses. Morphological analyses allowed the identification of eight distinct species on the European Alps, three already known - *Desoria saltans*, *Vertagopus alpinus and Gnathisotoma bicolor*, while five were undescribed so far: *D. orobica* sp. nov., *V. glacialis* sp. nov., *V. psychrophilus* sp. nov., *V. glacieinigrae* sp. nov. and *V. fradustaensis* sp. nov. (Table A1). Species descriptions and morphological notes are reported at the end of the results.

3.2. Genetic Analysis. The phylogenetic tree based on combined sequences of *cox1* and *16S* (Figure 3) is well structured, with long internal nodes and clusters characterised by a limited differentiation in the terminal ones. Internal nodes are usually supported by medium–high bootstrap values (99–100 for basal nodes, 83–98 for the other internal nodes, except for one with 51). Clusters are uniform, and there is a complete correspondence of these groups with species as morphologically identified. The basal branch separates *Desoria saltans* and *D.* cf. *saltans* from all other ice-dwelling springtails. *G. bicolor* and *D. orobica* constitutes two well separate clades, while *D. calderonis*, *V. glacieinigrae*, *V. alpinus*, Isotomidae sp., *V. psychrophilus*, *V. glacialis* constitute the apical branch of the tree. Two previously known species *D. saltans* and *V. alpinus* display intra-specific variability. Within *G. bicolor*, the individuals sampled in the Alps are reciprocally monophyletic with those from the Pyrenees. For Fellaria (FEL2), Miage (MIA1,5), Peirabroc (PEI4) and Scais (SCA1-5), we have a few specimens with only genetic data: SCA1-5 and FEL2 sequences correspond to *D. saltans*, PEI4 sequence is close to *D. saltans* but with a distance of 12.4%–13.6% (thus called *Desoria* cf.*saltans*), while MIA1,5 sequences were not closely related to any other known sequences and was nominated *Isotomidae* sp. (Figure 3).

Species delimitation analyses, performed using bPTP and ASAP, produced identical results identifying 17 different partitions with medium/high bPTP bayesian support (Figure 3). Eight out of 11 species (*D.* cf.*saltans*, *D. orobica*, *D. calderonis*, *V. glacieinigrae*, *Isotomidae* sp., *V. psychrophilus*, *V. fradustaensis* and *V. glacialis*) were retrieved as separate partitions that perfectly match the morphological and phylogenetic results. Three species (*D. saltans*, *G. bicolor* and *V. alpinus*, as identified based on morphology and phylogeny) were further split in 2–5 partitions each. These do not contrast with species attribution but may suggest additional internal diversification within named species (Figure 3).

Genetic distances from *cox1* reference sequences identified a distribution that spans between 0 and 1.8% for intra-specific comparisons and a spectrum higher than 14.2% (with a long tail in the upper range) for inter-specific comparisons (Figure 4). Some outliers of intra-specific diversity were identified around 14.5–20% and probably arise from unexpected divergence within named BOLD species that probably represent undescribed cryptic diversity. Within our dataset, intra-specific divergence ranges between 0 and 2.2%, while inter-specific divergence is higher than 10.6% in all inter-specific comparisons, showing a good correspondence to BOLD

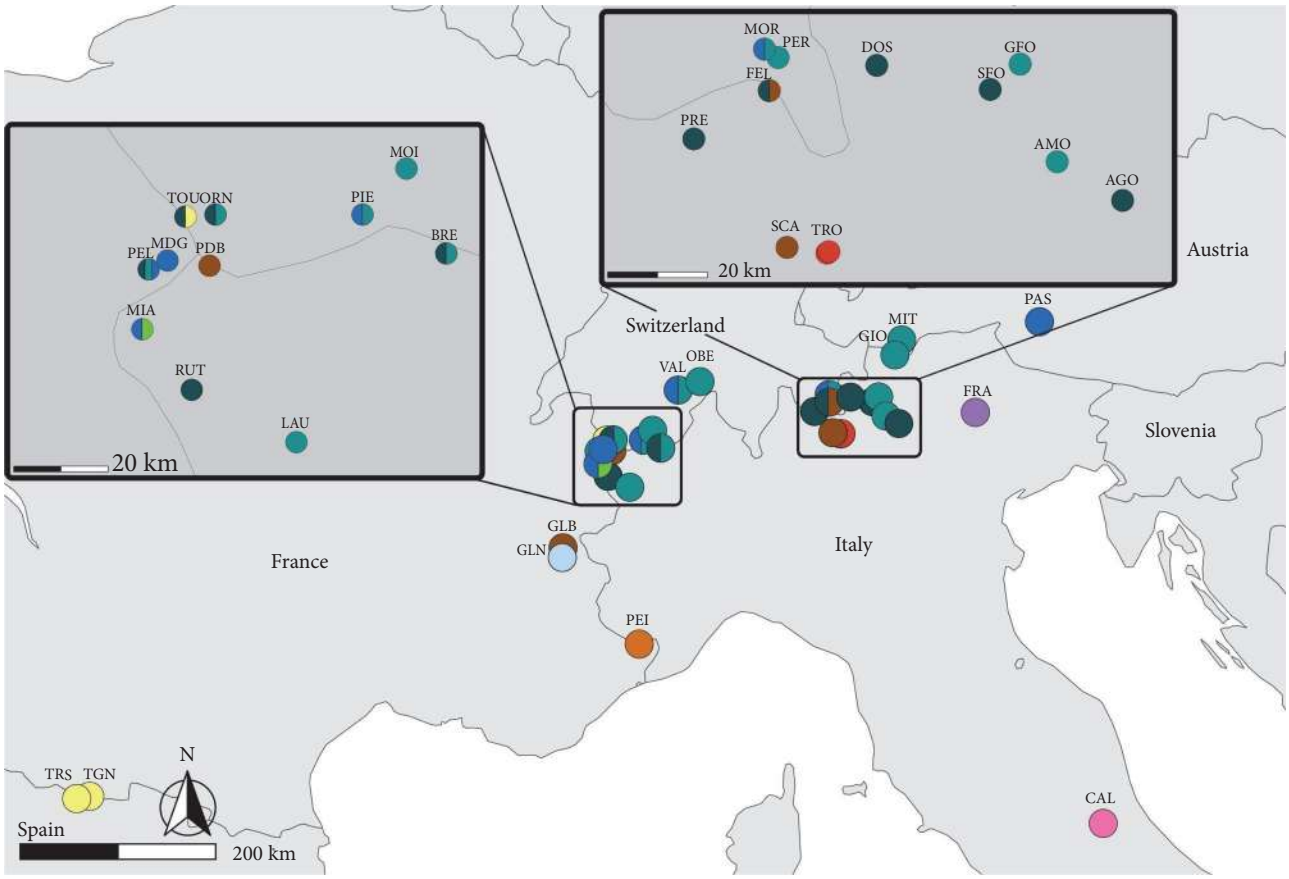

FIGURE 2: Distribution of the ice-dwelling springtail species found in this work on European Alps, Apennines, and Pyrenees.

divergences and support for the species distinctions (Figure 4). A limited number of species show very high intra-specific divergence (around 4.4%–6%; red arrow in Figure 4): they correspond to *D. saltans*, *G. bicolor* and *V. alpinus* that display variability within species (visible also in the phylogenetic tree and in the species delimitation analyses, Figure 3). Overall, the level of differentiation, both within and among clusters, is fully in line with that observed for named species of the same genera in the BOLD database, supporting the subdivision of specimens collected among clusters. The distribution of intra- and inter-specific distances for *cox1* visualised as a heatmap (Figure 5) shows the high intra-specific variability of *D. saltans* and *V. alpinus*, and identifies a close relationship among all *Vertagopus* species, including the unidentified *Isotomidae* sp. samples and *D. calderonis*.

Based on genetic analysis and morphologic description, paraphyly of *Desoria* is evident, from which a node emerges for *Gnathisotoma*, and another one includes the five *Vertagopus* species found in this work.

The phylogenetic investigation of the collocation of the new species described within the Isotominae tree is not possible because of the difficulty of collecting genetic information for such a large group of species. However, a preliminary investigation performed with the available data was conducted comparing our sequences (*cox1* only) with the BOLD database, currently the most complete available dataset (Figure S1). Although the tree shows low bootstrap values and a polyphyletic distribution of the genera, the glacial species found in this work emerged as three separate clusters. This suggests that the ice-dwelling *Vertagopus* species, *D. calderonis* and *D. orobica* represent a monophyletic group, separated from *D. saltans* and *G. bicolor*.

From the specimens of *V. alpinus*, *V. glacialis* sp. nov. and *V. psychrophilus* sp. nov. for which the barcode was obtained after removing the head for morphological identification, it is possible to affirm that *e7* seta on labium is always present in *V. alpinus* and *V. psychrophilus*, while on *V. glacialis* it could be missing in either one or both sides of the labium. The seta

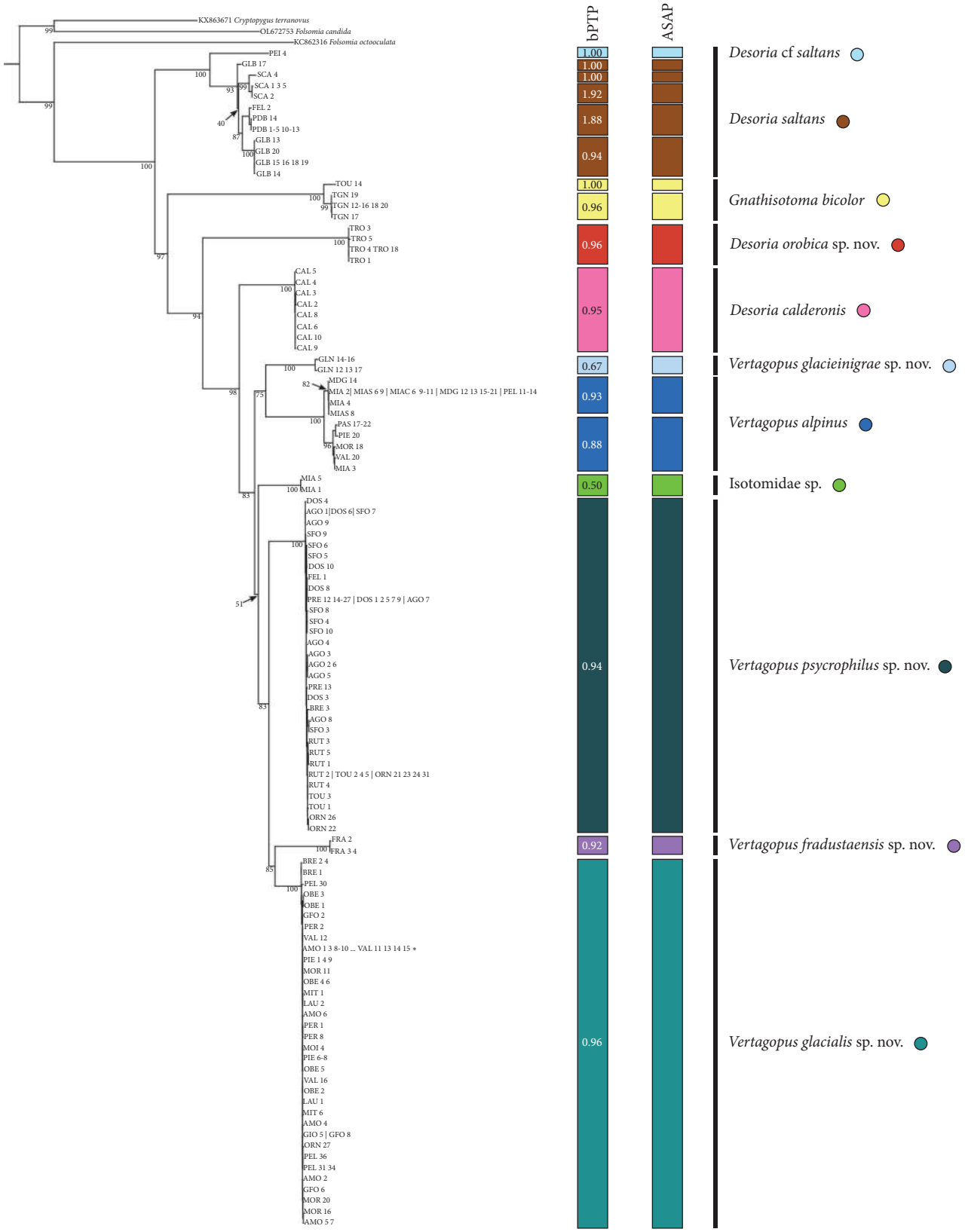

FIGURE 3: Maximum likelihood phylogenetic reconstruction of ice-dwelling springtail species included in this work performed using IQTREE2. The tree was generated using a concatenated matrix of *16S* and *cox1* markers. Bootstrap values are reported as numbers at nodes only for inter-species relationships. The phylogenetic tree was rooted using the three outgroups *C. terranovus*, *F. candida* and *F. octooculata*. Asterisk-tagged sequence haplotype corresponds to the following specimens: AMO 1, 3, 8–10 | GFO 1 3 7 10 | GIO 4, 10–16 | LAU 3–5 | MIT 2–5 | MOI 1–3, 5–10 | MOR 19 | PER 3–7 | PIE 2, 3, 5, 10 | VAL 11, 13, 14, 15 (all haplotypes are reported in Table ST1). Black bars show monophyletic clades, corresponding to the new taxonomic assignments of the individuals. The coloured circles correspond to colour legend in Figure 2 representing the geographic distribution of the species (see Table A1 for individual codes). The two coloured bars describe the results of the species delimitation analysis. bPTP's bayesian support values are indicated within the relevant bar.

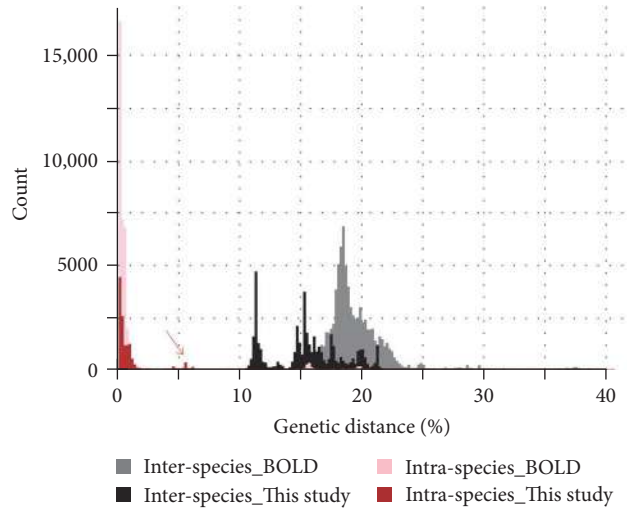

FIGURE 4: Frequency distribution histogram of genetic distances based on *cox1* sequences for 1173 species belonging to the genera *Desoria*, *Gnathisotoma* and *Vertagopus*. Grey and pink columns show inter- and intra-specific genetic distance frequencies of the BOLD system dataset; black and red columns show the inter- and intra-specific genetic distance frequencies of the ice-dwelling springtail species included in this work. The red arrow indicates the group of outliers for the intra-specific distance that corresponds to *D. saltans*, *G. bicolor* and *V. alpinus*.

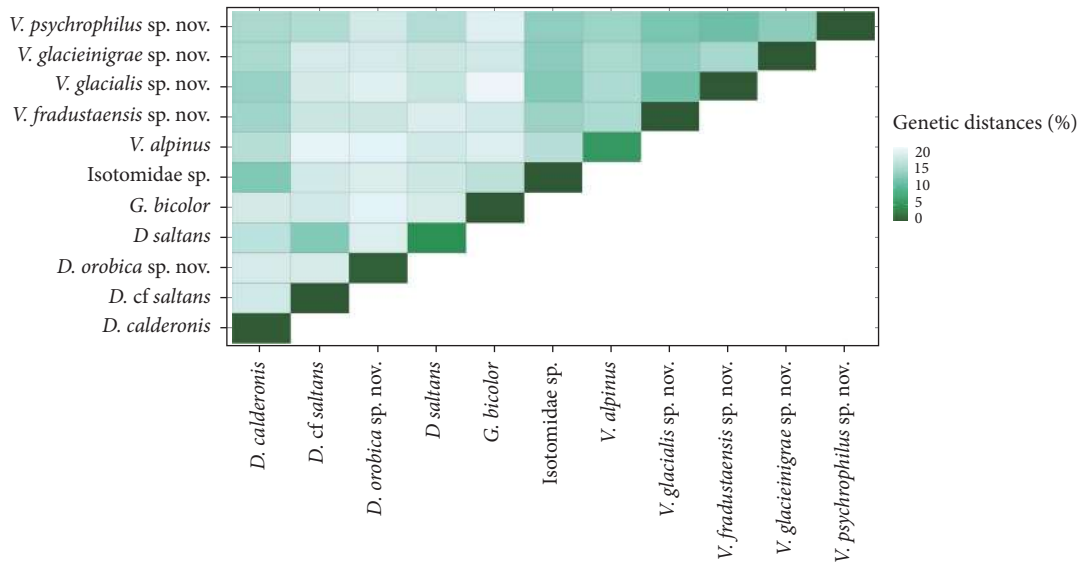

FIGURE 5: Pairwise genetic distance heatmap for the Alpine species (*cox1*). Dark and light shades of green indicate a low and high genetic distance between samples. Value ranges are detailed in Figure S2.

among ommatidia is absent in *V. alpinus* and *V. glacialis* sp. nov. and present in *V. psychrophilus* sp. nov.

3.3. Species Descriptions. From both morphological and phylogenetic analyses, *Vertagopus glacialis*, *V. psychrophilus*, *V. glacieinigrae* and *V. fradustaensis* (Figures 6A,B,C,F) resulted to be species new for science belonging to the genus *Vertagopus* genus, while *Desoria orobica* (Figure 6I) is a new species morphologically belonging to the *Desoria nivalis* complex. For the already described *D. saltans*, *Gnathisotoma bicolor*, *V. alpinus* and *V. helveticus* (Figures 6D,E,H,L), we present taxonomic, morphological and ecological notes for the Alpine populations. Genetic evidence indicates that *Desoria* cf. *saltans* found on Peirabroc ('PEI4'; Maritime Alps) and Isotomidae sp. on Miage ('MIA1,5'; Mont Blanc) could likely represent undescribed species; however, an insufficient number of specimens was found for their proper description.

4. *Vertagopus psychrophilus* Valle sp. nov. Figures 1F, 6B, and 7A–I

4.1. Material Examined. Holotype: ITALY • ♂; Lombardia, Valfurva (Sondrio province), European Alps, Ortles-Cevedale massif, Sforzellina Glacier ('SFO'); under stones on bare ice; on supraglacial snow and in supraglacial debris, 46°20′58.7"N, 10°30′44.1"E, 2860 ma.s.l.; 5 Aug. 2020, B. Valle leg.; B. Valle det., collected with flotation method; Genbank (NCBI)

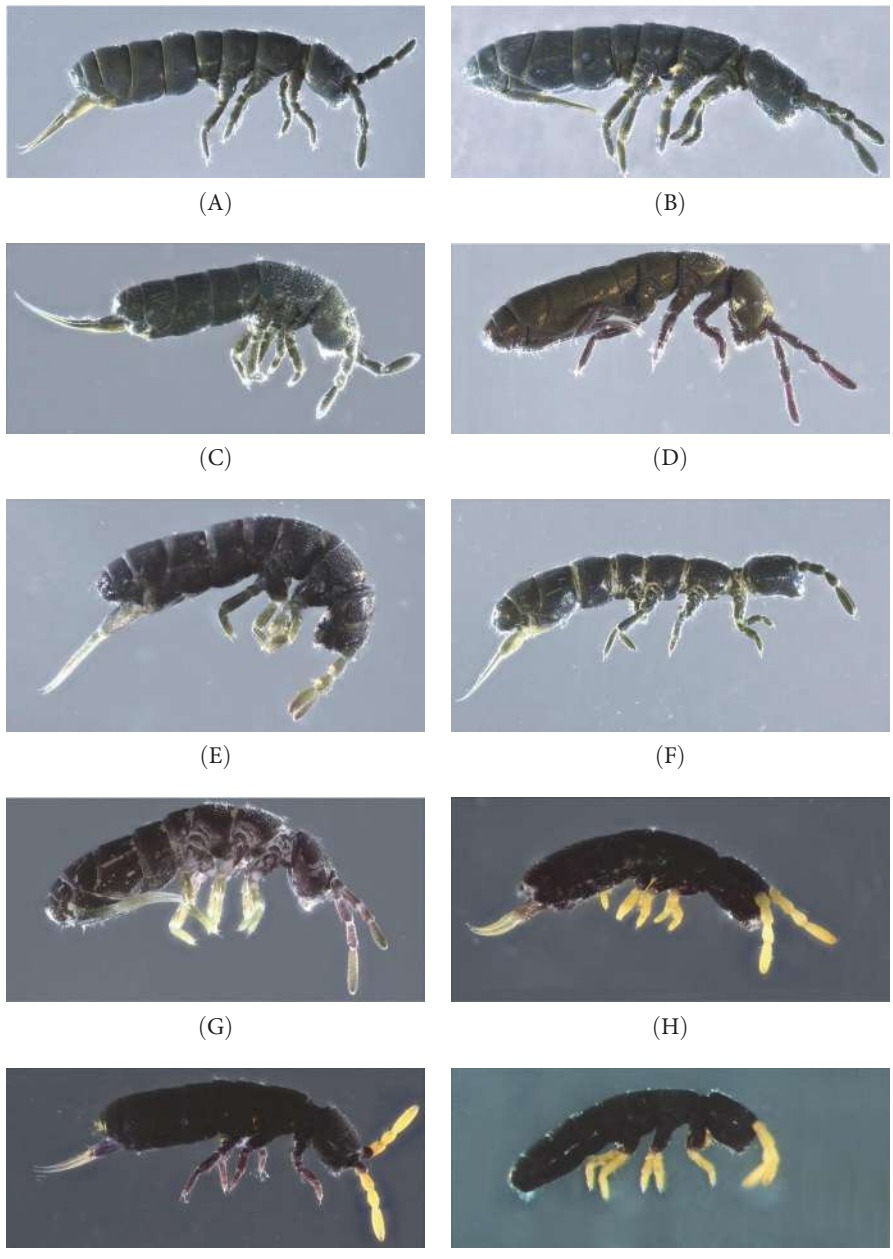

FIGURE 6: A–J. Colour pattern of the species reported in this work. (A) *Vertagopus glacialis* sp. nov, (B) *Vertagopus psychrophilus* sp. nov, (C) *Vertagopus glacieinigrae* sp. nov, (D) *Desoria saltans*, (E) *Vertagopus alpinus*, (F) *Vertagopus fradustaensis* sp. nov, (G) *Desoria calderonis*, (H) *Gnathisotoma bicolor* from Pyrenées, (I) *Desoria orobica* sp. nov, (J) *Gnathisotoma bicolor* from Mont Blanc.

PP441886-63 (*cox1*), PP465408−15 (*16S*); (SMNG-APT-AA04468).

Paratype: ITALY • 2 ♂ 6 ♀; same collection data as for holotype; (MCSNBG-205 to 208, SMNG-APT-AA04469 to 72); ITALY • 1 ♂ and 2 ♀; Lombardia, Valdidentro (Sondrio province), European Alps, Dosdè Glacier ('DOS'); under stones on bare ice; 46°23′34.8"N, 10°12′58.9"E, 2760 ma.s.l.; 5 Aug. 2020, R. Ambrosini, R.S. Azzoni leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441719-28 95 (*cox1*), PP465241−50 (*16S*); (SMNG-APT-AA04473 to 75). ITALY • 1 ♂ and 2 ♀; Trentino, Stenico (Trento province), European Alps, Brenta Dolomites, Agola Glacier ('AGO'); on supraglacial snow and ice, 46°08′57.9"N, 10°51′27.0"E, 2580 ma.s.l.; 10 Aug. 2020, M. Gobbi leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441687-95 (*cox1*), PP465209-17 (*16S*); (MUSE-INV-c001 000414 to 416).

4.2. Description. Body: Mean body length (with antennae) 1.9 (standard deviation: 0.3 mm on 11 specimens, see Table A2). Colour totally black, also juvenile, but the basis of the furca can be paler (Figure 6B). Cuticle granulation fine and regularly distributed; all dorsal tergites clearly separated from each other (Figures 7D, S3A). Abd. III and IV of approximately the same width.

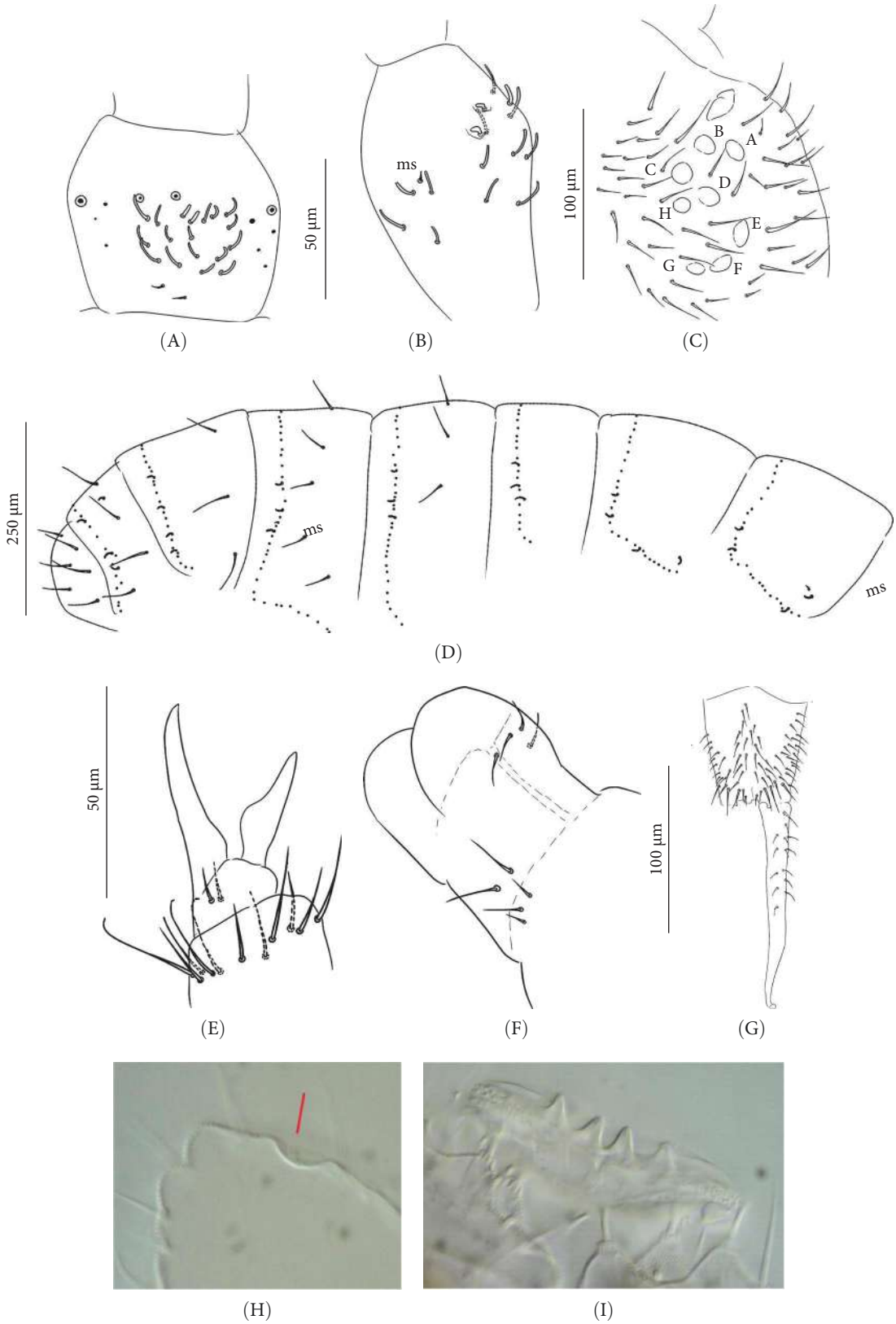

FIGURE 7: A–I: *Vertagopus psychrophilus* sp. nov. (A) Ant. I, ventral side, (B) Ant III, (C) ocular plate (A–H: ommatidia), (D) *accp* sens and macrosetae, (E) Claw III, (F) latero-posterior side of VT, (G) ventral side of furca, (H) Ant. IV subapical organite (red arrow) and pin-like seta, (I) apical folds of labrum.

Chaetotaxy: Terga consist of micro-, meso- and macrosetae, these latter well differentiated on last abdominal tergites (Abd. IV–VI, in median position) but not well distinguished from ordinary setae on other tergites (Figure 7D). No setae in the ventral side of Th. II–Th. III. All setae smooth. Macrosetae on Abd. V 0.6 times the median length of tergite and 1.4 times as long as the inner edge of Claw III (Table A2). Sensory chaetotaxy constituted by *ms*-setae, *accp*-, *al*- and *as*-setae. Only Th. II and Abd. III have *ms*-setae (formula 10/001). Dorsal *s*-setae constituted by single *al*-seta on Th. II and Th. III, single *as*-seta on Abd. V and by *accp*-setae normally set within *p*-row (Figure 7D). The number of *accp*-setae can be expressed as (2)3, (2)3/ (2)3, (2)3, 3, 4(5), 3(4).

VT with 0 + 0 anterior, 4 + 4 latero-distal and (4)5-6 posterior setae with 2 in apical transverse row (Figure 7F). Retinaculum with 4 teeth and 4-7 setae.

Head: Antennae slightly longer than cephalic diagonal (D/A = 0.38, Antennae = 0.43 mm; Table A2). Ratio among Ant. I/Ant. II/Ant. III/Ant. IV is 1/1.5/1.4/2.7 (Table A2). There are often cases of asymmetry among *s*-setae between antennae of the same specimen. Ant. I has 12–23 *s*-setae in ventro-lateral position, (1)-2-(3) of them are shorter and in distal position; two microsetae in ventro-proximal position (Figure 7A). Ant. II 4–6 *s*-setae in distal position. Ant. III has a sensory field that includes two inner *s*-setae of AO III, one lateral *ms*-seta and about 11–15 *s*-setae (Figure 7B). Ant. IV has one simple small subapical, peg-shaped organite and a bifurcate pin-like seta (Figure 7H). Eye spots strongly dark pigmented with 8 + 8 ocelli (G and H smaller; Figures 7C, S3B). Presence of a seta among ommatidia A, B and C, D (Figure 7C); sometimes there is asymmetry and only one side has the seta among ommatidia (important to check several specimens). PAO elongated about two times as long as the diameter of the nearest ocellus (Figure 7C). Prelabral setae 4. Labral formula as 5,5 and 4 and 4 sharp apical folds (Figures 7I, S3I). Maxillary palp bifurcated and maxillary outer lobe with four sublobal hairs. Labial palp with 5 papillae and a total of 16 guard setae [62] distributed as: A1, B1–4, C0, D1–4, E1–7 (like in Figure S3I). Hypostomal papilla with H shorter than h1/h2. Proximal (px), basomedian (bm) and basolateral (bl) fields of labium with 4, 4 and 5 setae, respectively, and 4–8 postlabial setae. Mandible with well-developed molar plate.

Furca: Furca almost as long as the antenna (see Table A1; Figure 7G); ratio of mucro/dens/manubrium = 1/25/16. Maximum number of ventral setae on manubrium about 38–40 (less in juveniles, and it is possible to confuse them with species A), including ventral–apical setae ((8)−10) that are usually larger than the others (Figure 7G) except for 1/2 + 1/2 shorter setae in apical–medial position; about 40 dorsal setae. Dens with dorsal crenulations, more than 90 ventral and 12–14 dorsal setae. Mucro quadridentate with apical tooth much smaller than subapical one (Figure S3C).

Legs: Eleven setae in the distal apical ring of Tita; 2-3-3 very weakly clavate tenent hairs (Figure 7E), shorter than the inner edge of Cl. Claw of normal shape without lateral and inner teeth; empodium sometimes with a small inner tooth; pretarsus with a pair of setae (Figures 7E, S3E).

Distribution and habitat: V. psychrophilus has been found in 10 Alpine glaciers from Western to Eastern Alps. Most records are in Southern Rhaetian Alps in Italy, three are in Graian Alps and one in Pennine Alps (Figure 2; Table A1 for more information). In general, past records of *D. saltans* should be revised and could probably be attributed to *V. glacialis* and *V. psychrophilus* (see notes in *D. saltans*).

Ecology: V. psychrophilus is a strictly ice-dwelling springtail that can be found on bare ice under stones, within supraglacial continuous stony debris (like medial moraines), on supraglacial snow (Figure 1F), and under stones on bare ice (Figure 1C). Valle et al. [41] noted its distribution on supraglacial debris and its rapid disappearance just moving from the ice front (as '*Vertagopus* sp. nov.'). Its life cycle and behaviour on the ice may be similar to that of *D. saltans* (see notes for this species); however, *V. psychrophilus* is probably more linked to stony microhabitat on the ice.

Some past ecological studies on *D. saltans* or ice-dwelling species in general could be referred to this species, but it is not verifiable if morphological notes are not reported.

Etymology: the epithet refers to the ecology of the species that is a strictly ice-dwelling cryophilic species.

Remarks: V. psychrophilus belongs to this new group of closely related, strictly ice-dwelling *Vertagopus* species that includes also *V. glacialis*, *V. glacieinigrae* and *V. fradustaensis*. These species are close to *V. alpinus* for the absence of setae on the anterior side of the VT and a small apical tooth on mucro but differ in the presence of only weakly clavate tenent hairs (strongly clavate in *V. alpinus*). Among the group of strictly ice-dwelling *Vertagopus* species, *V. psychrophilus* is differentiated from *V. glacialis* by a higher number of anterior setae on Man (on average 38–42, 18−22 in *V. glacialis*), a higher number of *accp*-setae (usually 3 from Th. II to Abd. III, less in *V. glacialis*), the presence of *e7* seta on labium (that could be absent in *V. glacialis*) and the presence of a seta among ommatidia A, B and C, D (usually absent in *V. glacialis*). *V. psychrophilus* is differentiated from *V. glacieinigrae* for having Abd. V-VI well separated by a narrowing (they are not clearly separated in *V. glacieinigrae*) and from *V. fradustaensis* from the higher number of anterior setae on Man (38–40 in *V. psychrophilus*, 26–30 in *V. fradustaensis*).

5. *Vertagopus glacialis* Valle sp. nov. Figures 1C,H, 6A, 8A–I

5.1. Material Examined. Holotype: ITALY • ♀; Lombardia, Valfurva (Sondrio province), Ortles-Cevedale massif, European Alps, Forni Glacier ('GFO'); under stones on bare ice; 46°23′ 42.95"N, 10°35′24.79"E, 2630 ma.s.l.; 3 Aug. 2020; B. Valle leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441735−41 (*cox1*), PP465257−63 (*16S*); (SMNG-APT-AA04476).

Paratype: ITALY • 2 ♂ and 1 ♀; same collection data as for holotype; (MCSNBG-209 to 211). ITALY • 3 ♀; Alto Adige, Senales (Bolzano province), European Alps, Giogo Alto Glacier ('GIO'); under stones on bare ice; 46°46′52.1"N, 10°48′20.7"E, 2840 ma.s.l.; 21 Aug. 2020, B. Valle, M. Caccianiga leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441742−50 (*cox1*), PP465264−72 (*16S*); (MCSNBG-212 to 214). SWITZERLAND

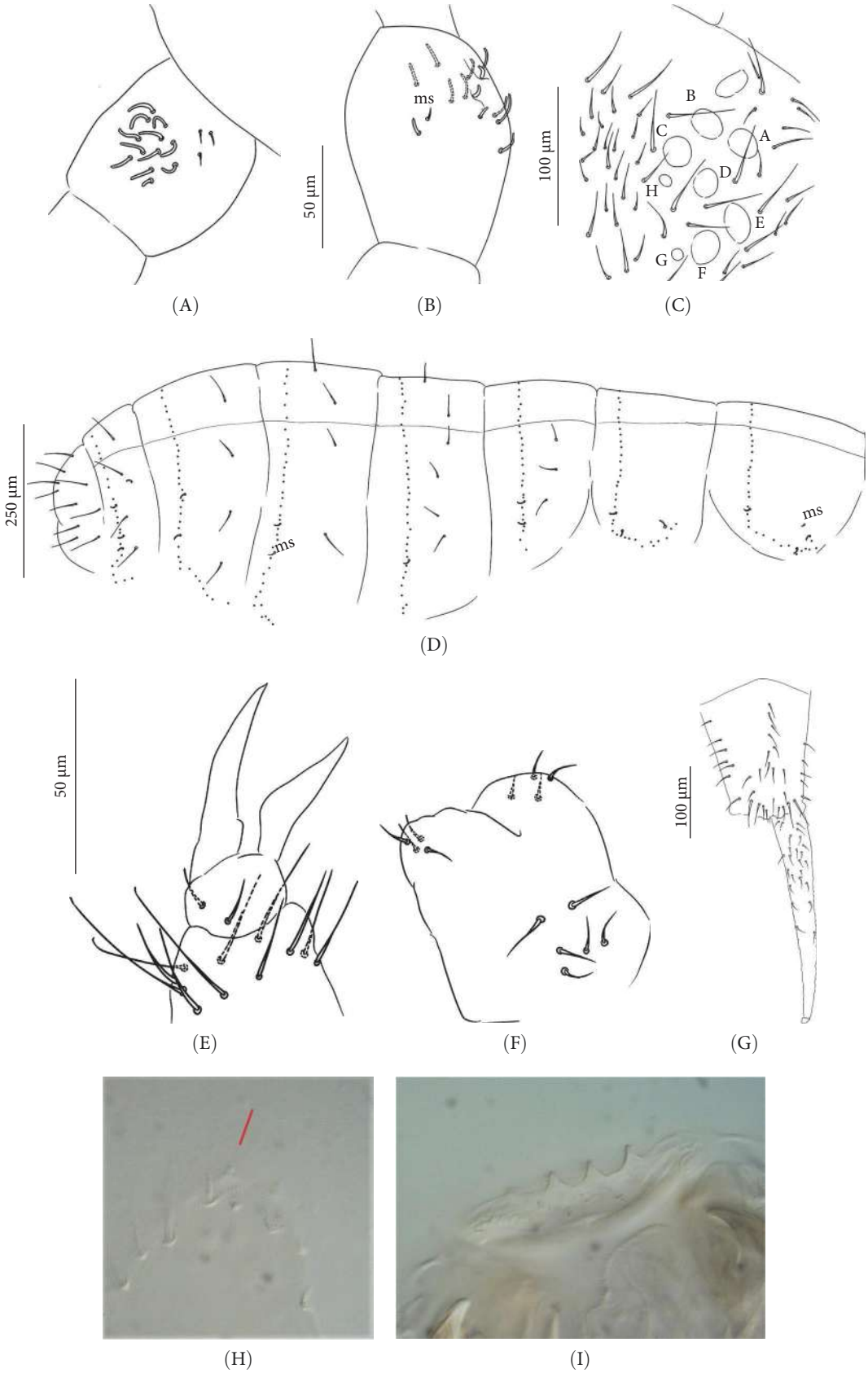

FIGURE 8: A–I: *Vertagopus glacialis* sp. nov. (A) Ant. I, ventral side, (B) Ant. III, (C) ocular plate (A–H: ommatidia), (D) *accp* sens and macrosetae, (E) Claw III, (F) latero-posterior side of VT, (G) ventral side of furca, (H) Ant. IV subapical organite (red arrow) and pin-like seta, (I) apical folds labrum, I.

• 2 ♂ and 1 ♀; Hérens, Evolène, European Alps, de Pièce Glacier ('PIE'), under stones on bare ice, 45°59′59.7"N, 7°28′12.2"E, 2750–2800 ma.s.l.; 17 Aug. 2021, J. Buda leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441859−61,63−69 (*cox1*), PP465381−91 (*16S*); (SMNG-APT-AA04477 to 79). SWITZERLAND • 2 ♂; Maloja, Pontresina, European Alps, Bernina massif, Pers Glacier ('PER'); under stones on bare ice, 46°24′25.9"N, 9°57′32.4"E, 2670–2700 ma.s.l.; 30 Jul. 2021, J. Buda leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441851−58 (*cox1*), PP465373−80 (*16S*); (MUSE-INV-c001 000417–418). SWITZERLAND • 1 ♀; Bezirk Westlich Raron, Blatten, European Alps, Lang Glacier ('VAL'); under stones on bare ice, 46°27′28.3"N, 7°55′55.8"E, 2450–2550 ma.s.l.; 10 Aug. 2021, J. Buda leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441914−20 (*cox1*), PP465436−42 (*16S*); (SMNG-APT-AA04480). SWITZERLAND • 1 ♂; Oberland, Guttannen, European Alps, Oberaar Glacier ('OBE'); under stones on bare ice, 46°32′08.6"N, 8°13′12.4"E, 2380–2440 ma.s.l.; 14 Aug. 2021, J. Buda leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441813−18 (*cox1*), PP465335−40 (*16S*); (SMNG-APT-AA04481).

5.2. Description. Body: Mean body length (with antennae) 2.2 mm (standard deviation: 0.2 mm on 29 specimens, Table A2). Colour totally black, also juvenile, but the basis of the furca could be paler (Figure 6A). Cuticle granulation fine and regularly distributed; all dorsal tergites clearly separated from each other (Figures 8D, S4A). Abd. III and IV of approximately same width.

Chaetotaxy: Terga consist of micro-, meso- and macrosetae, these latter well differentiated on last abdominal tergites (Abd. IV–VI, in median position) but not well distinguished from ordinary setae on other tergites (Figures 8D, S4A). No setae in ventral side of Th. II–Th. III. All setae smooth. Macrosetae on Abd. V 0.5 times median length of tergite and 1.2 times as long as the inner edge of Claw III (Table A2). Sensory chaetotaxy constituted by *ms*-setae, *accp*-, *al*- and *as*-setae. Only Th. II and Abd. III have *ms*-setae (formula 10/001). Dorsal *s*-setae constituted by single *al*-seta on Th. II and Th. III, single *as*-seta on Abd. V and by *accp*-setae normally set within *p*-row (Figure 8D). The number of *accp*-setae can be expressed as 2, 2/1(2), 1, 1–3, 2–3, 3(4).

VT with 0 + 0 anterior, 4 + 4 latero-distal and (4)5–9 posterior setae with 2 in apical transverse row (Figure 8F).

Head: Antennae slightly longer than cephalic diagonal (D/*A* = 0.42, Antennae = 0.45 mm; Table A2). Ratio among Ant. I/Ant. II/Ant. III/Ant. IV is 1.0/1.6/1.5/3.0 (Table A2). There are often cases of asymmetry among *s*-setae between antennae of the same specimen. Ant. I has 8–24 *s*-setae in ventro-lateral position, 1-(2) of them are shorter and in distal position; (1)-2-(3) microsetae in ventro-proximal position (Figure 8A). Ant. II 6 *s*-setae in distal position. Ant. III has a sensory field that includes 2 inner *s*-setae of AO III, one lateral *ms*-seta and about 10–13 *s*-setae (Figure 8B). Ant. IV with one simple small subapical, peg-shaped organite and a bifurcate pin-like seta (Figure 8G). Eye spots strongly dark pigmented with 8 + 8 ocelli (G and H smaller; Figures 8C, S4B). Usually no setae among OMMA A, B and C, D. PAO elongated (sometimes a weak median constriction is visible) about 1.5 times as long as the diameter of the nearest ocellus (Figure 8C). Prelabral setae 4. Labral formula as 5,5 and 4 and 4 sharp apical folds (Figure 8I). Maxillary palp bifurcated and maxillary outer lobe with 4 sublobal hairs. Labial palp with 5 papillae and a total of 15–16 guard setae [62] are distributed as A1, B1–4, C0, D1–4, E1–6/7 (Figure S4D). Characteristic of this species is the variability in number of guard setae. Usually, *e7* is not present but it could be present (like in Figure 8E) and frequently the same individual has one labium with *e7* and the other one without (see remarks). Hypostomal papilla with H shorter than h1/h2. Proximal (px), basomedian (bm) and basolateral (bl) fields of labium with 4, 4 and 5 setae, respectively. 4–7 postlabial setae. Mandible with well-developed molar plate.

Furca: Furca almost as long as antenna; ratio of mucro/dens/manubrium = 1/24/16 (Figure 8G. Table A2). Maximum number of ventral setae on manubrium about 18–20-(28), including ventro-apical setae (8) larger than the others (Figure 8G) except for 1(3) + 1(3) shorter setae in apical–medial position; about 50 dorsal setae on Man. Dens with dorsal crenulations, more than 60 ventral and 14–22 dorsal setae. Mucro quadridentate with apical tooth much smaller than subapical one (Figure S4C). Retinaculum with 4 teeth and 5–7 setae.

Legs: Eleven setae in the distal apical ring of Tita; 2–3–3 very weakly clavate tenent hair, shorter than the inner edge of Cl (Figures 8E, S4E). Claw of normal shape without lateral and inner teeth; empodium sometimes with a small inner tooth (Figures 8E, S4E); pretarsus with a pair of setae.

Distribution and habitat: V. glacialis has been found on several Alpine glaciers from Western to Eastern Alps. *V. glacialis* is present in the Rhaetian, Bernese, Pennine and Graian Alps (Figure 2, Table A1 for more information).

According to the distribution of this species, some past records — 'Isotomidae sp.' in Buda et al. [63] and '*Desoria glacialis*' in Stoppani [64] on Forni Glacier, and '*Isotoma* sp. G' in Zettel [24] on Oberaar glacier (Bernese Alps, Switzerland) — could be attributed to this species. Buda et al. [63] provided *cox1* sequence that corresponds to that of *V. glacialis*. Zettel [24] specified that it was a 'still undescribed species of the *fennica-propinqua* group' *sensu* Gisin (1960); Gisin [65] gives few characteristics for these two species but compatible with *V. glacialis* (in particular, mucro with four teeth, the apical one very small). In general, many other past records of *D. saltans* should be revised and could probably be attributed to *V. glacialis* and *V. psychrophilus* (see notes for *D. saltans*).

Ecology: V. glacialis is a strictly ice-dwelling springtail that in summer can be found on bare ice under stones (Figure 1C, H), within supraglacial continuous stony debris (like medial moraines; Figure 1B) and on supraglacial snow (for example, Stoppani [64]: see notes in 'Distribution and habitat'; Figure 1F). Checking under the debris just in front of the glaciers, this species disappears moving out from the ice (like for *V. psychrophilus* in Valle et al. [41]). Its life cycle and behaviour on the ice may be similar to that of *D. saltans* (see notes for this species). However, *V. glacialis* is probably more linked to stony microhabitat on the ice.

Buda et al. [63] probably studied the distribution of *V. glacialis* under stones along with a transect starting in the foreield toward the centre of Forni Glacier ablation zone (see notes on distribution). Individuals were more abundant under stones on bare ice than under those belonging to the

central moraine; however, the authors probably underestimated abundances inside the moraines since they did not use the flotation method [41]. In fact, we observed high abundances of this species within the supraglacial debris. In Zettel [24], analyses of the thermal hysteresis of a '*Isotoma* sp. G' attributable to *V. glacialis* are reported (see notes in 'Distribution and habitat'). Jaroměřská et al. [66] studied the isotopic composition of an ice-dwelling Isotomidae attributable to *V. glacialis*.

Etymology: the epithet refers to the typical habitat of the species that are Alpine glaciers.

Remarks: V. glacialis belongs to the new group of closely related, strictly ice-dwelling *Vertagopus* species that also includes *V. psychrophilus*, *V. glacieinigrae* and *V. fradustaensis*. These species are close to *V. alpinus* for the absence of setae on the anterior side of the VT and a small apical tooth on mucro but differ by the presence of only weakly clavate tenent hairs (strongly clavate in *V. alpinus*). Among the group of strictly ice-dwelling *Vertagopus* species, *V. glacialis* is characterised by a reduced number of anterior setae on Man (on average 18–22, 26−40 in the others) reduced number of *accp*-setae (only 2 on each thoracic terga and 1 on first abdominal terga, less than in other species). Other important features are the absence of seta among ommatidia A, B and C, D absent (Figure 8C) and the absence of *e7* seta on labium in some specimens: in 31 specimens checked (3–4 from each population), 18 specimens don't have *e7* (58%), 7 specimens have *e7* only on one side (23%) and 6 have *e7* on both sides (19%). This characteristic is equally distributed among the populations checked (Table A1).

6. *Vertagopus glacieinigrae* Valle sp. nov. Figures 6C, 9A−I

6.1. Material Examined. Holotype: FRANCE • ♀; Provence-Alpes-Côte d'Azur, Vallouise-Pelvoux, European Alps, Écrins massif, Glacier Noir ('GLN'); in supraglacial debris 44°55′ 11.3"N, 6°23′37.8"E, 2240 ma.s.l.; 9 Aug.2021, B. Valle leg.; B. Valle det., collected with flotation method; Genbank (NCBI) PP441759−64 (*cox1*), PP465281−86 (*16S*); (SMNG-APT-AA04482).

Paratype: FRANCE • 2 ♂ and 2 ♀; same collection data as for holotype; (SMNG-APT-AA04483 to 86).

6.2. Description. Body: Mean body length (with antennae) 1.7 mm (standard deviation: 0.1 mm on 10 specimens, Table A2). Colour black with appendages a little brownish (Figure 6C); juveniles are brownish. Cuticle granulation fine and regularly distributed; all dorsal tergites separated from each other, but Abd. V-VI not clearly separated by a narrowing (Figure 9D). Abd. III and IV of approximately the same width.

Chaetotaxy: Terga consist of micro-, meso- and macrosetae, the latter well differentiated on last abdominal tergites (Abd. IV–VI, in median position), but not well distinguished from ordinary setae on other tergites (Figure 9D). No setae in the ventral side of Th. II–Th. III. All setae smooth. Macrosetae on Abd. V is equal to the median length of tergite and 2.2 times as long as the inner edge of Claw III (Table A2). Sensory chaetotaxy constituted by *ms*-setae, *accp*-, *al*- and *as*-setae. Only Th. II and Abd. III have *ms*-setae (formula 10/001). Dorsal *s*-setae constituted by single *al*-seta on Th. II and Th. III, two *as*-seta on Abd. V and by *accp*-setae normally set within *p*-row (Figure 9D). The number of *accp*-setae can be expressed as 3, 3/3, 3, 3, 5, 3.

VT with 0 + 0 anterior, 4 + 4 latero-distal and 4 posterior setae with 2 in apical transverse row (Figure 9F).

Head: Antennae longer than cephalic diagonal (D/*A* = 0.33, Antenna = 0.38 mm; Table A2). Ratio among Ant. I/Ant. II/Ant. III/Ant. IV is 1/1.7/1.6/2.9 (Table A2). There are often cases of asymmetry among *s*-setae between antennae of the same specimen. Ant. I has 8–13 *s*-setae in ventro-lateral position, 1–3 of them are shorter and in distal position; 2 microsetae in ventro-proximal position (Figure 9A). Ant. II has 6–11 *s*-setae in distal position. Ant. III has a sensory field that includes 2 inner *s*-setae of AO III, one lateral *ms*-seta and about 18 *s*-setae (Figure 9B). Ant. IV has one big subapical, spherical organite and a bifurcate pin-like seta (Figure 9H). Eye spots strongly dark pigmented with 8 + 8 ocelli subequal and very small (Figure 9C). Presence of a seta among ommatidia A, *B*, C, D (Figure 9C) PAO little elongated, about three times as long as the diameter of the nearest ocellus (Figure 9C). Prelabral setae 4. Labral formula as 5–5–4 and 4 apical folds squared at the apex (Figure 9I). Maxillary palp bifurcated and maxillary outer lobe with four sublobal hairs. Labial palp with five papillae and a total of 16 guard setae [62] distributed as: A1, B1–4, C0, D1–4, E1–7 (like in Figure 9E). Hypostomal papilla with H shorter than h1/h2. Proximal (px), basomedian (bm) and basolateral (bl) fields of labium with 4, 4 and 5 setae, respectively. 4–5 postlabial setae. Mandible with a well-developed molar plate.

Furca: Furca longer than antenna; ratio of mucro/dens/manubrium = 1/25/13 (Table A2). Ventral setae on manubrium about 36, including ventro-apical setae (8) larger than the others with the exception of 1 + 1 short setae in apical–medial position; more than 40 dorsal setae on Man. Dens with dorsal crenulations, more than 60 ventral and 11–12 dorsal setae. Mucro quadridentate with apical tooth much smaller than subapical one (Figure 9G). Retinaculum with 4 teeth and 5–6 setae.

Legs: 11 setae in the distal apical ring of Tita; 2–3–3 very weakly clavate tenent hairs (Figure 9E), shorter than the inner edge of Cl. Claw of normal shape without lateral and inner teeth; empodium without inner tooth; pretarsus with a pair of setae (Figure 9E).

Distribution and habitat: V. glacieinigrae is currently known only for the type locality, Glacier Noir in French Alps (Figure 2, Table A1).

Ecology: V. glacieinigrae is an ice-dwelling species that inhabits deep supraglacial stony debris (like Figure 1A) of a debris-covered glacier.

Etymology: the epithet literally means 'of black ice' and refers both to its occurrence on supraglacial debris of a debris-covered glacier ('glacier noir', black glacier, in French) and to the name of the glacier where the species lives (Glacier Noir).

Remarks: V. glacieinigrae belongs to the new group of closely related, strictly ice-dwelling *Vertagopus* species that also includes *V. psychrophilus*, *V. glacialis*, *V. fradustaensis*. These species are close to *V. alpinus* for the absence of setae

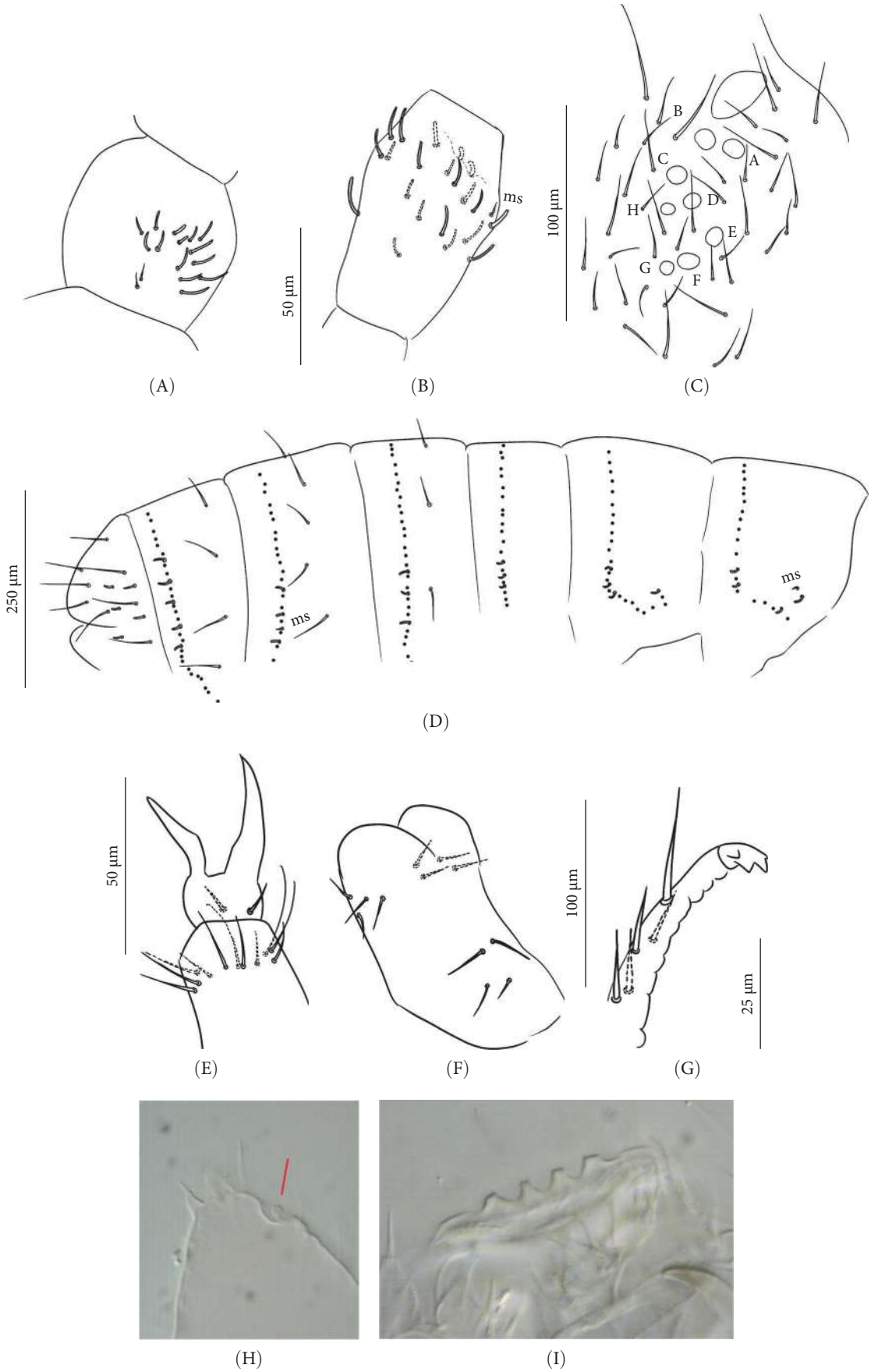

FIGURE 9: A–I: *Vertagopus glacieinigrae* sp. nov. (A) Ant. I, ventral side (B) Ant. III, (C) ocular plate (A–H: OMMA), (D) *accp* sens and macrosetae, (E) Claw III, (F) latero-posterior side of VT, (G) mucro (H) Ant. IV subapical organite (red arrow) and pin-like seta, (I) apical folds labrum.

on the anterior side of the VT and a small apical tooth on mucro but differ by the presence of only weakly clavate tenent hairs (strongly clavate in *V. alpinus*). Among the group of strictly ice-dwelling *Vertagopus* species, *V. glacieinigrae* is differentiated from all other species for having a big and spherical organite on Ant. IV (small and peg-shaped in the others) and the PAO 3 times as long as the nearest ocellus (<2 in the others). It is differentiated from *V. glacialis* even by a higher number of anterior setae on Man (on average 36, 18–22 in *V. glacialis*) and a higher number of *accp*-setae (usually 3 from Th. II to Abd. III, less in *V. glacialis*).

7. *Vertagopus fradustaensis* Valle sp. nov. Figures 6F, 10A–I

7.1. Material Examined. Holotype: ITALY • ♂; Trentino, Tonadico (Trento province), European Alps, Pale di San Martino, Fradusta Glacier ('FRA'); under stones on bare ice and in ice fractures; 46°15′06.8" N, 11°52′21.4" E; 2845 ma.s.l.; 8 Aug. 2023; M. Gobbi leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441732−34 (*cox1*), PP465254−56 (*16S*). (SMNG-APT-AA04487).

Paratype: ITALY • 5 ♂; same collection data as for holotype; (SMNG-APT-AA04488 to 89, MUSE-INV-c001 000419–421).

7.2. Description. Body: Mean body length (with antennae) 2.0 mm (standard deviation: 0.1 mm on seven specimens, see Table A2). Colour black (also juvenile) but with intermediate cuticle between body segments paler and weakly corrugate (Figures 6F, 10D), at least in specimens preserved in alcohol. Cuticle granulation fine and regularly distributed; all dorsal tergites clearly separated from each other (Figure 10D). Abd. III and IV of approximately the same width.

Chaetotaxy: Terga consisting of micro-, meso- and macrosetae, these latter well differentiated on last abdominal tergites (Abd. IV–VI, in median position), but not well distinguished from ordinary setae on other tergites (Figure 10D). No setae in the ventral side of Th. II–Th. III. All setae smooth. Macrosetae on Abd. V 0.6 times the median length of tergite and 1.8 times as long as the inner edge of Claw III (Table A2). Sensory chaetotaxy constituted by *ms*-setae, *accp*-, *al*- and *as*-setae. Only Th. II and Abd. III have *ms*-setae (formula 10/0010). Dorsal *s*-setae constituted by a single *al*-seta on Th. II and Th. III, a single *as*-seta on Abd. V and by *accp*-setae normally set within *p*-row (Figure 10D). The number of *accp*-setae can be expressed as (33/33343).

VT with 0 + 0 anterior, 4 + 4 latero-distal and 4–5 posterior setae with 2 in apical transverse row (Figure 10G).

Head: Antennae longer than cephalic diagonal (D/*A* = 0.8, Antennae = 0.4 mm. Table A2). Ratio among Ant. I/Ant. II/Ant. III/Ant. IV is 1/1.7/1.6/3.1 (Table A2). There are often cases of asymmetry among *s*-setae between antennae of the same specimen. Ant. I has 11–15 *s*-setae in ventro-lateral position, two of them are shorter in the distal position; 2 microsetae in ventro-proximal position (Figure 10A). Ant. II has 5–8 *s*-setae in distal position. Ant. III has a sensory field that includes 2 inner *s*-setae of AO III, 1 lateral *ms*-seta and about 9–13 *s*-setae (Figure 10B). Ant. IV has one simple small subapical, peg-shaped organite and a bifurcate pin-like seta. Eye spots strongly dark pigmented with 8 + 8 ocelli (G and H smaller). Seta among ommatidia A, B and C, D usually present (Figures 10H–I). PAO elongated about 1.5 times as long as the diameter of the nearest ocellus (Figures 10H–I). Prelabral setae 4. Labral formula as 5, 5 and 4 and 4 apical folds squared at the apex (like in Figure 10I of *V. glacieinigrae*). Maxillary palp bifurcated and maxillary outer lobe with four sublobal hairs. Labial palp with five papillae and a total of 16 guard setae [62] distributed as: A1, B1–4, C0, D1–4, E1–7 (Figure 10E). Hypostomal papilla with H shorter than h1/h2. Proximal (px), basomedian (bm) and basolateral (bl) fields of labium with 4, 4 and 5 setae, respectively, and 7–9 postlabial setae. Mandible with well-developed molar plate.

Furca: Furca longer than antenna (see Table A2); ratio of mucro/dens/manubrium = 1/30/17. Maximum number of ventral setae on manubrium about 26–30 (less in juveniles), including four ventro-apical setae that are usually larger than the others except 1(2) + 1(2) shorter setae in apical–medial position; more than 30 dorsal setae. Dens with dorsal crenulations, more than 80 ventral and with 10- (14) dorsal setae. Mucro quadridentate with apical tooth much smaller than subapical one (Figure 10C). Retinaculum with 4 teeth and 4–8 setae.

Legs: Eleven setae in the distal apical ring of Tita; 2–3–3 very weakly clavate tenent hairs (Figure 10F), shorter than the inner edge of Cl. Claw of normal shape without lateral and inner teeth; empodium sometimes without inner tooth; pretarsus with a pair of setae (Figure 10F).

Distribution and habitat: V. fradustaensis is currently known only for the type locality, Fradusta glacier ('FRA'; Dolomites, Italy) (Figure 2, Table A1).

Ecology: V. fradustaensis seems to be a strictly ice-dwelling springtail that could be found on bare ice under stones and in ice fractures. Its life cycle and ecology on ice may be similar to that of *D. saltans*, but ecological studies are needed.

Etymology: the epithet refers to the name of the single glacier where the species was found, Fradusta Glacier in Dolomites Mts, Italy.

Remarks: V. fradustaensis belongs to the new group of closely related, strictly ice-dwelling *Vertagopus* species that also includes *V. psychrophilus*, *V. glacialis* and *V. glacieinigrae*. These species are close to *V. alpinus* for the absence of setae on the anterior side of the VT and a small apical tooth on mucro but differ by the presence of only weakly clavate tenent hairs (strongly clavate in *V. alpinus*). Among the group of strictly ice-dwelling *Vertagopus* species, *V. fradustaensis* is differentiated from *V. glacialis* by a higher number of anterior setae on Man (on average 26–30, 18−22 in *V. glacialis*), a higher number of *accp*-setae (3 from Th. II to Abd. III, less in *V. glacialis*) and for usually having the seta among ommatidia A, B and C, D (Figure 10H). *V. fradustaensis* is differentiated from *V. glacieinigrae* for having Abd. V–VI clearly separated by a narrowing (not clear in *V. glacieinigrae*), a small, peg-shaped organite on Ant. IV (big and spherical in *V. glacieinigrae*) and the PAO only 1.5 times as long as the nearest ocellus (not 3 times as in *V. glacieinigrae*). From *V. psychrophilus*, *V. fradustaensis* differs in the different pigmentation patterns, being *V. fradustaensis* characterised by having paler cuticle in the area between body tergites.

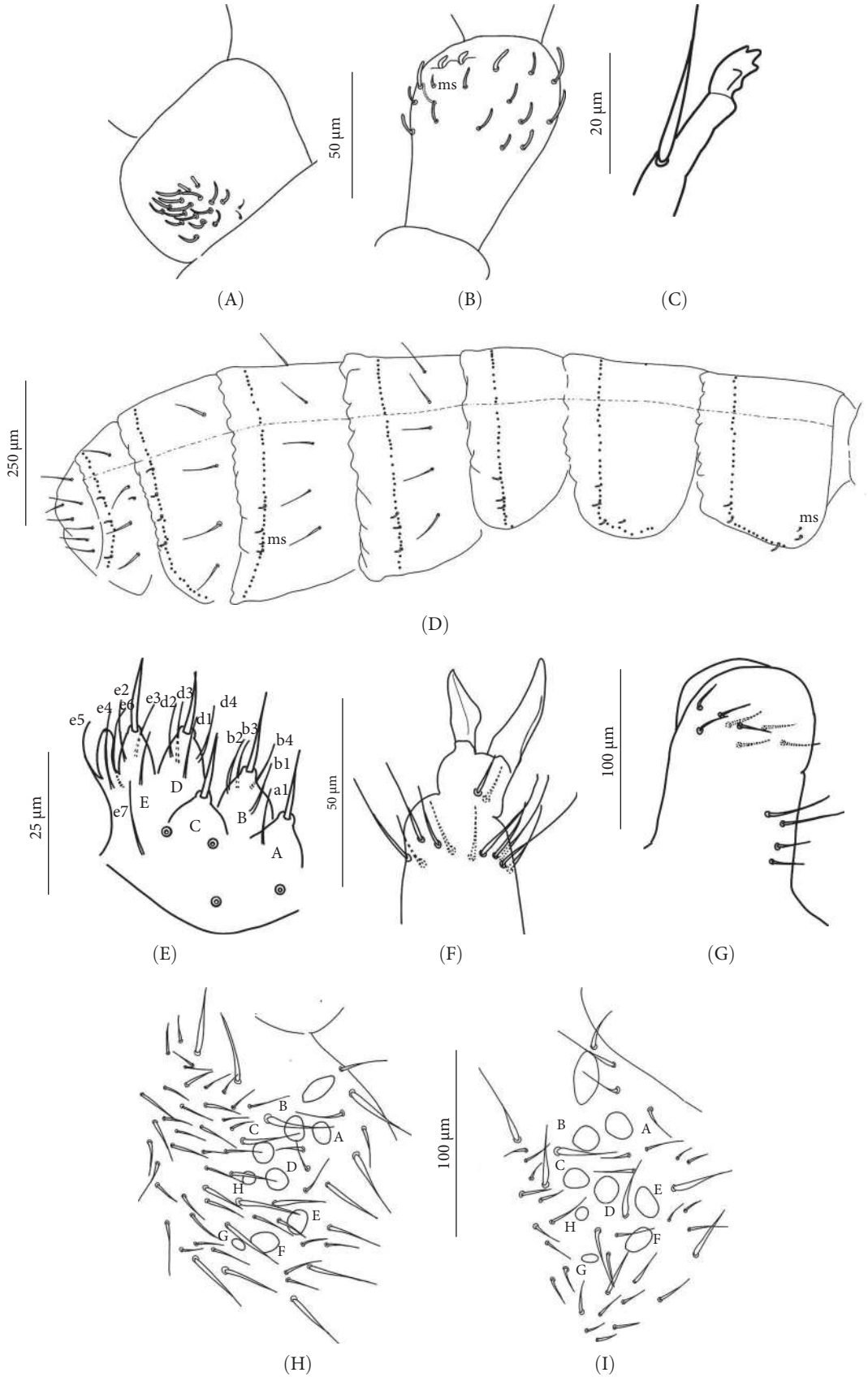

FIGURE 10: A–I: *Vertagopus fradustaensis* sp. nov. (A) Ant. I, ventral side, (B) Ant. III, (C) mucro, (D) *accp* sens and macrosetae, (E) labial palps, (F) Claw III, (G) lateral side of VT, (H) Ocular plate (A–H: ommatidia) with seta among A, B, C, D, (I) Ocular plate (A–H: ommatidia) without seta among A, B, C, D.

8. *Desoria orobica* sp. nov. Valle B. Figures 1I, 6I, 11A–L

8.1. Material Examined. Holotype: ITALY • ♀; Lombardia, Valbondione (Bergamo province), European Alps, Orobian Alps Trobio Glacier; inside cold, wet and inorganic stony debris at the border of a snow-patch; 46°03′25.5"N, 10°05′ 30.0"E, 2700 ma.s.l.; July 18, 2020; B. Valle, M. Caccianiga leg.; B. Valle det., collected with flotation method; Genbank (NCBI) PP441909−13 (*cox1*), PP465431−35 (*16S*). (SMNG-APT-AA04490).

Paratype: ITALY • 5 ♀; same collection data as for holotype; (SMNG-APT-AA04491 to 92, MCSNBG-215 to 217).

8.2. Description. Body: Mean body length (with antennae) 1.4 mm (standard deviation: 136 mm on 7 specimens, see Table A2). Colour violet-black, with Ant. II–IV yellow, except for a black spot on the top of Ant. IV (Figures 1H, 6I); juveniles have the same pattern but with a paler body. Cuticle granulation fine and regularly distributed; all dorsal tergites clearly separated from each other, except for Abd. V–VI that are only slightly fused (Figures 11D, S5A). Abd. III and IV approximately the same width (Figure 11D).

Chaetotaxy: Terga plurichaetotic, consisting of micro-, meso- and macrosetae, the latter well differentiated on last abdominal tergites (Abd. IV–VI, in median position), but not well distinguished from ordinary setae on other tergites (Figures 11D, S5A). No setae in ventral side of Th. II–Th. III. All setae smooth. Macrosetae on Abd. V 0.6 times the median length of tergite and 2 times as long as the inner edge of Claw III (Table A2). Sensory chaetotaxy constituted by *ms*-setae, *accp*-, *al*- and *as*-setae. Only Th. II and Abd. III have *ms*-setae (formula 10/0010). Dorsal *s*-setae constituted by single *al*-seta on Th. II and Th. III, single *as*-seta on Abd. V and by *accp*-setae, normally set within *p*-row (Figure 11D). The number of *accp*-setae can be expressed as 34/44442 (Figure 11D).

VT with 1 + 1 anterior, 3 + 3 latero-distal and 4–6 posterior setae with 2 in apical transverse row (Figure 11F).

Head: Ratio among Ant. I/Ant. II/Ant. III/Ant. IV is 1/1.5/1.4/3. Ant. I has 3–7 *s*-setae in ventro-lateral position and 3(−4) thicker and shorter sens in the most distal part; 2 microsetae in ventro-proximal position (Figure 11A). Ant. II has about 5 *s*-setae in distal position. Ant. III has a sensory field that includes 2 inner *s*-setae of AO III, one *ms*-seta and 6–7 *s*-setae (Figure 11B). Ant. IV with one simple subapical, peg-shaped organite and a bifurcated pin-like seta (Figure 11I). Eye spots strongly dark pigmented with 6 + 6 ocelli visible (Figures 11C, S4B). PAO elongated, with a weak median constriction, about two times as long as the diameter of the nearest ocellus (Figures 11C, S5B). Prelabral setae 4. Labral formula as 5, 5 anf 4 and 4 apical folds squared at the apex (Figures 11L, S5C). Maxillary palp bifurcated and maxillary outer lobe with 4 sublobal hairs. Labial palp with 5 papillae and a total of 16 guard setae [62] distributed as: A1, B1–4, C0, D1–4, E1–7 (like in Figures 10E, S5). Hypostomal papilla with H shorter than h1/h2. Proximal (px), basomedian (bm) and basolateral (bl) fields of labium with 4, 4 and 6 setae, respectively. 4–6 postlabial setae. Mandible with a well-developed molar plate.

Furca: Furca longer than antennae; ratio of mucro/dens/manubrium = 1/29/15 (Figure 11G). Ventral setae on the manubrium numerous (about 90), including about 10 ventro-apical setae larger than the others (Figure 11G), except for 2 + 2 short apical setae; more than 30 dorsal setae. Dens with dorsal crenulations, more than 80 ventral and 8–11 dorsal setae (Figure 11G). Mucro quadridentate with apical tooth much smaller than subapical one (Figure 11H). Apical seta on dens two times as long as mucro. Retinaculum with 4 teeth and 4–5 setae.

Legs: 11 setae in the distal apical ring of Tita; 2–3–3 very pointed tenent hairs (Figure 11E), shorter than the inner edge of Claw. Claw of normal shape without lateral and inner teeth; empodium without a small inner tooth; pretarsus with a pair of setae (Figures 11E, S5D).

Distribution and habitat: Desoria orobica is currently known only for the type locality, Trobio glacier ('TRO'; Orobian Alps, Italy) (Figure 2, Table A1).

Ecology: D. orobica is a cryophilic firn-dwelling species (*sensu* Eisenbeis & Meyer [11]), also able to colonise supraglacial habitat. In summer, it lives at the borders of snow patches, in wet and mineral soils: performing flotation methods along a linear transect getting farther from the snow, the abundance decreased and after a distance of 1 m, on average, *D. orobica* disappeared (unpublished data; Figures 1D,H).

Etymology: the epithet refers to the name of the mountains — Orobian Alps — where this species was discovered.

Remarks: Desoria orobica belongs to the *D. nivalis*-complex sharing some important characteristics with other species of the complex (*D. nivalis* [Carl, 1910], *D. duodecimoculata* [Denis, 1927]): 'the low number of sens on Ant. I and body tergites, the shape of mucro, oligochaetotic VT and dens, and long apical seta near mucro' [43]. *D. orobica* shares also the peculiar pigmentation with *D. nivalis* (even if usually *D. nivalis* has also the distal part of its legs yellow, while *D. orobica* does not), but differs from this species for the different number of setae on VT (*D. orobica* has 1 + 1 anterior, 3 + 3 latero-distal, *D. nivalis* 4 + 4 anterior, 4–5 + 4–5 latero-distal), Claw without inner tooth (small but present in *D. nivalis*), the number of sensilla Ant. I (3–4 short and 3–7 thin sensilla in *D. orobica*, only 4–5 short and thin sensilla in *D. nivalis*), the smooth Mac (that are slightly serrate in *D. nivalis*).

The only record of a cryophilic ice-dwelling species for this area was by Facchini Pajetta et al. [67] who reported '*Isotoma saltans*', but this record does not seem reliable because of its habitat (on the grass below 1900 ma.s.l.) and the absence of taxonomic notes; since the work referenced to Gisin [65], it is probably not associated with *D. saltans* sensu Potapov [43] (see notes for *D. saltans*).

9. Notes on *Vertagopus alpinus* Haybach, 1972 and *V. helveticus* Haybach, 1980

V. helveticus probably is not a valid species, as sampled population on Morteratsch (the type locality of *V. helveticus*) is genetically identical to *V. alpinus* and, following the descriptions reported in Potapov [43], morphologically they are not clearly different. We only have *cox1* and *16s* sequences of one specimen from the type locality of *V. helveticus* to strongly support this finding, and further investigation is suggested.

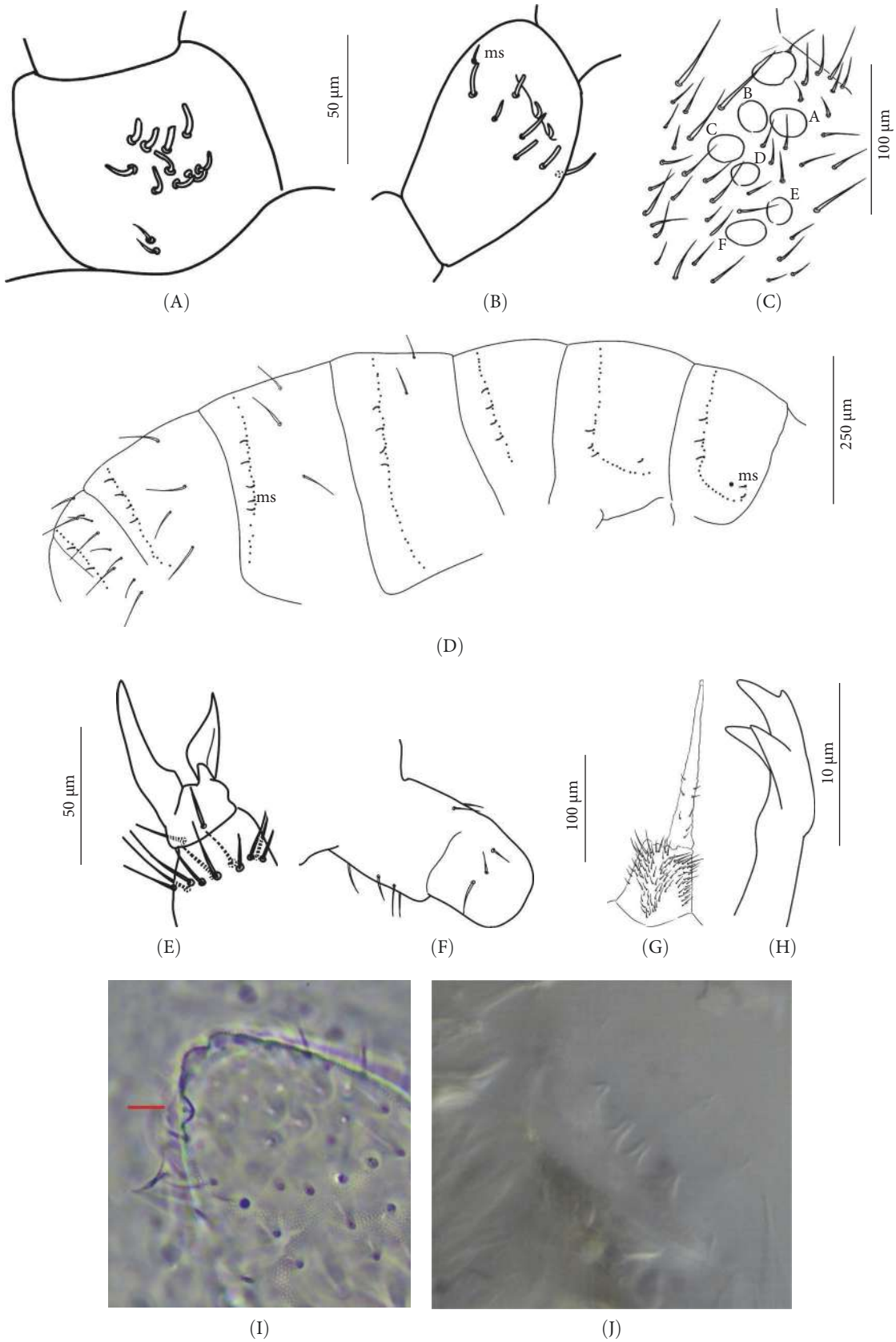

FIGURE 11: A–J *Desoria orobica* sp. nov. (A) Ant. I, ventral side, (B) Ant. III, (C) Ocular plate (A–F: ommatidia) with seta among A, B, C, D, (D) *accp* sens and macrosetae, (E) Claw III, (F) lateral side of VT, (G) ventral side of furca, (H) mucro, (I) Ant. IV organite, (J) apical folds on labrum.

9.1. Vertagopus alpinus Haybach, 1972. Figure 6E. Syn: Isotoma (*Vertagopus*) *alpina* Haybach, 1972.

Isotoma (*Vertagopus*) *helvetica* Haybach, 1980.

Material examined: ITALY • 6 ♂ and 6 ♀; Valle d'Aosta, Courmayeur (Aosta province), European Alps, Mont Blanc PP465302 Miage Glacier ('MIA/MIAC/MIAS'); in supraglacial stony debris; 45°46′52.6"N, 6°52′02.3"E, 2080 ma.s.l.; 8 Sep. 2020, B. Valle leg.; B. Valle det., collected with flotation method; Genbank (NCBI) PP441780−86; PP441788−90 (*cox1*), PP465302−08; PP465310−12 (*16S*); (SMB). FRANCE • 5 ♂ and 9 ♀; Auvergne Rhône-Alps, Chamonix-Mont-Blanc, European Alps, Mont Blanc massif, Mer de Glace glacier ('MDG'); in supraglacial stony debris; 45°54′44.5"N, 6°56′10.4"E, 2040 ma.s.l.; 12 Aug. 2021, B. Valle leg.; B. Valle det., collected with flotation method; Genbank (NCBI) PP441770−79 (*cox1*), PP465292−301 (*16S*); (SMNS). AUSTRIA • 2 ♂ and 7 ♀; Carinthia, Heilgenblut, European Alps, Großglockner, Pasterze glacier (type locality; 'PAS'); in supraglacial stony debris; 47°05′13.9"N, 12°43′24.9"E, 2340 ma.s.l.; 1 Aug. 2021, B. Valle leg.; B. Valle det., collected with flotation method; Genbank (NCBI) PP441826−31 (*cox1*), XPP465348−53 (*16S*); (SMNS).

Chaetotaxy: Sensory chaetotaxy is very constant and is constituted by ms-setae, *accp*-, *al*- and *as*-setae. Only Th. II and Abd. III have *ms*-setae (formula 10/001). Dorsal *s*-setae constituted by single *al*-seta on Th. II and Th. III, single *as*-seta on Abd. V and by *accp*-setae normally set within p-row. The number of *accp*-setae can be expressed as 3, 3/3, 3, 3, 3–4, 3. The number of anterior setae on Man is very variable, from 20 to 32.

Head: Ant. I has 9–13 *s*-setae in ventro-lateral position, 1–2 (3) of which are shorter in the distal part; 2(3) microsetae in ventro-proximal position. Ant. II has about 5 *s*-setae in distal position. Ant. III has a sensory field that includes 2 inner *s*-setae of AO III, one lateral *ms*-seta and 8–10 *s*-setae. Ant. IV with one simple subapical, peg-shaped organite and a bifurcate pin-like seta. Absence of a seta among ommatidia A, B and C, D.

For body measurements see Table A2.

Notes on distribution: V. alpinus was known to have an Alpine distribution [43]. We could confirm an Alpine distribution with new records from Western to Eastern Alps: Graian Alps (Mont Blanc), Bernina (Morteratsch glacier) Bernese (Lang glacier) and Pennine Alps (Piéce glacier).

Notes on ecology: V. alpinus seems to be a cold-adapted, high-mountain, not strictly ice-dwelling species able to colonise both the glacier forelands [68] and the supraglacial debris (our data) (Figure 1A). On Pasterze (Grossglockner), Mer de Glace and Miage (Mont Blanc) glaciers, it has been only found in thick layer of fine supraglacial debris but not on bare ice. On Morteratsch (Bernina), Pèlerins (Mont Blanc) glaciers, it was sampled on bare ice, under stones. According to our data, *V. alpinus* seems more linked to thicker supraglacial debris than other ice-dwelling species.

10. Notes on *Desoria saltans* Nicolet, 1841 sensu Potapov (2001). Figure 6D

Syn: Desoria glacialis Nicolet, 1841.

D. saltans was described for the Bernese Alps (Lauteraar and Finsteraar according to Desor [36]) by Nicolet [69] but with an incomplete description that does not include the diagnostic morphological characteristics of the species; therefore, considering the high diversity of ice-dwelling springtails as presented in this work, it is not possible to state which ice-dwelling species Nicolet referred to. In the following decades, several authors based the reports for this species only on its peculiar ecology, including some identification keys (for example, Gisin [65]). In particular, Handschin [70] and Gisin [65] probably considered a species of the ice-dwelling *Vertagopus* group (as suggested in particular by the mucro shape). However, from their description, it is not possible to know which species they refer to, so these works cannot be used as valid descriptions. The same applies to many other records of *D. saltans* (see notes on distribution and ecology). Only Potapov [43] for the first time provided a complete and unequivocal description of *D. saltans* based on specimens collected by Kopeszki and Zettel; for this reason, we referred to Potapov's [43] description for the identification of *D. saltans*.

Material examined: ITALY • 5 ♂ and 4 ♀; Valle d'Aosta, Courmayeur (Aosta provice), European Alps, Mont Blanc massif, Pré de Bar Glacier ('PDB'); under stones on bare ice, 45°54′ 08.9"N, 7°03′04.2"E, 2700 ma.s.l.; 25 Jul. 2021, R. Ambrosini, F. Pittino leg, B. Valle det., collected by hand; Genbank (NCBI) PP441832−41 (*cox1*), PP465354−63 (*16S*); (SMB). FRANCE • 8 ♂ and 10 ♀; Provence-Alpes-Côte d'Azur, Vallouise-Pelvoux, European Alps, Écrins massif, Glacier Blanc ('GLB'); on bare ice; 44°56′46.6"N, 6°23′48.8"E, 2970 ma.s.l.; 8 Aug. 2021, B. Valle leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441751−58 (*cox1*), PP465273−80 (*16S*); (SMNS). Head Ant. I has 2–5 *s*-setae in ventro-lateral position, and 5–7 shorter and thicker s-setae in the distal part; 2/3 microsetae in ventro-proximal position. Ant. II has about 8–11 *s*-setae in distal position. Ant. III has a sensory field that includes 2 inner *s*-setae of AO III, one lateral *ms*-seta and 7–11 *s*-setae. Ant. IV with one simple subapical, slightly bifurcated rod-shaped organite and a bifurcate pin-like seta (basal process very small).

For body measurements see Table A2.

Note on pigmentation: Desoria saltans is clearly distinguished from other ice-dwelling species by the purple reflections (Figure 6D), especially in juveniles, visible at the stereomicroscope.

Notes on taxonomy, past records and distribution: D. saltans is reported in Potapov [43] as an Alpine species (Austria, Switzerland, Italy); beyond the type locality of the original description —Lauteraar and Finsteraar glaciers, Switzerland (Desor [36], Nicolet [69])—it was recorded also in other localities. However, we hypothesise that several past records must be referred to other species. This is probably true for '*Desoria glacialis*' found by Stoppani [64] on Forni Glacier—without providing morphological evidence—where we found *V. glacialis* sp. nov. In Orobian Alps (Italy), close to Scais glacier where *D. saltans* was found (SCA), Facchini Pajetta et al. [67] reported '*Isotoma saltans*', but this report seems not reliable (see *D. orobica* notes).

Even some taxonomic texts report for *D. saltans* characters that are more similar to those of ice-dwelling *Vertagopus* group: for example Handschin [70]—material examined from different sites—and Gisin [65]—material examined from Gornergletscher (Monte Rosa)—affirm that *D. saltans* has the mucro

similar to *D. nivalis* and Gisin [65] states that Abd. V-VI are not well distinguished.

In contrast, other reports could be probably attributed to *D. saltans*, like '*Isotoma* sp. G' [11, 71] from Rotmoos and Gaisenberg glaciers (Ötzal Alps, Austria), according to what is deducible from SEM photos reported in Eisenbeis and Meyer [71] (for example, the big apical tooth on mucro); however, Eisenbeis and Meyer [11, 71] wrote that their species did not fit with *D. saltans* in the taxonomic key reported in Gisin [65]: this was due to the fact that Gisin [65] reported ice-dwelling *Vertagopus* in place of *D. saltans*. *Isotoma saltans* reported by Kopeszki [21] for Mittelberg glacier (Ötzal Alps, Austria) is confirmed as *D. saltans* according to the morphological information reported.

Therefore, *D. saltans*' Alpine distribution is confirmed, altough fragmented (Figure 2), adding new records collected with this work from Western to Eastern Alps: from Dauphiné Alps (Glacier Blanc), Graian Alps (Mont Blanc, Prè de Bar), Bernina group (Fellaria Ovest) and Orobian Alps (Scais Glacier).

The population on Peirabroc Glacier (Maritime Alps, Italy), according to the genetic data of the present work, could be attributed to a still unknown species close to *D. saltans*, but this point must be explored further.

Notes on ecology: D. saltans is a strictly ice-dwelling species [43]. Several studies were performed on *D. saltans*, but only Kopeszki's [21] record was taxonomically verified as *D. saltans*. This species is pluriannual and spends its entire life within the upper layers of the glaciers (Figure 1E), where it inhabits ice pores [72]: in winter, it remains in the layer between the snow and ice and only in summer, where the snow melting rate is high, it moves on the snow [21, 27, 28]. The ablation period is a critical phase of its life, since many individuals perish after being transported by turbulent melting water out of their microhabitat; the daily cycle of these organisms is defined by melting water [21]. *D. saltans* could be found also under stones on the bare ice. The diet consists primarily of inblown organic material, like pollen granules [28]. The reproduction period probably corresponds to winter, when the proper conditions for spermatophore dispersion occur (see Kopeszki [21] for detailed information on reproduction and embryonal development). The author also provided some physiological data on this species. Some other studies on physiology (for example: the measurement of respiration rate by Block and Tillbrook [73] and the finding of large amounts of long polyunsaturated fatty acids by Zinckler and Schaller [74] should be taxonomically verified.

11. Notes on *Gnathisotoma bicolor* Cassagnau, 1957 From European Alps. Figure 6H, J

Material examined: FRANCE • 1 ♂ and 1 ♀; Auvergne Rhône-Alps, Chamonix-Mont-Blanc, European Alps, Mont Blanc massif, Tour glacier ('TOU'); under stones on bare ice, 45°59′44.8"N, 6°59′07.4"E, 2660 ma.s.l.; August 24, 2022, A. Zimmer leg.; B. Valle det., collected by hand; Genbank (NCBI) PP441904 (*cox1*), PP465426 (*16S*). (SMNS).

The population of *Gnathisotoma bicolor* from Mont Blanc (Figure 6J) is the first report of this genus for the Alps. From the morphological identification based only on two specimens, it shows a characteristic maxilla with all lamellae elongated and a mucro with an apical dens only slightly smaller than the subapical one. Characteristic of *G. bicolor* from Mont Blanc is the presence on the ventral tube of 4 + 4 lateral, 3 posterior and 1 + 1 anterior setae, unlike *G. bicolor* from Pyrenées (Figure 6H), which lacks anterior setae on VT and has 5 + 5 laterodistal setae. Also genetically, *G. bicolor* from Mont Blanc shows a certain difference from Pyrenean population (4.48%). *Gnathisotoma bicolor*, with the characteristic yellow appendages, is a typical firn-dwelling species [11], like *Desoria orobica* and *D. nivalis*.

12. Distribution Pattern of Ice-Dwelling Species

Ice-dwelling springtails show contrasting distribution patterns in the European Alps. *Vertagopus alpinus* and *Desoria saltans* can be observed throughout the whole chain but with relatively low frequency and a very fragmented distribution. Two of the newly described species, *Vertagopus glacialis* and *V. psychrophilus*, exhibit a wide and almost continuous range distribution; the remaining species are confined to local endemic distribution on single glaciers often occurring the peripheral Alpine sectors. (Figure 2). *Desoria orobica* sp. nov. was found only on the Trobio Glaciers in the Orobian Alps (Southern Alps), *V. glacieinigrae* sp. nov. only on Glacier Noir in Western Alps and *V. fradustaensis* sp. nov. only on Fradusta Glaciers in Dolomites (Eastern Alps). *Gnathisotoma bicolor* record from Mont Blanc is the first record of the genus *Gnathisotoma* for the European Alps. Some important glacial areas, particularly Mont Blanc, host several species, often co-occurring on the same glacier, while elsewhere each glacier typically hosts a single species. On the Apennines, the only ice-dwelling species — *D. calderonis* (Figure 6G) — occurs on the single glacier still present in this mountain chain (Figure 2).

13. Discussion

13.1. Unveiling the Biodiversity of Ice-Dwelling Springtails. This study emphasises the previously unexplored biodiversity of ice-dwelling springtails on the European Alps, introducing five new species beyond the known *Desoria saltans* and *Vertagopus alpinus*. Such diversity is also supported by morphological, phylogenetic and species delimitation analyses. We provide evidence suggesting the potential existence of two additional new species on Peirabroc and Miage glaciers and clarify relationships among the taxa described. Our findings indicate that *Vertagopus helveticus* does not constitute a valid species, as the specimens collected from its type location are genetically and morphologically indistinguishable from *V. alpinus*. This study also provides the first report for the genus *Gnathisotoma* on the European Alps. Intra-specific genetic variability within *D. saltans*, *G. bicolor* and *V. alpinus* as morphologically defined is observed. Comparing our findings with the historical data, we also suggest an updated attribution of the past records (see notes for each species).

Phylogenetic analysis shows that the five new species belong to a group that includes both *Vertagopus* and *Desoria*. These genera thus need to be revised, especially *Desoria* which is clearly a paraphyletic genus, as observed in previous works [18, 75–77]. The extended paraphyly observed in all the three

observed genera suggests the presence of weak morphological characteristics for their discrimination: the only morphological trait that differentiates *Vertagopus* from *Desoria* (the presence of clavate tenent hairs) probably does not have a strong phylogenetic significance since this structure has an ecological implication concerning locomotion on wet surfaces [19, 78] highlighting that also the morphological features that distinguish *Gnathisotoma* from *Desoria* — the reduced number of sensilla on the tergites, the modified maxilla and the absence of frontal setae on the ventral tube — are not unique to the genus and show a gradual transition between the two genera.

Our preliminary phylogenetic analysis suggests that all the ice-dwelling *Vertagopus* species, *D. calderonis* and *D. orobica* represent a monophyletic group that could result from a unique adaptation event to glacial environment. Conversely, *D. saltans* and *G. bicolor* appear to have adapted independently; further investigation is needed to confirm these hypotheses.

13.2. Distribution Pattern. The currently known distribution patterns of the Alpine ice-dwelling springtails stand out for their striking diversity. *Vertogopus glacialis* and *V. psychrophilus* exhibit a wide, nearly continuous distribution range paralleled by a general genetic uniformity within species, with *V. psychrophilus* more linked to the Southern Alps and almost absent from the Northern side of the chain (except for two occurrences on the French slope of the Mont Blanc massif). The outlined distribution pattern supports the hypothesis of a relatively recent differentiation of these species. In contrast, *V. alpinus* and *D. saltans* present a fragmented distribution and a marked genetic variability, corroborating the hypothesis, arising from the phylogenetic evidence, of an independent and more ancient adaptation to the ice-dweller condition for *D. saltans* (while *V. alpinus* is not a truly ice-dwelling species). The low occurrence of *D. saltans* in our samplings is in contrast with many historical reports for the whole Alpine chain: while the *locus typicus* of Lauteraar and Finsteraar in Switzerland [69] cannot be unequivocally referred to any of the known species, many old reports actually refer to other ice-dwelling species: for instance, Stoppani [64] likely refers to *V. glacialis* (see notes on *D. saltans*).

The remaining species apparently have point-like distribution on single glaciers; this evidence could be related to the limited knowledge of their actual distribution ranges. On the other hand, these species are mainly located on glaciers in peripheral/southern subchains of the Alps like Maritime Alps, Orobian Alps, Écrins and Dolomites, with the significant addition of Calderone glacier on the Apennines. Such areas are well-known biodiversity hotspots due to glaciation history and existence of refugia [79–81]. The glaciation in these areas is generally reduced and often isolated from the main Alpine glaciers. Although the dispersal potential of ice-dwelling springtails is still not known, this isolation could be a driver for speciation; at the same time, it could be a reason for concern for the survival of the exclusive biodiversity of these areas under the present climate warming [2, 14]. On the other hand, inner Alpine areas with extensive glacial cover, like Mont Blanc, show a marked species richness, with the co-occurrence of many taxa on the same site; these areas could have acted as refugia for this cryophilic biodiversity during past warm periods [13, 14, 82, 83].

The compresence of different species on the same glacier is an important phenomenon that emerges from our data. Probably this could be favoured by the niche separation of species (for example, *D. saltans*, more linked to bare ice than *Vertagopus* species — see next paragraph). However, our qualitative sampling does not allow a quantification of this phenomenon and further studies are needed, focusing separately on different microhabitats.

13.3. Ice-, Firn- and Debris-Dwelling Species and Other Ecological Considerations. From the ecological point of view, the true ice-dwelling springtails seem to include a well-defined cryophilic group of springtails, including in the Alps the new *Vertagopus* species and *Desoria saltans* and, on the Apennines. *D. calderonis*. As suggested by Eisenbeis and Meyer [11], ice-dwelling species live only where ice is present, and may occur on snow or stony debris only where underlying ice occurs. Such species are differentiated from firn- and debris-dwelling cryophilic springtails that, in the Alps, include *Gnathisotoma bicolor*, *Desoria orobica* and *Vertagopus alpinus*. Eisenbeis and Meyer [11] hypothesised that the intense body pigmentation (Figures 6A–F) of ice-dwelling springtails is a functional adaptation to protect the animal against the high UV radiation on the glacier surface, while firn- and ground-dwelling springtails are less pigmented, especially on appendages. Our data confirm this observation, with all ice-dwelling species completely dark, while *G. bicolor* and *D. orobica* show lighter appendages (Figures 6H–J). In addition, *D. calderonis*, which permanently lives on a glacier totally covered by stony debris [14] presents paler appendages (Figure 6G). This pigmentation pattern can thus be a good indicator of the ecology of these species.

Within true ice-dwelling species, a slight ecological difference could occur between *D. saltans* and *Vertagopus* species (including *D. calderonis*), as the former seems to prefer clean ice avoiding thick debris, while the latter could be regularly observed in supraglacial debris. If confirmed, this phenomenon could imply an underrepresentation of *D. saltans* in our sampling, as the species could be linked to the less accessible accumulation areas of the glaciers, where almost no debris occurs.

Ice-dwelling springtails were not found on rock glaciers: this is probably due to the depth at which ice is located inside the stony debris. The probability of rock glaciers as habitats for these organisms is high, but their detection must overcome the difficulty in sampling at the rock–ice interface that lays at a significant depth in the soil. Underground ice associated with voids between stones like that of rock glaciers could represent an important refugium for ice-dwelling organisms during warm climatic periods [84].

14. Conclusions

This work highlights the need for a deep understanding of the still poorly known biodiversity of glacial habitats. The knowledge of the ecology and distribution of ice-dwelling organisms, many of which are likely to become extinct before their discovery, represents a fundamental key for understanding the biogeography of mountain systems of the Earth. Being intimately linked to the occurrence of glaciers, the distribution of these organisms may

improve our knowledge of the role of past and present climatic fluctuations in shaping the biodiversity of the Alpine regions.

We observed many species endemic of small glaciers of the Alps that are deemed to extinction in the next decades. Understanding how these unique components of biodiversity can be preserved under ongoing climate change should be a conservation priority in all glacier areas of the Earth. To this purpose, a deep taxonomic knowledge, combining morphological and genetic approaches, remains an indispensable tool.

14.1. Identification Key of Cryophilic, Ice-Dwelling Species Studied in This Work. As a general remark, for ice-dwelling springtails, we suggest the use of integrative taxonomy, especially for distinguishing some species of the ice-dweller *Vertagopus*. In addition to standard chaetotaxy, body colour pattern is a very important character. It is important to point out that, in addition to these cryophilic, ice-dwelling dominant species, cold-loving springtails of different genera and families can frequent supraglacial habitats, especially where debris is continuous and coarse.

1. Anterior setae on VT present, Tita tenent hairs pointed (Figure 11E), apical tooth on mucro small or big, apical folds on labrum present or not 2
   – Anterior setae on VT absent, Tita tenent hairs at least weakly clavate, apical tooth on mucro very small (Figures 10G, 8C), apical folds on labrum present 5
2. Body colour violet-black, with purple reflections (Figure 6D), few maxillary lamellae slightly but visibly elongated, maxillary palp simple, apical tooth on mucro equal or slightly smaller to the subapical one, apical folds on labrum absent . . . . . . *D. saltans*
   – Body colour violet-black but with paler appendages, apical folds on labrum present. 3
3. Appendages brownish-yellow (Figure 6G). High number of *accp* sens (5–6,6/5–6,5,7,6–7,4). Known distribution range limited to Calderone Glacier (Apennines) . . . . . .*D. calderonis*
   – Appendages yellow, body strongly dark 4
4. Maxillary lamellae not elongated, Antennae (Ant. II–IV) yellow (Figure 6I), apical tooth on mucro very small . . . .*D. orobica*
   – Maxillary lamellae strongly elongated, legs and Antennae (Ant. I–IV) yellow (Figures 6H,L), apical tooth on mucro equal or slightly smaller to the subapical one. . . . .*G. bicolor*
5. Tenent hairs strongly clavated (2–3–3). Seta *e7* on labium present . . . .*V. alpinus*
   – Tenent hairs very weakly clavated (2–3–3) (Figures 7E, 9E). 6
6. Abd V-VI not clearly separated by a narrowing (Figure 9D). Anterior setae on Man 36. Known distribution range limited to Glacier Noir (Ècrins, European Alps) . . . .*V. glacieinigrae*
   – Abd V-VI clearly separated by a narrowing 7
7. Seta among ommatidia A, B and C, D absent* (Figure 8C). Seta *e7* on labium could lack. Anterior setae on Man 18–22 (rarely till 28). Usually 2 *accp*-setae on Th. II-III and 1 on Abd. I–III. . . . . *V. glacialis*
   – Seta among ommatidia A, B and C, D present* (Figure 7C). Seta *e7* on labium present. Higher number of anterior setae on Man. Usually 3 *accp*-setae from Th. II to Abd. III. 8
8. Max number of anterior setae on Man 26–30. Known distribution range limited to Fradusta Glacier (Dolomites) . . . .*V. fradustaensis*
   – Max number of anterior setae on Man 38–40. . . . . ..*V. psychrophilus*

* *For this character it is important to compare several specimens, since it could present variation, even within different sides of the same specimens*

Nomenclature

Abd:	abdominal segment
accp-setae:	accessory p-row *s*-setae
al-setae:	antero-lateral *s*-setae
Ant:	antennal segment
AOIII:	antennal organ III
as-setae:	anterosubmedial *s*-setae
bl:	basolateral field of labium (mentum)
bm:	basomedian field of labium (submentum)
ms-setae:	micro *s*-setae
PAO:	postantennal organ
Px:	proximal field
Th:	thoracic segment
Tita:	tibiotarsus
VT:	ventral tube.

Appendix

Table A1: Sampled sites, including those where no ice-dwelling springtails were found ('NF').

Site names	Geographic area	Land form	Code	Historical reports for the same glaciers	Species name	Country	Sampling date	Microhabitat	Latitude	Longitude	Sampling altitude [m asl]	Morphology	Specimens sequenced
Sforzellina	Ortles-Cevedale, Southern Rhaetian Alps	G	SFO		*Vertagopu psychrophilus* sp. nov.	IT	August 5, 2020	On supraglacial snow and in supraglacial debris (FM)	46°20′58.7"N	10°30′ 44.1"E	2,860	Morph. Descr.	8
Dosdè	Ortles-Cevedale, Southern Rhaetian Alps	G	DOS		*Vertagopu psychrophilus* sp. nov.	IT	August 5, 2020	Under stones on bare ice	46°23′34.8"N	10°12′ 58.9"E	2,760	Morph. Descr.	10
Agola	Brenta Dolomites, Southern Rhaetian Alps Eastern Alps	G	AGO		*Vertagopu psychrophilus* sp. nov.	IT	August 10, 2020	On supraglacial snow and ice	46°08′57.9"N	10°51′ 27.0"E	2,580	Morph. Descr.	9
Predarossa	Valtellina, Southern Rhaetian Alps	G	PRE		*Vertagopu psychrophilus* sp. nov.	IT	August 19, 2021	Under stones on bare ice	46°15′37.4"N	9°44′21.8"E	2,850	Morph. Descr.	6
Fellaria Ovest	Bernina, Wester Rhaetian Alps	G	FEL*		*Vertagopu psychrophilus* sp. nov.	IT	September 14, 2022	Under stones on bare ice	46° 20′ 51" N	9°56′09" E	2,840	—	1
Rutor	Rutor-Léchaud chain, Graian Alps	G	RUT		*Vertagopu psychrophilus* sp. nov.	IT	June 30, 2023	In supraglacial debris (FM)	45°39′57.7"N	7°00′09.3"E	2,670	—	5
Valtournanche	Breuil basin, Pennine Alps	G	BRE*		*Vertagopu psychrophilus* sp. nov.	IT	July 23, 2023	Under stones on bare ice	45°55′33.0"N	7°41′58.8"E	3,100	—	1
Tour*	Mont Blanc, Graian Alps	G	TOU*		*Vertagopu psychrophilus* sp. nov.	FR	Augut 24, 2022	Under stones on bare ice	45°59′44.8"N	6°59′07.4"E	2,660	—	5
Orny	Mont Blanc, Graian Alps	G	ORN*		*Vertagopu psychrophilus* sp. nov.	CH	August 17, 2022	Under stones on bare ice	45°59′59.8"N	7°04′03.7"E	2700	—	6
Forni	Ortles-Cevedale, Rhaetian Alps	G	GFO	Isotomidae sp (Buda et al. 2020), *Desoria glacialis*" (Stoppani, 1876)	*Vertagopus glacialis* sp. nov.	IT	August 3, 2020	Under stones on bare ice	46°23′42.95"N	10°35′ 24.79"E	2630	Morph. Descr.	7
Vedretta d'Amola	Adamello, Southern Rhaetian Alps	G	AMO		*Vertagopus glacialis* sp. nov.	IT	July 21, 2020	On supraglacial snow and in supraglacial debris (FM)	46°13′8.95"N	10°41′ 12.25"E	2700	Morph. Descr.	10
Giogo alto	Val Senales, Southern Rhaetian Alps	G	GIO		*Vertagopus glacialis* sp. nov.	IT	August 21, 2020	Under stones on bare ice	46°46′52.1"N	10°48′ 20.7"E	2840	Morph. Descr.	9
Pers	Bernina, Wester Rhaetian Alps	G	PER		*Vertagopus glacialis* sp. nov.	CH	July 30, 2021	Under stones on bare ice	46°24′25.9"N	9°57′32.4"E	2670–2700	Morph. Descr.	8
Morteratsch*	Bernina, Western Rhaetian Alps	G	MOR*		*Vertagopus glacialis* sp. nov.	CH	July 10, 2021 and August 25, 2021	Under stones on bare ice	46°24′55.5"N	9°56′04.5"E	2200–2300	Morph. Descr.	4
Mittelberg	Ötztal Alps, Southern Rhaetian Alps	G	MIT		*Vertagopus glacialis* sp. nov.	AT	August 20, 2021	Under stones on bare ice	46°55′09.5"N	10°53′ 43.7"E	2680–2700	Morph. Descr.	6
Lang	Bernese Alps	G	VAL*		*Vertagopus glacialis* sp. nov.	CH	August 10, 2021	Under stones on bare ice	46°27′28.3"N	7°55′55.8"E	2450–2550	Morph. Descr.	6

Table A1: Continued.

Site names	Geographic area	Land form	Code	Historical reports for the same glaciers	Species name	Country	Sampling date	Microhabitat	Latitude	Longitude	Sampling altitude [m asl]	Morphology	Specimens sequenced
Oberaar*	Bernese Alps	G	OBE	Isotoma 'sp. G' (Zettel 1984). *Desoria saltans* (Nicolet, 1841)	*Vertagopus glacialis* sp. nov.	CH	August 14, 2021	Under stones on bare ice	46°32′08.6"N	8°13′12.4"E	2380–2440	Morph. Descr.	6
Moiry	Pennine Alps	G	MOI		*Vertagopus glacialis* sp. nov.	CH	August 11, 2021	Under stones on bare ice	46°05′15.5"N	7°35′25.3"E	2620–2660	Morph. Descr.	10
de Pièce	Pennine Alps	G	PIE*		*Vertagopus glacialis* sp. nov.	CH	August 17, 2021	Under stones on bare ice	45°59′59.7"N	7°28′12.2"E	2750–2800	Morph. Descr.	10
Valtournanche	Breuil basin, Pennine Alps	G	BRE*		*Vertagopus glacialis* sp. nov.	IT	July 23, 2023	Under stones on bare ice	45°55′33.0"N	7°41′58.8"E	3100	—	3
Lauson	Valnontery, Gran Paradiso Graian Alps, Western Alps	G	LAU		*Vertagopus glacialis* sp. nov.	IT	August 11, 2023	In supraglacial debris (FM)	45°33′58.6"N	7°17′20.6"E	3070	—	5
Pelerins	Mont Blanc, Graian Alps	G	PEL*		*Vertagopus glacialis* sp. nov.	FR	August 23, 2022	Under stones on bare ice	45°53′43.9"N	6°53′04.9"E	2320	—	4
Orny	Mont Blanc, Graian Alps	G	ORN*		*Vertagopus glacialis* sp. nov.	CH	August 17, 2022	Under stones on bare ice	45°59′59.8"N	7°04′03.7"E	2700	—	1
Miage*	Mont Blanc, Graian Alps	G	MIA/ MIAS		*Vertagopus alpinus* Haybach, 1972	IT	September 08, 2020	In supraglacial debris (FM)	45°46′52.6"N	6°52′02.3"E	2080	Morph. Ident. and notes	10
Mer de Glace	Mont Blanc, Graian Alps	G	MDG		*Vertagopus alpinus* Haybach, 1972	FR	August 12, 2021	In supraglacial debris (FM)	45°54′44.5"N	6°56′10.4"E	2040	Morph. Ident. and notes	10
Pasterze	Grossglockner, Western Tauern Alps	G	PAS	*Vertagopus alpinus* Haybach, 1972	*Vertagopus alpinus* Haybach, 1972	AT	August 01, 2021	In supraglacial debris (FM)	47°05′13.9"N	12°43′ 24.9"E	2340	Morph. Ident. and notes	6
Morteratsch	Bernina, Wester Rhaetian Alps	G	MOR*	*Vertagopus helveticus* (Haybach, 1980)	*Vertagopus alpinus* Haybach, 1972	CH	August 25, 2021	Under stones on bare ice	46°24′55.5"N	9°56′04.5"E	2200–2300	Morph. Ident. and notes	1
Pelerins	Mont Blanc, Graian Alps	G	PEL*		*Vertagopus alpinus* Haybach, 1972	FR	August 23, 2022	Under stones on bare ice	45°53′43.9"N	6°53′04.9"E	2,320		4
Lang gletscher	Bernese Alps	G	VAL20*		*Vertagopus alpinus* Haybach, 1972	CH	August 10, 2021	Under stones on bare ice	46°27′28.3"N	7°55′55.8"E	2450–2550	—	1
Pièce	Pennine Alps	G	PIE20*		*Vertagopus alpinus* Haybach, 1972	CH	August 17, 2021	Under stones on bare ice	45°59′59.7"N	7°28′12.2"E	2750–2800	—	1
Glacier Noir	Ecrins, Dauphiné Alps	G	GLN		*Vertagopus glacieinigrae* sp. nov.	FR	August 9, 2021	In supraglacial debris (FM)	44°55′11.3"N	6°23′37.8"E	2240	Morph. Descr.	6
Vedretta della Fradusta	Pale San Martino Dolomites, Southern-Eastern Alps	G	FRA		*Vertagopus fradustaensis* sp. nov.	IT	August 8, 2023	Under stones on bare ice and in ice fractures	46°15′06.8"N	11°52′ 21.4"E	2845	Morph. Descr.	3
Pré de Bar	Mont Blanc, Graian Alps, Western Alps	G	PDB		*Desoria saltans* Nicolet, 1841	IT	July 25, 2021	Under stones on bare ice	45°54′08.9"N	7°03′04.2"E	2700	Morph. Ident. and notes	10
Glacier Blanc	Ecrins, Dauphiné Alps	G	GLB		*Desoria saltans* Nicolet, 1841	FR	August 08, 2021	In ice fractures	44°56′46.6"N	6°23′48.8"E	2970	Morph. Ident. and notes	8
Scais	Orobian Alps	G	SCA		*Desoria saltans* Nicolet, 1841	IT	September 18, 2020	In supraglacial debris (FM)	46°03′52.8"N	9°58′56.1"E	2820	—	5

Table A1: Continued.

Site names	Geographic area	Land form	Code	Historical reports for the same glaciers	Species name	Country	Sampling date	Microhabitat	Latitude	Longitude	Sampling altitude [m asl]	Morphology	Specimens sequenced
Fellaria Ovest	Bernina, Wester Rhaetian Alps	G	FEL*		*Desoria saltans* Nicolet, 1841	IT	September 14, 2022	Under stones on bare ice	46° 20′ 51” N	9°56′09” E	2840	—	1
Peirabroc	Maritime Alps	G	PEI		*Desoria* cf. *saltans*	IT	September 14, 2020	In supraglacial debris (FM)	44°07′18.7"N	7°24′51.1"E	2540	—	1
Trobio Ovest	Orobian Alps	G	TRO		*Desoria orobica* sp. nov.	IT	August 10, 2023	In supraglacial debris (FM) and under stone on bare ice	46°03′16"N	10°05′11"E	2550	Morph. Descr.	—
Trobio Est	Orobian Alps	G	TRO		*Desoria orobica* sp. nov.	IT	July 18, 2020	In debris at the snow patch border (FM)	46°03′25.5"N	10°05′ 30.0"E	2700	Morph. Descr.	5
Tour	Mont Blanc, Graian Alps	G	TOU*		*Gnathisotoma bicolor* Cassagnau, 1957	FR	August 24, 2022	Under stones on bare ice	45°59′44.8"N	6°59′07.4"E	2660	Morph. Ident. and notes	1
Miage	Mont Blanc, Graian Alps	G	MIA		*Isotomidae* sp.	IT	September 8, 2020	In supraglacial debris (FM)	45°46′52.6"N	6°52′02.3"E	2080	—	2
Aletsch	Bernese Alps	G			NF	CH	August 1, 2021	Under stones on bare ice	46°26′36.2"N	8°04′41.5"E	2340	—	—
Passo Valsecca	Orobian Alps	N			NF	IT	September 19, 2020	On snow and in the stony debris on the border of the snow (FM)	46°02′22.8"N	9°54′22.2"E	2390	—	—
Lago della Malgina	Orobian Alps	N			NF	IT	July 17, 2020	On snow and in the stony debris on the border of the snow (FM)	46°04′49.5"N	10°03′ 01.8"E	2570	—	—
Secreti	Orobian Alps	N			NF	IT	September 18, 2020	On snow and in the stony debris on the border of the snow (FM)	46°03′28.3"N	9°58′54.8"E	2700	—	—
Mandrone	Adamello, Southern Rhaetian Alps	G			NF	IT	August 25, 2020 and August 2023	Under stones on bare ice	46°11′5.80″N	10°33′ 34.67″E	2606	—	—
Marmolada	Marmolada Dolomites, Southern-Eastern Alps	G			NF	IT	September 3, 2020	Under stones on bare ice	46°26′21.3"N	11°51′ 49.4"E	2900	—	—
Vedretta Piana	Ortles-Cevedale, Southern Rhaetian Alps	G			NF	IT	August 19, 2020	Under stones on bare ice	46°30′29.6"N	10°28′ 06.7"E	3200	—	—
Ventina	Valmalenco, Southern Rhaetian Alps	G			NF	IT	September 5, 2020	Under stones on bare ice and in supraglacial debris (FM)	46°17′08.9"N	9°46′52.2"E	2100	—	—
Belvedere	Monte Rosa, Pennine Alps	G			NF	IT	August 10, 2020	In supraglacial debris (FM)	45°57′33.5"N	7°54′47.0"E	2040	—	—
Cima Uomo	Marmolada Dolomites, Southern Rhaetian Alps	G			NF	IT	August 25, 2020	In supraglacial debris (FM)	46°24′33.5"N	11°48′ 21.1"E	2550	—	—
Lazaunkar	Val Senales, Southern Rhaetian Alps	RG			NF	IT	August 6, 2020	Ice not found, too deep	46°44′50.3"N	10°45′ 23.1"E	2540	—	—
Rock glacier Amola	Adamello, Southern Rhaetian Alps	RG			NF	IT	August 22, 2020	Ice not found, too deep	46°12′11.5"N	10°42′ 11.0"E	2460	—	—

Table A1: Continued.

	Site names	Geographic area	Land form	Code	Historical reports for the same glaciers	Species name	Country	Sampling date	Microhabitat	Latitude	Longitude	Sampling altitude [m asl]	Morphology	Specimens sequenced
Ap	Calderone	Gran Sasso, Apennines	G	CAL	*Desoria calderonis* Valle, 2021	*Desoria calderonis* Valle, 2021	IT	July 8, 2020	In supraglacial debris (FM)	42°28′16.2″ N	13°34′05.8″ E	2700	(Valle et al. 2021, [18])	8
Py	Troumouse	Gavarnie-Gédre, Pyrenées	N	TGN	*Gnathisotoma bicolor* Cassagnau, 1957	*Gnathisotoma bicolor* Cassagnau, 1957	FR	July 8, 2021	Stony debris at the border of a snowpatch, along the glacial stream (FM)	42°43′14.3"N	0°07′32.8"E	2500	Morph. Ident. and notes	9
	Tourettes	Gavarnie-Gédre, Pyrenées	G	TRS	—	*Gnathisotoma bicolor* Cassagnau, 1957	FR	August 2023	Supraglacial debris (FM)	42°41′59.1"N	0°02′ 41.4"W	2470	Morph. Ident.	—

Abbreviations: Al, European Alps; Ap, Apennines; Descr., description; FM, sampled with flotation method; G, glacier; Ident., Identification; Morph., morphological; N, snow-patch; Py, Pyrenées; RG, rock glacier. '*' is associated with glaciers where more than one species occurs. Nomenclature of Alpine sectors follows SOIUSA [85].

Table A2: Body measurements (μm). Mandible was measured from apex to molar plate, included.

		Head (dorsal)	Head and body	Ant I	Ant II	Ant III	Ant IV	Antenna	Head, body and antenna	Cephalic diagonal	Furca	Man	Dens	Mucro	Mac (Abd. V)	Abd. V	Inner edge of Claw3	Mandible
	Mean value	314	1490	64	97	89	176	427	1922	381	447	168	267	11	53	92	39	85
Vertagopus psychrophilus sp. nov.	St.dev	46	291	7	9	9	16	36	308	20	30	19	30	1	5	12	2	8
	N° of measurements	12	11	13	13	13	13	13	11	3	11	12	12	11	13	13	13	7
	Mean value	341	1729	64	102	94	190	451	2175	416	447	172	267	11	51	96	42	88
Vertagopus glacialis sp. nov.	St.dev	31	188	10	11	11	16	42	210	36	38	22	24	1	6	11	4	9
	N° of measurements	29	29	33	33	33	33	33	29	5	29	31	29	31	30	28	29	14
	Mean value	284	1296	53	90	85	153	380	1676	335	461	151	297	12	62	63	29	69
Vertagopus glacieinigrae sp. nov.	St.dev	22	100	5	12	11	14	37	118	18	42	15	28	1	7	9	4	8
	N° of measurements	10	10	10	10	10	10	10	10	3	10	10	10	10	9	9	8	7
	Mean value	403	1864	68	119	109	233	529	2393	484	665	230	421	15	59	102	50	123
Desoria saltans	St.dev	38	178	3	7	5	18	21	183	52	59	25	40	1	5	6	3	6
	N° of measurements	10	10	10	10	10	10	10	10	4	10	10	10	10	10	10	10	6
	Mean value	288	1420	53	76	71	142	342	1757	330	473	154	304	11	36	73	33	77
Vertagopus alpinus	St.dev	24	157	5	3	3	12	17	173	17	62	28	37	1	5	14	5	9
	N° of measurements	12	11	9	9	9	9	9	11	3	12	9	9	9	9	9	9	4
	Mean value	293	1584	55	92	89	171	407	1992	323	503	182	311	10	55	88	32	85
Vertagopus fradustaensis sp. nov.	St.dev	16	96	5	8	6	19	34	132	18	26	18	17	—	4	7	2	8
	N° of measurements	9	9	7	7	7	7	7	7	9	9	9	9	1	6	5	8	5
	Mean value	275	1463	50	75	72	152	341	1812	—	415	140	265	9	58	98	28	77
Desoria orobica sp. nov.	St.dev	17	136	7	9	6	11	20	161	—	27	20	13	1	9	12	2	2
	N° of measurements	7	7	7	7	7	7	7	6	—	7	7	7	7	6	7	7	2

Table A3: GenBank accession numbers of each specimen and species sequenced for this work (*cox1* and *16S*).

Specimen	Species	*cox1*	*16S*	Specimen	Species	*cox1*	*16S*
AGO_1	*Vertagopus psychrophilus*	PP441687	PP465209	MOI_6	*Vertagopus glacialis*	PP441804	PP465326
AGO_2	*Vertagopus psychrophilus*	PP441688	PP465210	MOI_7	*Vertagopus glacialis*	PP441805	PP465327
AGO_3	*Vertagopus psychrophilus*	PP441689	PP465211	MOI_8	*Vertagopus glacialis*	PP441806	PP465328
AGO_4	*Vertagopus psychrophilus*	PP441690	PP465212	MOI_9	*Vertagopus glacialis*	PP441807	PP465329
AGO_5	*Vertagopus psychrophilus*	PP441691	PP465213	MOR_11	*Vertagopus glacialis*	PP441808	PP465330
AGO_6	*Vertagopus psychrophilus*	PP441692	PP465214	MOR_16	*Vertagopus glacialis*	PP441809	PP465331
AGO_7	*Vertagopus psychrophilus*	PP441693	PP465215	MOR_18	*Vertagopus alpinus*	PP441810	PP465332
AGO_8	*Vertagopus psychrophilus*	PP441694	PP465216	MOR_19	*Vertagopus glacialis*	PP441811	PP465333
AGO_9	*Vertagopus psychrophilus*	PP441695	PP465217	MOR_20	*Vertagopus glacialis*	PP441812	PP465334
AMO_1	*Vertagopus glacialis*	PP441696	PP465218	OBE_1	*Vertagopus glacialis*	PP441813	PP465335
AMO_10	*Vertagopus glacialis*	PP441697	PP465219	OBE_2	*Vertagopus glacialis*	PP441814	PP465336
AMO_2	*Vertagopus glacialis*	PP441698	PP465220	OBE_3	*Vertagopus glacialis*	PP441815	PP465337
AMO_3	*Vertagopus glacialis*	PP441699	PP465221	OBE_4	*Vertagopus glacialis*	PP441816	PP465338
AMO_4	*Vertagopus glacialis*	PP441700	PP465222	OBE_5	*Vertagopus glacialis*	PP441817	PP465339
AMO_5	*Vertagopus glacialis*	PP441701	PP465223	OBE_6	*Vertagopus glacialis*	PP441818	PP465340
AMO_6	*Vertagopus glacialis*	PP441702	PP465224	ORN_21	*Vertagopus psychrophilus*	PP441819	PP465341
AMO_7	*Vertagopus glacialis*	PP441703	PP465225	ORN_22	*Vertagopus psychrophilus*	PP441820	PP465342
AMO_8	*Vertagopus glacialis*	PP441704	PP465226	ORN_23	*Vertagopus psychrophilus*	PP441821	PP465343
AMO_9	*Vertagopus glacialis*	PP441705	PP465227	ORN_24	*Vertagopus psychrophilus*	PP441822	PP465344
BRE_1	*Vertagopus glacialis*	PP441706	PP465228	ORN_26	*Vertagopus psychrophilus*	PP441823	PP465345
BRE_2	*Vertagopus glacialis*	PP441707	PP465229	ORN_27	*Vertagopus glacialis*	PP441824	PP465346
BRE_3	*Vertagopus psychrophilus*	PP441708	PP465230	ORN_31	*Vertagopus psychrophilus*	PP441825	PP465347
BRE_4	*Vertagopus glacialis*	PP441709	PP465231	PAS_17	*Vertagopus alpinus*	PP441826	PP465348
CAL_1	*Desoria calderonis*	PP441710	PP465232	PAS_18	*Vertagopus alpinus*	PP441827	PP465349
CAL_10	*Desoria calderonis*	PP441711	PP465233	PAS_19	*Vertagopus alpinus*	PP441828	PP465350
CAL_2	*Desoria calderonis*	PP441712	PP465234	PAS_20	*Vertagopus alpinus*	PP441829	PP465351
CAL_3	*Desoria calderonis*	PP441713	PP465235	PAS_21	*Vertagopus alpinus*	PP441830	PP465352
CAL_4	*Desoria calderonis*	PP441714	PP465236	PAS_22	*Vertagopus alpinus*	PP441831	PP465353
CAL_5	*Desoria calderonis*	PP441715	PP465237	PDB_1	*Desoria saltans*	PP441832	PP465354
CAL_6	*Desoria calderonis*	PP441716	PP465238	PDB_10	*Desoria saltans*	PP441833	PP465355
CAL_8	*Desoria calderonis*	PP441717	PP465239	PDB_11	*Desoria saltans*	PP441834	PP465356
CAL_9	*Desoria calderonis*	PP441718	PP465240	PDB_12	*Desoria saltans*	PP441835	PP465357
DOS_1	*Vertagopus psychrophilus*	PP441719	PP465241	PDB_13	*Desoria saltans*	PP441836	PP465358
DOS_10	*Vertagopus psychrophilus*	PP441720	PP465242	PDB_14	*Desoria saltans*	PP441837	PP465359
DOS_2	*Vertagopus psychrophilus*	PP441721	PP465243	PDB_2	*Desoria saltans*	PP441838	PP465360
DOS_3	*Vertagopus psychrophilus*	PP441722	PP465244	PDB_3	*Desoria saltans*	PP441839	PP465361
DOS_4	*Vertagopus psychrophilus*	PP441723	PP465245	PDB_4	*Desoria saltans*	PP441840	PP465362
DOS_5	*Vertagopus psychrophilus*	PP441724	PP465246	PDB_5	*Desoria saltans*	PP441841	PP465363
DOS_6	*Vertagopus psychrophilus*	PP441725	PP465247	PEI_4	*Desoria* cf *saltans*	PP441842	PP465364
DOS_7	*Vertagopus psychrophilus*	PP441726	PP465248	PEL_11	*Vertagopus alpinus*	PP441843	PP465365
DOS_8	*Vertagopus psychrophilus*	PP441727	PP465249	PEL_12	*Vertagopus alpinus*	PP441844	PP465366

Table A3: Continued.

Specimen	Species	*cox1*	*16S*	Specimen	Species	*cox1*	*16S*
DOS_9	*Vertagopus psychrophilus*	PP441728	PP465250	PEL_13	*Vertagopus alpinus*	PP441845	PP465367
FEL_1	*Vertagopus psychrophilus*	PP441729	PP465251	PEL_14	*Vertagopus alpinus*	PP441846	PP465368
FEL_2	*Desoria saltans*	PP441730	PP465252	PEL_30	*Vertagopus glacialis*	PP441847	PP465369
FEL_3	*Vertagopus psychrophilus*	PP441731	PP465253	PEL_31	*Vertagopus glacialis*	PP441848	PP465370
FRA_2	*Vertagopus fradustaensis*	PP441732	PP465254	PEL_34	*Vertagopus glacialis*	PP441849	PP465371
FRA_3	*Vertagopus fradustaensis*	PP441733	PP465255	PEL_36	*Vertagopus glacialis*	PP441850	PP465372
FRA_4	*Vertagopus fradustaensis*	PP441734	PP465256	PER_1	*Vertagopus glacialis*	PP441851	PP465373
GFO_1	*Vertagopus glacialis*	PP441735	PP465257	PER_2	*Vertagopus glacialis*	PP441852	PP465374
GFO_10	*Vertagopus glacialis*	PP441736	PP465258	PER_3	*Vertagopus glacialis*	PP441853	PP465375
GFO_2	*Vertagopus glacialis*	PP441737	PP465259	PER_4	*Vertagopus glacialis*	PP441854	PP465376
GFO_3	*Vertagopus glacialis*	PP441738	PP465260	PER_5	*Vertagopus glacialis*	PP441855	PP465377
GFO_6	*Vertagopus glacialis*	PP441739	PP465261	PER_6	*Vertagopus glacialis*	PP441856	PP465378
GFO_7	*Vertagopus glacialis*	PP441740	PP465262	PER_7	*Vertagopus glacialis*	PP441857	PP465379
GFO_8	*Vertagopus glacialis*	PP441741	PP465263	PER_8	*Vertagopus glacialis*	PP441858	PP465380
GIO_10	*Vertagopus glacialis*	PP441742	PP465264	PIE_1	*Vertagopus glacialis*	PP441859	PP465381
GIO_11	*Vertagopus glacialis*	PP441743	PP465265	PIE_10	*Vertagopus glacialis*	PP441860	PP465382
GIO_12	*Vertagopus glacialis*	PP441744	PP465266	PIE_2	*Vertagopus glacialis*	PP441861	PP465383
GIO_13	*Vertagopus glacialis*	PP441745	PP465267	PIE_20	*Vertagopus alpinus*	PP441862	PP465384
GIO_14	*Vertagopus glacialis*	PP441746	PP465268	PIE_3	*Vertagopus glacialis*	PP441863	PP465385
GIO_15	*Vertagopus glacialis*	PP441747	PP465269	PIE_4	*Vertagopus glacialis*	PP441864	PP465386
GIO_16	*Vertagopus glacialis*	PP441748	PP465270	PIE_5	*Vertagopus glacialis*	PP441865	PP465387
GIO_4	*Vertagopus glacialis*	PP441749	PP465271	PIE_6	*Vertagopus glacialis*	PP441866	PP465388
GIO_5	*Vertagopus glacialis*	PP441750	PP465272	PIE_7	*Vertagopus glacialis*	PP441867	PP465389
GLB_13	*Desoria saltans*	PP441751	PP465273	PIE_8	*Vertagopus glacialis*	PP441868	PP465390
GLB_14	*Desoria saltans*	PP441752	PP465274	PIE_9	*Vertagopus glacialis*	PP441869	PP465391
GLB_15	*Desoria saltans*	PP441753	PP465275	PRE_12	*Vertagopus psychrophilus*	PP441870	PP465392
GLB_16	*Desoria saltans*	PP441754	PP465276	PRE_13	*Vertagopus psychrophilus*	PP441871	PP465393
GLB_17	*Desoria saltans*	PP441755	PP465277	PRE_14	*Vertagopus psychrophilus*	PP441872	PP465394
GLB_18	*Desoria saltans*	PP441756	PP465278	PRE_15	*Vertagopus psychrophilus*	PP441873	PP465395
GLB_19	*Desoria saltans*	PP441757	PP465279	PRE_16	*Vertagopus psychrophilus*	PP441874	PP465396
GLB_20	*Desoria saltans*	PP441758	PP465280	PRE_17	*Vertagopus psychrophilus*	PP441875	PP465397
GLN_12	*Vertagopus glacieinigrae*	PP441759	PP465281	RUT_1	*Vertagopus psychrophilus*	PP441876	PP465398
GLN_13	*Vertagopus glacieinigrae*	PP441760	PP465282	RUT_2	*Vertagopus psychrophilus*	PP441877	PP465399
GLN_14	*Vertagopus glacieinigrae*	PP441761	PP465283	RUT_3	*Vertagopus psychrophilus*	PP441878	PP465400
GLN_15	*Vertagopus glacieinigrae*	PP441762	PP465284	RUT_4	*Vertagopus psychrophilus*	PP441879	PP465401
GLN_16	*Vertagopus glacieinigrae*	PP441763	PP465285	RUT_5	*Vertagopus psychrophilus*	PP441880	PP465402
GLN_17	*Vertagopus glacieinigrae*	PP441764	PP465286	SCA_1	*Desoria saltans*	PP441881	PP465403
LAU_1	*Vertagopus glacialis*	PP441765	PP465287	SCA_2	*Desoria saltans*	PP441882	PP465404
LAU_2	*Vertagopus glacialis*	PP441766	PP465288	SCA_3	*Desoria saltans*	PP441883	PP465405
LAU_3	*Vertagopus glacialis*	PP441767	PP465289	SCA_4	*Desoria saltans*	PP441884	PP465406
LAU_4	*Vertagopus glacialis*	PP441768	PP465290	SCA_5	*Desoria saltans*	PP441885	PP465407

Table A3: Continued.

Specimen	Species	*cox1*	*16S*	Specimen	Species	*cox1*	*16S*
LAU_5	*Vertagopus glacialis*	PP441769	PP465291	SFO_10	*Vertagopus psychrophilus*	PP441886	PP465408
MDG_12	*Vertagopus alpinus*	PP441770	PP465292	SFO_3	*Vertagopus psychrophilus*	PP441887	PP465409
MDG_13	*Vertagopus alpinus*	PP441771	PP465293	SFO_4	*Vertagopus psychrophilus*	PP441888	PP465410
MDG_14	*Vertagopus alpinus*	PP441772	PP465294	SFO_5	*Vertagopus psychrophilus*	PP441889	PP465411
MDG_15	*Vertagopus alpinus*	PP441773	PP465295	SFO_6	*Vertagopus psychrophilus*	PP441890	PP465412
MDG_16	*Vertagopus alpinus*	PP441774	PP465296	SFO_7	*Vertagopus psychrophilus*	PP441891	PP465413
MDG_17	*Vertagopus alpinus*	PP441775	PP465297	SFO_8	*Vertagopus psychrophilus*	PP441892	PP465414
MDG_18	*Vertagopus alpinus*	PP441776	PP465298	SFO_9	*Vertagopus psychrophilus*	PP441893	PP465415
MDG_19	*Vertagopus alpinus*	PP441777	PP465299	TGN_12	*Gnathisotoma bicolor*	PP441894	PP465416
MDG_20	*Vertagopus alpinus*	PP441778	PP465300	TGN_13	*Gnathisotoma bicolor*	PP441895	PP465417
MDG_21	*Vertagopus alpinus*	PP441779	PP465301	TGN_14	*Gnathisotoma bicolor*	PP441896	PP465418
MIAC_10	*Vertagopus alpinus*	PP441780	PP465302	TGN_15	*Gnathisotoma bicolor*	PP441897	PP465419
MIAC_11	*Vertagopus alpinus*	PP441781	PP465303	TGN_16	*Gnathisotoma bicolor*	PP441898	PP465420
MIAC_6	*Vertagopus alpinus*	PP441782	PP465304	TGN_17	*Gnathisotoma bicolor*	PP441899	PP465421
MIAC_9	*Vertagopus alpinus*	PP441783	PP465305	TGN_18	*Gnathisotoma bicolor*	PP441900	PP465422
MIAS_6	*Vertagopus alpinus*	PP441784	PP465306	TGN_19	*Gnathisotoma bicolor*	PP441901	PP465423
MIAS_8	*Vertagopus alpinus*	PP441785	PP465307	TGN_20	*Gnathisotoma bicolor*	PP441902	PP465424
MIAS_9	*Vertagopus alpinus*	PP441786	PP465308	TOU_1	*Vertagopus psychrophilus*	PP441903	PP465425
MIA_1	*Isotomidae* sp.	PP441787	PP465309	TOU_14	*Gnathisotoma bicolor*	PP441904	PP465426
MIA_2	*Vertagopus alpinus*	PP441788	PP465310	TOU_2	*Vertagopus psychrophilus*	PP441905	PP465427
MIA_3	*Vertagopus alpinus*	PP441789	PP465311	TOU_3	*Vertagopus psychrophilus*	PP441906	PP465428
MIA_4	*Vertagopus alpinus*	PP441790	PP465312	TOU_4	*Vertagopus psychrophilus*	PP441907	PP465429
MIA_5	*Isotomidae* sp.	PP441791	PP465313	TOU_5	*Vertagopus psychrophilus*	PP441908	PP465430
MIT_1	*Vertagopus glacialis*	PP441792	PP465314	TRO_1	*Desoria orobica*	PP441909	PP465431
MIT_2	*Vertagopus glacialis*	PP441793	PP465315	TRO_18	*Desoria orobica*	PP441910	PP465432
MIT_3	*Vertagopus glacialis*	PP441794	PP465316	TRO_3	*Desoria orobica*	PP441911	PP465433
MIT_4	*Vertagopus glacialis*	PP441795	PP465317	TRO_4	*Desoria orobica*	PP441912	PP465434
MIT_5	*Vertagopus glacialis*	PP441796	PP465318	TRO_5	*Desoria orobica*	PP441913	PP465435
MIT_6	*Vertagopus glacialis*	PP441797	PP465319	VAL_11	*Vertagopus glacialis*	PP441914	PP465436
MOI_1	*Vertagopus glacialis*	PP441798	PP465320	VAL_12	*Vertagopus glacialis*	PP441915	PP465437
MOI_10	*Vertagopus glacialis*	PP441799	PP465321	VAL_13	*Vertagopus glacialis*	PP441916	PP465438
MOI_2	*Vertagopus glacialis*	PP441800	PP465322	VAL_14	*Vertagopus glacialis*	PP441917	PP465439
MOI_3	*Vertagopus glacialis*	PP441801	PP465323	VAL_15	*Vertagopus glacialis*	PP441918	PP465440
MOI_4	*Vertagopus glacialis*	PP441802	PP465324	VAL_16	*Vertagopus glacialis*	PP441919	PP465441
MOI_5	*Vertagopus glacialis*	PP441803	PP465325	VAL_20	*Vertagopus alpinus*	PP441920	PP465442

Data Availability Statement

Specimens collected for this work and preserved in alcohol are available in Museum collections (see Material and Methods). Genetic sequences are available on GenBank (see Material and Methods and Appendices).

Conflicts of Interest

The authors have no conflicts of interest to declare.

Author Contributions

Barbara Valle conceived of the idea, directed the project, collected samples, performed morphological and molecular analysis and wrote the draft. Marco Caccianiga and Mauro Gobbi conceived of the idea, collected samples and wrote the draft. Claudio Cucini, Francesco Nardi, Sara Boschi and Giovanni Barbon performed molecular and genetic analysis. Roberto Ambrosini, Jakub Buda, Silvio Marta, Virginia Toscano Rivalta, Riccardo Scotti and Anaïs Zimmer collected samples. Ľubomír Kováč supervise the morphological analysis. Roberto Ambrosini, Marco Caccianiga, Gentile Francesco Ficetola, Francesco Frati and Ľubomír Kováč gave financial support to the project. All authors discussed the results and contributed to the final manuscript.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*) Figure S1: Maximum likelihood phylogenetic tree of newly described Alpine's springtail species using the BOLD dataset available for *cox1* gene. Figure S2: Pairwise genetic distance heatmap with values reported. Figures S3–S5: scanning electron microscopy pictures of *Vertagopus psychrophilus*, *V. glacialis* and *Desoria orobica* respectively. Table ST1: collapsed haplotype.

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